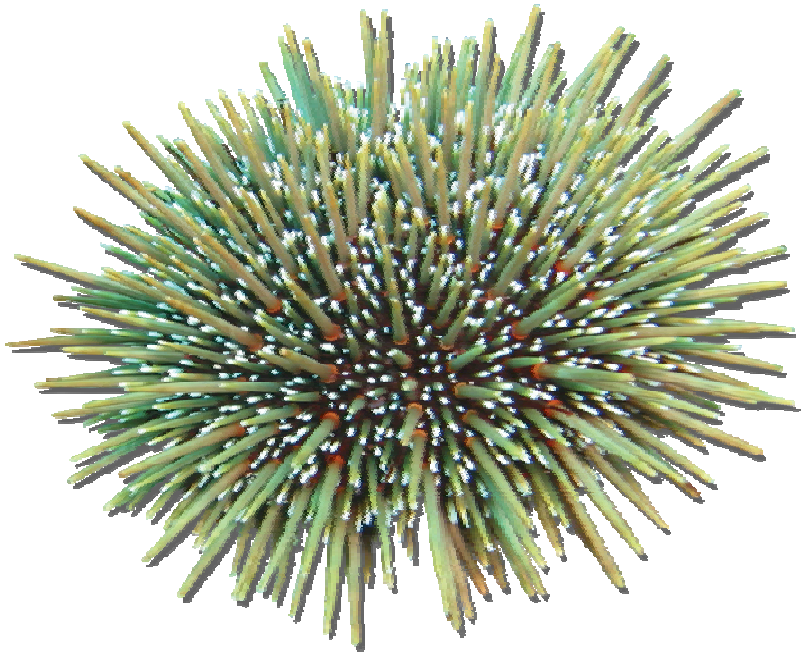
The Effects of Environmental Factors and Husbandry Techniques on Roe Enhancement of the New Zealand Sea Urchin, *Evechinus chloroticus*

By

Philip James



A thesis submitted to the Victoria University of Wellington
in fulfilment of the requirements for the degree of
Doctor of Philosophy in Marine Biology

Victoria University of Wellington
2007

ABSTRACT

The roe of sea urchins (Echinodermata: echinoidea) is a prized seafood in a number of countries around the world, including New Zealand. Increasing fishing pressure on world sea urchin stocks has failed to meet demand. This has led to increasing worldwide interest in roe enhancement of sea urchins. In New Zealand kina (*Evechinus chloroticus*) have also been heavily fished. However, there are large numbers of poor quality (low gonad index or GI) kina found in kina barrens which are uneconomic to harvest due to low returns. The primary aim of this research was to identify the key holding and environmental conditions for roe enhancement of *E. chloroticus* to assist in the development of a roe enhancement industry for *E. chloroticus* to utilise this resource.

A series of experiments testing the optimal holding conditions for *E. chloroticus* in both land- and sea-based holding systems showed that culture depth (3 and 6 m) and removal of the urchins from the water three times per week had no significant effect on gonad growth or urchin mortality. However, exposing *E. chloroticus* to increased water movement resulted in significantly greater gonad growth in 12 weeks. Increasing water movement is believed to increase the available dissolved oxygen and facilitate the removal of metabolites from around the urchins. Gonad development was not negatively impacted at the maximum stock density tested (6 kg urchin m^{-2} of internal surface area) and this density is recommended.

There are significantly lower running and maintenance costs when *E. chloroticus* are enhanced in sea-based compared to land-based systems but a full economic analysis is

required to assess which is likely to be the more economical option for future roe enhancement. A period of 9 to 12 weeks appears to be the optimal period for roe enhancement in terms of the maximum increase in GI in the shortest time period.

Repeated experiments over a 12 month period showed that food availability was the primary driver of roe enhancement (i.e. increase in gonad size) in *E. chloroticus*. This is followed by seawater temperature, which drives much of the seasonal variation in the gonad size that is observed in wild urchins. This is likely to be due to increased food consumption at higher temperatures. The reproductive stage of *E. chloroticus* had very little effect on the increase in gonad size of enhanced urchins other than in autumn when gonad growth was slightly lower than in all other seasons. Optimal gonad growth in this study was obtained at 18^{o}C, which was the highest temperature tested. Higher temperatures also resulted in an increase in the rate of progress of the gametogenic cycle of *E. chloroticus* whilst lower temperatures tended to slow the rate of progress. The effects of temperature on gonad growth (i.e. increased growth at higher temperatures) were consistent across seasons. Photoperiod had minimal effect on gonad growth and the reproductive stage of the urchins over periods of 12 weeks. Photoperiod may still affect gametogenesis of *E. chloroticus* over longer periods. Low GI kina appear to be capable of significantly larger increases in GI in 10-week periods than high GI kina, as a result of their higher tolerance to stress.

This thesis has contributed to improving the technical and economic feasibility of roe enhancement of kina (*E. chloroticus*) in New Zealand.

ACKNOWLEDGEMENTS

It is difficult to adequately thank all those who contributed to the completion of this project but I hope the following goes some way towards expressing my thanks.

Firstly a huge thank you to my co-supervisor Phil Heath (NIWA, Mahanga Bay); his on-going encouragement and assistance was well above and beyond my expectations of a co-supervisor. I thank you for your time, patience and expertise. Without your steady guidance this project may not have had the successful outcome that it has.

I have had a number of mentors in my time at NIWA both prior to and during this project and I thank Len Tong for being a true friend and father figure during a time when I desperately needed one. Thanks to Andrew Jeffs for the initial enthusiasm, encouragement and assistance in getting through the NIWA administration required to be able to undertake this PhD project, and also for reading a draft of Chapter 6. Andrew also encouraged and assisted me to apply for a NIWA Technical award that enabled me to visit Fiskeriforskning in Tromsø, Norway where I was first introduced to sea urchin roe enhancement. Thanks also to Sten Siikavuopio from Fiskeriforskning for his infectious enthusiasm for sea urchins and roe enhancement.

Over the course of the PhD I have been fortunate to have had two principal supervisors from Victoria University (Doug McNaught until August 2006 and subsequently Simon Davy). I thank Simon for 'stepping into the breach' and both supervisors for their time, expertise and assistance with my PhD project. Particular thanks to Simon for wading through a number of thesis and manuscript drafts. Thanks

to Colleen Kelly in the VUW Statistics department for her advice on analysis of the histology data in Chapter 5.

My thanks to Greg Senior and Sarah MacKenzie from the Otago School of Medicine (Wellington Hospital) for their timely and professional histology slide preparation.

In the background of most scientific projects there is an enormous amount of work preparing holding systems collecting urchins, feeding and maintaining urchins and a plethora of other less savoury tasks. My heartfelt thanks go to all the staff at the NIWA Mahanga Bay facility who all, in some way, contributed to this project. Particularly to John Illingworth for his invaluable technical advice, Chris Woods and Cedric Simon for statistical advice, Johnny Wright, Graeme Moss and Bob Hickman for their assistance throughout the many experiments and all for their continued friendship. Thanks again to Bob Hickman for reading a final draft of the thesis. Thanks also to Martin Unwin of NIWA Christchurch for his statistical expertise, and patience, during the data analysis of Chapter 6. Thanks to Rowan Wells, NIWA Hamilton for use of the urchin image on the cover page.

I have learnt a great deal about kina and the kina fishing industry from Herb (Peter Herbert) the owner/operator of Sea Urchin New Zealand. His enthusiasm and knowledge of kina has been inspirational. Thanks again to Herb and also to Neville, the skippers of the boats used for kina collections. The Cook Strait is always an interesting stretch of water, particularly on the good ship ‘Pamir’ and the small ship ‘L’.

This research was carried out with funding provided to NIWA from the Foundation for Research, Science and Technology (FRST) (Contract C01X0207). The funding and the opportunity to utilise it in the fulfilment of a PhD thesis is gratefully acknowledged.

My thanks to the stars of this project, the spiny and humble kina, which are both fascinating and yet so very frustrating. Oh to have such purpose, and yet no brain!

I would like to thank my mother, brother and sisters for their encouragement throughout this project. Special thanks to my dad for those early fishing trips off the Petone wharf as a child, they obviously inspired a lasting fascination with the sea and I know he would have been proud of this project. To Alison Morton who inspired in me a love and interest of both nature and science. I began my scholarly scientific journey with her as an undergrad and only wish we could have completed the journey together.

My family are my inspiration:

For Meta, Finn, Luca and Matthis,

"Love, deeper than the oceans".

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Chapter 1 General Introduction

1.1 General introduction and overview

The successful culture of any marine species requires a thorough understanding of the biology of that species. Much is known about the anatomy, taxonomy and ecology of the New Zealand sea urchin *Evechinus chloroticus* (kina), commonly known by its Maori name 'Kina', and this is summarised in this chapter. The gametogenic and reproductive cycles are described and the key factors that influence kina roe enhancement are also summarised. These include roe enhancement diets, the reproductive condition of the urchins and the availability of suitable holding conditions. There have been a number of previous roe enhancement experiments and trials using *E. chloroticus* in New Zealand and these are described, together with the history and current status of both the New Zealand kina fishery and markets, and sea urchin fisheries and markets worldwide. Finally, a summary of the objectives and aims of the current study are listed.

1.2 The New Zealand Sea urchin *Evechinus chloroticus*

1.2.1 Taxonomy of *E. chloroticus* and other New Zealand sea urchins

The New Zealand sea urchin, *E. chloroticus* (Valenciennes 1836), is a regular echinoid (Class: Echinoidea, Phyllum: Echinodermata) (Barker, 2007; Schultz, 2005). Regular echinoids are characterized by a globose test and pentameral symmetry (Schultz, 2005). Dix (1970a,b,c, and 1972) and more recently Barker (2007) and

Andrews (2003) provide a detailed and comprehensive description of the biology and ecology of *E. chloroticus*.

Although there are approximately 70 species of sea urchin living in New Zealand waters (Andrews, 2003) only 15 species live on rocky reefs in shallow coastal waters (Schultz, 2005). Five of these species are also found in Australia and the Indian and Pacific oceans, and are mainly restricted to the warmer northern waters of New Zealand (Schultz, 2005; Andrews, 2003). There are relatively large numbers of the black urchin (*Centrostephanus rodgersii*) and red urchin (*Heliocidaris tuberculata*) in northern New Zealand, but kina is by far the most common sea urchin found throughout New Zealand waters (Andrews, 2003) and is the only species commercially fished (Barker, 2007). All six sea urchin species found south of East Cape (North Island) are endemic (Andrews, 2003) but, apart from kina, these species are cryptic and not often seen.

1.2.2 Sea urchin anatomy

Sea urchins all have a basic five part body and unlike other echinoderms they have a very simple body structure. They are encased in a hard spherical test or shell constructed from calcium carbonate. The most obvious external feature of kina is the spines that cover the test. There are two types of spines, long (primary) and short (secondary), that are equally distributed over the body (Fig. 1.1, 1.2 and 1.3). In addition to the spines are the tube feet that are more difficult to see when the urchin is removed from water but can be easily seen when the urchin is submerged. The tube feet are used for movement, holding on to the substrate, and for collecting and manipulating food items (Fig. 1.3). Other external features are the dorsal anus and the ventral mouth (Fig. 1.1, 1.2 and 1.3). Kina have a basic nervous system but no brain.

