STUDIES ON THE REPRODUCTIVE BIOLOGY OF
NEW ZEALAND FRESHWATER EELS

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ABSTRACT

Macroscopic and histological observations of the gonads from 1,739 non-migrant freshwater eels, the shortfin Anquilla australis schmidtii Phillipps and the longfin A. dieffenbachii Gray, showed that they pass through seven stages of development. Shortfins become sexually differentiated at body lengths of 35.0cm to 56.9cm and longfins at lengths of 50.0cm to 67.0cm. No intersexual stage was present, as in A. anquilla L., and although 1% of 350 migrating longfin males examined contained ribbon-like testes, the typical lobed organ of Syrski (testis) can be used as diagnostic of maleness.

Histologically, the maximum stage of development attained in the non-migrant, immature stage, was spermatogonia in the males and vacuolated oocytes in females. At the time of seaward migration, based on gonad histology, gonadosomatic indices and ova diameters, migrating longfins were more sexually developed than shortfins. These differences may relate to the location of different oceanic spawning areas: that for the longfin possibly being closer to New Zealand.

The autumnal migratory runs, from March to May, of the sexually maturing adults in the Makara stream showed no particular species or sex sequence. The movement of eels was coincident with a rise in stream level and the second half of the lunar cycle. Other relevant environmental factors are discussed. In Lake Onoke peak catches of seaward migrating shortfins were made before the longfins and movements of eels occurred throughout the lunar cycle. Once at sea, the eels apparently disappear. A published note is included on the first eel of the New Zealand species, a longfin female, to be caught at sea.

Age determinations from 995 eels were made by otoliths, which were burnt lightly to intensify the growth zones for reading purposes. Shortfin males are younger than females at migration. Longfins are older than shortfins at migration but the males are younger than the females. In the non-migrant stage, sexually undifferentiated shortfins grow more slowly relative to the males, and males relatively more slowly than the females. Similar but less significant differences in growth occur in longfins.

Migrant males held in seawater were induced to mature and spawn with injections of mammalian hormones or carp pituitaries, over temperatures of 11.8°C to 28°C. The maturation period was dependent on temperature. Testes of experimental eels that survived maturation regressed to the pre-migrant or migrant stage. Two eels that had regressed were induced to mature a second time.

Females held at 20°C and injected with mammalian hormones showed significant increases in sexual development but died before maturity. Females injected with carp pituitaries matured and spawned. Mature longfin eggs, 0.9mm to 1.2mm in diameter, and mature shortfin eggs, 0.9mm to 1.2mm in diameter, are translucent and contain one to many oil globules. A blastodisc formed in water hardened eggs but attempts at fertilization were unsuccessful.

Gametogenesis, observed from non-migrant, migrant and hormone injected eels is similar to that described for other teleosts.

Electron microscope observations showed parallel features of spermiogenesis in both species. Mature spermatozoa have crescent shaped heads with an anteriorly placed mitochondrion. A flagellum of the unusual 9 + 0 pattern arises from the posterior region of the head, and a short, striated rod-like structure is positioned adjacent to the main flagellum. A complex of subfibrils which extend along either side of the head to the mitochondrion arise from the proximal centriole.

INTRODUCTION

The genus Anquilla Shaw is represented throughout the world by approximately 15 species of temperate and inter-tropical fresh-water eels. The systematics of this genus have been extensively studied by Ege (1939) who recognized six species present in the south-west Pacific, including three Australasian temperate species.

Archey (1924) recognized two species of eels in New Zealand but later Phillipps (1926) recognized four. Schmidt (1927), based on the number of vertebrae and the origin of the dorsal fin relative to the position of the vent, recognized only two species, the long-finned Anquilla aucklandii Richmond and the short-finned Anquilla australis Richmond forma orientalis Johns Schmidt. Griffin (1936) revised the classification and recognized that two species occur in New Zealand, the Chatham Islands and Auckland Islands: the short-finned Anquilla australis schmidtii Phillipps and the long-finned Anquilla dieffenbachii Gray. A. australis schmidtii also occurs in Norfolk Island, New Caledonia, Fiji and possibly Tahiti. The Australian subspecies A. australis australis Richardson occurs in New South Wales, Victoria, Tasmania and Lord Howe Island (Castle 1963). A key to the two New Zealand species is given by Griffin (1936) and Cairns (1941) lists the distinguishing features of the two species.

Schmidt (1928: 382) suggested that the English names long-finned eel and short-finned eel be used when referring to the two New Zealand species. For brevity the names longfin and shortfin are used in this study.

Within New Zealand the shortfin eel is typically found in the more stable streams, coastal lagoons, estuaries and some inland lakes. It appears to favour areas where rapid temperature changes do not occur (Cairns 1941). In contrast, the longfin eel occurs almost everywhere. Burnet (1969c) considers that the longfin eel is the most widely spread and the most abundant of the two species, and that this distribution appears to be related to temperature tolerance.

The basic biology of the two species is similar to that of the other temperate species, the European eel Anquilla anquilla (L.), the American eel Anquilla rostrata (LeSuer) and the Japanese eel Anquilla japonica Temminck and Schlegel.

A detailed account of the biology of the European eel is given by Bertin (1956) and it is compared with that of the other species.

For pre-1967 references on eels of the genus Anquilla Eale's (1967) bibliography was useful. McDowall's (1964) bibliography gives pre-1964 references on New Zealand eels.

In contrast to the European eel, the biology of the New Zealand eels has not been extensively studied. Little contribution to the understanding of the biology was made by some early authors (e.g. Travers 1871, Duigan 1875 and Canavan 1892). Downes (1917) published the first substantial information on the biology which described in detail the autumn migration of eels to the sea. Schmidt (1925, 1927, 1928), the Danish eel researcher who located the spawning grounds of the European and American eels, made some contribution to the biology, in particular the taxonomy, after his visit to New Zealand and Australia in 1926. However, it was not until Cairns (1941, 1942a, 1942b) reported on the life history of New Zealand eels that details of the basic biology, including distribution, size range, migration, age and growth and feeding were established. Since then several studies on the biology have been undertaken. Hobbs (1947) studied the seaward migration of eels from a coastal lake and Burnet (1952a, 1952b, 1955, 1968, 1969a, 1969b, 1969c, 1969d) has reported on the ecology of the long-finned eel, the inter-relationship between eels and trout, growth studies and the downstream migration of maturing adults.

However, much of the emphasis of these studies has been with regard to the inter-relationship with and effect of eels on trout. The research by Cairns was principally conducted so that better management could be made of trout stocks. Subsequently extensive eel eradication campaigns were carried out to reduce the damage to trout stocks supposedly caused by eels.

Little study has been done on the reproductive biology of New Zealand eels. The downstream and seaward migration of maturing adults has been reported by Downes (1917), Cairns (1941), Hobbs (1947) and Burnet (1969a), but only the latter author relates this migration to the prevailing environmental factors that might affect it.

Cairns (1942b) gives the only, brief description of the gonads in eels of both sexes but he does not distinguish between the two species. Non-migrant eels have been sexed by Burnet (1952b) and

Woods (1964) but these identifications were not confirmed by microscopic or histological analyses. No histological studies have been made on the development of the reproductive organs in non-migrant eels or on the state of reproductive development of migrating adults.

Although the age and growth of eels has been investigated by McFarlane (1936), Cairns (1941) and Burnet (1969b), no specific study has been made of the age of migrating adult eels, nor has the growth of eels of each sex in the non-migrant stage been investigated.

The reproductive development of the European eel, which includes an intersexual stage (Bertin 1956), is complex. In contrast the Japanese eel lacks this stage (Sato, Nakamura and Hibiya 1962). The intention of this study was to establish the basic reproductive biology of both species, including the development of the gonads in non-migrant, immature eels, and the state of reproductive development of the migrating adults. The time of this migration and the environmental factors associated with it would also be investigated. The age and growth of eels of each sex would be examined and the age of migrating adults established. To study the complete reproductive cycle it was anticipated that migrant eels could be held in seawater aquaria and be induced to mature with hormone injections, following the technique used by Fontaine, Bertrand, Lopez and Callamand (1964) and Boëtius and Boëtius (1967a) on the European eel. By this method the mature spermatozoa and eggs might be observed and fertilization attempted.

The status of eels in New Zealand, at least amongst anglers and many laymen, has been that of a nuisance fish, and of no commercial value. Last century and early this century an intensive eel fishery was conducted by the Maoris. The Maori knowledge of the time of movements and migrations, methods of trapping and utilization of eels as a food resource was extensive. Schmidt (1925: 351) commented on the high degree of techniques developed by the Maoris for capturing eels. The Maori methods of fishing and utilization as a food have been described by Best (1902; 1929), Hamilton (1908), Downes (1917) and Schmidt (1925).

About the turn of the century the Maori fishery declined and until recently the eel stocks had remained virtually unexploited. The last ten years has seen a rapid increase in the development of the eel fishery. Apart from some catches immediately after the second world war the first annual eel catch recorded in the annual fishing statistics was 901 cwt in 1961. Exports remained static

until 1966 and then from 1967 they rose rapidly. By 1972 the eel catch reached 40,729 cwt and was the second most valuable wet fish export.

The rapid rise in the eel fishery prompted the need for further study of the basic biology of eels for the future management of the industry. This study was begun in April 1970, in part, for this purpose.

1. MATERIALS AND METHODS

This section describes the sampling areas methods of capture, handling of eels in the laboratory and the histological techniques used for the microscopic analysis of gonad samples. The materials and methods for each specific section, i.e. the technique used for ageing, the induction of maturation experiments, and the methods used in the electron microscope study of spermiogenesis are given separately.

The term eels in this study refers to fresh-water eels of the genus Anquilla unless otherwise stated. In table and figure captions A. dieffenbachii, and A. australis, when referring to A. australis schmidtii, are used. When discussing the other temperate species the common terms European, American or Japanese eel are quoted.

The term non-migrant eel is used when referring to feeding eels which do not, at this stage of the life cycle, migrate downstream and into the sea to spawn. Migrant eels is the term used for those eels that have ceased feeding and have undergone the distinctive changes, often referred to as the metamorphosis, to the migrating stage of the life cycle. That is they have attained the migration livery typical of this stage and migrate downstream and into the sea to spawn. The term mature eels used by some authors (e.g. Cairns, 1941, 1942a, 1942b; Shorland and Russell, 1948) when referring to migrating eels is misleading. The eels at migration are not mature as their gonads are in the early stage of development. Neither is the term immature eels correct when the same authors refer to non-migrant eels. Migrant eels, although in the first stages of rapid sexual development, could also be termed immature eels.

The terms immature and mature as used by Cairns, and Shorland and Russell mistakenly imply stages of sexual development which have not been adequately defined. Therefore these terms are considered to be misleading and incorrect. The use of the term "silver eel" or "silver belly" as used to describe migrating European eels and also used by many New Zealand fishermen is misleading. Longfin migrants are not silvery and although some shortfin migrants are silvery, many non-migrant shortfins also have this colouration.

1.1. Location of sampling areas

Samples of non-migrant eels of both species, ranging in body length from 10.0 cm to 145.0 cm were collected from three streams, the Makara stream, the Happy Valley stream and Waimeha stream. A sample of non-migrant shortfins was obtained from a coastal dune lake, Pukepuke Lagoon. Except for Pukepuke Lagoon, approximately 90 km from Wellington, the streams were situated within 60 km of the laboratory. Figs.1.1, 1.2 and 1.3 show the location of these sampling areas.

Migrant eels were caught in the Makara stream, Pukepuke Lagoon, and from another lake, Lake Onoke, which is also shown in Figs.1.1 and 1.2. Other samples of migrant eels were obtained from eel processing factories throughout New Zealand. In all but one instance these eels had been held in holding tanks and the exact time and body of water they were caught in could not be established. The location of the factories, which were within the general area of capture, is given in Fig.1.1

FIG.1.1. Location of sampling areas
within New Zealand.

Fig.1.2 Location of sampling areas
within the Wellington area.

Fig. 1.3. The Makara stream.

The Makara stream is a moderate sized stream running through steep hills and farmland to the west of Wellington (Fig.1.3). A brief description of the stream is given by McDowall (1965: 235). The stream has a catchment area of approximately 7802 ha which includes many small tributary streams. The largest part of the stream, approximately 7 km in length, flows through farmland to the sea. The flow is generally slow and uninterrupted, but is broken in parts by shallow rocky areas. The annual temperature variation is from 5.5°C in winter to 21.5°C in the summer. In parts there are deep pools with overhanging vegetation, and other areas with submerged weed, providing ideal habitats for eels. Eels of both species and all sizes were found in these different areas. The most accessible areas along the 7 km stretch of stream were fished.

The Happy Valley stream is a small rocky stream running through steep hills to the south of Wellington. It is similar to the Makara stream but is smaller and lacks the pools and overhanging and submerged vegetation. The lack of cover provides suitable habitat for small eels only. None larger than 57.0 cm in length were caught.

The Waimeha stream, situated approximately 60 km north of Wellington, is a slow flowing, muddy stream running through flat pasture and some residential land. A large swamp drains into it. There are deep pools and overhanging and submerged vegetation providing ideal cover for large eels. The largest eel, a 145.0 cm non-migrant longfin female, was taken from this stream.

As only two samples of non-migrant eels were obtained from the Happy Valley and Waimeha stream further details on their catchment area and physical features are not given.

The Waikato River is a large, slow flowing river in the North Island which drains Lake Taupo. It reaches the sea approximately 200 km north of its source.

Pukepuke Lagoon is a coastal dune lake of 15 ha situated approximately 90 km north of Wellington. The physical features and catchment area are given by Gibbs (1973).

Lake Raikokopu is a coastal dune lake similar to, and situated 5 km to the south of, Pukepuke Lagoon.

Lake Onoke is a coastal lake, 800 ha in area and with a catchment area of 341,794 ha, located to the west of Wellington. It has three main tributaries, the Ruamahanga River, the Turanganui River and the Ponui stream. The inflow from Lake Wairarapa, a large lake 10 km to the north, is controlled by flood gates. The lake is shallow, the depth ranging from 6m near the outlet to a few centimetres on the western mud flats. The average depth is only 0.5m at high water ordinary spring tide, but the depth varies depending on the tide and whether the outlet is blocked. On its southern edge the lake is separated from the Pacific Ocean by a narrow bank of gravel through which seawater percolates. During southerly storms waves break over the bar. Throughout the summer the outlet to the lake, situated about halfway along the bar, is closed. During the autumn when the lake level rises through increased inflow the outlet is opened mechanically. When opened the water flows swiftly out through a gap approximately 15m wide. During southerly storms the outlet usually closes. The outlet channel cut only halfway through the bar to the sea is shown in Fig.1.6.

Lake Ellesmere is a coastal lake similar to Lake Onoke, although larger, approximately 2590 ha. The physical features of the lake are given by Hobbs (1947).

1.2 Sampling technique

Non-migrant eels from the Makara, Happy Valley and Waimeha streams were caught using a pulsatory, direct current, earth return electric fishing machine. The design details and method of operation of this machine are given by Burnet (1967).

Single samples of eels of both species were taken from the

Happy Valley and Waimeha streams. For a period of 16 months, from August 1970 to December 1971, monthly samples of approximately 30 eels of each species were taken from various parts of the Makara stream, varying in distance from 2 km to 7 km from the sea. For the purposes of analysis these monthly samples were combined. One sample of shortfin non-migrants from the Pukepuke Lagoon was obtained from Dr.P.H.J.Castle. These eels had been caught in fyke nets. The number of eels caught from each locality and the method of capture is given in Table 1.1.

During the three migratory seasons, January to May 1971, 1972 and 1973, migrating eels were caught in different areas by several methods. The number of migrant eels of each sex and species from different areas is given in Table 1.2.

In the Makara stream migrant eels were caught by setting two fyke nets across the width of the stream (Fig.1.4), approximately 3 km upstream from the sea. The nets faced upstream to intercept the migrating eels moving downstream. Two wings attached to each net were placed at 45° to the net openings to direct the eels into the nets (Fig.1.5). The times the nets were set, and the number of visits and the number of eels caught is given in the section on migrant eels.

Migrant eels were caught in Lake Onoke by three methods. Two fyke nets were set in shallow areas nearest to the sea where the eels congregate during the migratory season. Other eels were caught by dragging a small beach seine net parallel to the beach in shallow areas of the lake nearest to the sea. The Maori fishing method of digging channels into the bar from areas of the lake nearest to the sea was also used. Short channels, approximately 1 m wide and 3m to 4m long, were dug into the bar where the outlet had been partly cut through to the sea (Fig.1.6). If the lake outlet was open the swift outgoing current prevented fishing in or near the outlet. The times and number of visits to Lake Onoke are given in the section on migrant eels.

Migrant eels were caught at Pukepuke Lagoon using a specially designed trap (Fig.1.7) which was set in the outlet drain immediately downstream from the spillway which controlled the lake level. As described in the migrant eel section, this trap was visited once a month during the 1971 migratory season.

TABLE 1.1 Number of non-migrant eels examined from each locality.

Species	Locality	Method of capture	Approximate distance from sea (km)	No. of eels
<u>A. australis</u>	Makara stream	E.F.M.	1 - 7	760
	Happy Valley stream	E.F.M.	1 - 2	45
	Waimeha stream	E.F.M.	0.5 - 1	60
	Pukepuke Lagoon	Fyke net	4	122
<u>A. dieffen- bachii</u>	Makara stream	E.F.M.	1 - 7	677
	Happy Valley stream	E.F.M.	1 - 2	40
	Waimeha stream	E.F.M.	0.5 - 1	35

E.F.M. - electric fishing machine.

TABLE 1.2 Number of migrant eels examined from each locality.

<u>Locality</u>	<u>A. australis</u>	<u>A. dieffenbachii</u>
	No. of eels	No. of eels
Waikato River	18	10
Pukepuke Lagoon	22	0
Lake Kaikokopu	15	0
Lake Onoke	167	6
Makara stream	104	417
Lake Ellesmere	60	2
<u>Eel Factories</u>		
Hokitika	22	3
Dunedin	1	7
Invercargill	9	7

Fig.1.4

Fyke nets set in the Makara stream

Fig.1.5

Fyke net set in the Makara stream

Fig.1.6

Outlet channel at Lake Onoke

Fig.1.7

Pukepuke Lagoon eel trap.

A small sample of shortfin migrant eels was obtained from Lake Kaikokopu from a fisherman who had caught them in a similar manner to the Pukepuke Lagoon migrants.

Samples of migrant eels from Lake Ellesmere and the Waikato River were obtained from eel processing factories during the 1972 migratory season. Samples of migrant eels from other areas were also obtained from eel processing factories but the exact location and time of capture could not be determined.

Migrant eels obtained from eel processing factories had all been caught in fyke nets. These eels were either air-freighted live or frozen to the laboratory. By freezing fresh specimens it was established that eels showed varying weight losses of up to 5% of the initial total body weight through freezing. No allowance was made for this weight loss in the age-weight relationship.

All non-migrant and migrant eels were handled in the laboratory. Some migrant eels of both species and sex were kept alive in fresh and salt water aquaria for experimental purposes.

For weighing and measuring live eels are difficult to handle, so they were first anaesthetised and then killed. Eels were anaesthetised by holding them for half an hour in a tightly secured bucket of water to which 50cc of 7% benzocaine solution had been added. The benzocaine solution was made by dissolving the required amount of benzocaine powder in 95% ethyl alcohol. Half an hour was usually sufficient to anaesthetize and kill the eels. Approximately 200cc of full strength 880 ammonia was then added to the water to deslime the eels. After two hours the eels were individually washed to remove the slime and they were then measured, weighed, and otolith and gonad samples taken. Eels were deslimed to facilitate handling. Unless deslimed, dead eels, particularly migrants, are difficult to handle and cut open, and the handling of a large number of eels becomes a difficult and messy task. The weight loss due to desliming was calculated to be less than 1% and therefore no allowance was made for this in the age-weight and length-weight analyses.

The total length of each specimen was measured, to the nearest mm, from the tip of the longest jaw to the tip of the tail. Eels were weighed on a Salter spring balance which weighed up to

5kg with 10gm divisions for the first $\frac{1}{2}$ kg, and 25gm divisions from 1kg to 5 kg. The gutted weight of all specimens and the total weight of all migrants and some non-migrants were taken. As explained in the length-weight relationship, gutted weights were used for analysis to eliminate the effect empty or full guts in non-migrants and gonad weight in migrants on the length-weight relationship. The gonads of some non-migrant and migrant weels were weighed to the nearest 0.1 gm on an Ohaus, dial-o-gram, beam balance.

Using a pair of adjustable dividers several morphometric measurements were made. The horizontal and vertical diameters of the left eye were measured to the nearest 0.5mm, and the pectoral fin length and the maximum gonad width were taken to the nearest 1mm.

1.3 Gonad samples and histological techniques

Gonad samples were examined both macroscopically, microscopically and histologically. Fresh ova measurements on the largest ova from non-migrant and migrant eels were made.

For microscopic and histological examination gonad samples were fixed in a number of fixatives. From some eels several gonad samples were fixed in different fixatives. Gonad samples from all non-migrant and some migrant eels were fixed in buffered formalin. These samples retained their fresh colour and texture, and were suitable for whole examination under a binocular microscope. Other non-migrant gonad samples were fixed in Bouin's fixative. Histological sections were prepared from both buffered formalin and Bouin's fixed material.

For gonad samples from migrant eels, several fixatives were used. Ovarian samples were fixed in buffered formalin, Bouin's fixative and Smith's fixative. Testes samples were fixed in buffered formalin, Bouin's fixative, Zenker's fixative, Helly's fixative, Carnoy fixative (formula B), Champy fixative and aqueous osmium tetroxide. One gonad sample from all the migrant eels was fixed in Bouin's fixative and for some eels other samples were fixed in one or more of the other fixatives. For best staining results, Bouin's fixative was found to be the most suitable fixative

for both ovarian and testes samples, although Zenker's fixative for testes was excellent for mitotic and meiotic details of cell division.

For sectioning tissue was embedded in 56°F melting point wax and sectioned at between 5 μ m and 8 μ m thickness on a rotary microtome.

For routine analysis, slides were stained in Heidenhain's iron haematoxylin and counterstained with eosin. Initially some sections were stained in Delafield's haematoxylin and eosin but the results, particularly nuclear detail in the testes, and ova membrane details in the females, were not as precise as in Heidenhain's stained material. To determine the location and distribution of connective tissue, Mallory's triple connective tissue stain was used. This was particularly useful for sections of gonads from non-migrant eels when the structure of the gonad was not clear.

To detect the presence of yolk in ova, the Periodic Acid-Schiff (PAS) (aqueous solution) reaction was used. This technique gave good results when used on slides from migrant and hormone injected females in which yolk deposition had begun.

To stain for interstitial cells in the testes of male eels Threadgold's (1957) technique was used. Of the two methods, method 1, using Zenker's fixative, gave the most satisfactory results.

Peacock (1966) and Humason (1967) were used as reference texts for all fixative and stain formulae and, except for the interstitial cell staining technique, for fixing and staining procedures.

2. NON-MIGRANT EELS

2.1 Introduction

The development of the sex organs and the identification and distribution of each sex of New Zealand eels has been referred to by several authors (Cairns 1941, 1942b; Hobbs 1947; Burnet 1952b, 1969a and 1969b; Woods 1964).

Cairns (1941: 66B; 1942B: 173B - 175B) records observations on the development of the sex organs and distribution of male and female eels, and found that males did not occur in freshwater. This distribution of the sexes, males in the estuaries and brackish waters, and females in freshwater, was considered to be the general rule for the distribution of the sexes of the European eel (Tesch 1928; Bertin 1956). However, Bertin (1956: 81) quotes several exceptions of males occurring in freshwater and females in brackish lagoons. For New Zealand eels, Cairns' (1941; 1942b) observations were not confirmed by Burnet (1952b, 1969a) and Woods (1964: 119), who found both longfin and shortfin males in freshwater.

The method of sex determination in the European eel has been the subject of much controversy. Bertin (1956) discusses the two theories of sex determination, one that it is syngamic, i.e., the sex is determined genetically, and that male and female choose the habitat suitable for their sex. Or secondly, that sex determination is metagamic, i.e., elvers arriving in freshwater are asexual and distribute themselves at random amongst the various habitats, and their subsequent sex is determined by the environment in which they grow. In summary Bertin (1956: 162) suggests that the sex determination for 90% of eels is metagamic, however 10% of the population are females in which sex determination was syngamic. D'Ancona (1959) however, considers that sex determination is syngamic, but is gradual and in some individuals can be influenced by environmental factors. Penáz and Tesch (1970: 308) suggest that sex determination is metagamic. In contrast Sinha and Jones (1966: 382) suggest that sex is genetically (syngamic) determined and the sex of elvers arriving in freshwater is predetermined.

Bertin (1956) gives experimental evidence of sex reversal under changed environmental conditions to support the theory of metagamic sex determination. Tesch (1928) took small eels from an area that contained almost exclusively male eels and placed them in running freshwater. After three years, half of them had developed into female eels. Hornyold (1932) describes a less conclusive

experiment in which after three years only 17% of 100 transplanted eels developed into females. Undifferentiated eels comprise 60% of the sample and 17% males. Sinha and Jones (1966: 372) criticize these experiments and consider that they were inconclusive.

Cairns (1942: 1748) describes developing male eels in a sample of eels that had just migrated upstream from tidal waters. After four weeks a sample of eels from the same area revealed that the characteristic appearance of the male gonad was disappearing. After three months the eels were all unmistakable females. Cairns concluded that this observation confirmed the hypothesis that the environment determines the sex and that sex reversal can be caused by a change in the environment. Cairns' observations however are inconclusive. He does not differentiate between species, and no histological examinations were made to confirm that sex reversal had occurred. Further, there is no evidence given to show that the population sample was static.

Before the development and distribution of sex can be determined a correct identification of the sex must be made. This, for the European eel, has been the subject of much discussion. Until the late eighteenth century the reproductive organs of the eels had not been found, and eels were variously believed to be viviparous or even the product of spontaneous generation. In 1777 Mondini discovered the female ovary, or frilled organ, and in 1874 Syrski described for the first time what he considered to be the testes or lobulate organ, later to be known as the organ of Syrski.

Briefly, the ovary is a wide, frilled or pleated ribbon-like organ and the testes a narrow organ comprising a number of lobes. These organs extend along the length of the body cavity on either side of the gut. The presence of these organs have been used as the basis of sex identification. The gonads in situ are shown in Fig.2.3.

Since these discoveries, much research and discussion has centered around the early development of the gonad and the true nature of the organ of Syrski, that is, if it is diagnostic of the male eel.

Bertin (1956) describes the early research on the development of the gonads in the European eel, particularly that by Rodolico (1933), and D'Ancona (1943) who studied the development of the gonads from the elver stage onwards. Rodolico established that in an eel up to 14cm in length the gonad is undifferentiated and resembles that of a miniature organ of Syrski. Nests of oogonia and some oocytes then develop in the peripheral region of the gonad, this stage is termed the phase of precocious feminisation.

Spermatogonia then develop in the central portion of the gonad in eels between 18cm and 30cm in length. This is the intersexual stage or stage of non-functional hermaphroditism. The gonad at this stage resembles the organ of Syrski, typical of male silver eels. From this stage of hermaphroditism either the spermatogonia, or oogonia and oocytes divide and multiply, while the other atrophies. This is termed the phase of masculinization or feminization.

The complete differentiation into either sex may occur when the eel is as large as 36 cm (Sinha and Jones 1966: 378), but sex differentiation usually takes place at a length between 24cm and 26cm (D'Ancona 1943) or 26cm and 28cm (Sinha and Jones). In males the gonad now appears as the typical organ of Syrski and in the females the ovaries appear as frilled ribbons. From this description it is apparent that the development of the ovary includes, in the hermaphrodite stage, a stage resembling that of the testes in male eels. This indicates that the organ of Syrski is not a diagnostic feature of the male European eel. Tesch (1928: 54) describes the large number of small eels, in areas which are occupied by female eels, that contained gonads resembling those of male eels but doubts if they are really males. Sinha and Jones (1966) found that the organ of Syrski contained oocytes and therefore was ambiguous in nature. It was thus difficult to determine the sex of eels with these organs. These authors also suggest that many workers have sexed incorrectly as males small undifferentiated eels with gonads resembling the organ of Syrski.

In contrast to the European eel, Satoh, Nakamura and Hibiya (1962) found no stages of precocious feminization or phase of juvenile hermaphroditism in the Japanese eel. Conversely, in the American eel, Huver (1966) found that the organ of Syrski appeared histologically as an immature testis but it developed into an ovary as the eel increased in size.

The identification of male and female shortfin and longfin eels by Cairns (1941, 1942a, 1942b), Hobbs (1947), Burnet (1952b, 1969a, 1969b) and Woods (1964) have been made on the basis of Syrski's description. There is no reason to doubt the correct identification of sex of migrant eels, because in the European migrant eel the organ of Syrski can be correctly used to identify migrant males.

Considering the ambiguous nature of the organ of Syrski in yellow (non-migrant) European eels however, the correct identification of non-migrant male longfin and shortfin eels by previous authors was treated with caution.

The purpose of this section of the study was therefore to describe the development of the gonads in non-migrant eels of both species, to establish the size at which the sexes became differentiated, and to determine if the lobed organ of Syrski could be used to correctly identify male eels.

2.2 Development of the gonads - the macroscopic appearance of the gonads in non-migrant eels

To determine the size at which the gonads became differentiated into recognizable male and female organs and to establish if the presence of a lobed gonad could be correctly used to identify the male sex, the gonads of 1,739 non-migrant and 870 migrant eels were examined. These comprised 987 non-migrant shortfins ranging in size from 8.0cm to 87.0cm and 752 non-migrant longfins ranging in size from 10.0cm to 145.0cm. These eels came from four areas. The number of non-migrant eels of each species examined, from different areas, has been given in Table 1.1. The number of migrant eels of each sex and species examined has been given in Table 1.2, and the size range of these eels is given in the section on migrant eels.

Gonad samples from non-migrant eels, preserved in buffered formalin, were examined under a binocular microscope against a black background, using a reflected light source. The gross structure and shape of the gonad was noted, whether it appeared as a smooth ribbon, a frilled ribbon (ovary), or lobed. The maximum width of the gonad was measured with a micrometer eye-piece and the measurements later converted to mm. Some gonad samples were also observed under a compound microscope. Selected gonad samples were examined histologically. Samples fixed in buffered formalin and Bouin's fixative were used for this purpose.

The appearance of the gonads in migrant eels were examined without a microscope. Their histological appearance is described in the section on migrant eels.

From the macroscopic appearance of the gonads it was found that they could be conveniently divided into seven stages, based on their shape and size. Gonads from different eels within each gonad stage had a characteristic appearance, and each stage occurred within a broad size range for each species. No distinctive differences could be established between the same gonad stages of the two species except that the stages in longfins were confined to a larger size range than the same stage for shortfins. Each gonad stage also had a certain range in gonad width. However, the range in gonad width and the range in total length of each eel in each gonad stage showed considerable overlap within each species.

The gonad stages are represented diagrammatically in Fig.2.1.

Gonad stage 1

The gonad appears as an undifferentiated ribbon, uniform in density (Fig.2.2) but it may be irregular in shape in eels under 15 cm. In small eels (<30cm) it appears thin and opaque, but is wider and thicker in larger eels. The gonad varies in width from 0.07mm to 0.7 mm in shortfins from 8.0cm to 45.6cm in total body length. In longfins the width varies from 0.08mm to 2.0mm in eels from 10.0cm to 61.8cm in length.

Gonad stage 2

The gonad appears as an undifferentiated lobed ribbon, undulating in appearance (Fig.2.2). The undulations are sometimes regular, giving the gonad a slightly lobed appearance, but the lobes are never distinct. The width varies from 0.33mm to 1.0mm in shortfins from 22.6cm to 46.9cm in length. In longfins the width varies from 0.4mm to 1.2mm in eels from 23.5cm to 59.0cm in length.

Gonad stage 3

The gonad appears as a differentiated lobed ribbon with distinct white opaque zones joined by clear areas of tissue (Fig.2.2). In shortfins the width varies from 0.37mm to 1.25mm in eels from 27.3 to 48.1cm in length. In longfins the width varies from 0.45mm to 1.7mm in eels from 33.5cm to 64.0cm in length.

Gonad Stage 4

Lobed gonad, but the lobes are joined at their bases by a clear web of tissue (Fig.2.2) and hence are not distinct lobes as in the organ of Syrski. In shortfins the lobes varied in width from 0.95mm to 1.7mm in eels from 35.8cm to 50.6cm in length. In longfins the lobe

FIG.2.1

A SCHEMATIC REPRESENTATION OF THE
GONAD STAGES IN NON-MIGRANT EELS

Gonad Stages

FIG.2.2

GONAD STAGES IN NON-MIGRANT EELS

1. Gonad stage 1. A. australis (shortfin)
22.5cm total length. x 12
2. Gonad stage 2. A. australis 33.0cm total length.
x 12
3. Gonad stage 3. A. dieffenbachii (longfin)
40.2cm total length. x 10
4. Gonad stage 4. A. australis 39.8cm total length.
x 5
5. Gonad stage 5. A. dieffenbachii 55.0cm total length.
x 6

1

2

3

4

5

width varied from 0.85cm to 2.0mm in eels from 46.7cm to 67.2cm in length.

Gonad Stage 5

Lobed gonad, with the lobes distinct and separate (Figs. 2.2 and 2.4). Conforms to the description of the organ of Syrski (Syrski 1876). In shortfins the lobe width varies from 1.1mm to 3.0mm in eels from 38.5cm to 56.9cm in length. All migrant shortfin males had gonads of this type. In these the gonad lobe width varied from 3.0mm to 7.5mm in eels from 31.0cm to 59.8cm in length.

In longfins the lobe width varied from 2.7mm to 5.0mm in eels from 51.7cm to 68.7cm in length. This stage occurred in 99% of all migrant longfin males. In these the gonad lobe width varied from 7.5mm to 21.0mm in eels from 48.2 to 73.6cm in length.

Gonad stage 6

The gonad appears as a ribbon, opaque in colour, with an anastomosing network of vein-like structures (Fig. 2.3). On the outer edge of the gonad it may be slightly lobed but the lobes are never distinct. The gonad is smooth on its inner (medial) side and transverse ridges may be present on the outer (lateral) side. In shortfins the gonad width varies from 0.63mm to 1.7mm in eels from 32.6cm to 49.6cm in length. In longfins the width varies from 0.7mm to 3.0mm in eels from 42.0cm to 65.8cm in length.

Gonad stage 7

The gonad appears as a frilled ribbon, pleated in larger eels (Fig. 2.4). It is smooth on the inner (medial) side, with transverse ridges or ovarian lamellae on outer (lateral) face. These are more pronounced in the gonads from larger eels. The gonad conforms to the description of the organ of Mondini (ovary) given by Syrski (1876).

In non-migrant shortfins the gonads at this stage vary in width from 2.0mm to 15.6mm in eels from 42.1cm to 89.0cm in length. All migrant shortfin females have gonads at this stage, varying in width from 17.0mm to 42.0mm in eels from 55.0cm to 102.3cm in length.

In non-migrant longfins the gonad width varies from 2.5mm to 22.0mm in eels from 55.2cm to 137.0cm in length. All migrant longfin females have gonads at this stage, varying in width from 39.0mm to 88.0mm in eels from 78.1cm to 133.3cm in length.

FIG.2.3

GONAD STAGES IN NON-MIGRANT EELS

1. Gonad stage 6, viewed under transmitted light,
from A. australis (shortfin) 40.0cm total length.
x 30

2. Gonad stage 6, viewed under reflected light.
The slightly lobed appearance can be seen and the
ovarian leaflets containing the developing oocytes
are visible, from A. dieffenbachii (longfin)
55.0cm total length. x 8

1

2

FIG.2.4

GONAD STAGES

1. Gonad stage 5. A single testis lobe, viewed under transmitted light, from A. dieffenbachii (longfin) 55.0cm total length. x 25
2. Gonad stage 7. A portion of an immature ovary viewed under reflected light. The slightly lobed appearance visible and the pleated appearance of the ovary is developing. From A. australis (shortfin) 48.0cm total length.x 10

1

2

From histological examination of gonad stages 5 and 7 in non-migrant and migrant eels, described later in this section, it is shown that gonad stage 5, the organ of Syrski, in non-migrants which contains spermatogonia and in migrants other recognizable cellular elements of spermatogenesis, is definitely the male sex organ, and can be used as a diagnostic feature of males. Gonad stage 7, containing oogonia and oocytes in non-migrants and ova in later stages of development in migrants, can be used to recognize the female eel.

In 1% of the migrant longfin males examined the testes appeared as a ribbon-shaped gonad with no lobular structure (Fig.3.16 page 106a). The texture and colour of the testis was identical to that of the lobed testes and histologically they were identical. Although testes of this type do not conform to the organ of Syrski they were definitely male gonads and could not be confused with ovaries. No non-migrant longfin males were observed with ribbon-shaped testes but it is likely that the larger eels classified as gonad stage 1 included some males.

In detail the ovaries in females and the testes in males appear as follows:

In females the two ovaries are ribbon-shaped structures that extend along either side of the gut (Fig.2.5). The left ovary commences from just behind the level of the gall bladder, and extends along the gut and past the urogenital aperture by 2.0cm to 8.0cm, depending on the size of the eel, and into the caudal region of the body. The left ovary then doubles back on itself for 1.0cm to 4.0cm forming what Syrski (1876: 730) describes as an accessory ovary. The right ovary commences just anterior to the gall bladder and origin of the left ovary, and extends posteriorly to just past the urogenital aperture. In contrast to the description given by Syrski the right ovary does not extend far into the caudal region of the body and there is no right accessory ovary. The ovaries are widest about mid-way along their length, anteriorly they are slightly less in width but posteriorly they terminate in a thread-like form. In less developed non-migrant females the ovaries are colourless but in more developed non-migrants and migrants they are white.

When fully developed the ovaries appear as frilled or pleated ribbons, smooth on their inner face and with folds or ovarian leaflets on their outer face (Fig.3.17. page 106b). In larger non-migrant

and migrant females individual ova can be distinguished within the ovarian leaflets.

In males the testes appear as two longitudinal rows of lobes extending along either side of the gut (Fig.2.5). The size of the lobes vary according to the size of the eel and the stage of development. However, in a few longfin migrant males the testes appeared as a ribbon-like organ. Each testis originates and extends posteriorly alongside the gut in a similar manner to each ovary. In fully developed males of both species the lobes are distinct. They are smooth on both surfaces and are dense in appearance. In non-migrant males they are colourless but in migrants they appear pink. In earlier stages of development, as previously described, the lobes are not distinct and are joined together by clear areas of tissue.

From the total body length at which the gonad stages were observed, shortfin males may become sexually differentiated at 35.0cm, but it may be delayed until 56.9cm. Longfin males become sexually differentiated at 50.0cm but they may be as large as 67.0 cm. Shortfin females may become sexually differentiated at 40.0cm and longfin females at 55.0cm. It is shown in the migrant eel section that migrant males attain a maximum length of 59.8cm for shortfins and 73.6cm for longfins, therefore all non-migrant eels above these lengths for each species are females.

Cairns (1942b: 174b) states that sexual development is first seen at 40.0cm in shortfins and 45.0cm in longfins, but the sex is not specified. These lengths are greater than the minimum size at which the male organ becomes defined in each species. The length given by Cairns for shortfins is the size at which the ovary becomes recognizable, but the length for longfins is smaller than the minimum size for longfins at which the ovary is recognizable.

Woods (1964: 118) gives the size ranges within which male and females were recognizable. Longfin males measured from 36.0cm to 68.0cm, and females from 37.0cm to over 100.0cm. Shortfin males ranged in size from 43.0cm to 47.5cm, and females from 43.0cm to 81.0 cm. The minimum sizes for recognizable shortfin males and females are larger than found in this study, but the minimum sizes at sex differentiation for longfin males and females are considerably smaller. Woods does not give the criteria by which the eels were

FIG. 2.5

GONADS 'IN SITU'

1. The ovaries 'in situ' as seen in A. australis
(shortfin) female, 67.0cm total length.
2. The testes 'in situ' as seen in A. australis
migrant, male 45.0cm total length.

t - testes

o - ovaries

1

2

sexed, and it is possible that undifferentiated ribbon like gonads in longfins may have been mistaken for ovaries. To identify males the organ of Syrski was probably used, and lobed gonads in which the lobes were not yet distinct may also have been classified as males.

The change in the external appearance of the gonad in the European eel during development given by Bertin (1956) has already been described. In particular the gonad in eels of 6.0cm to 14.0cm in length is described as appearing as a miniature organ of Syrski. Thus the gonad in the female European eel first appears lobed and then becomes pleated (Bertin 1956: 77).

No such miniature organ of Syrski was observed in small shortfin and longfin eels. The gonad stage 2 described sometimes had a slightly lobulate appearance but it could not be described as a miniature organ of Syrski. It could be argued that the gonad stages 2 and 3 represent the equivalent stage of the lobed organs in the European eels, but in larger eels. However, the histological evidence given later in this section does not indicate this.

Evidence that shortfin females do not pass through an early lobulate stage is shown by the Pukepuke Lagoon sample of migrant and non-migrant eels. Of 122 non-migrants and 22 migrants, only 2% were males, these were two non-migrants with gonads at stage 4. This would indicate that the shortfin eels in the Pukepuke Lagoon are almost exclusively female. Of the 122 non-migrants, except the two described, all had gonads at stages 1, 6 or 7. None of these had gonads that could be confused with the organ of Syrski. As previously mentioned, Tesch (1928: 54), found a large number of small European eels with lobulate type gonads in areas almost exclusively occupied by females.

Table 2.1 gives the percentage of eels in each gonad stage from different areas. Few eels were found in gonad stages 3 to 5 compared to stages 1, 2, 6 and 7. In most areas the largest number of eels occurred in gonad stages 1 and 7. If the large number of female eels passed through a lobulate gonad stage, a high proportion of these would be found in the lobular or developing lobular gonad stages 3 to 5. In fact the percentage of eels occurring in these stages is similar to the proportion of male eels in each area, as

TABLE 2.1
Percentage of eels in each gonad stage

Area	Percentage of eels in each gonad stage							Number of eels
<u>A. australis</u>								
	1	2	3	4	5	6	7	
	%	%	%	%	%	%	%	
Makara stream	34	9	4	6	3	8	36	760
Happy Valley stream	16	15	8	57	4	0	0	45
Waimeha stream	25	5	0	5	10	15	40	60
Pukepuke Lagoon	36	0	0	2	0	9	53	122
<u>A. dieffenbachii</u>								
Makara stream	31	16	5	6	5	12	25	677
Happy Valley stream	48	22	4	15	11	0	0	40
Waimeha stream	27	0	5	10	0	26	32	35

discussed in the distribution of sex. It is therefore concluded that the gonads in female longfin and shortfin eels do not undergo a change from a lobulate type gonad to a frilled gonad, as described for the European eel.

2.3 Development of the gonads - the microscopic appearance of the gonads in non-migrant eels

A total of 410 gonads, from each of the seven gonad stages from non-migrant and migrant eels were examined histologically. This sample included 195 organs of Syrski.

The histological descriptions of the gonads from migrant eels is given separately in the section on migrant eels. Cytological details of the various cell types present in the gonads of each stage are given in the section on gametogenesis. In the following description only the presence or absence of the various cell types and the gross histological structure of the gonad is given.

Gonad stage 1

The gonad is composed of a dense connective tissue matrix containing isolated, large (15 μ m) cells. These cells stained faintly with all stains and were difficult to distinguish from the surrounding connective tissue. They are termed primary germ cells.

Gonad stage 2

An irregular connective tissue network is present similar to gonad stage 1. Isolated, faintly staining cells are dispersed amongst the connective tissue network, but they are more numerous than in the first gonad stage. Groups of two to four cells, similar in appearance to the isolated cells, are also dispersed amongst the connective tissue. Histologically this gonad stage is very similar to gonad stage 1.

Fig.2.6 shows the structure of a gonad between stages 1 and 2.

Gonad stage 3

The gonad consists of an irregular connective tissue network containing a few isolated cells, and groups of four to eight cells arranged in cysts (Fig.2.6). The cells in cysts are faintly staining and measure 8 μ m to 11 μ m in diameter.

FIG. 2.6

SECTIONS OF THE GONADS FROM STAGES 1 - 5

1. LS of the gonad in stage 1 - 2 from non-migrant A. dieffenbachii (longfin), 40.2 cm total length, showing the primary germ cells. x 450.
2. LS of the gonad in stage 3 from non-migrant A. dieffenbachii, 59.1 cm total length, showing germ cells arranged in clusters. x 450.
3. TS of the gonad in stage 4 from non-migrant A. dieffenbachii, 60.2 cm total length, showing the early development of the tubules. x 375.
4. TS of the gonad in stage 5 from migrant A. australis (shortfin) 42.0 cm total length, showing the development of the tubules containing spermatogonia and spermatocytes. x 250.

c - connective tissue
g - germ cells
sg - spermatogonia
sc - spermatocytes
it - intertubular connective tissue

1

2

3

4

Gonad stage 4

Similar to gonad stage 3 but there is less connective tissue and the cysts of cells ^{are} more distinct and numerous (Fig.2.6). In one eel, a longfin of 61.6 cm, a definite tubular structure was developing. Mitotic divisions were observed with ~~w~~ cells in some cysts.

Gonad stage 5

Less connective tissue present than in stage 4 but the amount varies with individual eels. The tubular structure in some gonads is well developed. In these, groups of cells are surrounded by a band of connective tissue (Fig.2.6). Two distinct cell types were observed. Larger, 8 μ m to 11 μ m in diameter, faintly staining germ cells as previously described, and secondly smaller, 5 μ m to 7 μ m in diameter, more darkly staining spermatogonia. They contain nuclei 4 μ m to 6 μ m in diameter, with a prominent nucleolus.

Isolated large germ cells are still present dispersed amongst the connective tissue in some eels with a less defined tubular structure. Spermatogonia are more numerous in gonads with a well defined tubular structure, particularly in longfins.

The testes of migrant males of both species are at a more advanced stage of development, with later stages of spermatogenesis present: spermatocytes, and in some longfin eels, spermatids and spermatozoa.

Gonad stage 6

The gonad consists of an irregular connective tissue network, containing three distinct cell types (Fig.2.7). Large, faintly staining primary germ cells, 8 μ m to 15 μ m in diameter, and larger more darkly staining oogonia, 10 μ m to 25 μ m in diameter, with nuclei containing distinct chromatin material. Thirdly, isolated oocytes 15 μ m or larger in diameter containing basophilic, darkly staining cytoplasm and nuclei, 14 μ m or greater in diameter. The germ cells and oogonia are more numerous than oocytes.

Gonad stage 7

There is little connective tissue and the gonad contains only oogonia and oocytes (Fig 2.8). In the smallest gonads at this stage (gonad width 5mm) oogonia and oocytes from 16 μ m to 30 μ m in diameter are present. As the gonad increases in width the number of oogonia decrease and the oocytes predominate. In larger gonads, oocytes up to 100 μ m in diameter contain more darkly staining cytoplasm. The oocyte diameter increases with increasing gonad width. In larger oocytes, greater than 100 μ m in diameter, vacuoles develop

FIG. 2.7

TRANSVERSE SECTIONS (TS) OF THE OVARIES
FROM NON-MIGRANT EELS, GONAD STAGE 6.

1. TS of the gonad from A. australis (shortfin),
40.4cm total length, showing isolated primary
oocytes and oogonia. The ovarian lamellae are
just appearing in the upper left margin of the gonad.
x 240

2. TS of the gonad from A. dieffenbachii (longfin),
58.0cm total length, showing the developing ovarian
lamellae containing isolated primary oocytes, and
oogonia. x 280

o - oogonia

p - primary oocytes

1

2

FIGURE 2.8

TRANSVERSE SECTIONS (TS) OF THE OVARIES
FROM NON-MIGRANT EELS, GONAD STAGE 7

1. TS of the ovary from an A. dieffenbachii (longfin),
60.8cm total length, showing the ovarian lamellae
containing the developing oocytes. x 100

2. TS of the ovary from an A. australis (shortfin)
54.0cm total length, showing primary oocytes and
vacuolated oocytes. x 100

p - primary oocytes
v - vacuolated oocytes

1

2

the
in cytoplasm. Non-migrant eels contained few oocytes at this stage and approximately 75% or more of oocytes were at earlier stages of development.

The ovaries of migrant females of both species are at a more advanced stage of development, with the oocytes at the stage of vacuolation or in the early stages of yolk deposition (Vitellogenesis).

D'Ancona (1960: 66) notes that the prevalence of growing oocytes is the essential characteristic of the female European eel and the appearance and persistence of the vas deferens and the cordonal (tubular) arrangement of the germ cells identifies the developing male eel. However, in the European eel these characteristics coexist in 90% of the eels at the intersexual stage.

In the gonads of longfin and shortfin non-migrant and migrant eels none were observed with oocytes, and cysts of cells containing spermatogonia characteristic of male eels, in the same gonad. Hence there is no evidence of an intersexual stage in either longfin or shortfin eels.

Sinha and Jones (1966) made histological examinations of the organ of Syrski from 200 European eels and found that 7% of them contained oocytes. They concluded that the lobed organ of Syrski could not be regarded as a diagnostic feature of maleness.

Huwer (1966) found that the organ of Syrski in the American eel appears histologically as an immature testis but it develops into an ovary as the eel increases in size. In contrast Satoh, Nakamura and Hibiya (1962) give a schematic representation of the development of the gonad in the Japanese eel which shows that the lobed gonad is diagnostic of male eels and they state that no intersexual stage was found.

In this study a total of 195 lobed organs of Syrski, from non-migrant and migrant eels of both species, were examined histologically. All were distinct males containing either spermatogonia or more advanced stages of spermatogenesis. None contained oocytes. In addition, as described in detail in the section on migrant eels, 1% of the longfin male migrants contained testes that

were not lobed. These gonads, although appearing as a ribbon gonad, could not be confused with an ovary. Therefore, the lobed organ of Syrski, although not found in all longfin males, can be used correctly to identify male eels of both species.

From the histological appearance of the gonads from various gonad stages it is possible to determine at what size the sexes become histologically sexually differentiated. The sex of an eel as represented by each gonad stage can also be determined.

It was not possible to differentiate between male and female germ cells. Therefore eels with gonad stages 1 and 2 are classified as undifferentiated eels which will develop as either males or females, according to their sex. Gonad stage 3 also contained only germ cells, but the external slightly lobed appearance of the gonad indicates that it is a developing organ of Syrski. Gonad stage 4 contained germ cells and some spermatogonia. The external appearance of this gonad stage also indicates that it is a developing organ of Syrski. Therefore, from the external appearance and histological structure of these gonads stages, the eels could be classified as developing males. As no oocytes were found in gonads from these stages they cannot be regarded as an intersexual stage. Eels with gonad stage 5 containing spermatogonia or more advanced stages of development are definite males.

The appearance of oogonia or oocytes in gonad stage 6 is the first indication of definite female development. Macroscopically, gonads at this stage have ridges (ovarian leaflets) developing laterally along the gonad. Eels at this stage can be called definite females. Eels with gonad stage 7 are also definite females.

From histological observations the minimum size at which the sexes differentiate can be found. Shortfin males may become sexually differentiated at 27.3cm but it may be delayed until 48.1cm. Shortfin females become sexually differentiated at 32.6cm but it may be delayed until 49.6cm. In some shortfins sexual differentiation into either recognizable sex is delayed up to 46.9cm.

Longfin males become sexually differentiated at 33.5cm but development may be delayed up to 64.0cm. Longfin females become sexually differentiated at 42.0cm but it may be delayed up to 65.8cm. In some longfins sexual differentiation into either recognizable sex is delayed up to 61.8cm.

Sex differentiation occurs at a smaller size than is recognizable by the external appearance of the gonad. Eels with gonad stages 3 and 4, although not possessing the true lobed gonad, can be classified as males.

Females with gonad stage 6 are difficult to differentiate from eels with gonad stage 1. Only microscopic examination reveals the developing ovarian leaflets. Therefore gonad stage 6 would not be a satisfactory diagnostic feature for identifying female eels and only those with gonad stage 7 could be classified as females.

In the European eel, as previously mentioned, sex differentiation usually occurs at a length between 24cm and 26cm (D'Ancona 1943) or 25cm to 28cm, but it may be delayed up to 36cm (Sinha and Jones 1966). This minimum size is similar to the size (28.4cm) at which sex differentiation may begin in the shortfin eel, but the size up to which it may be delayed (46.9cm) is larger. In the longfins the minimum size at which sex differentiation occurs (33.7cm) and the maximum size (61.8cm) up to which it may be delayed are much larger. In contrast, histological differentiation in the Japanese eel may occur at length 18cm but the ovaries and testes cannot be distinguished by their external appearance until the eels are about 30cm in length (Satoh, Nakamura and Hibya 1962). This is similar to New Zealand eels. Histologically sex differentiation occurs at a smaller size than that at which the gonads become externally visible as males or female (35cm for shortfins and 50cm for longfins).

Non-migrant longfin females may grow to a large size and there are several records (Downes 1917; Best 1929; Graham 1956) of these eels exceeding 130cm in length and 14kg (30 lb) in weight. Best (1929) discusses reports of eels of up to 43.6kg (96lb) and states that there are even reports of eels up to 59kg (130 lb). However, these records are not well documented and it appears from the more reliable sources quoted by Best that these large eels usually weight from 10kg to 14kg and rarely exceed 18kg (40 lb).

Cairns (1942b: 175b) suggests that these eels remain sterile for many years and become what are known as 'monster' eels. It is commonly suggested by eel fishermen that these eels grow to a large size and become sterile because they are landlocked. However, Downes (1917) and Cairns (1941), as discussed in the migrant eel section, record that these large eels do migrate. The use of the term sterile to describe these eels, even if they do migrate, is considered to be incorrect. In this study two large non-migrant longfin females of 135.0cm and 145.0cm in length from the Waimeha stream were examined. The ovaries in each appeared normally developed. Histologically the oocytes were at the stage of early vacuolation, typical of the oocytes in large non-migrant females of both species. There was no evidence of any regression of the gonad which one would associate with sterility. Thus the large longfin eels are non-migrant longfin females with normal gonad development. These may be eels in which the gonads did not become differentiated until the eel reached a large size. There is no evidence that sterility or hormonal imbalance, as commonly suggested, causes these eels to reach such a large size.

2.4 Distribution of the sexes

Cairns (1942b: 174b) found no male eels in fully freshwater but Burnet (1925b, 1969a) and Woods (1964) have established that males do occur in freshwater and that longfin males form a large percentage of the upstream population of this species. This study confirms these observations. Males of both species were caught upstream beyond the tidal influence in the Makara, Happy Valley and Waimeha streams. Male shortfins were also found in the Pukepuke Lagoon.

The number of eels caught of each species and the distance from the sea has been given in Tables 1.1, and 1.2. Table 2.2 gives the proportion of undifferentiated, male and female eels from each area. The length-frequency histograms of these groups of eels from each area are given in Figs. 2.9 to 2.12.

All areas contain a similar and high proportion of undifferentiated shortfins. The proportion of non-migrant males from each area varies. The Happy Valley stream contained the highest proportion of males and no females. These eels were caught from

MAKARA STREAM

A. australis

MAKARA STREAM

A. dieffenbachii

FIG.2.11

Length-frequency histograms of undifferentiated,
male and female A. australis (shortfin) and
A. dieffenbachii (longfin) from the Waimeha stream.

WAIMEHA STREAM

A. australis

female

male

undifferentiated

A. dieffenbachii

female

male

undifferentiated

Length (cm)

FIG. 2.12

Length-frequency histograms of undifferentiated and male A. australis (shortfin) and A. dieffenbachii (longfin) from the Happy Valley stream, and undifferentiated male and female A. australis from Pukepuke Lagoon.

Happy Valley Stream

Pukepuke Lagoon

TABLE 2.2

Proportion of undifferentiated, male and female eels from each area.

<u>A. australis</u>	Total no. eels	Smallest female (cm)	Smallest male (cm)	% undif- ferentia- ted	% male	% female
<u>Makara stream</u>						
non-migrants	760	36	28	38	19	43
migrants	104			0	67	33
<u>Happy Valley stream</u>						
	45	-	23	30	70	0
<u>Waimeha stream</u>						
	560	36	43	30	15	55
<u>Pukepuke Lagoon</u>						
	122	31	44	36	2	62
<u>A. dieffenbachii</u>						
<u>Makara stream</u>						
non-migrants	677	42	33	43	22	35
migrants	417			0	95	5
<u>Happy Valley stream</u>						
	40	0	44	70	30	0
<u>Waimeha stream</u>						
	35	46	46	26	16	58

1km to 2km from the sea and it indicates that female shortfins may migrate further upstream. However, this is not supported by the proportion of males and females in the Waimeha stream at a similar distance from the sea. Larger eels were caught, and 65% of the shortfins were females. This suggests that in this stream the shortfin females do not migrate upstream. The differences between the proportions of males and females in these two streams can be explained by the different stream types. The Happy Valley stream sampling area was shallow and rocky, offering little cover for larger female eels. The Waimeha stream is deeper, slow flowing and with overhanging and submerged vegetation offering suitable habitats for larger eels. Pukepuke Lagoon contained the lowest proportion of shortfin males. The only explanation offered for the low number of males is that of habitat preference, females may migrate into the lake and the males may remain in the outlet nearer the sea.

In the Makara stream the high proportion of male shortfin migrants compared with the lower proportion of non-migrant male shortfins indicates that a higher proportion of the male population migrates to the sea than migrant shortfin females.

The proportion of undifferentiated longfin eels varies in each area. In the Happy Valley stream it is suggested that only the smaller undifferentiated and male eels can find suitable habitats. This could explain the high proportion of undifferentiated eels and the absence of females.

In the Makara stream there is a high proportion of male longfin migrants compared with the proportion of non-migrant males. The reverse occurs with the migrant and non-migrant females. The high proportion and large number of migrant males indicates that there is a large upstream population of longfin males, a high proportion of which migrate. The smaller proportion and number of non-migrant longfin males is probably due to sampling bias and the distribution of non-migrant males in areas not fished.

2.5 Discussion

The apparent absence of the intersexual stage in New Zealand eels and the Japanese eel represents a fundamental difference in the reproductive biology of these species compared to the European eel.

It could be argued that the lobed or partially lobed organs observed in non-migrant eels in this study represent a stage in the development of the female gonad. As previously discussed, such stages are present in the European and American eels. However, in the European eel 90% of individuals pass through this stage, so that in a sample of non-migrant New Zealand eels of various sizes one could expect to find a large percentage of these with lobed gonads, if a similar intersexual stage was present. Sinha and Jones (1966) in a total sample of 8235 non-migrant European eels, found 66% had lobed organs. In this study only 19% of the non-migrant shortfins and 22% of the non-migrant longfins contained lobed organs. This indicates that the female gonad does not undergo a metamorphic change from a miniature organ of Syrski to a typical ovary. Gonads in stage 1 and 2 may have an irregular shape or even a slightly lobed appearance, but as previously stated they could not be called miniature lobed organs.

Histologically there was also no evidence of an intersexual stage. The criteria for this stage in the European eel is the co-existence of oocytes and the cordonal arrangement of germ cells (D'Ancona 1960). No such stage was observed histologically in New Zealand eels. Oocytes, as described, only appeared in gonad stages 6 and 7 and no oocytes were found in any lobed organs.

The distribution of the sexes of eels is closely linked with the question of sex determination. Bertin (1956: 79 - 80) states that 'it has often been suggested, in an attempt to solve this problem, that the geographical distribution of eels is a function of sex. The law is generally propounded as follows: Males occur in the littoral estuaries and lagoons; females are found in freshwater only. In proportion as one moves away from the sea, the number of males diminishes and the number of females increases'.

Penáz and Tesch (1970: 308) suggest phenotypic sex determination to explain the distribution of the male European eel in the Elbe estuary. Cairns (1942: 1738 - 1748) suggests that for New Zealand eels the 'law' holds true and that the environment determines the sex and hence distribution of the eels. The eels in fresh-running water become females and those in brackish or salt water become males. However, it has been clearly established that male

eels do occur in freshwater and that longfin males comprise a large percentage of the upstream population of that species. Woods (1964: 118) found that up to 35% of the upstream population of longfins may be males.

From recent investigations on other species it has been established that the generally accepted law of distribution of the sexes is not valid. Sinha and Jones (1966) found male and female European eels in fresh and brackish water. Matsui (1952) records the presence of male Japanese eels in freshwater and Huver (1966) found females of the American eel in brackish water. Vladykov (1966: 1013) notes that males of the American eel are found almost exclusively in salt or brackish water, but quotes an exception of a lake containing a number of male eels.

To explain the occurrence of large proportions of males and females of the European eel in different areas D'Ancona (1960: 67) suggests that these differences in distribution are a result of the different migratory tendencies in elvers with as yet undifferentiated gonads. Sinha and Jones (1966: 382) criticize this suggestion as it means that elvers with undifferentiated gonads have a migratory tendency based on their future sex. However, D'Ancona assumes that sex differentiation is generally under genetic control but is not always well defined. His suggestion is therefore tenable as the sex of those elvers may have been previously determined.

D'Ancona (1949 and 1959) considers that sex determination in the European eel is genotypic but it is labile and can be modified by environmental factors. Sex reversal may occur in small eels with apparently differentiated gonads (D'Ancona 1960: 47). Cairns (1942: 174b) considered that his observation on the apparent change of the lobed gonad to a female gonad was an example of sex reversal. However, this observation lacked any histological confirmation that sex reversal occurred and it was not confirmed that all the eels examined were of the same stock.

Svardson (1949a: 127), discussing the sex and distribution of the European eel, comments that it is important to distinguish between sex differentiation and sex determination. He considers that the confusion over sex differentiation has been interpreted as if there was no genotypic sex determination, but that there is little evidence

to suggest any phenotypic sex determination.

The weight of opinion as regards sex determination in the European eel suggests that it is under genetic control. Bertin (1956: 162) states that sex determination is syngamic in a few individuals but metagamic as a rule. However, D'Ancona (1966: 68 - 69) concludes from numerous observations that sex determination is genetically controlled but may be changed by environmental conditions. Sinha and Jones (1966: 382) suggest that the sex of the European elver is predetermined when it enters freshwater, and the distribution of the sexes is random as the eels grow.

To account for the distribution of male and female eels in New Zealand a similar explanation is offered. It is suggested that the sex of eels of both species is genetically determined and the sexes, immediately after entering freshwater, are randomly distributed. The eventual distribution of the sexes is determined by available habitats and habitat preference, and the different migratory tendencies of the sexes. The shortfins prefer the more stable streams, estuaries and coastal lakes. Longfins may be less selective, and are distributed in all waters, particularly the faster flowing, rocky bottomed streams and rivers. Both sexes of each species occur in all areas where that species is found, although some areas contain high proportions of female shortfins. This may arise from the habitat preferences of each sex, and also the different migratory tendencies, such that females penetrate further inland and males tend to remain in the lower reaches of the water system. The late sex differentiation in some New Zealand eels does not imply any lability in sex determination, and there is no evidence to suggest that the sex can be modified by environmental factors.

3. MIGRANT EELS

3.1 Introduction

Until late last century the European eel was not recognized as one species and many types of eels were distinguished by their different shape, size and colour. Later authors recognized only one species, A. anguilla (L.), but as Schmidt (1906: 139) points out, they did not relate the different types of eels previously described to the different stages of the life cycle. Petersen (1894) was the first author to link some of these types to specific stages of the life cycle. He showed that the feeding yellow eel changes to a silver eel when migrating to the sea to spawn. He called the dress of the silver eel the 'breeding dress' but this is now more commonly termed the migration livery.

It is recognized that other species of freshwater eels take on a migration livery and migrate to sea to spawn. Jubb (1961) describes this for the tropical eels occurring in South Africa, A. mossambica Peters, A. marmorata McClelland, Quoy and Gaimard, A. nebulosa labiata Peters and A. bicolor bicolor McClelland. Further, Bertin (1956) discusses all the temperate species of eels, A. anguilla, A. rostrata, A. japonica, A. dieffenbachii and A. australis schmidtii and concludes that their biology is comparable in this respect.

For New Zealand eels, early authors did not recognize that eels migrated downstream and out to sea to spawn. Duigan (1975) considered that access to the sea was not necessary to eels and Canavan (1892) considered that eels bore their young alive in the lower reaches of the river. The Maoris were astute observers of nature, and, as noted by Downes (1917: 297), they recognized that eels migrate to sea. However, it was Downes who first described the autumn migration of eels. His description, and the Maori descriptions he records of the eels, clearly establish that New Zealand eels migrate to sea to spawn at similar times and under similar conditions as the European eel. Cairns (1941: 68b and 1942b: 175b) describes the migration of mature eels and gives a short description of the sex organs but does not distinguish between the two species.

Hobbs (1947) gives the first detailed account of the seaward migration of eels and Burnet (1969a) discusses the downstream

migration in relation to environmental factors, such as the moon and rainfall.

The discovery of the spawning grounds of the European and American eels by Johannes Schmidt earlier this century is recognized as one of the classic discoveries in biology. However, the spawning grounds of other temperate species have not been discovered. Matsui (1957) proposed that the most probable area for the spawning ground of the Japanese eel is situated near Taiwan and Okinawa (Ryukyu Islands) but this still remains to be proved.

The fate of New Zealand eels once they leave freshwater similarly remains a mystery. Only one specimen has been caught at sea (Todd 1973), a female longfin of length approximately 100 cm. The location of the spawning grounds has received little attention but it has been assumed by Schmidt (1925; 1928) and Castle (1963) that they are in the south-west Pacific Ocean.

The migration of maturing New Zealand eels has been only briefly investigated and important aspects of the biology, such as the state of reproductive development at the time of migration, need investigating.

The purpose of this section of the study was to observe the downstream and seaward migration of maturing eels and to determine which environmental conditions, if any, influenced the migration. This was done by monitoring the downstream migration of maturing eels in the Makara stream and examining the catch figures of seaward migrating eels from Lake Onoke. In the Makara stream water temperature, water level, rainfall, phase of moon, barometric pressure and general weather conditions were recorded to ascertain the controlling conditions.

Further, the study of migration was undertaken to determine the state of reproductive development of migrating eels from as many areas of New Zealand as possible. A comparison could then be made between the stage of reproductive development of each species and from different areas. In addition it was expected that the migrant eels could be kept alive in salt water and, using the techniques of Boëtius, Boëtius, Hemmingsen, Bruun and Møller-Christensen (1962), Fontaine, Bertrand, Lopez and Callamand (1964) and Boëtius and Boëtius (1967a), be injected with hormones to induce them to mature. In this way the

complete cycle of gametogenesis could be observed by histological analysis and the final products of this process viz. ripe eggs and spermatozoa could be examined. Any changes in the morphology of the eels undergoing maturation could also be noted.

Thus the capture, examination and holding alive of migrating eels was an important part of this research project.

The method of capture, the location of the sampling sites, the methods of handling eels in the laboratory and the histological techniques used on gonad samples is described in the materials and methods section.

A 'run' of eels is the terminology accepted by both eel fishermen and scientists to describe the downstream or seaward movement of migrating eels during any particular time interval. A 'run' of eels in this section refers to the number of migrating eels on any particular night and the run may be referred to as being large or small depending on the number of eels caught.

3.2 Periodicity, time and sequence of migration in the Makara stream

The fyke nets set in the Makara stream were visited three times a week between 1 December 1970 and 11 June 1971, from 1 February 1972 to 9 June 1972 and from 14 February 1973 to 23 May 1973. In between each set of dates the nets were lifted. During the fishing periods when the stream level was rising due to rain the nets were visited every day until the stream level dropped. In this manner most of the runs of eels were noted within approximately 12 hours of their occurrence.

Migrant eels were identified by the enlarged eyes and the colour differences. A full description of the colouration and morphometric changes is given later in this section.

During the three years of investigation the nets were visited 181 times. On 60% of these visits no migrant eels were caught. For 29% of these visits one to four migrant eels were caught, and for 11% of these visits five or more migrant eels were caught. An analysis of the number of visits for each year is given in Table 3.1. A large number of visits were made in the 1971 migratory season because it was

TABLE 3.1

The number of visits to the fyke nets set in the Makara stream during migratory seasons 1971, 1972 and 1973

The figures in brackets are the percentage of the total number of visits.

Year	Total no. visits	No. visits yielding no eels	No. visits yielding one to four eels	No. visits yielding five or more eels
1971	92	58	27	7
1972	59	40	13	6
1973	30	10	13	7
Total	181	108 (60%)	53 (29%)	20 (11%)

then not known when the migration would occur. The nets were therefore set from early summer through to early winter. During the following 1972 migratory season, based on the previous year's results, the nets were set from 1st February through to early June. The total number of visits for that year was thus reduced. In view of the previous year's results the nets in 1973 were set for a similar time but because the time of the runs could now be judged the number of visits was further reduced. However, despite the different number of visits for each season, as seen in Table 3.1, there is a similarity between the number of visits when five or more eels were caught for each season. The large number of visits in 1971 arose from the visits made during January and February, when only single migrant eels were caught. As January was not fished during the succeeding two seasons the number of visits yielding one eel was consequently less. This was not considered significant as the catches of single eels did not constitute a run and they accounted for only 5% of the total number of eels caught. Thus for each of the migratory seasons the major runs of eels occurred on only six or seven nights of each season.

By examining the number of eels of each sex of each species in the three largest runs for each year, and for the three years data combined, it was possible to establish the periodicity of the movements. In Table 3.2 the number of eels of each sex of each species for the three largest runs are set out in order and expressed as a percentage of the total number of eels caught for the three year period. For each sex of each species, 40% or more of the eels migrated in one run. The second largest run is much less when expressed as a percentage of the total catch of that sex. The third largest run is similar to the second. Thus both sexes of each species show a restricted migration period. This is shown more clearly in Table 3.3. This gives the percentage of the total catch of each sex of each species for the three largest runs combined for each year and for the three years combined. The total number of nights when any eels of that sex were caught is also given. For each year over 60% of the eels of each sex, except shortfin males in 1972, migrated on only three nights. However, the females of each species, except for the longfin females in 1973, show a more restricted migration period than do the males.

To compare the species periodicity alone the number of eels of each sex of each species was combined and analysed in a similar manner to the data given in Table 3.3. Table 3.4 gives the percentage

TABLE 3.2

The number of eels of each sex of each species
in the three largest runs in the Makara stream
for the three migratory seasons 1971, 1972 and 1973
and for the three seasons combined

The figures in brackets represent the percentage of
the total catch

A. australis

	<u>Catch 1</u>		<u>Catch 2</u>		<u>Catch 3</u>	
	<u>male</u>	<u>female</u>	<u>male</u>	<u>female</u>	<u>male</u>	<u>female</u>
1971	17 (48%)	9 (39%)	7 (20%)	5 (22%)	2 (6%)	4 (17%)
1972	6 (27%)	4 (100%)	4 (18%)	0	3 (14%)	0
1973	5 (38%)	2 (29%)	2 (15%)	2 (29%)	2 (14%)	1 (14%)
N	28	13	13	7	7	5
Total catch for 3 years	70	34	70	34	70	34
% total catch for 3 years	40%	44%	19%	21%	10%	15%

A. dieffenbachii

	<u>Catch 1</u>		<u>Catch 2</u>		<u>Catch 3</u>	
	<u>male</u>	<u>female</u>	<u>male</u>	<u>female</u>	<u>male</u>	<u>female</u>
1971	106 (46%)	10 (71%)	38 (17%)	1 (7%)	30 (13%)	1 (7%)
1972	25 (37%)	1 (33%)	19 (28%)	1 (33%)	10 (15%)	1 (33%)
1973	45 (46%)	1 (20%)	14 (14%)	1 (20%)	10 (10%)	1 (20%)
N	176	12	71	3	50	3
Total catch for 3 years	395	22	395	22	395	22
% total catch for 3 years	45%	55%	18%	14%	13%	14%

TABLE 3.3

Percentage of the total catch of each sex of each species
in the Makara stream for the three largest runs combined
for the three migratory seasons 1971, 1972 and 1973
and for the three seasons combined.

The figures in brackets are the total number of nights when
any eel of that sex was caught

	<u>A. australis</u>				<u>A. dieffenbachii</u>			
	<u>male</u>		<u>female</u>		<u>male</u>		<u>female</u>	
1971	74%	(11)	78%	(7)	76%	(18)	86%	(5)
1972	59%	(10)	100%	(1)	79%	(6)	100%	(3)
1973	69%	(6)	71%	(5)	70%	(11)	60%	(5)
Total	69%	(27)	79%	(13)	75%	(35)	82%	(11)

TABLE 3.4

Percentage of the total catch of each species in the
Makara stream for the three largest runs combined
for the migratory seasons 1971, 1972 and 1972 and for
the three seasons combined

	<u>A. australis</u>	<u>A. dieffenbachii</u>
1971	76%	77%
1972	65%	80%
1973	60%	70%
Total	72%	76%

of the total catch of each species for the three largest runs combined for each year and for the three years combined. Again the figures show the very restricted period of migration. Over 60% of the eels of each species migrated on only three nights. However, the figures are slightly lower when compared with the percentage figures for the males and females in Table 3.3. This is because the largest runs of each sex of each species did not coincide and, as explained above, the females have a slightly more restricted migration period than do the males. Burnet (1969a: 231) found that shortfin migrants had a greater tendency to move at one time than the longfin migrants, probably because of the more restricted migration period of the shortfins. The Makara data as presented in Table 3.4 show that both species have a similar tendency to move at one time.

Migrant eels were caught between the 24 January and 4 May 1971, and from 2 February to 14 May 1972 and from 4 February to 13 May 1973. The catch of migrant eels by day for the first five months of each year is given in Fig.3.1. For convenience the 29 February for the leap year 1972 has been omitted. No migrant eels were caught on that day. This shows a maximum period of migration of approximately three and a half months. Fig.3.1 shows that the major period of the migration is restricted from approximately the end of March to the end of April. The most striking feature of Fig. 3.1 is that the bulk of the eels migrated on very few nights of the season, as described earlier.

The total catch of migrant eels of each species for the three migratory seasons is given in Table 3.5. The catch of each species expressed as a percentage of the total catch is also shown. The species composition of the catch is similar for each of the seasons, the longfin migrants constituting over 70% of the catch in any one year. Table 3.6 gives the number of migrant eels of each sex of each species for the three migratory seasons. The catch of each sex expressed as a percentage of the total catch of that species is also shown. Within each species the number of male migrants exceeds the number of female migrants. However, longfin males are more predominant over longfin females than shortfin males are over shortfin females.

FIG.3.1

Total catch of migrant eels per day
in the Makara stream for the three
migratory seasons 1971, 1972 and 1973.

MAKARA STREAM

TABLE 3.5

Total number of migrant eels of each species
caught in the Makara stream during
the 1971, 1972 and 1973 migratory seasons

The figures in brackets are the percentage of the total catch for
that year and for the three years combined.

<u>Year</u>	<u>A. australis</u>		<u>A. dieffenbachii</u>		<u>Totals</u>
1971	58	(19%)	243	(81%)	301
1972	26	(27%)	71	(73%)	97
1973	20	(16%)	103	(84%)	123
Totals	104	(20%)	417	(80%)	521

TABLE 3.6

Total number of migrant eels of each sex of each species
caught in the Makara stream during
the 1971, 1972 and 1973 migratory seasons

The figures in brackets are the percentage of the total catch for
that year and for the three years combined.

<u>Year</u>	<u>A. australis</u>			<u>A. dieffenbachii</u>		
	<u>male</u>	<u>female</u>	<u>totals</u>	<u>male</u>	<u>female</u>	<u>totals</u>
1971	35 (60%)	23 (40%)	58	229 (94%)	14 (6%)	243
1972	22 (85%)	4 (15%)	26	68 (96%)	3 (4%)	71
1973	13 (65%)	7 (35%)	20	98 (95%)	5 (5%)	103
Total	70 (67%)	34 (33%)	104	395 (95%)	22 (5%)	417

These differences in the species and sex composition of the migrants will be discussed later in this section.

The total catch of migrant eels per year shows considerable fluctuations. For 1972 it is approximately only one third of that for 1971, while 1973 shows some increase but it is still considerably less than 1971.

No species sequence could be established between the migrating eels. Fig.3.2 shows the period of migration of each species for the three migratory seasons. In each of the three years, eels of both species were caught throughout the whole of the migratory season. Except for the end of the 1971 season and the beginning of the 1973 season, the first and last migrants to be caught were shortfins.

Even for the two exceptions mentioned they were ^{the} first and last migrants to be caught but in these instances they were caught with longfin migrants.

The only sequence of migration that could be established was between longfin males and longfin females. Fig.3.3 gives the period of migration of each sex of each species for the three migratory seasons. The number of eels of each sex is expressed as a percentage of the total catch of the sex for that year. The peak run of the longfin males occurs in April of each year, followed by the longfin females in late April and early May. At no time did the peak run of the longfin females occur before the males. No such distinction could be made between shortfin male and females, both sexes intermingling throughout the migratory season.

During the three migratory seasons 187 non-migrant eels were caught. Table 3.7 gives the total number of non-migrant eels caught during the three migratory seasons. For the three years combined, 76% of the non-migrant eels were caught during the first migratory season, 1971. The catch of non-migrant eels by day for December 1970 and for the 1971 migratory season is set out in Fig.3.4. The greatest movement of non-migrant eels occurred between December 1970 and the beginning of March 1971, before any runs of migrant eels. None of the largest catches coincided with runs of migrant eels. No species periodicity within the movements of the non-migrant eels could be determined, both the species intermingling. Burnet (1969a) found

FIG.3.2

Period of migration of each species
in the Makara stream for the three
migratory seasons 1971, 1972 and 1973.

RA STREAM

FIG.3.3

Period of migration of each sex of each species in the Makara stream for the three migratory seasons 1971, 1972 and 1973. The number of eels of each sex is expressed as a percentage of the total catch of that sex.

TABLE 3.7

Numbers of non-migrant eels caught in the Makara stream
during the migratory seasons 1971, 1972 and 1973

The figures in brackets are the percentage of the total catch of
non-migrant eels for that species for each year, and for the three
years combined

<u>Year</u>	<u>A. australis</u>	<u>A. dieffenbachii</u>	<u>Totals</u>
1971	41 (28%)	104 (72%)	145
1972	14 (42%)	19 (58%)	33
1973	5 (56%)	4 (44%)	9
Total	60 (32%)	127 (68%)	187

FIG.3.4

Catch of non-migrant eels per day in
the Makara stream and the daily rainfall (mm).

MAKARA STREAM

n = 187

than runs of maturing and immature eels coincided six times for 23 major runs, indicating that runs of immature and mature eels were probably affected by similar conditions. Further, he found that runs of immature eels occurred for each of the six years sampled, indicating that the runs of immature eels were annual. In the Makara stream it was noticeable that when runs of migrant eels occurred, only one or two non-migrants were caught. This would suggest that either the runs of migrant and non-migrant eels were affected by different conditions or that the non-migrants moved during different times in the season. It is not possible to determine if the movement of non-migrant eels was annual in occurrence. The catch of non-migrants for the three years is not comparable because of the different fishing times. During the 1971 season 82% of the non-migrant eels were caught before the end of February. Fishing did not start until the beginning of February for the 1972 and 1973 seasons, but for the 1972 season 50% of non-migrants were caught during February. During the 1973 season all of the non-migrant eels were caught during February. Therefore, there is probably a downstream movement of non-migrant eels during December, January and February of each year, before the runs of migrant eels.

3.3 Periodicity, time and sequence of migration from Lake Onoke.

Five visits were made to Lake Onoke during the three years of this study. The distance from Wellington, approximately 100 km, and the lack of transport prohibited more frequent visits. Further, the migration times in the Makara stream coincided with those from the lake and there were insufficient fyke nets to fish in the two areas at the same time. No trips were made during the migratory season of 1970 at the commencement of this study because of the lack of equipment and knowledge of the migration from the lake. During the 1971 migratory season three visits were made, the last being unsuccessful as the outlet to the lake was open and no eels could be caught. When the lake is open the migrating eels do not congregate in the shallow water near the sea, but remain in the deeper waters of the lake and then move directly to the sea. It was not possible to fish in the outlet because of the swift outward-flowing current. Two visits were made to the lake during the 1972 migratory season. In 1973 no visits

were made, the outlet was open throughout the migratory period, February-May, and no fishing was possible. The times of the visits to Lake Onoke and the catch of migrant eels obtained is given in Table 3.8. Few longfin migrants were caught because, as explained later, these are generally the last to migrate in April and May. If the lake is open by this time few are caught.

The shortfin migrant eels obtained during these visits yielded sufficient data for length frequency, length-weight relationships, ageing and gonad analysis but did not provide enough to determine the periodicity time and sequence of migration to sea. However, catch and processing figures for Lake Onoke migrant eels obtained from a local eel processor provided a comprehensive record of the time and sequence of movement. During the processing of migrant eels at the factory they are sorted by species into lb. weight groups, 1-2 lbs, 2-3 lbs, etc., up to 12 lbs. Any large eels are called 'over-size' eels. The small shortfin migrants are graded into $\frac{1}{2}$ lb or 150-250 g categories. Knowing the length and weight range of the sexes of migrant eels from Lake Onoke the sex of the eels in each weight range could be determined i.e. approximately all shortfin migrants under 1 lb were males and all migrant longfins under 3 lbs were males. The eels in the heavier weight categories were all females.

The total catch of migrant eels by day for the migratory seasons of 1971 and 1972 is given in Fig.3.5. The migration period is from late March to late April or mid-May. For each of the years the migration was well advanced by late March, as indicated by the high catch figures on the first part of each graph. Catch figures were not available for the earlier part of March for each year but local fishermen state that the migration begins in late February or early March. My own catch figures support this, shortfin migrant males were caught in late February 1971. Migrant eels were caught through to the end of April as in 1971 or mid-May as in 1972. Each year shows an abrupt stoppage because the lake was opened at both of these times and fishing could not be continued. Local fishermen had found that in previous years, when the lake remained closed, the migration continued through until mid-or late May.

Fig.3.6 gives the catch sequence of each sex of each species for the 1972 migratory season. The catch of each sex is expressed as a percentage of the total catch of that sex. The peak catch of

TABLE 3.8

Lake Onoke - Number of visits and number of migrant eels
caught per visit

<u>Year</u>	<u>No. visits</u>	<u>A. australis</u>		<u>A. dieffenbachii</u>	
		<u>males</u>	<u>females</u>	<u>males</u>	<u>females</u>
1971	1	46	4	0	0
	2	1	18	0	0
	3	0	0	0	0
1972	1	70	13	0	0
	2	0	7	2	4
Total		117	42	2	4

FIG. 3.5

Total catch of migrant eels from Lake Onoke
per day related to the phase of the moon for
the 1971 and 1972 migratory seasons.

new moon	●
full moon	○
first quarter	◐
last quarter	◑

LAKE ONOKE

FIG.3.6

Period of migration of each sex of each
species in Lake Onoko for the 1972
migratory season.

LAKE ONOKE

shortfin males occurred first, in late March, followed by the peak catch of longfin males in early April. Shortfin female migrants reached a peak shortly after the longfin males. The longfin migrant female catches reached a peak last, in late April. Each sex of each species was caught throughout the period that data was available for. Shortfin males, longfin males and longfin females show distinct peaks in their catch figures. Shortfin female catches show a less distinct peak, being caught in similar quantities throughout the period.

The 1971 catch figures are less comprehensive. The period that data were available was shorter and figures for several days during the season are missing. However, the catches for each sex show a similar trend, the peak catch of shortfin males occurring first, in late March. Then shortfin females followed closely by the longfin males. This sequence of shortfin females and then longfin males is the reverse of the 1972 catches. As the two peaks are so close together in each year no significance is attached to this reversal. The ^{last} peak catch is for the migrant longfin females.

The peak catches of the sexes in either year constitute less than 20% of the total catch of that sex. As the sexes of both species are caught throughout the season, the sequence of migration is not definite when the data are examined at any one time during the migratory period. This is supported by local fishermen who state that on one night they may catch mainly longfin migrant males, while the next night the catch may be predominantly shortfin males. However, the catch figures for the whole migration period show an overall sequence of shortfin males followed by shortfin females and longfin males, or vice versa, close together, and finally longfin females.

3.4 Migration times from other areas

Accurate times of the migration of eels from other areas could not be obtained. Migrant eels were obtained from the areas given in the materials and methods section. The eels, except those from Lake Ellesmere, Lake Kaikokopu and the Pukepukelagoon, were obtained from eel processors after they had held them in their holding tanks. The times of migration could not be obtained from the eel processors to accompany these samples. The Lake Ellesmere migrants were obtained

during a field trip in February 1972 to the South Island, when the migration of the shortfin males occurred, as shown in Table 3.9. However, the migration from Lake Ellesmere has previously been studied by Hobbs (1947). The migrant eels from the Pukepuke Lagoon were caught in a trap which was left unattended for up to a month at a time during the 1971 migratory season. Consequently, of the few migrants that were caught, the time of the migration could not be accurately determined. However, a small sample of shortfin migrant females was obtained from the nearby Lake Kaikokopu in early June 1970, from a local fisherman. These eels were caught migrating down to the sea along a drain leading from the lake. Their access to the sea had previously been blocked by a spillway but with a rise in the water level of the lake it overflowed the spillway enabling the eels to migrate to sea. From the migration times in different areas given in Table 3.9 it would appear that the eels in Lake Kaikokopu were delayed in their migration because of the particular prevailing conditions.

3.5 Discussion of the times and sequence of migration

The migration times and the sequence of migration of downstream and seaward migrating eels from different areas as recorded by different authors is set out in Table 3.9. The months, in brackets, give the duration of the migration for that sex, the other month(s) the peak of migration. The seaward migrating eels are those caught in coastal lakes congregating near the sea where their access has been blocked by sand or shingle bars. Downstream migrants are those eels caught during their downstream migration to the sea in streams and rivers where their access is not blocked.

The only previous detailed study of the downstream migration of maturing eels is that of Burnet (1959a), who found that in the South Branch stream migrating longfin males were caught first, with a peak run in December, although the run extended from October to February. Longfin females migrated slightly later than the males. Shortfin migrating males had a peak run in February, two months after the longfin males. The migration of shortfin females coincided with that of the males. As shown in Table 3.9 the migration times and sequence of migration of Makara downstream migrants are much later. Makara longfin migrants

TABLE 3.9

The migration times, and sequence of downstream and seaward migrating eels recorded by different authors

TABLE 3.9

The migration times, and sequence of downstream and
 (The figures in brackets are the peak migration times)

Author	<u>A. australis</u> males	<u>A. australis</u> females
Downes (1917) downstream migrants sequence	early April <u>A. australis</u> males	mid-April <u>A. Australis</u> females
Cairns (1941) downstream migrants sequence	February <u>A. australis</u> males	late February <u>A. australis</u> females
Hobbs (1947) seaward migrants sequence	March - April <u>A. australis</u> males	March - April <u>A. australis</u> females
Burnet (1969a) downstream migrants sequence	January - March (February) <u>A. dieffenbachii</u> males	January - March (February) <u>A. dieffenbachii</u> females
Makara stream downstream migrants sequence	January - May (Mid-April) No definite sequence but	January - April (Mid-April) <u>A. dieffenbachii</u> females
Lake Onoke seaward migrants sequence	late March - early May (late March) <u>A. Australis</u> males	late March - early May (mid-April) <u>A. australis</u> females

seaward migrating eels recorded by different authors

	<u>A. dieffenbachii</u> males	<u>A. dieffenbachii</u> females
	mid-April	May
	<u>A. dieffenbachii</u> males	<u>A. dieffenbachii</u> females
	late March	late April - May
	<u>A. dieffenbachii</u> males	<u>A. dieffenbachii</u> females
	April - May	May - June
	<u>A. dieffenbachii</u> males	<u>A. dieffenbachii</u> females
	October - February (December)	October - February (December)
	<u>A. australis</u> males	<u>A. australis</u> females
generally last	January - April (mid-April)	April - May (late April - early May)
	Late March - early May (mid-April)	late March - early May late April
← (may be reversed) →	<u>A. dieffenbachii</u> males	<u>A. dieffenbachii</u> females

migrate some four months later than recorded by Burnet and the shortfin migrants two months later. The sequence of migration of each species from the two areas are in reverse. Burnet found that male and female longfin migrants moved downstream before the shortfin migrants. In the Makara stream, although no species sequence could be clearly determined, the large longfin females were the last to migrate.

The migration times of downstream and seaward migrating eels from different areas recorded by the other authors are very similar to those for the Makara stream and Lake Onoke in this study. The peak migration time for all migrants from all areas is April, except Cairns (1941), who indicates that March would be the peak period. They are all later than the times given by Burnet (1969a).

The sequence of migration of each sex from the different areas is also similar. All show the migration sequence of shortfin males, shortfin females and longfin males close together, and finally longfin females. The Makara data, with no clear sequence, however does have the similarity of the longfin females migrating last.

The differing migration times shown by Burnet (1969a) and this study, supported by other authors, are difficult to explain. The sampling site used by Burnet was situated on the South Branch stream, a tributary of the the Waimakariri River, 18 km from the sea. In contrast, the Makara sampling was 3 km upstream, just beyond the tidal influence. The location of the Makara sampling site closer to the sea does not explain the four month delay in the migration of the longfins and the two month delay of the shortfin migrants, as compared with the earlier migration of the South Branch eels. It could be expected that the South Branch migrants spend some time in the estuarine waters of the Waimakariri River and then migrate out to sea at a later date. In discussion, Burnet (1969a: 243) considers that the date of migration into the sea can be expected to be later than the dates of the downstream migration. However, it is difficult to explain a delay of up to four months in the estuarine waters if the South Branch eels migrate to sea at a similar time to the Makara migrants. The Makara migrants were caught just beyond the upper limit of the tidal influence and would be expected to migrate downstream and then move to sea within a few days. Further, these eels were caught at the same time as the seaward migrating eels from Lake Onoke and Lake Ellesmere, so it is assumed

that they spend very little time in the estuarine areas and soon migrate to sea. Thus the downstream migration of eels in the Makara stream would be close to the time of the seaward migration from the stream estuary into the sea.

Burnet (1969a; 1969c) notes that in the migrant eels caught earlier in the season the enlargement of the eye was only just visible but as the summer progressed they assumed a more distinct migratory livery. This implies, from the reproductive state of migrants described later in the section, that these earlier migrating eels were less sexually developed than the later migrants. These earlier eels may spend some time in estuarine waters to undergo further sexual development before migrating to sea. This would account for some of the difference between the earlier part of the migration recorded by Burnet (1969a) and those migration times recorded by other authors. Nevertheless, the peak of the migration given by Burnet for migrants with a distinct migratory livery are still much earlier. It is shown later in this section that the state of sexual development within each sex at migration varies little in migrant eels of the same sex and species from different areas. There is no evidence to suggest that the migrant eels observed by Burnet at the peak of the migration were any less sexually developed than those eels from other areas. Thus, as previously stated, it is difficult to explain the differences in the migration times.

A possible explanation between the differing migration times from the two areas is the difference in latitude. The South Branch site is $2\frac{1}{2}^{\circ}$ of latitude, approximately 322 km, south of the Makara sampling site. It is assumed that the spawning grounds of New Zealand eels lie to the north of New Zealand in the south-west Pacific Ocean (Schmidt 1925; 1928). Migrating eels from areas in the South Island may move out to sea before those in the North Island, so as to arrive at the spawning grounds at the same time. However, this is not substantiated by the migration times from Lake Ellesmere which are the same as those from Makara and Lake Onoke.

The peak of the migration of the European eels occurs from October to November in most regions. In the low countries of North western Europe however, the migration may start in June and be well advanced by July (Deelder 1970: 3,25). July may even be the peak of the migration time in some small bodies of water according to Deelder (Deelder 1957 in Deelder 1970; 3,25). The European eel has earlier

migration times from the more northern limit of its distribution comparable with the differences discussed here.

To explain the difference in the sequence and time of migration between the South Branch eels and the Lake Ellesmere migration time and sequence given by Hobbs (1947), Burnet (1969a: 243) states 'Possibly, there is a longer lapse between the stimulus to migrate downstream and the stimulus to migrate to sea in A. dieffenbachii because this species is generally found further inland than A. australis. Conversely, because A. australis is usually found in streams near the coast, it probably does not take as long to move downstream; there may be only a short time between the stimulus to move downstream and the stimulus to move into the sea.' This may explain the differing migration time and sequence between the South Branch eels and the Lake Ellesmere eels but it is not supported by the Makara downstream migration sequence nor by the sequence of migration that can be deduced from Downes (1917). It is established later in this study that in the Makara stream both species respond to the same stimuli and migrate downstream at the same time. It is also assumed, as discussed earlier, that they migrate to sea at the same time with little delay. Thus the delay for the longfins between the stimulus to migrate downstream and the stimulus to move out to sea that Burnet proposes would probably not occur in the Makara stream. Downes made his observations on the sequence of migration from a site at least 32 km from the sea on the Waitotara river in the North Island. From his descriptions of the migrant eels, the species and sex sequence of the migration can be calculated, as given in Table 3.9. Again, from the sequence and the time of the downstream migration the eels of both species respond to the same stimulus at the same time. The downstream migration time is the same as that of seaward migrating eels from other areas so there would be little delay before the stimulus to move out to sea.

The above discussion compares areas that may have a different species composition of the upstream population. Burnet (1969a: 243) mentions that A. dieffenbachii is generally found further inland than A. australis. Streams and rivers with a large inland population of A. dieffenbachii at considerable distances from the sea may show a time and sequence of migration similar to the South Branch stream. In these situations upstream migrant longfin

eels would respond earlier to the stimulus to migrate downstream, before the longfin eels located nearer to the sea. For the earlier responding upstream migrants there would therefore be a delay between the initial stimulus to move downstream and the stimulus to migrate out to the sea. Conversely, in streams and rivers with inland populations closer to the sea, there would be little delay between the stimulus to migrate downstream and the stimulus to migrate out to the sea, as in the Makara stream.

Conversations with eel processors and eel fishermen throughout New Zealand revealed that few migratory eels are caught in river systems in New Zealand. Most processors estimated that migrant eels constituted no more than 5% of the total amount of eels they processed. Of these migrants most would come from the coastal lakes, Lake Ellesmere and Lake Onoke, and would be processed in eel factories close to these areas. Eel factories relying only on eels from rivers and inland lakes process far fewer migrant eels.

As described later, the migration of eels is closely associated with rainfall and the consequent rise in water level and flow. Few fishermen are willing to risk the loss of their nets under such conditions to catch the migrants and consequently few are caught. Thus a large percentage of the migrant eels come from the two lakes mentioned, Lakes Ellesmere and Lake Onoke.

Both of these present a unique situation in the New Zealand eel fishery. These lakes and one other, Lake Forsyth, adjacent to Lake Ellesmere, all have similar physical characteristics. They are brackish, shallow, separated from the sea by a shingle or sand bar and in the cases of Lakes Ellesmere and Onoke, have a large freshwater system draining into them. All three lakes provide ideal fishing for migrating eels. As described earlier, the bar blocks the access to the sea for the migrating eels and they congregate in areas of the lakes nearest to the sea. Large quantities of eels can thus be caught. Later during the migratory season, usually in May or June, when the water levels have risen due to the autumn rains the lakes are opened to prevent the flooding of surrounding farmlands. This situation is more frequent in Lake Onoke than in the other two lakes.

Fishing in Lake Forsyth is restricted to the Maoris so that the commercial catches come from the other lakes. It is

of interest that in 1973 a new flood control system was in operation at Lake Onoke. The lake level remained high throughout the migratory season and was sufficient to keep the outlet open throughout the period. In previous years when the lake level was lower the outlet often closed due to the wave action building up the bar again. Consequently in 1973 the migratory eels did not congregate in areas that could be readily fished and they moved straight out to sea. Only approximately two metric tons were caught in 1973 compared with 20 metric tons the previous year. The local body controlling the flood control system is likely in future years to keep the lake outlet open, as in 1973. One of two major migrant eel fisheries will thereby be severely reduced.

Further research on monitoring the downstream migration of eels at points further inland and in different areas of New Zealand could explain the differences in the migration times discussed and may help to increase the catch of migrant eels in river systems to compensate for the loss of the migrant eel fishery at Lake Onoke.

3.6 Correlation of the migration with environmental factors

Earlier research on New Zealand eels has established the relationship between the migration of maturing eels and certain environmental conditions. Downes (1917: 299) recorded that the largest catches of migrating eels caught by the Maoris were associated with an increase in the water level and moonless nights. Burnet (1969a) made the first detailed study of the environmental factors affecting the downstream migration. A definite correlation was found between the lunar cycle and rainfall with the downstream migration.

The environmental factors that were associated with the runs of eels in the Makara stream are discussed separately below. Where the data are available, the factors affecting the seaward migration of eels from Lake Onoke are also discussed.

Rainfall

The total catch of migrant eels by night, both species combined, for the three migratory seasons 1971, 1972 and 1973 is given

in Fig.3.7. The daily rainfall and the phase of the moon are also given. Both species are combined to give the total catch of migrant eels by night as no sequence of migration between the species could be established.

One of the most obvious environmental factors associated with each run of migrating eels was the increased rainfall, resulting in an increased water level and flow in the stream. The relationship between rainfall and the migration is also shown in Table 3.10. The terms rainfall and water level are both used in the text as synonyms. Increased rainfall always resulted in increased water level and water flow. All runs of five or more eels are associated with some rainfall and hence increased water levels. However, as shown in Fig.3.7, there were several nights when an increase in water level during the migratory period was not associated with a run of eels. This occurred when a rise in water level occurred very early in the season, as in February 1971, or when associated with a full moon as on 12 March 1971. Runs did occur immediately before or after the full moon if the water level was raised, as on 12 April and 22 April 1973. The largest catches of eels were not always associated with the highest rainfall, as on 5 May 1971, as other conditions were not favourable, in this case a nearly full moon. The largest runs were associated with a combination of factors: time of the season, water level, general weather conditions on that night and the phase of the moon. The combined effect of these environmental factors will be discussed separately.

The catches of single migrant eels in the early part of 1971 migratory season are not related to increased water level and their movements appear to be random. The movement of these few individual eels does not appear to be associated with any specific environmental factor.

The coincidence of runs of eels with rainfall has been established by previous research. Burnet (1969a) concluded that heavy rainfall, in association with the correct phase of the moon and season, stimulated the larger runs of eels. He also found instances of heavy rainfall coincident with small runs of eels when the lunar and seasonal conditions were not favourable. No correlation could be found between rainfall and the smaller runs.

FIG.3.7

Total catch of migrant eels in the Makara stream per day related to rainfall (mm) and the phase of the moon, for the three migratory seasons 1971, 1972 and 1973.

new moon

full moon

first quarter

last quarter

TABLE 3.10

The rainfall (mm) and the number of migrant eels caught per day in the Makara stream for the three migratory seasons, 1971, 1972 and 1973

<u>1971</u>			<u>1972</u>			<u>1973</u>		
Date	Rainfall (mm)	no. eels	Date	Rainfall (mm)	no. eels	Date	Rainfall (mm)	no. eels
24/1		1						
29/1		1						
4/2		1	2/2		1			
9/2		1	7/2		1			
14/2		1	9/2	2.6	2			
18/2		1	17/2		5			
21/2		1	24/2	3.4	6			
25/2		1						
7/3		1	25/3	12.6		3/3	3.2	
14/3		1	26/3	1.5	11	4/3		2
18/3		1	27/3		11	5/3	11.2	9
21/3		2	28/3		1	12/3	24.0	
25/3	4.5	19				13/3		3
26/3	2.8	43				23/3	17.2	
27/3	1.0	5				24/3		11
4/4	1.0	1	6/4	7.2	21	1/4	6.4	49
6/4		1	7/4	13.3	33	2/4	4.1	10
11/4		4	14/4	3.8	1	10/4	2.5	
13/4		1				11/4		5
15/4	2.0	112				13/4	7.0	
16/4	1.0	19				14/4		2
17/4		3				18/4		1
20/4	10.0	2				21/4	5.9	17
21/4	2.5	66				23/4	1.9	9
22/4		1				16/4		2
3/5	15.0	3	1/5		1	10/5		1
4/5	2.8	8	4/5	4.7	1	13/5		2
			13/5	27.9	1			
			14/5	9.4	1			
N = 301			N = 97			N = 123		

For the European eel Frost (1950) Jens ^{1952/}(1953) and Lowe (1952) all found that increases in catches of migrant eels were made when there was an increase in the water level. Deelder (1954: 179; 1970: 3: 31) however considers that it is the increase in water flow and not water level that encourages the eel to migrate. His suggestion is based on observations on the movement of migrant eels in Dutch 'polders' where there was water flow but constant water level. The eels were stimulated to move by the water movement. In rivers and lakes it is not possible to separate the effect of rainfall, and increased water level and flow. Frost (1950: 370) however, does show a correlation between catch increase and increased water flow.

In the Makara stream, as described in the Materials and Methods section, two fyke nets were set across the width of the stream to catch the downstream migrants. Although no measurements were made of the water flow during the migration, one net fished the deepest and fastest flowing portion of the stream and caught 99% of the eels. This would indicate that the eels movements were influenced by the greater water flow in that portion of the stream.

The eels rarely migrated when the water level was low and no peak runs occurred under these conditions. This may be due to the low water level hindering the movement of the eels. Under low water level conditions sections of the stream are very shallow, less than 0.25 m deep and the flow of water does not take a direct course in the stream bed but meanders, often into blind backwaters. However, at no stage prior to the migration was the stream dry in any place, so access to the sea was always available, although often partially obstructed. In flood conditions the water flow downstream is direct and unhindered. Thus, in a flood, access to the sea would be direct and the eels could move downstream unhindered. This suggests that a rise in water level has a mechanical effect on the migrating eel as proposed by Lowe (1952: 298). The rise in water level, the increased flow and the direction of the water flow may guide the eels downstream and increase their speed of movement.

Although no accurate measurements of water level were recorded throughout the three migratory seasons it was noted that the eels often migrated after the initial increase in water level, often when it was dropping. In some instances there appeared to be

approximately a 12 hour delay in the response of the eels. When it rained heavily during the daylight hours the water level increased rapidly. By that night the water level would have risen considerably but on several occasions the eels did not migrate until the following night. Often the water level was dropping by this time if the rain had ceased. If the level remained high good catches were made the following night and a few more migrants might be caught on the third night. Fig.3.7 and Table 3.10 both show that the best runs occurred over three nights and even if the water level remained high no runs occurred after that three night period. This 'migration-period' is discussed separately.

Jens (1952/1953:109) studied catch figures for migrating European eels and found that the intensity of the migration was usually greater with rising water levels than with falling water levels. This is the reverse of the behaviour of migrating eels in the Makara stream. Jens also noted that the intensity of the migration increased with rising water levels. However, as noted for migrating Makara stream eels, some of the best runs occurred when the water level was not at its highest and the intensity of the migration is dependent on a combination of environmental factors.

There may be other factors, chemical and physical, associated with a flood that may effect the migrating eel. A drop in temperature was one factor associated with each rise in water level and is discussed separately.

On some nights with high water level the water was very turbid but it did not appear to affect the movement of the eels as runs occurred under these conditions. Downes (1917: 299) recorded that large numbers of eels migrated when the water was turbid. Under these conditions the Maoris caught migrating eels during the day as well but in smaller numbers than at night. Frost (1950: 370) mentions that fishermen on the River Bann found fishing for migrating European eels better under these conditions. As migrating eels move in the darkest periods and are sensitive to light they probably do not rely solely on visual orientation to guide themselves downstream. Instead the water flow directs them downstream. Turbid conditions would therefore not be expected to affect them.

Other chemical and physical factors coincident with increased water levels, such as pH and oxygen levels may affect the migration of the eels in some way. No attempt was made in this study to measure these factors, and their role in influencing the migrating, if any, has not been ascertained.

Moon and lunar rhythm

The other most obvious environmental factor associated with the runs of eels was the phase of the moon. Fig.3.7 shows that most of the runs of eels appear to be associated with one phase of the moon, the last quarter. As shown in Table 3.11, 47% of the runs of more than five eels occurred during the last quarter of the moon.

To determine if the downstream migration of eels showed a correlation with the lunar cycle the total catch of eels by night was set out in lunar months. This was done by taking the day of the new moon immediately preceding the first catch of a migrant eel for each migratory season as day one. The following catches of eels per day were then plotted according to the number of days after the new moon, until the end of that lunar month. For the following lunar months this procedure was repeated until the catch of migrant eels for the whole migratory season was covered. For each of the three migratory seasons 1971, 1972 and 1973 the eels migrated over a maximum period of five lunar months. The number of eels caught per day of each lunar month was then combined for each of the migratory seasons separately and then for the three years combined. This gave the total number of migratory eels caught each day per lunar month for the three migratory seasons and is set out in Fig.3.8.

Fig.3.8 clearly shows that there was a period of maximum activity just before the last quarter of the moon. There is greater activity during the latter half of the lunar cycle, but definite movements do occur throughout the whole period. Overall there is a definite correlation of runs with the lunar cycle and the migrating eels exhibit a definite lunar rhythm.

The correlation of the catch figures from Lake Onoke with the lunar cycle was calculated in the same way as the Makara catch

TABLE 3.11

Migration dates and associated weather conditions
when more than five migratory eels were caught in
the Makara stream during the three migratory seasons,
1971, 1972 and 1973.

TABLE 3.11

Migration dates and associated weather conditions when
during the three

Migration date	No. eels	Moon phase	Rainfall (mm)	Water T ^o (C)	Wind direction
25/3/71	19	last $\frac{1}{4}$ - new	4.5	16.0	N/W
26/3/71	43	new	2.8	18.0	N/W
15/3/71	112	full - last $\frac{1}{4}$	2.0	11.2	S
16/4/71	19	full - last $\frac{1}{4}$	1.0	12.0	S
21/4/71	66	last $\frac{1}{4}$ - new	2.5	14.5	N/W
4/5/71	8	last $\frac{1}{4}$ - full	28.0	14.5	S
24/2/72	6	1st $\frac{1}{4}$ - full	3.4	14.6	S
26/3/72	11	1st $\frac{1}{4}$ - full	1.5	13.0	N/W
27/3/72	11	1st $\frac{1}{4}$ - full	-	13.0	N/W
6/4/72	21	last $\frac{1}{4}$	7.2	13.0	S
7/4/72	33	last $\frac{1}{4}$	13.3	13.0	S
5/3/73	9	new - 1st $\frac{1}{4}$	11.2	14.8	N/W
24/3/73	11	last $\frac{1}{4}$	17.2	13.5	S
1/4/73	49	new	-	12.5	S
2/4/73	10	new	-	12.1	S
21/4/73	17	full - last $\frac{1}{4}$ -	-	14.0	N/W
23/4/73	9	full - last $\frac{1}{4}$ -	-	-	N/W

Wind direction

N/W = north west

S = south

more than five migratory eels were caught in the Makara stream
migratory seasons 1971, 1972 and 1973

Barometric pressure (mb)	Rise in stream water level (cm)	General weather conditions
3.0	7	showers, dark night, depression near
3.2	5	showers, dark night, depression near
4.3	3	
3.5	2	showers, deep depression near
1.9	3	showers, deep depression near
-	18	Rain, depression near
3.4	10	Rain, no depression near
-	5	Rain, deep depression near
-	10	Rain, deep depression near
1.6	8	Rain, deep depression near
0.9	10	Rain, deep depression near
-	8	Rain, no depression near
-	5	Rain previous night, depression near
-	8	Showers, southerly front
-	5	Showers, southerly front
8	8	Showers, no depression near
-	3	Showers, no depression near.

FIG.3.8

Total catch of migrant eels of both species
in relation to the lunar cycle.

A. The Makara stream

B. Lake Onoke

new moon

full moon

first quarter

last quarter

MAKARA STREAM

LAKE ONOKE

figures. The total catch of migrant eels (in lbs) per night is shown in Fig.3.8. The relationship of the peaks to the lunar cycle vary considerably, and the pattern of activity is less definite than the Makara lunar rhythm. The graph however does show that there was minimum activity on and just after the full moon, but definite movements occur throughout the lunar cycle. There is a much greater spread of activity through the lunar cycle than is shown by the Makara migrants and the lunar rhythm is consequently less definite.

Previous research, (Burnet 1969a and Downes 1917), has shown that migrating eels are influenced by the moon and exhibit a lunar rhythm. Downes records that the Maoris knew that it was almost useless to fish for migrating eels on a moonlight night. Burnet found a correlation of down-stream migration with the lunar cycle and calculated a lunar rhythm very similar to that for the Lake Onoke eels (Fig.3.8). There was a minimum of activity at the full moon but otherwise movement occurred throughout the lunar cycle. Hobbs (1947: 230) did not calculate a lunar rhythm for the Lake Ellesmere migrating eels but it was noted that on dark nights, especially from two to three nights after the full moon, the eels congregated in the shallow areas of the lake nearest to the outlet beach.

The lunar activity found for the migrating Makara eels is very similar to that of the migrating European eel. Frost (1950: 370) and Lowe (1952: 280) show that the peak catches and runs of eels coincided with the last quarter and new moon phases of the lunar cycle. A statistical analysis of the catches of migrating eels and the phase of the moon by Jens (1952/1953: 109) showed that the best catches were made during the last quarter. Similarly, Deelder (1954: 180) found that the best catches of migrating eels in Holland were made during the last quarter and new moon phases of the lunar cycle. This time of activity is supported by experimental evidence. Boëtius (1967) found that under experimental conditions the maximum escape activity of captive migrant eels occurred during the third (last) quarter of the moon.

Frost (1950: 370) and Lowe (1953: 299) consider that the migration of the eels is probably influenced by the phase of the moon through the effect of the light, rather than by the periodic effect of the lunar phases. However, this assumption is not accepted by all

authors. Jens (1952/1953: 110) considers that for the European eel the relation of the migration to the moon phase is not affected by the moonlight. Deelder (1970: 3: 39), discussing the influence of the moon on migration, considers that this assumption may not be correct. He considers that during an eel's lifetime an inner rhythm is set up by the influence of the moonlight. This rhythm operates at the migratory stage whether moonlight is present or not. This assumption is supported by Boetius (1967) who conducted experiments on migrant European eels in permanent darkness and concluded that the nocturnal, moon phase dependent, autumnal migratory activity of silver eels is independent of light, daylight as well as moonlight.

Although eels may possess an endogenous lunar rhythm as indicated by Boetius, field observations and laboratory experiments indicate that under normal conditions this rhythm is controlled by the effect of light. Lowe (1952) determined that the movement of the European eel in the laboratory and runs in the field were stopped by a light barrier.

Under certain circumstances migratory eels will run during a full moon period. Lowe reported that in one instance eels migrated during a full moon when the water was turbid. In this study a similar run of eels occurred. On 4 and 5 May 1971 eels in the Makara stream migrated during a full moon period in a flood and overcast conditions.

This and Lowe's evidence suggests that the migration of the European and New Zealand eels is influenced by the phase of the moon through the effect of the light, with the bright periods of the lunar cycle inhibiting movement. However, runs may occur if overcast and flood conditions occur during the least favourable part of the lunar cycle.

Observations on migrating eels in New Zealand by Downes (1917) also indicate that their movement is affected by light. As discussed, Downes (1917: 299) recorded that the Maoris knew that fishing on a moonlit night resulted in poor catches. Fishing in daylight was equally ineffective but if the water was turbid migrating eels could be caught under these conditions.

When fishing at Lake Onoke using channels cut into the bar (see Materials and methods section) I observed that the eels swimming in the shallow water and into the channels would swim off into the darkness if a light was shone at them. The period of maximum movement of the Makara eels was in the second half of the

lunar cycle and concentrated about the last quarter of the moon. The last quarter of the moon is the part of the lunar cycle during which the earlier part of the night is dark, the moonlight failing early. Further, as discussed under diurnal rhythm, the eels migrate in the earlier part of the night. It is logical to assume that it is the absence of moonlight which acts as a stimulus to migrate and conversely moonlight and daylight would have an inhibitory effect on the movement of the eels. The period of maximum activity exhibited by the Makara migratory eels is more restricted than that of the Lake Onoke migrants. The latter show migratory activity over a much greater period of the lunar cycle. Deelder (1970: 3: 29) and Jens ¹⁹⁵² (1953) found that the moon's effect on the European eel does not appear to be felt everywhere at the same time. In the Upper Rhine the migration occurs before the last quarter of the moon whereas in Holland and the Baltic it occurs after the last quarter.

When comparing the lunar activity exhibited by the Makara and Lake Onoke migrants it is not that the runs from either area are restricted to different phases of the moon, but that the Lake Onoke migrants show a less definite lunar rhythm. In the latter, movement occurs throughout the lunar cycle. This can be explained in two ways, by sampling bias and the different sampling areas. The Lake Onoke data are from catches made by fishermen who fish throughout the lunar cycle. Thus a certain catch level is maintained throughout the lunar cycle.

Secondly, as previously discussed, it is likely that the moonlight exerts an inhibitory effect on the migration of the eels. The Makara stream in many stretches is less than 0.5 m deep and the moonlight would penetrate to the stream bottom in these areas. These stretches of shallow water could act as a barrier to the migrating eels when the moon is in the first half of the lunar cycle, that is, when the moonlight occurs in the earlier part of the night. Lake Onoke on the other hand is deeper (0.5 to 6 m) than the Makara stream and the movement of migratory eels may be less affected by the moonlight except at the brightest period, the full moon, when the activity is less (Fig.3.8). However, migratory eels congregating in the shallow areas of the lake would be expected to be inhibited by the moonlight in the first half of the lunar cycle as the Makara

eels may be. Nevertheless, the deeper water of Lake Onoke would allow for more movement during the greater part of the lunar cycle without being inhibited by the moonlight and thus a certain minimum catch level would be maintained throughout the lunar cycle.

This explanation for the difference in the two lunar rhythms is supported by observations on the migrating European eel. Petersen (1906) suggested that the full moon affected the depth at which eels migrated in Danish waters. From field and laboratory observations, and experiments, Lowe (1952: 307) concluded that light will have a relatively greater effect on eels in shallow or clear water. This evidence supports the suggestion that eels are less inhibited by moonlight in Lake Onoke than they are in the shallower Makara stream.

Diurnal rhythm

It has been established from fishermen's observations that migrating eels usually only run at night. Downes (1917) observed that the migration started at dusk and reached a peak between 2000 hr and 0002 hr, then slowly decreased. The Maoris found that migrant eels could not be caught during the day except if the water was turbid. Under these conditions migrants were caught during the day but in much smaller numbers. Migrant longfin females were the only migrants seen at daytime by Hobbs (1947). At dusk he found that migrant eels approached the surface and continued to appear for up to eight hours.

In the Makara stream no migrants were caught in the fyke nets during the day. However, a few migrant eels were caught when electric fishing during the day in the migratory season. No observations were made to determine at what time during the night the run occurred in the stream but it was definitely during darkness. When fishing at Lake Onoke the fyke nets were set two to three hours before dusk but no migrants were caught before dark. With the onset of darkness the first eels appeared in the shallow water nearest the sea. The best catches were made between 2000 hr and 2200 hr, and sometimes until midnight, after which time the catch dwindled.

The nocturnal behaviour of migrant eels is similar to that observed for migrating European eels. Deelder (1970:3:35) found that it can be shown from fishermen's catches that the migration is limited to the night. Further, he states that Brautigam (1961), from experimental evidence, found the daily migration occurred mainly between sunset and midnight.

Water Temperature

The daily water temperature recorded in the Makara stream during the three migratory seasons 1971, 1972 and 1973 is plotted in Fig.3.9. A drop in water temperature was associated with a rise in water level and all runs of three or more eels. However, a drop in water temperature occurred on all occasions when the water level rose, even when the eels did not migrate. This suggests that water temperature was not a significant factor influencing the migration.

Deelder (1970: 3: 25) mentions that the migration of the European eel is interrupted by cold spells or freezing periods. Frost (1950 : 371) records that fishermen on the River Bann observed that the eels did not run on frosty nights. Frosts are usually associated with clear starry nights and in this case light, especially moonlight, may inhibit the migration, not the low temperature.

During the migratory periods studied in the Makara stream no frosts were recorded and the stream water temperature did not drop below 9.0°C . The largest run of eels recorded occurred when the water temperature was 11.25°C . The low temperatures recorded in European waters which interrupt the migration of eels do not occur in the Makara stream.

Lowe (1952: 298) however does consider that a drop in temperature associated with floods may be a factor triggering the migration. This suggestion is supported by observations on migrating eels held in a sink. The eels escaped from the sink more often on dark nights with a drop in temperature of approximately 1°C . As no rise in water level was associated with the temperature drop, the latter may be considered to have a stimulatory effect.

However, as previously mentioned, the influence of the temperature drop could not be disassociated from the accompanying rise in the water level of the stream, and no similar behaviour by captive migrant eels was observed.

FIG.3.9

Daily water temperature in the Makara
stream for the three migratory seasons
1971, 1972 and 1973.

MAKARA STREAM

Wind

There was no relationship between the runs of eels in the Makara stream and the wind direction and force. The prevailing wind in the Wellington area is from the north-west. Southerly changes are common and are often associated with a cold front and rain. Wind from either direction was usually associated with rain-fall and, as shown in Table 3.11, the eels migrated under both these conditions. No runs of eels occurred on windy nights with no rain. Wind is therefore not regarded as a significant factor influencing the migration of eels in the Makara stream.

The wind direction is regarded as a factor influencing the migration of the European eel in lakes (Deelder 1970). Frost (1950: 371) discusses this factor and mentions that fishermen on the River Bann make good catches when a southerly wind is blowing down Lough Neagh. The wind disturbs the lough in the direction of the River Bann and the fishermen suggest that this movement created by the wind stimulates the eels to move in that direction. Frost further mentions that a northerly wind has a similar effect on Lake Windermere.

Deelder (1970: 3: 33 - 3: 35) discusses the influence of the wind on migrating eels from his own and other authors observations. He concludes that the migration in broad open water such as lakes is influenced by the wind direction. No data were available to compare the Lake Onoke catch figures with the wind direction. However, the prevailing wind on Lake Onoke is from the north-west, blowing down towards the lake outlet. This would set up a movement of water in the direction of the outlet and it would probably stimulate the eels to move in that direction. However, this suggestion cannot be supported by any observations. Hobbs (1947: 230) gives conflicting observations on the movement of eels in relation to the wind in Lake Ellesmere. The movement of eels offshore from the outlet beach was irrespective of the wind direction, while the movement inshore was usually with the wind.

Seawater

The Makara sampling site where the downstream migrating eels were caught was beyond the tidal influence. Therefore seawater could have no influence on the downstream migration.

In coastal lakes such as Lake Ellesmere and Lake Onoke however, the inflow of seawater into the lake could be a factor that causes the eels to congregate in the shallow water nearest the sea. Ryan (1972) established that a salinity gradient exists in Lake Ellesmere, rising towards the blocked outlet area nearest the sea. The migratory eels appeared to be attracted by the higher salinity and congregated in the shallow area. No salinity measurements were made in Lake Onoke so that any response by the eels could not be determined. However, during one visit to the lake there was a rough sea running and at one place waves were breaking over the bar and flowing into the lake. Eels were congregating around the area where the seawater was flowing in and some attempted to swim over the bar and into the sea. Many managed to get halfway over the bar, partly aided by the backflow of the waves as they receded, but they could not withstand the force of the following waves and they were pushed back into the lake.

Cairns (1941: 69b) records a similar occurrence at Lake Onoke when the eels were able to struggle across the bar as large waves broke over it. The Maoris took advantage of the unusual conditions and caught many eels for food. In Lake Ellesmere, Hobbs (1947: 230) and Cairns (1941: 69b) noted that the migrating eels attempted to climb over the bar when waves broke over it. Cairns mentions that many thousand of eels became stranded in a belt of sand that runs across the top of the bar.

The behaviour of the eels under these conditions suggests that they are affected by the inflow of seawater, either by inflow over the bar or by percolation through the bar into the lake. Hobbs (1947: 230) however, considers that the migratory eels were repelled when seawater percolated in through channels but adds that the eels would attempt to climb over the bar if waves were breaking over it. In the latter case, and as described for the attempted movement of eels over the Lake Onoke bar, the movement is against the inflow of seawater. This is the reverse of the behaviour of the downstream migrating eels that move in the direction of the flow of water. The eels in these instances may be influenced by the

seawater flow even though it is the reverse of the freshwater, rather than by the salinity.

As described in the Materials and methods section a common method of fishing is to cut channels into the bar towards the sea. Little movement of water occurs in these channels on a calm night yet the eels swim into them in an attempt to reach the sea. It is presumed, despite the observation by Hobbs (1947: 230) that they are repelled by seawater, that the seawater percolating into these channels stimulates the eels and gives them a direction in which to move, towards the increasing salinity.

According to Deelder (1970: 3: 33) the European eel fishermen find that migrant eels in freshwater are greatly stimulated by inflowing seawater. Deelder suggests that inflowing seawater into lagoons and river mouths where this behaviour is observed occurs mainly during storms. Therefore the eels may be activated by either the storms or the saltwater. However, the fishermen state that seawater artificially introduced into freshwater stimulates the migration and Deelder concludes that inflowing seawater most likely exerts a stimulatory effect.

Observations on New Zealand migrating eels would support this assumption. The increasing salinity in estuarine areas and coastal lakes may stimulate the eels to move towards the areas of increasing salinity and hence out to sea.

Barometric pressure

The meteorological records of the daily barometric pressure recorded during the three migratory seasons were examined to determine if there was a relationship between a drop in barometric pressure and the runs of eels. The barometric pressure varied throughout the three seasons and no relationship could be established. As shown in Table 3.11, the barometric pressure varied considerably between different runs. Most runs were immediately preceded by, or associated with, a drop in barometric pressure but these changes also occurred when no runs of eels were recorded. Although lower barometric pressures are associated with deep depressions, several shallow depressions with no large drop in barometric pressure coincided with runs of eels. For the Makara stream there was a definite correlation between runs of eels and the passage of depressions, as discussed below. It was other factors such as rainfall and general weather conditions associated with the depressions rather than the change in barometric pressure that influenced the migration. However, as discussed later, it is difficult to separate these combined factors to establish which has the most significant influence on the migration.

The direct influence of barometric pressure on the migration of the European eel is not clear. Frost (1950: 370-371) records that good catches were made under stormy conditions and presumably therefore at times of lower barometric pressure. From the Table given by Frost (1950: 282) the best runs occurred under overcast conditions and, although not given, probably lower barometric pressure. Lowe (1952: 302) had insufficient data to establish if this factor was important.

Passage of depressions and general weather conditions

The dates of runs of more than five eels and the associated environmental conditions, moon phase, rainfall, rise in stream water level, barometric pressure, water temperature and general weather conditions, are listed in Table 3.11. With few exceptions most of the runs coincided with the location of a depression close to New Zealand.

The data on depressions were obtained from daily meteorological weather maps. The depressions varied in intensity and the largest run of eels occurred on the night of 15 April 1971, when a deep depression, with a centre pressure of 1005 millibars, was located to the north-west of New Zealand. On some nights runs occurred when no depression was near but when a southerly low pressure front with associated rain was moving over the sampling area. The other runs listed in Table 3.11 vary according to the different factors. However, most occurred during the last half of the lunar cycle. Rainfall and stream level varied but all runs were associated with some rainfall and rise in water level. Barometric pressure and temperature also varied and no correlation of these two factors with the migration was established.

Burnet (1969a: 235) found a similar relationship between the passing of a depression and a large percentage of the runs, and concluded that the runs could be influenced by a depression or the associated changes in the weather, such as rainfall.

The best runs in the Makara stream were clearly associated with stormy conditions (Table 3.11), as shown by the location of a depression close to the sampling area and the cloudy and rainy nights on which the runs occurred.

These general weather conditions are similar to the conditions under which the European eel migrates in large numbers. Both Frost (1950: 370) and Lowe (1952: 281) describe stormy conditions with rain, ^{and} dark and moonless periods as being the times at which major runs occur.

Migrant eels were kept in seawater aquaria during the migration period and their activity was normally very low, especially during the day. However, on stormy nights, noticeably in the evenings

even under artificial lighting, the eels showed much greater activity, swimming restlessly around the tanks and occasionally escaping if the lids were not secure. Some of these stormy nights coincided with runs of eels in the Makara stream. Some factor associated with the stormy conditions must have stimulated the eels but none could be determined. Barometric pressure was one of the conditions common to both the captive and wild eels, but as previously discussed the influence of this factor is difficult to ascertain.

This behaviour of captive migrant eels is similar to that observed by Lowe (1952: 302). Migrant European eels held in aquaria became active at the same time as eels ran in the field. Temperature drop, darkness and barometric pressure were the only factors influencing both the wild and captive eels. However, Lowe could not establish which of these factors, if any, was acting as a stimulant.

Microseisms

Deelder (1954: 185) noted that the intensity of the migration of silver eels in Holland increased under weather conditions associated with passing depressions. On several occasions however, he found that an examination of the usual conditions associated with the depressions such as wind, rainfall, cloud, barometric pressure and air temperature could not be correlated with the migration. One factor which however was common to all these instances was increased microseismic activity.

Deelder (1954: 181) defines microseisms as vibrations caused by the passage of depressions over the ocean. These vibrations pass through the sea floor, into the land masses and hence into bodies of fresh water.

Vibrations in bodies of fresh water can be detected by eels, probably through the lateral line organs. According to Lowe (1942: 299), Wunder (1927) showed that eels are very sensitive to vibrations in the water. Deelder (1954: 184) established that large catches of European migratory eels were made after the occurrence of microseisms with a three second period. Microseisms of this period were only associated with passing depressions and all these periods were followed by a distinct increase in catches. Deelder concluded

that microseisms were acting as a stimulant causing the increased activity. Lowe (1952: 302), as previously discussed, observed that captive European migrant eels showed increased activity only on nights when eels in the field migrated in large numbers. Darkness, temperature drop and barometric pressure were the only common factors, none of which could be shown to be the stimulating influence. Deelder (1970: 3: 33) however, considers that microseisms must be the factor that caused this increased activity.

Burnet (1969a: 243) and this study have established the correlation between the passage of depressions and the runs of eels. This, and the behaviour of eels in captivity, as previously discussed, is similar to that described by Lowe (1952) and Deelder (1954). Burnet however found that the weather records were insufficient to determine if microseisms were possibly acting as a stimulant for migrating eels.

For this study seismograph records were examined for the duration of the three migratory periods, 1971, 1972 and 1973. No increase in microseismic activity occurred during any of these periods, even when a deep depression was located close to New Zealand. Such a night occurred on 15 April 1971, when a large run of eels occurred and a deep depression was located close to New Zealand. As stated, there was no increase in microseismic activity.

It is possible that the Makara stream migration data is not sufficient to establish any relationship between microseismic activity and increased migratory activity. A more extensive study would be needed to determine if there is any relationship between microseismic activity and the migration.

However, in the Wellington area at least, it is considered doubtful if any increase in microseismic activity would stimulate the migration of eels as suggested by Deelder (1954). The average daily microseismic activity in this area has periods of between three to four seconds. Thus the migratory eels in the Makara stream are continually subjected to microseisms which would be of sufficient magnitude to stimulate the migration of the European eel. No rise in the level of microseismic activity above the three to four second period occurred in the Wellington area during the three migratory seasons. This may not be the situation for other areas of New Zealand but discussions with Mr. G. Eiby, a seismologist, revealed that the general level of microseismic activity throughout New Zealand is higher

than that in Europe, and in many areas occurs at the level which is regarded as having a stimulating effect on the migratory activity of the European eel. It is therefore doubtful if migratory New Zealand freshwater eels are activated by increased microseismic activity.

Earthquakes are the only other factor which cause vibrations in the water that could have a stimulatory effect on migrating eels. These vibrations could be as low as the background level of microseismic activity or be many times greater. Seismograph records were examined of both small local earthquakes and deeper, more widely felt earthquakes occurring in Wellington area for the first five months of 1971, 1972 and 1973. The dates of these earthquakes were then compared with the dates of migration in the Makara stream. In only one instance did an earthquake immediately precede a run of eels. Consequently it is assumed that vibrations which are caused by earthquakes have no influence on the migration of eels.

Combined effect of environmental factors

From Fig.3.7 and the list of environmental conditions prevailing at the time of migration of more than five eels given in Table 3.11, it is clear that several environmental factors were coincident on many of the nights when large runs of eels occurred. This is not revealed when each of the factors is discussed separately.

Fig.3.7 and Table 3.11 show that the best runs occurred on dark, moonless nights during rainfall and when the stream level was raised. Analysing the catch data by lunar months (Fig.3.8) does not show this combined effect of increased water level and moonless or dark periods. Nor is it apparent that on some nights eels migrated in small numbers during a flood when the effect of the moon would be expected to be adverse. Such a run occurred on the night of 5 May, 1971 when a small run of eels occurred during a flood between the first quarter and full phase of the moon. The largest runs of eels occurred when a rise in water level coincided with either the last quarter or a new moon.

It is difficult to separate the combined effect of these factors influencing the migration. However, Fig.3.7 and Table 3.11 do show that an increase in water level is the factor associated with all runs of more than five eels, whereas the phase of the moon is more variable (Fig.3.7). No runs were recorded on dark moonless nights with no increase in the water level. It is therefore assumed that the increase in water level is the most important factor affecting the migration. This influence can be modified under certain circumstances by the moon, as described for the run on the night of 5 May, 1971. When the two most favourable conditions for migration coincide, i.e. increased water level and a new or last quarter phase of the moon, the largest runs occur. Such a combination occurred on the nights of 15,16 and 21 April 1971, when some of the largest runs took place. Although the rainfall was not as high as on other nights the combined effect of a favourable moon phase and increased water level stimulated the largest runs. Such conditions occurred at other times in the season but with smaller runs. The period of mid-April would therefore seem to be the optimum time of migration, provided that environmental conditions are favourable.

The combined influence of environmental factors on the larger runs of eels has previously been discussed by Burnet (1969a: 242). Large runs showed a correlation with the lunar cycle and they were also influenced by rainfall, water volume, or the passage of a depression.