The inside of a kina is dominated by the five gonads, situated evenly around the interior of the test, the gut, and the complex Aristotle's lantern that extends through the mouth and makes up the chewing apparatus of the kina (Fig. 1.4, 1.5 and 1.6). The lantern contains five teeth that scrape food particles into the mouth and open and close to chew and form balls of food that are then passed into the gut. The gut is directly connected to the anus on the dorsal surface (Fig. 1.4).

1.2.3 Geographic distribution

Kina are widespread throughout New Zealand, as well as the Chatham Islands, and on a number of the northern and southern offshore islands from Snares Island in the south, to the Three Kings in the north (Dix, 1970a; Barker, 2007; Andrews, 2003). A study of *E. chloroticus* collected from various locations around New Zealand showed little genetic variation throughout the geographical range other than slight differences found in a population in Fiordland in the south of the South Island (Mladenov et al., 1997).

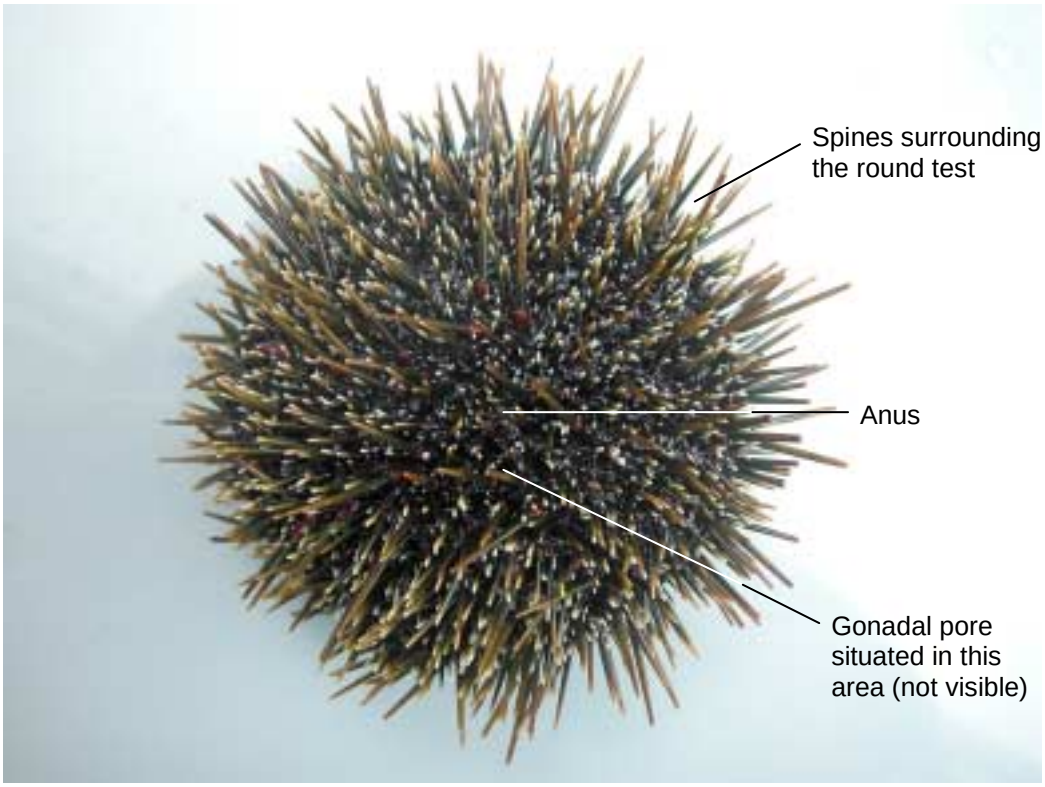


Figure 1.1 Aboral (dorsal) view of *Evechinus chloroticus.*

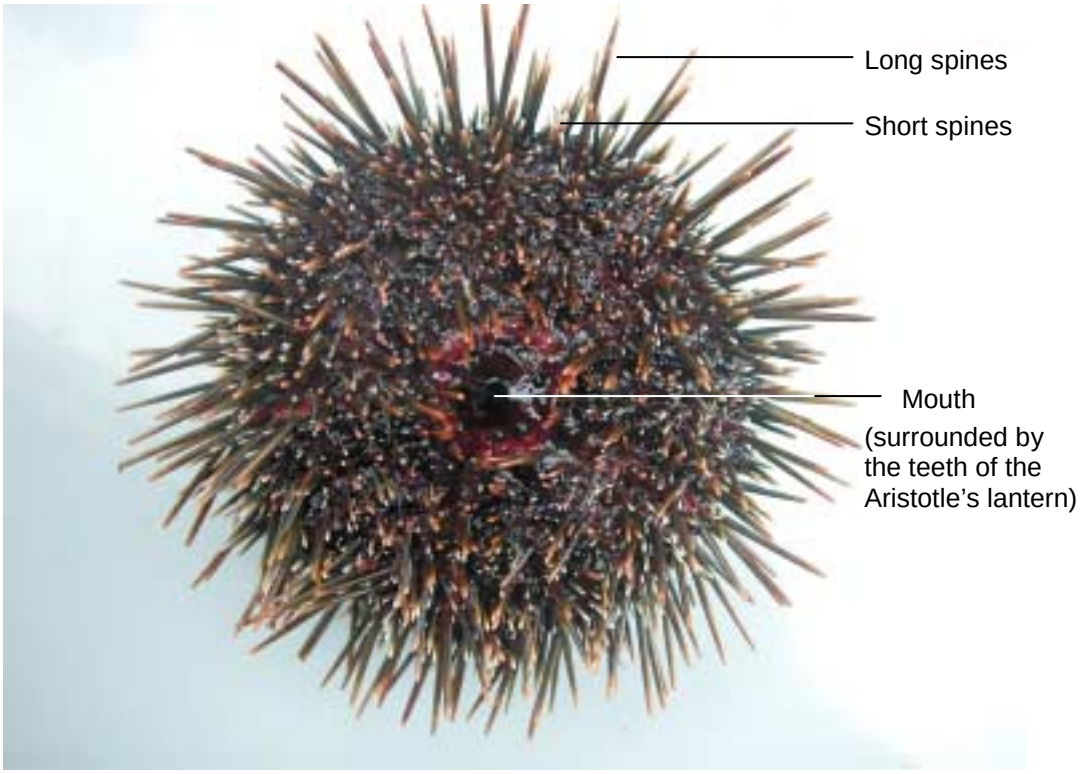


Figure 1.2 Oral (ventral) view of *Evechinus chloroticus.*

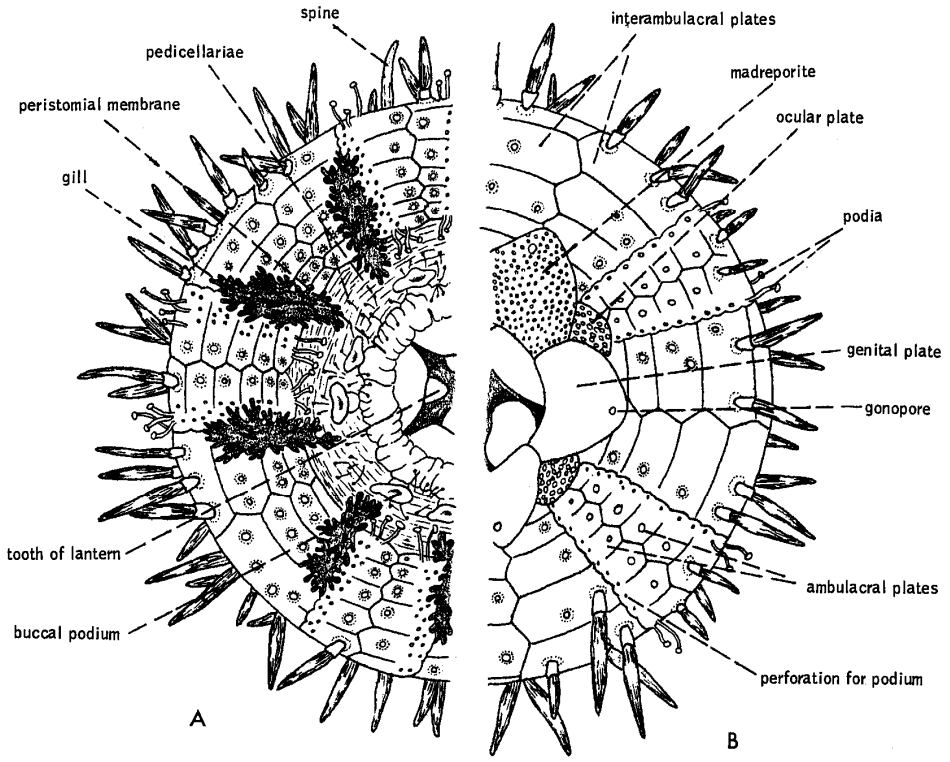


Figure 1.3 The external anatomy of a regular sea urchin; A. Oral view, B. Aboral view (adapted from Barnes 1974).

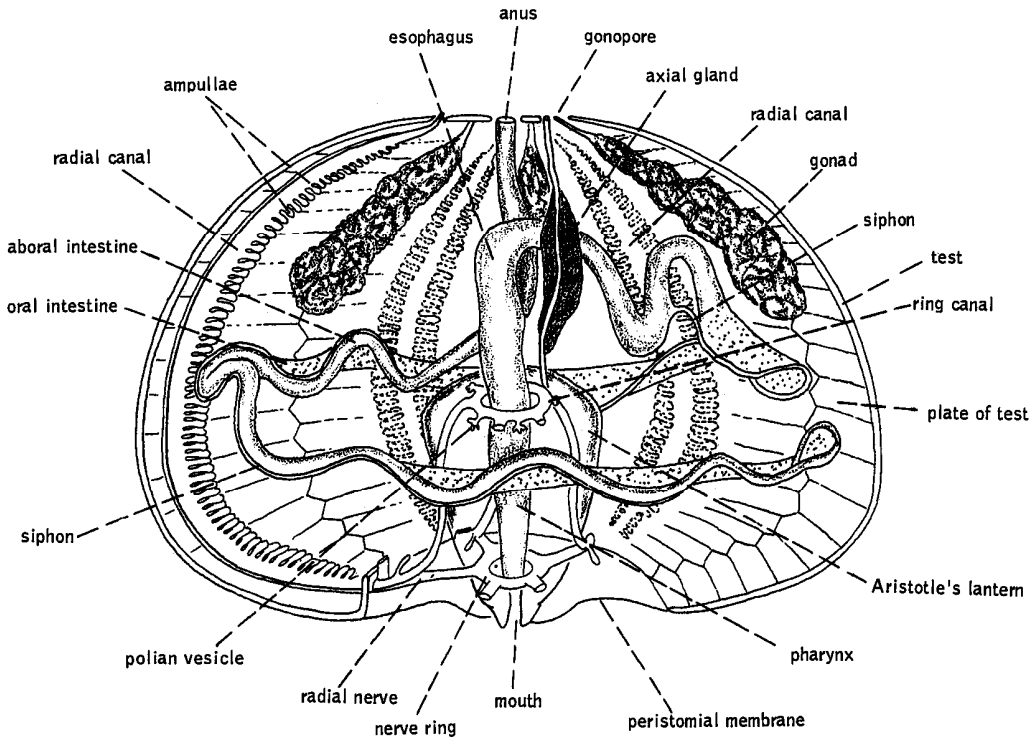


Figure 1.4 The internal anatomy of a regular sea urchin (adapted from Barnes 1974).