The combined effect of environmental factors influencing the migration of the European eel has been recognized by Frost (1950: 370) who states that, "The moon's light, although exerting a great influence on the run of eels, cannot always be isolated from other factors affecting the run". Lowe (1952: 281) found that the largest runs of eels occurred when a rise in water level coincided with moonless periods. In these instances the two most favourable conditions for migration coincided, resulting in large runs. However, Lowe (1952: 281-2) noted that when a flood coincided with a full moon, the run of eels was usually delayed. A similar coincidence of factors occurred in the Makara stream on the night of 5 May 1971, as previously discussed. In both these instances a favourable condition of higher water level had been

modified by an unfavourable condition, a full or nearly full moon. The result is either a delayed run as Lowe (1952: 282) records, or a reduced run as in the Makara stream.

The combined effect of environmental factors on the migration of eels is also discussed below.

3.7 Discussion of environmental factors affecting the migration

One of the obvious features about the migration of eels in the Makara stream is that it is limited to very few nights of the season. As established, each run is associated with one or more environmental factors. As shown in Fig.3.7, the runs are of varying intensities, the largest runs coinciding with the increased water level and moonless or dark nights.

It is apparent that external conditions are an important factor in triggering the migration. These factors act as stimuli and may occur singly or in combination. The stimuli vary in intensity. The factor with the greatest stimulatory effect is the increased water level. A small rise in water level has less affect than a large rise in water level. When stimuli coincide the biggest runs occur. When they do not coincide the migration is less intense. Hence the eels migrate in response to a number of stimuli, each stimulus varying in intensity and at times acting in unison.

Lowe (1952), discussing the varying responses of migrating European eels to different stimuli, considers that the stimulus to migrate is one factor or a combination of factors each varying in intensity. These stimuli have no threshold value above or below which the eel will or will not respond, but instead the migration is dependent on the responsiveness of the eel to the stimulus.

In this study any factor, water level, moon phase, passage of a depression and stormy weather conditions, that initiates a migration is considered as a stimulus. Thus a combination of factors act as a number of separate stimuli, as each is capable of triggering a migration alone.

Deelder (1954: 178) suggests that the large fluctuations in the catches of migrating European eels are due to changes in the behaviour of migrating eels in response to several factors. Lowe (1952: 297) considers that a 'migration-mood' is produced in eels which are in stages in physiological preparedness for migration. Lowe supports this suggestion with evidence of the capture during high floods of migrating eels that were not fully silver.

In this study not enough non-migratory eels were caught in the runs to draw similar conclusions. On the contrary, it was noticeable that the migratory eels were all in a very similar condition. Eels of each sex of each species were very similar in length, weight, colour and gonad state, indicating that they were all in a similar physiological condition. It is assumed that it is only when the eels are in this physiological state or 'migration-mood' that they are able to respond to the environmental stimuli.

From the limited size range and the similar reproductive state of the migrant eels of each sex of each species, as shown later in this section, the correct physiological state is reached at a certain minimum length. The minimum lengths for each sex from different areas, and those recorded by different authors, are very similar. However, many eels migrate at a length greater than the minimum for that particular sex and, as shown in the age and growth section, are considerably older than the smaller eels. This suggests that these larger eels have a slower growth rate and become greater in length before reaching the optimum physiological condition. However, Frost (1945b), discussing a similar situation with the European eel, suggests that these older, larger migrant eels had a greater preceding growth rate.

Deelder (1970: 3: 1), from observations on the size of migrating European eels, concluded that the attainment of maturity is related to length and is therefore primarily a matter of growth

rate in the preceding period. Similarly, for migrating New Zealand eels, the attainment of the correct physiological condition is related to length and is also dependent on the growth rate prior to migration.

It may also be argued that the correct physiological condition is attained when a certain weight is reached. However, in the analysis of length/weight data, weight is considered to be the dependent variable in the independent variable, length. Thus, the physiological condition could be attained at a certain length/weight combination but it is more common to state that it is attained at a certain length.

As the eels migrate during a limited period the 'migration-mood' is only reached at this time, or the environmental factors stimulating the migration are only favourable at this time. However, this latter explanation is unlikely as these factors, rainfall, moon phase etc., occur throughout the year. Therefore in the autumn the correct physiological state is coincident with favourable environmental conditions. Further, the eels may need to migrate at this time in order to reach the spawning grounds at the right season.

Thus the migration is a result of a combination of an internal migration urge triggered by an external stimulus.

It has been established that an external stimulus or stimuli triggers the downstream migration. The largest runs of eels lasted for no more than three nights, even though in some instances the water level and moon phase were the same as at the beginning of the run. Either the effect of the stimulation did not last or all the eels that were responsive at that time migrated within three days of the first stimulus being received.

Runs of eels can occur close together, such as on the nights of 15 and 16 April, 1972 followed by a gap, then a run on the night of 21 April, 1972.

This indicates that all the eels that were in the correct migration mood migrated in the first period. A short interval occurred when there were no external stimuli to trigger a migration but by the time a renewed stimulus occurred more eels were in the correct physiological state to respond.

It is not known how long the effect of the stimulation lasts on an individual eel. One stimulation may be sufficient for the eel to migrate all the way downstream or it may need successive stimuli as it moves to keep it activated. Lowe (1952: 300), discussing how long the stimulus may last, gives evidence that suggests that the initial trigger stimulus lasts for several nights. Lowe also suggests that the initial stimulus does not last throughout the downstream migration and the eels need successive stimuli to complete their migration. For eels migrating from well upstream it seems unlikely that one stimulation would be sufficient to induce them to move all the way downstream to the sea. Daylight would stop the migration after two to three days, as suggested by the three day migration period, and the eels would need restimulating. This would apply to eels migrating from upstream but eels migrating from areas near the sea may need only one stimulation. Lowe (1952: 303) calculated that a downstream migrating European eel moves at a rate of 2.43 ft/sec. Trybonn and Schneider (1908) in Lowe (1952: 303), found that migrating eels in the Baltic sea had a maximum movement of 50 km in 24 hours. It is assumed that migrating New Zealand eels would swim downstream at similar rates. Consequently migrating Makara eels caught 2 km from the sea probably move downstream and to sea on the same night. This suggestion has previously been discussed under the time and sequence of migration.

3.8 Mean length, length range and length-frequency of migrant eels

The mean length, range and standard deviation for each sex of each species by area and year is given in Tables 3.12 to 3.15. The size of migrant eels from other authors is also included. The length/weight relationship for migrant eels is given in the age and growth section.

TABLE 3.12

Mean length, length range and standard deviation of migrant A. australis males from different areas. The size of migrant eels recorded by different authors is also included

A. australis males

Area and Year	Mean length (cm)	Length range (cm.)	Standard deviation	No. eels
Makara stream 1971	45.9	38.1 - 53.2	3.97	32
Makara stream 1972	47.0	41.8 - 57.1	4.23	20
Makara stream 1973	47.1	41.5 - 59.8	5.36	13
All years combined	46.5	38.1 - 59.8	4.32	65
Lake Onoke 1971	45.3	38.8 - 54.9	3.97	47
Lake Onoke 1972	44.3	37.0 - 51.5	3.51	73
All years combined	44.7	37.0 - 54.9	3.71	120
Lake Ellesmere 1972	44.1	38.4 - 50.1	2.77	49
Hokitika 1972	45.4	41.7 - 50.4	2.87	13
Invercargill 1972	51.9	51.2 - 52.7	1.06	2
Dunedin 1971	43.7			-
Waikato River 1972	44.0	40.2 - 47.5	3.35	5
Lake Ellesmere (Hobbs 1947)	48.0	38.0 - 58.0	-	69
South Branch (Burnet 1969a)	43.3	33.0 - 58.0	4.0	194

TABLE 3.13

Mean length, length range and standard deviation of migrant A. australis females from different areas. The size of migrant eels recorded by different authors is also included

Area and Year		Mean length (cm)	Length range (cm)	Standard Deviation	No. eels
Makara stream	1971	74.1	56.3 - 93.3	8.95	23
Makara stream	1972	74.6	71.8 - 80.6	4.08	4
Makara stream	1973	73.2	58.0 - 92.4	11.77	7
All years combined		74.0	56.3 - 93.3	8.98	32
Lake Onoke	1971	75.4	55.0 - 99.5	10.4	22
Lake Onoke	1972	77.2	58.8 - 102.3	10.58	25
All years combined		76.4	55.0 - 102.3	10.42	47
Lake Ellesmere	1972	71.9	63.0 - 87.5	7.17	11
Hokitika	1972	69.3	60.0 - 85.7	9.41	9
Invercargill	1972	74.9	55.1 - 66.8	14.84	5
Waikato River	1972	82.9	74.1 - 100.3	8.9	11
Pukepuke Lagoon	1971	80.3	73.0 - 85.0	3.68	15
Lake Ellesmere (Hobbs 1947)		68.8	55.0 - 81.0	-	253
South Branch (Burnet 1969a)		64.1	53.0 - 81.0	4.9	56

TABLE 3.14

Mean length, length range and standard deviation of migrant A. dieffenbachii males from different areas. The size of migrant eels recorded by different authors is also included

Area and Year		Mean length (cm)	Length range (cm)	Standard Deviation	No. eels
Makara stream	1971	62.6	48.2 - 73.1	4.65	221
Makara stream	1972	62.8	52.1 - 72.9	5.29	46
Makara stream	1973	62.1	53.7 - 73.6	3.96	95
All years combined		62.5	48.2 - 73.6	4.63	378
Lake Onoke	1972	67.0	64.6 - 69.5	3.46	2
Lake Ellesmere	1972	66.0	62.7 - 69.2	4.59	2
Hokitika	1972	61.7	59.3 - 64.1	3.39	2
Invercargill	1972	67.0	62.3 - 72.6	5.2	3
Dunedin	1971	63.9	56.3 - 69.8	5.12	6
Lake Ellesmere (Hobbs 1947)		64.5	57.0 - 73.0	-	51
South Branch (Burnet 1969a)		59.1	48.0 - 74.0	4.0	758

TABLE 3.15

Mean length, length range and standard deviation of migrant A. dieffenbachii females from different areas. The size of migrant eels recorded by different authors is also included

Area and Year		Mean length (cm)	Length range (cm)	Standard deviation	No. eels
Makara stream	1971	107.7	88.0 - 133.3	16.89	14
Makara stream	1972	113.8	93.0 - 132.8	19.96	3
Makara stream	1973	97.7	78.1 - 110.1	11.96	5
All years combined		106.3	78.1 - 133.3	16.4	22
Lake Onoke	1972	91.3	83.6 - 98.0	5.92	4
Hokitika	1972	137.0			1
Invercargill	1972	106.6	102.5 - 110.8	5.87	2
Dunedin	1971	93.3			1
Waikato River	1971	93.8			1
Lake Ellesmere (Hobbs 1947)		119.4	81.0 - 137.0		177
South Branch (Burnet 1969a)		120.8	102.0 - 132.0	8.5	11

Within each area for the Makara and Lake Onoke samples for each year there is little variation between the means. The mean sizes between different areas however do show a marked variation. Mean values for shortfin males for the bigger samples vary from 43.4 cm (Burnet 1969a) to 48.0 cm (Hobbs 1947). Shortfin females vary from a mean of 64.1 cm (Burnet 1969 a) to 76.4 cm (Lake Onoke). Longfin males mean values vary from 59.1 cm (Burnet 1969a) to 64.5 cm (Hobbs 1947). The mean values for longfin females cannot be accurately compared because of the small sample sizes. The smaller mean sizes for migrants from some areas is probably associated with a slower growth rate in those areas, as suggested by Burnet (1969a: 243).

Males of both species from all areas show a smaller size range than do the females. Calculated from the larger samples, shortfin males have an average range of 19.3 cm and longfin males 22.0 cm. Shortfin females have an average range of 33.0 cm and longfin females 47.0 cm. The upper size limit of 102.3 cm recorded for a Lake Onoke female shortfin is appreciably greater than the maximum of 92.5 cm previously recorded by Cairns (1942b: 175B). The size ranges of male and female shortfins overlap, as shown by Hobbs (1947) and Burnet (1969a). No overlap of the size ranges for longfin males and females occurs.

Longfin females attain a large size. The largest specimen, from Hokitika, measured 137.0 cm. Hobbs (1947: 229) records 137 cm as the maximum length. Downes (1917: 301) describes two large migrant eels which could only have been longfin females. One measured 185.4 cm (6ft 1in) and calculated from the length/weight equation in the age and growth section this eel would have a weight of 29 kg (64 lb). The second eel weighed 17.2 kg (38 lb) and the calculated length would have been 165.0 cm (5 ft 5 in).

Cairns (1941: 688) also mentions large longfin female migrants of up to 180.0 cm and states that these large eels are the last to migrate in a separate run following the smaller longfin female migrants. Hobbs (1947: 229) found no change in the size of longfin females as the run progressed. In the Makara stream it was the largest female longfins, up to 133.3 cm, that migrated last.

It is interesting that in the European eel the largest eels migrate first. Deelder (unpublished) in Deelder (1970: 3: 25) found that the average lengths of migrating males decreased during the season.

The large size of longfin female migrants indicates a delay in development as suggested by Cairns (1941: 688) but the fact that these large eels apparently do eventually migrate does not support the idea of Cairns (1942b: 1758) that the large non-migrant longfin females are sterile.

Length-frequency polygons for the Makara migrants of each species are given in Fig.3.10. The length-frequency polygons calculated for the non-migrant eels of each species over the three year period is also shown. Few non-migrant eels were caught which were larger than the smallest migrant females. All the non-migrants over the maximum size for the migrant males for each species were females. Fig.3.11 gives the length frequency polygons for migrant shortfin male and female eels from Lake Ellesmere and Lake Onoke. The length-frequency polygons for males of each species show only one peak. The sample sizes of migrant females of each species were too small to show an even length-frequency distribution.

Of the total number of migrant shortfins caught (104) in the Makara stream for the three years, 67% were males. Of the migrant longfins (417), 95% were males. For the total number of migrant eels caught, 80% were longfins. Burnet (1969a: 242) found a similar high percentage of males among the migrants of each species.

The occurrence of the migrants of each sex in the upper limits of the size reached by non-migrants of that sex clearly shows that the migrant stage represents the final growth phase of the life cycle. This is to be expected as the migrant stage is the final stage in the life cycle.

The restricted size ranges of the migrants, in particular the males of each species, indicates that the size at migration is connected with length and is dependent on the growth rate in non-migrant stage of the life cycle.

The size range at migration for other species is listed in Table 3.16. The size range for both sexes of the European eel are similar to the shortfins but smaller than for the longfins.

FIG.3.10

Distribution of length frequencies for
non-migrant and migrant male and female
eels of each species.

A. A. dieffenbachii

B. A. australis

MAKARA STREAM

FIG.3.11

Distribution of length frequencies for migrant,
A. australis male and female eels.

- A. Lake Ellesmere
- B. Lake Onoke

TABLE 3.16

Mean size and size range at migration of the different species
of freshwater eels

Species	Male (cm)	Female (cm)
<u>A. australis</u> (Migrants)	33.0 - 59.8 Mean 45.8	53.0 - 102.3 Mean 73.9
<u>A. dieffenbachii</u> (Migrants)	48.0 - 74.0 Mean 63.7	78.1 - 137.0 Mean 108.1
<u>A. anguilla</u> (Frost 1945)	35.0 - 43.0 Mean 40.6	44.0 - 95.0 Mean 60.8
<u>A. rostrata</u> (Bigelow and Schroeder 1953) (Gray and Andrews 1971)	28.0 - 46.0	46.0 - 122.0 Average 61.0 - 107.0 53.5 - 93.1
<u>A. japonica</u> (Matsui 1952)	45.5 - 57.5	40.5 - 129.7
<u>A. mossambica</u> (Jubb 1961)	34.5 - 51.5	77.5 - 114.0
<u>A. marmorata</u> (Jubb 1961)		134.0
<u>A. bicolor</u> bicolor (Jubb 1961)		64.0
<u>A. nebulosa labiata</u> (Jubb 1961)	59.0	135.0

The mean lengths however for each sex of the European eel are smaller than for the shortfins.

Only limited data are available on the size of migrating American eels. Bruun (1963: 148) made comparisons between the size of American and European eels and concluded that there was not a great size difference between the two species. The size of the American eel is similar to that of the shortfin, although the maximum size of females is greater.

Few data have been published in English on the size of Japanese eels. Matsui (1952; 1957: 159) gives data indicating that the Japanese eel may be slightly larger at migration than the European eel and approximating that of the shortfins. The females, like that of the American eel, have a larger maximum size than the female shortfins.

The size range of the South African eel A. mossambica (Jubb 1961: 39) is similar to that of the shortfin eel although the females grow larger. Individual lengths of other species listed by Jubb are also listed in Table 3.16. From these figures and the accompanying photographs of A. marmorata and A. nebulosa labiata these species appear to be large eels similar to the longfin. The A. bicolor bicolor female appears to be of a similar size to shortfin females.

3.9 Colouration of and morphometric changes in migrating eels

The distinctive changes in the morphology and colour which occur in New Zealand migratory eels have previously been described, briefly by Cairns (1952b: 688) and later, in more detail, by Hobbs (1947: 229). Earlier, Downes (1917: 299) described the migrating eels using the Maori names for the eels, but it is possible from the descriptions to distinguish the sex of each species to which he refers.

A more detailed description of the migrant eels is given here. Figs. 3.12 to 3.15 show migrant and non-migrant eels whole and dissected.

Shortfinned migrant male and female eels (Fig. 3.12) have a similar migration livery and changes in morphology. The head becomes flattened dorsally and the snout appears slightly chisel shaped. The lips become thin and the lower jaw is often slightly longer than the upper jaw. In some large females the lower jaw may be noticeably longer and terminally hooked shaped. The lateral line pores are prominent, especially those of the head, just under the lower jaw and above the upper jaw under the eye.

In contrast a non-migrant shortfin (Fig. 3.14) has a more bulbous rounded head and prominent lips. The lateral line pores are less conspicuous. Migrant shortfins, particularly the larger females, have prominent scales whereas in the non-migrants they are less distinct.

In general body proportions, the migrant shortfins are rounder and thicker in the caudal region than the non-migrants. In particular the shorter migrant males become noticeably round in the tail region, giving the overall body shape the appearance of a cigar.

The enlarged eye and the change in iris colour is one of the most striking features of the migrant shortfin. The exact morphometric changes in the eye size are discussed separately.

Both the orbit and the pupil enlarge. The migrant eye has a prominent jet black pupil surrounded by a golden iris. This in turn is surrounded by a thin ring of blue tissue, the sclerotic layer. In contrast the non-migrant eye is smaller and less prominent. Although the pupil is black, the iris is less distinct in colour and the surrounding ring of sclerotic tissue is reduced.

The change in the colouration and shape of the pectoral fins is a prominent feature of the migrant shortfins. They become slightly pointed but there is no increase in relative length. Colouration is black or darkish green fringed with black. The darker colours

FIG.3.12

MIGRANT EELS - A. australis (shortfin)

1. Migrant shortfin male, 46.0cm total length
2. Migrant shortfin male - head region, 45.0cm
total length
3. Migrant shortfin female, 68.0 total length
4. Migrant shortfin female - head region, 72.5cm
total length

1

2

3

4

FIG.3.13

MIGRANT EELS - A. dieffenbachii (longfin)

1. Migrant longfin male, 65.0cm total length
2. Migrant longfin male - head region 65.0cm
total length
3. Migrant longfin female, 98.0cm total length
4. Migrant longfin female - head region, 110.0cm
total length

1

2

3

4

FIG.3.14

MIGRANT AND NON-MIGRANT EELS

1. Migrant and non-migrant A. australis (shortfin)
and A. dieffenbachii (longfin)

Top to bottom:

longfin non-migrant 60.0cm total length
longfin migrant male, 67.0cm total length
shortfin non-migrant, 43.0cm total length
shortfin migrant male, 45.5cm total length

2. Migrant and non-migrant female A. dieffenbachii

Top to bottom:

longfin non-migrant, 96.0cm total length
longfin migrant, 98.0cm total length

3. Migrant and non-migrant A. dieffenbachii

Top to bottom:

longfin non-migrant female head region,
92.0cm total length
longfin migrant male head region,
62.0cm total length

1

2

3

FIG. 3.15

MIGRANT EELS - DISSECTED

1. A. australis (shortfin) male, 46.0cm total length
2. A. dieffenbachii (longfin) male, 65.0cm total length
3. A. australis female, 68.0cm total length
4. A. dieffenbachii female, 111.0 cm total length

t - testes

o - ovaries

2

3

4

of the pectoral fins make them prominent and appear broader in shape. By comparison the pectoral fins of non-migrants are usually pale green and they are more rounded. This makes them appear less prominent and narrower.

Colour changes over the whole body are distinct in most migrant shortfin eels. The dorsal surface becomes a dark brown/black or a dark greenish shade. The sides of the body are a prominent metallic bronze, especially in the largest females. Others are greenish laterally. The ventral surface is a metallic silver. In eels with more greenish colours, the ventral surface is a bright silver. Eels with bronze colouration have a dull metallic silver ventral surface. In these darker coloured eels, which are usually the large females, the pectoral fins are jet black and prominent.

From the description of the colouration of the migrant shortfin eels it is apparent that there is a range in colouration from the lighter greenish colours to the darker bronze eels. These differing colours occur in eels of all sizes, both male and female. No difference in the reproductive state, as shown by the gonads, could be established for eels of these different colours. However, these are not two distinctive colour combinations, as many migrant eels of intermediate colouration were observed.

In most cases the migratory colouration is distinctive enough to distinguish a migrant from a non-migrant eel. However, many non-migrant eels have colouration very similar to the lighter coloured migrant eels. For example the silver ventral surface is a colour common to both migrants and many non-migrant shortfin eels. The only distinguishing feature in these cases is the different eye size and colour. As mentioned earlier, it is therefore misleading to refer to migrating shortfin eels as 'silver eels or silver bellies' as these colours are common in non-migrant eels.

The dermis of migrants is thicker than in non-migrants and tough to cut. Internally there is a reduction in the size of the stomach and the intestine. In the females there are no fat deposits around the gut, whereas the males retain these around the stomach and the lower intestine. No food was present in the gut and it was clear that the eels had ceased feeding.

The gonads, especially in the ovaries in the females, are prominent. Their macroscopic appearance is discussed separately.

The migration livery and the morphological changes in migrant longfin eels (Fig.3.13) are more prominent and are markedly distinct from the non-migrant stage. The head becomes flattened and the snout is distinctly chisel shaped. In the females the large muscular domes on top of the head, a prominent feature of large non-migrant females (Fig.3.14), is lost. The lips become thin, in contrast to the thick fleshy lips of the non-migrants. In most males the jaws are equal in length, although in some the upper jaw is slightly longer, emphasizing the chisel shaped appearance of the snout. The females, in contrast, often have the lower jaw slightly longer. The lateral line pores are prominent, especially under the lower jaw, above the upper jaw and under the eye and along the side of the head. Unlike the shortfin migrants the scale pattern is not noticeable. Further, the general body proportions are not obviously different from the non-migrants, although the migrants may be a little fatter in the tail region.

The most distinctive morphological change in longfin migrants is the enlargement of the eye and the associated colour change. The change in eye size from the non-migrant to the migrant eels is discussed separately. Both the orbit and the pupil are enlarged. The large black pupil is surrounded by the bright golden coloured iris. A prominent ring of dark blue tissue of the sclerotic layer orbit surrounds the iris, making eyes appear distinctly blue. This contrasts strongly with the surrounding dark skin. By comparison the eye of a non-migrant is small, the iris is less prominent in colour, and the surrounding ring of blue sclerotic tissue is reduced.

Like the shortfin migrants, the change in the colouration and shape of the pectoral fins is obvious. They are slightly pointed but no increase in the relative length occurs. Colouration is jet black, with often a bronze sheen at the base of the fin. The dark colour of the pectoral fins make them prominent and appear broader in width. In contrast the pectoral fins of a non-migrant longfin are a pale green and are rounded, making them appear less prominent than in the migrants.

The overall dark body colouration in all specimens, unlike the shortfins, is distinctly different from the non-migrant phase. Dorsal colouration is a sooty black, extending down the sides to a dark black/brown around the lateral line area. The ventral surface is paler in colour, being a mottled dark grey and white. Some males may be grey on the dorsal surface and white underneath. Females may have a bronze sheen along the sides of the body and extending along

the head. Overall there is much less variation in colour than shown by the shortfin migrants. Their colouration and enlarged eyes make the longfin migrants easily distinguishable from the non-migrants. At no stage can the longfin eel be called a silver eel or a 'silver belly' so that no confusion arises as with the shortfins.

The flesh of longfin migrants is firmer to the touch than a non-migrant, but it still retains a 'flabby', soft feel. Like the shortfins, the dermis is thicker than in the non-migrants and the body is tough to cut open.

Internally, the stomach and the intestine appear reduced in size, and in the females there are no fat deposits around the gut. Feeding has obviously ceased, the gut being completely empty.

In the males the fat deposits around the gut, especially the lower intestine, are retained and become prominent.

The gonads, in particular the testis, are more prominent than in the shortfin migrants. Both the testis and ovaries are noticeably greater in width, even allowing for the larger size of the eels. The macroscopic appearance of the gonads is described separately.

All the morphometric changes that occur in migrant eels have been mentioned in the general description of the migrant eels. The most significant changes are discussed in greater detail here, except the change in eye size which is given separately.

To determine if there was any change in pectoral fin length between migrant and non-migrant eels of the same species, a scatter diagram of pectoral fin length against total body length was made. It showed that the pectoral fin increased in length in direct proportion to the increase in total length. No distinction was apparent between the scatter for non-migrant and migrant eels. It was not considered necessary to confirm the conclusion by a statistical analysis. Accordingly it is assumed that no increase in pectoral fin length occurs in migrant eels.

Hobbs (1947: 229), describing shortfin migrants, states that they have greatly enlarged pectorals but gives no measurements. The change in colour, as previously mentioned, may make the fin appear larger. Further, they do become slightly pointed, as mentioned by Hobbs (1947: 229), but this is a change in shape, not in overall length. There may be a difference in width of the pectoral fin between

migrant and non-migrant eels of the same size but this measurement was not taken. However, as previously discussed, the difference in colour may make the fin appear larger although there is no difference in size.

For the Japanese eel, Matsui (1952: 106) showed a similar linear relationship between the length of pectoral fin and the total length of the body. However, he found this character to be distinctly different in male and female eels but did not make a comparison between migrant and non-migrant eels.

Although Matsui found that the length of the pectoral fin is shorter in females than in males, I consider it doubtful that this character could be used to distinguish individual male and female eels. The scatter diagram for New Zealand migrant eels showed no difference between the length of the pectoral fin in relation to the total body length between the sexes for each species.

For the European eel the change in colour and shape of the pectoral fin is a distinguishing feature of the migrants. Bertin (1956: 95) describes the nearly black pectoral fins and D'Ancona (1960: 68) notes the change in shape of the fin to a lanceolate form. No mention is made by these authors of an actual increase in pectoral length at the silver eel stage. However, Svardson (1949b: 130) describing eels in partial nuptial dress caught in Sweden, describes the pectoral fins as being very pointed and lengthened. Similarly, Rasmussen (1951: 37) notes the prolonged and pointed pectoral fins of two female eels in partial spawning dress. From these descriptions it is apparent that the pectoral fin increases in length in the more sexually advanced stages of the European eel.

Only one New Zealand freshwater eel, in a slightly more advanced sexual state than normal migrating eels, has been caught at sea (Todd: 1973). In this specimen the pectoral fin was relatively no larger than in normal migrating eels of that sex and species.

It is of interest that the pointed, lengthened pectorals recorded by Svardson and Rasmussen from eels in nature were not observed under experimental conditions. Boëtius, Boëtius, Hemmingsen, Bruun and Møller-Christensen (1962) observed no increase in pectoral fin length in eels in which increased sexual development had been induced using hormone injections. Similarly, as described later in this study, no change in the shape and length of the pectoral fin was

observed in New Zealand eels in which maturation had been induced by hormone injections.

The reduction in the size of the gut and the cessation of feeding in migrants of both species has been mentioned as a prominent migratory feature. Cairns (1942b: 688) mentions that the gut of migrants atrophies and shrivels to a thin strand of tissue.

In many shortfin migrants, from external observations, the anus appeared constricted but was definitely not blocked. Schnakenbeck (1934) in Bertin (1956: 98) observed the complete occlusion of the anus in a migrating European eel caught in the North Sea. Bertin considers it possible that this anal occlusion may be normal in migrating European eels. However, D'Ancona (1959b: 1405) considers this to be an exceptional occurrence.

Said (1972: 32), from morphological and histological observations of the different gut regions and associated organs of migrating shortfin eels, observed a general degeneration of the gut. The superficial examination of the gut of the first migrant longfin to be caught at sea (Todd 1973) indicated that the gut resembled that of non-migrant eels. However, it would be erroneous to draw conclusions about the changes in the gut of migrant longfins from this one observation. My observations on migrant longfins is that the gut is reduced from the non-migrant stage.

Said (1972: 32) uses the term degenerate to describe the gut of shortfin migrants. This term implies that the changes are irreversible, but migrant eels that I have kept in freshwater for two years during this study readily accept food. Superficial examination of the guts of some of these eels revealed no change from the migrant stage, except for the complete absence of internal fat deposits. This implies that the changes in the gut of migrant eels is one of reduction or regression, rather than degeneration.

Bertin (1956: 96) describes the changes in the gut of the migrating European eel, and discusses what is termed the regression of the tissues of the digestive tract. D'Ancona (1960: 68) similarly lists the reduction of the digestive tract as one of the changes in migrating European eels.

This reduction or regression of the gut has been one of the subjects of discussion following Tucker's (1959) hypothesis on the Atlantic eel problem. His thesis, in part, is that the European eel does not return to the Sargasso sea to spawn, as suggested by

Schmidt (1922: 205-6). To support this, Tucker (1959: 496) lists what he terms the degeneration of the gut and the occlusion of the vent, the latter observed in only one specimen as previously mentioned, as one of the characters of a degenerate fish unable to feed. However, captive migrant European eels have been observed to feed, as described by D'Ancona (1959: 1404) and Boëtius and Boëtius (1967a: 345). These observations and those on feeding captive New Zealand migrant eels shows that the gut, despite its regression, is still functional. Therefore, the possibility that migrating eels might resume feeding at sea cannot be discounted.

It is of interest that Jubb (1961: 38-9) found that a migrating male A. mossambica had been feeding prior to capture and he does not list the reduction of the gut as one of the migratory features of this species.

Other morphometric changes have already been mentioned in the overall description of the migrant eels. Namely, the change in body shape, the extension of the lower jaw in shortfins, and the change in head and snout shape, the latter two being more prominent in the long-fins. The change in snout shape is similar to that described for migrating European eels (D'Ancona 1960:68), and the South African migrating eels A. mossambica, A. marmorata and A. bicolor bicolor (Jubb 1961: 38-43).

3.10 Change in eye size in migrant eels.

The most obvious morphometric change in the metamorphosis from the non-migrant to the migrant stage in both species is the enlargement of the eye and the change in eye colouration. This change in eye size and colouration has previously been noted by Downes (1917: 299), Cairns (1941: 688), Hobbs (1947: 229) and Burnet (1969a: 230).

In non-migrant eels the eye is round in shape and the iris is dull in colour. In contrast, in the migrant eels, the eye becomes slightly elliptical in shape and the iris, as described earlier, becomes bright gold.

To compare the eye size of migrant and non-migrant the horizontal and vertical diameter of the eye was measured. The product of these two measurements gives the eye-index in mm^2 . This follows the method used by Boëtius and Boëtius (1967a: 354) to overcome the problem of the elliptical shape of the eye in the immature European eel.

The reverse applies in this study. This method makes possible a comparison between the round eyes in the immature eels and the slightly elliptical eye shape in the migrant eels.

The relationship between the total length and the eye-index of non-migrant, migrant male and female eels of both species is given in Fig.3.16. Female and male non-migrant eels were combined but for the migrant eels the sexes were kept separate. This enabled a direct comparison to be made between the sexes for migrant and mature eels which is given later in this study.

The regression equations for eye-index on total length for non-migrant eels of both species, and migrant female and male eels of both species are given in Table 3.17. There is a positive linear relationship, significant at the 1% level, between the eye-index and the total length for the non-migrant, migrant male and female eels of both species.

To calculate the growth of the eye from the non-migrant to the migrant stage, the eye-index at 45cm and 65cm body length, was calculated for non-migrant, migrant male and female shortfins. These values are given in Table 3.18. The eye-index of a 45cm shortfin male increases by a factor of 2.0 over that of a non-migrant of the same length. However, the eye-index of a 65cm migrant female increases by a smaller factor of 1.65 over that of a non-migrant of the same length. A similar difference occurs in the factors of increase of eye-index for longfin eels of 65cm and 95cm as shown in Table 3.18.

Thus female migrants of both species show a smaller increase than migrant males in the eye-index over non-migrants of the same size.

Fig.3.16 shows that non-migrant and migrant longfins have a larger eye-index than non-migrant and migrant shortfins of the same size. This is clearly seen when handling migrant male longfins, which as noted earlier, have conspicuously large eyes. Further, the factor of increase of the eye-index in longfins is greater than in shortfins of the same length.

Thus longfins, as compared with shortfins of the same length, have a larger eye-index at the non-migrant and migrant stage. They also show a larger factor of increase of the eye-index at these stages in eels of the same length.

The increase in the eye size of migrating freshwater eels of other species has been well documented. Jubb (1961) found that the five species of freshwater eel in South Africa, A. mossambica,

FIG.3.16

Regression of eye-index (mm^2) on total length (cm) for non-migrant and migrant male and female A. australis (shortfin) and A. dieffenbachii (longfin)

TABLE 3.17

Regression equations of eye-index (mm^2), y , on total body length (cm), x , for non-migrant, and male and female migrant eels of each species

Species	Regression equation	Correlation coefficient (r)	n
<u>A. australis</u>			
non-migrant	$y = 0.936x - 19.38$	0.82	159
migrant male	$y = 1.215x - 9.45$	0.52	72
migrant female	$y = 1.493x - 28.47$	0.75	71
<u>A. dieffenbachii</u>			
non-migrant	$y = 1.176x - 25.86$	0.89	179
migrant male	$y = 1.591x - 3.62$	0.60	170
migrant female	$y = 1.505x - 1.01$	0.83	21

(All r values are significant at the 1% level)

TABLE 3.18

Eye-index (mm^2) of non-migrant and migrant eels 45cm, 65cm
and 95cm body length.

(Values calculated from regression equations given in Table 3.17)

Species	Eye-index	Factors of increase of eye-index
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A. australis

A. marmorata, A. nebulosa labiata and A. bicolor bicolor, all had enlarged eyes accompanying the migratory livery. A. japonica similarly shows an increase in eye size at migration. Data on the increase in eye size at migration is available for only A. rostrata (Boëtius and Boëtius 1967a) and A. anguilla (Boëtius and Boëtius 1967b; Stramke 1972). From the data given by Boëtius and Boëtius (1967a: 1967b) the eye-index of a migrant A. rostrata of 45cm in body length increases by a factor of 1.53 over that of a non-migrant of the same size. The eye-index of a migrant A. anguilla of similar length is the same but the eye increases by a factor of 2.4. This is due to the smaller eye of a non-migrant A. anguilla of the same length.

Non-migrant shortfin and longfin eels have a smaller eye-index than A. rostrata at the same length. Compared with the European eel the eye-index increases more rapidly with increasing length, giving a smaller eye-index below approximately 30cm body length, and a greater eye-index above approximately 45cm body length. Comparing the eye-index of migrant shortfin and longfin, the shortfin eye-index is similar to that of the European and American migrant eels, with a factorial increase between the values for these two species. The eye-index of a migrant longfin is greater than that of a European or American eel of the same size, but the factorial increase is between the values for these two species. The larger eye-index but similar factorial increase is due to the rapid increase of eye-index with increasing length in the non-migrant eels.

D'Ancona (1927) in Bertin (1956: 96) made histological observations on the eyes of yellow and silver European eels and showed that in the silver eel the eye diameter doubled, making the retinal surface four times as large. However, he found that the number of visual cells remained constant. Bertin (1956: 96) drew the conclusion from D'Ancona (1927) that these changes indicated a degeneration of the eye. Later research has shown this conclusion to be incorrect. It has since been established by Carlisle and Denton (1959), that during metamorphosis from the yellow to the silver eel stage of the European eel, the eye enlarges and the retinal pigments change from purple to golden, like that of the photosensitive chrysopsins of deep-sea fish. The larger eye and the relatively even larger pupil of the migrant eel eye make it a more efficient light collecting organ. Further, the maximum absorption of light by the eye shifts towards the shorter wavelength of the spectrum. It is light of the shorter wavelengths that penetrates

deepest into the ocean to depths where the eels may swim. The retina would therefore be more efficient in usefully absorbing the light falling on it. Stramke (1972: 116) agrees with Carlise and Denton (1959) and states 'the large increase in the diameter of the eye in combination with physiological changes during the metamorphosis must be interpreted as functional improvements which raise the effectiveness of the eye'.

Thus the enlargement of the eye and the associated changes in the retinal pigments is a functional adaptation to the conditions under which the eel makes its spawning migration.

3.11. The state of reproductive development of migrating eels

Macroscopic appearance of the gonads

A detailed description of the macroscopic appearance of the gonads in migrating eels has not previously been given. Cairns (1942b: 174B - 5B), as previously discussed, gives descriptions and photographs of the gonads of a male and female eel but does not distinguish between those of the two species. Boëtius and Boëtius (1967a: 381) incorrectly assumed that the photograph of the testes represented the stage of development at migration in both species. Cairns does not state whether the photographs are of migrant eels or not, although they appear to be of the gonads in a male and female migrant longfin. Hobbs (1947: 229) mentions that he could readily distinguish the gonads of migrating eels except in some shortfin males. He gives no description of the appearance of the gonad or any details of the gonad width.

This sub-section describes in detail the macroscopic appearance of the gonads, and discusses the size of the gonad as given by its width. An adequate description is considered important before describing and discussing the other features of the gonad, namely the gonadosomatic index, ova size and the microscopic degree of development, all given in the following sub-sections.

The gonad morphology and development in non-migrant eels has already been described. A brief description of the position of the gonads in the body cavity is repeated here.

The gonads (Fig.3.15) extend along each side of the body cavity, on either side of the gut. In an eel of 50cm body length the left gonad begins just posterior to the level of the gall-bladder, and

extends posteriorly past the vent for several centimetres and then doubles back on itself for 2-3cm. The right gonad begins just anterior to the level of the left gonad, just anterior to the gall-bladder. It extends posteriorly to the anus and does not double back on itself as does the left gonad.

The left gonad is usually longer than the right gonad, by approximately 1-2 cm in shortfin males, 4-6cm in shortfin females and longfin males, and by up to 8cm in longfin females. The maximum width of each gonad is the same, and they attain their greatest width in the middle of the body cavity. All gonad measurements were made in this region.

The appearance of the gonads in migrant shortfin males does not differ greatly from those in the most developed non-migrant males previously described. A portion of the testis from a migrant shortfin male is shown in Fig.3.17. The gonad has the distinct lobes characteristic of gonad stage five, the organ of Syrski. In migrant males the testis lobes appear pale or slightly pink in colour, and the blood vessels are usually prominent. The vas deferens is not distinguishable. The maximum width of the testis in non-migrant shortfins was 3.0mm, compared with a mean testis width of 5.0mm in the migrants. The mean testis width, range and standard deviation for shortfin male migrants is given in Table 3.19. In migrant shortfin females the ovaries appear as creamy white, frilled ribbons. Blood vessels are prominent. A portion of an ovary from a migrant shortfin female is shown in Fig. 3.18. The ovaries do not fill the body cavity and no individual ova are discernable to the naked eye.

Some non-migrant shortfin females have ovaries similar to the ovaries in migrant females, but they lack the distinctive white creamy colour. The maximum width of the ovary in non-migrant female shortfins was 15.6mm, compared with a mean ovary width of 27.0^{mm} in the migrants. The mean ovary width, range and standard deviation for shortfin female migrants is given in Table 3.19.

The gonads in migratory longfins show a much greater degree of development and differ greatly from the maximum degree of development seen in non-migrants. In the males the lobes of the testes are large and pale pink to blood red. Blood vessels are prominent. In most males the lobes are distinct, (Fig.3.17), the diagnostic character

FIG.3.17

TESTES FROM MIGRANT EELS

1. Portion of the testis from a migrant A. australis
(shortfin), 45.0cm total length. x 5
2. Portion of the lobed testis from a migrant
A. dieffenbachii (longfin), 65.0cm
total length. x 4
3. Portion of the ribbon-like testis from a migrant
A. dieffenbachii, 62.3cm total length.
x 4

1

2

3

FIG. 3.18

PORTIONS OF THE OVARY FROM A
MIGRANT A. australis (shortfin),
68.0cm TOTAL LENGTH

1. View of the medial, smooth surface of the ovary. x 2
2. View of the lateral surface of the ovary showing the lamellae containing the developing ova. x 2

1

2

TABLE 3.19

Mean gonad width, range and standard deviation of
migrating male and female eels (all areas combined)

	<u>A. australis</u>		<u>A. dieffenbachii</u>	
	<u>male</u>	<u>female</u>	<u>male</u>	<u>female</u>
Mean gonad width (mm)	5	27	12	60
Range (mm)	3 - 7.5	17 - 42	7.5 - 21	39 - 88
Standard deviation	1.07	6.00	2.86	18.0
n	97	85	79	18

of the organ of Syrski as previous described. However, in some males the lobes were joined, either partially or completely, to form what appears to be a ribbon gonad characteristic of the female gonad (Fig.3.17). However, no confusion arises as to the correct identification of the sex, as the gonad retains the colour and firm texture of the testis. The maximum width of the testis in non-migrant male longfins was 5.0mm compared with a mean testis width of 12.0mm in the migrants. The mean testis width, range and standard deviation for longfin male migrants is given in Table 3.19.

In migrant longfin females the creamy white, frilled ovaries appear considerably more developed than those in any non-migrant longfin, and migrant shortfin females.

Individual ova are distinguishable to the naked eye. In some well developed specimens the ova on the outer edge of the ovary were held in a clear gelatinous matrix, loosely connected to each other. It is of interest that a similar observation of 'loose ova' was made (1949:222) in the European eel by Bruun, Hemmingsen and Møller-Christensen[^] in which increased sexual development had been induced by hormone injections. The ova diameter of 0.35mm at which this condition occurred in the European eel approximates the mean ova diameter of longfin females at migration. (See section on ova diameter).

The ovaries were not firm and had no structure, readily breaking up when they were removed. The maximum width of the ovary in non-migrant female longfins was 22.0mm compared with a mean ovary width of 60.0mm in the migrants. The mean ovary width, range and standard deviation for longfin female migrants is given in Table 3.19.

A scatter diagram of gonad width against total length for the female migrants of each species shows that the width of the ovary increased in direct proportion to the increase in total length, that is, a positive linear relationship exists between the width of the ovary and total length in female migrants. Calculations for both species gave a correlation coefficient significant at the 5% level. Fig.3.19 gives the regression of gonad width on total length for migrant longfin and shortfin females. From Fig.3.19 it is seen that the width of the ovary in longfin females shows a higher increase per cm total length than the ovary width in shortfin females.

A scatter diagram of the gonad width against total length for male longfin and shortfin migrants showed no correlation.

FIG.3.19

Regression of gonad width (mm) on total length (cm)
for migrating female A. australis (shortfin) and
A. dieffenbachii (longfin)

It was concluded that for the male migrants the width of the testes was not dependent upon the total length. Examining the data of testes width at different body lengths showed that some of the smallest male migrants of each species had wider testes than the eels in the larger length groups.

It is of interest to know the width of the gonad at migration, and what relationship it has with the total length of the eel. The gonad width in female migrants has been shown to be directly proportional to the body length. In male migrants it is not. This is not considered significant, as the width of the gonad at migration for either sex represents only one stage in the development of the gonad. It is shown in the section on the induction of maturation that the gonad increases rapidly in width as it develops. Thus, at least in males, the width of the gonad is related more to the stage of sexual development than to the body length of the eel. However, it is not convenient to express the degree of sexual development by the gonad width. Instead, the weight of the gonad expressed as a percentage of the total body weight of the eel is used. This is discussed separately, and gives a more accurate indication of the degree of sexual development of an individual eel.

The width of the gonad in the migrating European eel is considerably less than that for both the shortfin and longfin migrants. Bruun et al. (1949: 214) mentions that the testes rarely exceed 1.5mm in width, and the ovaries are from 5.0mm - 20.0mm in width. Schmidt (1906: 143), in a footnote, states that the lobes of the testes do not exceed 3.0mm in width. This latter figure is similar to the width of the testes in shortfin migrants. Matsui (1957: 159) found that the maximum width of the testes in migrating males of the Japanese eel was from 2.0mm - 3.0mm, similar to that for the shortfin males. Jubb (1961: 39 - 43) gives the gonad widths for three species of migrating South African eels. The gonad widths of 39.0mm (body length 77.5cm) and 50.0mm (body length 114.0cm) for migrant females of A. mossambica and 51.0mm (body length 134.0cm) for A. marmorata approximate those for migrant longfins of similar size. However, the gonad width of 12.0mm (body length 64.0cm) for a female of A. bicolor bicolor is smaller than that for a shortfin of similar size.

Gonadosomatic index

A convenient and well established method for determining the stage of reproductive development of a fish is to express the weight of the gonad as a percentage of the total body weight. By examining this ratio, the gonadosomatic index or GSI, of both male and female migrant eels from different areas the degree of reproductive development can be determined, and comparisons made between different areas and species.

When comparing the state of sexual development at migration as shown by the GSI values it is important to know the GSI at maturity. By inducing maturation with hormone injections, described later in this study, it was established that the maximum GSI values for male shortfin and longfin eels at maturity are 22.0 and 17.0 respectively. At maturity longfin females have a maximum GSI of 30.0 and shortfin females 45.0. The GSI at maturity is discussed in more detail in the section on the induction of maturation.

Few data are available on the GSI values for migrating New Zealand eels. Only two authors make brief reference to them. Shorland and Russell (1948: 178) calculated that the ovary comprised 3.5% of the total body weight in migrating shortfin females, and 10.7% in migrating longfin females. Hobbs (1947: 229) calculated that for Lake Ellesmere migrant longfin females the ovaries comprised 10% of the total body weight. Nothing is known of the GSI values for migrant male eels.

Tables 3.20 and 3.21 give the mean GSI, range and standard deviation for migrants of both sexes and species from different areas. Where data were available, the values for different years is also shown. Because of the small number of longfin migrants obtained from areas other than Makara, data on the GSI values for longfins are scarce.

The mean GSI values for each sex, area, and year show some variation. In particular the mean values for Lake Onoke shortfins for each year are higher than those for Makara and Lake Ellesmere. However, the range in values for each sex, area and year is approximately the same.

For some areas the sample size is too small to make a comparison with other areas. Further, the range of values between different areas is no greater than the range within any one area. For this

TABLE 3.20

Mean gonadosomatic index (GSI), range and standard deviation for migrating male and female A. australis from different areas.

Area and year		Mean G.S.I.	Range	Standard Deviation	No. eels
<u>A. australis</u> males					
Makara stream	1971	0.34	0.17 - 0.7	0.15	9
Makara stream	1972	0.14	0.04 - 0.22	0.09	3
Years combined		0.29	0.04 - 0.7	0.17	12
Lake Onoke	1973	0.43	0.38 - 0.49	0.07	2
All areas combined		0.31	0.04 - 0.7	0.16	14
<u>A. australis</u> females					
Makara stream	1971	3.93	1.99 - 5.57	0.98	16
Makara stream	1972	3.74	2.7 - 4.79	0.9	4
Makara stream	1973	3.86	3.29 - 4.9	0.62	6
Years combined		3.84	1.99 - 5.57	0.86	26
Lake Onoke	1971	4.33	3.24 - 5.29	0.62	19
Lake Onoke	1972	4.43	3.09 - 5.63	0.69	24
Years combined		4.4	3.09 - 5.63	0.52	43
Lake Ellesmere	1972	2.67	1.22 - 4.0	0.34	10
Hokitika	1972	3.72	2.85 - 4.42	0.63	8
Invercargill	1972	4.16	3.7 - 5.17	0.64	5
Pukepuke Lagoon	1971	2.67	1.85 - 3.4	0.46	9
Waikato River	1972	2.18	2.01 - 2.36	0.24	2
All areas combined		3.82	1.22 - 5.64	0.98	103

TABLE 3.21

Mean gonadosomatic index (G S I), range and standard deviation for migrating male and female A. dieffenbachii from different areas.

Area and year		Mean GSI	Range	Standard deviation	No. eels
<u>A. dieffenbachii</u> males					
Makara stream	1971	1.46	0.51 - 2.73	0.46	63
Makara stream	1972	0.92	0.52 - 1.17	0.26	6
Years combined		1.32	0.51 - 2.73	0.38	69
Lake Onoke	1972	1.86			1
Lake Ellesmere	1972	0.8	0.53 - 1.06	0.37	2
Hokitika	1972	1.51	1.6 - 1.52	0.12	2
Invercargill	1972	1.71	1.37 - 2.29	0.5	3
All areas combined		1.41	0.51 - 2.73	0.47	77
<u>A. dieffenbachii</u> females					
Makara stream	1971	8.77	6.49 - 11.12	1.5	10
Makara stream	1972	8.59	7.04 - 10.26	1.61	3
Makara stream	1973	7.4			
All years combined		8.73	7.04 - 11.12	1.46	14
Lake Onoke	1972	9.67	8.36 - 11.53	1.42	4
Hokitika	1972	6.75			1
Invercargill	1972	7.15	6.85 - 7.46	0.43	2
All areas combined		8.6	6.49 - 11.53	1.51	21

reason, and also because of the small sample size for some areas, the differences between the mean GSI values for different areas within each species are not considered to be biologically significant. However, the differences between each species are considered to be biologically significant.

It is shown in the sub-section on the histological analysis of the gonads of migrant eels, that the higher GSI values represent a more advanced state of sexual development.

The GSI values at migration show an advance in sexual development over the most advanced non-migrant eels. Only five GSI values were calculated for non-migrant females and none exceeded 1.0. The increased sexual development as shown by the higher GSI values in migrant longfin females represents a considerable increase in gonad development over the non-migrant stage. Migrant shortfin females, although more sexually advanced than the non-migrants, show a smaller increase in sexual development, as shown by the smaller GSI values.

Tables 3.20 and 3.21 clearly show that both male and female migrating longfins have a greater GSI than the shortfins of the same sex. The GSI range for females of each species do not overlap. This indicates a considerable difference in the state of sexual development at migration between the two species.

The GSI value of 45.0 at maturity for shortfin females shows that at migration (GSI 3.8) the ovaries are relatively immature. To reach maturity the GSI has to increase by a factor of 12 during the sea journey to the spawning grounds. Migrating longfin females, based on the GSI values, show a much greater increase in the development of the ovary at the time of seaward migration, and could be called partly mature. The GSI at maturity indicates that the GSI increases by a factor of only approximately 3.5 compared with 12 for the shortfin females during the sea journey.

The male migrants show a similar pattern. Based on a GSI value of 22.0 at maturity, the GSI value for shortfin males increases by a factor of approximately 71.0. For longfin males with a GSI of 17.0 at maturity it increase by a factor of approximately 12.

No relationship could be established between the GSI and body length or gonad width. Therefore, the degree of sexual development at migration, as represented by the GSI, is independent of the total length of an eel. Examination of the data showed that some of the

smallest migrants of each sex and species had the largest GSI values. Similarly, eels with the widest gonads did not have the largest GSI values. As explained previously, an increase in gonad width is associated with increasing maturity during the whole development of the gonad. But at the migrant stage, which represents only a short period of this development, this relationship cannot be seen. Therefore the width of the gonad does not adequately represent the development of the gonad as does the GSI value.

It is of interest to compare the GSI values of other species of eels at migration with those of New Zealand eels. As will be discussed separately, such a comparison may help to indicate more precisely the spawning grounds of the New Zealand species. Before direct comparisons are made between species on the state of sexual development at migration as indicated by the GSI values, account must be taken of the GSI at maturity for each species.

For the European eel Fontaine, Bertrand, Lopez and Callamand (1964: 2908-9) calculated a GSI value of 31.8% for a female eel that spawned artificially. The values for three other females which died before spawning were 21%, 37% and 40%. Since these experiments Fontaine (pers. comm.) has calculated gonadosomatic indices of over 40.0 for mature females. These values approximate those for mature shortfin and longfin females. Boëtius and Boëtius (1967a: 351) found that the maximum GSI value for a mature male European eel under artificial conditions was 13.9%, considerably less than for male shortfin and longfin eels at maturity. However, from the GSI values at maturity for the female European eel and for the two species of New Zealand eels it is safe to assume that the GSI values at maturity for other species of temperate freshwater eels will be approximately the same. On this basis direct comparisons can be made between species on the degree of sexual development at migration.

The GSI values for other species of temperate eels are set out in Table 3.22, which includes the mean GSI values for New Zealand eels, calculated from all areas and years combined.

The GSI values for the European eel are considerably lower than those for New Zealand eels, indicating very little increase in sexual development at the time of seaward migration. Indeed Sinha and Jones (1967b: 72) state that migrating European eels are sexually no more advanced than the larger non-migrants. Migrant female American eels appear to show a considerable increase in development at migration, although it is not known what the greatest value of the GSI is in non-migrant eels.

TABLE 3.22

Mean gonadosomatic index (GSI) and range at migration for
other species of temperate freshwater eels

<u>Species</u>	<u>Male</u>		<u>Female</u>	
	<u>Mean</u>	<u>Range</u>	<u>Mean</u>	<u>Range</u>
<u>A. australis</u>	0.31	0.04 - 0.7	3.82	1.22 - 5.64
<u>A. dieffenbachii</u>	1.41	0.51 - 2.73	8.6	6.49 - 11.53
<u>A. anguilla</u> (Boëtius and Boëtius 1967a)	-	0.05 - 0.11	1.65	1.1 - 2.3
<u>A. anguilla</u> (Sinha and Jones 1967b)	-	-	2.5	-
<u>A. rostrata</u> (Boëtius, Boëtius, Hemmingsen, Bruun and Møller-Christensen 1962)	-	-	5.9	4.5 - 6.8
<u>A. japonica</u> (Ochiai, Umeda and Ōta 1972)	-	-	2.57	1.0 - 3.3
<u>A. japonica</u> (Honma 1966)	0.65	-	4.0	2.7 - 5.5

Few data are available on the GSI for the Japanese eel at migration. Ochiai, Umeda and Ōta (1972) give the GSI values for seven females which range from 1.0 to 3.3 (mean 2.6). Honma (1966: 242) lists some eels caught at sea, and the GSI values I calculated from his data range from 2.7 to 5.5 (mean 4.0). The GSI value calculated for a single male was 0.6. The values for the female eels caught at sea calculated from Honma are larger than those given by Ochiai et al., probably because of the increased sexual development which had occurred since they left freshwater.

The comparison with other species clearly shows that the European eel is the least sexually developed at the time of migration. The shortfin eel is slightly more advanced, with a mean value at migration for the females of 3.82. From the values given by Ochiai et al., the state of sexual development of the Japanese eel as reflected by the GSI value approximates that of the shortfin. The American eel is considerably more advanced than either the European, Japanese or shortfin eels. Boëtius, Boëtius, Hemmingsen, Bruun and Møller-Christensen (1962: 185) state that the data, quoted in Table 3.22, shows the more advanced state of sexual development in the American eel as compared to the migrant European eel. Lastly, the longfin eel is in a much more advanced state of sexual development at the time of seaward migration than any of the other species.

Ova Diameter

Another convenient indication of the state of sexual development at migration attained by females is given by the maximum ova diameter.

Nothing is known of the ova size at migration for female eels of either species. Cairns (1942b: 175B) makes the only reference to ova when he notes that ova in well developed eels could be seen with a magnifying glass.

In order to compare species and accurately determine the state of sexual development at migration, as indicated by the maximum ova diameter, it is important to know the size of the ova at maturity. It is shown in the section of induction of maturation that the mean ova diameter for shortfin females is 1.0mm and for longfin females 0.92mm.

In longfin females, the largest ova are easily removed from a fresh piece of ovary by teasing them off the ovarian tissue with a scalpel. Individual ova are easily observed. Shortfin ova adhere to the ovarian tissue more strongly and are less easy to separate; they dislodge in clumps when teased off the ovary.

Viewed under a binocular microscope, the ova of both species appear similar, except for the noticeably larger size of the longfin ova. Fig.3.20 shows the macroscopic appearance of the largest ova for each species at migration. The ova are spherical and white. The yolk is granular in appearance, very dense and occupies almost the complete volume of the ovum. The yolk is composed of many small spherical globules and individual yolk globules are distinguishable on the edge of the yolk mass. The perivitelline space is very narrow. There are apparently no oil globules.

Table 3.23 gives the mean ova diameter, range and standard deviation for each species from different areas. Where data were available the mean ova diameter for different years is also shown. The mean ova diameter within each species for different areas and years show some variation, although the range for each is similar. In particular the mean ova diameter for Makara 1973 shortfins is greater than for other areas. However, the range of values between different areas is no greater than the range within any one area for each species. For this reason, and also because of the varying sample sizes, the differences between the mean ova diameter for different areas within each species are not considered to be biologically significant. However, the differences between the species are considered to be significant.

The mean ova diameter at migration is considerably greater than the maximum ova diameter measured in non-migrant females. In non-migrants of both species no ova were observed with diameters larger than 0.1mm.

The mean ova diameter for each species shown in Table 3.23 clearly show that migrating longfin females have larger ova than shortfin females.

The mean ova diameter of 1.0mm for mature shortfin females shows that during the sea journey to the spawning grounds the ova increase in volume about x156. Based on the mature ova diameter of 0.92mm, ova of migrating longfin females increase in volume by only approximately

FIG.3.20

IMMATURE OVA FROM MIGRATING EELS

1. Fresh, immature ovum from a migrant A. australis (shortfin), 65.5cm total length. x 300
2. Fresh, immature ovum from a migrant A. dieffenbachii (longfin), 98.0c, total length. x 200

1

2

TABLE 3.23

Mean ova diameter and range (fresh ova measurements) for migrating females of each species from different areas.

<u>Area</u>		<u>Mean ova diameter (mm)</u>	<u>Range (mm)</u>	<u>Standard deviation</u>	<u>No</u>
<u>A. australis</u>					
Makara stream	1971	0.27	0.25 - 0.3	-	-
Makara stream	1972	0.31	0.2 - 0.45	0.07	4
Makara stream	1973	0.33	0.3 - 0.4	0.04	5
Lake Onoke	1972	0.27	0.2 - 0.32	0.01	14
Hokitika	1972	0.28	0.2 - 0.4	0.05	8
Invercargill	1972	0.25	0.21 - 0.32	0.03	5
All areas		0.28	0.2 - 0.45	0.04	37
<u>A. dieffenbachii</u>					
Makara stream	1971	0.37	0.35 - 0.45	-	-
Makara stream	1972	0.38	0.32 - 0.42	0.02	3
Makara stream	1973	0.4	0.35 - 0.48	0.05	2
Lake Onoke		0.39	0.35 - 0.45	0.02	4
Hokitika		0.38	0.3 - 0.4		1
Invercargill		0.38	0.35 - 0.42	0.02	2
All areas combined		0.38	0.3 - 0.48	0.02	13

x34 during the sea journey to the spawning grounds.

There is no correlation between ova diameter and the body length of the eel. This is contrary to Gray and Andrews (1970: 485) who established a correlation between ova diameter and length for the migrating American eels.

However, oocyte diameter increases with increasing GSI, as shown for the mature eels in the section on induction of maturation. Therefore, the state of sexual development at migration, as represented by the GSI and ova diameter in females, and GSI values in males, is independent of total length.

The ova size at the time of seaward migration for other species of temperate eels is reasonably well documented. Table 3.24 lists the mean ova diameter and/or range at migration for other species, and includes the mean ova diameter and range for New Zealand eels calculated from all areas and years combined.

Before direct comparisons are made between species on the state of sexual development at migration, it is important to know the diameter of the mature ova of each species. Fontaine, Bertrand, Lopez and Callamand (1964: 2909) found that the diameter of mature ova from the European eel varied from 0.93 mm to 1.4mm. For the Japanese eel, Ishida and Ishii (1970: 267) found that the mature ova measured from 0.8mm to 1.0mm in diameter. Considering the mean ova diameters of 0.92mm and 1.0mm for the longfin and shortfin eels respectively, the ova diameters of mature ova from all temperate species so far observed are similar. It is therefore reasonable to assume that the ova diameters for other species in which maturity has not been observed will be approximately the same. In particular I refer to the American eel. Fish (1926: 455, and 1927: 290) described eel ova, 3.3mm in diameter, in which a very early stage of development had been reached. She considered that these were the ova of the American eel, but as Bruun (1963: 155) notes, she expresses some hesitation about her identification. Schmidt (1929: 16) ^{in Bruun (1963) also} considered her identification to be incorrect, and Bigelow and Schroeder (1953: 153) also doubt it. All doubt was removed by Eldred (1968) who identified them as those of the pygmy moray eel, Anarchias yoshiae Kanazawa (Muraenidae).

The state of sexual development at the time of seaward migration as shown by ova diameters approximates that shown by the GSI values. The European eel has the least developed ova at migration.

TABLE 3.24

Mean ova diameter and range at migration of other species of temperate freshwater eels.

<u>Species</u>	<u>Mean ova diameter</u> <u>(mm)</u>	<u>Ova diameter</u> <u>(mm)</u>
<u>A. australis</u>	0.28	0.2 - 0.45
<u>A. dieffenbachii</u>	0.38	0.3 - 0.48
<u>A. anguilla</u> (Bruun, Hemmingsen and Møller-Christensen 1949)		0.1 - 0.2
<u>A. rostrata</u> (Bruun 1963)		0.2 - 0.35
<u>A. rostrata</u> (Gray and Andrews 1970)		0.109 - 0.214
<u>A. japonica</u> (Matsui 1957)	0.22	0.17 - 0.27
<u>A. japonica</u> (Ochiai, Umeda and Ōta 1972)	0.35	0.24 - 0.59

The Japanese eel, from Matsui's (1957) data, has a similar range of ova diameter to the shortfin. However, the values given by Ochiai, Umeda and Ota (1972) are larger and are similar to those of the longfin. The shortfin eel, and the American eel as shown by Bruun (1963: 149), have a similar range in ova diameter at migration, although the American eel has a higher mean GSI value.

The range of ova diameters at migration for the American eel given by Gray and Andrews (1970: 486) is less than those given by Bruun (1963: 149), and is the same as those given by Bruun, Hemming-sen and Møller-Christensen (1949: 218) for the European eel.

Microscopic appearance of the gonad

A total of 254 migrant eel gonad samples were examined histologically from the different sampling areas. The histological methods used to examine the gonads of migrants are described in the Materials and methods section. The location of these areas and the number of migrants obtained is given in Fig.1.1 and Table 1.2. The gonads of most migrant eels were examined macroscopically and a large number of these were then sectioned for histological analysis. The number of migrant eels from different areas that were sectioned is included in Tables 3.25 and 3.26. The total number of migrant eels sectioned is not large,^{but} because of the similarity in the state of sexual development within each sex of each species this sample was considered to be representative of all the migrants of each sex.

The purpose of the histological examination was to determine the state of sexual development of the gonads at the time of seaward migration, by identifying the stages of spermatogenesis in the males and the stages of oocyte development in the females.

The state of sexual development was based on the presence or absence in the gonad of the following cellular elements. In the males: primary germ cells, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. In the females: the presence or absence of primary oocytes, oocytes in the stage of vacuolation and oocytes in the stage of vitellogenesis. The presence of the latter stages of sexual development, viz. spermatocytes, spermatids and spermatozoa in the males, and oocytes in vitellogenesis in the females, indicating a more advanced stage of development.

The nuclear and cytoplasmic detail of these various cell types is given in the section on gametogenesis. The state of development in males was also based on the size and degree of development of the tubules. The presence or absence of interstitial cells was also noted.

A preliminary qualitative examination of the histological sections revealed that specimens of each sex of each species from all areas were in very similar stages of sexual development. It was also apparent that there was a significant difference in the state of sexual development between the two species. Once the various cell types were identified in the males and the stage of oocyte development determined in the female migrants, a quantitative analysis of the sections was made to determine more accurately if there were any differences within each sex of each species from different areas.

An accurate measure of the differences between each species could also be made.

Ahsan (1966: 151) describes a method used for the quantitative analysis of sections of the testis of the Lake chub Covesius plumbeus Agassiz. This method involves the use of a camera lucida to draw on to paper the outlines of selected lobules and the various cell types within them. The names of the various cell types were then noted on the projection. Using a planimeter the areas of the various cell types were determined, and the percentage composition in terms of relative abundance calculated.

This method was initially used to make a qualitative analysis of slides of the testis, but was found to be unsatisfactory. The method was laborious, and the previously determined similarity shown by the sections of the testis within males of each species did not necessitate such detailed analysis.

A simple and more rapid method was devised for a qualitative analysis of sections. For the sections of the testis a squared eyepiece was used to determine by eye the percentage composition of the various cell types in each of ten randomly selected lobules. This method was found satisfactory, and accurately measured the differences between males of each species.

A quantitative analysis between females of each species from different areas was also made. Using a micrometer eye-piece the greatest oocyte diameter and nuclear diameter was measured on the ten largest oocytes. Three or four random selections of the 10 to 15

oocytes were made, and the number of oogonia, resting oocytes, and oocytes in the stages of vacuolation or vitellogenesis counted. The number of oogonia or oocytes in these various stages were then expressed as a percentage of the total number of oogonia and oocytes counted in one section.

By these methods a quantitative comparison could be made between eels of each species from different areas, to detect any variability in the state of gonad development at the time of seaward migration. An accurate measure of the differences between each sex of each species could also be made.

Matsui (1957: 160) found that migrating eels from the same area differed in the stage of sex development. Examination of shortfin and longfin migrants of each sex showed little variation. The slide analyses were therefore combined for eels within each area. Only comparisons between areas were made.

In the following cytological descriptions of migrants what are considered to be significant differences between each sex of each species may not be regarded as significant in other species of teleosts. For example, Bowers and Holliday (1961) describe gonad stages for male herring, Clupea harengus L., (Clupeidae), based on the International (Hjort's) scale of maturity, that includes stages that incorporate the presence of spermatogonia, spermatocytes, spermatids and spermatozoa. Similarly, Tong and Vooren (1972) describe maturity stages for both male and female tarakihi, Cheilodactylus macropterus (Block and Schneider)(Cheilodactylidae), single stages of which include all the differences considered significant between males and females of each species of eel. However, the migratory eels represent a short stage in the process of sexual development, the remainder of which occurs at sea. Therefore, a more detailed scale of comparison is necessary to determine any differences between the eels at the time of seaward migration.

Migrant shortfin males

In migrant shortfin males the testes lobes are composed of a number of tubules, each containing the spermatogonial elements. Fig.3.21 shows, in cross section, the microscopic appearance of the tubules under low power. Each tubule is surrounded by a band of

FIG. 3.21

TRANSVERSE SECTIONS (TS) OF THE TESTES
FROM MIGRATING A. Australis (shortfin)
EELS FROM THE MAKARA STREAM

1. TS of the testis from a 43.2cm eel to show the tubular structure and the presence of spermatogonia and spermatocytes. Only isolated lumina of the tubules can be seen. x 250
2. TS of the testis from a 46.5cm eel to show details of the spermatogonia, dividing spermatogonia and spermatocytes. x 700

sg - spermatogonia

sp - spermatocytes

l - lumen of tubule

it - intertubular connective tissue

1

2

connective tissue, and in the centre of each tubule is a small lumen. The tubular structure, as shown in Fig.3.21, is well developed in most shortfin migrant males, although three Makara specimens had poorly developed tubules and a large amount of connective tissue, similar to many non-migrant males. The tubules varied in size from 40 μ m to 68 μ m in greatest diameter, with the lumina measuring from 5 μ m to 12 μ m in greatest diameter. The intertubular connective tissue was well developed, measuring from 5 μ m to 12 μ m in width. Thin strands of this connective tissue were seen extending into the tubules amongst groups of cells, forming thin intratubular connective tissue septa.

In longitudinal sections it was observed that the lumina of each tubule interconnect to form a network of efferent ducts which converge at the main efferent duct, the vas deferens. In some slides the ducts could be seen opening into the vas deferens, but in most specimens the vas deferens appeared closed and restricted.

The tubules are composed of groups or cysts of germ cells in various stages of maturation surrounding the lumen.

Around the periphery of each tubule lie the spermatogonia (Fig.3.21). They are readily recognized by their large nuclear size, nuclear detail, the presence of a nucleolus and their position around the periphery of the tubule. The cell outline can be easily distinguished and the cytoplasm stains faintly. The spermatogonia form a layer of cells four to six cells thick around the tubule. They were often seen in active mitotic division. Primary spermatocytes (Fig. 3.21), when present, were observed on the inner margin of the tubule. These were recognised by their smaller nuclear size, darkly staining chromatin material, and their position in the lobule. The cell outline was not distinguishable. No secondary spermatocytes, spermatids or spermatozoa were found in the tubules of any eels examined. In 10 specimens with open vas deferens, small clusters of spermatids and spermatozoa were observed in the lumen of the vas deferens. The spermatids were recognized by their small size and dense, darkly staining chromatin material; spermatozoa were distinguished by their elongate, crescent-shaped heads and darkly staining chromatin.

The presence of isolated spermatids and spermatozoa in the lumen of the vas deferens was independent of the state of development

in the tubules. The percentage composition of the tubules in these specimens was no different than for some eels with closed vas deferens.

Testes lobes were sectioned from the anterior, middle and posterior portions of both the left and right gonad from four specimens. No difference in the percentage composition of the various cell types within tubules from these areas could be detected. In any one section of an individual gonad lobe no difference was observed in the percentage composition between tubules in the vas deferens region and tubules on the opposite edge of the gonad lobe.

The small amount of variation in the percentage composition of each cell type within tubules from different areas of the gonad and within each lobe indicates that the development is synchronous throughout the gonad.

Table 3.25 gives the mean percentage composition and range of spermatogonia and primary spermatocytes in the tubules for 95 migrant shortfin males from different areas. The mean percentage composition and range of values for each of the two cell types show little difference for each area. No significance could be attached to these differences, and it was concluded that migrant shortfin males from different areas were in the same stage of sexual development at the time of seaward migration.

Only two specimens, 2% of the total number examined, had tubules containing on an average less than 50% spermatogonia, and more than 50% primary spermatocytes. This was the most advanced state of sexual development observed. It was the two specimens with the highest GSI, 0.7, which showed the greatest development.

Only three specimens (3%) of the total number of eels examined had tubules containing only spermatogonia and no primary spermatocytes. These specimens showed nests of large primary spermatogonia within an irregular connective tissue network. No proper tubular structure was evident. These three eels had the lowest GSI values. This was the most immature stage of sexual development observed.

From Table 3.25 it is seen that the migrating shortfin males, for all areas combined, contained in their tubules 73% spermatogonia and 27% primary spermatocytes. Only 2% of the eels were more developed, containing more than 50% primary spermatocytes, and only 3% were less developed, containing no primary spermatocytes. The migrant

TABLE 3.25

Histological analysis of migrant males. Percentage composition of the various cell types within the testes of migrating males of each species.

Area	Spermato- gonia %	Range %	Sperma- tocytes %	Range %	Sperma- tozoa %	No. eels
<u>A. australis</u>						
Makara	75	30-100	25	0-70	0	30
Lake Onoke	70	40-80	30	20-60	0	30
Lake Ellesmere	78	60-100	22	0-40	0	20
Hokitika	80	70-90	20	10-30	0	8
Invercargill	90	85-95	10	5-15	0	2
Waikato River	90	80-100	10	0-20	0	5
All areas combined	73	30-100	27	0-70	0	95
<u>A. dieffenbachii</u>						
Makara	25	5-75	75	25-95	1	61
Lake Onoke	10	5-15	90	85-95		2
Lake Ellesmere	25	20-30	75	70-80		2
Hokitika	10	5-15	90	85-95		2
Invercargill	13	5-20	87	80-95	1	3
All areas combined	23	5-75	77	25-95		70

shortfin males at the time of seaward migration are therefore classified as being at the 'spermatogonia-primary spermatocyte' stage. The spermatogonia preceding the primary spermatocyte indicating that the former cell type is the most abundant.

Migrant longfin males

As in the migrant shortfin males, the testes lobes of the longfin migrant males are composed of a number of tubules, each surrounded by a band of connective tissue. Fig.3.22 shows in cross section the microscopic appearance of the gonads under lower power. The tubular structure is better developed in the longfin male migrants than in the shortfins. The tubules are larger in their greatest diameter, measuring from $68\mu\text{m}$ to $100\mu\text{m}$ and the lumen of each tubule measures from $12\mu\text{m}$ to $20\mu\text{m}$ in greatest diameter. The connective tissue network is well developed, but is thinner ($5\mu\text{m}$) than in the shortfins. Thin strands of connective tissue extend into the tubules amongst groups of cells, forming thin intratubular connective tissue septa.

Similar to the shortfins, the lumen of the tubules are seen as efferent ducts when the tubules are sectioned longitudinally. These efferent ducts converge at the main efferent duct, the vas deferens.

Unlike the shortfins in which the vas deferens usually appeared closed and constricted, the vas deferens in the longfins is open, and the efferent ducts can be seen opening into it.

Around the inside periphery of each tubule is a layer of spermatogonia one cell layer thick (Fig.3.22). The spermatogonia were readily recognized by their large nuclear size, nuclear detail, the presence of a nucleolus and their position around the periphery of the tubule. They were often observed in active mitotic division. On the inner margin of the tubule were approximately six layers, each one cell thick, of primary spermatocytes (Fig.3.22). These cells were recognized by their smaller nuclear size, darkly staining chromatin material, and their position in the tubule. The number of layers of cells of each cell type varied according to the degree of development of each eel. In the least developed specimens there were two to three cell

FIG.3.22

TRANSVERSE SECTIONS (TS) OF THE TESTES FROM
MIGRATING A. dieffenbachii (LONGFIN) EELS FROM
THE MAKARA STREAM

1. TS of the testis of a 60.0c, eel to show the tubular structure and the presence of spermatogonia and spermatocytes. The lumina of the tubules are clearly visible. x 250
2. TS of a tubule of a 65.0cm eel to show details of the spermatogonia and spermatocytes. A group of spermatogonia undergoing mitotic division are visible in the upper middle portion. x 700

sg - spermatogonia

sp - spermatocytes

it - intertubular connective tissue

l - lumen of tubule

1

2

layers of spermatogonia and fewer layers of primary spermatocytes. Some of the more developed eels had only isolated spermatogonia around the periphery of the tubule and no continuous cell layer. No secondary spermatocytes were observed. Small clusters of spermatids were present in the tubules of 10 of the eels examined. These were positioned in isolated groups on the inner margin of the tubule adjacent to the lumen. The spermatids were recognized by their small size and dense, darkly staining chromatin material.

Isolated spermatozoa were seen in the lumina of the tubules of 17 of the eels examined (Fig.3.23). In two specimens in particular a large number of spermatozoa were observed. Spermatozoa were recognized by their elongate, crescent-shaped, darkly staining chromatin material, and when present in the tubules they were always free in the lumen. It was the eels with the largest GSI values that contained the spermatids and spermatozoa in the tubules.

In those specimens which were sectioned through the open vas deferens, groups of spermatids and spermatozoa were present in the lumen of the vas deferens (Fig.3.23). These eels did not in all cases have spermatids or spermatozoa in the tubules. As in the shortfins, the presence of spermatids and spermatozoa in the vas deferens was independent of the stage of development of the tubules.

Testis lobes were sectioned from the anterior, middle and posterior portions of both the left and right gonad from four specimens. No difference in the percentage composition of the various cell types within the tubules from these areas could be found. In any one section of an individual gonad lobe no difference was observed in the percentage composition between tubules in the vas deferens region and tubules on the opposite edge of the lobe.

The small amount of variation in the percentage composition of each cell type within the tubules from different areas of the gonad, and within each lobe, indicates that the development is synchronous throughout the gonad.

Table 3.25 gives the mean percentage composition and range of spermatogonia and primary spermatocytes in the tubules for 70 migrant longfin males from different areas. The mean percentage composition and range of values for each of the two cell types show some variation between each area. In particular one of the three specimens from Invercargill was one of the most sexually advanced eels observed,

FIG.3.23

TRANSVERSE SECTIONS (TS) OF THE TESTES FROM
MIGRATING A. dieffenbachii (LONGFIN) EELS

1. TS of the vas deferens from a 62.0cm eel to show the presence of spermatocytes, spermatids and spermatozoa. x 250
2. TS of the testis from a 65.0cm eel from the Makara stream to show the presence of spermatids, and spermatozoa in the lumen of a tubule. x 700
3. TS of the testis of a 69.0cm eel from the Invercargill area to show the presence of large numbers of spermatozoa in the lumina of the tubules. x 250

sg - spermatogonia
sc - spermatocytes
sp - spermatids
s - spermatozoa
l - lumen of tubule
vdl - lumen of vas deferens

1

2

3

although two of the Makara eels were almost equally well developed.

The small sample sizes from areas other than Makara would preclude any statistical comparison being made between all areas. However, the mean percentage and range of values are similar for all areas, and it was therefore concluded that migrant longfin males from different areas were in the same stage of sexual development at the time of seaward migration.

All specimens except one had tubules containing less than 50% spermatogonia and more than 50% primary spermatocytes. Twenty-one percent (15) of the eels examined contained isolated groups of spermatids and spermatozoa in the lumen of the tubules away from the vas deferens. Two specimens were in a particularly advanced state of development. Each displayed a large number of spermatozoa in the lumen of the tubules. The tubules and the lumen of each had enlarged, but the tubular structure was still well developed. Despite the large number of spermatozoa there were few spermatids, and the most dominant cell type was the primary spermatocyte. These eels represented the most advanced state of sexual development observed. It was these two eels that had the highest GSI values of 2.4 and 2.3.

Only one eel had tubules containing less than 50% primary spermatocytes and more than 50% spermatogonia. This specimen was the most immature stage of sexual development observed.

From Table 3.25 it is seen that the migratory longfin males, for all areas combined, contained in their tubules 23% spermatogonia and 77% primary spermatocytes. Twenty four percent were more developed, containing less than 50% primary spermatocytes. The migrant longfin males at the time of seaward migration are therefore classified as being at the 'primary spermatocyte - spermatogonia' stage. The primary spermatocyte preceeding the spermatogonia as the former cell type is the most abundant.

Migrant shortfin females

Developing ova, as described for the non-migrant females, are found in lamellae or ovarian leaflets that project out from the lateral surface of the ovary into the abdominal cavity. The ova are embedded in a thin stroma of ovarian tissue that is thickest on the inner, smooth side of the ovary.

The oocytes appear in three stages of development, as primary oocytes, vacuolated oocytes and oocytes in the stage of vitellogenesis or yolk formation. A transverse section of an ovary containing primary oocytes and vacuolated oocytes is shown in Fig.3.24.

Primary oocytes are distinguished by their small size, darkly staining cytoplasm and a small number of vacuoles in the cytoplasm. The vacuolated oocytes contain a large number of vacuoles throughout a less darkly staining cytoplasm. There is a considerable variation in the size of the vacuolated oocytes. The smaller ones, 64 μ m in diameter, have few vacuoles and the area of cytoplasm greatly exceeds that of the vacuoles. As the size of the oocyte increases the number of vacuoles increases. In the larger oocytes, 150 μ m in diameter, the amount of cytoplasm is small compared to the large number of vacuoles.

In some eels a few of the largest oocytes were at the stage of early vitellogenesis. In these, a number of fine, darkly staining PAS-positive yolk globules were seen around the periphery of the oocytes, similar to those shown for the longfin in Fig.3.25.

From five specimens ovarian samples were sectioned from the anterior, middle and posterior region of each gonad. Measurements of the oocyte diameter in the largest oocytes, and the number of oocytes in the various stages of development showed that there was no difference in the development in different regions of the gonad within each eel. It was concluded that the development within the ovaries of each eel is synchronous.

Table 3.26 gives the percentage composition of oocytes observed in the stages of resting oocytes, vacuolated oocytes and oocytes in vitellogenesis seen in migrating females from each area. Table 3.26 shows that there is some difference between the mean values for each oocyte stage for each area, but the range of values for each area is similar. In particular the Invercargill eels had a higher percentage of oocytes in vitellogenesis than for other areas, although the percentage of resting oocytes is similar. However, the range of values between different areas is less than the range within any one area. Therefore the differences between areas are not considered to be significant, and it is concluded that there is no difference in the state of sexual development in migrant shortfin females from different areas.

FIG. 3.24

Transverse section of an ovary from a
migrating A. australis (shortfin) eel 65.0cm total length
from the Makara stream to show the primary oocytes and
vacuolated oocytes. x 140

p = primary oocytes
v - vacuolated oocytes
n- nucleus
nl - nucleoli

TABLE 3.26

Histological analysis of migrant females.

TABLE 3.26 HISTOLOGICAL

Area	Mean oocyte diameter (μm)	Range	Standard deviation	Mean nuclear diameter (μm)	Standard deviation
<u>A. australis</u>					
Makara	198	176-288	25.0	63.4	6.1
Lake Onoke	212	176-256	18.5	65.1	4.8
Lake Elles- mere	184	144-240	17.8	60.9	5.7
Hokitika	193	176-240	20.8	67.2	6.3
Invercargill	213	160-256	28.1	62.4	2.3
All areas	203	144-288	24.2	63.8	5.1
<u>A. dieffenbachii</u>					
Makara	292	224-368	24.9	70.5	6.2
Lake Onoke	288	208-384	49.1	78.5	13.2
Hokitika	274	256-304	—	70.6	1.9
Invercargill	301	256-368	7.9	70.6	1.9
All areas	274	208-368	15.6	70.9	4.5

ANALYSIS OF MIGRANT FEMALES

Percentage of oocytes in three stages of development						No. eels
Oocytes	range	vacuolation	range	vitello- genesis	range	
%	%	%	%	%	%	%
19	0-30	71	30-96	10	0-60	20
12	0-27	72	13-90	16	0-63	25
14	5-23	85	82-95	1	0- 6	10
16	4-27	69	28-85	15	0-67	8
19	13-28	45	12- 8	36	0-71	5
16	0-28	68	12-96	16	0-71	68
4	0- 7	25	14-40	71	58-80	13
2	0- 6	21	17-31	77	62-83	4
5	--	16	--	79	--	1
8	0-17	14	8-20	77	75-80	2
4	0-17	21	8-40	75	58-83	20

Seven eels, 10% of the total, contained more than 50% of their oocytes at vitellogenesis. In all of these the yolk globules were confined to the periphery of the oocyte, indicating that they were in the very early stages of vitellogenesis. These eels were the most advanced migratory shortfin females observed.

The majority (90%) of the eels examined contained more than 50% of their oocytes in the vacuolation stage. No eels contained more than 30% of the oocytes in the primary oocyte stage. Migrant shortfin females are therefore classified as being at the vacuolation stage of development.

Table 3.26 gives the mean oocyte diameter, standard deviation, and the mean nuclear diameter and standard deviation of oocytes from eels from all areas. In only two samples (Lake Onoke and Invercargill) is the mean oocyte diameter greater than 200 μ m, although the range of oocyte diameter in all areas is over 200 μ m. The differences in the mean oocyte diameters between areas were therefore not considered to be biologically significant and a mean value was calculated for all areas combined and is listed in Table 3.26.

Similarly, the mean oocyte nuclear diameter between different areas show little difference. A mean value was calculated for all areas combined, and is also listed in Table 3.26.

The nuclei of oocytes in all stages of development each contained a number of nucleoli, lying around the periphery of the nucleus next to the nuclear membrane (Fig.3.24). They were too numerous to count accurately, being in excess of 20 or 30.

In summary, of all the oocytes examined from eels from different areas combined, 16% measured between 200 μ m and 288 μ m in diameter and were at the stage of early vitellogenesis. Oocytes at the stage of vacuolation comprised 68% of the oocytes examined, and measured from 176 μ m to 240 μ m in diameter. Primary oocytes, or those oocytes in the early stages of vacuolation measuring under 176 μ m, comprised 16% of the oocytes examined.

Migrant longfin females

The developing ova appear in the ovarian lamellae as described for the shortfin female migrants.

Oocytes in section appear in the three stages of development as described for the shortfin females. Transverse sections of ovaries containing oocytes in these stages is shown in Fig.3.25. Oocytes in vitellogenesis contain a large number of darkly staining, PAS-positive yolk globules throughout the cytoplasm. This is a more advanced stage of development than seen in shortfins where the oocytes in vitellogenesis, if present, have yolk globules only around the periphery of the oocyte.

From three specimens ovarian samples were taken from the anterior, middle and posterior regions of each gonad. The oocyte diameter of the largest oocytes was measured, and the number of oocytes in the three stages of development counted. No difference in the diameter of the largest oocytes and the number of oocytes in different stages could be found, and it was concluded that the development within the ovaries of each eel is synchronous. Therefore sections of a sample from the middle region of either gonad, which was the standard practice in this study, gave an accurate picture of the state of development of both the gonads.

Table 3.26 gives the percentage composition of oocytes observed in the three stages seen in migrating eels from each area. The percentage composition is similar for the different areas. The range of values between the areas is less than the range within the Makara and Lake Onoke samples. It was therefore concluded that there is no difference in the stage of sexual development in migrating longfin females from different areas.

The most sexually advanced eels contained 80% of their oocytes in vitellogenesis, and no eels contained less than 58% of the oocytes in this stage. The least advanced specimen contained 40% of its developing ova as vacuolated oocytes. No eel contained more than 8% of its oocytes in the primary stage.

All eels examined contained more than 50% of the oocytes in the stage of vitellogenesis. Migrant longfin females are therefore classified as being at the stage of vitellogenesis at the time of seaward migration.

Table 3.26 gives the mean oocyte diameter, standard deviation and the mean nuclear diameter and standard deviation of oocytes from eels from all areas. The mean oocyte diameter for all areas, except Southland, is under 300µm. However, the ranges of values, except for the single specimen from the Hokitika are all similar. The

FIG.3.25

TRANSVERSE SECTIONS (TS) OF THE OVARIES FROM
MIGRATING A. DIEFFENBACHII (LONGFIN) EELS FROM
THE MAKARA STREAM

1. TS of the ovary from a 108.0cm eel to show a primary oocyte and oocytes in the stage of advanced vitellogenesis. Darkly staining (black) yolk globules are scattered throughout the cytoplasm. x 130

2. TS of the ovary from a 88.0cm eel to show vacuolated oocytes and oocytes in the stage of early vitellogenesis. Darkly staining (black) yolk globules are present around the periphery of the oocyte. A few migrating shortfin eels contained oocytes at this stage of vitellogenesis. x 320

n - nucleus
nl - nucleoli
vt - oocytes in vitellogenesis
v - vacuolated oocytes
p - primary oocytes
y - yolk globules

1

2

mean oocyte diameters between different areas was therefore not considered to be biologically significant and a mean value was calculated for all areas.

The mean nuclear diameters between different areas show some variation. In particular the single West Coast specimen has a much smaller mean nuclear diameter than shown by other areas. However, little importance can be attached to the difference shown by one specimen. A mean value was calculated for all areas and is listed in Table 3.26.

The nuclei of oocytes in all stages of development each contained a number of nucleoli, arranged around the periphery of the nucleus (Fig.3.26.). As in the shortfin oocyte nuclei, the nucleoli were too numerous (20 to 30) to count accurately.

In summary, of all the oocytes examined from eels from different areas combined, 75% measured between 384 μ m and 208 μ m and were at the stage of vitellogenesis. Oocytes at the stage of vacuolation measured from 250 μ m to 192 μ m and comprised 21% of all oocytes. Resting oocytes or those oocytes in the early stages of vacuolation, measuring under approximately 180 μ m, comprised 4% of all oocytes examined.

3.12 Discussion on the state of sexual development at migration.

The differences in the state of sexual development between the sexes of each species at the time of seaward migration is marked. The presence of large numbers of primary spermatocytes in all longfin male migrants, and the occurrence of spermatids and spermatozoa in some, is a significant increase in development over the predominantly spermatogonia stage of development described in shortfin migrant males. Similarly, in migrant longfin females, the 75% of oocytes in the stage of vitellogenesis represents a significant increase in development when compared with only 16% of the oocytes at this stage in migrant shortfin females.

In the males of each species the presence of spermatids and spermatozoa in the lumen of the vas deferens in 10% of the shortfin migrants and all longfin migrants is not considered significant. In these specimens the bulk of the gonad is at a much earlier stage of development, in shortfins the 'spermatogonia - primary spermatocyte'

and in longfins the 'primary spermatocyte' - spermatogonia stage. In longfins and shortfins spermatozoa in the vas deferens is considered to be no more than abortive prespermatogenesis similar to that occurring in other fish. D'Ancona (1960: 70) uses prespermatogenesis to explain the presence rarely of small nests of spermatids and spermatozoa reported by several authors in migrating males of the European eel.

The male European eel at migration is at the spermatogonia stage (D'Ancona 1960: 69) and cells in more advanced stages are rare. Boëtius and Boëtius (1967a: 347 - 8) describe the gonad of European migrant male eel as being only slightly differentiated, with spermatogonia separated by interstitial connective tissue. The spermatid tubuli are little developed. This contrasts with the more sexually advanced migrant shortfin males in which the tubular structure is well developed and the predominant cell type is represented by the spermatogonia, although primary spermatocytes are common. Longfin migrant males are clearly further advanced than migrant male European eels, with large numbers of primary spermatocytes and some spermatids and spermatozoa.

For migrant males of the Japanese eel, Matsui (1952: 233) found that the most advanced eels were at the spermatocyte - spermatid stage but most were at the spermatocyte stage. Spermatozoa were rare. Satoh, Nakamura and Hibiya (1962) state that a number of spermatocytes are found and that spermatids are rare. From these descriptions the Japanese male eel at migration is at approximately the same stage as the longfin male at the time of seaward migration and more advanced than the shortfin.

Little information is available on the state of sexual development in migrating males of the American eel. According to Boëtius and Boëtius (1967a: 379), Ryder (1885) illustrates a section of a testis which has a well developed lobular structure. This would make the American male eel at least as developed as the shortfin eel at the time of seaward migration.

No cytological descriptions of males of other species of eels at migration are available.

Comparing females of different species at migration, D'Ancona (1960: 69) states that the oocytes in migrant females of the European eel are at different growth stages. From the ova size at migration of 0.1mm to 0.2mm and the GSI value of 2.5% it is doubtful if any of the oocytes are beyond the stage of vacuolation. In this respect

they are less advanced than shortfin eels with 16% of the oocytes beyond this stage and in vitellogenesis.

Matsui (1952: 233 and 1957: 159) found that 16% of the oocytes in migrating females of the Japanese eel were at the stage of ooplasm. Oocytes with few yolk granules and measuring over 0.1mm constituted 31% of the oocytes^{and} oocytes below 0.1mm 53%. Satch~~et~~ al. (1962) found that the oocytes develop up to the yolk vesicle stage at migration. Ochiai, Umeda and Ōta (1972) examined histologically the oocytes from seven migrating females and found that the majority, about 0.3mm in diameter, were at the early stage of vitellogenesis. Some oocytes however measured from 0.4mm to 0.59mm in diameter and were in more advanced stages of vitellogenesis, or the primary yolk stage as they describe it, with yolk globules in the outer half of the cytoplasm. From these descriptions, the stage of sexual development of the Japanese female eel is therefore more advanced than that of the shortfin, but is slightly less advanced than the migrant longfin females.

From the comparisons between species based on the GSI values and the state of development as revealed by cytological analysis, it is apparent that males of all species are relatively immature at the time of seaward migration. Little development towards sexual maturation has occurred in freshwater and consequently there must be a very rapid development during the sea journey to the spawning ground.

Female eels on the other hand, except the European eel as discussed by Sinha and Jones (1967b: 72), do show some degree of sexual development in freshwater before the time of seaward migration. In longfin females this pre-migratory development is considerable. Consequently, although the development to reach maturity in female eels must be rapid, it is perhaps a little less so than in males during the sea journey to the spawning grounds.

The colour and morphometric changes associated with the change from the non-migrant to migrant eel and the increased gonad development, especially in the longfins, represents the beginning of a different stage in the life cycle. In the European eel this change from the non-migrant or yellow eel to the migrant or silver eel stage is considered to be a metamorphic change (Bertin 1956). On this basis the change in the New Zealand species also represents a metamorphic change. D'Ancona (1960: 69) suggests that in the life cycle of the

European eel there are two successive metamorphic changes, from the leptocephalus to the elver, and from the yellow to the silver eel. Bertin (1956: 163) also considers that there are two metamorphoses, the final change resulting in the silver eel stage or phase of reproduction.

Svårdson (1949b) however, describes four eels caught in the Baltic Sea that were more advanced than the normal silver eel. They had enormously large eyes, black, pointed pectoral fins and were reddish-brown or bronze. He suggests that the colouration and changes in these eels represent the nuptial dress or stage, and this is the final reproductive stage, not the silver eel stage as suggested by Bertin (1956: 163) and D'Ancona (1960: 69). Svårdson further suggests that the change from the silver eel stage to the nuptial eel stage represents another metamorphosis, making three metamorphoses during the four stages (leptocephali, yellow eel, silver eel, nuptial eel) of the life cycle. Bruun (1963: 148) supports Svårdson's recognition of four stages, but does not discuss them as metamorphic changes. The reddish-brown or bronze colouration and the enlarged eyes observed in some European eels in which maturity or partial maturity had been induced by hormone injections (Tuzet and Fontaine 1937: Boëtius, Boëtius, Hemmingsen, Bruun and Møller-Christensen 1962: 197; Fontaine, Bertrand, Lopez and Callamand 1964) supports Svårdson's (1949: 133) suggestion of a third metamorphic change.

There is no evidence that New Zealand eels have a fourth, nuptial stage in the life cycle and consequently a third metamorphosis. Migrant shortfin eels show some colour variation at migration but they are in general a bronze colour on the dorsal surface and sides with a metallic silver ventral surface. Migrant longfins are predominantly a brown/black. In eels of both sexes of both species in which maturation had been induced by hormone injections, as described later in this study, no significant changes in colouration were observed. Eye size however did increase. The migrant longfin female caught at sea (Todd 1973) showed no colour change from normal seaward migrating longfin females. This, and the lack of change of maturity, suggests that no fourth stage is present in the life cycle. The migrant eel stage for New Zealand eels, which could be called the bronze eel stage for shortfins and the black eel stage for longfins, represents the nuptial stage or final

reproductive phase for these two species. Therefore there are only two metamorphoses during the life cycle, that from the leptocephalus stage to the elver or glass eel stage, and secondly from the non-migrant or feeding eel stage to the migrant eel stage.

Tucker (1959: 496) in his hypothesis refuting Schmidt's (1922) theory that the European eel returns to the Sargasso sea to spawn, supports his argument by stating that 'the European eel is well advanced towards being a reproductive oceanic fish and this despite the long journey which supposedly lies before it. The American eel, with a much smaller distance/time to go, is yet relatively retarded. The two conditions are the reverse of what a reasonable consideration of adaptive characters and natural selection would predict.'

Boëtius, Boëtius et al. (1962: 185) and Bruun (1963: 149) both present data on ova diameters of migrating American eels and Boëtius et al. give ova diameters and GSI values of the European eel that clearly show that the American eel is more sexually advanced than the European eel at migration. The evidence therefore supports what Tucker (1959) would consider to be logically expected, that the species with its distribution nearest to the spawning grounds in the Sargasso Sea is the most sexually advanced at the time of seaward migration. Thus the two conditions are what reasonable consideration of adaptive characters and natural selection would predict.

With this evidence from two northern hemisphere species it is reasonable to suggest that the degree of sexual development at the time of seaward migration is proportional to the distance from the spawning grounds.

Little information exists on the proposed spawning grounds of New Zealand eels. Schmidt (1925: 374) considered that the temperate species of eels inhabiting Australia and New Zealand have their spawning grounds in the warmer waters of the South Pacific Ocean near the tropics. Schmidt (1928: 210) qualified this by further suggesting that the short-fin species has its breeding ground in the basin to the east of the New Caledonian submarine ridge. Castle (1963: 13), based on the evidence of anguillid leptocephali from the south-west Pacific, suggests that the spawning ground for both species is in a slightly different area, to the east of New Caledonia, between Fiji and Tahiti. Hydrographic data given by Schmidt (1925: 373) and Sverdrup, Johnson and Fleming (1942) show conditions in these two areas to be similar

to the known spawning conditions for the two Atlantic species.

Thus both authors suggest that the spawning grounds for both species lie in the south-west Pacific ocean and the inference by both is that ^{both} species spawn in the same area.

The location of the spawning grounds in the areas suggested would entail a sea journey of approximately 2000 miles, less than for the European eel, but approximating that for the American, and the Japanese eel (calculated from Matsui 1957). The reproductive state of the shortfin eel, as represented by its ova diameter and GSI values, approximates that of the American and Japanese eels at migration as previously discussed. The European eel is the least developed at migration, in keeping with the much longer journey to the spawning grounds. Thus, considering the reproductive condition of the shortfin eel, the location of the spawning grounds as suggested by Schmidt (1925 and 1928) and Castle (1963) would be in keeping with its degree of sexual development.

The more advanced state of sexual development at migration in the longfin eel however causes a problem. If we accept that the degree of sexual development at the time of seaward migration is proportional to the distance from the spawning grounds, the longfin eel would not undertake such a long migration as the shortfin, and would therefore have its spawning ground located closer to New Zealand. It is therefore suggested that the longfin eel may spawn in the areas suggested by Schmidt (1925 and 1928) or Castle (1963) and the shortfin species undertakes a longer migration to spawn in an area further from New Zealand. Alternatively, the shortfin may spawn in the areas previously mentioned, and the longfin spawns in an area closer to New Zealand. Unfortunately there are insufficient hydrographic data to suggest any areas closer to New Zealand with the correct temperature and salinity, approximating the known spawning conditions for the Atlantic species, that may be suitable spawning grounds.

One further possible location of separate spawning grounds may be considered. Longfins may spawn in the basin to the east of the New Caledonian submarine ridge, as suggested by Schmidt (1928: 210) for the shortfin species, and the shortfin species may spawn at a further distance from New Zealand, well to the east of New Caledonia

between Fiji and Tahiti as suggested for both species by Castle (1963).

However, these suggestions are considered to be very tentative, as further hydrographic information is essential before any accurate study of the possible location of the spawning grounds can be made. Nevertheless, the advanced state of sexual development in longfins, as compared with shortfins and other species, does suggest spawning grounds in different areas, with the longfin species spawning closer to New Zealand than the shortfin.

4. AGE AND GROWTH

4.1 Introduction

Age and growth studies are of obvious value in the correct understanding of the biology of a fish and for the management of the fishery. From age determinations, the growth rate, population structure, age at maturity and lifespan can often be calculated.

Age can be determined in several ways; by length-frequency analysis (Petersen's Method) or by tagging. Other ageing methods are based on the growth zones or checks that appear in the hard parts of fishes such as the vertebrae, opercula, scales and otoliths. The latter two structures are the most frequently used. It has been established for many temperate species of fish that the annuli or rings on the scales represent an annual winter cessation or decrease in growth. By counting these rings the age of the fish can be determined. This method is best suited for fish in which the scale's formation commences when the fish is very small. In most species of eels the scales are formed when the fish has attained a considerable length, often up to 20cm. Thus the earlier growth is not reflected in the scale.

Age determination by otoliths is similarly based on the appearance of rings or zones. These appear as zones of differing optical densities when the otolith is viewed under transmitted or reflected light. If the otolith is burnt in a bunsen flame the zones appear as alternating black and white rings. The object of this section of the study was to examine the age and growth of eels of each sex in the non-migrant stage, and to determine the age of the eels at migration.

Age and growth studies on New Zealand eels are limited, and all have been conducted on commercially unexploited eel populations. MacFarlane (1936) obtained age determinations using both scales and otoliths. Cairns (1941) used otoliths for ageing but concluded that scales were also satisfactory for age determination. Unfortunately Cairns' (1941) age determinations and consequent growth rate curves were calculated from eels from many areas in New Zealand combined. Burnet (1969b) made a more detailed study of the age and growth in three separate streams, using both otoliths and tagging returns. Only Cairns (1941) differentiates between the

age of eels of each sex. No ageing studies have been conducted on migrant eels of either sex or species.

In contrast to the limited age and growth investigations on New Zealand eels, many studies have been undertaken on the European eel. Sinha and Jones (1967a) list the most important authors. More recently Penáz and Tesch (1970), Monarty (1972) and Voronin and Rusetskaya (1971) have studied the age and growth of the European eel. Scales or otoliths, or a combination of both, have been used in these studies.

No ageing investigations on the wild populations of the Japanese eel, published in English, are available. Matsui (1952) gives only fragmentary data on the age of pond cultured eels.

The age and growth of the American eel has been studied by Smith and Saunders (1955), Gunning and Shoop (1962), Boëtius and Boëtius (1967b), Ogden (1970) and Gray and Andrews (1971). Tagging, scales and otoliths were used in these investigations.

Age determinations of the Indian eels, A. nebulosa by Pantulu and Singh (1962) using otoliths, and for A. bengalensis Pantulu (1957) using scales, have been conducted.

4.2 Use of scales for ageing eels

The use of scales for ageing fish is well established. Lagler (1956), Nikolsky (1963) and Tesch (1968) adequately outline the theory and different methods for the use of scales for ageing.

According to MacFarlane (1936) and Cairns (1941: 56B), the scales of New Zealand eels are satisfactory for ageing. Both authors found that scales were formed regularly in the seventh year of life. Burnet (1955) however, considered scales were unsuitable for ageing. MacFarlane's (1936) and Cairns' (1941) conclusions are **contrary** to what is accepted by those investigating the age of the European eel. Ehrenbaum and Maukawa (1913) showed that the time of scale formation is dependent on the length of the eel rather than on its age. This invalidated Gamzoe's (1908) earlier conclusion that the rings on the scales were annual, and that the scales are formed in the third year of life in freshwater. Tesch (1928) determined that scales are formed at between body lengths of 16cm to 18cm, which may be attained at between two and six years

of life. Tesch recommended that ageing was best achieved by using both otoliths and scales, as otoliths in large eels were unsatisfactory for ageing because the rings could not be accurately counted. Frost (1945) found a variation in the number of scale rings on scales from different areas of the body and that the size of the scale bore no relationship to the number of rings.

In consideration of the inconsistencies that scales show Bertin (1956: 38) concluded that they were unsatisfactory for age determination. Sinha and Jones (1967a: 100) similarly concluded that scales of the European eel are not accurate indicators of age. Despite the conclusions by these authors, Voronin and Rusetskaya (1971) found scales satisfactory for ageing and for the back calculation of growth of eels in Russian waters.

Smith and Saunders (1955), studying the age and growth of the American eel, found that scales first formed in the caudal region of the body at between lengths of 16cm to 20cm during the third year of life in freshwater. By allowing for the growth not represented by rings on the scales, nine age groups were recognised. There was a variation in the number of rings on scales from the same area, so that the maximum number of ring was not always recognised.

Pantulu (1957) found that in the Indian eel A. bengalensis scales were first formed in the anal area at lengths of 6cm and age six months. Age was calculated from the number of rings on the scales but the number of rings varied on different scales taken from the same region of the body. The increasing number of rings was not always in proportion to the increasing size of the scale. Pantulu (1957: 273) concluded that the scales are satisfactory for ageing if some caution is exercised, but that they are not suitable for growth rate determinations.

It is clear from these studies on other species that the use of scales for ageing has many disadvantages and therefore MacFarlane's (1936) and Cairns' (1941) assertions that scales may be used to determine the age of New Zealand eels is treated with caution.

MacFarlane (1936: 46) established that in the longfin eel scales were first formed at a length of 23cm and in the eighth year of life, in a region along the lateral line in the posterior third of the body. In larger eels scales from this region showed the highest number and least variation in the number of scale rings.

In this study, to determine the body length at which scales are first formed, 20 eels of each species of lengths between 14cm and 25cm were examined. Eels were deslimed as described in the Materials

methods section and examined under a binocular microscope.

The smallest shortfin on which scales had formed was 16.5cm in total length. Scales were arranged in two rows of approximately five scales each along either side of the lateral line, three-quarters of the way along the body from the anterior end. The smallest longfin on which scales were observed was 19.0cm in total length. Scales were similarly arranged as two short rows on either side of the lateral line, threequarters of the way along the body. Two shortfin of 17.6cm total length had small areas of scales two thirds of the way along the body about the lateral line, indicating that the scales may have been first formed at a size slightly less than 16.5cm in total length. Longfin eels of a slightly larger size than 19.0cm total length were observed with scale areas that indicated scale formation also occurred at slightly smaller size than 19.0cm total length. Hence the body lengths of 16.5cm and 19.0cm as the size at scale formation for shortfin and longfin eels respectively is only an approximation. However, both sizes are less than the 23cm body length at the time of scale formation given by MacFarlane (1936).

The scales cover the whole body and are arranged obliquely or at right angles to one another, which gives the scale pattern the appearance of parquet flooring. The appearance and structure of a scale, shown in Fig.4.1, is identical to that described by Waly (1940). Briefly, each scale is oval and is composed of a number of platelets or loculi arranged in concentric rows. Zones of platelets are separated by a narrow band of clear fibrous tissue. The wide zones of platelets correspond to the period of scale growth. The narrow clear zone corresponds to the period of minimum scale growth.

In this study the number of clear narrow zones or growth checks were counted and compared with the total length of the eel and the number of growth checks on the otolith.

Scale samples were taken from the area of first scale formation. They were removed by scraping the skin of a deslimed eel with the blade of a scalpel and washing the scales in a small vial of 70% isopropyl alcohol mixed with a drop of 'Dettol' antiseptic solution. Scales were stored for up to two years with no apparent deterioration. Scale samples were taken from non-migrant and migrant of varying sizes from both species and sexes.

FIG.4.1

1. Scale from a 75.6cm total length
A. dieffenbachii (longfin) female
showing five scale rings. x 35
2. Sagittal otolith from a 6.2cm total
length A. australis (shortfin) glass
eel. x 130
3. Burnt otolith from a 40.0 total length
migrant A. australis male showing the
glass eel centre and 14 black (winter)
rings. x 25

a - annual rings

g - glass eel nucleus

1

2

3

Scales were examined under a binocular microscope at x40 using a transmitted light source. By altering the light intensity the rings on the scales could be counted. No staining was necessary to show the rings more clearly. The maximum number of rings occurring on the scales were counted. Scale shape and size varied considerably, from dumbbell or T-shapes to round scales. The scales with the maximum number of rings were the largest, and oval in shape. Some larger, less regular shaped scales possessed fewer rings. The lengths of the scales with the maximum number of scales were measured using a micrometer eyepiece and the measurements converted to mm.

Scale length - fish length relationship.

Part of the technique in the use of scales for age determination, as outlined by Lagler (1956), is to establish the relationship between scale growth and increasing body length. It is necessary to establish if the scale growth is in direct proportion to the increasing body length. Although this is more important if back calculations are to be made, for which eels scales are not suitable, it was considered of use to determine this relationship for the eels. It would show whether the scales continue to grow throughout the life of the eel, and by extrapolation could confirm the body size at the time of scale formation as previously observed.

To establish the relationship between total fish length and scale length Whitney and Carlander (1956) suggest that the best regression to use is that of body length on scale length, represented by the equation,

$$y = mx + c \dots\dots\dots (1)$$

where y is the fish length, x is scale length, m and c are constants and a, the intercept of the line on the y axis, may be interpreted as the fish length at the time of scale formation.

This formula can only be used if a linear relationship exists between body length and scale length. A scatter diagram of body length on scale length for both species showed that scale length increased in direct proportion to increasing body length. Equation (1) was therefore used to calculate the regression.

Regressions of total length on scale length were calculated by the least squares method. The equations for these calculated regressions are:

$$y = 20.82 + 17.25$$

The regression coefficient (r) = 0.91 is significant at the 5% level, and n , the number of observations = 49.

Longfin:

$$y = 25.53x + 18.77$$

where r = 0.94, significant at the 5% level, and

$$n = 45.$$

The regression equations are represented graphically in Fig.4.2.

In the longfin eel scale length increases at a slightly greater rate than in the shortfin. The intercept c on the y - axis for each species, 17.25 for shortfins and 18.77 for longfins, approximates the body length at which scales were observed to be first formed.

Therefore the intercept a can be interpreted as being the body length at which scales are first formed.

Number of scale rings to total body length relationship.

The mean fish length per number of scale rings was calculated for each species and is shown graphically in Fig.4.3. The number of scale rings increases in direct proportion to the increase in total length.

For analysis, a mean length was calculated from all fish within each scale group. Scale group *one* being all those eels with only one ring on the scales. The maximum number of scale rings recorded for otolith aged eels was nine for both species. The regression analysis, calculated by the least squares method, of the number of scale groups on mean total length gave the following equations, where y is the total length and x the number of scale rings.

Shortfin:

$$y = 7.8x + 21.56$$

Regression coefficient (r) = 0.97 is significant at the 5% level, and n , the maximum number of scale rings = 9.

Longfin:

$$y = 12.96x + 18.7$$

Regression coefficient (r) = 0.96, is significant at the 5% level, and n = 9.

Fig.4.3 shows that longfins are larger than shortfins at the time of ring formation.

FIG. 4.2.

Regression of total fish length (cm) on scale length (mm) of A. dieffenbachii (longfin) and A. australis (shortfin) eels.

FIG. 4.3

Regression of total fish length (cm)
on the number of scale rings.

Number of scale rings compared with the otolith calculated age

The number of scale rings per age group calculated from otolith readings is given in Table 4.1.

The smallest age groups include eels with the least number of scale rings. As age increases the number of scale rings increases until approximately age group 20 for shortfins and age group 26 for longfins. In higher age groups there is little increase in the number of scale rings. The maximum number of scale rings observed for either species was nine, which occurred in the largest eels.

It is established later in this section that otolith readings give valid indications of age. However, Table 4.1 shows that the number of scale rings bears little relationship to age based on otolith readings.

The number of scale rings does increase with increasing age in so much as increasing age does represent an increase in total length. But, as shown later, the length in any one age group is so variable that the number of scale rings bears no relationship to the age of the eel.

The analysis of scale length and number of scale rings in relation to body length has shown that both are dependent on length which is not an accurate indicator of age. Therefore, contrary to MacFarlane's (1936) and Cairns' (1941) conclusions, the scales of New Zealand eels are not considered to be suitable for age determinations.

4.3 Use of otoliths for ageing eels

Age determination by otoliths is based on the theory that checks in growth are reflected in the chemical composition of the otoliths. Dannevig (1956) and Irie (1960) established that the zones of different optical densities were composed of differing amounts of organic material. These zones exhibit different optical properties depending on the light source used. When viewed under reflected light the zones appear as alternating wide, white opaque, and narrow, black transparent zones. Under transmitted light each opaque zone appears as a dark zone. The dark transparent or hyaline zone seen under reflected light becomes a light transparent zone under transmitted light. For many species of fish confusion has arisen over the interpretation of these zones, as several authors have not defined the type of light source used. For the eel otolith however there is

TABLE 4.1

Mean number of scale rings per age group

Age Group (by otolith readings)	<u>A. australis</u>	<u>A. dieffenbachii</u>
	Mean number of scale rings	Mean number of scale rings
5	1.5	
6	2.3	0
7	3	1
8	2	
9	4	2
10	3	1
11	4.5	1
12	5	3
13	5	3
14	4	3.5
15	4	2.5
16		2.5
17	5.7	3
18	6.5	3
19	6	3
20	6	3.3
21	5.5	3
22		3.5
23	6	4
24	7	4
25	9	5
26	7	5
27		
28		6
29	9	
30		6
31		
32		6
33		6
34		5
35		7
36		5
37		
38		9
41		5
47		5

no confusion over the interpretation of the white opaque and transparent hyaline zones as all researchers have used reflected light.

For the European eel it has been established and confirmed by many authors, including Hornyold (1922), Frost (1945) and Sinha and Jones (1967a) that the white opaque zone represents the period of summer growth and the narrow, black hyaline zone the period of winter growth.

The zones in the otoliths of New Zealand eels are similar in appearance to those of the European eel. MacFarlane (1936) established that in the longfin eel the alternating opaque and hyaline zones corresponded respectively to periods of summer and winter growth. Based on this interpretation of the otolith, Cairns (1941) aged eels and Burnet (1969b), although not stated, presumably used the same interpretation of otolith zonation in small eels. However Burnet (1955) earlier expressed reservations about the use of otoliths for ageing.

Preparation of otoliths for age determination

The sagittal otolith was used for age determination. The sagittal otolith is the largest of the three otoliths occurring in the labyrinth, although in eels it is small compared with the otoliths of other fish. In size the sagittal otolith varies from approximately 1.8mm long and 1.2mm wide in a 22cm eel, to 7.0mm long and 4.5mm in width in a 132 cm longfin female.

The otolith is white, almost oval, concave medially and convex laterally. On the convex side is a groove, the sulcus. The anterior end of each otolith has two projections, the rostrum and antirostrum. These are more prominent in larger otoliths.

Otoliths were removed by making a sagittal incision along the length of the head with a heavy bladed knife. The two halves of the cranium were then separated and the otoliths removed from the saccular cavities in the basal region of the cranium with forceps. They were stored in numbered vials in a solution of 70% isopropyl alcohol in which a drop of antiseptic solution ('Dettol') had been added to prevent fungal growth. Scales from the same eel, as described previously, were stored in the same vial. Otoliths were not stored dry, as is normal for many species, because of their small size and because they are easily lost.

Otoliths from eels other than glass eels and elvers, when viewed under reflected light, do not show the alternating opaque

and transparent zones and need some preparation to make these zones visible. Various techniques of preparing the otoliths by grinding have been described for the European eel (Hornoyold 1922; Frost 1945; Sinha and Jones 1967a), the American eel (Gray and Andrews 1971) and New Zealand eels (Cairns 1941). This general method is satisfactory for eels less than 35cm to 40cm, as suggested by Frost (1945: 31), but for larger eels the otoliths are more or less readable. Deelder (1971) considers that it is very difficult or even impossible to age eels over 10 years old by the otolith.

The various grinding techniques outlined by the above mentioned authors for preparing otoliths were tried, in particular the 'acid-contact method' described by Sinha and Jones (1967a: 101). However, all methods of grinding otoliths were found unsatisfactory for eels over 30cm and no accurate assessment of the number of rings could be made. The otoliths broke easily when grinding, particularly the large more convex ones from big eels, and the shape of the otolith meant that it was difficult to grind it down to a uniform thickness.

Other methods of grinding were tried to prevent breakage. Otoliths were embedded in a clear polyester resin and sectioned transversely and longitudinally with a fine toothed saw. The cut exposed surfaces were then ground but the zones were no clearer. Opuszynskii (1965) describes a method of embedding the otolith in a drop of Canada balsam on a glass slide and heating the slide until the balsam thickens, firmly attaching the otolith to the slide. The slide was then turned over and the otolith ground. This method was tried but found unsatisfactory. The zones were no clearer and the method was time consuming, unsuitable for a large number of otoliths.

Wiedeman Smith (1968) describes a method of examining otoliths by surface microscopy, and satisfactory results were obtained for the European eel. However, the otolith needs considerable preparation involving embedding, cutting and polishing which would be too time consuming. Therefore this method would also be unsuitable for examining a large number of otoliths.

Christensen (1964) describes a method for age determination of the sole Solea solea L. involving burning and breaking the otoliths which reveals a series of alternating narrow black and broad white rings. The black zones, as Christensen describes, appeared between the transparent and opaque zones seen in whole otoliths under

reflected light. As previously mentioned, it has been established by Dannevig (1956) and Irie (1960) that zones or rings in the otoliths are caused by the different proportions of calcium carbonate and protein or organic matter layed down during the different growth periods. However, as outlined by Elder (1972), there is much confusion in the literature as to the correct interpretation of these rings. Dannevig (1956) considered the black rings correspond to the opaque zones, the period of summer growth in the cod otolith. Blacker (1969) however found that the organic matter forming the black ring in burnt otoliths corresponds to the black, transparent hyaline zone seen under reflected light. Christensen (1964: 81) concluded that the black rings indicated a growth check and the white rings the period of growth. He found the burning technique also proved successful on the European eel otolith.

In this study it is established that the black rings correspond to the dark transparent winter zone and the broad white rings represent the white opaque summer zones.

Various techniques of burning otoliths were tried before a suitable method was selected. The exposed surface of the embedded otoliths that were used for grinding were heated in a bunsen flame. The black and white rings were visible but the embedding resin was not heat resistant. The technique described by Christensen (1964: 73) was also tried, that is breaking the otolith after it has been burnt. This technique, although found suitable for the European eel, was not found satisfactory. The burnt otoliths broke into many small fragments which were difficult to handle. It was also difficult to break the otolith through the nucleus and no one radius, from the nucleus to the outer edge, could be used consistently with each otolith for age determination. Moriarty (1973) describes a burning technique, similar to that of Christensen (1964), for otoliths of the European eel, which involves embedding the burnt otolith in a clear resin through which the rings can be observed. The otoliths can be stored and conveniently read at a later date. The burning technique used in this study was developed before Moriarty (1973) published. For future research a combination of the method described here and Moriarty's (1973) would be most suitable.

For this study the most satisfactory method of preparing otoliths was to break the otoliths in half, transversely, before burning. The otoliths were broken by placing them convex side uppermost between the folds of a small (2cm x 2cm) piece of clear thin plastic, and pressing across the centre of the otolith in a transverse direction with the edge of a scalpel/blade. In most cases a clean break was obtained and the plastic covering prevented the otolith halves from scattering as the otolith was broken. Both sagittal otoliths from each eel were used, and the broken halves were stored in a plastic dish, 15cm x 6cm, with 30 small numbered compartments, one per eel. In this manner a large number of otolith halves could be handled without confusion.

The otoliths halves were burnt by holding the broken edge against the edge of an incandescent bunsen flame until the broken edge turned a light brown. The correct amount of heating was critical for later reading and it was easy to overburn the otoliths, particularly the small ones. Otoliths were not heated to an ash grey as described by Christensen (1964: 73) because they crumbled easily and the zones became less distinct. After burning the otolith half, the burned edge uppermost, was embedded in a piece of plasticine, placed in the bottom of a small petrie dish, and viewed in a bath of beechwood creosote oil under a binocular microscope at x400. A reflected light source was used. All four halves were examined in this manner. Before viewing the otolith in oil, embedding it in a resin as suggested by Moriarty (1973) would be useful so that the otolith could be easily re-read.

Interpretation of the otolith rings

By comparing burnt and ground otoliths from smaller eels it was found that the black rings corresponded to the dark, hyaline winter zones in ground otoliths, and the broad white zone to the opaque white zone.

Along the two shortest radii across the thinnest part of the otolith through the nucleus, and along the shorter transverse radius, the black rings were close together and difficult to distinguish. The rings were best observed along the longest transverse radius, as shown in Fig.4.1. It was difficult to photograph an otolith that showed all the rings clearly without refocusing.

Therefore only one burnt otolith is illustrated.

Along the longest radius the black winter rings were spaced apart nearer to the nucleus but on the outer edge they were more closely spaced. In the centre of the largest transverse radial region the black rings tended to split, but nearer to the lateral edge the rings were more distinguishable.

Age determinations were made by counting the number of black winter rings along the longest transverse radius.

The otoliths of the smallest eels, the shorter longfin and shortfin non-migrants below 45cm, and the migrant shortfin males, were generally easy to read. In otoliths from larger eels the rings were crowded together in the outer half of the otolith and were difficult to count. All four otolith halves were read in these instances and the readings averaged to obtain the age. In a few otoliths a variation of up to six rings was found between the four readings so the accuracy of the age determination was plus or minus three years in some eels.

In order to establish the reliability and variation in the age determination all otoliths were assigned a readability figure based on a five figure scale. The scale is purely subjective and is based on the ease with which the rings could be distinguished and counted, and the variability that was found between the counts from each otolith half.

Readability scale:

1. Unreadable. Rings too closely spaced to count.
Multiple rings present.
2. Difficult to read. Rings crowded and indistinguishable near outer edge. Variation of ± 3 rings between otolith halves.
3. Readable. All rings distinguishable but near to edge crowded and less distinct. Variation of up to 1 ring between otolith halves.
4. Reasonably easy to read. All rings distinguishable. Variation of up to ± 1 ring between otolith halves.
5. Very easy to read. All rings easily distinguishable. No variation in number of rings between otolith halves.

For eels from different areas it was necessary to read a number of otoliths before the ring pattern for that area could be established. The otoliths for each species from different areas were of similar readability. However, some difference in readability was found between species. As previously mentioned, smaller non-migrant and migrant shortfin males were easiest to read, and were placed in categories four and five of the readability scale. The larger, older migrant longfin males and females were most difficult to read, falling into categories one and two of the scale. In general for both species readability decreased with increasing age.

The results of the readability allocations for each species are given in Table 4.2. Eels from different areas, and migrant and non-migrant eels are combined. Table 4.2 shows that a higher percentage of longfin age determinations were placed in the first two categories. A slightly higher percentage of age determinations for shortfins were placed in category three. In categories four and five the percentage of age determinations for both species is similar. Overall, 60% of the age determinations for both species fall into categories three and four, that is readable and easy to read, and a variation of up to $\pm$ two winter rings, although 70% of these were found to be $\pm$ one ring.

Considering the great range of ages attained by eels and the variability of ages in eels of the same length, the variation of up to $\pm$ two winter rings in 60% of the age determinations, although taken into account later, is not considered greatly significant. Nevertheless, the variability in age determinations shows that the

age is only an approximation

TABLE 4.2

Readability Scale

Percentage of otoliths in each readability category

Scale	<u>A. australis</u>	<u>A. dieffenbachii</u>
1	3%	11%
2	16%	18%
3	55%	48%
4	16%	14%
5	10%	9%

the otolith as having two dark opaque rings or seawater rings. I presume one of these dark rings refers to the black otolith centre. Sinha and Jones (1967a: 103) describe the freshly arrived elver otolith as having, under reflected light, a black centre sometimes containing a white spot, surrounded by a white ring in turn surrounded by a thin black ring. The latter ring may be incomplete or absent. Surrounding the black ring is a thick white ring, and between this and the outer edge of the otolith is a clear area. Bertin (1956: 41) states that the elver (glass eel) otolith of the European eel represents a sea life of three years. MacFarlane (1936) and Cairns (1941: 568) interpret Hornyold's (1922) description of elver otolith as representing a sea life of only two years, and apply this assumption to New Zealand eels. However, neither author gives a description of the otoliths of freshly arrived glass eels.

To determine the correct appearance of the glass eel otolith several glass eels were obtained from Mr.D.J.Jellyman. The sagittal otoliths from five shortfin (size range 5.2cm to 6.2cm) and five longfin glass eels (size range 6.0cm to 5.2cm) were dissected out from the caranium, cleared in beechwood creosote oil, and viewed under a binocular microscope against a black background. A reflected light source was used.

The glass eel otolith is identical in appearance for both species and is shown in Fig.4.1. A black centre, in some otoliths containing a white centre spot, measures approximately 1.7mm in diameter and is surrounded by an opaque white ring approximately 0.7mm in width. On the edge of the otolith and surrounding the white ring is a thin transparent or hyaline ring approximately 0.4mm wide. Based on the interpretation of the thin, black, hyaline winter rings, and opaque,white, summer rings in adult otoliths, the glass eel otolith shows a centre formed in winter surrounded by a summer ring, in turn surrounded by a narrow white ring. The dark centre may contain a summer white spot. This is one black, hyaline ring less than is usually present in the European elver otolith as described by Sinha and Jones (1967a: 103) and drawn by Bertin (1956: 40).

In burnt adult otoliths the glass eel otolith, or nucleus as it is called, appears as a black centre zone surrounded by a broad white ring (Fig.4.1). Surrounding this is a narrow black ring. Around this last ring is the first white zone or summer ring formed in the first summer of freshwater life immediately after entry into freshwater. This zone is always very wide, indicating rapid growth

during the first summer period. During the succeeding first winter in freshwater a narrow black ring is formed. The black winter rings in burnt otoliths were easier to count than the broader white summer rings. Age determination was calculated from the first winter ring formed in freshwater. The complete formation of this winter ring and the beginning of the second summer ring in freshwater represents the first complete year of freshwater life.

In accordance with most studies, including those of Ehrenbaum and Maikawa (1913), Frost (1945) and Sinha and Jones (1967a), investigating the age and growth of the European eel, and Gray and Andrews (1971) the American eel, the sea life is not included in the age determination. The age therefore refers to the time spent in freshwater and not total life.

The assumption by MacFarlane (1936) and Cairns (1941) that the otolith of the New Zealand glass eel is the same as the European eel and represents a sea life of two years is incorrect if Bertin's (1956: 41) sea life of three years is accepted.

New Zealand eels migrate out to sea to spawn during the autumn as does the European eel. Considering the similarities of the basic biology of temperate species of eels as outlined by Bertin (1956) it is reasonable to assume that New Zealand eels spawn in the spring and summer as does the European eel. The white centre spot and the dark centre of the New Zealand glass eel otolith probably represent the first spring, summer and winter periods of sea life, depending on the time of spawning. The white spot seen in the centre of some glass eel otoliths may be a spring or summer spot. The opaque white ring represents the first complete summer at sea, and the surrounding dark ring (black in burnt otoliths) represents the second winter of sea life. At this point, the end of winter and early spring, from October to December, the glass eels enter freshwater (Cairns 1941: 62B). Therefore the glass eel otolith represents a probable sea life of slightly under two years, with a minimum of 18 months if it was spawned in summer. The proposed sea life based on the interpretation of the glass eel otolith is summarised below.

<u>Glass eel</u>	White spot and	Opaque ring	Black trans-	Entry
<u>Zones</u>	dark centre		parent ring.	into
<u>Season</u>	Part of spring,			fresh-
	summer and	First complete	Second	water
	winter	summer at sea if	winter of	spring
		spawned during	sea life	
		preceding		
		summer		

Thus MacFarlane's (1936) and Cairns' (1941) assumption of a sea life of two years is essentially correct, but is based on an incorrect comparison with the European eel.

Time of formation of rings and validity of ageing technique

It was difficult to establish the time of formation of the winter and summer rings as they were not distinguishable on the edge of the otolith when they were first formed. A thin black ring was present on the outer edge of the otolith at all times and was not counted as an annual ring. This ring appeared to be a carbonized outer coating of the otolith. On the few easily read otoliths it was observed that the broad white summer zone on the edge of the otolith appeared widest in May, the beginning of winter. The exact time of formation of the next black winter ring could not be determined, but it appeared closest to the edge of the otolith in October, indicating that the winter ring forms during the winter between May and October. Jellyman (pers. comm.) examined the otoliths from smaller eels below 20cm in length and determined that the winter ring is formed in September or October. No other black growth checks were observed forming at any other time of the year, so it is reasonable to assume that in eels of all sizes the black rings represent annual winter growth checks. The growth rates shown later in this section compare favourably with those calculated by Burnet (1969b) based on tagging returns. Hence, despite Burnet's (1955) reservations on the use of otoliths for ageing, the technique given in this study for the age determination is considered valid.

Similar difficulty in determining the exact time of formation of the rings for the European eel is described by Frost (1945: 30) and Sinha and Jones (1967a). Both authors found that the rings were not easily recognised when first formed. Gray and Andrews (1971: 22), for the American eel, established that the opaque ring formed in summer during June, but the exact time of formation of the transparent ring could not be determined, although it definitely formed in winter.

Multiple rings and false rings

As previously mentioned, the winter rings often split in

the centre of the longest lateral radial region of the otolith and the rings were more easily distinguished towards the lateral edge. In some otoliths the rings were split in all areas. These otoliths were classed as unreadable. A pattern of ring formation could be established which was similar for eels of each species from any one area. The general spacing of the rings was important and any irregularly spaced black rings were called false rings and not counted. Despite the variation in readings for the same eel as previously described, the exclusion of false rings removed one source of error.

Definition of age groups

As stated, age determinations were made by counting the number of black winter rings formed in freshwater. The freshly arrived glass eel has an outer black ring on the outer edge of the otolith, the succeeding first summers growth in freshwater is represented by a broad white zone. Complete formation of the first winter ring in freshwater was recognized as the completion of the first year of life in freshwater. By accepted nomenclature the first year of life in freshwater was classified as age group 0. After complete formation of the second winter ring the eel is classified as belonging to age group 1. In this classification the sea life is incorporated into age group 0. The practice of assigning the first year of freshwater life to age group 0 is used by Frost (1945) and Sinha and Jones (1967) for the European eel. Arabic numerals are used for age group numbers in this study instead of Roman numerals as used by European authors.

Classification of the eels into age groups and incorporation of the sea life into age group 0 is different to the age groupings used by MacFarlane (1936), Cairns (1941) and Burnet (1969b). All three authors use age in years as their groupings, and MacFarlane (1936) and Cairns (1941) clearly designate ages one and two as the period of sea life. By their classification an eel that has completed its first year in freshwater is three years old. In this study it is only one year old and belongs to age group 0. To make a direct comparison of this study with MacFarlane (1936), Cairns (1941) and Burnet (1969b) it is necessary to subtract three from the ages given by them.

Some variation in the definition of age groups occurs in the European literature as discussed by Frost (1945: 29-31) and Sinha and Jones (1967a: 102-105). Frost aged eels by counting the winter rings and assigned eels caught between the beginning of each spring, defined as May 1, as belonging to one particular age group. An eel caught before May 1, in March or April, with eleven winter zones would still be in its tenth year of life and is placed in age group 10. If caught after May 1, in June or July, and still having eleven winter zones, it would be in its eleventh year of life and be placed in age group 11. May 1 was selected as the 'birth date' as it is the general time of arrival of the European elvers into freshwater.