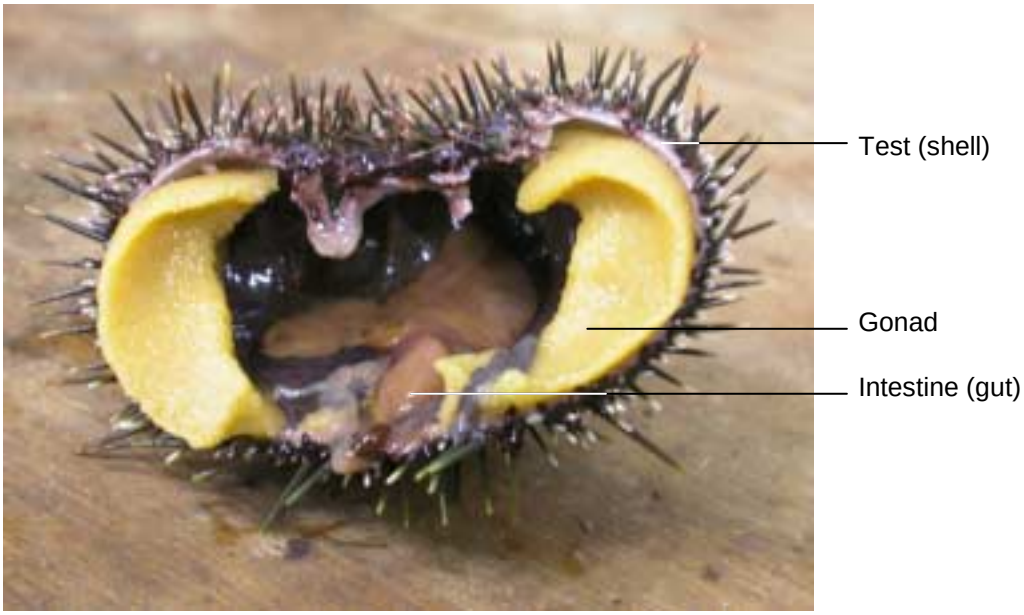


Figure 1.5 Internal view of *Evechinus chloroticus* split along the middle of the test on the vertical axis showing bright yellow gonads (roe) Note the kina is lying upside down (Photo by C. Woods).

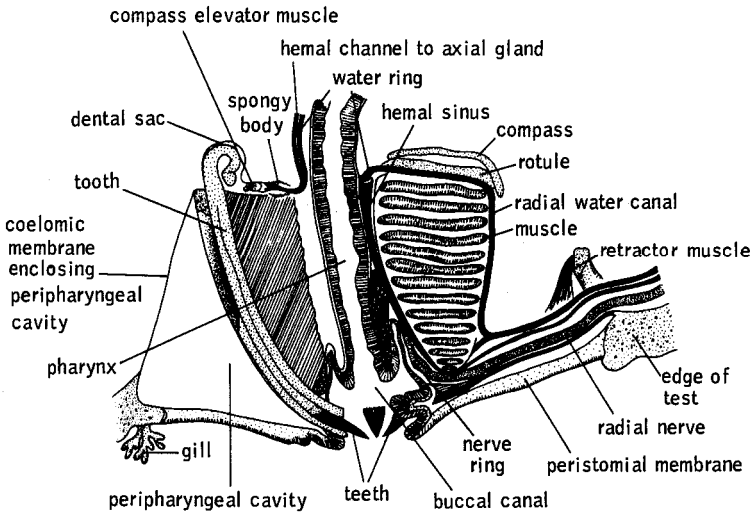


Figure 1.6 Vertical section through the Aristotle's lantern and peristomial region of a regular sea urchin (adapted from Barnes 1974).

1.2.4 Habitat and population structure

Kina are mainly found on hard substrates (Fig. 1.7), although they sometimes occur on sandy and shingle areas (Dix, 1970a; Barker, 2007; pers obs.). They are generally found at depths of less than 15 m (Andrews, 2003) but have been collected from depths as great as 60 m (Barker, 2007). Juvenile kina (< 40 mm) are cryptic and are found in very sheltered, shallow habitats (Dix 1970a; Barker, 2007). They often cover themselves with small stones and gravel making them even more difficult to see (Andrews, 2003). Adult kina are normally found in areas of moderate to strong currents (Barker, 2007; P. Herbert, Sea Urchin New Zealand, pers comm.; pers obs.) but are seldom seen in areas of extreme wave exposure (Barker, 2007). Kina occur at varying densities ranging from solitary urchins to barrens with up to 20-40 urchins m^{-2} (depending on location). Barker (2007) described the size structure of kina populations as typically unimodal in distribution and dominated by larger individuals, although size structure of kina populations may differ significantly over very short distances. This unusual population distribution is thought to be due to wide dispersal of the larvae resulting from the 3-4 week larval period and the cryptic nature of young juveniles (Barker, 2007; P, Bremen, Otago University, pers com.) but there is no published research to support this. Wing et al. (2003) showed that most variability in the size of kina within any given site is likely to be due to differences in growth as a result of nutritional history, rather than mortality or recruitment. Dix (1970b) reported that in two study sites there was little evidence of large scale movements by kina, with movement of urchins being related to food availability. Andrews (2003) suggested that although kina are relatively mobile animals their movements are mainly restricted to short range nocturnal foraging for food.

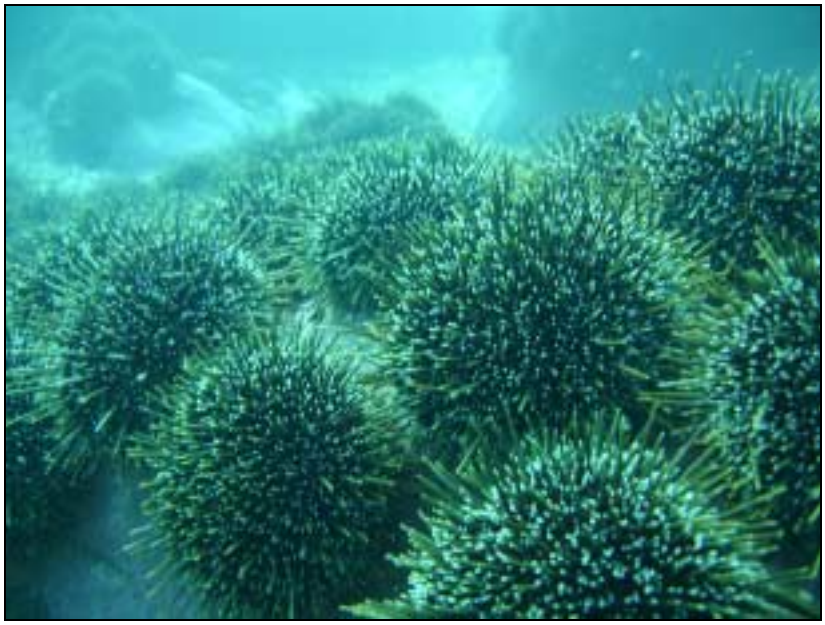


Figure 1.7 A typical picture of wild *Evechinus chloroticus* forming a large group (commonly known as a barren) at very high density on a hard substrate.

1.2.5 Diets and feeding

Kina are mainly herbivorous, actively feeding on a variety of red, brown and green algal species but show clear preferences for fucoid algae such as *Ecklonia radiata*, *Macrocystis pyrifera* and *Carpophyllum* sp. (Barker et al., 1998; Barker, 2007; Fell, 2002) as do a number of other sea urchin species (Andrews, 1986). Kina will also forage any other available food sources when algae are scarce (Dix, 1970a; Barker, 2007; Andrews, 2003) and will sometimes simply rely on catching passing drift algae as a food source (Dix, 1970a; Andrews, 2003). Although kina appear to have preferences for particular algal species it is unclear whether they selectively graze on particular species to maximize reproductive output (Barker, 2007). Previous experiments have indicated that the feeding preference of kina held in land-based tanks differs from that of urchins in their natural environment (Schiel, 1982). There is evidence that kina also scavenge dead animals (Jonathon Gardener, Victoria

University of Wellington, pers com.) and they are also capable of excellent gonad growth on high protein diets (James, 2006c).

At high densities, kina are capable of influencing the mix of algae that are present on shallow water reefs and consequently they can also affect the ecology of other species, such as the abalone (or paua), that are present (Barker, 2007; Andrews, 2003). Aggregations of kina are capable of destructively grazing the entire algal flora from a reef and the resulting kina 'barren' may persist for several years despite the subsequent lack of adequate food supply (Dix, 1970a; Barker, 2007; Fell, 2002; Andrew, 2003, P. Herbert, Sea Urchin New Zealand, pers comm.). This generally results in large numbers of kina in relatively poor reproductive condition (low gonad index) which are unsuitable for use in the kina fishery.

1.2.6 Growth and life cycle

Adult kina are broadcast spawners (Dix, 1970c). Spawning is synchronised, so that as many animals as possible release eggs and sperm together to maximise their chance of achieving fertilisation. Spawning normally occurs in the austral summer (Barker, 2007). The larvae are free swimming for 4-6 weeks and feed on phytoplankton and zooplankton as they drift with the tides. The larvae settle onto a suitable substrate and are then cryptic for the first 2-3 years of their life. The survival of the urchins during this period is thought to depend mainly on the type of habitat in which they settle. Juveniles emerge when they are larger than 30 mm or approximately 2-3 years old (Andrews, 2003). Lamare and Mladenov (2000) modelled the somatic growth of *E. chloroticus* from two separate populations and estimated that the test diameters of individuals at ages 1 to 6 years are approximately 10, 25, 40, 55, 65 and 72 mm test diameter respectively. The size of *E. chloroticus* at sexual maturity varies markedly

between populations, depending primarily on their geographic location and food availability, but is believed to range between 35-75 mm in diameter (McShane et al. 1994; Barker, 2007). However, kina fed artificial pellet diets have been observed to be sexually mature at 30 mm test diameter, showing that sexual maturity is determined primarily by nutritive input and not size (Barker, 2007). The life expectancy of a kina is difficult to determine but it is estimated that some of the largest specimens found (190 mm test diameter) could be as much as 50 years old (Andrews, 2003).

1.2.7 Gametogenesis and the reproductive cycle

The gonad of kina is made up of two main types of cells: germ cells and nutritive phagocytes. The germ cells are the oogonium, oocyte and ovum in females and the spermatogonium, spermatocyte, spermatid and spermatozoon in males (Yokota et al., 2000). Nutritive phagocytes are somatic cells that store the nutrients, such as proteins, carbohydrates and lipids that are necessary for gametogenesis (Walker, 1982). The percentage of the two types of cells present in the gonad varies throughout the reproductive cycle and has a significant effect on the size and quality of the gonad (Yokota et al., 2000; Brewin, 1994; Buisson, 2001; Fell, 2002). Gonad development of *Paracentrotus lividus* was described and allocated to six stages by Byrne (1990) and this classification has been used in a number of subsequent studies of *E. chloroticus* (Brewin, 1994; Barker et al., 1998; Buisson, 2001; Fell, 2002, James et al., 2004) and throughout the current study (details of each reproductive stage are given in Section 2.3.2).