In contrast Sinha and Jones (1967a: 103-4) calculated ages from the first summer ring formed in freshwater. The completion of the second summer ring completed the first year in freshwater, and the eel was assigned to age group 0. Marcus (1919) assigned eels to new age classes when a new winter ring was formed, but the classification of age groups is the same as Sinha and Jones (1967a). This is the same method as used in this study, the completion of a new winter ring places the eel in the next age group.

Sinha and Jones (1967a: 104) note that the time of immigration of the European elver varies in different countries, and it is advisable to use the number of otolith rings when assigning eels to different age groups, rather than select a general date for glass eel entry into freshwater as does Frost (1945).

This is applicable to New Zealand eels. The time of entry into freshwater may vary from July to October (Jellyman pers. comm.) and as the age determinations of many eels over approximately 30cm can be calculated with an accuracy of only ± 1 year or more, therefore the allocation of a birth date such as Frost (1945) uses is unnecessary.

4.4 Age and length relationship of non-migrant eels

From otolith readings a total of 318 non-migrant shortfin and 228 non-migrant longfin eels were aged from the Makara stream. Shortfin eels ranged in total length from 22.5cm to 85.1cm, and in age from age group 3 to 29. Eels under 20cm were not aged.

Longfins ranged in size from 21.5cm to 106.0cm and age from age groups 5 to 36.

Three sex categories were recognised:

1. Definite females with recognisable developing or developed ovaries (gonad stages 6 and 7 as previously described).
2. Males with definite lobulate organs (gonad stages 3, 4 and 5).
3. Undifferentiated eels, those with gonads unrecognisable as either male or female (gonad stages 1 and 2).

Table 4.3 shows the number of eels aged belonging to each sex category and species.

The age and length data for each species was first sorted into length for age, with the sex category for each eel noted with its length. It was immediately apparent that the largest eels in each age group, particularly the lower age groups, were females. The smallest eels in each age group, in particular in the higher age groups, were undifferentiated eels. This was more apparent in the shortfins than in the longfins. Because of this obvious difference in the lengths of eels from different sex categories within the same age group, the data for each sex category was analyzed separately. From this it was clear that eels of each sex category, although in the same environment, showed different rates of growth.

The range in length within each age group for either sex category varied greatly. Some age groups, as shown in Tables 4.4 to 4.9, have a length range of up to 20cm. Hence, within each sex category for each species, eels of the same sex show different rates of growth. The length range for each successive age group shows considerable overlap. An undifferentiated shortfin eel of length 40cm could be in age groups 7 to 18, a male of the same length could belong to age groups 6 to 17, and a female of 40cm could be in age groups 6 to 14. An undifferentiated longfin eel of 40cm could be in age groups 10 to 16, a male of 40cm from age groups 5 to 14. No longfin females of 40cm were aged, but a 50cm longfin female could be in age groups 11 to 17. Thus eels of either sex category within each species and having the same length can vary in age by up to nine years.

TABLE 4.3

Number of non-migrant and migrant eels aged

	Undifferentiated	Male	Female
<u>A. australis</u>			
non-migrant	119	53	146
migrant	--	152	116
<u>A. dieffenbachii</u>			
non-migrant	98	48	74
migrant	--	173	23

From the extreme variability in the age of eels within each sex category, and the size overlap of length range within each age group, it is clear that length is an unsatisfactory indicator of age.

Sinha and Jones (1967a: 105) found a similar but less extreme variability in the age-length relationship for the European eel. For this reason they analysed the age and length data in two ways. Firstly, length for age where the mean length for each age group was calculated, and secondly age for length, where the mean age for each 2cm length group was determined. Frost (1945: 106) interpreted her ageing data for the European eel in a similar manner but for different reasons. She regarded the length for age analysis as the best expression of the data because age determinations are only estimates, whereas length measurements are constants. Using this method she concluded that age determinations could be made when only length data was available.

While it is true that age is only an approximation, at least for New Zealand eels, data on the age and length for other species is usually presented as length for age only. For the New Zealand eels Cairns (1941) and Burnet (1969b), and for the American eel, Gray and Andrews (1971), analyse their data in this manner. Frosts's (1945: 106) contention that age can be calculated from length data is not tenable for New Zealand eels because of the range of age an eel of one particular length may have.

This section on age and growth is not presented as a detailed age and growth study, and is given only to show the differences in age and growth between the sex categories. The length for age and the weight for age data is sufficient to demonstrate these differences, therefore age for length analyses are not given.

Length for age

Shortfin

The growth curves for each sex category calculated from the length for age data is given in Fig.4.4. The length range and standard deviation of each age group are not included on the graph because of the confusion of lines that would result. Tables 4.4, 4.5 and 4.6 give the mean length, length range and standard deviation for each age group of each sex category.

FIG. 4.4

Age - length relationship of the undifferentiated, male and female, A. australis (shortfin) and A. dieffenbachii (longfin) eels from the Makara stream.

TABLE 4.4

Mean length (cm), range in length (cm), standard deviation, mean weight (g), range in weight (g) and standard deviation of eels of different age groups.

A. australis undifferentiated eels - Makara stream.

Age group	3	4	5	6	7	8	9	10
Mean length (cm)	24.4	25.9	27.9	27.5	32.8	33.1	34.3	36.3
Length range (cm)		23.7- 29.6	22.6- 37.2	22.5- 34.3	25.5- 43.7	23.3- 42.6	26.2- 39.9	27.3 49.1
Standard deviation		3.17	5.26	3.89	5.11	5.28	3.81	5.98
Mean weight (g)	20	31	44	44.16	72.5	78.3	77.9	101.3
Weight range (g)		25- 38	14- 110	18- 80	25- 175	19- 145	40- 125	35- 200
Standard deviation		6.6	31.7	18.5	39.9	40.6	27.7	50.4
No. eels	1	3	8	11	13	13	20	15

A. australis undifferentiated eels - Makara stream

Age group	11	12	13	14	15	16	17	18
Mean length (cm)	37.2	37.3	38.7	32.7	38.5	35.6	43.6	42.9
Length range (cm)	31.2- 44.8	30.9- 43.9	31.5- 44.5	31.5- 34	37.1- 40.0			
Standard deviation	2.67	3.76	4.73	1.76	2.05			
Mean weight (g)	109.6	118.6	117.0	65	120.0	95	163	170
Weight range (g)	52- 150	68- 215	60- 175	60- 70	100 140			
Standard deviation	28.4	47.0	42.8	7.10	28.3			
No. eels	12	11	5	2	2	1	1	1

TABLE 4.5

Mean length (cm), range in length (cm), standard deviation, mean weight (g), range in weight (g), standard deviation and calculated weight (g) of eels of different age groups.

<u>A. australis</u> non-migrant males - Makara stream							
Age group	5	6	7	8	9	10	11
Mean length (cm)	37.9	41.7	36.6	38.1	38.5	39.8	40.2
Length range (cm)	37.4- 38.5	40.8- 44.8	28.6 43.8	35.8 41.1	32.8 43.5	29.4- 45.3	34.4 42.8
Standard deviation	0.77	2.80	5.78	2.21	3.81	5.2	2.95
Mean weight (g)	95	153.3	114.3	103.7	118.3	139.8	132.0
Weight range (g)	75- 115	110- 212	75- 155	95- 115	55- 175	35- 278	70- 162
Standard deviation	28.3	52.7	38.3	8.5	40.3	63.19	38.5
Calculated weight (g)	104.3	141.1	93.5	105.2	109.6	121.7	124.6
No. eels	2	3	5	4	7	10	7

<u>A. australis</u> non-migrant males - Makara stream						
Age group	12	13	14	15	16	17
Mean length (cm)	41.3	45.5	48.7	49.3	43.5	44.7
Length range (cm)	37.2- 43.9	42.6- 50.5	40.7- 56.9	48- 50.6	41- 46	
Standard deviation	3.18	4.37	8.10	1.84	3.54	
Mean weight (g)	141.3	201.7	231.7	242.5	154	200
Weight range (g)	105- 175	150- 265	130- 350	210- 275	120- 188	
Standard deviation	30.4	58.4	110.9	46.0	48.1	
Calculated weight (g)	136.8	185.0	229.0	281.4	161.0	175.3
No. eels	4	3	3	2	2	1

TABLE 4.6

Mean length (cm), range in length (cm), standard deviation, mean weight (g), range in weight (g), standard deviation and calculated weight (g) of eels of different age groups

A. australis non-migrant females - Makara stream

Age group	6	7	8	9	10	11	12	13
Mean length (cm)	46.5	42.1	39.0	43.1	46.4	46.6	48.9	50.44
Length range (cm)	40.6- 52.5	32.8- 51.9	32.6- 46.7	35.8- 49.5	37.7- 54	39.6- 65.1	36.3- 60.8	38.3- 65.6
Standard deviation	8.41	8.13	5.71	5.07	5.77	6.37	6.95	8.28
Mean weight(g)		142.8	130.0	180.1	256.2	307.2	283.9	289.8
Weight range (g)		75- 212	70- 225	90- 310	120- 390	135- 990	90- 575	110- 575
Standard deviation		60.1	64.2	76.0	97.7	264.2	136.1	146.6
Calculated weight (g)	199.3	145.5	114.2	156.7	197.5	199.8	231.9	256.1
No. eels	2	6	5	8	10	13	14	18

A. australis non-migrant females - Makara stream

Age Group	14	15	16	17	18	19	20	21
Mean length (cm)	53.9	54.9	54.9	53.0	54.9	50.8	57.1	64.1
Length range (cm)	39.8- 69.4	44.1- 63.3	53.7- 56.2	42.3- 64.2	42.1- 63.5	43.7- 64.8		58.3- 72.4
Standard deviation	8.33	8.15	1.25	7.43	9.95	8.43		6.1
Mean weight (g)	390	399.8	403.3	352.9	351.7	321.2	400	635.5
Weight range (g)	125- 725	165- 640	380- 482	164- 600	180- 565	160- 625		455- 925
Standard deviation	181.2	180.53	38.7	151.2	195.8	210.8	378.3	541.7
Calculated weight(g)	315.7	333.8	336.4	299.4	334.0	262.5	378.3	541.7
No. eels	16	8	3	11	4	5	1	4

A. australis non-migrant females - Makara stream

Age group	22	23	24	25	26	27	28	29
Mean length (cm)	68.7	67.8	77.1	81.5	72.9	75.6	83.5	76.4
Length range (cm)		57.3- 76.8	70.8- 81.4		66.9- 77.6			67.7- 85.1
Standard deviation		8.3	4.55		5.52			12.3
Mean weight (g)	780	694.3	996.2	1110	869.5	1050	1275	1105
Weight range (g)		360- 1012	700- 1175		628- 1050			700- 1510
Standard deviation		269.2	210.29		193.9			572.8
Calculated weight (g)	676.9	650.4	971.2	1158	815.1	913.9	1249	944.8
No. eels	1	4	4	1	4	1	1	2

Fig.4.4 shows that the undifferentiated eels have the slowest growth rate, and there is a fairly constant increment in growth up to age group 13. Age groups 14 to 18 show some fluctuation in mean sizes, but this is probably due to the small numbers of eels in these age groups. Male eels become sexually differentiated in age group 5. After an initial faster increase in length than the undifferentiated eels the growth increment for males is fairly constant until age group 12. From age groups 13 to 17 some fluctuation in the growth increment occurs, probably due to small sample size (Table 4.5).

Female eels become sexually differentiated in age group 6 at a slightly greater age than the males. Females show a faster growth rate than the undifferentiated and male eels. From age group 8 to 19 female eels have a fairly constant increment in growth after which time the growth increment increases rapidly. The significance of this is discussed later.

Tables 4.4, 4.5 and 4.6 show that the length range within the same age group for undifferentiated and male eels is similar. Females in the same age groups have a similar minimum length to the undifferentiated and male eels, but the maximum length is always much greater. However, despite the similar length ranges within each age group for each sex category the mean lengths are clearly different in most age groups.

Longfin

The growth curves for each sex category, calculated from the length for age data, is shown in Fig.4.4. As with the shortfin graph, the length range and standard deviation for each age group are not included on the graph. Tables 4.7, 4.8 and 4.9 give the mean length, length range and standard deviation for each age group of each sex category.

Fig.4.4 shows that the undifferentiated eels have a constant increase in growth to age group 18, after which some fluctuation occurs. Male eels show some fluctuation in growth increments throughout the growth curve, in some cases showing slower growth than the undifferentiated eels. Overall, for all but two age groups, the mean lengths of males are larger than for undifferentiated eels of the same age group. Therefore, although less distinct than the shortfin males, the longfin males do have a faster growth than the undifferentiated eels. Females also show some fluctuation in

TABLE 4.7

Mean length (cm), range in length (cm), standard deviation, mean weight (g), range in weight (g), and standard deviation of eels of different age groups.

A. dieffenbachii undifferentiated eels - Makara stream

Age group	5.....7	8	9	10	11	12	
Mean length (cm)	25.6	30.0	32.1	33.8	36.6	34.8	39.6
Length range (cm)	21.5- 31.3	27.9- 35.7	28.0- 35.0	25.7- 39.2	31.1- 43.3	31.8- 38.0	31.4- 50.3
Standard deviation	3.68	3.2	2.63	4.68	3.73	2.44	6.84
Mean weight(g)	25.2	62.0	73.8	103.2	125.1	92.1	173.7
Weight range (g)	15- 35	49- 105	48- 130	30- 165	65- 215	60- 120	70- 355
Standard deviation	9.53	24.11	31.37	45.9	48.5	20.0	112.3
No. eels	5	5	6	9	7	6	9

A. dieffenbachii undifferentiated eels - Makara stream

Age group	13	14	15	16	17	18	19
Mean length (cm)	43.0	45.2	47.7	46.6	50.3	55.4	52.0
Length range (cm)	35.0- 54.7	31.8- 59.4	37.0- 56.7	33.5- 58.8	48.4- 52.3	48.6- 64.0	51.1- 52.9
Standard deviation	5.82	8.17	9.11	12.67	2.75	6.37	1.27
Mean weight (g)	225.6	257.6	310.5	356.2	373.4	459.1	397.3
Weight range (g)	110- 420	77- 650	120- 562	80- 575	272- 435	312- 675	332- 460
Standard deviation	106.5	172.6	192.5	220.4	69.76	144.4	64.0
No. eels	11	10	4	3	2	5	2

A. dieffenbachii undifferentiated eels - Makara stream

Age group	20	21	22	23	24	25	26
Mean length (cm)	50.4	56.4	53.6	54.3	44.6		59.1
Length range (cm)	41.4- 55.9	49.2- 59.5					
Standard deviation	5.35	4.85					
Mean weight (g)	343.5	510.7	385	400	305		625
Weight range (g)	180- 475	331- 650					
Standard deviation	123.5	134.7					
No. eels	6	4	1	1	1		1

TABLE 4.8

Mean length (cm), range in length (cm), standard deviation, mean weight (g), range in weight (g), standard deviation and calculated weight (g) of eels of different age groups.

A. dieffenbachii - non-migrant males - Makara stream

Age Group	12.....14	15	16	17	18	19	20	
Mean length (cm)	44.7	46.4	53.0	53.3	54.1	53.5	54.7	56.6
Length range (cm)	33.8- 61.6	39.6- 55.0	50.5- 55.5	51.1- 55.5	45.4- 67.2	47.6- 61.0	53.2- 56.1	50.0- 63.4
Standard deviation	12.46	6.77	3.53	3.11	8.56	4.5	1.45	6.7
Mean weight (g)	278	326.6	409	410	524.8	442.1	432.3	609.3
Weight range (g)	75- 625	175- 420	335- 483	400- 420	225- 975	285- 650	382- 475	328- 775
Standard deviation	216.0	132.5	104.6	?	307.1	135.6	46.9	244.9
Calculated weight(g)	223.4	251.9	387.7	394.7	414.5	400.1	429.1	478.9
No. eels	4	5	2	2	6	8	3	3

A. dieffenbachii - non-migrant males - Makara stream

Age Group	21	22	23	24	25	26	27	28
Mean length (cm)	54.4	60.7	60.7	64.7	59.7	61.1	63.0	51.7
Length range (cm)	53.3- 56.3	52.0- 66.1	60.2- 61.2		59.4- 60.1			
Standard deviation	1.62	6.26	0.7		0.49			
Mean weight (g)	390.0	660	656	900	612.5	675	775	262
Weight range (g)	335- 415	395- 950	625- 687		575- 650			
Standard deviation	47.69	244.9	43.8		53.0			
Calculated weight (g)	421.6	600.2	600.2	737.2	569.0	612.8	676.4	357.8
No. eels	3	4	2	1	2	1	1	1

TABLE 4.9

Mean length (cm), range in length (cm), standard deviation, mean weight (g), range in weight (g), standard deviation and calculated weight of eels of different age groups

<u>A. dieffenbachii</u> non-migrant females - Makara stream								
Age group	10	11	12	13	14	15	16	17
Mean length (cm)	47.4	54.0	51.2	55.8	62.2	53.2	54.9	52.7
Length range (cm)			42.0- 60.4	53.6- 59.2	57.2- 67.2		51.0- 59.7	47.4- 58.5
Standard deviation			13.01	2.96	7.07		3.65	4.50
Mean weight (g)	260	446	370	431	550	378	408.3	390
Weight range (g)			190- 550	372- 490	450- 550		340- 500	255- 505
Standard deviation			254.5	83.4			82.5	126.1
Calculated weight (g)	270.5	411.6	346.8	458.1	649.4	392.2	436.2	381.1
No. eels	1	1	2	3	2	1	4	5
<u>A. dieffenbachii</u> non-migrant females - Makara stream								
Age group	18	19	20	21	22	23	24	25
Mean length (cm)	57.3	55.8	58.7	63.7	63.6	59.7	68.3	66.8
Length range (cm)	51.8- 63.0	52.5- 61.0	50.2- 64.9	58.1- 72.3	52- 72.2	53.7- 65.8	61.8- 75.0	55.4- 79.0
Standard deviation	3.68	3.4	5.64	5.79	9.46	8.55	4.69	8.93
Mean weight (g)	475	477.5	608.5	785.3	995.0	880	937.5	887.8
Weight range (g)	380- 570	345- 700	490- 775	570- 1150	835- 1075	760- 1000	720- 1225	405- 1350
Standard deviation	67.2	154.6	174.9	226.6	138.5	169.7	211.2	374.0
Calculated weight (g)	497.5	458.1	537.4	701.5	696.3	571.1	878.4	819.1
No. eels	6	5	9	6	5	2	5	6
<u>A. dieffenbachii</u> non-migrant females - Makara stream.								
Age group	26.....	28.....	30.....	32.....	34	35	36	
Mean length (cm)	64.56	83.1	97.6	91.2	71.8	101.3	106.0	
Length range (cm)	58.7- 67.9	75.6- 92.6						
Standard deviation	5.09	8.66						
Mean weight (g)	720	1655.6	2750	2235	1012	2835	3300	
Weight range (g)	550- 860	1137- 2230						
Standard deviation	157.1	548.6						
Calculated weight (g)	732.3	1654	2773	2229	1031	3218	3620	
No. eels	3	3	1	1	1	1	1	

growth increments throughout the growth curve, although from age group 15 to 26 it is fairly constant. From age group 27 to 26 there is considerable fluctuation in the growth curve due to the small number of eels within each age group. After an initial faster growth rate than undifferentiated or male eels to age group 14, the growth increment for all eels is similar. In only three age groups do males have similar mean lengths to the females, in all other age groups females are larger, indicating a faster growth rate than males and undifferentiated eels. From age group 26 females show an increased growth increment similar to the shortfin females.

Tables 4.7, 4.8 and 4.9 show that the length ranges for undifferentiated and male eels, and the mean values up to age group 21, are similar. The length ranges for females up to age class 20 are similar to that for males of the same group, after which the maximum length for the females is greater. The mean lengths for females up to age group 23 are little different from the mean values for males of the same age group. From age group 24 the mean values for females is greater than that for males. Overall there is less distinction between the growth rates for each sex category than is shown in the shortfins. However, in most age groups the mean length for females is greater than that of males, and males greater than that of undifferentiated eels of the same age group.

The mean lengths and length range for each age group and sex category for each **species** are generally similar, and growth rates, where they overlap, are little different. The growth increments for undifferentiated eels for each species up to age group 12 are similar. Undifferentiated longfin eels then show better growth in length, the mean lengths for the higher age groups being greater. Little comparison can be made between the growth increments for male eels of each species, because of the small overlap of age groups that occurs. In the age groups that do coincide the mean values for longfin males is greater than that for shortfin males, except for age group 14 where the reverse occurs. The larger size of longfin males in the common age groups does indicate a slightly faster growth rate than shown by shortfin males. Longfin females in the lower age groups, below age group 20, are slightly larger than shortfin females of the same age.

After age group 20 the shortfin females tend to show better growth in length than the longfin females.

Fig.4.4 shows that there is a distinct difference between the species in the age at which the majority of the males and all females become sexually differentiated. Shortfin males and females are smaller and younger at the time of sex differentiations than the longfins.

The extreme variability of lengths within each age group, and the great range of ages shown by eels of the same length, means there are relatively few eels within each age group. The analysis of age and length data shows the irregularity of growth within each sex category for each species. There is also an overlap of length ranges within the same age group between each sex category. This overlap may be considered justification for combining all observations and constructing a growth curve for all eels of each species. However, it is considered that the mean lengths for each sex category are biologically significantly different.

✓ Females of each species grow faster than males and undifferentiated eels, because they are usually larger at the same age. This is more apparent in shortfins than in longfins. Similarly males of both species grow faster than undifferentiated ^{eels}, although this is less distinct for longfin males.

Cairns (1941) presents data in tabular form that can be directly compared with Tables 4.4 to 4.9, if his age in years are each reduced by three to correspond with the age groups used in this study. The mean length and length range given by Cairns for each corresponding age group for each species are all larger than for eels in the Makara stream of the same age. There is considerable difference between the size of male and female longfins within each age group. Cairns records male longfins in age groups 7 to 10 and females from age groups 7 onwards. In this study no longfin males were recorded in these age groups, and the youngest recognisable females were placed in age group 10. Cairns does not give ages for shortfin males, but the shortfin females recorded are placed in age groups 7 to 11. This is similar to the shortfin females in this study of which the youngest occur in age group 6, but the oldest females were placed in age group 29, considerably older than Cairns' maximum age. Length ranges given by Cairns for longfin males are greater than those for

longfin females, indicating a faster growth rate in males. This is contrary to the faster growth in longfin females shown in this study.

Cairns shows that the length of shortfins exceeds that of longfins in each age group after age group 6. Data presented here shows little difference between the mean lengths of shortfin and longfin eels of the same sex category within the same age group.

Burnet (1969b) gives growth curves for both species from three streams based on otolith readings and tag returns. The Makara longfin females show much greater growth than the South Branch longfins, being much larger at the same age. The growth rate for Makara longfin females is only slightly better than Burnet's (1969b) Main Drain longfin growth curve.

Makara shortfin females are larger than Burnet's (1969b) South Branch shortfins at the same age group, but smaller than the Doyleston Drain shortfins.

Data given by Cairns (1941), Burnet (1969b) and this study show that the growth rate in all areas studied is slow, but that there are clear differences in the growth rates between different eel populations.

The growth rate of the Makara eels is slower than that of other temperate species in which the growth has been studied. Sinha and Jones (1967a) found that the female European eel grew faster than eels with lobulate gonads, that is males, and undifferentiated males and females. Bertin (1956: 43-46) also discusses the faster growth rate of females. The difference in growth between the sexes in New Zealand eels is similar to that found in the European eel.

Sinha and Jones (1967a) give data on the mean length of eels for each age group which are all greater than for shortfin and longfin eels of the same age. Apart from the faster growth, European female eels become sexually differentiated at an earlier age, age group 3 and 4, than either shortfin or longfin female eels. New Zealand eels also attain a much greater age than the European eel. Apart from the exceptional cases of longevity given by Bertin (1956: 46), Sinha and Jones (1967a) give ages of the non-migrant European eel up to age group 13, compared with age group 29 for shortfins and 36 for longfins in this study. However, migrating European eels

do reach ages of up to 19 years (Frost 1945) and therefore non-migrants in some populations must attain greater ages than shown by Sinha and Jones (1967a).

The American eel shows ~~better~~ growth than either New Zealand species. The mean lengths for the American eel for each age group (Gray and Andrews 1971) are all greater than for shortfin or longfin eels of the same age group.

Weight for age relationship

Shortfin eels ranged in weight from 14g to 1510 g and longfins from 15g to 3300 g. The mean weight and weight range for each age group for each sex category and species is given in Tables 4.4 to 4.9.

As is shown by the weight range and large standard deviations for many age groups, eels of the same age differ widely in their weights. For this reason the mean weights are misleading and therefore the calculated weights are also given. From the mean length for each age group, the weight was calculated from the length-weight regression equations given later in this section. These calculated weights give a more accurate indication of the weight for age relationship than do the observed means.

The growth in weight for each sex category is similar to that shown by the increase in length. For shortfins, the males and females are heavier than undifferentiated eels within the same age group. In turn the females are heavier than the males. In the longfins, females are generally heavier than males within the same age group, and both are heavier than undifferentiated eels. However, like the difference in length, the weight difference between the three sex categories within the same age group is not as great as shown by shortfins.

There is little difference in the mean lengths in the same age group for each sex category between each species. However, there is a considerable difference in the calculated weights. The calculated weights for longfins in each sex category are greater than for shortfins within the same age group. This shows that while there is a similar increase in length for each species, the longfins have a greater increment in weight. The greater weight of longfins compared with shortfins of the same length is discussed separately in the length-weight relationship given later in this section.

4.5 Age of migrant eels

A total of 464 migrant eels were aged from different areas. The majority of migrants aged were from the Makara stream, Lake Onoke and Lake Ellesmere. The number of eels aged from each sex and species has been given in Table 4.3 and is included in Tables 4.22 and 4.23. The location of the areas where migrants were obtained is given in the materials and methods section.

The ages of eels from each migratory season are combined.

Age for length relationship

Male shortfins

Tables 4.10 to 4.13 give the mean length range and standard deviation for each age group for different areas.

Shortfin males ranged in age from age group 5 to age group 22, and in size from 33.0cm to 59.7cm. Within the numerically largest age groups for any area there is a wide range in lengths, almost as large as the total length for all eels from that area. There is some fluctuation in the mean lengths between different age groups for each area, but in general they are within ± 5 cm of the mean length for all migrants from that area. The largest mean lengths tend to occur in the higher age groups, but the length range for each age group does show that the largest individual migrants are not necessarily the oldest. Because of the small sample size and wide variation in lengths within age groups, little comparison can be made between the mean lengths for the same age group from different areas.

The mean lengths for each age group are similar to the mean migratory length. This indicates that growth has ceased and eels gain little in length by remaining in freshwater. This indicates that the time of migration is more dependent on the attainment of a certain length than it is on age.

Female shortfins

The mean length, length range and standard deviation for each age group and area is given in Tables 4.14 to 4.17.

Shortfin females ranged in age from age group 9 to 35, and in size from 53.0cm to 102.3cm. There is a great variation in lengths within the numerically largest age groups. The largest mean lengths tend to occur in the higher age groups, particularly for the Lake Onoke females. However, considerable fluctuation between the mean lengths for each age group occur, due to the wide range of lengths and,

TABLE 4.10

Mean length (cm), range in length (cm), standard deviation, mean weight (g), weight range (g), standard deviation and calculated weight (g) of eels of different age groups.

A. australis - migrant males

Makara stream - mean migratory length 46.5 cm

Age group	5	6	7	8	9	10	11	12
Mean length (cm)	46.9		50.1	48.2	49.8	47.3	46.9	49.0
Length range (cm)			47.7- 52.5		44.4- 54.1		41.8- 52.0	45.7 53.9
Standard deviation			3.39		4.94		3.45	3.75
Mean weight (g)	195		193.5	195	218.3	180.0	179.9	198.7
Weight range (g)			175- 212		140- 275		140- 235	145- 275
Standard deviation			26.2		70.1		38.4	55.6
Calculated weight (g)	203		251	222	246	209	204	234
No. eels	1		2	1	3	1	7	4

A. australis - migrant males

Makara stream - mean migratory length 46.5 cm

Age group	13	14	15	16	17	18	19
Mean length (cm)	43.6	53.2	49.3		45.5	43.2	43.8
Length range (cm)	42.7- 44.5		48.8- 49.8		43.2- 47.1		42.4- 45.1
Standard deviation	1.27		0.71		1.44		1.35
Mean weight (g)	136.0	340	158.5		148.4	145	150
Weight range (g)	112- 160		105- 212		112- 165		142- 162
Standard deviation	33.9		75.7		19.2		10.6
Calculated weight (g)	162	303	238		185	157	163

A. australis - migrant males

Makara stream - mean migratory length 46.5 cm

Age group	20	21	22
Mean length (cm)	53.1	45.3	57.1
Mean weight (g)	265	165	240
Calculated weight (g)	301	182	378
No. eels	1	1	1

TABLE 4.11

Mean length (cm), length range (cm), standard deviation, mean weight (g), weight (g), weight range (g), standard deviation and calculated weight of eels of different age groups.

A. australis - migrant males

Lake Onoke - mean migratory length 44.7 cm

Age Group	7	8	9	10	11	12	13	14
Mean length (cm)	47.1	43.0	41.8	45.0	45.3	44.1	44.1	43.3
Length range (cm)			39.8- 43.9	39.1- 49.6	39.5- 53.0	40.1- 48.6	37.0- 53.5	38.7 58.8
Standard deviation			2.9	4.55	4.52	3.14	4.96	3.09
Mean weight (g)	202	120	125	145	150.5	138.2	139.4	120.9
Weight range (g)			90- 160	110- 202	120- 215	110- 182	110- 225	110- 180
Standard deviation			49.5	47.5	37.1	28.9	46.1	29.2
Calculated weight (g)	172.1	129.3	118.7	148.8	151.6	140.2	140.2	132.0
No. eels	1	1	2	6	11	11	15	9

A. australis - migrant males

Lake Onoke - mean migratory length 44.7 cm

Age Group	15	16	17	18	19	20	21
Mean length (cm)	45.6	46.7	43.8	46.4	49.8		44.7
Length range (cm)	39.4- 49.7	41.6- 51.2	37.5- 48.0	44.0- 51.5			
Standard deviation	4.49	3.8	4.04	3.13			
Mean weight (g)	154.5	161.8	126	173.4	147.5		145
Weight range (g)	110- 210	130- 205	108- 185	170- 245			
Standard deviation	55.4	37.2	40.7	42.1			
Calculated weight (g)	55.7	167.0	136.9	163.7	204.1		145.7
No. eels	4	5	7	5	1		1

TABLE 4.12

Mean length (cm), length range (cm), standard deviation, mean weight (g), weight range (g), and standard deviation of eels of different age groups.

A. australis migrant males

Lake Ellesmere - mean migratory length 44.1 cm

Age group	8	9	10	11	12	13
Mean length (cm)	42.9	46.85	41.7	45.4	43.9	46.5
Length range (cm)		44.0- 49.7	41.3- 42.2	45.5- 46.6		42.9 50.1
Standard deviation		4.03	0.64	1.31		5.09
Mean weight (g)	125	157.5	115.2	192.3	172.0	227.0
Weight range (g)		120- 195	88- 142	180- 275		149- 305
Standard deviation		53.0	38.2	77.2		110.3
No. eels	1	2	2	3	1	2

A. australis migrant males

Lake Ellesmere - mean migratory length 44.1 cm

Age group	14	15	16	17	18
Mean length (cm)	42.0	43.7	46.4		47.9
Length range (cm)	38.8- 48.2		44.9- 47.9		
Standard deviation	3.74		2.12		
Mean weight (g)	128.6	156	229		150
Weight range (g)	90- 230		223- 235		
Standard deviation	67.1		8.5		
No. eels	5	1	2		1

A. australis migrant males

Waikato River - mean migratory length 44.0 cm

Age group	10	11	12
Length (cm)	40.2	46.2	47.5
Weight (g)	110	160	180
No. eels	1	1	1

TABLE 4.13

Mean length (cm), length range (cm), standard deviation, mean weight (g), weight range (g), and standard deviation of eels of different age groups

A. australis migrant males

Hokitika - mean migratory length 45.4

Age group	10.....	12.....	14.....	16	17	18	19
Mean length (cm)	49.9	42.3	45.7	42.8	44.5	46.5	46.2
Length range (cm)			43.2- 47.1		42.4- 46.2		43.2 49.2
Standard deviation			1.44		1.27		
Mean weight (g)	212	145	148.4	140	139.0	185	160.2
Weight range (g)			110- 155		115- 163		120- 200
Standard deviation			15.3		33.9		34.2
No. eels	1	1	3	1	2	1	2

A. australis migrant males

Invercargill - mean migratory length 51.9 cm

Age group	9	10	11	12	13	14	15	16
Length (cm)	51.2							52.7
Weight (g)	220							228
No. eels	1							1

TABLE 4.14

Mean length (cm), length range (cm), standard deviation, mean weight (g), weight range (g), standard deviation and calculated weight (g) of eels of different age group.

A. australis migrant females

Makara stream - mean migratory length 74.0cm

Age group	9.....	13.....	17	18	19	20
Mean length (cm)	67.3	73.3	76.9	82.5	69.0	73.5
Length range (cm)	56.3- 78.4	66.1- 80.6	67.4- 86.4		65.1- 74.3	65.0- 81.5
Standard deviation	15.63	10.25	13.44		4.74	8.26
Mean weight (g)	615	885	820	1212	586.7	666.7
Weight range	330- 900	665- 1105	460- 1180		470- 775	465- 900
Standard deviation	403.1	311.1	509.1		163.3	219.2
Calculated weight (g)	634.5	829.7	964.1	1203	685.2	837.2
No. eels	2	2	2	1	3	3

A. australis migrant females

Makara stream - mean migratory length 74.0cm

Age group	21	22....	25.....	28
Mean length (cm)	73.9	70.4	72.6	93.3
Length range (cm)		69.0- 71.8		
Standard deviation		1.98		
Mean weight (g)	665	618.5	668	1290
Weight range (g)		587- 650		
Standard deviation		44.5		
Calculated weight(g)	851.1	731.2	804.1	1770
No. eels	1	2	1	1

TABLE 4.5

Mean length (cm), length range (cm), standard deviation, mean weight (g), weight range (g), standard deviation and calculated weight of eels of different age groups.

A. australis migrant females

Lake Onoke - mean migratory length 76.4cm

Age group	11	12	13	14	15	16	17
Mean length (cm)	67.2	71.5		67.2	76.5	64.4	64.1
Length range (cm)				65.3- 69.1		55.0- 72.4	66.6- 70.6
Standard deviation				2.69		8.78	8.1
Mean weight (g)	435	630		607.5	850	491.7	481.6
Weight range (g)				445- 770		235- 775	235- 720
Standard deviation				229.8		271.0	242.6
Calculated weight(g)	516.9	630.1		519.7	778.2	454.8	448.4
No. eels	1	1		2	1	3	3

Age group	18	19	20	21	22	23	24
Mean length (cm)	70.4	81.1	62.3	73.3	72.2	76.8	76.7
Length range (cm)	58.8- 77.0	72.6- 86.1		70.6- 75.5	72.2- 72.3	69.0- 91.0	73.9- 81.5
Standard deviation	10.08	7.4		2.49	0.07	9.94	4.18
Mean weight(g)	668.3	951.7	360.0	752.3	852.5	882.5	850
Weight range (g)	275- 900	605- 1200		700- 795	700- 1005	600- 1350	750- 950
Standard deviation	267.3	309.4		57.5	215.7	332.5	100
Calculated weight (g)	600.5	933.0	410.3	681.0	650.3	788.9	784.3
No. eels	3	3	1	3	2	4	3

Age group	25	26	27	28	29.....33	34	
Mean weight (cm)	77.0	79.1	92.4	83.5	99.5	90.1	102.3
Length range (cm)	63.8- 91.6	76.0- 84.2	92.0 92.8				
Standard deviation	13.96	4.47	0.57				
Mean weight (g)	886.3	863.8	1381	1025	1450	1325	1812
Weight range (g)	410- 1312	755- 1075	1337- 1425				
Standard deviation	453.1	146.7	52.2				
Calculated weight (g)	793.9	863.4	1402	1022	1764	1295	1923
No. eels	3	4	2	1		1	1

TABLE 4.16

Mean length (cm), length range (cm), standard deviation, mean weight (g), weight range and standard deviation of eels of different age groups.

A. australis migrant females

Pukepuke lagoon - mean migratory length 80.3 cm

Age group	12	13	14	15	16	17....
Mean length (cm)	57.9		59.2	78.7	73.0	76.2
Length range (cm)						
Standard deviation						
Mean weight (g)	356		410	855	865	850
Weight range (g)						
Standard deviation						
No. eels	1		1	1	1	1

Age group	...20	21	22	23	24	25	26....	29
Mean length (cm)	81.8	82.0	82.5	78.8	79.1	81.9	84.2	80.2
Length range (cm)			78.0- 85.0	74.7- 82.9		80.2- 83.5		
Standard deviation			3.9	5.1		2.6		
Mean weight (g)	1095	1120	1000	837.5	960	1056	1000	1002
Weight range (g)			900- 110	775- 900		900- 1212		
Standard deviation		100	170.9		229			
No. eels	1	1	3	2	1	2	1	1

A. australis - migrant females

Waikato River - mean migratory length 82.9

Age group	21	22	23	24
Mean length (cm)	97.8		89.7	80.0
Length range (cm)			74.1- 100.3	
Standard deviation			8.9	
Mean weight	1988		1650	1020
Weight range (g)			750- 1812	
Standard deviation				
No. eels	1		3	1

TABLE 4.17

Mean length (cm), length range (cm), standard deviation, mean weight (g), weight range (g) and standard deviation of eels of different age groups

A. australis migrant females

Lake Ellesmere - mean migratory length 71.9 cm

Age group	12.....	16.....	19....	21	22	23....	26	27	28
Mean length (cm)	84.65	75.3	64.9	62.0	58.7	68.8	69.76	66.4	66.5
Length range (cm)	81.8- 87.5		58.0- 71.9	57.1- 68.4	54.5- 63.0	63.4- 71.0	69.3- 70.2		
Standard deviation	4.03		9.82	5.78	6.01	4.72	0.64		
Mean weight(g)	1075	860	487.5	389.3	400.5	650	625	405	585
Weight range(g)	176.8 950		375- 600	350- 418	325- 476	475- 725	525- 725		
Standard deviation	1200		159	35.2	106.7	152.6	141.4		
No. eels	2	1	2	3	2	3	2	1	1

Hokitika - mean migratory length 69.3 cm

Age group	17....	23	24	25	26	27	28
Mean length (cm)	85.7	66.2	60.6	65.0		67.2	72.2
Length range (cm)						65.3- 70.0	60.6- 84.3
Standard deviation						8.2	10.25
Mean weight (g)	1050	530	425	550		481.6	900
Weight range (g)						235- 720	670- 1120
Standard deviation						242.6	311.1
No. eels	1	1	1	1		3	2

Invercargill - mean migratory length 74.9 cm

Age group	17	18	19.....	24	25
Length (cm)	91.6	55.1	86.9	66.8	74.1
Weight (g)	1640	305	1355	575	725
No. eels	1	1	1	1	1

because of the great variation in ages, the small number of eels within each age group.

Because of the small number of eels within each age group few comparisons can be made between areas. However, the Lake Onoke data show that up to age group 26 the eels gain little in length by remaining in freshwater. From age group 27 to 34 there is a considerable increase in size with the largest eels being the oldest. Similarly the largest Makara female migrant was the oldest. For other areas the largest eels were not the oldest. In contrast to the younger migrants, the older females gain a considerable increase in length by remaining in freshwater. Although the mean lengths for the age groups indicate that migration is dependent on length rather than age, the much larger size of the eels in the higher age groups shows that some other factor may also influence migration. This may be the attainment of a certain length weight combination, or the migration may be dependent on the growth rate in the preceding non-migrant stage. This is discussed separately later in this section.

Male Longfins

Tables 4.18 to 4.19 give the mean length, length range and standard deviation for each age group from different areas. Ages ranged from age group 11 to 34 and in size from 48.0cm to 72.7cm. As with the male and female shortfin migrants there is a great range in lengths within each age class. However, the large sample size of the Makara longfin male migrants meant that there were more eels within any one age group. Consequently the mean sizes for each age group are a more reliable indication of the length attained at each age. Except for age groups 11, 13 and 34, the mean length for each age group is very close to the mean size for all Makara longfin male migrants. For age groups 11 and 13 the lengths are smaller, but these are based on only three eels. The largest eel occurred in age group 34. However, other eels of over 70cm were placed in lower age groups, so the largest eels were not necessarily the oldest. Like the shortfin males, the similarity of the mean lengths for each age group with the mean length for all migrants shows that little is gained in length by remaining in freshwater, and that growth in the migrants has ceased. This indicated that migration is dependent on length and not age.

TABLE 4.18

Mean length (cm), length range (cm), standard deviation, mean weight (g), weight range (g), standard deviation and calculated weight (g) of eels of different age groups.

A. dieffenbachii migrant males

Makara stream - mean migratory length 62.5 cm

Age groups	13	14	15	16	17	18	19
Mean length (cm)	53.5	65.1	62.3	62.4	63.4	59.0	60.7
Length range (cm)		57.2- 73.0	53.7- 69.0	56.7- 67.3	55.1- 71.1	51.3- 64.4	54.0- 67.1
Standard deviation		11.17	6.96	4.5	5.83	3.74	3.79
Mean weight (g)	310	620	533.8	581.6	525.0	415.9	480.6
Weight range (g)		365- 875	280- 775	300- 760	390- 676	370- 572	385- 650
Standard deviation		360.6	240.7	122.3	154.2	89.0	98.7
Calculated weight(g)	308.3	585.2	506.9	509.5	528.3	424.4	465.6
No. eels	1	2	4	5	6	14	11

Age groups	20	21	22	23	24	25
Mean length (cm)	62.2	61.4	62.7	62.1	65.3	61.9
Length range (cm)	53.6- 72.9	48.2- 71.8	51.4- 70.5	51.4- 67.6	56.5- 73.1	56.9- 70.2
Standard deviation	5.54	5.67	4.98	4.38	4.8	5.35
Mean weight (g)	499.4	501.1	532.7	536.5	595.7	477.1
Weight range (g)	280- 837	250- 750	270- 675	270- 700	300- 885	335- 730
Standard deviation	168.8	143.9	133.5	131.9	151.1	141.3
Calculated weight(g)	505.4	483.1	479.0	499.4	591.4	422.4
No. eels	15	18	17	15	15	10

Age groups	26	27	28	29	30.....34
Mean length (cm)	62.8	66.2	65.1	64.6	65.6 72.7
Length range (cm)	56.9- 66.8	62.6- 72.1	63.6- 67.6	59.8- 69.4	62.0 67.8
Standard deviation	3.69	5.05	1.95	6.79	3.06
Mean weight	524.5	582.8	607.6	559.0	531.0 802.0
Weight range (g)	300- 625	435- 738	500- 738	370- 748	500- 625
Standard deviation	105.7	152.4	131.6	267.3	133.7
Calculated weight (g)	520.0	619.5	585.6	570.5	594.0 820.4
No. eels	10	4	5	2	3 1

TABLE 4.19

Length (cm) and weight (g) of eels of different age groups

A. dieffenbachii migrant males

Lake Onoke - mean migratory length 67.0cm

Age group	20	21	22
Length (cm)	64.6		69.5
Weight (g)	655		825
No. eels	1		1

Lake Ellesmere - mean migratory length 66.0cm

Age group	20.....30	
Length (cm)	69.2	62.7
Weight (g)	650	454
No. eels	1	1

Hokitika - mean migratory length 61.7cm

Age group	26.....28	
Length (cm)	64.1	59.3
Weight (g)	500	412
No. eels	1	1

Dunedin - mean migratory length 63.9cm

Age group	20	21....	27.....	33	34
Length (cm)	66.7	62.0	60.0	62.8	69.8
Weight (g)	838	620	500	635	900
No. eels	1	1	1	1	1

Invercargill - mean migratory length 67.0cm

Age group	20.....28	29	
Length (cm)	72.6	62.3	66.1
Weight (g)	662	445	612
No. eels	1	1	1

Female longfins

The mean length, length range and standard deviation for each age group and area is given in Tables 4.20 and 4.21. The ages ranged from age group 19 to 47 and in size from 83.6cm to 131.6 cm. Because of the small sample sizes little can be said about the age of longfin migrant females. As in the shortfin migrant females there is a great range in age, and the largest eels tend to be the oldest, although some of the eels over 100cm in length were placed in the lower age groups. Unlike the males, the larger eels do gain more in length by remaining in freshwater, indicating that migration is dependent on length associated with some other, undetermined, factor.

Age at migration

Tables 4.22 and 4.23 give the mean age, age range and standard deviation of migrants for each sex, species and area.

The mean ages of shortfin migrant males from each area are identical except for the West Coast and Waikato samples. The number of eels aged from these two areas is however too small for any valid comparison to be made.

Shortfin females show some variation in the mean age at migration from different areas. Because of the wide range in age at migration and the smaller sample sizes for some areas, the differences between the means are not considered to be significant.

No valid comparisons can be made here between different areas on the age at migration of longfin male and females. The sample sizes from all areas other than Makara are too small. For both species males are younger than females at migration.

Although, as mentioned, little significance can be attached to the differences in the mean age at migration for shortfins, the variability in the mean age of the females may reflect differences in the growth rates between areas. In particular the Hokitika migrant shortfins have the highest mean age and the single migrant longfin female was the oldest aged. This indicates that the Hokitika eels could have a slower growth rate than eels from other areas.

The ages of migrant eels studied are less than MacFarlane's (1936) age at migration of 12-14 years (age group 9-11) for longfin females. Cairns (1941) does not record the exact age of migrant eels but the oldest eels he records are shortfin females, 14 years

TABLE 4.20

Mean length (cm), length range (cm), standard deviation, mean weight (g), weight range (g), standard deviation and calculated weight of eels of different age groups.

A. dieffenbachii migrant females

Makara stream - mean migratory length 106.3cm

Age groups	24	25	26	27	28	29.....32	33
Mean length	115.7		95.4		88.0	100.3 94.4	126.6
Length range			93.0- 97.7			84.4- 101.0	
Standard deviation			3.32			8.95	
Mean weight	3237		1775		1335	2450 1780	5050
Weight range			1700- 1850			1250- 2212	
Standard deviation			106.1			488.6	
Calculated weight	2989		1879		1547	2260 1832	3438
No. eels	1		2		1	1 3	1

Age groups	34	35	36	37	38.....41.....44.....47
Mean length		108.0	96.1		133.3 129.7 132.8 131.6
Length range					
Standard deviation					
Mean weight		3350	1600		5670 5120 5824 4000
Weight range					
Standard deviation					
Calculated weight		2533	1913		4207 3838 4167 4075
No. eels		1	1		1 1 1 1

TABLE 4.21

Length (cm) and weight (g) of eels of different age groups

A. dieffenbachii migrant females

Waikato River - migratory length 93.8 cm

Age group	19
Length (cm)	93.8
Weight (g)	1792
No. eels	1

Lake Onoke - mean migratory length 91.3 cm

Age group	25	26	27	28
Length (cm)	83.6	98.0	91.5	92.2
Weight (g)	1075	2525	1825	1550
No. eels	1	1	1	1

Hokitika - migratory length 137.0cm

Age group	47
Length (cm)	137.0
Weight (g)	7635
No. eels	1

Invercargill

Age group	33.....39
Length (cm)	102.5 110.8
Weight (g)	2385 3550
No. eels	1 1

TABLE 4.22

Mean age group, range in age and standard deviation
of migrant eels from different areas

Area	<u>A. australis</u>		<u>A. dieffenbachii</u>	
	male	female	male	female
<u>Makara</u>				
Mean age	13	18	22	33
Range in age	5 - 22	9 - 29	11 - 34	24 - 47
Standard deviation	4.34	4.94	3.88	6.74
No. eels	36	18	160	15
<u>Lake Onoke</u>				
Mean age	13	21	22	37
Range in age	7 - 21	11 - 34	21 - 23	25 - 43
Standard deviation	2.76	5.25	1.5	8.42
No. eels	79	43	2	4
<u>Lake Ellesmere</u>				
Mean age	13	22	25	
Range in age	8 - 18	12 - 20	20 - 30	
Standard deviation	2.68	4.74	4.92	
No. eels	20	21	2	
<u>Invercargill</u>				
Mean age	13	20	25	36
Range in age	9 - 16	17 - 25	20 - 28	33 - 39
Standard deviation	3.58	4.25	4.25	2.48
No. eels	2	5	2	2

TABLE 4.23

Mean age group, range in age and standard deviation
of migrant eels from different areas

Area	<u>A. australis</u>		<u>A. dieffenbachii</u>	
	male	female	male	female
<u>Hokitika</u>				
Mean age	14	25	27	47
Range in age	10 - 19	17 - 28	26 - 28	—
Standard deviation	3.75	4.15	1.5	—
No. eels	12	9	2	1
<u>Waikato River</u>				
Mean age	11	23		34
Range in age	10 - 12	21 - 34		—
Standard deviation	1.23	4.31		—
No. eels	3	5		1
<u>Pukepuke Lagoon</u>				
Mean age		21		
Range in age		15 - 28		
Standard deviation		3.81		
No. eels		15		
<u>Dunedin</u>				
Mean age			27	
Range in age			20 - 34	
Standard deviation			7.5	
No. eels			5	

(age group 11), longfin males 13 years (age group 10), and longfin females 19 years (age group 16). All ages are considerably less than the ages attained by migrant eels in this study. Burnet (1969b) gives no age at migration, but in the South Branch stream the oldest non-migrant shortfin eels shown in the growth curves are 30 years (age group 27) and for longfin non-migrant females 60 years (age group 57). From the size at these ages, both below the migratory size for that stream (Burnet 1969a) it could be expected that the age at migration would be greater than for the Makara, Lake Onoke and Lake Ellesmere migrant females. This would indicate a slower growth rate in the non-migrant stage in the South Branch stream. The differences in the growth rates of non-migrant eels recorded by different authors has been discussed earlier in this section.

Several authors (Frost 1945 and 1950; Rasmussen 1952; Deelder 1957) have studied the age of migrating European eels from different areas. All give similar ages at migration for males, ranging from 6 to 12 years, mean 9 years, and females 9-19 years, mean 12 years. These ages are considerably less than the age at migration for New Zealand male and female eels of both species.

The American eel is also younger at migration than New Zealand eels. Gray and Andrews (1971: 125) record that migrating females ranged in age from 9 to 18 years with a mean age of 12.28 years.

Weight for age

The mean weight, weight range and standard deviation for each age group of each sex by species and area is given in Tables 4.10 to 4.21. The weight range and mean weight show a similar trend to the length range and mean length. There is a wide range in weight within each age group, which, in the numerically largest age groups is the same as the total weight range for all migrants of that sex. As with the mean lengths, the mean weights show that male eels of both species gain little in weight by remaining in freshwater. The mean weights within each age group for shortfin females vary, although the Lake Onoke eels up to age group 26 have similar mean weights. From age group 27 to 34 there is an increase in the mean weights. Similarly the oldest longfin females in Makara stream, unlike the males, show an increase in mean weight.

As in the non-migrant eels, eels of the same age differ widely in their weights. The calculated weights therefore give a better indication of the weight for each age class than the mean weights

do. From the mean length for each age group, the weight was calculated for the Makara and Lake Onoke eels from the length-weight regression equation given for these eels later in this section.

4.6 Length-weight relationship

The term length-weight relationship means the mathematical relationship between the length and weight of a fish. This, for most fish, can be represented by the equation,

$$W = aL^n \dots\dots\dots(1)$$

Where W = weight, L = length, a is a constant and n the exponent. (Lagler 1956; Le Cren 1951). The value of n for different fish can vary between two and four but is usually close to three (Tesch 1968). If $n = 3$ the fish is said to grow symmetrically or growth is isometric, if n is greater than three growth is said to be allometric, the fish becomes heavier for its length as it grows larger. It has been established by Burnet (1952) and Woods (1964) that the length-weight relationship of New Zealand eels can be represented by equation (1) and that the growth is allometric. Cairns (1942) found that longfin females were heavier than shortfin females of the same size, showing that longfin females increase in weight more rapidly than shortfins. The larger growth exponent, n , calculated by Woods (1964) for the longfin eel, also shows that they increase in weight more rapidly as they grow larger.

The growth exponent, n , can be different for fish from different areas, for each sex and for immature and mature fish (Le Cren 1951: 202). The purpose of examining the length-weight relationship in this study was to determine if n for each species varied between male and female in the migrant and non-migrant stages, and if n varied between non-migrant and migrant eels with the sexes combined. The length-weight relationship may be represented graphically by plotting as a scatter diagram the observed lengths and weights on logarithmic paper. The points for fish having the same length-weight relationship will lie on a straight line. To calculate the values of a and n the length and weight data are converted to logarithmic values and the regression line to fit the data is calculated by the method of least squares using the logarithmic form of equation (1),

$$\log W = \log a + n \log L \dots\dots\dots(2)$$

where W = weight, L = length, n is the slope of the regression line (regression coefficient) and $\log a$ its position.

The length-weight data, for male and female eels for each species separately, were plotted graphically on double logarithmic paper. By fitting a line by eye it was apparent that there was no difference between the length-weight relationship for non-migrant male and female eels of each species. For migrant eels no difference was apparent between shortfin male and female eels or between non-migrant and migrant male and female eels. The length-weight relationship for migrant longfins however indicated a difference between the sexes, and between migrant and non-migrant eels.

To establish if there were any differences in the length-weight relationships between the sexes within each migratory state the length-weight data were analysed by the statistical method of covariance (Snedecor 1956: 394 - 400). This method was used by Le Cren (1951) to study the length weight relationship and condition of the perch.

In his study Le Cren (1951: 204) states that "if it is expected that a large group, of fish containing a number of smaller groups, the condition of which latter it is desired to compare is..... homogeneous in its length weight relationship, then a length weight relationship can be calculated (formula 2) for each subgroup. The values of n determined are tested for homogeneity, and if no significant difference exists between them a pooled regression can be calculated for the whole group combined, and the values of $\underline{a}$ adjusted for this pooled length-weight relationship. These adjusted values of $\underline{a}$ are accurate measures of the relative condition of the subgroups, and the significance of the differences between them can be subjected to statistical tests." However, the use of the analysis of covariance of the length-weight relationship as a method of directly comparing condition is confined to comparisons between fish which are homogeneous for n in their length-weight relationships and to fish that are the same size. Non-migrant and migrant male and female eels are of different sizes, and there is little overlap of size ranges between the sexes at migration. Therefore, as Le Cren (1951: 205) suggests, differences between condition factors would be difficult to interpret. Hence for this

study only the differences in the values of n were examined.

The analysis of length-weight data by covariance involves a comparison of the subgroup regression equations calculated from the logarithmic length-weight relationship (equation 2). The regression lines may differ in slope, in elevation or in residual variance. The purpose of this study was to examine the differences in slope (n), and for this the differences between the regression coefficients and a plot of the regression equation expressing the length-weight relationship of each subgroup were used.

The calculations to give the length-weight regression equations in the form of equation 2 for each subgroup and group, the regression coefficients, and the values of F for testing the significance of differences between regression coefficients in the analysis of covariance, were made by computer. The computer programmes used are given in Appendices A and B.

The regression equations for each subgroup (sex or migratory state) and group (sexes combined or migratory state) were plotted graphically by calculating values of $\log W$ using selected values of $\log L$ (equation 2). For testing the homogeneity of the subgroup regression coefficients, the F value between regression coefficients, a set of statistical tables of F -distribution were used (Hayslett 1967: 234). If the differences between the regression coefficients were not significant at either the 1% or 5% level, and the plot of length-weight relationships for each subgroup were not obviously different, the common regression equation was accepted as representing the length-weight relationship of both subgroups.

Gutted weights only were used for the length-weight analyses to eliminate the possible fluctuations in weight the stomach contents and gonads would produce. It is suggested that the gutted weight gives a better measure of the length-weight relationship. By eliminating the weight of the gut contents in non-migrants, and the gonad weight in migrants, particularly longfin females, a more accurate comparison of the length-weight relationships can be made. Sufficient data were only available to examine the length-weight relationship of Makara migrant and non-migrant eels and Lake Onoke shortfin migrants.

For length-weight analyses, the following comparisons between subgroups were made:

1. Between sexes within each migratory state (migrant and non-migrant). This was done for each migratory state and species for the Makara and Lake Onoke data. This tested whether the length-weight data for each sex and migratory state could be combined within the one regression equation. Computer programme A was used for this analysis.
2. Between migratory states, sexes combined. For each species the sexes were combined and tested to see whether the length-weight data for each migratory state (migrant or non-migrant) could be combined within the one regression equation. Only the Makara data was used in this analysis. Computer programme B was used for this analysis.

The first analysis tested for homogeneity of male and female regression coefficients, and provided regression equations for each sex within each migratory state and area, and a common regression equation for the subgroups combined. The second analysis tested for homogeneity between migrant and non-migrant regression coefficients and provided regression equations for each migratory state for each species and a common regression equation for all data combined (by species).

Table 4.24 gives the F value between regression coefficients and degrees of freedom for each group for the analysis of covariance by sex within each migratory state, and for the analysis of covariance, sexes combined, between migratory states.

Depending on the tests of significance between the regression coefficients, the equations expressing the length-weight relationships for each subgroup or group were either accepted or rejected. Table 4.25 gives the accepted $\log a$, $\underline{a}$ and n values for each accepted group or subgroup. The values of $\log a$, $\underline{a}$ and n for longfin non-migrant males and females combined is also given.

The regression equations for each accepted group or subgroup are discussed separately below, where r = correlation coefficient; sd = sample standard deviation; W = weight (g); L = length (cm). The equations are plotted graphically in Figs. 4.5 and 4.6.

TABLE 4.24

Group	F-value between regression coefficients	Degrees of freedom	Homogeneity within subgroups at 5% level
<u>Analysis of covariance by sex within migratory state</u>			
<u>A. australis</u>			
<u>Makara stream</u>			
non-migrant male and female	2.1488	1,239	Yes
migrant male and female	0.1466	1,92	Yes
<u>Lake Onoke</u>			
migrant male and female	0.0559	1,168	Yes
<u>A. dieffenbachii</u>			
<u>Makara stream</u>			
non-migrant male and female	6.2799	1,194	No, but homogeneity at 1% level
migrant male and female	9.6644	1,355	No
<u>Analysis of covariance by migratory state</u>			
<u>Makara stream</u>			
<u>A. australis</u>	1.1709	1,336	Yes
<u>A. dieffenbachii</u>	19.4467	1,714	No

TABLE 4.25

Group or subgroup	n	Length range (cm)	Weight range (gm)	log a	a	n
<u>Calculated log a, a and n values for accepted groups or subgroups by sex</u>						
<u>Makara stream</u>						
<u>A. australis</u>						
non-migrant	240	28.6-85.1	35-1510	-2.778	0.001679	3.0619
migrant	92	38.1-93.3	98-1290	-2.8268	0.00149	3.0332
<u>A. dieffenbachii</u>						
non-migrant male	54	33.8-66.1	75-950	-3.3094	0.0004906	3.4263
non-migrant female	146	42.0-106.0	190-3300	-2.8766	0.001328	3.1694
non-migrant, sexes combined	200	33.8-106.0	75-3300	-2.9696	0.001072	3.2234
migrant males	337	48.2-73.1	250-802	-3.1572	0.0006963	3.2667
migrant females	22	88.0-133.3	1355-5824	-1.4895	0.03239	2.4064
<u>Lake Onoke</u>						
<u>A. australis</u>						
migrant	170	38.8-102.3	95-1812	-2.9795	0.001048	3.1165
<u>Calculated log a, a and n values for groups or subgroups by migratory state.</u>						
<u>Makara stream</u>						
<u>A. australis</u>						
(non-migrant and migrant combined)	340	28.6-93.3	35-1290	-2.9423	0.001142	3.1425
<u>A. dieffenbachii</u>						
non-migrant	200	33.8-106.0	75-3300	-2.9217	0.001198	3.1967
migrant	557	48.2-133.3	250-5824	-2.6638	0.002169	2.9915

FIGURE 4.5

Length-weight relationship of A. australis
(shortfin) non-migrant male and female, and
migrant male and female from the Makara stream,
and migrant male and female from Lake Onoke.

A. australis

FIG. 4.6

Length-weight relationship of A. dieffenbachii (longfin). Non-migrant male and non-migrant female; and non-migrant male and female, and migrant male and migrant female from the Makara stream.

A. dieffenbachii

Shortfin non-migrant male and female eels, and migrant male and female eels, have subgroup regression coefficients which are not significantly different. The length-weight relationships for migrant and non-migrant eels, sexes combined, can therefore be represented by the equations;

non-migrant male and female

$$\log W = 2.7748 + 3.0619 \log L \quad (r = 0.9819, \text{sd} = 0.0380)$$

or $W = 0.001679 L^{3.0619}$

migrant male and female

$$\log W = 2.8268 + 3.03321 \log L \quad (r = 0.9246, \text{sd} = 0.1295)$$

or $W = 0.00149 L^{3.0332}$

when plotted, the regression lines ran approximately parallel to one another, showing that non-migrant male and female eels were slightly heavier than migrant eels of the same length.

The test of significance established that there was no significant difference between the subgroup regression coefficients for the migrant and non-migrant subgroups. Therefore the length weight relationship for all shortfin male and female eels can be represented by the equation,

$$\log W = 2.9423 + 3.1425 \log L \quad (r = 0.98669, \text{sd} = 0.0282)$$

or $W = 0.001142 L^{3.1425}$

and is represented graphically in Fig.4.5.

Longfin non-migrant male and female eels have subgroup regression coefficients significantly different at the 5% level only. The length-weight relationships for non-migrant male and female eels can be represented by the equations,

non-migrant males

$$\log W = -3.3094 + 3.4263 \log L \quad (r = 0.9787, \text{sd} = 0.1006)$$

or $W = 0.0008306 L^{3.4263}$

non-migrant female

$$\log W = -2.8766 + 3.1694 \log L \quad (r = 0.9858, \text{sd} = 0.0451)$$

or $W = 0.001328 L^{3.1694}$

The length-weight relationships for longfin non-migrant male and female eels is represented in Fig.4.6 It is seen that between

lengths 40cm to 60cm there is little difference in the weight of male and female eels of the same size. Males from 60cm to 70cm in length, approximately the maximum length for males at migration, are heavier than females by approximately 100g. Larger, non-migrant females clearly show a different length-weight relationship than males. However, the observed lengths for non-migrant males ranged from 33.8cm to 67.2cm and Fig.4.6 shows that within this size range the calculated weights are similar to the calculated weights of females of the same size. Therefore the difference between the length weight relationships of non-migrant male and female eels, significant at the 5% level only, is not considered to be biologically significant. Hence the length-weight relationship of non-migrant male and female eels can be represented by the equation,

$$\log W = -2.9696 + 3.2234 \log L \quad (r = 0.9836, \text{sd} = 0.0423)$$

$$\text{or } W = 0.001072 L^{3.2234}$$

and is represented graphically in Fig.4.6.

The subgroup regression coefficients for longfin migrant male and migrant female eels are significantly different. The length-weight relationship for longfin migrant male and migrant female eels can be represented by the formulae,

migrant male

$$\log W = -3.1572 + 3.2667 \log L \quad (r = 0.8729, \text{sd} = 0.9975)$$

$$\text{or } W = 0.0006963 L^{3.2667}$$

migrant female

$$\log W = -1.4895 + 2.4064 \log L \quad (r = 0.6278, \text{sd} = 0.6669)$$

$$\text{or } W = 0.03289 L^{2.4064}$$

The length-weight relationships for migrant longfin male and female eels is represented graphically in Fig.4.6.

No direct comparisons can be made between the calculated weights of males and females because their size ranges do not overlap. Migrant longfin females show a considerable loss in weight when compared with non-migrant females of the same size. Migrant longfin males also show a small loss in weight when compared with non-migrants of the same size.

Longfin non-migrant male and female, and migrant male and female eels have significantly different subgroup regression

coefficients. Although the migrant male and female eels have different length-weight relationships, the length weight data were combined for the analysis of covariance between migratory states. The length-weight relationships for non-migrant longfins, sexes combined, and migrant longfins sexes combined, can be represented by the equations,

non-migrant

$$\log W = -2.9696 + 3.2234 \log L \quad (r = 0.9850, \text{sd} = 0.0401)$$

$$\text{or } W = 0.00172 L^{3.2234}$$

migrant

$$\log W = -2.6638 + 2.9915 \log L \quad (r = 0.9313, \text{sd} = 0.0619)$$

$$\text{or } W = 0.002169 L^{2.9915}$$

The length-weight relationship for longfin non-migrant and migrant eels is represented graphically in Fig.4.6. It shows that non-migrant longfins are heavier than migrant longfins of the same size. This loss in weight is probably exaggerated by the greater loss in weight by migrant females, but, as noted, migrant males also show a loss in weight.

There was no significant difference between the subgroup regression coefficients for Lake Onoke shortfin migrant male and migrant female eels. The length-weight relationship for all Lake Onoke migrant shortfins can therefore be represented by the equation,

$$\log W = -2.9795 + 3.1165 \log L \quad (r = 0.9411, \text{sd} = 0.0861)$$

$$\text{or } W = 0.001048 L^{3.1165}$$

The length-weight relationship for migrant Lake Onoke eels is represented graphically in Fig.4.7.

4.7. Discussion

The slow and varying growth rates in the New Zealand species shown by this study and that of Burnet (1969b) are similar to the varying growth rates in the European eel in various waters. Frost (1945), discussing the environmental conditions affecting growth of the European eel, suggests that population density and available food supply are factors influencing the growth rate.

Sinha and Jones (1967a) suggest that temperature and day-length may be the limiting factors for growth. Bertin (1956), discussing the influence of sex, temperature, population density and food, concluded that: 'the increase in the size and weight of eels is more a question of food than anything else'.

Insufficient data are available to establish which environmental factors affect the growth rates of New Zealand eels. Burnet (1969b) suggests that as females grow faster than males (Bertin 1956) and that sex determination is probably metagamic, i.e. determined by factors other than the genes, then the faster growing population will contain a higher proportion of females. He suggests that this could explain the growth rate differences between the three streams, the Cust Main Drain, the Doyleston Drain and the South Branch, in his study. For example, the South Branch stream had the slowest growth rate and contained a high proportion of males. In the present study it has been suggested that sex is determined genetically. There is a clear difference in the growth rate between the sexes, particularly in the shortfins, but I am unable to account for this sex difference in growth rate.

Burnet (1969b: 382) also suggests that greater population densities can explain the slower growth rates. I suggest that the differing population densities, available food supply and temperature all influence the growth rate in different waters. However, there are few specific data to support this suggestion. Cairns (1942a), and observations made during this study show that a high proportion of eels had empty stomachs. Further, Cairns (1941) found that eels hibernated during the winter months when water temperatures are low. These observations indicate that population density, food supply and temperature are probably the main limiting factors of growth.

The growth rate of non-migrant eels (Fig.4.4) shows that females of each species above the maximum length of migrant males increase in growth rate. These larger females as shown by the length frequency graphs (Fig.3.10) constitute a small percentage of the total sample. Cairns (1942a), for longfin and shortfin eels, and Burnet (1952a) for longfins, found that there is a change in the feeding habits of these larger eels. It is suggested that the increase

in growth rate in the larger females arises from the change in feeding habits. Because of the fewer eels in this size range there is less competition for the available food.

The range in length and ages of migrant eels and the similar mean lengths of each age class indicates that the migration is dependent on length and not age. The variation in mean ages and lengths at migration for different areas indicates different growth rates in these areas.

Migrant male eels gain little in length by remaining in freshwater, the mean lengths of the higher age groups being similar to the lower age groups and to the mean length of all migrants for that sex. For female migrants however, there is some increase in length in the higher age groups. Frost (1945) similarly found that a minority of migrant females were longer and older than the average migrants. She suggests that there are two types of yellow eels: one, with a minority of eels, grow faster than the second group, which contains the majority of slower growing eels. This faster rate of growth may have been present during the whole life, or may have begun at a certain age and length. These eels remain longer in freshwater, and migrate at an older age and greater length. The second group of slower growing eels migrate at a smaller size and younger age.

Frost (1945) supports her suggestion by comparing the mean lengths of silver and yellow eels of the same age, and found them to be similar, showing that silvering did not occur first amongst the smaller, faster growing yellow eels. Insufficient numbers of eels in each age class were available to make similar comparisons between the mean lengths of migrant and non-migrant females of the same age. Hence Frost's (1945) suggestion cannot be supported by the present study. However, the large size of some non-migrant females in the lower age groups indicates that there is a number of more rapidly growing females, and it would be logical to assume that these eels attain the migrating length earlier and migrate at an earlier age. A minority of slower growing females may not migrate when the optimum length is attained and remain in freshwater for a longer period. The slower growth rate may be associated with some physiological and endocrinological differences which prevent the eels changing into migrants at the optimum length. They therefore remain in freshwater for a longer period. The reproductive cycle of fish is controlled by the endocrine system influenced by the internal state of the fish, and indirectly by environmental factors. The transformation of a non-

migrant to a migrant eel is under the control of this system. If such endocrine activity is associated with slow growth rates, this could explain the extreme age and large size of some non-migrant and migrant longfin females.

No statistical comparisons were made between the length-weight relationships for each species. However the length-weight regression equations for non-migrant eels clearly shows that non-migrant longfins increase in weight more rapidly than non-migrant shortfins, and are heavier than the latter at the same size.

Woods (1964: 103) calculated the length-weight relationship for non-migrant longfin and shortfin eels, and found $n = 3.18$ for shortfins and $n = 3.36$ for longfins, showing that longfins increase in weight more rapidly than shortfins. Burnet (1952a:50) calculated the length-weight relationships for longfin eels from different areas and found that n varied from 3.13 to 3.31. The differences were considered small and Burnet (1952a: 50) suggested that the similarity showed that environmental conditions may not affect the growth pattern. The differences between the values of n were not statistically tested for significance. Shortland and Russell (1948), analysing the length-weight relationship of non-migrant longfins, found a point of inflection at 89cm which they considered was due to the development of the ovary. No similar point of inflection was found in this study, as gutted weights were used. Burnet (1952a: 50) also found no similar point of inflection.

Sinha and Jones (1967a), analysing the length-weight relationship of the European eel, found n varied for undifferentiated and male eels, and for female eels, and concluded that for the former group the similar values of n represented similar growth patterns in different environments.

Frost (1945) calculated the length-weight relationship for European female yellow and silver eels, and found a statistically significant difference between yellow and silver eels 45cm to 70cm in length. The value of $n = 3.4709$ for yellow eels dropped to $n = 2.7628$ for silver eels.

A similar change in the value of n was found in this study for non-migrant and migrant longfin eels. The value of n is often found to differ for different stages of the life history of a fish, and it is not surprising to find a difference between non-migrant

and migrant eels and between the sexes within these groups. Shortfin eels are little more sexually developed than non-migrant eels, and the difference in the length-weight relationship between the sexes and between migratory groups is not significant. The more advanced state of sexual development in migrant longfin male and female eels has altered the length-weight relationship, and a significant difference is found between male and female migrants, and between migrant and non-migrant eels. In migrant longfin females this change in the length-weight relationship is shown by the low value of $n = 2.4064$. It is considered that this considerable loss in weight is due to the development of the ovary. Shorland and Russell (1948: 175) state that longfin eels show a 10% loss in weight during the change from the non-migrant to the migrant stage. They found changes in the weight of oil which included a reduction of the weight of oil in the trunk region and a rise of weight in oil in the ovary. The use of gutted weights in this study excluded the direct effect the inclusion of the weight of the gonad may have on the length-weight relationship. However, the increased development of the ovary in longfin migrant females, by its increased oil content, affects the length-weight relationship as calculated from the gutted weights.

5. INDUCTION OF MATURATION AND GAMETOGENESIS

51. Introduction

The sexual development of New Zealand eels, once they leave freshwater on their journey to the spawning grounds, has not yet been observed. It has been established previously in this study that migrant eels, particularly shortfins, are not greatly sexually advanced. Further sexual development and any associated changes in morphology occur at sea. Only one migrant eel, a longfin female, has been caught at sea (Todd 1973), and this was but slightly more sexually advanced (ova diameter 0.45mm to 0.50mm) than seaward migrating females of that species (ova diameter 0.35mm to 0.45 mm).

The purpose of this section of the study was, by the use of hormone injections, to obtain the complete maturation of male and female eels of both species. By this method the complete process of gonad development could be studied, both macroscopically and by histological methods. Any changes in morphology associated with maturity could be observed and the fertilization of mature ova could be attempted. By combining the results of gonad observations on non-migrant, migrant and hormone injected eels an almost complete account of the reproductive cycle could be obtained.

Several authors have induced maturation in the male European eel, as listed by Boëtius and Boëtius (1967a: 340), using non-piscine conadogrophins. Since, Meske and Cellarius (1973) have induced maturation in male European eels raised from the glass eel stage.

With similar hormones several attempts have been made to induce maturation in the female European eel (Boucher, Boucher and Fontaine 1934; Bruun, Hemmingsen and Møller-Christensen, 1949; Møller-Christensen, Bruun and Hemmingsen 1958; Boëtius, Boëtius, Hemmingsen, Bruun and Møller-Christensen 1962), but only limited increased development was achieved. Fontaine, Bertrand, Lopez and Callamand (1964), using carp pituitary extract, successfully produced the experimental spawning of the female eel and described the mature ovum. No mature males were available to attempt fertilization, but subsequent experiments have produced fertilized ova up to the second cell stage (Fontaine, pers. comm.). Maturation of the Japanese eel has been achieved by Ishida and Ishii (1970) using fish pituitaries and mammalian hormones, and partial

maturation by Ochiai, Umeda, and Ōta (1972) using mammalian hormones. Hansen (1939) unsuccessfully attempted to induce maturation in the American eel using mammalian hormones.

The use of mammalian hormones, mainly chorionic gonadotrophins (CG) and fish pituitary extracts, to induce maturation and spawning in fish for fish culturing purposes is well established, and there are several descriptions of the techniques used. (Ball and Bacon, 1954; Palmer, Burrows, Robertson and Newman 1954; Pickford and Atz 1957; Sneed and Clemens 1959; Clemens and Sneed 1962; Haydock, 1971).

The effects of hormones on the reproduction of fish is described and reviewed by Pickford and Atz (1957); Ball (1960); Hoar (1965, 1969) and Liley (1969). Yamazaki and Donaldson (1968), Pandey (1969a, 1969b) and Liley and Donaldson (1969) describe the effects of mammalian and fish pituitaries on hypophysectomized fish. Ashan and Hoar (1963) describe the effects of gonadotrophic hormones on the threespine stickleback Gasterosteus aculeatus. These experiments were conducted to establish the specific effects of various hormones on the sexual cycle of the fish. As discussed by Ball (1960: 109) and Ashn and Hoar (1963: 1045-6), many previous attempts to induce maturation in sexually immature fish using mammalian hormones and pituitary extracts from closely or distantly related fish species have been conducted without adequate controls. Further, highly purified pituitary extracts have usually not been used and the exact effects of the hormones cannot be determined.

More recent research (Sinha 1971) has been involved in the separation of the fish pituitary fractions possessing the hormonal activity and, as listed by Sinha, several authors have obtained potent gonadotrophic preparations.

For culturing purposes the induced spawning of fish is usually conducted on fish that are near maturity but do not spawn in captivity, or so that the spawning of a large number of fish may be synchronized. In most instances the fish spawn or can be stripped of eggs within a few days.

Eels however, as discussed earlier in this study, are relatively immature when caught migrating to sea. Thus the hormone

induced maturation of eels involves a considerable amount of sexual development before full maturation.

The purpose of this section of the study was not to determine the direct effect of specific hormones on the maturation of eels, but only to use different hormones to obtain maturation so that the sexual development could be observed. Non-injected control eels were used only to determine if any sexual development occurred without the use of hormones.

5.2 Materials and methods

The experiments were carried out from May 1971 to May 1973 at the Marine Laboratory, Victoria University of Wellington.

Migrant eels of both species and sexes, caught in the Makara stream and Lake Onoke during the 1971, 1972 and 1973 migratory seasons, were used for experimental purposes. The methods of capture have previously been described. The sexes of each species were recognized by their size, the smaller migrant eels of each species being males. Longfin females of a suitable size (under 100cm in total body length) were difficult to obtain and keep alive. Only during 1973 were they successfully kept alive and induced to spawn.

Experimental eels were kept in five glass aquaria, similar to that shown in Fig.5.1, and varying in size from 200 litres to 375 litres, through which seawater was circulated. The seawater temperature varied from 9.0°C in the winter to 24°C in the summer. The mean monthly temperature and monthly range for the seawater aquaria during part of the experimental period, from May 1971 to March 1973, is given in Fig.5.2. Most experiments were conducted during the winter in heated tanks at a constant temperature ($\pm 1.5^{\circ}\text{C}$). The water was heated with a 1500 watt immersion heater placed in one end of the aquarium and covered with wire netting to prevent the eels burning themselves against the element. Water was constantly circulating through the aquaria and the temperature was controlled by a thermostat. Some experiments were conducted in aquaria at ambient temperature. Although the yearly temperature range was 15.0°C , the temperature fluctuation during the experiments conducted at ambient temperature was no more than $\pm 3.5^{\circ}\text{C}$.

FIG.5.1

SEAWATER AQUARIA USED FOR THE INDUCTION
OF MATURATION EXPERIMENTS

1. A heated aquarium containing experimental eels. The immersion heater covered with wire netting is seen in the bottom right hand side of the aquarium.

2. The refrigeration unit and aquarium used for experiments below ambient temperature. The larger, outer tank contained freshwater in which part of the refrigeration unit was immersed. The smaller, inner aquarium contained the eels in seawater.

1

2

FIG. 5.2

Mean monthly temperature and range in temperature
for the seawater aquaria during part of the
experimental period from May 1971 to March 1973.

Live eels were put directly into the saltwater aquaria. Some of the shortfin males died within 24 hours, possibly from the effect of sudden immersion in saltwater. However, sufficient numbers remained alive for experimental purposes. Some shortfin males were first placed in an aerated mixture of half salt and half freshwater for approximately 12 hours before being placed in saltwater. This reduced the mortality amongst these eels.

Prior to the injections eels were held in saltwater at ambient temperature. Before being placed in heated aquaria, the eels were acclimatized to the experimental temperature for 48 hours.

To conduct experiments at temperatures below the minimum ambient temperature, a plastic tank containing the eels in aerated seawater was partially immersed in a bath of freshwater that was chilled with a refrigeration unit (Fig.5.1). The seawater temperature was held at $9.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

The oxygen content of the seawater was constantly at approximately 95% saturation, and the salinity 35 ‰. Light conditions were not regulated, the aquaria being exposed to diffuse daylight and fluorescent light. Boëtius and Boëtius (1967a) established that salinity and light did not affect the hormone induced maturation of the male European eel. Consequently in this study these factors were not controlled.

Several different hormones were used in attempts to induce maturation. Male eels were injected with human chorionic gonadotrophin (HCG) and acetone dried pituitaries from mature or maturing carp, Cyprinus sp. (Cyprinidae). Female eels were injected with varying doses of HCG and oestradiol benzoate, a synthetic oestrogenic hormone. Other females were injected with acetone dried carp pituitaries from mature or maturing tarakihi, Cheilodactylus macropterus (Cheilodactylidae), groper, Polyprion oxygeneios (Serranidae), and from carp. Heads from mature groper and tarakihi were obtained from local fishermen and the pituitaries removed and preserved in acetone as described by Haydock (1971). Acetone dried carp pituitaries were obtained from Stoller Fisheries, Iowa, U.S.A., and mammalian hormones from a local agent for the manufacturers, Organon Laboratories Limited.

The experimental procedure in general follows that described by Boëtius, Boëtius et al. (1962), Boëtius and Boëtius (1967a) and Fontaine et al. (1964).

The mammalian hormones were injected intramuscularly at a site in the dorsal muscle mass about the origin of the dorsal fin. Pituitaries were injected directly into the coelomic cavity (after Fontaine et al. 1964). Injections were administered with a 2 ml hypodermic syringe graded into 0.1 ml divisions. For mammalian hormones a 22 gauge, 3cm long needle was used. For pituitaries a larger 20 gauge, 4cm long needle was necessary. Mammalian hormones, except the oestradiol benzoate which was supplied in liquid form (oestradiol benzoate in oil), were available in a freeze dry form, which necessitated the mixing of the dried hormone with a saline solution before injecting. Pituitaries were finely macerated and injected in suspension in a teleost marine (Young) solution (Hale 1958). The larger needle was used to inject the pituitaries to prevent clogging. The dosages and combinations of hormones used are given separately in the relevant sections. Each injected eel was assigned a number, and these are listed in Appendix C with the hormone dosage, experimental life and maturation period.

For injections the eels were anaesthetized in a solution of benzocaine and seawater, following the method outlined in the Materials and methods section. However, care had to be taken not to kill the eels in the anaesthetic solution. They were removed when they had stopped moving, which was after approximately three to four minutes. They were then injected as described. Eels were returned immediately to the aquaria and recovered within approximately two minutes, depending on the temperature. Eels from the cold tank had to be revived at a temperature above that of 9.5°C.

Any colour and morphometric change during development were noted, and the eye-size of eels when anaesthetized was measured. Male eels were killed and examined at various intervals after the date of first injection to observe the development of the testis. No female eels were killed during development because of the limited number of females available. Macroscopic and histological examinations of the gonads were made. The histological techniques used for examination of the gonads is described in the Materials and Methods section.

Male eels were considered mature when milt was exuded from the urogenital opening on stripping. ✓ Eels were stripped when anaesthetized. Females were considered mature when ova were released through the urogenital aperture when stripped, or when

the eel actually spawned. The maturation period is defined as the number of days between the date of the first injection and the date of maturation. When maturity is approaching, which could be observed from the increasing girth of the eel, male eels were stripped twice a week, and the maturation period could be calculated with an error of about ± 1 day. Female eels were injected three times per week with pituitary extract and the maturation period could be calculated at ± 1 day.

Non-injected control eels were held in the same tanks as injected eels to determine if any sexual development occurred without hormonal stimulation. Insufficient numbers of longfin females were available to be kept as controls but four eels that died after being held in saltwater for two and a half months before injections could be administered were used as controls.

For identification individual eels were fin clipped. Reclipping was frequently necessary because of the rapid regeneration of cut fins.

Hormone dosages and action of hormones

The hormone dosages were calculated from those given to the European eel by Boëtius, Boëtius et al. (1962), Fontaine et al. (1964) and Boëtius and Boëtius (1967a). Allowances were made for the greater body weight of the New Zealand eels.

The dosages of HCG were measured in international units (IU), dosages of oestradiol benzoate and pituitaries were measured in milligrams (mg).

For the male European eel Boëtius and Boëtius (1967a) used a standard dosage of 250 IU of HCG injected weekly until maturity was reached. These authors established that for males with an average weight of 83g, single dosages of up to 100 IU of HCG did not produce complete maturation. A minimum of three 250 IU doses at weekly intervals were needed to produce maturation. The shortfin males used in this study weighed up to 190 gm with an average weight of 126gm SD ± 30.3 gm. Instructions on the dosages of HCG for veterinary purposes showed that the dosage is dependent on the body weight of the animal. However, although the average weight of the experimental male shortfins was almost twice that of the experimental European male eel a dosage of 250 IU was considered sufficient. The male longfins however were considerably heavier, the average

weight of the experimental eels ($535.7 \text{ gm SD} \pm 116.5 \text{ gm}$) and it was therefore considered necessary to increase the dosage. To determine the optimum dosage for longfin males, three eels (eels No 1, 2 and 3) were injected weekly with 250 IU, 500 IU and 750 IU of HCG at 12°C . The eel injected with 500 IU weekly matured first in 51 days, followed by the eel given 750 IU weekly in 58 days. The eel injected with 250 IU weekly matured last in 75 days. A weekly dosage of 500 IU of HCG until maturity was reached was therefore used as the standard dosage for longfin males. However, this experiment to determine the optimum dosage is not considered to be conclusive. Subsequent experiments showed that at temperatures of 12°C and 15°C the variation between the maturation periods for different male longfins was as large as for the experiment to determine the optimum dosage. Nevertheless the dosage of 500 IU weekly for longfin males, although not in direct proportion to the greater weight than shortfins, was considered to be an appropriate dosage. Further, larger dosages would have greater quantities of hormone, making the cost of the experiment prohibitive.

Thus the standard dosages for male eels injected with HCG were 250 IU for shortfins and 500 IU for longfins, injected at weekly intervals until maturity was reached.

The dosages of HCG and oestradiol benzoate for females were based on the dosages used by Boëtius et al. (1962) for the female European eel. Varying dosages of 250 IU to 1000 IU of HCG in combination with 2 mg of oestradiol benzoate were injected at weekly or three weekly intervals. The exact dosages for individual eels is given in the section describing the injection of female eels with mammalian hormones.

Pituitaries were injected at the dosage of 2mg of dry pituitary powder per 100gm of the initial body weight of the eel. Injections were given three times per week until maturity was reached. Pituitary injections were given to male shortfins and female longfin and shortfin eels. The numbers of eels of each sex and species injected with either HCG or pituitaries is given in Table 5.1. The exact dosages for each eel is given in the relevant sections and Appendix C.

The purpose of these experiments was to induce maturation in the eels and to observe the process of maturation of the gonads.

It is beyond the scope of this study to discuss in detail the internal mechanism by which injections of the various hormones induce sexual maturation.

Boëtius and Boëtius (1967a: 363), after hypophysectomy, induced normal maturation with HCG in male European eels, indicating that the action of the HCG was direct and not acting via the pituitary. The effect of HCG is usually considered to be similar to that of lutenizing hormone (LH) (Pickford and Atz, 1957: 201; Boëtius and Boëtius 1967a: 363; Hoar 1969: 50) which in tetrapods stimulates ovulation and the formation of corpora lutea in females and the secretion of androgen in males. Many of the experiments using HCG, particularly for fish culturing purposes as discussed, have been conducted on fish that were mature or nearly mature. However, the experimental evidence given by Boëtius and Boëtius (1967a: 363) and this study, in which complete maturity and spawning males was induced by HCG, indicates, as suggested by Boëtius and Boëtius, that HCG also exhibits follicle stimulating hormones (FSH) like properties.

The experimental evidence from hypophysectomized fish, summarised by Pickford and Atz (1957: 178 - 203) and Ball (1960: 107), similarly shows that the pituitary injections act directly or at least not via the pituitary.

In tetrapods the two separate pituitary gonadotrophins FSH and LH are physiologically distinct (Hoar 1969: 48). The experimental evidence discussed by Hoar (1969: 48 - 50) suggests that in fish there is only one pituitary hormone, which exhibits principally LH properties but also contains some FSH like properties.

Thus it is considered that the gonadotrophin more closely resembles LH but it also contains other physiological properties.

5.3 Mortality, general behaviour and spawning behaviour of experimental eels

No accurate count was kept of the number of migrant eels held in the holding tanks prior to injections, but it was estimated that the mortality rate was approximately 10% for males of both species, and 20% and 50% for shortfin and longfin females respectively. No satisfactory explanation can be given for the higher mortality rate amongst females but it is suggested that their larger size,

particularly longfin females, in the size of the aquaria available may have been a contributing factor.

Most of the eels died from gas embolism. Externally the symptoms were small bubbles under the skin along the margin of the fins, mainly in the tail region. Internally the blood vessels were full of gas bubbles. The exact cause of the embolism could not be established, but it was possibly due to a gaseous imbalance in the water. However, increased aeration and water flow, and reduced aeration and water flow, appeared to have no effect. This type of mortality occurred on two specific occasions and most of the eels died within one week.

Other eels died from a fungal infection that first attacked the dorsal surface and then spread over the rest of the body. Few eels died from this infection. If the aquaria were kept clean and the body surface of infected eels scraped clean the eels usually survived.

In total 85 eels were injected with hormones. The number of eels of each sex and species injected, and the number that matured, died or were killed for gonad analysis is given in Table 5.1. The causes of death of the few hormone injected eels that died before maturation were ulcerations over the body, swelling of the body and gas embolism. Of the four male eels, three longfin and one shortfin, that were observed spawning, three died within 12 hours. One longfin (eel no.8) survived spawning. Males that matured but did not spawn lived for varying periods. The cause of death of these eels could not be established. The females injected with mammalian hormones died before complete maturation was achieved. Most of these died after an experimental life of 102 days to 108 days. Neither the exact cause of death nor the reason for the similar experimental life of these eels could be determined.

All pituitary injected females except one died immediately after reaching maturity or spawning. One shortfin (eel no.66) matured but survived. It was later killed for examination.

Generally all eels were inactive during the day, hiding in earthenware pipes placed in the bottom of the aquaria, or burrowing in stones surrounding the heating elements. Any swimming activity was slow and the eels appeared to glide around the aquaria. Activity increased at dusk, particularly on stormy overcast nights during the migratory season. At night the eels were extremely sensitive to light. If the light was suddenly turned on at night the eels

TABLE 5.1

Number of eels injected with hormones
and mortality rate

	<u>A. australis</u>		<u>A. dieffenbachii</u>	
	<u>male</u>	<u>female</u>	<u>male</u>	<u>female</u>
No. injected with mammalian hormones	33	8	28	3
No. injected with fish pituitaries	2	7	0	3
No. that died during the experimental period	4	11	5	4
No. killed for gonad analysis.	5	0	7	0
No. that matured	27	5	16	2
Total no. injected	36	15	28	6
Mortality rate (%)	11	68	18	66

would erupt into a frenzy of activity. A few hormone injected eels showed abnormal activity by swimming ceaselessly around the aquaria during the day and hitting the sides, but this behaviour was unusual. Swimming activity was clearly related to temperature. Eels below 12°C were noticeably less active than eels at higher temperatures.

Only four male eels (nos 1, 6, 8 and 30) and two females, one shortfin and one longfin (nos 65 and 67) were observed spawning.

The spawning of males of each species was unobtrusive. The eel would either lie upright on the bottom of the aquarium or hang suspended in the water in an inclined position with the head towards the surface. White milt was emitted from the urogenital opening in a continuous stream for periods of about five minutes, with intervals of several minutes to approximately one hour between emissions. The whole spawning process lasted approximately six hours, although one male longfin emitted milt at intervals for 12 hours. Milt rapidly dispersed in the water and did not appear to affect the other eels. As the spawning of males was remarkably unobtrusive it is likely that other eels that matured may have spawned unnoticed.

The spawning behaviour of females was more obvious, partly because of their larger size. They either swam around the tank emitting ova, or lay, sometimes ventral surface uppermost, in an inclined position with the head towards the surface. From the urogenital opening ova were emitted in small clusters which immediately dissociated in the water. Large numbers of ova were distributed throughout the water depending on their state of maturity. Ripe, transparent ova floated to the surface, and partially ripe ova floated in suspension in the water. Immature ova with opaque white yolk sank to the bottom. Those from one longfin female turned the water opaque and completely covered the surface of the water in the aquarium. The eels were not active when spawning but they did appear to be under some stress, as indicated by the rapid respiration rate and the upside-down position sometimes assumed.

No difference was observed between the spawning behaviour of each species. Mature males, present in the aquarium when females of each species spawned, showed no interest in the spawning activity of the females.

The spawning behaviour of the eels is similar to that described by Fontaine (1936: 1313) and Fontaine et al. (1964), for the male and female European eel respectively. For the male European eel, Fontaine noted that the spawning process lasted for 24 hours and the eels then died within 12 to 24 hours after the last emission of milt.

The inclined position of the spawning female European Fontaine et al. (1964) is similar to that observed in some instances for spawning longfin and shortfin eels. Fontaine et al. note that this behaviour is similar to that observed in mature conger eels, Conger conger L. (Congridae).

Boëtius and Boëtius (1967a: 352) could not determine any special behaviour of mature European male eels that could be interpreted as spawning behaviour. As noted, the spawning of male longfin and shortfin eels was unobtrusive and little different from normal activity. Nevertheless, although the spawning process has few special behavioural patterns different from normal activity, it has been interpreted as spawning behaviour so that a definite distinction can be made between spawning and normal activity. However, it is not suggested that the spawning behaviour of male and female eels as observed in the aquaria can be interpreted as being similar to that occurring in nature.

It is generally assumed that eels in nature die soon after spawning. This is indicated by the death of conger eels after spawning, and the fact that adult freshwater eels do not return to freshwater after having migrated to the sea to spawn.

In this study four males were observed spawning, three of which died within 24 hours. The third survived and lived for a further 115 days. In addition, of the eels that matured, some of which may have spawned unobserved, 13 survived for more than 200 days. The two females that spawned died within 48 hours, but one shortfin female that matured but did not spawn survived.

Fontaine (1936: 1313) records that the male European eel died after spawning. From the date given by Fontaine et al. (1964) it is apparent that the European female eel survived spawning but was later killed for examination. Boëtius and Boëtius (1967a: 352 - 4) found that the experimental life of hormone injected eels did not differ essentially from that of the non-injected controls. However, they give no data on the exact survival times of eels that spawned. They consider that as the testes in eels that have survived maturation

return to the Syrski or early stage of development, this raises the question as to whether eels in the wild spawn more than once. The survival of the female European eel after spawning (Fontaine et al. 1964), and the survival of the male longfin after spawning in this study, similarly indicates that eels may survive spawning in the wild. Further, as described later in this section, the testes of male longfin and shortfin eels that survived maturation returned to the earlier pre-migrant or migrant stage of development. In two male eels, one longfin and one shortfin, maturation was induced for a second time after the gonads had returned to the early stages of development. This clearly indicates that eels in nature, at least as far as males are concerned, may spawn more than once. However, the survival of eels after maturation or spawning, and the induced maturation for a second time, all in an artificial environment, should not be regarded as sufficient proof that eels in the wild survive spawning, and may even spawn a second time.

5.4 Morphometric changes in hormone injected eels

No colour changes from the migrant stage previously described were observed in mature eels. This is contrary to the colour changes observed in maturing European eels described by Boetius et al. (1962: 197). Other changes however were evident. The head and snout of mature eels becomes more narrow and pointed, and the lower jaw, particularly in males, appeared smaller and receded under the upper jaw. The pectoral fins in shortfin males, if not previously black and pointed as in some migrants, appear this colour and shape. General body proportions change significantly. The increased volume of the gonad, particularly in mature females, greatly distends the gut region, making the eel appear quite fat. In mature females the tail region becomes laterally very thin. Mature whole and dissected eels are shown in Figs. 5.3 and 5.4.

Internally the gut was greatly reduced in mature eels, and there was a complete absence of fat deposits. The body wall around the gut region was noticeably thin, compared with the firm, thick flesh in the same region of migrant eels.

Hormone injected male eels showed a considerable weight loss during the experimental period. The greatest weight loss

FIG. 5.3

MATURE AND MIGRATORY EELS

1. From top to bottom: Mature male A. dieffenbachii
(longfin) 65.5cm total length

Mature male A. australis
(shortfin) 42.2cm total length

Migrant male A. australis
40.5cm total length

2. From top to bottom: Mature female A. australis
70.0cm total length

Migrant female A. australis
76.5cm total length

1

2

FIG.5.4

MATURE FEMALE CARP PITUITARY INJECTED EELS

1. Mature A. australis (shortfin) 75.5cm total length
2. Mature A. dieffenbachii (longfin) 98.0cm total length and 101.0cm total length

1

2

occurred in eels that survived the longest. Three male eels (eel nos 2, 3 and 25) which survived for periods of 427 to 534 days showed weight losses of 44%, 50% and 43% respectively.

Hormone injected female eels on the other hand showed a weight increase during the experimental period, with the greatest weight gain occurring at maturity. In females injected with mammalian hormones one eel (eel no. 50) showed a weight gain of 36%. Carp pituitary injected females showed maximum weight gains of 21% for a shortfin (eel no. 83) and 26% for a longfin (eel no. 96). The smallest weight gain was 6% (eel No. 84). Only one female (eel no. 66) that survived maturity and in which the gonads regressed, showed a weight loss of 4%.

The weight increase in the females was probably due to hydration, which also occurs in other species of fish which are induced to spawn with hormone injections. For example Haydock (1971) describes hydration of the gulf croaker Bairdiella icistia (Jordan and Gilbert), which occurred immediately after injection in 7 to 30 hours and preceded ovulation. However, the hydration observed in the eels occurred over a longer period of time, up to 30 to 40 days in some eels.

The most obvious morphometric change in mature eels was the increase in eye size. A detailed description and discussion on the enlargement of the eye during the metamorphosis from the non-migrant to migrant stage for both species has been given in the migrant eel section. The relationship between the total length and the eye-index of non-migrant, migrant male and female, and mature male and female eels of both species is given in Fig.5.5. For mature eels there was only sufficient data to calculate the regression equations for the eye-index on total length for males of each species.

Table 5.2 gives the regression equations, calculated by the method of least squares, for the regression of eye-index (mm^2), y , on total fish body length x , for mature male longfin and shortfin eels. Table 5.2 also includes the eye-index and factor of increase of eye-index for non-migrant, migrant and mature males of a selected length for each species. The eye-index for non-migrant and migrant males were calculated from regression equations given in Table 3.17.

FIG .5.5

Regression of eye-index (mm^2) on total length (cm)
for non-migrant, migrant male and females and mature
males A. australis (shortfin) and A. dieffenbachii
(longfin) eels. Individual values of the eye-
indices for mature females are also plotted.

TABLE 5.2

Eye size of non-migrant, migrant and mature eels

		Eye-index (mm^2)	Factors of increase of eye-index	
<u>A. australis</u>				
Non-migrant eel	45 cm	22.7	} 2.1 } 1.5	} 2.9
Migrant eel	45 cm	45.2		
Mature eel	45 cm	66.0		
<u>A. dieffenbachii</u>				
Non-migrant eel	65 cm	50.6	} 2.1 } 1.2	} 2.4
Migrant eel	65 cm	98.8		
Mature eel	65 cm	120.7		

Regression equations of eye-index (mm^2) y on total length (cm) x for
mature male eels of both species

A. australis schmidtii $y = 1.709x - 10.86$
 $r = 0.59^*$
 $n = 25$

A. dieffenbachii $y = 2.268x - 26.28$
 $r = 0.577^x$
 $n = 16$

* significant at 1% level

x significant at 5% level only

Table 5.2 shows that there is a smaller increase in the eye size from the migrant to the mature stage for each species than there is from the non-migrant to the migrant stage.

Overall, from the non-migrant to the mature stage, shortfins show a greater increase in eye-index than longfins. During the metamorphosis from the non-migrant to the migrant stage both species show a similar increase in eye-index. During maturation however shortfins show a greater increase in eye-index from the migrant to the mature stage.

The increase in eye size of mature eels is similar to that described for the mature male European eel (Boëtius and Boëtius 1967a). They calculated that the increase in eye size from the yellow to the silver eel stage was greater than the increase from the silver eel to the mature eel. In total, from the yellow eel to the mature eel, the eye size increased by a factor of 3.9, greater than for either longfin or shortfin eels.

During the post maturation stages Boëtius and Boëtius (1967a: 356) observed a decrease in the eye size accompanying the regression of the gonads. In this study no reduction in the eye size was observed in eels that had matured and in which the gonads were regressing.

In the Japanese male eel Boëtius and Boëtius (1967a: 380) observed no change in the eye size of hormone injected eels over that of the non-injected eel controls.

Only limited increase in the eye size of female European eels in which increased sexual development had occurred is reported by Boëtius et al. (1962: 195 - 196). However, Fontaine et al. (1964) noted that the eyes of the mature female European eel that spawned were greatly enlarged. In this study some increase in the eye size of mature females of both species was observed, but the data was insufficient to calculate the factors of increase in eye-index or the regression of eye-index on total length.

5.5 Changes in the testis associated with maturation

The appearance and size of the gonads in the non-migrant and migrant stages have been described. Considerable change in the appearance and size of the gonads occurs during maturation.

Male eels were killed at various stages of development

to observe changes in the gonad, both macroscopically and histologically. Insufficient female eels were available to kill eels at various stages of development.

The development of the testes in both species is similar, except for the greater size of the testes lobes in longfins. In mature males the gonad lobes are large and white in colour (Fig.5.3), in contrast to the smaller pink or colourless lobes in migrant eels. The vas deferens is prominent and full of spermatozoa. During development from the migrant to the mature stage the width of the gonad lobes and the vas deferens increase. In shortfin males the maximum gonad width attained was 19mm and the vas deferens width 6mm. Mature male longfins attained a gonad width of 26mm and the vas deferens 8mm.

The ovaries in mature females appear as a pale pink gelatinous mass (Fig.5.3). Individual ova could be distinguished with the naked eye. The gonad width of mature females could not be accurately measured because the ovaries lose their structure as maturation proceeds, and at maturity each ovary dissociates as ovulation occurs.

The development and regression of the testes in male eels can be divided into several convenient stages. Boëtius and Boëtius (1967a: 346 - 349) describe seven stages for the complete cycle of development in the male European eel. This cycle was determined from observations on a large number of eels at different stages of development. In this study insufficient eels were available to establish a similar cycle. The state of development was also different in the migrant stages to make direct comparisons with the seven stages possible. However, the different stages of development can be conveniently divided into five stages, which correspond to some of the stages given by Boëtius and Boëtius.

The gonad stages are described separately below and include stages of development observed in non-migrant and migrant eels. Histological details of the various cell stages is given in the description of gametogenesis. These gonad stages are distinct from the gonad stages describing the development of the gonads in immature, non-migrant eels. (Fig.2.1).

Fig.5.6 gives a schematic reconstruction of the development and regression of the testes from ^{the}organ of Syrski in the non-migrant eel to the mature and regressed testes observed in hormone injected eels.

vd—vas deferens

connective tissue

spermatic tissue

spermatozoa

Gonad stage 1

This stage is observed in non-migrant males and some migrant shortfin males. The lobes of the testis are fully differentiated into the organ of Syrski. Histologically the gonad is differentiated (Fig.5.7, 1). Clusters of spermatogonia are grouped into a distinct tubular structure and each tubule is surrounded by a band of connective tissue. The vas deferens is well defined but not open. Boëtius and Boëtius describe this stage as characteristic of non-injected control eels so it can therefore be considered representative of migrant European silver eels. The gonad at this stage in the European eel is only slightly differentiated, with spermatogonia in clusters separated by connective tissue. Although the tubular structure of the gonad is not developed, this stage in the European eel is not far removed from that for non-migrant longfin and shortfin male eels.

Gonad stage 2

This stage is observed only in migrant eels. The tubular structure is well developed, and within the tubules in shortfin male migrants spermatogonia are predominant, with some primary spermatocytes present (Fig.5.7, 2). The vas deferens is well developed but in most specimens the lumen is closed. In those eels in which the tubules were observed opening into the vas deferens isolated spermatids and spermatozoa were observed.

In longfins the tubules contain predominantly primary spermatocytes with some spermatogonia. A few specimens contained spermatozoa in the lumen of the tubules. In the majority of eels the lumina of the tubules were observed opening into the vas deferens, the lumen of which contained small clusters of spermatids and spermatozoa.

This stage occurs naturally in longfin and shortfin eels but the similar stage in the European eel is only observed at about 50 days after the first injection. Boëtius and Boëtius (1967a: 381), from a photograph of the gonads of a male eel published by Cairns (1942b: 174B), concluded that the testes of both species of New Zealand eels were near that of gonad stage 3 (but within gonad stage 2).

This would be partially correct for migrating longfin males but incorrect for migrating shortfin males. The latter could only be described as being at the beginning of stage 2. As previously discussed in the migrant eel section, Cairns (1942b: 174B - 175B)

FIG. 5.7

TRANSVERSE SECTIONS OF THE TESTES

1. Non-migrant A. dieffenbachii (longfin) 56.4cm total length, to show the beginning of the arrangement of the testicular elements into a tubular pattern. x 500

2. Migrant A. australis (shortfin), 42.1cm total length, to show the tubular arrangement of the testis. Each tubule, bounded by connective tissue, contains spermatogonia and isolated spermatocytes. x 250

3. Hormone (HCG) injected A. australis (eel no.20), 42 days after the first injection. The tubules have greatly enlarged and are beginning to break down. The lumina of each tubule is filled with spermatozoa. The inter-tubular connective tissue is greatly reduced and only spermatocytes, spermatids and spermatozoa are present. x 250

4. Hormone (HCG) injected A. australis (eel no.22) 63 days after the first injection, to show the complete breakdown of the tubular structure of the testis. Isolated groups of spermatocytes and spermatids are present. x 250

it - intertubular connective tissue

l - lumen of tubule

s - spermatozoa

sc - spermatocytes

sp - spermatids

1

2

3

4

did not differentiate between the species in his photograph or description of the migrant eel testis, and Boëtius and Boëtius assumed the photograph represented the normal developmental stage for both species at migration.

Gonad stage 3

This is the stage of maturity when spermatozoa can be stripped from the eel and it is only obtained by injecting the eels with hormones. The time taken to reach this stage depends on the temperature. The relationship between maturation period and temperature is discussed separately.

The gonad lobes are large and white. Histologically (Fig.5.7, 3) the tubules are enlarged with the lumen full of spermatozoa. A few isolated spermatogonia may be present around the outer margin of the tubule but the predominant cell types are primary spermatocytes, spermatids and spermatozoa. The intertubular connective tissue is reduced. The vas deferens is open and spermatozoa fill the lumen.

This stage is similar to stage 3 described by Boëtius and Boëtius. The essential difference is the time taken to reach this stage. At 20°C it varies from approximately 26 days to 33 days, whereas for New Zealand eels at the same temperature it varies from 23 days to 30 days for longfins and 31 days to 40 days for shortfins.

Gonad stage 4

This is the stage of maximum development. The gonad lobes obtain their maximum width (19mm in shortfins, 26mm in longfins) and the vas deferens is wider and packed full of spermatozoa. The maximum gonadosomatic index is attained at this stage. This stage is reached in approximately 135 days and may last for approximately 250 days. The eels appear fat at this stage with distended gut regions. Spermatozoa are released when the eel is stripped.

In testes at various phases of development in this stage the progressive breakdown of the tubules can be observed (Fig.5.7, 3 and 4). At first the tubules, outlined by thin strands of intertubular connective tissue, can be distinguished. Small clusters of primary spermatocytes and spermatids are closely associated with the connective tissue (Fig.5.7, 3). The rest of the testis is packed with spermatozoa. As development proceeds the connective tissue strands break down and only small remnants are seen scattered amongst

the mass of spermatozoa (Fig.5.7, 4). Few spermatocytes and spermatids are present. The vas deferens is distended and packed full of spermatozoa. Histologically it is difficult to distinguish the vas deferens from the lower portion of the testis.

This stage corresponds to stages 4, 5 and 6 described by Boëtius and Boëtius. Their stage 4 corresponds to the beginning of stage 4 in this study, except that only spermatozoa are present. Stage 5 includes the maximum expansion of the vas deferens and a reduction in the size of the testes lobes. The mass of the spermatozoa are now stored in the lumen of the vas deferens. The vas deferens contracts and the lobes decrease in size. This latter stage was not observed in this study. Stages 4 and 5, although observed, were not considered to be sufficiently different to separate into two stages. Further, insufficient eels were observed to warrant a division of gonad stage 4 into separate stages.

Gonad stage 5

This is the stage of regression and recovery to pre-migrant or migrant stage. The gonad lobes and vas deferens progressively reduce in size, and eventually cannot be distinguished by colour or size from the pre-migrant or migrant stage. This stage is reached after approximately 200 days to 438 days. The regression and recovery is more rapid at higher temperatures, above 20°C. The eels do not appear fat and will not yield spermatozoa when stripped.

Histologically the connective tissue is observed regenerating (Fig.5.8,2) Phagocytes appear, and there is a progressive reduction in the amount of spermatozoa (Fig.5.8,1). The connective tissue regenerates, and a tubular structure develops (Fig.5.8,3). Small pockets of spermatozoa persist and spermatogonia begin to appear, closely associated with the connective tissue of the developing tubules. The spermatozoa remnants eventually disappear, the spermatogonia increase in number and the tubular structure becomes well developed (Fig.5.8,4). At this stage it is difficult to distinguish the gonad from the pre-migrant or migrant stage of either species (Fig.5.8,4).

FIG.5.8

TRANSVERSE SECTIONS OF THE TESTES
FROM HORMONE INJECTED EELS

1. Mature A. australis (shortfin, eel no.4), 286 days after the first injection, to show the appearance of the phagocytes. x 250

2. Regressing A. dieffenbachii (longfin, eel no.7) 136 days after the first injection to show the regeneration of the intertubular connective tissue. x 250

3. Regressing A. dieffenbachii (eel no.3) 427 days after the first injection, to show the reformation of the testis into the tubular arrangement. An isolated group of relic spermatozoa remain. The developing tubules contain spermatogonia. x 250

4. Recovered A. australis (eel no.56) 294 days after the first injection to show the complete recovery of the testis to the migrant stage. A few relic spermatozoa are present in the lumina of the tubules. x 250

it - intertubular connective tissue
l - lumen of tubule
ph - phagocyte
s - spermatozoa
sc - spermatocytes
sg - spermatogonia

1

2

3

4

The gonadosomatic index progressively reduces during this stage, and in eels that have gonads similar to pre-migrant or migrant stage the GSI value is slightly below that for migrants of that species. The GSI value for hormone injected eels is discussed separately.

Two eels, one longfin and one shortfin (eel nos 2 and 25) were presumed to have reached gonad stage 5 in 438 days and 337 days respectively. These eels appeared thin and did not yield spermatozoa when stripped. A second series of injections induced maturation for a second time but the eels died within two days. At death the gonads had reached the beginning of Stage 3 (Fig.5.9,1). This induction of maturation for a second time is discussed separately.

Gonad stage 5 corresponds to gonad stage 7 described by Boëtius and Boëtius, a brief description of which includes the resorption of the spermatogonia and the regeneration of the spermatogenic tissue (spermatogonia). However no mention is made of any phagocytic like cells as observed in this study.

No definite sequence of stages of development in the ovaries in experimental female eels could be identified. In the eels that died during development it was observed that the ovaries increased in width, and in mature eels completely filled the body cavity, distending the gut region. The well defined pleated appearance of the ovaries in migrant eels was progressively lost and the ovary at maturity became a loose gelatinous mass (Fig.5.3). The degree of ovarian development achieved with different hormone injections is described separately. Only one eel (eel no. 66) showed any regression of the ovary after maturity had been reached. The ovaries were reduced in size, white, and had a fibrous texture. Histologically they showed a large number of resorbing atretic oocytes (Fig.5.9,2). These oocytes are described in detail in the section on gametogenesis.

5.6 Gonadosomatic indices (GSI) of hormone injected eels

Males

At migration the mean GSI value for shortfin migrant males is 0.31 and for longfins 1.41. During maturation the GSI value increases rapidly to reach a maximum at gonad stages 3 and 4. As the gonad regresses the GSI value decreases to a value slightly lower than that at migration. The maximum GSI value for a mature shortfin male was 22.0 (eel no. 87). Thus the GSI increases by a factor of

FIG. 5.9

1. Transverse section of the testis from eel no.2 (A. dieffenbachii, longfin) that was induced to mature for a second time. x 250

2. Transverse section of the ovary from an A. australis (shortfin, eel no.46) injected with mammalian hormones. One oocyte is in an advanced stage of vitellogenesis. The other oocytes (atretic oocytes) are being resorbed. x 75

at - atretic oocyte

vt - oocyte in vitellogenesis

s - spermatozoa

sc - spermatocytes

1

2

64 from the migrant to the mature stage. The maximum GSI attained by a longfin male was 17.0 (eel nos 7 and 52). This represents an increase by a factor of 14 from the migrant stage.

In contrast, the maximum GSI value recorded for a mature European male eel was 13.9% (Boëtius and Boëtius 1967a: 350). These authors conducted an experiment with a number of eels at the same temperature to determine the weight change of the testes during development. In this study insufficient numbers of male eels were available to conduct a similar experiment. However, the increase in GSI during development could be determined from the eels that died or were killed during development, during the experiments conducted to determine the maturation period in relation to temperature. Fig. 5.10 shows the increase in GSI during development at temperatures from 15°C to 23.7°C.

Gonad development, as shown by the increasing GSI values, increases rapidly during the maturation period, but the maximum GSI occurs after maturity has been reached. The period of maximum development attained in gonad stage 4, as shown in Fig. 5, shows that it may last for a considerable time after maturity has been reached. This phase of development may last up to 138 days in longfins and 109 days for shortfins, and persisted longer in eels at lower temperatures. However eel no. 7, a shortfin was an exception, which, at a temperature of 23.5°C, reached the maximum GSI value (22.0) in 203 days.

Following the long period of maximum development (Fig. 5.10) the GSI value rapidly decreases during gonad stage 5 as the gonad regresses.

Females

Migrating shortfin females have a mean GSI value of 3.82 and migrant longfin females 8.6. During maturation there is a considerable increase in the GSI. At maturity, in carp pituitary injected females, the GSI value reached a maximum of 45.0 in shortfins and 30.23 in longfins. In shortfins this represents an increase by a factor of 10 - 12 and in longfins by a factor of five to six, from the migrant to the mature stage. Table 5.3 gives the GSI values for females injected with mammalian hormones and carp pituitaries. The GSI values varied accordingly to the dosages and type of hormone used. This is discussed in more detail in the relevant sections. The highest values for the mammalian hormone injected eels of both species approximate that obtained at maturity with carp pituitary injections.

FIG. 5.10

The increase in the gonadosomatic index during the development of the testes in hormone injected A. australis (shortfin) and A. dieffenbachii (longfin)

TABLE 5.3

GSI values for hormone injected females

	Eel no.	Hormone	Experimental life (days)	GSI
<u>A. australis</u>	37	Mammalian	62	11.62
	38	"	102	9.66
	43	"	50	5.85
	45	"	105	25.3
	46	"	105	13.77
	49	"	108	21.0
	50	"	105	40.85
	51	"	105	44.14
	64	Carp pituitary	80	22.5
	65	"	41	45.0
	66	"	147	9.0
	82	"	4	6.0
	83	"	31	31.3
	84	"	31	14.63
<u>A. dieffenbachii</u>	36	Mammalian	100	28.77
	39	"	117	6.48
	44	"	19	5.03
	96	Carp pituitary	25	30.23
	97	"	26	20.23

However, the former eels, although showing a considerable increase in development, were not fully mature, and the high HSI values were reached in a longer period of time than in carp pituitary injected eels.

Once ovulation had occurred in the carp pituitary injected eels the GSI value decreased rapidly. This may account for the lower GSI values recorded for mature longfin females. One of these spawned and the other was stripped of some ova before they died. It is therefore assumed that the GSI value for longfin females prior ovulation is larger. One shortfin female (eel no. 66) matured but did not spawn. It was killed after 147 days and the ovaries had regressed, as did the testes in males. The GSI value was greatly reduced to nine.

The GSI value for the mature European female eel is 31.8% (Fontaine et al. 1964). This eel had spawned and therefore the GSI would have been greater just prior to ovulation. Fontaine (pers. comm.) has since obtained GSI values of greater than 40%. These values for the GSI of mature female European eels are similar to that for mature longfin and shortfin females. Unfortunately no data published in English was available on the GSI value of the mature Japanese eel described by Ishida and Ishii (1970).

5.7 Effect of temperature on the maturation period in male eels

A series of experiments, involving the injection of male eels at temperatures varying from 9.5°C to 28.0°C, was conducted over a period of two years to determine the effect of temperature on the maturation period. The method of cooling and heating the aquaria has been described. Prior to the injections the eels were held in holding aquaria at ambient temperature. Before experiments in either heated or cooled water were begun, the eels were acclimatized by slowly raising or lowering the water temperature, over 48 hours, to the required temperature. Injections were started a week later.

Tables 5.4 and 5.5 list the eels injected, the temperature and maturation period. The longfin eels injected with different dosages are also included. Eels were injected at different times of the year, from May to October 1971, and from July 1972 to January 1973. That is, from immediately after the migration period and during the winter, and during the spring and summer when eels are presumed to spawn at sea.

Four eels, two longfins and two shortfins (eels nos. 75, 76, 78 and 79) were injected after being acclimatized to a temperature of

TABLE 5.4

Relationship between temperature and maturation period

A. australis

Eel no.	Mean daily T. °C ($\pm 1.5^{\circ}\text{C}$)	Maturation period
78	9.7	-
79	9.7	-
4	11.9	93
6	12.0	100
80	12.8	90
57	14.3	61
55	15.0	74
56	15.0	105
9	17.2	47
61	18.6	40
63	18.6	46
20	20.0	40
21	20.0	35
22	20.0	35
23	20.0	40
24	20.0	39
25	20.0	35
31	20.0	40
62	20.0	31
87	23.5	31
88	23.5	36
90	25.7	31
91	25.5	23
93	28.0	23
94	28.0	23
95	30.0	-

(- did not mature)

TABLE 5.5

Relationship between Temperature and maturation period

A. dieffenbachii

Eel no	Mean daily T. °C ($\pm 1.5^{\circ}\text{C}$).	Maturation period
75	9.5	-
76	9.8	-
2	11.8	75
3	12.0	58
1	12.6	51
60	14.1	42
52	14.3	53
53	14.3	66
54	15.1	23
7	17.0	44
8	17.0	44
29	20.0	30
30	20.0	30
58	20.1	23
59	20.1	28
85	23.7	23
86	23.7	20
89	25.9	24
92	28.0	-

(- did not mature)

9.5°C. The two longfins died after 20 days and 90 days respectively from the date of the first injection. Neither showed any gonad development beyond the migrant stage. One shortfin was killed after 136 days and it also showed no gonad response to the hormone injections. The second shortfin (eel no. 79) showed no outward signs (increasing fatness) of approaching maturity and it was transferred to an aquarium at ambient temperature (20°C). With no further injections it matured in 30 days.

The experiments at between temperatures of 11.8°C and 15.0°C were conducted in aquaria at ambient temperatures and at different times of the year. The daily temperatures recorded did not fluctuate more than 2°C during the maturation periods. The maturation temperature was calculated from the daily temperatures recorded during the maturation period for each eel.

The lowest temperature at which maturation occurred in longfin eels was 11.8°C (maturation period 74 days) and for shortfins 11.9°C (maturation period 93 days). At temperatures of 15°C and below the maturation period varied, up to 105 days for one shortfin. Tables 5.4 and 5.5 show that at these temperatures longfins mature more quickly than shortfins.

Experiments at temperatures above 15°C were conducted in heated aquaria at the prescribed temperature, $\pm 1^\circ\text{C}$.

The maturation period steadily decreased with increasing temperature, until 23.7°C for longfins (maturation period 20 days) and 25.5°C for shortfins (maturation period 23 days). At temperatures above these for each species the maturation period increased slightly. The maximum temperature at which maturation occurred in longfins was 25.9°C (maturation period 24 days), and the maximum temperature for shortfins was 28°C (maturation period 27 days).

Fig. 5.11 represents graphically the relationship between maturation period and temperature. The curves were fitted by eye to the scatter diagram of maturation period against temperature by calculating the mean maturation period at each 5°C rise in temperature.

At temperatures below 20°C the scatter diagram shows that there is considerable variation in the maturation period for each species. There is less variation at temperatures above 20°C. From 11.8°C to 20°C there is a rapid decrease in the maturation period. At temperatures above 20°C it decreases further, but less rapidly. The rate of decrease for each species is similar.

At each temperature longfins mature more rapidly than

FIG. 5.11

The relationship between the maturation period (days) and temperature ($^{\circ}\text{C}$) for hormone injected A. australis (shortfin) and A. dieffenbachii (longfin)

shortfins. This is more apparent at temperatures below 16°C . At higher temperatures the difference in maturation periods between the species is less.

It has been established earlier in this study that longfin migrants are more sexually developed at migration than shortfins and therefore could be expected to mature more rapidly in response to the hormone injections. However, as shown in the discussion on the GSI values in hormone injected eels, the response to the hormone injections is rapid, particularly at temperatures above 15°C , and the difference in the state of sexual development at migration may not be significant when compared with this rapid response. The response to hormone treatment at temperatures below 15°C is slower than at higher temperatures. Therefore any difference in the state of sexual development prior to the injections may be more significant. Hence it is suggested that the differing maturation periods between each species is due to the difference in the state of gonad development at migration and that this difference is more significant at lower temperatures, when response to the hormones is slower than at higher temperatures, when it is more rapid.

Boëtius and Boëtius (1967a: 369 - 77) conducted a series of experiments on male European eels at different temperatures and made a detailed statistical analysis of their data to establish the mathematical relationship between the temperature and maturation period. Their experiments fell into two periods of the year, from September to November immediately following the migration of the silver eels, and from December to March during the winter. Untreated eels showed gonad regression before injections in the latter period and response to the hormone slowed during the winter. Differences in the maturation times at the same temperature occurred between the two groups, with the September to November group maturing sooner. The December to March period of experiments was considered to be nearer to the natural spawning time but because of the gonad regression and the reduced response to the hormones, the September to November results were considered to be a more accurate reflection of the influence of temperature on maturation.

In this study insufficient numbers of eels were available to establish if a similar difference occurred. Data from experiments conducted during the winter and spring were combined. Non-injected control eels kept during the winter showed no significant gonad regression, and it was not apparent that eels injected during the winter showed a reduced response to the hormones.

The differences in the maturation rate between the species may be explained by suggesting a difference in the temperature response between the two species. That is longfins may mature more rapidly at lower temperatures than shortfins do, because of a lower temperature threshold or biological zero.

Blaxter (1956: 9) defines the biological zero as the point at which, as the rate of development decreases with falling temperature, development will cease. Extrapolation of the curves for each species in Fig.5.11 to a point where the maturation period reaches infinity gives a biological zero between 8°C and 11°C for both species. The upper limit of this range approximates the minimum temperatures at which maturation was experimentally obtained, 11.8°C for longfins and 11.9°C for shortfins. Therefore the true biological zero may lie between 8°C and 11.9°C for both species.

Although longfins matured more rapidly at lower temperatures than shortfins, the minimum temperatures at which maturation occurred for each species are similar. Thus the experimental evidence does not suggest that longfins have a lower biological zero than shortfins.

Blaxter (1956) and Boëtius and Boëtius (1967a) both discuss the concept of a biological zero and use mathematical formulae to calculate it. Blaxter indicates the danger of extrapolation, but suggests that it is the only method of obtaining a value for the biological zero without carrying out tedious experiments at low temperatures. His method of extrapolation involved plotting as separate graphs the reciprocal values of temperature against maturation period. Boëtius and Boëtius plotted the reciprocal values of the maturation rate $\frac{1}{T}$ against temperature and obtained a slightly sigmoid curve. This was done for the longfin and shortfin eels but there was insufficient data to determine if the plot was sigmoid, linear or curvilinear. Boëtius and Boëtius calculated mathematically a biological zero of 11°C for the European eel, which agreed well with the experimentally calculated value of about 10°C (range 9.6°C to 13.2°C). The data in this study were considered insufficient to make a similar mathematical analysis. However, the experimental value for the biological zero for New Zealand eels approximates that for the European eel.

The rate of increase of the maturation period at temperatures above approximately 20°C decreases. At the highest temperature (25.9°C) at which maturation occurred in longfins there is a slight increase in the maturation period. For shortfins however there is no similar clear increase in the maturation period at the highest temperature

(28°C) at which maturation occurred. The maximum rate of development, as indicated by the shortest maturation period, occurs at 23.7°C for longfins, and between 25.5°C and 28°C for shortfins. Blaxter (1956) states that at a theoretically maximum temperature the rate of development will be greatest. This is easier to calculate than the biological zero as the organism usually dies within a few degrees of the maximum temperature. In the case of the eels in this study development ceases at the maximum temperature. The data indicate that longfins have a lower maximum temperature (approximately 24°C) at which maturation occurs than shortfins (25.5°C to 28°C). For the European eel Boëtius and Boëtius calculated that the maximum temperature at which normal maturation occurred was 20°C. The data in this study were insufficient to make a similar analysis. However, the least variation in the maturation periods occurred at 20°C, and the slope of the curves in Fig. 5.11 show that the rate of increase in the maturation period is at its maximum at approximately this temperature. Above this temperature the rate of increase decreases. Thus the data indicate that both longfin and shortfin eels have a similar optimum temperature of approximately 20°C at which maturation occurs.

5.8 Non-injected control eels

Male non-injected control eels were held in the holding tanks at ambient temperature and in the heated aquaria at 20°C. Four eels, two longfins and two shortfins were kept at 20°C with hormone injected eels. During their experimental life these eels showed no gonad development past that of the migrant stage. The GSI values of 0.88 and 1.0 after an experimental life of 137 days for the longfins, and GSI values of 0.2 and 0.12 after an experimental life of 194 days for the shortfins, fall within the GSI values recorded for migrant eels of each species. Histologically these eels showed no sign of regression from the typical migrant stage of development described earlier. Males that died in the holding tanks or were killed after varying periods similarly showed no signs of either gonad development or regression from the migrant stage.

Insufficient numbers of female eels were available for any to be held as control eels with the hormone injected eels. However, females that died in the holding tanks after being held for varying periods were used as control eels. Table 5.6 gives the GSI values, mean ova diameter and the ova diameter range of the non-injected females.

The GSI values and mean ova diameters of the five shortfin eels all occur within the range recorded for shortfin migrant females. The GSI values and the mean ova diameters for the three longfins also occur within the range recorded for migrating longfin females.

The absence of development in non-injected control males and shortfin and longfin females in which the migration of the eel has been prevented is similar to that observed by Boëtius et al. (1962) and Boëtius and Boëtius (1967a) for the female and male European eel.

In contrast, an increase in the ova diameter of the migrant American female eel held in captivity has been recorded by Fish (1927) and Boëtius et al. (1962). Fish (1927) found that the ova diameter of American eels increased from 0.25mm to 0.4mm in one year. Boëtius et al. (1962: 185) similarly found an increase in ova diameter from 0.33mm to 0.45mm but within a shorter period of time, approximately one month. These authors suggest that the growth in the gonads may have occurred because the experimental animals were held in comparatively warm water, this condition favouring the development of the gonads of the American eel, but not those of the European eel.

The low water temperature in the holding tanks in this study may have inhibited the further development of the gonads in captive eels. The mean monthly temperature of 13°C for the period in which these eels were held is considerably below the presumed spawning temperature of 20°C.

5.9 Maturation of male eels for a second time

The experimental life of 10 eels exceeded 450 days. These eels are listed in Table 5.7. Four of them were still mature and could be stripped of milt. The remainder had regressed to the migrant or pre-migrant stage. This was shown by their reduced girth and the absence of milt when stripped. Histological examination of the gonads of two of these eels showed that the gonads had completely regressed and were almost indistinguishable from the migrant stage.

TABLE 5.6

GSI and ova diameter of non-injected female
A. australis and A. dieffenbachii that died
in the holding tanks

	No. days in captivity	GSI(%)	Mean ova diameter (mm)	Ova diameter range (mm)
<u>A. australis</u>	60	4.1	0.26	0.2-0.35
	60	4.56	0.27	0.2-0.3
	45	5.6	0.28	0.25-0.3
	40	4.5	0.28	0.25-0.3
	30	4.2	0.28	0.25-0.35
Migratory eels		3.82 (1.22-5.64)	0.28	0.2-0.45
<u>A. dieffenbachii</u>	40	10.0	0.41	0.4-0.45
	40	11.5	0.41	0.4-0.45
	30	7.6	0.38	0.35-0.4
Migratory eels		8.6 (6.49-11.53)	0.38	0.3-0.48

TABLE 5.7

HCG injected male eels that survived for over 250 days

Eel No.	Maturation period 1 (days)	Maturation period 2 (days)	Gonad stage	GSI	Experi- mental life (days)	% weight loss
<u>A. australis</u>						
4	93	-	3	2.8	286	24
24	39	-	5	0.42	262	25
25	35	91	3	3.0	429	43
55	105	-	5	8.2	294	29
80	90	-	4-5	12.0	252	30
81	32	-	5	2.8	262	23
<u>A. dieffenbachii</u>						
2	75	77	5	2.0	534	44
3	58	-	2	1.06	427	50
29	30	-	5	5.67	287	23

Three of the eels, two longfins (eel nos 2 and 3) and one shortfin (eel no.25) that had clearly regressed were acclimatized to 20°C, and after one week a series of second injections was begun in an attempt to induce maturation for a second time. Eel no. 3 died within 14 days and showed no sign of gonad response to the hormones. The remaining two eels matured in 77 days and 99 days, but both died within five days of maturation. Macroscopically and histologically (Fig.5.9,1) the gonads were at an early stage of maturity (gonad stage 3).

Eels that survived for more than 250 days showed a weight loss of up to 50% of the initial total body weight. None of the eels took any food during the period of captivity.

Boëtius and Boëtius (1967a: 349) indicate that in the European eel it may be possible to induce spawning in the same eel more than once, and suggest that biopsy would show if the eel had regressed to the migrant stage before a second series of injections commenced. In this study it was not considered necessary to confirm by biopsy that the gonads had regressed to the migrant stage. Their external appearance, absence of milt and the regressed state of the gonads in other eels with similar experimental lives was considered sufficient evidence that regression had occurred.

As previously discussed, the second hormone induced maturation of the males raises the question as to whether the eels in nature are capable of spawning more than once. Under experimental conditions the eels showed a considerable weight loss. At sea, after travelling to the spawning grounds, the weight loss at maturity would probably be greater than that observed in the aquaria, and it is considered doubtful if the eels would therefore survive a second spawning. Further, from these experimental results obtained under artificial conditions it is considered erroneous to suppose that similar events occur under natural conditions.

5.10 Injection of female eels with mammalian (non-piscine) hormones

As previously noted several authors (Boucher et al. 1936; Bruun, et al. 1949, Møller-Christensen et al. 1958; Boëtius et al. 1962) have unsuccessfully attempted to induce ovulation in the female European eel using HCG and oestrogenic hormones. Injections of HCG alone are sufficient to induce maturation in the male European

eel. Alone or in combination with oestrogens they have produced only limited development in the female eel. Despite these results an attempt was made to induce maturation in female New Zealand eels using HCG and oestrogenic hormones.