Understanding the reproductive cycle of kina is important to the kina fishery in New Zealand, as it is in other parts of the world, as traditionally the gonad (roe) is the only part of the urchin that has any commercial value. The reproductive cycle

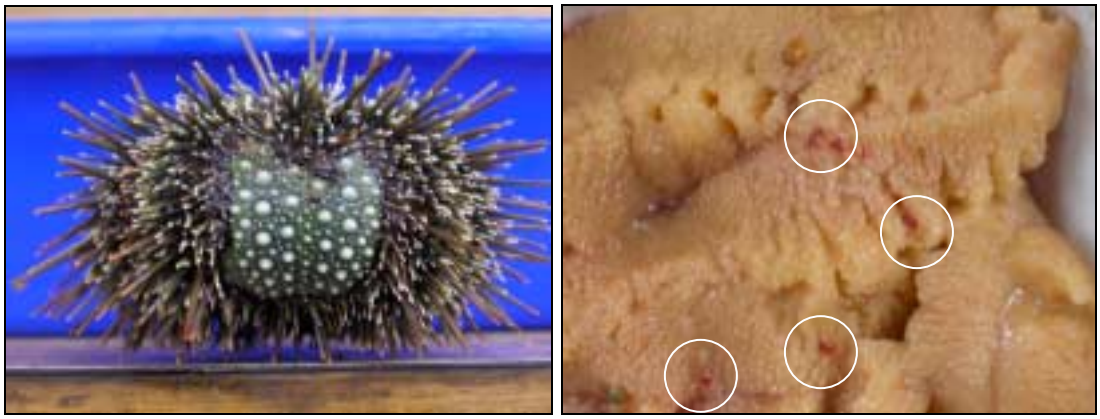
affects both the size and the quality of the gonad and subsequently its value. The reproductive cycle of wild kina from New Zealand mainland populations has been described in a number of studies (Walker, 1982; Brewin, 1994; Brewin et al., 2000; Barker, 2007; Buisson, 2001; Fell, 2002; Lamare et al., 2002; Andrews, 2003). Adult kina are either male or female, with a normal sex ratio of 1:1, and both are annual broadcast spawners (Dix, 1970c). Spawning normally occurs in the late summer and is generally complete by March (Barker, 2007). Following spawning in late summer/autumn the urchins go through a dormant stage in winter when the gonad is generally small and in poor condition. In late winter there is a build-up of storage cells in the gonad. In early to mid summer gametogenesis occurs and the number of storage cells in the gonad reduces to be replaced by reproductive cells. The cues that stimulate gametogenesis are not fully understood but the primary cue is believed to be changing photoperiod (Brewin, 1994; Walker et al., 1998; Lawrence and Guzman, 2004). The number of reproductive cells within the gonad builds up over summer until the urchin is once again in spawning condition in late summer to early autumn. The cues that trigger spawning are also unclear but the primary cues are believed to be temperature and environmental factors such as storms (Barker, 2007; P. Herbert, Sea Urchin New Zealand, pers comm.). Consequently, spawning events can vary widely between geographic locations and even within relatively limited areas (Barker, 2007). There is anecdotal evidence that the reproductive cycle of kina on some of the New Zealand offshore islands, such as the Chatham Islands, differs from that of populations on the mainland, despite similar latitudes and seawater temperatures (P. Thomas, Rekohu Seafoods, Chatham Islands, pers comm.). Food availability and urchin density have been shown to play an important role in the reproductive output of wild kina (Dix, 1970c; Andrews, 1986; Barker, 2007). When food is limited, the size of the gonad

decreases. This normally has a negative effect on the reproductive cycle and when the food supply is very low (e.g. as can occur in extensive kina barrens) the roe of the urchin may be too small to produce any reproductive cells (Andrews, 2003). There are studies showing that sea urchins can reabsorb the nutrients from the gonads in times of extreme starvation and they have even been known to decrease in body size, due to the calcium being reabsorbed from the test, as a result of long term starvation (Pearse and Cameron, 1991). In New Zealand there are a significant number of areas where wild kina are in very poor condition as a result of over population and low feed availability (Barker, 2007; Buisson, 2001; Fell, 2002; Andrews, 2003; James, 2005).

1.2.8 Disease and associated mortality

Little is known of any diseases that may affect *E. chloroticus* with the only described cases of disease occurring in wild kina being three cases of 'balding sea-urchin disease' where the urchins lost spines in patches around the test. The disease also caused kina mortality in two of the documented cases, particularly in areas where the urchins were found in high densities (Barker, 2007). This disease has also been observed in very small numbers in captive urchins held in land-based holding systems (Fig. 1.8a) and normally led to the death of the urchin.

A parasitic flatworm (*Syndesmis* sp.) is also commonly found in the gut of wild kina (P. James, pers obs.; B. Diggles, pers comm.) (Fig. 1.8b) but it is unclear whether this worm has any negative effect on kina growth or survival.



a) **b)**

Figure 1.8 Diseases and gut parasite of *Evechinus chloroticus*: a) bacterial infection and subsequent spine loss and b) the gonad (roe) of *E. chloroticus* with the small red flatworm (*Syndesmis* sp.) circled.

There are only two documented incidences of disease outbreaks in sea urchins from around the world. An acute infection by the pathogenic amoeba, *Paramoeba invadens*, occurred in the sea urchin *Strongylocentrotus droebachiensis*, in Nova Scotia causing mass mortalities, and an endoparasitic nematode *Echinomermalla matsi* was recorded in a *S. droebachiensis* population off the coast of Norway. The nematode affected the size of the urchin gonads and also caused increased mortality in infected populations (Tajima and Lawrence, 2001).

1.3 Roe enhancement (kina fattening)

1.3.1 Key factors influencing roe enhancement

Roe enhancement involves the collection and holding of mature urchins in land- or sea-based holding systems for a limited period (2-3 months) to increase the quantity and the quality of the roe. There has been extensive international research into the factors that affect sea urchin roe enhancement (Lesser and Walker, 1998; Lawrence,

2001a; Lawrence and Guzman, 2004). Key factors that have been identified are: the availability of an effective diet, the reproductive condition of the urchins and the availability of suitable holding systems.

1.3.2 Roe enhancement diets

There are a large number of published papers referring to roe enhancement of a range of sea urchin species on both natural and artificial diets, or a combination of the two (See Table 1.1). There is general consensus amongst researchers that artificial diets are much more effective at increasing the percentage of roe found within an urchin, generally measured as gonad index or GI, than are natural diets. There are now a number of effective artificial diets available worldwide.

Internationally, research into diet development has focused on semi-moist feeds prepared by extrusion cooking (Lawrence et. al., 2001). There have been a number of studies on *E. chloroticus* testing the efficacy of natural and moist extruded diets on roe enhancement (Brewin, 1994; Barker et al., 1998; Buisson, 2001; Fell, 2002; James et al., 2004). The extruded diet produced good results in terms of increases in GI but poor results in terms of roe colour (Buisson, 2001; Fell, 2002). Alternatively, natural algal diets produced better gonad colour but lower gonad indices (Barker et. al., 1998). At the Norwegian Institute of Fisheries and Aquaculture (Fiskeriforskning) in Tromsø, Norway, an artificial sea urchin diet has been developed which utilizes waste products from the fishing industry. The diet has proved to be very effective at enhancing the roe of wild caught S. *droebachiensis* (S. Siikavuopio, Fiskeriforskning, pers comm.; Mortensen et al., 2003). At the National Institute of Water and Atmospheric Research (NIWA) Aquaculture Research Facility at Mahanga

Bay, Wellington, a sea urchin roe enhancement diet has been developed, based on the Fiskeriforskning diet.

A description of experiments that utilised the NIWA diet (James 2003a, 2003b; James et al., 2004; James 2004b,c) is given in Section 1.4.

1.3.3 Reproductive condition of the urchins

Changes in the GI values of wild sea urchins throughout the seasons are thought to be driven by changes in reproductive condition of the urchins (Walker et al., 1998) (see Section 1.2.6). The reproductive condition of urchins is also recognised as a critical factor in roe enhancement (Lawrence and Bazhin, 1998; McBride et al., 1997; McBride et al., 1998; Walker et al., 1998; Walker and Lesser, 1998; Spirlet et al., 2000; Yokato, 2000; Buisson, 2001; Garrido and Barber, 2001; Kelly, 2001; Fell 2002; Shpigel et al., 2004a). Despite this there are only a handful of international studies (Spirlet et al., 2000; Garrido and Barber, 2001; Shpigel et al., 2004a; Siikavuopio et al., 2006) that repeat roe enhancement experiments throughout a 12 month reproductive cycle. Four such studies are on *E. chloroticus* (Brewin, 1994; Barker et al., 1998; Buisson, 2001; Fell, 2002) but there is still limited information on what effect roe enhancement has on gamete development in sea urchins, including *E. chloroticus*.

Table 1.1. A list of roe enhancement experiments (listed in the order they were published) undertaken between 1993 and 2007 on a range of sea urchin species in either land-based (L) or sea-based (S) holding systems, or both (L/S), and using either natural (Nat.) or artificial (Art.) or a combination of diets (Nat./Art.). The list is not exhaustive list but represents many of the experiments undertaken during this period (studies marked with * were conducted over a period longer than 10-16 weeks)

Authors	Date published	Sea urchin Species	Land- or sea-based	Natural or artificial diets
González, M.L.; Pérez,, M.C.; López, D.A.; Pino, C.A.	1993	*Loxechinus albus*	L	Nat.
Brewin, P.E.	1994	*Evechinus chloroticus*	L	Nat.
Cuthbert, F.M. and Hooper, R.G.	1995	*Strongylocentrotus droebachiensis*	L/S	Nat.
Hooper, R.G.; Cuthbert, F.M.; McKeever, T.	1997	*Strongylocentrotus droebachiensis*	S	Nat.
Lawrence, J.M.; Olave, S.; Otaiza, R.; Lawrence, A.; Bustos, E.	1997	*Loxechinus albus*	S	Nat./Art.
McBride, S.C.; Pinnix, W.D.; Lawrence, J.M.; Lawrence, A.L.; Mulligan, T.J.	1997	*Strongylocentrotus franciscanus*	L	Nat./Art.
Klinger, T.S.; Lawrence, J.M.; Lawrence, A.	1997	*Strongylocentrotus droebachiensis*	L	Nat./Art.
Robinson, S.M.C. and Colborne, L.	1997	*Strongylocentrotus droebachiensis*	S	Nat
Walker, C and Lesser, M.P.	1997	*Strongylocentrotus droebachiensis*	L	Art.
Barker, M.F.; Keogh, J.A.; Lawrence, J.M.; Lawrence, A.L.	1998	*Evechinus chloroticus*	L	Nat./Art.
Bridger, C.J.; Hooper, R.G.; McKeever, T.J.	1998	*Strongylocentrotus franciscanus*	S	Nat.
Cook, E.J.; Kelly, M.S.; McKenzie, J.D.	1998	*Psammechinus miliaris*	L	Nat./Art.