A total of 11 females, eight shortfins and three longfins, were injected with various dosages of HCG and a synthetic oestrogen, oestradiol benzoate, at weekly or three weekly intervals. The experimental procedure, including dosages, follows that of Boëtius et al. (1962). The number of eels injected, the dosage, experimental life and degree of development attained, is given in Table 5.8. Insufficient numbers of eels were available to be able to kill any during the experimental period to monitor any gonad development. Eels were therefore kept until they died, and this also ensured that the maximum development was reached. The degree of development was determined by measuring the diameter of the largest 10 fresh ova and calculating the GSI.

All eels died within 117 days from the date of the first injection. The greatest degree of development in shortfin eels, mean ova diameter 1.09 mm and GSI 44.14, was obtained in an eel injected at weekly intervals. The most developed longfin contained ova of 1.02 mm and a GSI of 28.77 in which injections had been given at ~~three~~ weekly intervals. Both these eels showed external visible signs of increased development. They were extremely fat, appearing similar to the mature eels injected with carp pituitaries (Fig. 5.4).

The GSI values and mean ova diameters are similar to those obtained in carp pituitary injected females. However, neither of these eels were mature. They could not be stripped of eggs and therefore ovulation had not occurred. Although the eggs were larger than mature eggs they were not fully developed. The yolk was dense, white and irregular in shape (Fig. 5.12). In some eggs the yolk was in the shape of a pyramid with the apex pointing towards the centre of the egg. A single, larger oil globule was present in some eggs.

Histologically the ova were in an advanced stage of vitellogenesis, with large PAS-positive yolk globules throughout the cytoplasm (Fig. 5.12). Others were being resorbed and had formed atretic eggs (Fig. 5.9, 2). In contrast, mature eggs from the carp pituitary injected eels were colourless and the yolk was spherical in shape.

The degree of development obtained in the eels was not consistent with the total dosages or the time difference between the

TABLE 5.8

A. australis and A. dieffenbachii female eels injected
with mammalian hormones

TABLE 5.8

A. australis and A. dieffenbachii female eels

Species	Eel No.	Hormone HCG (IU)	Dosage Oestra- diol benzoate (mg)	Interval between injections	Total HCG
<u>A. australis</u>					
	51	750	0.3	weekly	11,250
	50	750	0.3	three-weekly	3,750
	45	750	0.3	" "	3,750
	49	750	0.3	weekly	11,250
	46	750	0.3	"	11,250
	37	1000	0.3	three-weekly	2,000
	48	250	0.3	weekly	3,500
	43	500	0.3	"	3,500
<u>A. dieffenbachii</u>					
	36	750	0.3	three-weekly	3,000
	40	750	0.3	weekly	12,000
	44	750	0.3	weekly	1,500

injected with mammalian hormones

Dosage Oestra- diol benzoate (mg)	Experimental Life (days)	Mean Ova diameter (mm)	Ova diameter range (mm)	GSI
4.5	105	1.09	0.9 - 1.2	44.14
1.5	105	1.02	0.9 - 1.1	40.85
1.5	105	0.95	0.8 - 1.1	25.3
4.5	108	0.71	0.65- 0.8	21.0
4.5	105	0.7	0.6 - 0.8	13.77
0.6	62	1.0	0.85- 1.25	11.62
4.2	102	0.82	0.6 - 1.0	9.66
2.1	50	0.54	0.4 - 0.6	5.85
1.2	100	1.02	0.7 - 1.2	28.77
4.8	117	0.5	0.5 - 0.63	6.48
0.6	19	0.44	0.4 - 0.5	5.0

FIG. 5.12

IMMATURE OVA FROM EELS INJECTED WITH
MAMMALIAN HORMONES

1. A. australis (shortfin) ova. x 68
2. A. dieffenbachii (longfin) ova. x 80

1

2

injections. Table 5.8 shows that the greatest development (eel no. 51) in shortfins was attained with weekly injections, but the next most mature eel (eel no.50) was given injections at three weekly intervals. The total dosage was approximately one third of that of the most mature eel. Thus there appeared to be no relationship between total dosage and development. The degree of development, except for eel no. 48, was proportional to the experimental life, the least development occurring in eels with the shortest experimental life.

Only one longfin showed any development beyond the migrant stage as a result of the hormone treatment. The other two longfins showed no development beyond that recorded for the migrant stage.

The increase in gonad development beyond that of the migrant stage for eels of both species is considerably greater than that obtained in European and American eels (Boëtius et al. 1962), using similar hormones. In their experiments the experimental lives of the eels were longer but the maximum development attained was less. Considerably increased gonad development has been obtained in the Japanese eel by Ochiai, Umeda and Ōta (1972). The maximum egg diameter (0.98mm) is similar to that obtained in this study. However the mean egg diameters (0.48mm - 0.68mm) and the GSI values (8.6 - 21.9) are smaller. The maximum development of these species compared with the New Zealand eels is given in Table 5.9.

Boëtius et al. (1962) do not give detailed macroscopic or histological descriptions of the eggs obtained from the European and American eels. However, from the size of the eggs compared with this study it is likely that the largest ova were in the stage of vitellogenesis. Similarly Ochiai, Umeda and Ōta (1972) obtained what they describe as apparently mature eggs at the tertiary yolk stage. The accompanying histological photograph of a cross-section of one of these eggs shows that it is at an advanced stage of vitellogenesis, similar to that obtained in some eels in this study and shown in Fig.5.18,3.

From these experiments it is concluded that the injection of HCG and oestrogenic hormones into eels produces vitellogenesis but not ovulation. Ball (1960: 108), discussing the administration of non-piscine hormones such as HCG, notes that vitellogenesis may be produced but negative results are often obtained. Ball, and Pickford and Atz (1957) discuss the possible reasons for the lack of response of fish to non-piscine preparations. Generally the dosages of these hormones needed to obtain a response is greater than when fish pituitary is used. Pickford and Atz suggest that there are differences between

TABLE 5.9

Maximum development obtained in female eels of different species injected with mammalian (non-piscine) hormones

Species	Maximum ova diameter	G.S.I.	Experimental Life (days)
<u>A. australis</u>	1.25	44.14	105
<u>A. dieffenbachii</u>	1.63	28.77	100
<u>A. anguilla</u> (Boëtius <u>et al.</u> 1962)	0.56	12.6	250
<u>A. rostrata</u> (Boëtius <u>et al.</u> 1962)	0.6	15.8	100
<u>A. japonica</u> (Ochiai, Umeda and Ōta 1972)	0.98	21.9	approx. 126

vertebrate groups in the molecular structure of the carrier proteins which bind the active molecular groups of the gonadotrophins together. These differences in the carrier proteins, as discussed by Ball, may account for the phenomena of phylogenetic specificity. Briefly, hormones from different groups of vertebrates and pituitaries from other species of fish produce different responses in different fish. The injection of hormones from distantly related species or from species belonging to other vertebrate groups generally produce less response than those hormones from closely related species. This had led to the conclusion that there is evidence of a relative specificity of hormones, and in some cases correlated with the phylogenetic relationship of the species. This could explain the need for high dosages of foreign hormones to obtain a response.

Associated with the administration of large amounts of foreign hormones is the formation of antihormones (Pickford and Atz 1957). The injection of proteins into foreign species can be expected to produce antibodies which 'neutralize' the effect of the injected hormones. As noted by Ball, antibodies may be formed to attack the species-specific carrier proteins. However, in eels the significant increase in gonad development would indicate that any antibody formation did not affect the action of the injected hormone.

Ball (1960: 115) suggests that there are two gonadotrophins present in the fish pituitary, one which regulates follicular growth and the other ovulation. In this study the injection of mammalian hormones may have substituted for the role of the first gonadotrophin but not the second. This could account for the advanced stage of development reached in the eels and the absence of ovulation. However, the presence of two fish gonadotrophins has not been clearly established and Hoar (1969: 48) notes that there is much evidence to suggest that only a single gonadotrophin exists.

5.11 Injection of eels with teleost pituitaries

Fontaine et al. (1964), prompted by the failure of non-piscine hormone preparations to produce maturity in the European eel, conducted a series of experiments using carp pituitaries, peptone and nucleic acid. The spawning of an eel injected with only carp pituitaries was achieved in 89 days at 20°C. Maturation of the Japanese eel has also been

achieved using a combination of non-piscine hormones and trout pituitary (Ishida and Ishii 1970).

Using pituitaries from maturing or mature fish, a series of experiments was begun in 1971 to induce maturation and spawning in both longfin and shortfin female eels. Two shortfin males were also injected.

In the first attempts acetone dried pituitaries from ripe groper and tarakihi were used. The method of collecting, preparing and injecting, and the calculation of the dosages of these pituitaries has been described. Insufficient numbers of pituitaries were collected to inject those of just one donor species into an eel and they had to be combined.

One shortfin female (eel no.38) and one longfin female (eel no. 39) were injected with these pituitaries. The pituitary material only lasts for three weeks and from their external appearance the eels showed no signs of increased development. The eels were killed and the gonads examined. The mean ova diameter and GSI values recorded for each species are given in Table 5.10, and are within the range recorded for migrant females of that species.

A second series of injections was begun in 1972 using acetone dried carp pituitaries. Table 5.10 lists the number of eels injected, the dosages and maturation times. Ovulation was obtained in all but one of the female eels. However, only two eels, one longfin and one shortfin, spawned. Two shortfin males were also injected and are included in Table 5.10.

Longfin females matured more quickly than shortfin females. It is likely that the differences in the maturation times were due to the differing stages of sexual development at the time of migration, which have previously been described. In the shortfin eels the maturation time varies, and because of the varying weights of the eels the total dosage of pituitary varied. No relationship could be established between the total dosage and maturation time. It is suggested that the variation in maturation times amongst the shortfins may have been due to different individual states of reproductive development at the beginning of the experiment, and to the individual responses of the eels to the injections.

Maturation occurred in all but one eel, however only two eels spawned. Although the other eels could be stripped of eggs and therefore ovulation had occurred it is likely that these eels became 'egg-bound' and died. Pickford and Atz (1957: 210) note that 'egg-binding' is

TABLE 5.10

Hormone dosage, maturation period, spawning time,
experimental life, egg diameter and GSI of pituitary
injected A. australis and A. dieffenbachii held at
20°C.

TABLE 5.10

Hormone dosage, maturation period, spawning time,
injected A. australis and A. dieffenbachii

Species	Eel No.	Dosage per injection (mg)	No. injections	Total Dosage (mg)	Maturation Period (days)	Spawning Time (days)
<u>A. australis</u>	38*	14.0	9	126	-	-
females	64	11.0	14	154.0	45	-
	65	22.6	10	226.0	31	31
	66	15.0	12	180.0	43	-
	82	15.0	3	30.0	-	-
	83	9.6	10	96.0	26	-
	84	15.5	11	170.5	29	-
males	67	3.0	14	42.0	35	-
	81	3.0	12	36.0	32	-
<u>A. dieffenbachii</u>						
	39 ^x	42.0	9	378.0	-	-
females	96	42.0	8	336.0	22	-
	97	42.0	8	336.0	23	-

* Tarakini and groper pituitaries

experimental life, egg diameter and GSI of pituitary
held at 20°C

Experimental Life (days)	Mean Egg Diameter (mm)	Egg diameter range (mm)	GSI
44	0.35	0.3 - 0.4	4.2
80	0.95	0.9 - 1.2	22.5
41	1.0	0.9 - 1.2	45.0
147	0.35	0.3 - 0.4	9.0
4	0.4	0.35-0.45	6.0
31	0.88	0.85- 1.2	31.3
31	0.87	0.8 - 1.0	14.6
-	-	-	-
262	-	-	2.8
44	0.53	0.5 - 0.6	6.48
25	0.9	0.85- 1.2	30.23
26	0.95	0.9 - 1.0	20.23

a common cause of mortality in fish kept in aquaria, but dosages of HCG induce ovulation and thereby get rid of the eggs. Injections of 500 IU of HCG were given to mature eels to prevent egg binding, but the eels still died. Fontaine et al. (1964) injected the mature female European eel with DOCA (deoxycorticosterone) to induce spawning but there was no response. In this study no DOCA was available, but HCG as mentioned was used instead.

Table 5.11 gives the type of hormone used, the experimental temperature, and the maturation period, GSI and egg diameter range of the New Zealand, European and Japanese eels in which maturity has been induced. In all except the Japanese eel, maturity was induced with injections of acetone dried carp pituitaries. A combination treatment of hormones was used for the Japanese eel. The GSI values and egg diameter range are all similar, except for the lower GSI range for the mature longfin eel which has been discussed. However, there is a considerable difference in the maturation period between the species. These differences between the longfin and shortfin eels, and within the shortfin eels, have been ascribed to the different stages of sexual development at migration, and to the individual responsiveness of the eels to the hormone treatment. As maturation has only been induced in single specimens of the European and Japanese eel it is difficult to make comparisons between the maturation periods. Considering the variation that occurred in the five shortfin eels (Table 5.10) it is likely that similar variations in the maturation period would occur in the other species.

As previously discussed, the injection of pituitary material has in most cases been conducted on maturing or mature individuals and the injections have produced ovulation and spawning. In the eels considerable development of the oocytes, complete vitellogenesis in shortfin and the completion of vitellogenesis in longfins, has to occur before ovulation and spawning. In the eels that were stripped of eggs or those that spawned, 50% to 75% of the eggs were immature. These contained a dense, white yolk and sank to the bottom. Pickford and Atz (1957: 216) note that immature eggs are frequently ovulated following pituitary treatment.

TABLE 5.11

Hormone type, experimental temperature, maturation period, GSI and egg diameter of mature A. australis, A. dieffenbachii, A. anguilla and A. japonica.

Species	Hormones used	T°C	Maturation period (days)	GSI	Egg diameter range (mm)
<u>A. australis</u>	acetone dried carp pituitaries	20	26-45	14.6-45.0	0.8-1.2
<u>A. dieffenbachii</u>	acetone dried carp pituitaries	20	22-32	20.23-30.23	0.85-1.2
<u>A. anguilla</u> (Fontaine et al. 1964)	acetone dried carp pituitaries	24-25	89	40	0.93-1.4
<u>A. japonica</u> (Ishida and Ishii 1970)	trout pituitaries and mammalian hormones	20-23	92	-	0.8 - 1.0

A distinction is made here between ovulation and spawning. Ovulation is the release of the eggs from the follicle. It has been established that this had occurred in the eels when they were stripped of ova. However, ovulation was not complete, as when these eels died the ovaries were found to contain a large number of unovulated eggs. Spawning of the two eels occurred when the ovulated eggs were released from within the body cavity. Even these eels, at death, contained unovulated eggs.

Pickford and Atz suggest that eggs may not be ovulated spontaneously when mature, but are instead released in response to environmental stimuli or by the male partner. Liley (1969: 97) concludes that spawning only occurs after the ovulation of mature eggs. This close dependence of spawning on ovulation raises the question of the hormonal control of spawning. Ball (1960: 115) suggests that there are stimuli causing or influencing ovulation by stimulating the release of the appropriate gonadotrophin. As summarized by Liley (1969: 100) the evidence of several workers suggests that spawning is controlled by a hormone secreted by the neurohypophysis of the pituitary in response to sexual and environmental stimuli. Therefore the failure to spawn of all but two eels, even in the presence of mature males, is possibly caused by the lack of environmental stimuli under the experimental conditions.

The lack of development in female eels injected with tarakihi and groper pituitaries may have been due to the phylogenetic specificity of the gonadotrophins. There are several examples of pituitaries from one species inducing ovulation in closely related species, but not in distantly related species. Pickford and Atz (1957: 206) present data from Kazanskii (1940) who concluded that the most successful pituitary injections were limited to those when the donor and recipient belonged to the same family. Eels, groper and tarakihi are not closely related, and it is therefore not surprising that the injections were unsuccessful. However, carp pituitaries have been used successfully to induce maturation in eels, although the two species are not closely related. Pickford and Atz (1957: 205) note that some species seem to make better donors than others, and that ostariophysiid species have a higher success rate than other species. Of these species, the pituitaries from carp are the most frequently used with a high degree of success.

5.12 Descriptions of mature spermatozoa and eggs

Spermatozoa

Over 50 samples of spermatozoa were observed under the light microscope from mature eels of both species. Spermatozoa samples were taken from mature eels at different temperatures, and in shortfins from eels in which maturity had also been induced with carp pituitary injections. The eels had either just reached maturity or had been mature for varying periods of up to 200 days.

For observation a drop of milt was placed on a glass slide, covered with a cover slip and viewed under a phase contrast microscope. The spermatozoa showed varying degrees of activity. In approximately 95% of the samples they were completely inactive, in other samples approximately 25% of the spermatozoa were active. No samples contained spermatozoa that were all active. No correlation could be established between the amount of activity and the temperature the eels were held at or the type of hormone used. It was suspected that the inactivity of the spermatozoa may have been due to the action of the hormones or to the physical conditions under which the samples were examined.

Boëtius and Boëtius (1967a: 345) found similar varying degrees of spermatozoa activity in the European eel, and in the majority of samples all the spermatozoa were inactive. In contrast Fontaine (1936: 1313) noted that the spermatozoa were active. Similarly Meske and Cellarius (1972) describe the spermatozoa that they obtained as being strongly motile.

As some of the spermatozoa were active it is suggested that the lack of motility in others was either due to the physical conditions under which they were observed or to the effect of the hormones. Considering the unique spawning conditions of the European eel: depth approximately 400m, temperature 16°C to 17°C, (Bertin 1956: 161), it has, as previously discussed, been accepted that New Zealand eels spawn under similar conditions. The spermatozoa possibly have precise temperature and salinity requirements, which would be difficult to produce and control within narrow limits in the laboratory. Further, the depth at spawning, the pressure and light conditions, may exert an unknown effect on the activity of the spermatozoa. However, Fontaine (1936) and Meske and Cellarius (1973) both describe active spermatozoa, presumably observed under similar conditions to those in this study. The difference between these experiments and mine

is in the type of hormone used. All experiments used mammalian gonadotrophins but in different preparations. Fontaine used the urine from pregnant women which contains HCG during the first three months of pregnancy. Meske and Cellarius used a combination treatment of spleen extract (Solcosplen) and a mammalian gonadotrophin preparation (Synahorin). In contrast, HCG alone was used in this study, and by Boëtius and Boëtius (1967a) and in both cases only few active spermatozoa were observed. It is suggested that this lack of motility is due to the hormone preparations used. Further research using these different hormone preparations under the same experimental conditions may establish if this is correct.

Under the light microscope the spermatozoa of both species appear similar in size and shape. The heads of spermatozoa of the longfin eel appear as elongated, slightly recurved, crescent shaped bodies, 6 μ m to 8 μ m long and 3 μ m wide (Fig. 5.13). A slight bulbous projection appears at the anterior end of the head on the inner, convex side. From the posterior region of the head arises a flagellum, 30 μ m to 32 μ m long. A shorter, 2 μ m accessory flagellar-like structure was observed projecting from the posterior region of the head from the origin of the flagellum. The heads of the spermatozoa of the shortfin eel are smaller, 6 μ m long and 2 μ m wide (Fig. 5.13), and the flagellum is also shorter, 26 μ m to 30 μ m long. A similar short accessory flagellar like structure was also observed.

Few light microscope descriptions of eel spermatozoa are available. Tuzet and Fontaine (1936: 213) describe spermatozoa of the European eel and concluded that earlier descriptions applied to immature spermatozoa. However, their description is different than that given for New Zealand eels. They describe a similar elongated head of 6 μ m but state that it is capped, anteriorly, by an acrosome, 2 μ m long, strongly tapered and sometimes recurved. It has since been established that teleost spermatozoa do not possess an acrosome (Mattei 1970: 63), and Ginsburg and Billard (1972) suggest that the acrosome described corresponds to the mitochondria observed in electron microscope observations on eel spermatozoa. Tuzet and Fontaine also describe a posterior discoidal mitochondrial segment, but it has since been established that a single mitochondria is located in an anterior position (Ginsburg and Billard 1972). Meske and Cellarius (1973) only briefly describe the spermatozoa of the European eel

FIG. 5.13

MATURE SPERMATOZOA

1. A. australis spermatozoa. x 1700

2. A. dieffenbachii spermatozoon. The extra 'tail-like' structure can be seen projecting from the posterior end (left hand side in the photograph) of the head adjacent to the origin of the flagellum. x 1750

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as observed under the light microscope, and state that the flagella are 30 μ m long and the heads 9 μ m long. An accessory flagellar like structure is not described.

A detailed description of the spermatozoa as observed whole and in section under the transmission electron microscope is given in the next section.

Mature eggs

Mature eggs were only obtained from carp pituitary injected eels. From females injected with mammalian (non-piscine) hormones only immature eggs were obtained.

Fresh eggs from mature females of both species were collected by scooping them out of the aquarium while the eel was spawning or by stripping the eel.

Shortfin eggs

When the eels spawned or eels were stripped, two types of eggs were observed. Some ova contained opaque, white yolks and either sank or remained suspended in water. These eggs were not considered to be mature. Other eggs contained yolk that was completely transparent, making them difficult to see in the aquaria. These eggs floated to the surface of the water and were considered to be mature.

The following descriptions are of the mature eggs observed in sterilized saltwater (20°C) at varying periods of time after being released from the eel. Eggs were observed under a binocular microscope against a black background with a reflected and transmitted light source. Eggs were also examined and photographed using a compound microscope.

0 - 2 hours

When first released the eggs were irregular in shape and varied from 0.8mm to 1.0mm in diameter. After half an hour they had swollen slightly by the uptake of water through the chorion. They then appeared spherical in shape, measuring up to 1.1mm in diameter (Fig. 5.14,1). Only one egg membrane was observed. The perivitelline space was at first narrow (Fig. 5.14,1) but it increased in size as the egg swelled (Fig. 5.14,2). The central yolk mass, 0.9mm in diameter, was generally colourless although in some eggs it was slightly opaque. Under transmitted light it was granular in appearance (Fig. 5.14,2.). The number of oil globules varied. In many eggs only one central oil globule, 0.3mm in diameter, was present. When viewed under

FIG. 5.14

MATURE EGGS FROM CARP PITUITARY INJECTED

A. australis (shortfin)

1. 0 - 2 hrs. x 34
2. 2 - 4 hrs. The large perivitelline space is clearly visible. x 40
3. 4 hrs. The egg is water hardened and the perivitelline space has enlarged. The indentations on the surface of the chorion are visible. The dark black ring concentric to the chorion is the edge of the yolk mass. Within this the circular outline of the blastodisc is visible. A single central oil globule is seen. x 50
4. 4 - 6 hrs. The fine grained blastodisc is seen on the upper left margin of the coarser textured yolk mass. x 55
5. 4 - 6 hrs. Similar to 4. x 55

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reflected light the chorion, yolk and oil globule appeared as three concentric circles (Fig.5.14,1). Because of its high refractibility, the oil globule appears black around its periphery in the photographs.

In other ova two or many oil globules were present. These varied in size, but there was always one predominantly larger oil globule present. The oil globules appeared to be central in position, as the eggs always orientated themselves with the oil globule uppermost. Movement of the eggs showed that the oil globules occupied the upper pole of the sphere.

2-4 hours

After two hours the maximum size was attained and the eggs measured up to 1.2mm in diameter. The eggs were completely water hardened and no further increase in size occurred. The perivitelline space varied in size between ova, but it was usually large (Fig.5.14,3), and the chorion appeared sculptured, like the surface of a golf ball. After three hours the yolk mass began to appear pear shaped as a protuberance developed at one pole (Fig.5.14,4 and 5). This was the concentration of the cytoplasm into the blastodisc. The yolk in some eggs became eccentrically placed at this stage. Under transmitted light, the blastodisc appeared less granular in appearance than the yolk. The two regions were separated by a line or thin clear zone. In some eggs (Fig.5.14,3), the blastodisc was observed as a semicircle outlined against the yolk. This was observed when the eggs orientated themselves in such a way that the blastodisc occupied the upper pole of the egg.

4 - 7 hours

The blastodisc increased in size and measured up to 0.5mm in diameter. During this period the eggs died, becoming opaque in colour.

Several attempts were made to fertilize the eggs without success. Fresh eggs were mixed with fresh milt in a small amount of sterilized seawater at 20°C. After five minutes, more water was added and the eggs and spermatozoa left for half an hour. The eggs were then washed by mixing them with fresh sterilized water and decanting off the supernatant. They were then periodically examined under the microscope.

The same sequence of events as described for fresh 'unfertilized' eggs was observed, with the ova dying after eight to twelve hours. As no further development was observed, it was concluded that

fertilization had not occurred.

Other attempts at fertilization were made at different temperatures, from 15°C to 25°C, but no further development was obtained.

Longfin eggs

Two types of eggs from mature longfins were observed, similar to those from the shortfin females: immature eggs, containing opaque white yolk that sank to the bottom or remained suspended in the water, and mature transparent eggs that floated to the surface.

0 - 2 hours

Freshly released eggs were irregular in shape and measured from 0.9 - 1.0mm in diameter. After half an hour they became spherical in shape and increased slightly in size, up to 1.1mm in diameter, by the uptake of water through the chorion (Fig.5.15,1). The perivitelline space was narrower in width than in shortfin eggs of the same stage, but like the latter it increased in width (Fig.5.15,2). The granular yolk was usually central in position but in some eggs it was slightly eccentrically placed. Oil globules numbered one to many. In eggs with a single oil globule, 0.3mm in diameter, it occupied the upper pole of the egg, although it appeared central in position when the egg was normally observed. As in the photographs of the shortfin eggs the oil globule appears black around its periphery.

2-4 hours

The eggs attained their maximum size, up to 1.1mm in diameter, at this stage. The perivitelline space was usually smaller than in shortfin eggs of the same age (Fig.5.15,2). After 2 - 3 hours a protuberance developed at one pole of the yolk, making the yolk mass appear pear shaped (Fig.5.15,3). As in the shortfin eggs, the protuberance was recognized as being the formation of the blastodisc by the cytoplasm concentrating at one pole. The blastodisc was less granular in appearance than the yolk and a thin line or clear zone separated the blastodisc from the yolk (Fig.5.15,4).

4 - 7 hours

The blastodisc increased in size and measured up to 0.5mm in diameter. During this period the eggs died.

FIG. 5.15

MATURE EGGS FROM CARP PITUITARY
INJECTED A. dieffenbachii (LONGFIN)

1. 0 - 2 hrs. x 44
2. 2 - 4 hrs. The slight enlargement of the perivitelline space can be seen. x 40
3. 4 - 7 hrs. The prominent blastodisc is visible on the upper right edge of the yolk mass. x 40
4. 4 - 7 hrs. Similar to 3 but the egg contains only one oil globule. x 40

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Several attempts were made to fertilize the longfin eggs, as described for the shortfin eggs, but no further development than that described for the 'unfertilized' ova occurred.

The fresh eggs of each species were identical in appearance. However, after two hours some differences appeared. In shortfin eggs the perivitelline space became very large and the yolk mass usually remained in a central position. In longfin eggs the perivitelline space generally did not become as large and the yolk mass, after the development of the blastodisc, was usually eccentrically positioned.

The failure to fertilize the eggs was possibly due to two reasons, the first being the physical conditions under which fertilization was attempted. These conditions, as described for the spermatozoa, are not precisely known, and it is therefore impossible to reproduce these in the laboratory. Secondly, the eggs and spermatozoa may have been inviable. The lack of motility in the spermatozoa as previously described, possibly indicates that they are inviable and this may account for the failure of fertilization. The eggs may also have been inviable, but considering that the formation of the blastocisc occurred this is considered unlikely.

The success of any future attempts at fertilization would therefore depend on the ability to produce viable spermatozoa, and to carefully control the physical conditions near to those presumed to occur at the spawning grounds.

The concentration of the cytoplasm towards one pole of the egg to form the blastodisc is similar to the process of bipolar differentiation described by Smith (1957). In fertilized salmon eggs, the streaming of the cytoplasm to the zygote nuclei bringing about bipolar differentiation takes up to 6 hours to complete. Cleavage then begins. However, in other species, as in the eels in this study, bipolar differentiation may occur before fertilization. In the perch, Perca fluviatilis L. (Percidae), bipolar differentiation is begun before laying and it is completed on touching water, before fertilization (Thomopoulos 1953 in Smith 1957).

Few descriptions of mature eggs from other species of freshwater eels are available. Fontaine et al. (1964) describe the mature eggs of the European eel but no photographs are included. These eggs, 0.93mm to 1.4mm in diameter, contained an undivided yolk and several oil globules, and are similar in size and appearance

to mature longfin and shortfin eggs. However, the two outer membranes and a narrow band of cytoplasm surrounding the yolk as described by Fontaine et al. were not observed in this study. The authors similarly describe the concentration of the cytoplasm at one pole to form a swollen area, identical to the blastodisc described in longfin and shortfin eggs. Fontaine et al. did not attempt to fertilize the eggs, but since publication Fontaine (pers. comm.) has succeeded in rearing eggs to second cell stage.

Ishida and Ishii (1970) give photographs of mature eggs from the Japanese eel. The eggs measured 0.8mm to 1.0mm in diameter and contained numerous oil globules. As previously described, Ochiai, Umeda and Ota (1972) obtained what they describe as apparently mature eggs, larger than 0.95mm, in diameter, from a Japanese eel injected with non-piscine hormones. From the histological description of these eggs, the ova diameter size range (0.60 - 0.98mm) and the GSI value (21.9) of the eel from which they were obtained, it is considered unlikely that they were fully mature.

5.13 Gametogenesis and associated structural changes in the gonads

The process of gametogenesis in teleosts involves the formation and development of the sex cells, which, by a series of cytological changes result in the formation of the eggs and spermatozoa. This begins in embryonic life and continues through the sexual life of the fish. Primordial germ cells originate during gastrulation, and either migrate or are carried passively by formative processes to the region of the differentiating gonad (Hoar 1957: 289).

In male teleosts the formation of spermatozoa from primordial germ cells, through this series of cytological stages, is known as spermatogenesis. This process involves the formation and proliferation by mitotic division of spermatogonia from the primary germ cells which then differentiate into primary spermatocytes, and by meiotic division form secondary spermatocytes and then spermatids. By the process of spermiogenesis the spermatids metamorphose into spermatozoa.

Spermatogenesis occurs within testicular units which are either lobular or tubular in form (Hoar 1969: 9). The lobules or tubules are made up of the spermatogonial cells, in some fish in various stages of development, which surround a lumen. During spermiogenesis the spermatozoa are liberated into the lumina which inter-connect to form ducts, which converge on the main collecting duct, the vas deferens, which opens to the exterior. Associated with the spermatogonial cells are interstitial cells, Leydig cells and Sertoli cells, which have an endocrine function. Their role in the production of gonadal steroids has been clearly established (Hoar 1969) but their distribution and presence within the testis of different species is not uniform. As Hoar (1957: 293) notes they are inconspicuous or absent in many fish. Sertoli cells have also been identified in the testes of many fish, and are found in the layer of connective tissue surrounding each lobule or tubule (Hoar 1969: 13). As reviewed by Hoar they play a nutritive role during spermatogenesis, and may also be concerned with the phagocytosis of unused sperm and be involved in hormone production.

In female teleosts the process of oogenesis involves the formation of "nests" of oogonia from the mitotic division of the primary germ cells. During development oogonia becomes surrounded by follicle cells to form the ovarian follicle. Meiotic division begins but this is not completed until the egg has completed growth, (Richards 1931 in Tong and Vooren 1972: 47). Oogonia transform into oocytes which then pass through several stages of growth before becoming mature eggs.

Accompanying the growth of the oocyte is the development of the egg membranes: the theca, granulosa and zona radiata. The granulosa and thecal membranes are cellular layers, and the former is recognized as being responsible for the deposition of the yolk in the developing ovum (Hoar 1969: 18). The zona-radiata is a non-cellular layer separating the developing ovum from the granulosa.

Oocytes at advanced stages of development and mature ova not ovulated may be resorbed. The resorption of oocytes or ova, termed atresia, results in the formation of corpora atretica or corpora lutea. The physiology of preovulatory corpora atretica and postovulatory corpora lutea is still discussed, and the opposing views are summarized by Hoar (1969: 18 - 19). However, Hoar suggests that there is much

evidence to support the idea that they are the source of ovarian hormones. Ball (1960: 125) and Hoar (1965) both suggest that corpora lutea are a source of oestrogen production.

At maturity mature eggs are released from the ovarian follicle into the body cavity, or into the cavity of the ovary. In oviparous species the eggs are passed through the oviducts, pores or funnels to the exterior.

Gametogenesis in the European eel has been described by Rodolico (1933) but no observations were made on mature eels. Spermatogenesis in the male European eel has been described by Tuzet and Fontaine (1937) in eels induced to mature with hormone injections.

As previously discussed, it has been established that the European eel passes through an intersexual stage, when stages of spermatogenesis and oogenesis co-exist. No evidence has been found of a similar stage in New Zealand eels.

Descriptions of the development of the gonad in non-migrant longfin and shortfin eels, and the stage of sexual development attained in migrant eels has been described. The gonad changes associated with maturation in hormone injected eels has been given and brief descriptions were made of the associated histological changes. This section describes the process of gametogenesis in longfin and shortfin eels as observed in non-migrant, migrant and hormone injected eels. Details of the associated changes in the histological structure of the gonad, particularly in hormone injected eels, is also given. The process of regression of the testis in males and the formation of corpora atretica in the females is also described.

Gametogenesis in both species appeared identical. The descriptions therefore apply to both species, but some differences that were observed are noted.

5.14. Oogenesis

During the process of oogenesis the oocyte passes through two phases of growth. In the first phase, growth is slow and cytoplasmic, in the second phase growth is rapid and involves the deposition of large amounts of yolk in the oocyte (Ball 1960: 117). Yamamoto (1956) divides the process of oocyte development in the flounder Liopsetta obscura into eleven stages.

In this study it is convenient to divide the process of oogenesis into six stages of development: primary germ cells, oogonia, primary oocytes, vacuolation, vitellogenesis, and maturation. Similar divisions of development in oogenesis are used by Tong and Vooren (1972).

Primary germ cells

Primary germ cells were observed in gonad stages 1 and 2 of non-migrant eels. No distinction could be made between male and female primary germ cells. These cells were observed singularly or in groups of two or four scattered amongst an irregular network of connective tissue (Fig.5.16,1). The solitary germ cells were large, measuring up to $16\mu\text{m}$ in greatest diameter with nuclei $7\mu\text{m}$ in diameter. Germ cells in groups were slightly smaller. The cytoplasm and nuclei of the germ cells stained very faintly and little cytological detail could be observed. The chromatin material appeared in faint irregular threads and fragments were concentrated around the periphery of the nucleus. A slightly eccentrically placed more darkly staining nucleolus was present. The cytoplasm was colourless. No stages of nuclear division were seen.

Oogonia

These cells were observed singly or in groups in gonad stages 6 and 7 of non-migrant eels, in association with primary germ cells and primary oocytes. They varied from $10\mu\text{m}$ diameter for oogonia in early stages of development to $25\mu\text{m}$ in diameter for oogonia prior to transformation into primary oocytes. The nuclei measured between $5\mu\text{m}$ and $16\mu\text{m}$ in diameter and contained a prominent darkly staining nucleolus (Fig.5.16,3). Smaller oogonia were faintly staining and difficult to distinguish from the primary germ cells. Larger oogonia contained more darkly staining basophilic cytoplasm. The chromatin material was prominent, appearing as thin, darkly staining tangled threads (Fig.5.16,3). Darkly staining flattened follicle cells were observed associated with the nests of oogonia.

No oogonia were observed in ovaries of migrant eels of either species. In the non-migrant stage oogonia may be present in large eels. For example, ^alongfin non-migrant female of 70.1cm

FIG. 5.16

TRANSVERSE SECTIONS OF THE OVARY

1. Non-migrant A. dieffenbachii (longfin), 35.0cm total length, to show the primary germ cells. x 700

2. Non-migrant A. dieffenbachii, 71.9 cm total length, to show the development of ovarian lamellae (arrows). x 330

3. Non-migrant A. australis (shortfin), 51.2cm total length, to show a developing ovarian lamella containing three primary oocytes and many oogonia. x 550

g - primary germ cells
n - nucleus
o - oogonia
p - primary oocytes
l - lamellae

1

2

3

length and a shortfin non-migrant of 64.4cm in body length both contained oogonia.

Primary oocytes

The transformation of an oogonium into an oocyte is accompanied by the growth of the cell and changes in the nuclear arrangement. The smallest oocytes, 15 μ m in diameter, were difficult to distinguish from the larger, darkly staining oogonia.

As growth proceeds the cytoplasm becomes strongly basophilic, staining darkly with haematoxylin (Figs.5.17, 1 and 2). The nucleus also increased in size, and the arrangement of the nucleolus and chromatin material changes. In smaller oocytes only one darkly staining nucleolus is present. During growth the number of nucleoli increase and eventually become arranged around the periphery of the nucleus. The chromatin material is loosely arranged in thin darkly staining threads, at the mid-prophase of meiotic division.

Primary oocytes ranged from 15 μ m to 100 μ m in diameter. The smallest were associated with large numbers of oogonia and were observed in non-migrant eels at gonad stage 6, in which the ovarian leaflets were just forming (Fig.5.16,2).

As the oocytes increase in size, above approximately 50 μ m, the cytoplasm becomes less basophilic and stains grey with haematoxylin, in contrast to dark cytoplasm in smaller oocytes. In the largest oocytes the granulosa layer surrounding each oocyte becomes distinguishable.

Primary oocytes up to 100 μ m in diameter were predominant in ovaries of non-migrant eels at gonad stage 7.

Vacuolation

In oocytes larger than 100 μ m diameter small vacuoles appear in the cytoplasm. These first appear around the nuclear region and spread outwards towards the periphery of the cytoplasm in oocytes of 130 μ m to 180 μ m in diameter (Fig.5.17,1). The number and size of the vacuoles increase with the increasing size of the oocyte. Vacuolation involves a rapid increase in the size of the oocyte from 100 μ m to 225 μ m in diameter. The nucleus also increases in size, and many nucleoli are arranged around the periphery of the nucleus (Fig.5.17,1 and 2). As vacuolation proceeds the chromatin material becomes indistinguishable.

FIG. 5.17

TRANSVERSE SECTIONS OF THE OVARY

1. Non-migrant A. australis (shortfin), 65.0cm total length, to show the primary oocytes. x 550

2. Migrant A. australis, 72.1cm total length, to show the vacuolated oocytes and primary oocytes. x 350.

3. TS of an oocyte in vitellogenesis from a migrant A. dieffenbachii (longfin), 110.0cm total length. x 350

n - nucleus
nl - nucleoli
p - primary oocytes
v - vacuolated oocytes
vc - vacuolated cytoplasm
vt - oocytes in vitellogenesis
y - yolk globules

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3

Few non-migrant eels contained vacuolated oocytes. Only the largest females contained oocytes larger than $100\mu\text{m}$ in diameter. Oocytes in advanced stages of vacuolation were only observed in migrant eels of each species (Fig.5.17,2). As previously described, oocytes in the stage of vacuolation constituted 68% of all oocytes in migrant shortfins but only 21% of the oocytes in migrant longfin females.

Vitellogenesis

Vitellogenesis is the stage of yolk formation in the oocyte. When oocytes in advanced stages of vacuolation reach approximately $250\mu\text{m}$ in diameter, fine globules or droplets of yolk appear around the periphery. These yolk globules stain dark black with haematoxylin and bright red with Mallory's triple stain. Yolk at this stage gives a positive PAS reaction. As vitellogenesis proceeds the yolk globules increase in size and become distributed throughout the cytoplasm (Fig.5.17,3). Deposition of the yolk, like the formation of the vacuoles, is accompanied by a rapid growth of the oocyte, from $200\mu\text{m}$ to approximately $650\mu\text{m}$ in diameter. The nucleus also increases in size but stains faintly and loses its regular outline. The numerous nucleoli arranged around the periphery of the nucleus stain deeply with haematoxylin (Fig.5.17,3).

During vitellogenesis the three membranes surrounding the oocyte become clearly visible (Fig.5.18,1). The outer thin theca, only just visible, consists of flattened cells containing elongate flattened nuclei, $1.5\mu\text{m}$ long and $3\mu\text{m}$ wide. Internal to the theca is the granulosa, which first becomes distinguishable during vacuolation. It is composed of elongate cells, $5\mu\text{m}$ long and $3\mu\text{m}$ wide, containing flattened darkly staining nuclei. Internal to the granulosa is the zona radiata. In oocytes in advanced stages of vitellogenesis this acellular layer is $5\mu\text{m}$ in thickness and has prominent radial striations (Fig.5.18,2). During vitellogenesis both the granulosa and zona radiata have a strong positive PAS reaction, staining deep red with this stain and with Mallory's triple stain. Within the cytoplasm of the oocyte, adjacent to the zona radiata, is a clear zone of cytoplasm, the oolemma, $3\mu\text{m}$ to $5\mu\text{m}$ in width.

The oocyte membranes were difficult to observe clearly in migrant shortfins as the oocytes were only at the stage of vacuolation

FIG. 5.18

TRANSVERSE SECTIONS OF THE OVARY

1. TS of an oocyte in vitellogenesis from a migrant A. dieffenbachii, 98.5cm total length, to show the development of the oocyte membranes. The zona radiata appears narrow at this stage of development. x 750

2. TS of an oocyte in an advanced stage of vitellogenesis from a mammalian hormone injected A. australis (shortfin, no.46) to show the oocyte membranes. The zona radiata has prominent cross-striations. x 750

3. TS of oocytes from a mammalian hormone injected A. australis (eel no.48). One oocyte (arrowed) is almost mature and the yolk appears vesicular. The other oocyte is in an advanced stage of vitellogenesis. x 80

g - granulosa

n - nucleus

o - oolemma

vt- vitellogenesis

y - yolk globules

yk - yolk

z - zona radiata

1

2

3

or early vitellogenesis. They were better observed in hormone injected eels in which further development had occurred (Fig.5.18,2). In migrant longfin females the oocyte membranes were clearly distinguishable in oocytes in vitellogenesis (Fig.5.18,1).

Advanced stages of vitellogenesis in either species were only observed in hormone injected eels.

Mature eggs

In hormone injected females the largest oocytes in which vitellogenesis was observed measured from 200um to 650um in diameter (Fig.5.18,3). In the few larger oocytes sectioned, the darkly staining yolk globules were reduced to a peripheral ring around the oocyte. In other oocytes the yolk had become clear and vesicular in appearance (Fig.5.18,3). The nuclei were irregular and the nucleoli scattered through the nucleoplasm. The oocyte membranes were still distinct but the zona radiata was less prominent than in vitellogenesis. These ova had a less positive PAS reaction, particularly the zona radiata, which indicated that yolk deposition was less active than in vitellogenesis. It was difficult to section mature ova, and no satisfactory sections of mature ova from mature carp pituitary injected females could be made.

Ovulation

Ovulation is the process of release of the mature ovum from its follicle. The theca and granulosa rupture and the mature ovum is released into the lumen of the ovary or, as in eels, into the body cavity. Ovulation only occurred in carp pituitary injected eels of both species. The histological changes accompanying ovulation in these eels was not observed.

Resorption of ova

In some hormone injected eels with oocytes in advanced stages of vitellogenesis, several oocytes were observed (Fig.5.19,1) which had the appearance of resorbed ova or corpora atretica described by several authors (Bowers and Holliday 1961; Hoar 1969; Tong and Vooren 1972). Ova may be resorbed before or after spawning and are classified respectively as prevulatory or post ovulatory corpora atretica (Hoar 1969: 16). In the eels ovulation had not occurred and the resorbed ova are therefore classified as preovulatory corpora atretica.

FIG. 5. 19
ATRETIC OOCYTES

1. Section of the ovary from a mammalian hormone injected A. australis (shortfin, eel no.46) to show atretic oocytes. In two oocytes (numbered 1 and 2) the yolk is being resorbed. In another oocyte (arrow) the yolk has been completely resorbed. x 75

2. Section of an atretic oocyte from a mammalian hormone injected A. australis (eel no.48) to show the contents of the oocyte. The zona radiata and granulose (arrows) are deeply folded and the yolk is being resorbed. x 650

y - yolk remains

1

2

Resorbing ova were at first recognized by the changes in the zona radiata and granulosa which ruptured and became deeply folded and collapsed into the yolk mass (Fig.5.19, 1 and 2). Early stages of the formation of corpora atretica were later recognized by changes in the appearance of the yolk which stained less heavily and appeared globular in arrangement. Erythrocytes and other phagocytic cells, probably the granulosa cells, invade the oocyte as the zona radiata folds and ingests the yolk mass. This rapidly disappears and the atretic oocyte appears as a folded black mass (Fig.5.19,1).

5.15. Spermatogenesis

It is convenient to describe the process of spermatogenesis by separating it into the various cell types. Like oogenesis, this represents the sequence of development, but in the males, unlike oogenesis in females, several stages of spermatogenesis may be present at one time.

Primary germ cells

Primary germ cells that would give rise to spermatogonia could not be recognized in gonad stages 1 and 2 of non-migrant eels. However, these cells were recognized in male eels with lobed gonads in which spermatogonia was present (Fig.5.20). Primary germ cells in these eels were large, 14.5 μ m in diameter in shortfins, and 16 μ m in diameter in longfins. The nuclei measured 6 μ m to 7 μ m in diameter and contained a prominent central nucleolus which appeared to be connected to the periphery of the nucleus by thin faintly staining threads. The chromatin material appeared as irregular faintly staining fragments. The cytoplasm was colourless.

The largest primary germ cells were observed singularly, interspersed amongst an irregular network of connective tissue. Smaller germ cells, 8 μ m to 10 μ m in diameter, occurred in cyst like units of two, four or eight cells, each cyst surrounded by the network of connective tissue. No cell divisions were observed, but it was apparent that several generations of germ cells arose by mitotic division from the initial large solitary primary germ cells. The smaller germ cells were difficult to distinguish from some spermatogonia.

FIG. 5.20

TRANSVERSE SECTIONS OF THE TESTES

1. A. dieffenbachii (longfin) 59.1cm total length, to show the developing germ cells. x 500

2. A. australis (shortfin), 43.8cm total length, to show the developing germ cells and spermatogonia. x 750

ct - connective tissue

g - germ cells

sg - spermatogonia

1

2

Spermatogonia

Spermatogonia arise from the germ cells by mitotic division. The largest measured $8\mu\text{m}$ in diameter and contained a nucleus of $5\mu\text{m}$ in diameter (Fig.5.21,1). First formed spermatogonia are similar to smaller germ cells but were recognizable by the more compact and darkly staining nucleus, containing a darkly staining nucleolus and a faintly staining reticular network of chromatin. The cytoplasm stained faintly, but in smaller spermatogonia the nucleus and cytoplasm stained more heavily. Small clusters of spermatogonia were present in gonad Stage 4 in non-migrant eels, and large numbers in gonad Stage 5 of non-migrant eels. As previously established in this study, spermatogonia were the predominant cell type in migrant shortfin males but less frequent in migrant longfin males.

Spermatogonia were observed in various stages of mitotic division in some non-migrant longfin males at gonad Stage 5, and in migrant shortfin and longfin males (Fig.5.21,3). From the number of spermatogonia and spermatocytes in migrant eels it was apparent that up to eight generations of spermatogonia are formed. As division proceeds the size of the spermatogonia decreases. The smallest were $5\mu\text{m}$ in diameter and contained a darkly staining nucleus, $3\mu\text{m}$ to $4\mu\text{m}$ in diameter. In these, the cytoplasm stained heavily with eosin but the cell outline was difficult to distinguish.

In non-migrant eels the division of the spermatogonia was accompanied by the formation of the testis into a definite tubular arrangement (Fig.5.21,2), in contrast to the more cyst like appearance of germ cells and spermatogonia in earlier stages of development. Each tubule is surrounded by a band of connective tissue which forms a regular network of connective tissue throughout the testis. Small blood vessels are also present in the connective tissue network. Within each tubule (Fig.5.21,3), each group of spermatogonia is surrounded by a thin band of connective tissue. Around the periphery of each tubule isolated germ cells are present adjacent to the tubule connective tissue wall. Spermatogonia also lie around the periphery of the tubule and in successive layers towards the centre. As the tubule increases in size, several layers of spermatogonia are formed by successive mitotic divisions, and the tubule walls are stretched and appear thinner. During development the isolated germ cells disappear, and only spermatogonia are present.

FIG.5.21

TRANSVERSE SECTIONS OF THE TESTIS

1. Non-migrant A. dieffenbachii (longfin), 55.0cm total length, to show the spermatogonia. The tubular structure of the testis has not developed. x 750

2. Migrant A. australis (shortfin), 42.0cm total length, to show the tubular arrangement of testis. x 350

3. Migrant A. dieffenbachii 45.6cm total length, to show the cellular contents of a tubule. Spermatogonia are on the outer margin of the tubule adjacent to the intertubular connective tissue. One spermatogonia (arrow) is in the metaphase stage of mitosis. Spermatocytes are on the inner margin of the tubule. Each group of spermatocytes is surrounded by a thin band of intertubular connective tissue. x 750

c - connective tissue
it - intertubular connective tissue.
l - lumen of tubule
sg - spermatogonia
sc - spermatocytes
t - tubules
ita - intratubular connective tissue

1

2

3

Primary spermatocytes

Primary spermatocytes arise from late stage spermatogonia and represent the beginning of meiotic division. Spermatocytes when first formed are similar to the smallest spermatogonia. The cell outline and cytoplasm was indistinguishable but the nucleus was prominent. The nucleus, 3 μ m to 4 μ m in diameter, was densely staining and contained a darkly staining nucleolus amongst an irregular network of darkly staining chromatin (Fig.5.21,3). As meiosis proceeds the nucleolus disappears and the chromatin material becomes progressively darker. In the testis of some shortfin migrants and most longfin migrants groups of spermatocytes were associated with groups of spermatogonia within the tubule. In longfin migrants different groups of spermatocytes were in different stages of nuclear organisation. The groups of spermatocytes nearest to the tubular wall were in earlier stages of development with the nucleolus still visible. Groups nearer to the centre of the tubule had dense staining chromatin and no nucleolus. In some spermatocytes on the inner margin of the tubule the chromatin material was arranged in a cup-shape lying against one side of the nucleus, characteristic of the synizeis (leptotene) stage of meiosis. Other stages of meiosis could not be easily distinguished but the changes in appearance of the spermatocytes were associated with

~~maturation. Groups of spermatocytes of unequal maturation stages~~

Spermatids

Spermatids were recognized by their small size, $2\mu\text{m}$ in diameter, and their concentrated chromatin. Initially the chromatin is dense and spherical in shape (Fig.5.24,1), but during spermiogenesis it condenses further and becomes cup shaped, with an accompanying reduction in size of the nucleus to $1\mu\text{m}$. As metamorphosis proceeds the nucleus becomes elongate and measures up to $4\mu\text{m}$ in length.

As previously described, spermatids were present in the lumen of the vas deferens in some migrant shortfin males and many migrant longfins. They were more commonly observed in large clusters in hormone injected eels at gonad stages 3 and 4, either against the inner margin of the tubules in partially mature males (Fig.5.22,2), or against the connective remnants in more mature eels (Fig.5.24,1).

Spermatozoa

Spermatozoa have elongate crescent shaped heads, $5\mu\text{m}$ long in shortfins and $6\mu\text{m}$ long in longfins. The details described in the light microscope study of fresh spermatozoa were not visible in histological sections.

Spermatozoa were observed in the lumen of the vas deferens of a few shortfin migrants and most longfin migrants. As discussed, this was considered to be prespermatogenesis as the rest of the gonad was in a much earlier state of development. In a few longfin males however, spermatozoa were observed in the lumen of the tubules.

Spermatozoa were more common in hormone injected eels at various stages of maturation. Groups of spermatozoa first appeared in the lumina of the enlarging tubules (Fig.5.22,2). As maturation proceeds the tubules increase in size and the connective tissue walls break down. The masses of spermatozoa in the lumina of the tubules coalesce and a mass of spermatozoa results (Figs.5.22 and 5.23).

The process of spermiogenesis is accompanied by changes in the structure of the gonad that become more marked as maturation proceeds. The development of the tubular structure in non-migrant males, the appearance of the tubules in migrant males, and the changes in the structure of the testes at each stage of development during maturation have been described. A brief summary is given here before the post maturation changes in the testes are described.

FIG.5.22

TRANSVERSE SECTION OF THE TESTES FROM
HORMONE INJECTED EELS

1. A. dieffenbachii (longfin, eel no.11) 11 days after the first injection to show the tubular arrangement of the testis. x 350

2. A. dieffenbachii (eel no.13) 28 days after the first injection to show the enlargement of the tubules. Spermatocytes and spermatids are predominant. x 350

bv - blood vessel
it - intertubular connective tissue
l - lumen of tubule
sc - spermatocytes
sp - spermatids
s - spermatozoa
t - tubule

1

2

FIG.5.23

TRANSVERSE SECTIONS (TS) OF THE TESTES
FROM HORMONE INJECTED EELS

1. Mature A. australis (shortfin, eel no.20) 42 days after the first injection. The tubules are greatly enlarged and the lumen of each is full of spermatozoa. A few spermatogonia remain and there are large numbers of spermatoocytes and spermatids. x 600

2. Mature A. australis (eel no.22) 63 days after the first injection. No spermatogonia are present and the numbers of spermatoocytes and spermatids are reduced. Spermatozoa are predominant. x 500

it - inter~~t~~ubular connective tissue

sg - spermatogonia

sc - spermatoocytes

sp - spermatids

s - spermatozoa

1

2

FIG.5.24
TRANSVERSE SECTIONS (TS) OF THE TESTES FROM
HORMONE INJECTED EELS

1. Mature A. australis (shortfin, eel no.57), 100 days after the first injection. Two relic spermatogonia remain. Few spermatocytes and spermatids are present. x 750

2. Mature A. dieffenbachii (longfin, eel no.1) 106 days after the first injection. Few spermatocytes and spermatids remain, and the intertubular connective tissue has almost disappeared. x 350

3. Regressing A. australis (eel no.4), 286 days after the first injection. Some spermatids and intertubular connective tissue remain. The round or ovoid phagocytic cells are present. x 500

4. Regressing A. australis (eel no.4) 286 days after the first injection. The large, round or ovoid phagocytes are present amongst the spermatozoa. x 750

5. As in 4. x 750
 - it - intertubular connective tissue
 - ph - phagocytes
 - sc - spermatocytes
 - s - spermatozoa
 - sg - spermatogonia
 - sp - spermatids

1

2

3

4

5

During the proliferation of the spermatogonia the tubular structure of the testis develops. At migration, the tubules are well developed and contain spermatocytes and spermatogonia (Fig.5.21,1). In hormone injected eels the number of spermatocytes increases and they undergo reduction division to form spermatids (Fig.5.22). These divisions are accompanied by the enlargement of the tubule and a reduction in the width of the tubule walls (Fig.5.22,2). As development proceeds spermatozoa begin to fill the lumina of the tubules (Fig.5.23,1). Large numbers of spermatids, spermatocytes and some spermatogonia are present. As more spermatozoa develop most of the spermatogonia and spermatocytes disappear. The tubules become packed with spermatozoa and the tubule walls are reduced to thin strands of connective tissue, less than $1\mu\text{m}$ wide. These walls break down and the masses of spermatozoa coalesce (Fig.5.24,2). The connective tissue persists as thin broken strands amongst the spermatozoa. Small groups of primary and secondary spermatocytes, and spermatids, are found adjacent to the connective tissue strands, but these gradually disappear. The maximum stage of development is reached when there are few spermatocytes or spermatids present and the connective tissue is reduced to a minimum. The vas deferens at this stage is greatly enlarged and full of spermatozoa.

Post maturation changes in the testis

After the maximum stage of development has been attained regression of the gonad occurs. Regression occurs in eels that have spawned, or have matured and not spawned. The latter eels retain large amounts of spermatozoa in the testes.

The first sign of regression is the regeneration of the connective tissue and the appearance of phagocytes (Fig.5.24 and 5.25). Thin strands of connective tissue, present throughout the stage of maximum development, regenerate and branch out into the degenerating mass of spermatozoa (Fig.5.25,1). The spermatozoa lose the regular crescent shaped outline of the head and begin to undergo autolysis. Large, round or oval shaped bodies appear amongst the degenerating spermatozoa (Fig.5.24,3). These measured up to $17\mu\text{m}$ in length, and contained a faintly staining nucleus, $8\mu\text{m}$ in diameter (Fig.5.24, 4 and 5). Within the nucleus the chromatin material was diffuse and a faintly staining nucleolus was present. The cytoplasm

FIG. 5.25

TRANSVERSE SECTIONS (TS) OF THE TESTES FROM HORMONE INJECTED
EELS

1. Regressing A. australis (shortfin, eel no.23) 135 days after the first injection. The intertubular connective tissue is regenerating. x 350

2. Regressing A. australis (eel no.4) 286 days after the first injection. The intertubular connective tissue is regenerating and phagocytes are present amongst the spermatozoa. x 500

3. Regressing A. australis (eel no.91) 102 days after the first injection. Darkly staining interstitial cells are present adjacent to the regenerating connective tissue. x 500

it - regenerating connective tissue
its - interstitial cells
ph - phagocytes
s - spermatozoa
y - yellow body

1

2

3

contained numerous round, darkly staining inclusions which stained heavily with haematoxylin. From the position of these cells, their appearance, and the time of their formation in the testes, it was assumed that they had some phagocytic function.

The regeneration of connective tissue and the appearance of phagocytes occurred in some eels within 80 days from the date of first injection. In others it was not observed until after 200 days. Regression occurred more rapidly at higher temperatures, above 20°C.

The connective tissue developed rapidly throughout the testis and increased in thickness. The large phagocytes were closely associated with the developing connective tissue. Erythrocytes were also observed amongst the degenerating spermatozoa adjacent to the regenerating connective tissue. As the connective tissue increased in thickness yellow coloured bodies appeared amongst the connective tissue (Fig.5.25,2). These were granular in appearance and 8µm in diameter. Some larger, 35µm in diameter, yellow bodies were also observed. Small, darkly staining round or pyriform shaped cells developed along the edges of the connective tissue and projected out into the testis (Fig. 5.25,3). These cells measured 3µm to 6µm in height and contained a very darkly staining cytoplasm.

As the connective tissue developed and thickened, areas of degenerating spermatozoa were delimited (Fig. 5.26,1). These areas of spermatozoa reduced in size until only the cell debris remained. At this stage the large phagocytes disappeared.

The complete disappearance of the spermatozoa represented the stage of maximum regression. The arrangement of the connective tissue into a roughly tubular arrangement marked the beginning of the regeneration phase. (Fig.5.26,1).

At this stage large germ cells were observed amongst the connective tissue. These and smaller spermatogonia appeared along the margins, adjacent to the connective tissue, in the developing tubules. By a reduction of the connective tissue, multiplication of the spermatogonia and the development of the tubular structure, eels at this stage were hard to distinguish from migrant eels (Fig.5.26,2). It is likely that the two eels that matured for a second time (Fig.5.25,3) had reached this stage before the second series of injections commenced.

FIG.5.26

TRANSVERSE SECTIONS OF THE TESTES FROM
HORMONE INJECTED EELS

1. Regressing A. dieffenbachii (longfin, eel no.85) 203 days after the first injection. A large amount of connective tissue is present, surrounding a number of spermatogonia. A group of relict spermatozoa remains. x 350

2. Recovered A. australis (shortfin, eel no.94), 126 days after the first injection. The testis is indistinguishable from a migrant A. australis testis, except for a few relict spermatozoa that remain, and the thicker intertubular connective tissue walls. x 750

3. A. australis (eel no.25) that matured for a second time when given a second series of injections after the testis had regressed. x 500

bv - blood vessel

it - intertubular connective tissue

s - spermatozoa

sg - spermatogonia

sp - spermatids

1

2

3

Regression of the testes, unlike the stages of maturation, were not synchronous throughout the testis. From the macroscopic appearance of the gonad and histological examination it was established that regression occurred first in the posterior lobes of each testis and then progressed anteriorly.

Interstitial cells

In haematoxylin stained sections of testes from non-migrant, migrant and hormone injected eels prior to regression no cells were observed that could be described as interstitial cells. None were found either within the connective tissue walls of the tubules or within the tubules. According to Hoar (1969: 12) well vascularized clusters of cells appear between the tubules of many fish. In routine sections these cells appear large, with spherical or oval nuclei, and often appear vacuolated because of the removal of lipoidal substances. The presence of these cells can be detected by several methods using sudanophilic dyes to stain cytoplasmic droplets, which reveals their endocrine nature.

Most of these methods involve the freeze dry technique of sectioning. However, Threadgold (1956) gives a method of staining for interstitial cells which used wax embedded tissue. This method is convenient as tissue used for routine histological examination, if correctly fixed, can be used. This technique failed to reveal the presence of any interstitial cells in non-migrant, migrant or mature testis. Positive reactions to the staining technique however were observed in regressing testes. The developing connective tissue and associated round or pyriform shaped cells stained lightly with sudan B, indicating the presence of some interstitial cells in this area. However, it was difficult to determine the exact area and the slides were not suitable for photography.

5.16 Discussion

Spermatogenesis in longfin and shortfin eels is similar to that described by Tuzet and Fontaine (1937) for the European eel, except that many of the nuclear details described by these authors was not observed. Further, there are details of the structure of the testis and associated cells that were also not observed. Tuzet and Fontaine describe a cellular base that extends around the margin of each tubule. This forms a layer two cells thick in testes in the medium stage of development. Extensions of this layer protrude

into the tubule and surround the groups of germinal cells. This glandular layer remains in the mature and spent testis. These authors consider that the cells in this glandular layer are types of Sertoli cells nourishing the developing spermatogenic elements. According to Tuzet and Fontaine (1937), Rodolico (1933) confused this glandular layer with early stage spermatogonia lining the outer margin of the tubules. No similar continuous layer of glandular cells was observed around the margin of tubules in testes during this study. Spermatogonia were observed forming a continuous layer around the tubules in some eels in early stages of development, but they disappeared as maturation proceeded.

The presence and function of interstitial cells and Sertoli cells is reviewed by Hoar (1969). Endocrine producing interstitial cells in teleosts may be arranged in two ways. Typical interstitial cells (Leydig cells) are present in intertubular spaces, or the intertubular spaces are very narrow and contain only blood vessels and the nuclei of the fibroblasts (Ashan 1966: 158). In these fish the interstitial cells are located inside the tubule close to the tubular wall, and are termed tubule or lobule boundary cells. Hoar (1957: 293) in an earlier review, notes that the interstitial cells may be in conspicuous or absent in many fish. Marshall (1960: 140 - 144), discussing the two types of interstitial cells, states that lobule boundary cells are difficult to distinguish from the fibroblast cells of the lobule wall using normal histological techniques. Sudanophilic dyes demonstrate the presence of the lobule boundary cells when they are maturing or mature. Only during certain periods of the sexual cycle are boundary cells clearly evident.

In this study the walls of the tubules contained only fibroblasts and at the interstices of the tubule walls only blood vessels were present. There was no evidence of interstitial cells in routine histological slides or in sections stained with Sudan B. This suggests that interstitial cells, if present, would appear as tubule boundary cells. However, as stated these could not be found in non-migrant, migrant or mature eels. This may have been a result of the technique used or, because as Marshall (1960: 141) notes, it is only during certain periods of the sexual cycle that they are clearly evident. The positive reaction to Sudan B by cells within the connective tissue and the round or pyriform cells in regressing gonads, would indicate that interstitial cells are present at this stage of sexual cycle and their arrangement is like that of the lobule boundary cells.

The glandular layer in the testes of the European eel described by Tuzet and Fontaine (1937) may have some endocrine function and this would suggest that they also have interstitial cells present as lobule boundary cells.

The large, round to ovoid cells that appeared amongst the degenerating spermatozoa in regressing gonads appeared from their position and time of appearance to be phagocytic. Except for their regular shape, these cells are similar to those described by Bowers and Holliday (1961: 6) in the herring (Clupea harengus L.). These cells were also large, but irregular in shape, and were observed lying free in the lumina of empty tubules. Their function and origin could not be determined, but the authors suggest that they are the interstitial cells seen earlier in development in a different phase. In the eel testis these large cells appeared when the spermatozoa were still present. At this stage only small amounts of connective tissue, some containing small blood vessels, were present in the testes amongst the spermatozoa. It is unlikely that these large cells originated from cells within the connective tissue as only fibroblasts were observed in it. It is therefore suggested that the large cells originated from the blood vessels and may be modified granular leucocytes. In regressing testes erythrocytes were also observed amongst the degenerating spermatozoa and tissue debris, and they may have also been involved in the phagocytosis of the spermatozoa. Therefore, it appears likely that the resorption of spermatozoa involves phagocytosis by cellular elements of the blood. Sertoli cells have been recognized as phagocytes in some of the testes in some fish (Hoar 1969) but this type of cell was not observed in the eel testes.

The presence of the large cells amongst the degenerating spermatozoa may be considered as an abnormal phenomena. Eels in nature, if they survived spawning, would presumably have little, if any, spermatozoa remaining in the testes. When this stage was reached in hormone injected eels the large cells have disappeared. Their presence may therefore have been due to the unusual occurrence of large amounts of spermatozoa remaining in the testes.