Table 1.1 Continued. A list of roe enhancement experiments (listed in the order they were published) undertaken between 1993 and 2007 on a range of sea urchin species in either land-based (L) or sea-based (S) holding systems, or both (L/S), and using either natural (Nat.) or artificial (Art.) or a combination of diets (Nat./Art.). The list is not exhaustive list but represents many of the experiments undertaken during this period (studies marked with * were conducted over a period longer than 10-16 weeks)

Authors	Date published	Sea urchin Species	Land- or sea-based	Natural or artificial diets
Ferdenandez, C. and Pergent, G.	1998*	*Paracentrotus lividus*	L	Art.
Grosjean, P.; Spirlet, C.; Gosselin,P.; Vaïtilingon, D.; Jangoux, M.	1998*	*Paracentrotus lividus*	L	Nat.
Kelly, M.S.; Brodie, C.C.; McKenzie, J.D.	1998	*Psammechinus miliaris*	S	Art.
McBride, S.C.; Lawrence, J.M.; Lawrence, A.L.; Mulligan, T.J.	1998	*Strongylocentrotus franciscanus*	L	Art.
Motniker, S.; Marsans, R.; Tétrealt, F.	1998	*Strongylocentrotus franciscanus*	L	Art.
Walker, C.W. and Lesser, M.P.	1998*	*Strongylocentrotus droebachiensis*	L	Art.
Hagen, N.	1998*	*Strongylocentrotus droebachiensis*	L	Nat.
Watts, S.A.; Boettiger, A.; McClintock, J.B.; Lawrence, A.M.	1998	*Lytechinus variegatus*	L	Nat.
Kennedy, E.J.; Robinson, S.M.C.; Parsons, G.J.; Castell, J.	1999	*Strongylocentrotus droebachiensis*	S	Nat.
Vadas, R.L.; Beal, B.; Dowling, T.; Fegley, J.C.	1999	*Strongylocentrotus droebachiensis*	L	Nat./Art.
Spirlet, C.; Grosjean, P.; Jangoux, M.	2000	*Paracentrotus lividus*	L	Art.
Buisson, P.	2001	*Evechinus chloroticus*	L	Art.
Kelly, M.S.	2001*	*Psammechinus miliaris*	L	Nat.
McLaughlin, G. and Kelly, M.S.	2001	*Psammechinus miliaris*	L	Art.

Table 1.1 Continued. A list of roe enhancement experiments (listed in the order they were published) undertaken between 1993 and 2007 on a range of sea urchin species in either land-based (L) or sea-based (S) holding systems, or both (L/S), and using either natural (Nat.) or artificial (Art.) or a combination of diets (Nat./Art.). The list is not exhaustive list but represents many of the experiments undertaken during this period (studies marked with * were conducted over a period longer than 10-16 weeks)

Authors	Date published	Sea urchin Species	Land- or sea-based	Natural or artificial diets
Olave, S.; Bustos, E.; Lawrence, J.M.; Carcamo, P.	2001	*Loxechinus albus*	S	Nat./Art.
Garrido C.L. and Barber, B.J.	2001	*Strongylocentrotus droebachiensis*	L	Art.
Fell, J.	2002	*Evechinus chloroticus*	S	Nat./Art
Pearce, C.M.; Dagget, T.L.; Robinson, S.M.C.	2002a	*Strongylocentrotus droebachiensis*	L	Art.
Pearce, C.M.; Dagget, T.L.; Robinson, S.M.C.	2002b	*Strongylocentrotus droebachiensis*	L	Art.
Robinson, S.M.C.; Castell, J.D; Kennedy, E.J.	2002	*Strongylocentrotus droebachiensis*	L	Art.
Lawrence, J.M.; Plank, L.R.; Lawrence, A.	2003	*Lytechinus variegatus*	L	Art.
Mortensen, A.; Siikavuopio, S.I.; Raa, J.	2003	*Strongylocentrotus droebachiensis*	L	Nat./Art.
James, P.J.; Woods, C.M.; Illingworth, J.	2004	*Evechinus chloroticus*	L	Nat./Art.
Pearce, C.M.; Dagget, T.L.; Robinson, S.M.C.	2004	*Strongylocentrotus droebachiensis*	L	Nat./Art.
Shpigel, M.; McBride, S.C.; Marciano, S.; Lupatsch, I.	2004a	*Paracentrotus lividus*	L	Art.
Shpigel, M.; McBride, S.C.; Marciano, S.; Shiri, R; Ben-Amotz, A.	2004b	*Paracentrotus lividus*	L	Nat./Art.
Siikavuopio, S.I.; Dale, T.; Foss, A.; Mortensen, A.	2004a	*Strongylocentrotus droebachiensis*	L	Art.
Siikavuopio, S.I.; Dale, T.; Christiansen, J.S.; Nevermo, I.	2004b	*Strongylocentrotus droebachiensis*	L	Art.

Table 1.1 Continued. A list of roe enhancement experiments (listed in the order they were published) undertaken between 1993 and 2007 on a range of sea urchin species in either land-based (L) or sea-based (S) holding systems, or both (L/S), and using either natural (Nat.) or artificial (Art.) or a combination of diets (Nat./Art.). The list is not exhaustive list but represents many of the experiments undertaken during this period (studies marked with * were conducted over a period longer than 10-16 weeks)

Authors	Date published	Sea urchin Species	Land- or sea-based	Natural or artificial diets
Dale, T.; Siikavuopio, S.I.; Aas, K.	2005	*Strongylocentrotus intermedius*	L	Nat./Art.
Chang, Y; Lawrence, J.M; Cao, X.; Lawrence, A.	2005	*Strongylocentrotus droebachiensis*	L	Art.
Senaratna, M.; Evans, L.H.; Southam, L.; Tsvetnenko, E.	2005	*Heliocidaris erythrogramma*	L	Nat./Art.
Daggett, T.L.; Pearce, C.M.; Robinson, S.M.C.	2006	*Strongylocentrotus droebachiensis*	L	Art.
Hill, S. and Lawrence, J.M.	2006	*Arbacia punctulata* *Lytechinus variegatus*	L	Art.
James, P.J.	2006a	*Evechinus chloroticus*	L/S	Art.
James, P.J.	2006b	*Evechinus chloroticus*	S	Art.
James, P.J.	2006c	*Evechinus chloroticus*	S	Art.
Siikavuopio, S.I.; Christiansen, J.S; Dale, T.	2006a	*Strongylocentrotus droebachiensis*	L	Art.
Siikavuopio, S.I.; Dale, T., Mortensen, A.	2006b	*Strongylocentrotus droebachiensis*	L	Art.
Christiansen, J.S. and Siikavuopio, S.I.	2007	*Strongylocentrotus droebachiensis*	L	Art.
Siikavuopio, S.I.; Dale, T.; Foss, A.; Mortensen, A.	2007a	*Strongylocentrotus droebachiensis*	L	Art.
Siikavuopio, S.I.; Foss, A.; Dale, T.; Mortensen, A.	2007b	*Strongylocentrotus droebachiensis*	L	Art.
James, P.J.; Heath, P.; Unwin, M.	2007	*Evechinus chloroticus*	L	Art.

1.3.4 Availability of suitable holding systems

Most previous roe enhancement research has focused on land-based experimental scale trials (See Table 1.1), but more recently there has also been an increase in worldwide interest in sea-based urchin roe enhancement. In Canada (New Brunswick) an experiment to test the feasibility and efficacy of enhancing the roe of *S. droebachiensis* held in suspended sea-cages was successful (Robinson and Colborne, 1997). In Newfoundland, a roe enhancement ranching trial, holding the urchins in underwater corrals on the seafloor, was undertaken with some success (Bridger, et al., 1998). A study by Kelly et al. (1998) in Scotland measured the somatic and gonadal growth of the sea urchin *Psammechinus miliaris* maintained in polyculture with Atlantic salmon. An experiment holding *S. droebachiensis* in small sea-cages in Maine, northeast USA, showed it was feasible to enhance the gonads of 'post-spawned' urchins using a variety of natural algae as feed (Vadas, et al., 1999). Growth trials of *Loxechinus albus* fed natural algae have also been carried out in lantern nets in Chile with some success (Mendes and Becarra, 2004). In Norway, sea-cage technology has been developed for roe enhancement of *S. droebachiensis* with the urchins held at stocking densities of 35 kg m^{-2} in stacked baskets suspended beneath a supporting structure (Anon, 2000; Aas, 2004).

In New Zealand, a significant sea-based infrastructure has been developed to service the Greenshell™ mussel industry. However, there are a number of existing mussel farms that are not well suited to growing mussel (A. Parnell, Marlborough Mussels, pers comm.) that could be ideally suited for holding other aquaculture species such as algae (P. Heath, NIWA, pers comm.), lobsters (Jeffs and James, 2001), sponges (M. Page, NIWA, pers com.) or kina. Fell (2002) ran a series of experiments in cages

suspended from a small scale mussel line looking at the effects of diet and density on kina roe enhancement. However, there has been no previous research looking at the efficacy of roe enhancement of kina held in sea-based cages compared to kina held in land-based holding systems, and no studies investigating the factors that specifically affect kina roe enhancement in sea-cages suspended from mussel longlines.

1.4 Roe enhancement in New Zealand

In New Zealand, and elsewhere in the world, there are large congregations of sea urchins in poor condition, i.e., having a low GI (Andrew, 2003; James et al., 2004; S. Siikavuopiio, Fiskeriforskning, pers comm.). Although abundant and easily located, these urchins have not previously been fished because of the low economic returns resulting from their low GI values. The economic cut-off point for commercial fishers varies around New Zealand from a GI of 6% in the far north (P. Herbert, Sea Urchin New Zealand, pers comm.) to approximately 10% in the far south (C. McManaway, NZ Sea Products, pers comm.). Kina that fall below these levels, despite forming a significant proportion of the New Zealand kina population, are uneconomic to fish and are currently not utilized in the fishery. However they could form an ideal resource for use in a kina roe enhancement industry (Fig. 1.9).

1.4.1 Previous roe enhancement research

There have been a number of studies on the New Zealand sea urchin *E. chloroticus* testing the efficacy of natural diets and artificial diets on roe enhancement in land-based holding systems (Brewin, 1994; Barker et al., 1998; Buisson, 2001; James et al., 2004;

James, 2003a, 2003b; James 2004b,c). In addition, a study by Fell (2002) investigated the effects of holding kina in sea-cages and feeding a combination of artificial and natural diets. The results were comparable to previous land-based trials. James (2006c) also conducted sea-cage trials holding kina in commercial scale cages and using the NIWA artificial roe enhancement diet.

Barker et al. (1998) showed that kina size had a significant effect on roe enhancement when comparing three discrete size classes of urchin, including very small immature urchins and larger urchins (size classes studied were 30-40, 50-60 and 70-80 mm test diameter). This study also showed that feeding rates varied significantly with diets (natural vs. artificial) and that feeding rates appeared to correlate with seasonal changes in water temperature. Brewin (1994) found that feeding one of four algal species to captive *E. chloroticus* affected their GI values, gametogenic cycle and biochemical composition of the gonads in the experimental urchins. Brewin (1994) postulated that the cue for gametogenesis in kina was increasing photoperiod and that manipulation of photoperiods may influence the gametogenic cycle when urchins are collected prior to being exposed to the lengthening day cue in the wild. This study recommended that further research was required to understand the relationships between gonad growth, reproduction and the biochemical composition of the gonads of *E. chloroticus*. The author also urged that it was essential to understand how these characteristics can be utilised under artificial conditions to produce a constant supply of quality gonad. The two most important environmental factors recommended for further investigation were photoperiod and temperature.

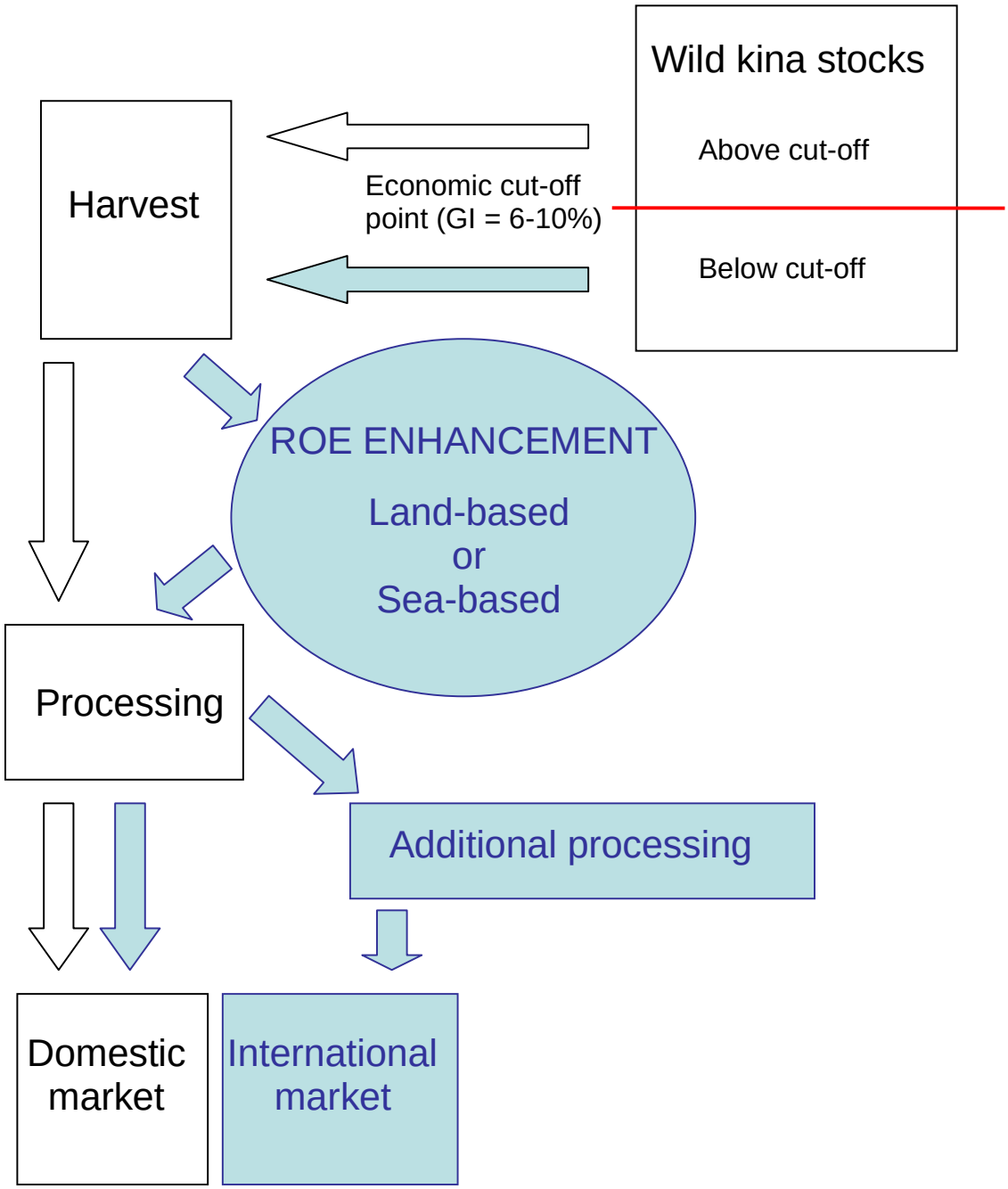


Figure 1.9 A flow chart showing how roe enhancement (shaded blue) could potentially complement the existing fishery process (un-shaded) by utilizing the currently unused low GI kina found in the New Zealand kina fishery.

Buisson (2001) described the changes that occur in terms of GI and gonad development in a wild *E. chloroticus* population throughout a 12 month cycle. This study also attempted to quantify the quality of the roe for export to international markets (with limited success) and also tested a range of diet combinations in a series of trials. The study concluded that the urchins from the study population were in suitable reproductive condition for gonad enhancement for six out of the twelve months of the year, that small kina produced brighter gonads, and that feeding rates were higher at warmer temperatures. In another experiment, photoperiod set six months out of phase with ambient conditions did not cause a significant change in gonad development, although this experiment was confounded by significantly different temperatures in the photoperiod treatments subsequently affecting the GI values. Buisson (2001) recommended further research to understand the effects of photoperiod on the gametogenic cycle of *E. chloroticus*.

Fell (2002) conducted three sea-cage experiments on a small scale mussel longline in Otago Harbour, Dunedin. In the trials the author tested a range of algae, artificial and combined diets, including switching from one to the other. The study showed that urchins fed the artificial diet (a variation of the Wenger diet, see James et al., 2004) had significantly higher growth in GI, in all three experiments, than those fed algae diets and that a combination of artificial diets followed by algal diets produced the best quality gonads. The study also suggested that the optimal period for sea-cage cultivation using Doubtful Sound urchins is between late January and May (when the urchins are laying down nutritive tissue) and results in superior gonad growth potential. The research by Brewin (1994), Barker et al. (1998), Buisson (2001) and Fell (2002) has all been

undertaken using urchins taken from populations of kina in Fiordland on the lower southwest coast of the South Island of New Zealand.

James et al. (2004) also tested a range of diets for roe enhancement of *E. chloroticus* in a land-based holding system. The diets tested were: *Macrocystis pyrifera,* the NIWA moist diet, the Fiskeriforskning moist diet and a moist extruded pellet diet (a variation of the Wenger diet produced and imported from the South Australian Research and Development Institute). The results of the trial showed that in terms of an increase in GI, the moist extruded diet and the diet produced by NIWA were equally effective at enhancing the roe of wild caught sea urchins after a period of 10 weeks. The poor performance of the Fiskeriforskning diet in this trial was probably due to the extended period (1 year) for which the diet sample had been frozen and the irradiation treatment the diet underwent on arrival in New Zealand, since it has shown to be very effective at increasing the gonad index of *S. droebachiensis* in previous trials in Norway (S. Siikavuopio, Fiskeriforskning, pers comm.; Mortensen et al., 2003). The very poor GI results from sea urchins fed the natural algal diet, *M. pyrifera,* indicated that this would not be a suitable diet for producing significant increases in the quantity of roe from sea urchins held in a land-based system.

1.4.2 Commercial roe enhancement trials

There has been a series of pilot scale commercial kina roe enhancement trials conducted by NIWA, in partnership with Sea Urchin New Zealand (James, 2006c). Four land-based trials were conducted at the Eastland Marine Farm (an existing paua farm) situated at Te Kaha, on the east coast of the North Island. The trials tested a range of diet types and

different combinations of diet types. Each trial utilised the results of the previous trial to improve the feed regime. The diets used in each of the trials significantly increased the roe yield of kina held in land-based tanks over a period of 10 weeks compared to those that were left in the wild for the same period of time. The kina roe from the final trial was of a suitable quality (in terms of size, taste, colour and texture) to be sold into the domestic market and showed a marked improvement in taste over previous trials. Although this trial established that it is biologically possible to increase the size of the roe of kina, and to retain the colour and taste quality suitable for the domestic market on a commercial scale, this study did not establish at what scale kina roe enhancement would be required to be undertaken to make it commercially viable.

Following the series of land-based commercial trials, NIWA conducted a commercial scale trial holding kina in commercial scale sea-cages at two mussel marine farms in the Marlborough Sounds (Apex Marine Farm and Tory Channel Kelp Products) (James, 2006c). A total of 600 kg (greenweight) of wild caught kina were held for 12 weeks and fed the NIWA artificial diet for 5 weeks and the alga *Macrocystis pyrifera* for the following 7 weeks. An initial census was made of the kina from the trial and kina were collected and assessed at the conclusion of the trial from the same area as the initial kina sample. The results of the experiment showed significant differences in the GI of kina held in cages at the two sites after 12 weeks, regardless of the cage type. The kina held at both sites had significantly higher GI values than kina taken from the wild at the beginning and conclusion of the trial. The combination of NIWA artificial diet and natural algal diet used in the trial was effective at significantly increasing the roe yield of kina held in sea-cages over a period of 12 weeks. In addition, all of the kina roe from the

trial were of a suitable quality for sale on the domestic market, despite the very poor quality of the wild kina taken at the beginning and conclusion of this trial. The experiment also showed that there can be variations in the GI of kina held in the same cages at different sites and that further investigations into the factors that influence gonad development in commercial scale sea-cages were required (James, 2006c) (Fig. 1.10). Indications from the trial were that sea-based kina roe enhancement with its reduced infrastructure, maintenance and labour costs would prove a more economically viable strategy than land-based kina roe enhancement.

1.5 Sea urchin fisheries and markets

1.5.1 The New Zealand kina fishery

Kina are fished in New Zealand by commercial, recreational and Maori customary fishers. In some areas the Maori customary catches have been reported to be up to 50% of the commercial catch (McShane, 1992; Andrew et al., 2001). Kina have been commercially fished since 1986 using breath-hold diving. The exception was in 1998-99 when approximately 10% of the total catch was collected by dredge. The kina fishery in New Zealand is divided into 10 fishing areas but commercial harvesting is concentrated in five of the ten areas (Fig. 1.11 and Table 1.2). Total allowable catches (TAC's) were set for each of the five fishing areas in 1988. Fishers were required to obtain permits to fish, or fish on behalf of permit holders, for all non-quota species, including kina. Annual catches in all areas have since varied erratically and there have been major declines in catch and effort in several of the fisheries since that time. In 2002 kina were introduced into the Quota Management System (QMS) along with a number of other species. The

TAC for any new species introduced into the QMS is normally set according to an assessment of stock sustainability. However, there was no reliable stock assessment information or estimates of biomass available for any of the kina fishing areas and the Total Allowable Commercial Catch (TACC) was set based on the average annual catch for the fishing years from 1993/4 to 2001/2 (Table 1.2). The amount of kina caught in the New Zealand fishery since the introduction of the species into the QMS has been variable with a peak occurring in the 2001/2 year (847 t).