In some teleosts that have a seasonal cycle of spermatogenesis germ cells and spermatogonia are present throughout the cycle (Bowers and Holliday 1961; Ashan 1966; Tong and Vooren 1972), although pycnotic degeneration of remnant spermatocytes and spermatogonia may occur (Ashan 1966). Tong and Vooren (1972) record the presence of primary germ cells throughout the year, and these would be a

source of new germ cells for each cycle of spermatogenesis. In the eel however development is synchronous and at maturity spermatogonia were rarely observed. These few would form new spermatogonia in the recovering testis by successive mitotic divisions.

The resorption of oocytes and the formation of corpora atretica has not previously been described in eels, and their formation raises the question of their function. The atretic oocytes have been described as preovulatory corpora atretica. Hoar (1969: 18 - 19) summarizes the two opposing views on the physiology of preovulatory and postovulatory corpora atretica. One view is that they are concerned with yolk phagocytosis in the preovulatory follicles or the removal of tissue fragments and blood cells in the postovulatory ones. The other view is that they both have an endocrine function. For some fish it is suggested by Hoar (1965) that the formation of corpora atretica is similar to the formation of corpora lutea in mammals, which produce the hormone progesterone and are under the control of the pituitary gland. Hoar (1965) suggests that the corpora atretica in some fish may function as corpora lutea but instead synthesizing oestrogen as their hormone.

In this study the endocrine function, if any, of the corpora atretica could not be determined. As only preovulatory corpora atretica were observed it is suggested that their formation may have only been concerned with the phagocytosis of yolk. It was noticeable that the largest number of atretic ova occurred in the eel in which maturity but not ovulation had occurred. Faleeva (1965) in Tong and Vooren (1972) suggests that the formation of corpora atretica is a sign of disturbance in the spawning conditions. This may account for the large number of atretic ova observed in the carp pituitary injected shortfin that matured but did not spawn.

This study of gametogenesis in the New Zealand eels shows that it is similar to that occurring in other teleosts. The post maturation changes in the testis and the formation of large numbers of corpora atretica in the ovaries may be abnormal as these were observed in eels kept under experimental conditions. The survival of eels in these conditions is not considered proof that these changes may also occur in eels in nature. Prior to this study no attempts had been made to induce the maturation of New Zealand eels. Although maturation of the male eel has been achieved by several authors

(Boëtius and Boëtius 1967a) only limited success has been achieved with the female eel. Injections of mammalian hormones have not produced a significant increase in the sexual development of the females, and, using carp pituitary extract, only one has been induced to spawn. Published reports also show that few Japanese eels, either male or female, have been induced to spawn. However, a significant increase in sexual development has been obtained with injections of mammalian hormones.

The hormone induced maturation and spawning of New Zealand eels, in particular the seven females, therefore represents a significant advance in the reproductive biology of the two species. The complete development of the gonads beyond the migrant stage has been observed and the mature spermatozoa and eggs described. These results, for practical reasons, could only be obtained in the laboratory by the artificial induction of maturation.

The mortality rate amongst the hormone injected eels of either sex was low. Male eels were relatively easy to induce to mature, and it has been suggested that by using different hormone preparations viable spermatozoa could be produced. A high success rate in maturation was also obtained with the female eels injected with carp pituitaries. With these results it is considered likely that the fertilization of the mature eggs could be easily obtained with further experimentation. Thus it may be ultimately possible to observe the early development of the egg, an event that has not yet been recorded for any anguillid species.

6. A COMPARATIVE ELECTRON MICROSCOPE STUDY OF SPERMIOGENESIS

6.1 Introduction

Teleost spermatozoa are diverse in their form and structure, varying from the biflagellate spermatids of Porichthys notatus Girard (Batrachoididae) (Stanley 1965) to the aflagellate spermatozoa of Gymnarchus niloticus Cuvier (Gymnarchidae) (Mattei, Boisson, Mattei and Reizer 1967). The absence of an acrosome, which occurs in all other vertebrate groups, is the only common feature (Mattei 1970 : 63).

Several electron microscope studies on teleost spermatozoa exist but as noted by Stanley (1969) most of these are of limited scope. Porte and Follenius (1960) and Stanley (1969) give the only detailed accounts of spermiogenesis in a teleost, while only two authors (Billard 1970; Mattei 1970), make a comparative study of teleost spermiogenesis. Mattei and Mattei (1972) describe the fine structure of the spermatozoa of the teleost Albula vulpes L. (Albulidae) which is similar to the spermatozoa of the European freshwater eel, a brief account of which is given by Ginsburg and Billard (1972). This latter study gives the only description of freshwater eel spermatozoa as seen in the electron microscope. However, Boisson, Mattei and Mattei (1967) describe the fine structure of the spermatids of the marine eel Lycondontis afer Bloch (Muraenidae). Two early papers, Rodolico (1933), and Tuzet and Fontaine (1937), describe the spermatozoa of the European freshwater eel as observed under the light microscope. These descriptions are discussed in the previous section on the induction of maturation.

This section describes the spermiogenesis of the two species of New Zealand freshwater eels.

6.2 Materials and Methods

Fresh spermatozoa were obtained from mature eels of both species in which maturity had previously been induced by hormone injections, as described in the section on the induction of maturation. Spermatozoa were obtained after anaesthetizing the eels and then stripping them of milt (see section 5). Spermatozoa were examined both whole and in section.

For the examination of whole spermatozoa two methods of fixing and drying were used. Drops of fresh milt were placed on a glass slide with an equal amount of 2% aqueous osmium tetroxide or 3% glutaraldehyde for 15 minutes. A drop of the sperm in suspension was then placed on a grid and air dried at room temperature for 30 minutes, or vacuum dried. The grids were then examined in a Zeiss EM-9A transmission electron microscope.

For sectioning two methods of fixation were used. In the first method small pieces of testis from freshly killed mature male eels were fixed in 0.1M Millonig's phosphate buffered glutaraldehyde at pH7.4 for three hours, then washed for three hours at 15 minute intervals in fresh 0.1M buffer solution at pH7.4. The material was post-fixed in 2% osmium tetroxide in phosphate buffer for three hours. The tissue was washed in distilled water and dehydrated in a graded ethyl alcohol series, followed by two changes of propylene oxide. Infiltration and embedding in Araldite was done according to Johnston and Loper (1970).

In the second method of fixation the tissue was fixed in 1% osmium tetroxide for three hours, washed in distilled water and dehydrated and embedded as previously described.

Sections were cut on a LKB ultramicrotome with a glass knife. Sections were stained using a modified method of Johnston and Loper (1970) for 30 minutes in aqueous lead nitrate at room temperature (see appendix for modified stain formula).

Sections were examined in a Zeiss EM-9A transmission electron microscope.

6.3 Observations

The heads of mature, whole spermatozoa of the longfin eel appear as electron dense, elongate, crescent-shaped bodies. Anteriorly a prominent bulbous projection is seen on the inner, concave surface of the head (Fig.6.1). At the posterior end of the head, close to the origin of the flagellum, is a constriction forming two prominent ridges (Fig.6.2). The heads are 8 μ m long and 3 μ m wide. The flagellum arises from the base end of the head, from the inner concave side relative to the shorter rod like structure, the auxiliary flagellum (Fig.6.1). The main flagellum 28 μ m to 44 μ m long, has nine

FIG.6.1 A. dieffenbachii (longfin) spermatozoon.
The short auxiliary flagellum (arrow)
projects from the posterior end of the head
adjacent to the main flagellum. x 4500

FIG.6.2 A. dieffenbachii spermatozoon. The
constriction in the neck region is arrowed.
x 12000

6.1

6.2

longitudinal filaments (Fig.6.3). This indicates an unusual 9 + 0 filament structure. The auxiliary flagellum, 2 μ m long, has cross striations (Fig.6.1). In Fig.6.1 it projects as a rod like structure at an acute angle from the posterior end of the head on the convex side relative to the main flagellum. In other spermatozoa it was observed projecting from the concave side of the head.

In contrast, the heads of the spermatozoa of the shortfin are slightly smaller and more slender (Fig.6.4). They measure 6 μ m long and 2 μ m wide. The anterior bulbous portion is less prominent and the ridges at the posterior end of the head are less noticeable. The flagella are shorter, measuring from 26 μ m to 30 μ m long. The auxiliary flagellum is similar in size (2 μ m long) and has prominent cross striations (Fig. 6.5) each at 0.08 intervals (periodicity 12 to 15 per 1 μ m).

Apart from the differences in size the spermatozoa of the two species are essentially similar.

All stages of spermiogenesis could be observed in both species, though identical sections of different structures in the spermatozoa of both species could not be obtained. However, the similarity of whole spermatozoa as well as their fine structure indicated parallel features of spermiogenesis in both species. The following observations are therefore considered to be representative of both species.

In early stage spermatids, in telephase of the last maturation division, the nucleus appears as a large round structure with a complete nuclear membrane and occupies most of the cell volume. The chromatin is irregularly dispersed in loosely packed electron dense areas. The cytoplasm, much less electron dense than the nuclear material, appears granular in structure. Several mitochondria are seen irregularly dispersed in the cytoplasm (Fig.6.6). The cell membrane encloses the developing spermatid. Some spermatids show cytoplasmic bridges between them where cytokinesis is not yet complete (Fig.6.7).

The distal centriole is seen in the early stages of spermiogenesis as a small cylindrical structure close to and at right angles to the cell membrane. A protuberance develops in the cell membrane as the distal centriole gives rise to the main flagellum (Fig.6.7).

The proximal centriole appears at right angles to and slightly to one side of the distal centriole in an indentation in the nuclear membrane (Fig.6.8). A series of subfibrils extend from

FIG. 6.3 Flagellum filaments - A. dieffenbachii
 (longfin) spermatozoa. Nine filaments
 are present. x 19000

FIG.6.4 A. australis (shortfin) spermatozoon. The
 short auxiliary flagellum (arrow) projects
 from the posterior end of the head adjacent
 to the main flagellum. x 5000

6.3

6.4

FIG.6.5 A. australis (shortfin) spermatozoon head.
Transverse cross-striations are present on
the auxiliary flagellum (arrow.). x 1750

FIG.6.6 Early stage spermatids. The cell outline
is clearly visible and several mitochondria(m)
are present in the cytoplasm. The distal
centriole(d) is adjacent to the cell wall,
n - nucleus - A. australis. x 12000

6.5

6.6

FIG.6.7 Spermatid. The auxiliary flagellum (arrow)
is within the cell adjacent to the mitochondrion(m).
The asterisk marks the cytoplasmic bridge
between two cells. f - flagellum, d - distal
centriole, p - proximal centriole -
A. australis (shortfin). x 7000

FIG.6.8 An early stage spermatid, to show the single
mitochondrion (m) and the distal (d) and
proximal (p) centrioles. The intracellular
filaments (arrow) extend from the proximal
centriole into the cytoplasm. The indentation
in the nucleus (n) is visible.
A. australis. x 19000

6.7

6.8

the proximal centriole to the cytoplasm (Fig.6.8). Prior to the formation of the proximal centriole several mitochondria occur in the cytoplasm (Fig.6.6) around the region of the developing distal centriole. These mitochondria fuse to form a single, large, elongate mitochondrion with a network of cristae. This fusion is complete by the time of the formation of the proximal centriole (Fig.6.7 and 6.8). The mitochondrion lies in an indentation of the nuclear membrane, closely appressed between this and the two centrioles (Fig.6.8).

The auxiliary flagellum develops as a rod-like structure 2 μm long and 0.2 μm wide, with prominent cross striations (12 to 15 per 1 μm), and lies close to the distal centriole and the elongated mitochondrion (Fig.6.7).

The chromatin material becomes increasingly electron dense during spermiogenesis and occupies the whole nucleus. The nucleus becomes crescent shaped as the indentation at its base becomes deeper (Fig.6.7). The centrioles maintain their same relative positions as they come to lie at one end of the crescent shaped nucleus. The mitochondrion is displaced to the opposite end of the crescent as the indentation deepens (Fig.6.9). The tubular cristae of the mitochondrion are now distinct. In the mature spermatozoa the mitochondrion lies against the concave face of the nucleus, forming the bulbous portion described in the whole spermatozoa (Fig.6.9). The convex side of the nucleus is pressed close to the cell wall with the cytoplasm confined to its concave side (Fig.6.9).

A complex arrangement of subfibrils develops in connection with the proximal centriole. In the centre of the proximal centriole an extension of the nuclear membrane separates the filaments into one group of five and one group of four filaments (Fig.6.10). The two sets of filaments diverge and extend along opposite sides of the nucleus (Fig.6.11). In the group of five filaments, each filament is composed of three sub-fibrils (Fig.6.12.) These five triplets extend parallel to one another along the inner, concave, curve of the nucleus (Fig.6.13) separated from the plasma membrane by a wide band of cytoplasm (Fig.6.13).

The four filaments of the second group have slightly modified triplets. The middle subfibril B of each triplet loses its tubular shape and forms a bar joining the two subfibrils A and C (Figs.6.12 and 6.13). These modified triplets appear in cross section as binocular shaped structures and extend up the outer, convex, side of the nucleus.

FIG.6.9 A section through a spermatid to show the development of the crescent shaped nucleus.
m - mitochondrion, p - proximal centriole,
d - distal centriole, pl - plasma membrane.
A. dieffenbachii (longfin) x 12000

FIG.6.10 Complex proximal centriole. The extension of the nuclear membrane (arrow) divides the proximal centriole filaments. In the centre of the complex, the binocular-shaped double subfibrils and the triple subfibrils are present.
A. dieffenbachii. x 40000

6.9

6.10

FIG.6.11 A longitudinal section through a late stage spermatid to show the two diverging sets of intracellular filaments (arrow), arising from the proximal centriole (p)
A. australis (shortfin). x 12000

FIG.6.12 A transverse section through a late stage spermatid to show the two sets of intracellular filaments. The set of five filaments (double arrows) are each composed of three subfibrils. The set of four filaments (single arrow) are each composed of two subfibrils (A and C) joined by the modified B subfibril.
A. australis. x 19000

6.11

6.12

FIG.6.13 Section through a late stage spermatid to show the set of five intracellular filaments (arrow) separated from the plasma membrane (pl) by a wide band of cytoplasm. The set of four modified intracellular filaments are close to plasma membrane (pl)
A. australis (shortfin). x 40000

FIG.6.14 An oblique section through a late stage spermatid to show the set of five intracellular filaments (arrow) extending around the mitochondrion (m).
A. dieffenbachii (longfin). x 19000

6.13

6.14

The two sets of filaments extend up to and around the mitochondrion. The outer set of four filaments extend around the upper curve of the nucleus and over the mitochondrion (Fig.6.14). The inner set of five filaments also extend around the mitochondrion, between it and the nuclear membrane. Both sets of filaments are seen to extend around the top of the mitochondrion (Fig.6.15) and to extend along one side of it (Fig.6.16). The two sets of subfibrils do not extend beyond the mitochondrion. In the narrow neck region of the mature sperm the distal and proximal centrioles lie close together (Figs.6.9 and 6.16). In longitudinal sections the proximal centriole lies at right angles to the distal centriole and the filaments from it project forward onto the nucleus. In the corner of the right angle is a rounded electron dense body (Fig.6.17). In other sections the centrioles are cut in a transverse direction (Fig.6.16). Rarely was the auxiliary flagellum visible in longitudinal section with the main flagellum (Figs.6.7 and 6.18). The position of the centrioles (Figs.6.7 and 6.17) would indicate that the auxiliary flagellum could be an extension of the distal centriole, or be associated with both centrioles.

The main flagellum consists of the unusual 9 + 0 filament complex surrounded by the plasma membrane of the cell (Fig.6.19). In cross-sections of the flagellum near to the distal centriole the two subfibrils of each filament are linked to the plasma membrane by radial Y-shaped densities (Fig.6.20), giving the appearance of a cartwheel arrangement. In sections further down the flagellum the links disappear and the subfibrils are closely surrounded by the plasma membrane (Fig.6.19).

6.4 Discussion

Though the spermatozoa of the European eel have been described only briefly by Ginsburg and Billard (1972), they appear similar in size, shape and general detail to those of New Zealand eels. The spermatozoa have a crescent-shaped head, a single anterior mitochondrion, a 9 + 0 filament type flagellum and a double set of four and five filaments around the head. However, an extra tail-like structure or auxiliary flagellum has not been described. A schematic reconstruction of an eel spermatozoa is given in Fig.6.21.

The spermatozoa of this study are also similar in shape and

FIG.6.15 An oblique section similar to Fig.6.14 to show the two sets of intracellular filaments (arrow) extending over the mitochondrion (m)
A. dieffenbachii (longfin). x 14000

FIG.6.16 A longitudinal section through a late stage spermatid. The large mitochondrion (m) is partly surrounded by the nucleus. The intracellular filaments (arrow) extend around the mitochondrion. The proximal (p) and distal (d) centrioles are at the posterior end of the nucleus.
A. dieffenbachii. x 12000

6.15

6.16

FIG.6.17 Longitudinal section through the 'neck' region of a sperm head. The set of intracellular filaments (arrow) extend from the proximal centriole (p). The distal centriole (d) is at right angles to the proximal centriole and the flagellum (f) arises from it. An electron dense body (asterisk) is present between the two centrioles.
A. australis (shortfin). x 19000

FIG.6.18 Longitudinal section through a late stage spermatid. The set of five intracellular filaments (arrow) extends from the proximal centriole (p). The flagellum (f) projects from the distal centriole (d). A portion of the auxiliary flagellum (asterisk) is adjacent to the main flagellum.
A. australis. x 12000

6.17

6.18

FIG.6.19 Transverse sections through two flagella showing the 9 + 0 filament pattern. The filaments lie close to the plasma membrane (pl) A. australis (shortfin). x 19000

FIG.6.20 Transverse sections through flagella. One, (arrow) is sectioned nearer to the distal centriole than in Fig.6.19. The subfibrils are joined to the plasma membrane (pl) by radial Y-shaped densities. x 19000

6.19

6.20

FIG.6.21

A schematic reconstruction of an eel spermatozoon.

A, B, C and D represent cross-sections of the spermatozoon.

af - auxiliary flagellum

dc - distal centriole

fl - flagellum

m - mitochondrion

n - nucleus

pc - proximal centriole

sb - subfibrils

fine structure to the spermatozoa of A. vulpes described by Mattei and Mattei (1972), though several differences exist. The set of five triplet subfibrils passes along the convex side of the nucleus in A. vulpes, while in eel spermatozoa the modified set of four subfibrils occupy a similar position. In A. vulpes the triplet and doublet subfibrils form a tubular structure with the two leaflets of the nuclear membrane, which can extend from the distal (anterior in this study) end of the spermatozoa and form a 'pseudo-flagellum'. No such extension could be seen from the anterior end of the eel spermatozoa. Mattei and Mattei (1972) also describe a rod of periodic structure associated with the distal centriole which projects at the anterior (posterior in this study) end of the spermatozoid. This is identical to the striated rod-like structure projecting from the posterior end of the eel spermatozoa.

The cross striated appearance of the auxiliary flagellum is similar to that described for ciliary rootlets which are closely associated with the cilia in ciliated epithelia (Fawcett and Porter 1954). These authors suggest that these rootlets are similar to protein fibres and therefore indicate that they are involved in a mechanical or contractile role, perhaps serving to anchor the basal body.

The spermatids of the teleost P. notatus are biflagellate (Stanley 1965) and presumably both are motile organelles. The eel spermatozoa however could not be classified as biflagellate. The auxiliary flagellum, as indicated by its size and striated appearance, probably does not function as a motile organelle. Its close association with the centrioles suggests that it may be a modified flagellum. However, its position in Fig.6.7 indicates that it originates from the basal body of the main flagellum. It is suggested that it has a mechanical function, perhaps anchoring the basal body and acting as a 'rudder-like' structure providing stability when the spermatozoa is moving.

The centriolar apparatus of the eel spermatozoa is similar to that described for A. vulpes (Mattei and Mattei 1972) and the muraenid eel L. afer (Boisson et al. 1967). The latter paper describes the fine structure of the spermatid and states that the proximal centriole develops intracellular filaments that disperse in the cytoplasm. A schematic representation of the spermatozoid of

L. afer (Mattei 1970 : 64) shows that this arrangement persists in the spermatozoa. In the eel spermatozoa these intracellular filaments extend further, along the total length of the head and in a definite arrangement before dispersing. In the biflagellate spermatozoa of P. notatus each of the two centrioles form a flagellum (Stanley 1965). In the eel spermatozoa the rod-like structure develops as an auxiliary flagellum and the filaments arising from the proximal centriole are intracellular. This suggests that the eel spermatozoa may have evolved from a biflagellate spermatozoa, with the second flagellum being reduced to form the auxiliary flagellum, or becoming modified to form the intracellular filaments.

Until recently the 9 + 2 arrangement of filaments was considered to be the uniform pattern for all motile cilia and flagella, and was held remarkable for its stability (Satir 1961). Several atypical arrangements have been shown to occur, mainly in the invertebrates, as listed by Boisson et al. (1967). Flagella of the type 9 + 0 are considered to be modified flagella which have lost their primary function of motility to become sensory (Boisson et al. 1967). However, in the eel spermatozoa the flagellum retains its motile function. These authors describe for the first time a vertebrate, L. afer, with spermatid flagella possessing a 9 + 0 filament structure. It is of interest that this unusual arrangement has been observed only in the spermatozoa of the order Anguilliformes: in L. afer (Boisson et al. 1967), in A. anguilla (Ginsberg and Billard 1972), in A. vulpes (Mattei and Mattei 1972), and in A. dieffenbachii and A. australis as shown in this present study. The morphological features of the spermatozoa of these species of the order Anguilliformes are listed in Table 6.1.

Teleost spermatozoa exhibit great variation in the position and number of mitochondria (Mattei 1970 : 64). The migration and fusion of several mitochondria as seen in the eel spermatozoa has been briefly shown by Mattei (1970) for two teleosts, Arapristipoma octolineatum (Pomadasidae) and Upeneus paryensis (Mullidae). Eel spermatozoa have no middle piece, the region of the spermatozoid behind the head usually containing the mitochondria(ion) (Farard and Andre 1970: 415). Most other teleost spermatozoa, except the European eel (Ginsburg and Billard 1972) and A. vulpes (Mattei and Mattei 1972), have one or more mitochondria distributed

TABLE 6.1

Morphological features of the
spermatozoa of the order
Anguilliformes

TABLE 6.1

Morphological features of the

Species	Head shape	Mitochondrion
<u>Albula vulpes</u> (Mattei and Mattei 1972)	Elongate, tapered anterior end	Single, anterior
<u>A. anquilla</u> (Ginsburg and Billard 1972)	Elongate, crescent- shaped 8 μm to 11 μm long.	Single, large anterior
<u>A. australis</u>	Elongate, crescent- shaped 6 μm long 2 μm wide	Single, large anterior
<u>A. dieffenbachii</u>	Elongate, crescent- shaped, 8 μm long, 3 μm wide	Single, large anterior
<u>Lycondontis afer</u> (Boisson, Mattei and Mattei 1967; Mattei 1970)	Rounded	Four, posterior region of head

spermatozoa of the order Anguilliformes

Centriolar apparatus	Intracellular sub fibril	Auxiliary flagellum	Flagellum
Complex. Distal and proximal centrioles located at posterior end of head.	One group of 5 and one group of 4 triplets modified. Sub fibrils may extend beyond anterior end of head as 'pseudo-flagellum.'	Present, periodic structure. Not called auxiliary flagellum.	9 + 0 filament pattern.
Complex. Distal and proximal centrioles located at posterior end of head.	One group of 5 and one group of 4 triplets around head.	Not recorded.	9 + 0 filament pattern 24 μ m-36 μ m long.
Complex. Distal and proximal centrioles at right angles, located at posterior end of head.	One group of 5 and one group of 4 triplets. Sub fibrils extend length of head.	Present, striated, rod-like. 2 μ m long.	9 + 0 filament pattern. 26 μ m - 30 μ m long.
Complex. Distal and proximal centrioles at right angles, located at posterior end of head.	One group of 5 and one group of 4 triplets. Sub-fibril B of 4 triplets modified. sub fibrils extend length of head.	Present, striated rod-like. 2 μ m long.	9 + 0 filament pattern. 28 μ m - 44 μ m long
Complex. Distal and proximal centrioles and right angles.	Nine groups of triplets. Scattered in cytoplasm, do not extend length of head.		9 + 0 filament pattern

symmetrically or asymmetrically around the centriolar apparatus in the middle piece. This concentration of energy producing organelles in the middle piece would allow for the rapid transfer of energy to the flagellum.

In contrast to this usual arrangement of the mitochondria, the anterior position of the large single mitochondrion in the eel spermatozoa presents a unique arrangement. The strongly tapered and sometimes recurved structure described as the acrosome in the European eel spermatozoa by Tuzet and Fontaine (1937), probably corresponds to the curving anterior portion of the head described here. Ginsburg and Billard (1972) consider that the acrosome described by Tuzet and Fontaine probably corresponds to the mitochondria. However, the latter structure is a large and round, unlike the description given by Tuzet and Fontaine.

The anterior position of the mitochondrion remote from the flagellum raises the problem of energy transfer. Although connected to the proximal centriole by the subfibrils along which the transfer of energy could take place, it would be expected that with the external fertilization in the eel the mitochondrion would be placed in close association with the flagellum for the rapid transfer of energy. The large mitochondrion with closely packed cristae indicates that it produces considerable amounts of energy. Further, the long flagellum would show that the spermatozoa are actively motile in nature. Energy could be transported along the subfibrils extending along the head to the proximal centriole. The close proximity of the centrioles would allow for the transfer of energy across a concentration gradient from the proximal to the distal centriole and hence to the flagellum. Of the five species listed in Table 6.1, only the spermatozoa of L. afer (Boisson et al. 1967) have a posteriorly located mitochondria and no subfibrils along the length of the head. All species whose spermatozoa have anterior mitochondria possess subfibrils linking the mitochondria to the proximal centriole. This would indicate that they are microtubules used for energy transport.

The absence of an acrosome in teleosts has been ascribed to the presence of micropyles in the eggs (Nicander 1970: 49). The anterior position of the mitochondria in eel spermatozoa, in the position occupied by the acrosome in other species, may indicate

that it is involved in the entry of the spermatozoa into the egg. Thus it may function as does the acrosome in other species during fertilization.

Another possible explanation for the isolation of the mitochondrion from the motile apparatus has been suggested by Robison in Billard (1970: 79). He suggests that as DNA is present in the mitochondria it may be involved in the transmission of hereditary material. Thus the anterior position of the mitochondrion in the eel spermatozoa may indicate that its role in supplying energy for movement has been partially replaced by that of transmitting hereditary material.

It is suggested that the mitochondrion in the eel spermatozoa may be fulfilling a dual function. Firstly, the production of energy for movement, as indicated by the dense cristae and the connection of the mitochondrion to the proximal centriole. Secondly, to facilitate the penetration of the spermatozoa into the egg, and the possible transmission of some hereditary material, as indicated by the anterior position of the mitochondrion.

As noted by Favard and Andre (1970: 424), the great variation in sperm mitochondria have probably little importance in evolution. However, Mattei (1970: 68) discussing the evolution of fish spermatozoa suggests that the teleosts present 'une sorte d'explosion en plusieurs types spermatiques simplifies'. Spermatozoa of the order Anguilliformes with their similar structures, such as the single mitochondrion and the 9 + 0 filament structure of the flagellum, may represent an evolutionary trend from a common, possibly biflagellate, ancestral spermatozoa type. The morphological differences in their spermatozoa (Table 6.1), may indicate that these cells may be useful in taxonomic studies as suggested by Dadone and Narbaitz (1967). Further, the variety shown in shape and structure of teleost spermatozoa, and the specialization of spermatozoa in species with internal fertilization, would indicate that the diversity is due to individual specialization of the spermatozoa in relation to the type of fertilization. Thus the unique and similar structure of eel spermatozoa could be explained as an adaption to its type of fertilization which, for the European eel, while presumed to be external (Deelder 1970: 3:1), remains unknown.

7. GENERAL DISCUSSION

This study has established for the first time the specific details of the reproductive biology of New Zealand freshwater eels. Macroscopic studies of the gonads in non-migrant eels, complemented with histological examinations, has shown a sequence of development of the gonads. From this evidence it is suggested that the intersexual stage of development, which occurs in the European eel (Bertin, 1956) but probably not the Japanese eel (Satoh, Nakamura and Hibiya 1962), is absent. Further, the lobed organ of Syrski, although absent in some longfin migrant males can be used as a diagnostic of maleness. This is in contrast to the European eel in which the lobed organ of Syrski may contain oocytes.

These differences represent a significant difference between the reproductive biology of the New Zealand eels and the European eel. For the latter species there are extensive histological studies on the development of the gonad (Rodolico 1933; D'Ancona 1943). Now that the general sequence of gonad development has been established for New Zealand eels, similar more detailed histological investigations should be conducted. In this study most of the eels examined were larger than 20cm in total length. It could therefore be argued that the intersexual stage may occur in eels under 20cm in length. However, from the smaller number of eels examined under this length there was no evidence that this occurred. Additional detailed macroscopic and histological studies should determine the development of the gonad from the glass eel stage in a similar manner to the previously mentioned European and Japanese studies. The absence of this part of the sexual development could be considered a limitation of this study. However, it was considered beyond the scope of this present investigation to include this.

The identification of male eels by previous authors (e.g. Burnet 1952b; Woods 1964) has been confirmed and extended. Compared with studies on the sex and distribution of the European eel (Tesch 1928; D'Ancona 1959a; Sinha and Jones 1966) that for New Zealand eels is not accurately known. In this study insufficient numbers of eels were available from different localities to determine the distribution of the sexes, but now that eels can be correctly sexed future research should establish the sex and distribution of eels in different bodies of water.

The downstream and seaward migration of eels from the Makara stream and Lake Onoke has been shown to be related to certain environmental factors, such as the increase in stream level and the darkest period of the lunar cycle. This confirms and extends Burnet's (1969a) earlier research and, in contrast, shows that migrating eels in the Makara stream migrate later than in the South-branch stream. As discussed, these differences in the migration times are possibly due to the earlier migration of the eels from further inland. It was not possible in this study to investigate in detail the migration times of eels from other areas. Further studies to determine this, and the reproductive state of eels in other streams and rivers, may explain these differences in migration times.

From the gonadosomatic indices, ova diameter and histology of the gonads it has been shown that longfin eels are significantly more sexually developed at the time of seaward migration than the shortfins. Prior to this study the state of reproductive development of the migrating eels had not been investigated. Thus this section of the study is a significant contribution to the reproductive biology of New Zealand eels.

Compared with other temperate species of freshwater eels, the shortfin is more sexually advanced than the European eel at the time of seaward migration. In contrast, the longfin is the more sexually developed than either the European, American or Japanese eels at migration. If it is accepted that the degree of sexual development at migration is proportional to the distance from the spawning grounds, the longfin eel probably spawns closer to New Zealand than does the shortfin. However, the known hydrographical data are insufficient to determine where these spawning grounds are. Thus, although this study has clearly defined the state of reproductive development at migration and indicated the possibility of separate spawning grounds, it has contributed little to determining where they are.

In this study a satisfactory technique for ageing eels, that of burning otoliths, has been established. Differences in the growth rate between the sexes have been found that are similar to those recorded for the European eel by Sinha and Jones (1967a)

Compared with extensive studies on the European eel there are only two significant age and growth studies on New Zealand eels (Cairns 1941; Burnet 1969b). This study, by differentiating between the sexes and in particular by determining the age of migrant eels, has therefore considerably advanced present knowledge of the age and growth of New Zealand eels.

In contrast to other temperate species, New Zealand eels, particularly the longfins, have extremely slow growth rates and attain great ages. This makes the eels vulnerable to overfishing and indications are (Burnet 1969c) that the recovery rate of exploited populations is slow. Future age and growth research therefore should determine the variability in the growth rates of different populations, and investigate the age and growth of commercially exploited populations.

To complete the study of the reproductive cycle, migrant eels of both sexes and species were injected with either mammalian hormones or carp pituitaries and induced to spawn. In addition, the maturing process and regression of the gonads was examined histologically. A limitation of this section of the study was that the histological techniques used did not reveal precisely the exact location and nature of the interstitial cells. Further histological studies using different techniques should establish this.

Experiments to induce maturation have not previously been attempted with New Zealand eels. The European male eel has been induced to mature by several authors (Boëtius and Boëtius 1967a), but only one female has been induced to spawn (Fontaine, Bertrand, Lopez and Callamand 1964). Only one Japanese eel has been induced to mature (Ishida and Ishii 1970).

None of these studies has given a detailed account of the histological changes in the gonad, including the regression and recovery of the testis, or given a detailed illustrated description of the mature eggs and spermatozoa. This study has included this and it has also described spermiogenesis as observed under the electron microscope. Therefore, the hormone induced spawning of both longfin and shortfin male and female eels represents a considerable advance in the study of their reproductive biology, and for anguillid eels in general.

One of the aims of inducing maturation in eels has been, from a commercial viewpoint, to ultimately obtain fertilized eggs and rear them through to the glass eel stage for farming purposes.

Because of the shortage of Japanese glass eels for restocking eel farms this apparently has been the aim of some research on the hormone induced maturation of the Japanese eel (Sanders 1971: 17).

From current research it seems probable that viable eggs and spermatozoa can be obtained from the European, Japanese and New Zealand eels, and successful fertilization of the eggs achieved. However, to rear developing eggs through the embryonic, pre-leptocephalus and leptocephalus stages will present major problems. Lack of knowledge of the precise conditions at spawning and development suggests that they would be difficult to duplicate. If reared to the leptocephalus stage the problem of feeding would arise. Further, this stage would need to be kept in controlled conditions for periods of perhaps two years before metamorphosis into the glass eel stage. Unless this metamorphosis could be induced earlier, a delay of up to two years would make the operation uneconomical. With these problems to overcome it is considered unlikely that the hormone induced maturation of eels will be of any commercial value in the foreseeable future.

This study has considerably advanced knowledge of the reproductive biology of New Zealand freshwater eels. New Zealand is unique in possessing two species of temperate freshwater eels and, although the basic biology of all temperate freshwater eels so far studied is essentially similar, several specific differences exist, such as the absence of the intersexual stage in New Zealand and Japanese eels. Other differences, such as in the growth rates are consequences of the different environments, while others such as the reproductive state at migration, are adaptations to the length of the journey to the oceanic spawning grounds.

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APPENDIX A

analysis of eel data , analysis of covariance (log wt/log length)
between sexes within migratory state, species and area:

```

begin
  real d, x, y;
  integer mig, area, sp, sex, i, j, k;
  real array sx, sy, ssx, ssy, sxy, cx, cv, cxy, Sx, Sv, Sxv, r, b, ss,
    df, mms, sb, jy, mv, mx, a[0:1, 0:1, 1:2];
  integer array n[0:1, 0:1, 1:2];

  real array smx, smy, smxx, smyy, smxv, crx, crv, crxv, Tx, Tv, Txv,
    tss, tdf, spx, spv, spxv[0:1, 0:1];
  real array se1, se2, se3, se4, ms1, ms2, ms3, ms4, ms7, f1, f2, dfa,
    dfb, dfd, dfc, DF, SB, B, Ybar, Xbar, A, R[0:1,0:1];
  integer array N[0:1, 0:1];
  switch sw:=L1;

  sameline;

  i:= j:= k:= 0;
  for i:= 0, 1 do for j:= 0, 1 do for k:= 1,2 do
    begin
      sx[i, j, k]:= sy[i, j, k]:= ssx[i, j, k]:= ssy[i, j, k]:=
        sxy[i, j, k]:= 0;
      n[i, j, k]:= 0;

    end;

  for i:= 0, 1 do for j:= 0, 1 do
    begin
      smx[i,j]:= smy[i,j]:= smxx[i,j]:= smyy[i,j]:=
        smxy[i,j]:= 0;
      Tx[i,j]:= Ty[i,j]:= Txy[i,j]:= tss[i,j]:= tdf[i,j]:= 0;
      N[i,j]:= 0;

    end;

  L1: read mig, area, d, d, d, sp, x, v, d, d, d, d, d, d, sex, d, d;

  for i:= 0,1 do begin if sp = 1 then
    for j:=0, 1 do begin if mig = 1 then
      for k:= 1, 2 do begin if sex = k then
        begin
          x:= ln(x) * .4342944819;
          y:= ln(y) * .4342944819;

```

```

sx[i,j,k]:=sx[i,j,k]+x;
sy[i,j,k]:=sy[i,j,k]+v;
ssx[i,j,k]:=ssx[i,j,k]+x*x;
ssy[i,j,k]:=ssy[i,j,k]+v*v;
sxy[i,j,k]:=sxy[i,j,k]+x*v;
n[i,j,k]:=n[i,j,k]+1;

```

end;

end;

end;

end;

if buffer $\neq$ 8 then goto L1;

comment calculates sums, squares and products by species migratory state and sex;

for i:=0,1 do for j:=0,1 do for k:=1,2 do if n[i,j,k] > 0 then

begin

```

smx[i,j]:=smx[i,j]+sx[i,j,k];
smy[i,j]:=smy[i,j]+sy[i,j,k];
smxx[i,j]:=smxx[i,j]+ssx[i,j,k];
smyy[i,j]:=smyy[i,j]+ssy[i,j,k];
smxy[i,j]:=smxy[i,j]+sxy[i,j,k];
N[i,j]:=N[i,j]+n[i,j,k];

```

comment calculates sums of squares and products for total by species and migratory state;

```

cx[i,j,k]:=sx[i,j,k]^2/n[i,j,k];
cy[i,j,k]:=sy[i,j,k]^2/n[i,j,k];
cxy[i,j,k]:=sx[i,j,k]*sy[i,j,k]/n[i,j,k];

```

comment calculates correction factors for subgroup sums of squares and products;

```

crx[i,j]:=smx[i,j]^2/N[i,j];
cry[i,j]:=smy[i,j]^2/N[i,j];
crxy[i,j]:=smx[i,j]*smy[i,j]/N[i,j];

```

comment calculates correction factors for total sums of squares and products of each subgroup;

```

Sx[i,j,k]:=ssx[i,j,k]-cx[i,j,k];
Sy[i,j,k]:=ssy[i,j,k]-cy[i,j,k];
Sxy[i,j,k]:=sxy[i,j,k]-cxy[i,j,k];

```

comment calculates corrected sums of squares and products for each subgroup;

```

Tx[i,j]:=Tx[i,j]+Sx[i,j,k];
Ty[i,j]:=Ty[i,j]+Sy[i,j,k];
Txy[i,j]:=Txy[i,j]+Sxy[i,j,k];

```

comment adds sums of squares and products for subgroups to give common sums of squares and products;

```

r(i,j,k):=Sxy(i,j,k)/(sqrt(Sx(i,j,k)*Sy(i,j,k)));
b(i,j,k):=Sxy(i,j,k)/Sx(i,j,k);
ss(i,j,k):=Sy(i,j,k)-Sxy(i,j,k)^2/Sx(i,j,k);
df(i,j,k):=n(i,j,k)-2;
mma(i,j,k):=ss(i,j,k)/df(i,j,k);

```

comment calculates correlation coefficient, regression coefficient, sums of squares of deviations from regression, d.f., and mean square for each subgroup;

$tss(i,j) := tss(i,j) + ss(i,j,k);$
 $tdf(i,j) := tdf(i,j) + df(i,j,k);$
 $sb(i,j,k) := (\sqrt{ss(i,j,k)/df(i,j,k)})/\sqrt{Sx(i,j,k)};$

comment calculates within sums of squares of deviations from regression, d.f. and standard deviation of subgroup regression coefficients:

$spx(i,j) := smxx(i,j) - crx(i,j);$
 $spy(i,j) := smyy(i,j) - cry(i,j);$
 $spxy(i,j) := smxy(i,j) - crxy(i,j);$

comment calculates corrected sums of squares and products for total:

$se1(i,j) := spy(i,j) - spxy(i,j)^2/spx(i,j);$
 $dfa(i,j) := N(i,j) - 2;$

comment calculates total sums of squares of deviation from regression and d.f.;

$se2[i,j] := Ty[i,j] - Txy[i,j]^2/Tx[i,j];$
 $dfb(i,j) := dfb(i,j) + (n(i,j,k)-1);$

end;

for i:= 0,1 do for j:= 0,1 do if N[i,j] > 0 then

begin

$dfb[i,j] := dfb[i,j] - 1;$

end;

comment calculates common sum of squares of deviations from regression and D.F.;

for i:= 0,1 do for j:= 0,1 do for k:= 1,2 do if n[i,j,k] > 0 then

begin

$ms1[i,j] := se2[i,j]/dfb[i,j];$

comment common mean square;

$dfc[i,j] := dfa[i,j] - dfb[i,j];$

comment adj. means d.f.;

$ms2[i,j] := (se1[i,j] - se2[i,j])/dfc[i,j];$

comment adj means mean square;

$SB[i,j] := (\sqrt{ms1[i,j]})/(\sqrt{Tx[i,j]});$

comment s.d. of common reg. coeff. ;

$f1[i,j] := ms2[i,j]/ms1[i,j];$

comment f value for testing elevations ;

$se3[i,j] := se2[i,j] - tan[i,j];$

$DF[i,j] := dfb[i,j] - tdf[i,j];$

$ms3[i,j] := tan[i,j]/tdf[i,j];$

comment within mean square;

$ms4[i,j] := se3[i,j]/DF[i,j];$

comment reg. coeff. mean square;

$f2[i,j] := ms4[i,j]/ms3[i,j];$

comment f value for testing reg. coefficients;

$my[i,j,k] := sy[i,j,k]/n[i,j,k];$

$mx[i,j,k] := sx[i,j,k]/n[i,j,k];$

$a[i,j,k] := my[i,j,k] - b[i,j,k]*mx[i,j,k];$

comment values for subgroup regression equations:

$B[i,j] := Txy[i,j]/Tx[i,j];$

$Ybar[i,j] := smy[i,j]/N[i,j];$

$Xbar[i,j] := smx[i,j]/N[i,j];$

$A[i,j] := Ybar[i,j] - B[i,j]*Xbar[i,j];$

comment values for common regression equation:

$se4[i,j] := se1[i,j] - se2[i,j];$

$R[i,j] := Txy[i,j]/(sqrt(Tx[i,j]*Ty[i,j]));$

comment common correlation coeff.;

$ms7[i,j] := se1[i,j]/dfe[i,j];$

comment total mean square;

$Jy[i,j,k] := (sy[i,j,k]/n[i,j,k]) - (((sx[i,j,k]/n[i,j,k]) - Xbar[i,j]) * B[i,j]);$

comment adjusted mean weight;

end;

print \$L3s39? analysis of covariance table. area?, digits(3).area,
\$L2?species \$s? state \$s?sex \$s5?ax2\$s6? sy2\$s6? sxv\$s7?b\$s8?df\$s6?
sdyx2\$s6?ms\$s8?r\$s9?sb\$L??;

for i:=0,1 do for j:=0, 1 do for k:=1,2 do if n[i,j,k] > 0 then

begin

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print \$L2?, digits(3), \$s3??, digits(2), j, \$s??:

if k=1 then print \$ female? else print \$ male ?:

print \$x[i,j,k], \$y[i,j,k], \$xv[i,i,k], \$b[i,j,k], \$df[i,i,k], \$s[i,j,k],
\$ms[i,j,k], \$r[i,i,k], \$rb[i,j,k]:

end of print list for subgroup data, analysis of covariance table:

for i:=0,1 do for j:=0,1 do if N[i,j] > 0 then

begin

print \$L2? within \$s53??. tdf[i,j], tss[i,j], ms3[i,j],
\$L? reg. coeff. \$s48??. DF[i,j], se3[i,j], ms4[i,j].
\$L? common \$s12??. Tx[i,j], Ty[i,i], Txy[i,i], B[i,j].
\$s??, dfb[i,j], se2[i,j], ms1[i,j], R[i,j], SB[i,j].
\$L? adj means \$s50??. dfc[i,j], se4[i,j], ms2[i,j].
\$L? total \$s13??. sdx[i,j], spy[i,j], sdxv[i,j], \$s11??.
dfa[i,i], se1[i,j], ms7[i,j].
\$L? F reg. coeff. = ? f2[i,j]. \$s3?d.f.?. DF[i,j]. \$.?.
tdf[i,j]. \$s8? f adj. means = ? f1[i,j],
\$s3?d.f.?. dfc[i,j]. \$.?. dfb[i,j]. \$L??:

end:

print \$L4? the regression equations for each subgroup are as
follows: \$L??:

for i:=0,1 do for j:=0,1 do for k:=1,2 do if n[i,j,k] > 0 then

begin

print \$L? species?, digits(2), i, \$s2? state?, digits(2), j, \$s2??:
if k=1 then print \$ female? else print \$ male ?:
print \$s6?y = ? b[i,j,k], \$x + ? a[i,j,k]:

end:

for i:=0,1 do for j:=0,1 do if N[i,j] > 0 then

begin

print \$L4? the common regression equation is y = ?, P[i,j].
\$x + ? A[i,j]. \$L2??:
\$L? adjusted mean weights for each subgroup with grand
mean length = ?.
Xbar[i,j], \$s? are as follows: ?;

end:

for i:=0,1 do for j:=0,1 do for k:=1,2 do if n[i,i,k] > 0 then

begin

print \$L? species?, digits(2), i, \$s2? state?, digits(2), j, \$s2??:
if k=1 then print \$ female? else print \$ male ?;
print \$s3? adjusted mean weight = ? jy[i,j,k];

end:

end;

APPENDIX B

analysis of eel data . Analysis of covariance (log wt/log length)
between migratory state, species and area:

begin

```

real d, x, y;
integer mig, area, sp, i, j;
real array sx, sy, ssx, ssy, sxx, syy, sxy, cx, cy, cxv, cyv, Sx, Sy, Sxx, Syy, r, b,
        ss, mms, sb, tv, mv, mx, a[0:1, 0:1];
integer array df, n[0:1, 0:1];

real array smx, smy, smxx, smyy, smxy, crx, cry, crxy, Tx, Ty, Txy,
        tss, spx, spy, spxy[0:1];
real array se1, se2, se3, se4, ms1, ms2, ms3, ms4, ms7, f1, f2, dfd,
        SB, B, Ybar, Xbar, A, R[0:1];
integer array tdf, dfa, dfb, dfc, DF, N[0:1];

switch sw:=L1;

```

same line;

i:= j:= 0;

for i:= 0, 1 do for j:= 0, 1 do

begin

```

sx[i, j]:= sy[i, j]:= ssx[i, j]:= ssy[i, j]:= sxy[i, j]:=
cx[i, j]:= cy[i, j]:= cxv[i, j]:= Sx[i, j]:= Sy[i, j]:= Sxx
[i, j]:= 0;
r[i, j]:= b[i, j]:= ss[i, j]:= mms[i, j]:= sb[i, j]:= tv[i, j]:=
mv[i, j]:= mx[i, j]:= a[i, j]:= 0;
df[i, j]:= 0;
n[i, j]:= 0;

```

end;

for i:= 0, 1 do

begin

```

smx[i]:= smy[i]:= smxx[i]:= smyy[i]:= smxy[i]:= crx[i]:=
cry[i]:= crxy[i]:= Tx[i]:= Ty[i]:= Txy[i]:= tss[i]:= 0;
spx[i]:= spy[i]:= spxy[i]:= 0;
se1[i]:= se2[i]:= se3[i]:= se4[i]:= ms1[i]:= ms2[i]:=
ms3[i]:= ms4[i]:= ms7[i]:= f1[i]:= f2[i]:= dfd[i]:= 0;
SB[i]:= B[i]:= Ybar[i]:= Xbar[i]:= A[i]:= R[i]:= 0;
tdf[i]:= dfa[i]:= dfb[i]:= dfc[i]:= DF[i]:= 0;
N[i]:= 0;

```

end;

L1: read mig, area, d, d, d, sp, x, y, d, d, d, d, d, d, d, d;

if x > 0 and y > 0 then begin

for i:= 0,1 do begin if ap = i then
for j:=0, 1 do begin if miq = j then

begin

x:= ln(x) * .4342944819;

y:= ln(y) * .4342944819;

sx[i,j]:=sx[i,j]+v;
 sy[i,j]:=sy[i,j]+ y;
 ssx[i,j]:=ssx[i,j] + x*x;
 ssy[i,j]:= ssy[i,j] + v * v ;
 sxy[i,j]:= sxy[i,j] + x * v ;
 n[i,j]:= n[i,j] + 1 ;

end;

end;

end;

end;

if buffer $\neq$ 8 then goto L1;

comment calculates sums, squares and products by species migratory state ;
for i:=0,1 do for j:= 0,1 do if n[i,j] > 0 then

begin

smx[i]:= smx[i] + sx[i,j];
 smy[i]:= smy[i] + sy[i,j];
 smxx[i]:= smxx[i] + ssx[i,j];
 smyy[i]:= smyy[i] + ssy[i,j];
 smxy[i]:= smxy[i] + sxy[i,j];

N[i]:= N[i] + n[i,j];

comment calculates sums of squares and products for total by species and migratory state;

cx[i,j]:= sx[i,j] $\uparrow$ 2/n[i,j];
 cy[i,j]:= sy[i,j] $\uparrow$ 2/n[i,j];
 cxy[i,j]:= sx[i,j] * sy[i,j] / n[i,j];

comment calculates correction factors for subgroup sums of squares and products;

crx[i]:= smx[i] $\uparrow$ 2/N[i];
 cry[i]:= smy[i] $\uparrow$ 2/N[i];
 crxy[i]:= smx[i] * smy[i] / N[i];

comment calculates correction factors for total sums of squares and products of each subgroup:

Sx[i,j]:= ssx[i,j] - cx[i,j];
 Sy[i,j]:= ssy[i,j] - cy[i,j];
 Sxy[i,j]:= sxy[i,j] - cxy[i,j];

comment calculates corrected sums of squares and products for each subgroup;

```
Tx[i]:= Tx[i] + Sx[i,j];
Ty[i]:= Ty[i] + Sv[i,j];
Txy[i]:= Txy[i] + Sxy[i,j];
```

comment adda sums of squares and products for subgroups to give common sums of squares and products:

```
r(i,j):= Sxy(i,j)/(sqrt(Sx(i,j)*Sv(i,j)));
b(i,j):= Sxy(i,j)/Sx(i,j);
ss(i,j):= Sy(i,j) - Sxy(i,j)2/Sx(i,j);
df(i,j):= n(i,j)-2;
mms(i,j):= ss(i,j)/df(i,j);
```

comment calculates correlation coefficient, regression coefficient, sums of squares of deviations from regression, d.f., and mean square for each subgroup:

```
tss(i):= tss(i) + ss(i,j);
tdf(i):= tdf(i) + df(i,j);
sb(i,j):= (sqrt(ss(i,j)/df(i,j)))/sqrt(Sx(i,j));
```

comment calculates within sums of squares of deviations from regression, d.f. and standard deviation of subgroup regression coefficients:

```
spx(i):= smxx(i) - crx(i);
spy(i):= smyy(i) - cry(i);
spxy(i):= smxy(i) - crxy(i);
```

comment calculates corrected sums of squares and products for total:

```
se1(i):= spy(i) - spxy(i)2/spx(i);
dfa(i):= N(i) - 2;
```

comment calculates total sums of squares of deviation from regression and d.f.;

```
se2[i]:= Ty[i] - Txy[i] 2 /Tx[i];
dfb(i):= dfb(i) + (n(i,j)-1);
```

end;

for i:= 0,1 do if N[i] > 0 then

begin

dfb[i]:= dfb[i] - 1;

end;

comment calculates common sum of squares of deviations from regression and D.F.;

for i:= 0,1 do for j:= 0,1 do if n[i,j] > 0 then

begin

```
ms1[i] := se2[i]/dfe[i];

comment common mean square;

dfe[i] := dfa[i]-dfb[i];

comment adj. means d.f.;

ms2[i] := (se1[i]-se2[i])/dfe[i];

comment adj means mean square;

SF[i] := (sqrt(ms1[i]))/(sqrt(Tx[i]));

comment s.d. of common reg. coeff. ;

f1[i] := ms2[i]/ms1[i];

comment f value for testing elevations ;

se3[i] := se2[i]-tas[i];
df[i] := dfb[i]-tdf[i];
ms3[i] := tas[i]/tdf[i];

comment within mean square;
ms4[i] := se3[i]/df[i];

comment reg. coeff. mean square;

f2[i] := ms4[i]/ms3[i];

comment f value for testing reg. coefficients:

my[i,j] := sy[i,j]/n[i,j];
mx[i,j] := sx[i,j]/n[i,j];
a[i,j] := my[i,j]-b[i,j]*mx[i,j];

comment values for subgroup regression equations:

B[i] := Txy[i]/Tx[i];
Ybar[i] := smy[i]/N[i];
Xbar[i] := smx[i]/N[i];
A[i] := Ybar[i]-B[i]*Xbar[i];

comment values for common regression equation:

se4[i] := se1[i]-se2[i];
R[i] := Txy[i]/(sqrt(Tx[i]*Ty[i]));

comment common correlation coeff.;

ms7[i] := se1[i]/dfa[i];

comment total mean square;

jy[i,j] := (sy[i,j]/n[i,j]) - (((sx[i,j]/n[i,j]) - Xbar[i]) * B[i]);

comment adjusted mean weight;
```

end;

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print \$\$L3s39? analysis of covariance table. area?. digits(3).area.
\$\$L2?species \$n? state \$a9?ax2\$a6? av2\$a6? axv\$a7?b\$a8?d1\$a6?
sdyx2\$a6?ms\$a8?r\$a8?sb\$L7?
for i:=0,1 do for j:=0, 1 do if n[i,j] > 0 then

begin

print \$\$L??, digits(3).\$\$s3??,i. \$\$s3??, digits(2).j.\$\$s??;
print Sx[i,j], Sy[i,j], Sxv[i,j].b[i,j],df[i,j].ss[i,j],
mms[i,j],r[i,j],sb[i,j];
end of print list for subgroup data. analysis of covariance table;

for i:=0,1 do if N[i] > 0 then

begin

print \$\$L2? within \$s53??, tdf[i]. tas[i]. ms3[i].
\$\$L? reg. coeff. \$s48??, DF[i].se3[i].ms4[i].
\$\$L? common \$s12??, Tx[i],Ty[i],Txy[i],R[i],\$\$s??,dfb[i],
se2[i],ms1[i],R[i],SB[i],
\$\$L? adj means \$s50??,dfc[i],se4[i],ms2[i].
\$\$L? total \$s13??, spx[i],spy[i],spxy[i],\$\$s11??, dfa[i].
se1[i],ms7[i].
\$\$L2? F reg. coeff. =?.f2[i]. \$\$s3?d.f.?, DF[i],\$,?,tdf[i].
\$\$s8? f adj. means =?.f1[i].
\$\$s3?d.f.?,dfc[i],\$.?.dfb[i].\$\$L??;

end;

print \$\$L4? the regression equations for each subgroup are as
follows:\$L??;

for i:=0,1 do for j:=0,1 do if n[i,j] > 0 then

begin

print \$\$L? species?. digits(2),i,\$\$s2?state?.digits(2).
j,\$\$s9??;
print \$\$s6?y = ?,b[i,j].\$x + ?.a[i,j];

end;

for i:=0,1 do if N[i] > 0 then

begin

print \$\$L4? the common regression equation is $y = ?$, R[i], \$x
+ ?, A[i],\$\$L2??,
\$\$L? adjusted mean weights for each subgroup with grand
mean length = ?.
Xbar[i],\$\$s? are as follows:?

end;

for i:= 0,1 do for j:= 0,1 do if n[i,j] > 0 then

begin

print \$\$L?species?,digits(2),i,\$\$s2? state?.digits(2).j.\$\$s8??;
print \$\$s3? adjusted mean weight = ?, jy[i,j];

end;

end;

APPENDIX C

Eel no.	Hor- mone	Temp. (°C)	Date 1st injection	Matura- tion period (days)	Experi- mental life (days)	No. in- jections	Total dosage (IW)	Spec- ies	Sex
1	HCG	12.6	26/5/71	51	106	7	3,500	LF	M
2	"	11.8	"	75	534	11	2,750	LF	"
3	"	15	"	58	427	10	7,500	LF	"
4	"	11.9	"	93	286	12	3,250	SF	"
5	"	12.0	"	—	142	19	4,750	SF	"
6	"	12.0	"	100	144	17	8,500	SF	"
7	"	17.0	19/10/71	44	136	7	3,500	LF	"
8	"	17.0	"	44	209	7	3,500	LF	"
9	"	17.2	"	47	49	7	1,750	SF	"
10	"	20	23/8/71	—	7	1	500	LF	" *
11	"	20	"	—	14	2	1,000	LF	" *
12	"	20	"	—	21	3	1,500	LF	" *
13	"	20	"	—	28	4	2,000	LF	" *
14	"	20	"	—	6	1	500	LF	" e
16	"	20	"	—	14	2	500	SF	" *
17	"	20	"	—	21	3	750	SF	" *
18	"	20	"	—	35	4	1,000	SF	" *
19	"	20	"	—	28	5	1,250	SF	" *
20	"	20	"	40	42	6	1,500	SF	"
21	"	20	"	35	49	7	1,750	SF	"
22	"	20	"	35	63	6	2,000	SF	"
23	"	20	"	40	135	6	1,500	SF	"
24	"	20	"	39	262	6	1,500	SF	"
25	"	20	"	35	429	6	1,750	SF	"
27	"	20	7/9/71	—	24	4	2,000	LF	
28	"	20	"	—	19	—	—	LF	dm
29	"	20	"	30	284	6	3,000	LF	
30	"	20	"	30	106	6	3,000	LF	
31	"	20	"	40	85	6	1,500	SF	
36	HCG + o.b.	20	30/8/71	—	100	4	3,000 1.2 mg	LF	F
37	"	"	"	—	62	2	2,000 0.6 mg	SF	F
38	pitu- itar- ies	"	6/9/72	—	44	9	126mg	SF	F
39	"	"	"	—	44	9	378mg	LF	F

* = killed. e = died dm = data missing

Eel No.	Hor- mone	Temp. (°C)	Date 1st injection	Matura- tion period (days)	Experi- mental life (days)	No. in- jections	Total dosage (IW)	Spec- ies	Sex
40	HCG + o.b.	20.0	7/9/71	-	117	16	12,000 4.8 mg	LF	F
43	"	"	19/10/71	-	50	7	3,500 2.1mg.	SF	F
44	"	"	16/11/71	-	19	2	1,500 0.6mg.	LF	F
45	"	"	"	-	105	5	3,750 1.5mg.	SF	F
46	"	"	"	-	105	15	11,250 4.5mg	SF	F
48	"	"	"	-	102	14	3,500 4.2mg	SF	F
49	"	"	"	-	108	15	11,250 4.5mg	SF	F
50	"	"	"	-	105	5	3,750 1.5mg	SF	F
51	"	"	"	-	105	15	11,250 4.5mg	SF	F
52	HCG	14.3	3/7/72	53	116	8	4,000	LF	M
53	"	14.3	"	66	119	10	5,000	LF	M
54	"	15.1	"	23	100	4	2,000	LF	M
55	"	15.0	"	74	235	11	2,750	SF	M
56	"	15.0	"	105	294	15	3,750	SF	M
57	"	14.3	"	61	100	9	2,250	SF	M
58	"	20.1	"	23	71	4	2,000	LF	M
59	"	20.1	"	28	79	4	2,000	LF	M
60	"	14.1	"	42	100	3	1,500	LF	M
61	"	18.6	"	40	79	6	1,500	SF	M
62	"	20.0	"	31	71	5	1,250	SF	M
63	"	18.6	"	46	107	7	1,750	SF	M
64	carp pitu- itary	20.0	"	45	80	14	154mg	SF	F
65	"	20.0	"	31	41	10	226mg	SF	F
66	"	20.0	"	43	147	12	180mg	SF	F
67	"	20.0	17/7/72	35	-	14	42mg	SF	F
68	HCG	13.2	"	-	10	2	1,000	LF	M
69	"	13.8	"	-	35	5	2,500	LF	M

Eel No.	Hormone	Temp. (°C)	Date 1st injection	Maturation period (days)	Experimental life (days)	No. injections	Total dosage (IW)	Species	Sex
70	HCG	14.0	17/7/72	-	65	10	2,500	SF	M
71	"	13.0	"	-	17	4	1,000	SF	M
72	"	13.9	"	-	66	10	2,500	SF	M
75	"	9.5	24/7/72	-	20	3	1,500	LF	M
76	"	9.8	"	-	90	13	6,500	LF	M
78	"	9.7	"	-	136	20	5,000	SF	M
79	"	9.7 12.5	"	136	196	20	5,000	SF	M
80	"		31/7/72	90	252	13	3,250	SF	M
81	carp pituitary	20.0	4/8/72	32	262	12	36mg	SF	M
82	"	20.0	8/9/72	-	4	2	30.0mg	SF	F
83	"	20.0	"	26	31	10	96.0mg	SF	F
84	"	20.0	13/9/72	29	31	11	170.5mg	SF	F
85	HCG	23.7	2/10/72	23	203	4	2,000	LF	M
86	"	23.7	2/10/72	20	203	3	1,500	LF	M
87	"	23.5	"	31	203	5	1,250	SF	M
88	"	23.5	"	36	78	6	1,500	SF	M
89	"	25.9	13/11/72	24	102	3	1,500	LF	M
90	"	25.7	"	31	-	5	1,250	SF	M
91	"	25.5	"	23	102	4	1,000	SF	M
92	"	28.0	19/12/72	-	126	7	3,500	LF	M
93	"	28.0	"	23	-	4	1,000	SF	M
94	"	28.0	"	27	126	5	1,250	SF	M
95	"	30.0	15/1/73	-	49	6	1,500	SF	M
96	carp pituitary	20.0	27/4/73	22	25	8	336mg	LF	F
97	"	20.0	1/5/73	23	26	8	336mg	LF	F

Species LF = A. dieffenbachii (longfin)

SF = A. australis (shortfin)

Sex M = male
F = female

pituitaries = groper and tarakihi pituitaries

HCG = human chorionic gonadotrophin

o.b. = oestradiol benzoate

APPENDIX D

Stain formula for electron microscope sections,
modified after Johnston and Loper (1970)

distilled water		60 ml
uranyl nitrate	AR	40.0 gm
citric acid	AR	0.5 gm
lead nitrate	AR	6.0 gm
lead citrate	AR	0.5 gm

First record of the freshwater eel *Anguilla dieffenbachii* Gray to be caught at sea

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A female *A. dieffenbachii* caught off the New Zealand coast has ova of 0.45 mm to 0.50 mm in diameter; these ova are larger than those observed in seaward migrating females of this species. The estimated percentage of gonad weight to total body weight of 11.2% is significantly greater than that observed in migrating females of the same size, but other migratory features, such as the eye size, colour, head and pectoral fin shape, are identical.

Several records exist of northern, temperate, freshwater eels *Anguilla anguilla* (L.) and *Anguilla japonica* Temminck and Schlegel caught at sea during their migration to the oceanic spawning grounds (Svardson, 1949; Rasmussen, 1951; Bertin, 1956; Honma, 1966). There are no similar reports for southern, temperate freshwater eels. The first record of a migrating adult New Zealand freshwater eel to be caught at sea was made on 10 May 1971. A female *Anguilla dieffenbachii* Gray was caught in a Granton trawl 17 miles off Cape Farewell, New Zealand, latitude 40°15' S, longitude 172°37' E, in 113 m. The spawning ground of this species is unknown, but it has been suggested that it is in the south-west Pacific Ocean (Schmidt, 1928; Castle, 1963).

The tip of the tail was missing, but the specimen was estimated to have been 100 cm in total length and an estimated total weight of 2.7 kg. The dorsal surface was black and the ventral surface and sides below the lateral line were a mottled grey-white. The pectoral fins were also black, pointed and measured 66 mm in length, with the width one-third of the length. The lower jaw was pointed, narrow and slightly longer than the upper jaw. The lips were thin. The head was flat and the snout narrow and rounded.

In comparison a non-migrant female *A. dieffenbachii* is black on the dorsal surface and a yellowish-brown on the ventral surface. The head is broad with a large muscular dome on the dorsal surface and the lips are thick.

The eyes of the migratory specimen were large and oval, measuring 13.5 mm in horizontal axis and 15.0 mm in vertical axis. The eyes of a non-migrant female *A. dieffenbachii* of similar size are round, measuring 10.0 mm in diameter. The greatest width of the ovaries of the migrant was 57 mm, with the right ovary weighing 153 g and the left ovary 149 g. The ovaries were estimated to represent 11.2% of the total body weight. The largest ova measured 0.45–0.50 mm. Histologically the ova showed large PAS-positive yolk vesicles and prominent PAS-positive yolk globules through the cytoplasm. In the largest ova the yolk was concentrated towards one pole. One to three oil globules were present. The specimen was frozen when received making it unsuitable for further histological examination.

The ova were slightly larger than those of seaward migrating female *A. dieffenbachii* (ova size 0.35–0.42 mm) and the percentage gonad weight was 4.2% greater than the mean of 7.0% s.d. 0.8 recorded for five seaward migrating females of 96–102 cm in total length (unpublished data). The specimen is not otherwise more sexually advanced. The eye size was within the range recorded for seaward-migrating female *A. dieffenbachii* and the colour, head and pectoral shape were identical.

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