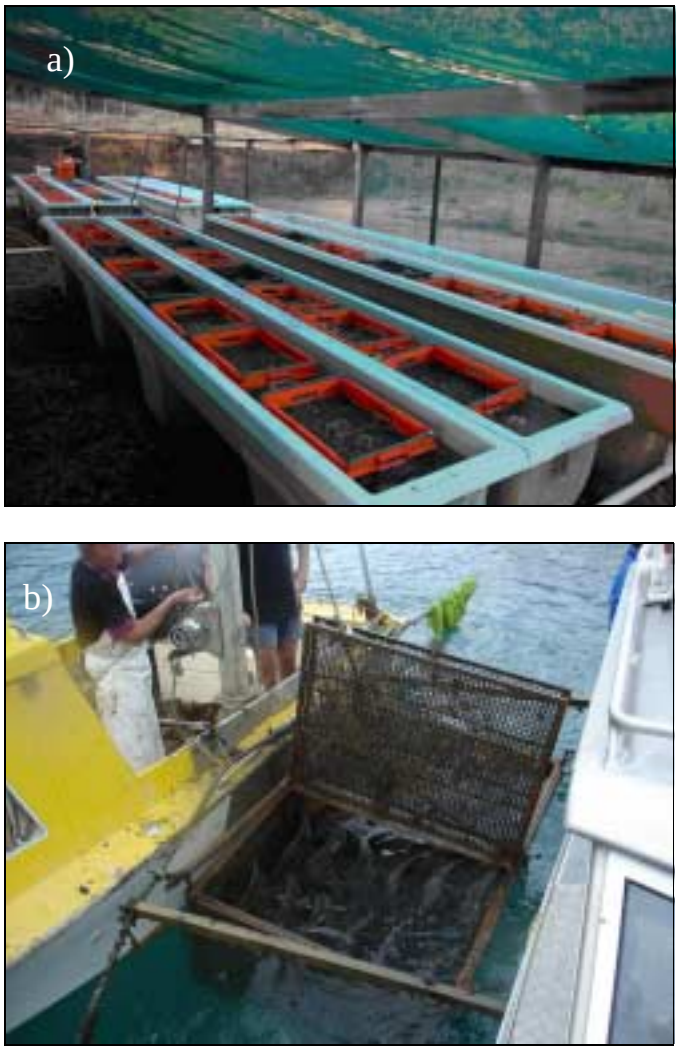


Figure 1.10 Commercial kina roe enhancement trials undertaken by NIWA and commercial clients in a) land-based and b) sea-based holding systems.

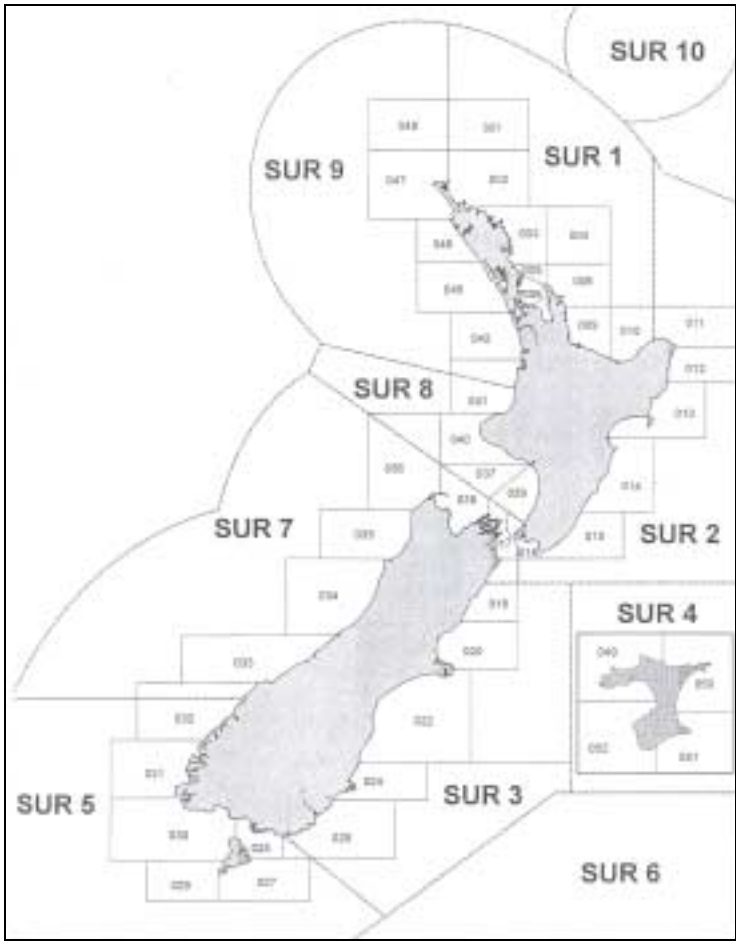


Figure 1.11 The fisheries management areas (FMA's) and Statistical Reporting Areas (SRA's) for the New Zealand kina fishery (Note the areas are designated as SUR 1-10 with SUR being the Ministry of Fisheries code for Sea Urchin or kina).

The TACC for the entire New Zealand fishery in 2003/4 was 937 t but only 58% of the TACC (548 t) was caught, primarily due to the low value of the landed product and the difficulty of consistently fishing good quality kina. The TAC since 2003/4 has increased dramatically in SUR 5 due to the reintroduction of the kina quota from the research area in Fiordland. Previously this area has been closed for commercial fishing and could only be fished under an experimental permit (McShane et al., 1993). In November 2004 this permit was withdrawn and 210 t (the estimated sustainable yield from this area) were

added to the existing TACC (245 t) in SUR 5 (Table 1.1). This has made a total TACC of 455 t available for fishing from SUR 5, dramatically increased the landings in this area, and has generated significant interest in establishing a more comprehensive and united kina fishery. This has seen the formation of the SUR 5 Kina Association. Attempts are now being made to incorporate SUR 3 and SUR 7 into the Kina Association and to create a nationwide association. This would focus on developing the kina fishery into a comprehensive and united organisation, capable of developing and supplying export markets with consistent quality product (all catch figures are taken from: 'The New Zealand Commercial Fisheries: The Atlas of Area Codes and TACC's', available online at www.fishinfo.co.nz).

1.5.2 The New Zealand kina market

The NZ kina market consists of retailers purchasing direct from kina fisher/processors but the majority of kina fishers are not involved in kina processing. Traditionally, the low landing price ($1.00-3.0 / kg greenweight) has meant that kina is often fished after the Annual Catch Entitlement (ACE) for more lucrative species (such as lobster and paua) has been reached. Consequently, kina has historically been considered a low value product to fish and low in terms of its market value. The processing of kina involves opening the test and extracting the roe by the traditional spooning method which is a labour intensive process. There are kina processing facilities in Whitianga on the North Island, Picton, Dunedin and Invercargill in the South Island and on the Chatham Islands. Once extracted from the test, the roe is packed and sold in plastic pottles that vary in size from 200 to 400 g (Fig. 1.12).

Figure 1.12 Kina for sale in 200 and 400 g pottles (NZ$13.95/200g) on the domestic market: a) packed and ready for shipment from a processing plant in Dunedin and b) presented at Deep Blue Seafood in Wellington

Table 1.2 Total Allowable Catch, customary allowance, recreational allowance, Total Allowable Commercial Catch and other mortality (all given in tonnes) set for kina stocks in the five main areas (SUR 3, 4, 5, 7A and 7B) when the species was introduced into the QMS system in 2002, and for all areas in the year 2006/7.

Area	TAC (t)	Customary allowance (t)	Recreational allowance (t)	TACC (t)	Other mortality (t)
		2002/3 Introduction to QMS			
SUR 3	76	10	5	60	1
SUR 4	255	20	7	225	3
SUR 5	263	10	5	245	3
SUR 7	264	90	25	145	4
		2006/7			
SUR 1	496	155	155	180	6
SUR 2	306	95	95	110	6
SUR 3	42	10	10	21	1
SUR 4	255	20	7	225	3
SUR 5	480	10	10	455	5
SUR 7	264	90	25	145	4
SUR 8	26	12	12	1	1
SUR 9	33	11	11	10	1

Historically, there has been no quality grading of processed kina roe in New Zealand with no differentiation between large or small roe, good or poor coloured roe (see Fig 1.13 for examples), or fresh and previously frozen product. Nor is there any quality control in terms of taste other than consumer feedback. Retail prices for roe vary in the New Zealand market from as low as $25/kg to as high as $70/kg. Some fishers have built a reputation with suppliers for only supplying kina roe of acceptable quality (light coloured, yellow or orange, and sweet tasting roe as defined by P. Herbert, Sea Urchin New Zealand, Whitianga) over a number of years, consequently they consistently receive the maximum wholesale price ($55/kg) for their product (P. Herbert, Sea Urchin New Zealand, pers comm.).

There is a limited market for whole kina supplied fresh to the Auckland seafood market. In recent years the domestic market for kina has changed significantly. Kina roe has been introduced into supermarket chains and a range of other fish retail outlets, increasing the demand for consistent quantities of suitable quality sea urchin roe. This demand, and a surge of interest in the fishery, has meant that the value of sea urchin quota has increased markedly. Surprisingly, the domestic retail value of sea urchin roe has remained relatively constant. This is most likely to be due to the large quantities of product being delivered to the market from SUR 5 where the TACC has been significantly increased. There are a number of processors that are now beginning to vary the packaging of kina to improve the market perception of kina roe and add value to the product (C. McManaway, NZ Sea Products, pers comm.). As the fishery in SUR 5 becomes more established and kina landings settle at a sustainable level the catch rates

are likely to drop from their present levels (M. Barker, Otago University, pers comm.) and the value of kina roe on the domestic market is likely to rise.

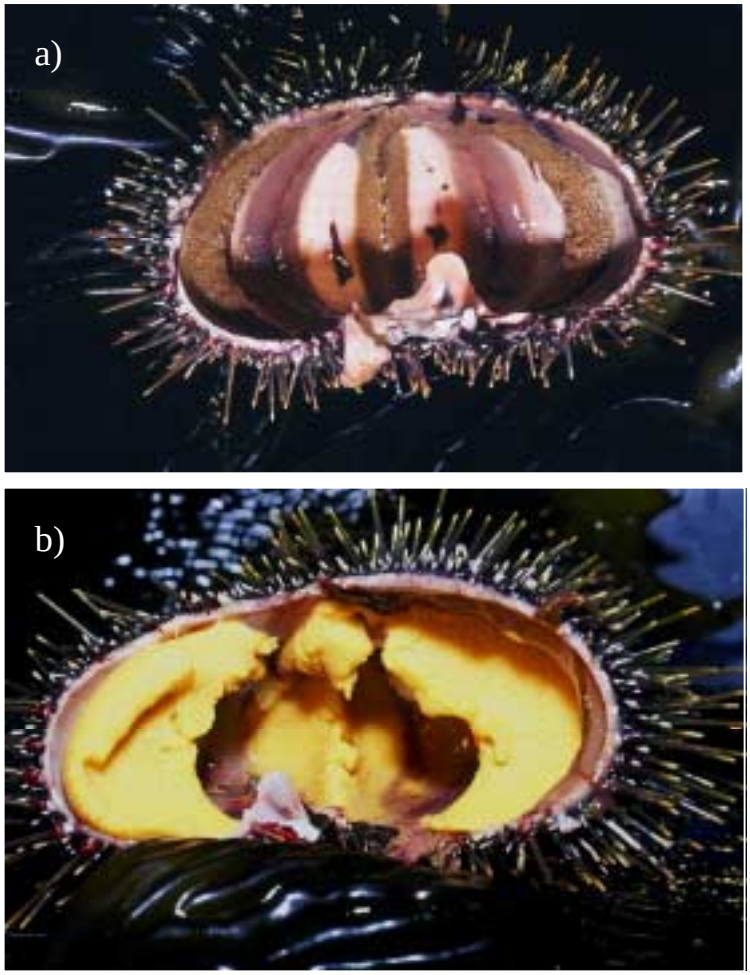


Figure 1.13 Examples of the variation in the colour of kina roe sold on the domestic market: a) poor and b) good colour quality roe.

1.5.3 International sea urchin fisheries

The world production of sea urchin roe is dominated by Chile, with more than half the world's landings (Andrews et al., 2001). Other important fisheries are found in Japan,

USA, South Korea, Russia and Mexico, and a number of other countries that produce relatively small amounts. New Zealand, Japan, Chile and the Philippines are the only countries in the world to have fisheries that support domestic sea urchin markets. Worldwide catches steadily increased in the later half of the last century but the increases were mainly due to the expansion of regional fisheries. There has been relatively little research into the sustainability of many of these fisheries and since the worldwide catch peaked at 120,306 tonnes in 1995, catches have been declining. By 1998 the total world catch was only 75% of its peak, three years earlier (Andrews et al., 2001). Evidence indicates that a number of the larger fisheries are likely to be over-fished. Most of the sea urchin roe harvested around the world goes to the Japanese market, arriving as fresh or frozen product and in various stages of processing, with most of the roe arriving from Chile, USA, South Korea and Canada. France is the world's second largest consumer of sea urchin roe and there are a number of countries that consume much smaller quantities (Sonu, 2003).

1.5.4 International sea urchin markets

A comprehensive study of the Japanese kina market undertaken in 2003 showed that Japan was by far the greatest importer of kina roe (Sonu, 2003). However, the wild sea urchin stocks in Japan have reduced dramatically since 1984 when they were the world's largest harvester of sea urchins. In 2002 Japan imported 18,525 metric tonnes of roe valued at 247 million US dollars, a ten-fold increase from 1975 resulting from increasing demand, decreasing local catches and a consequent rise in price for sea urchin roe. Thirty nine percent of the 2002 imported product volume consisted of fresh product and the

remainder was frozen product. Most fresh sea urchin roe in Japan is sold through auction at the Tokyo Central Wholesale Market and the prices are controlled primarily by the quality of the product but also by the total (local and imported) supply available. The months of highest prices of Japanese roe were January and September, reflecting the low availability of the product during these times. There are tariff costs associated with selling sea urchin product in Japan and these must be included in any economic models for establishing export markets (Sonu, 2003). The Japanese taste for sea urchin roe is different to that in New Zealand where the creamy, pre-spawning condition is preferred. In Japan consumers prefer firm roe, so all the local fisheries are closed during the spawning season (Andrew et al., 2001) and this is another important factor to consider for the export of kina roe to Japan. Wholesale prices in the Japanese market are also reliant on the quality of the roe with premium prices obtained for roe that is bright yellow or orange, firm, unbroken and packed neatly in traditional wooden trays (Sonu, 2003) (Fig. 1.14).

There have been a number of attempts to export urchin roe from New Zealand to Japan, but only a small amount has been exported due to the roe having a bitter taste, poor colour and inconsistent or low yields (McShane et al., 1994). Also the nature of the Japanese sea urchin market is complex. Most export attempts have concentrated on fishing Fiordland stocks (SUR 5) under an experimental permit. The urchins in this area have subsequently been shown to have higher levels of bitterness than those from other areas of New Zealand. There is little doubt that the kina from other areas produce roe of suitable quality for the Japanese market (C. McManaway, NZ Sea Products; P Herbert, Sea Urchin New Zealand; P. Thomas, Rekoha Seafoods Ltd, pers comm.). There is

currently a research project currently underway at Otago University attempting to identify the taste parameters that are acceptable to the Japanese market and how various roe enhancement diets affect these parameters (M. Barker and P. Bremer, Otago University, Otago, pers com.).

Figure 1.14 High quality sea urchin roe for sale, packaged in typical wooden boxes in the Tsukuji Seafood Market in Tokyo, Japan.

1.6 Objectives and aims of the current study

Previous studies have shown that there is potential to develop a roe enhancement industry in New Zealand (to service the domestic or export markets, or both) based on fishing low quality kina from easily accessible kina barrens that are currently uneconomic to fish (Section 1.4). However, there are still a number of key areas of knowledge that have not

been investigated that will have a significant impact on the development of such an industry.

There has been very little study on holding system design with only one published study comparing different land-based designs (Dagget et al., 2006). There has been no comparative study of the efficacy of land vs. sea-based holding systems, and no study of the factors that will affect optimal holding conditions in sea-based holding systems or sea-cage design.

Seasonal variations in gonad index have been widely reported and temperature is recognised as having a significant effect on sea urchin roe enhancement. However, there is little information on whether seasonal differences in gonad development and GI are driven by ambient seawater temperatures or changes in the reproductive stage of the urchins. For *E. chloroticus* there is also a paucity of information regarding the effects of varying temperatures and photoperiods.

1.6.1 Aims

The overall aim of the current study was to describe and quantify the effects of a range of holding systems and environmental factors on roe enhancement of *E. chloroticus*. Specific aims were as follows:

1. Describe the optimal holding conditions for roe enhancement of kina in terms of land-based vs. sea-based, water depth and stock density.
2. Measure the effects of wave and feeding disturbance on roe enhancement of captive kina in sea-cages suspended from a mussel longline.

3. Measure the gonad development of captive kina over an extended period to determine the optimal roe enhancement period.
4. Measure and differentiate between seasonal (environmental conditions such as temperature) and temporal (reproductive condition) effects on kina roe enhancement.
5. Compare the effects of initial gonad condition on roe enhancement (i.e. high initial GI vs. low initial GI kina.
6. Describe the effects of varying photoperiods and temperatures on roe enhancement across seasons and gonad developmental stages.

Chapter Two General Methodology

2.1 General

The following section describes standardised methodology that was used throughout the experiments in the current study. An additional 'Materials and methods' section is provided for each experimental chapter (Chapters 3-7).

2.2 Experimental diet

The importance of a suitable roe enhancement diet is now widely recognised and there have been sufficient experiments carried out in New Zealand and overseas, on a range of artificial diets and urchin species, to show that there are a number of suitable diets available. In order to exclude the effects of diet variability in the current study, the NIWA moist roe enhancement diet (see Table 2.1 and 2.2) was used exclusively in all of the experiments. This enabled the experiments to differentiate the effects of a range of holding conditions and environmental factors on roe enhancement without being compromised by the efficacy of different, or varied, feeds.

The diet has been developed and refined over a number of years and has been shown to be equally, or more effective than other artificial diets tested and significantly more effective than a diet of the algae *M. pyrifera* (James et al., 2004). The percentage composition and proximate analysis of the diet are described in Table 2.1 and Table 2.2. The diet was produced at the NIWA Aquaculture Facility shortly before each trial (Fig.

2.1) and frozen at –20^{o}C. For feeding it was removed from the freezer, allowed to defrost at room temperature and cut into approximately 20 mm cubes. The standard feeding rate used throughout the trials (except where stated in the 'Materials and methods' sections) was 1.5% of the urchin wet weight/day. This level of feeding proved to be above satiation levels for the urchins held in all the experiments in this study.

Proximate analyses of the diet were carried out on 100 g samples by a commercial analytical laboratory (Massey Nutrition Laboratory, Palmerston North, New Zealand) (Table 2.2) using the Association of Official Analytical Chemists standards (AOAC) for: protein (Leco, total combustion method AOAC 968.06), fat (Soxhlet extraction AOAC 991.36), moisture (convection oven 105°C AOAC 930.15, 925.10), ash (furnace 550°C AOAC 942.05), and gross energy (bomb calorimetry).

2.3 Histology samples

2.3.1 Collection and staining

Previous research has shown there is homogeneity in the reproductive stage throughout a single gonad of *E. chloroticus* and also between each of the five gonads found within an individual urchin (Brewin, 1994; Buisson, 2001; Fell, 2002). In this study individual histology samples were collected from the mid point of a single randomly chosen gonad from an individual urchin. Each sample consisted of an approximately 2-3 mm perpendicular slice of the gonad which was removed using clean forceps and a scalpel, placed in a marked histology cassette and stored in 10% buffered formalin prior to staining. Histology samples were stained at the Otago School of Medicine (Wellington)

using standard Harris's haematoxylin stain, counterstained with eosin (Bancroft and Stevens, 1990).

a) **b)** **c)**

d)

Figure 2.1 Manufacture of the NIWA kina roe enhancement diet: a) mixing cooked with uncooked ingredients, b) forming the mixed ingredients into 20 mm thick blocks before the binder ingredient causes the diet to set, c) blocks of diet which have set ready for freezer storage and transport, and, d) kina feeding on cube of NIWA diet cut from a block of food whilst holding another cube for later consumption (photos by C. Woods).

Table 2.1 Percentage ingredient composition of the NIWA diet used in all roe enhancement experiments in the current study.

Ingredient	% inclusion
Hoki skins (*Macruronus novaezelandiae*)	79.9
Kelp meal	12.0
Algro™ (*Dunaliella* sp., 2% active Beta-carotene)	1.0
Dry mix	1.9
Transglutaminate binder	0.5
Greenshell™ mussel meat (*Perna canaliculus*)	4.8

Table 2.2 Proximate composition of the NIWA diet used in all roe enhancement experiments in the current study. Values given for protein, fat, carbohydrate and ash are dry weight values. B-carotene content estimated from the inclusion rate of Algro.

Variable	NIWA
Protein % m/m	14.0
Fat % m/m	6.5
Saturated fat g/100g	1.6
Carbohydrate g/100g	8.4
Ash g/100g	3.7
Moisture g/100g	67.4
Energy GE kJ/100g	621
B-carotene mg/100g	1.5

2.3.2 Staging

The sex ratio and reproductive condition of the initial urchin sample was assessed using the six reproductive stages described in Walker (1982) and Byrne (1990). Staging and classification of the gonad reproductive state follows that of Byrne (1990) and of Buisson (2001) who gives a photographic description of each of the stages for *E. chloroticus*.

For males, 6 gonad stages are classified as follows:

1) Recovering testes: with a thin band of primary spermatocytes along the ascinal wall. Relict spermatozoa may be present and are surrounded by a meshwork of nutritive phagocytes.
2) Growing testes: with increasing thickness of the primary spermatocyte band.
3) Pre-mature testes: with columns of spermatocytes projecting centrally and spermatocytes accumulating in the lumen. Nutritive phagocytes are still present, although they are displaced from the centre by spermatozoa.
4) Mature testes: packed with spermatocytes with nutritive phagocytes limited to the periphery.
5) Partially spawned testes: with spaces vacated by spermatozoa.
6) Spent testes: with thin ascinal walls largely devoid of contents and a pale network of nutritive phagocytes around the periphery.

For females, 6 gonad stages are classified as follows:

1) Recovering ovaries: with nutritive phagocytes forming a meshwork that projects into the lumen creating a vacuolated appearance. Small, pre-vitellogenic oocytes

occur along the ovary wall. Relict globules of lysed material and unspawned ova may be present.

2) Growing ovaries: with pre-vitellogenic oocytes tightly packed along the ascinal wall. Early vitellogenic oocytes with distinct nucleus are also present and nutritive phagocytes become increasingly dense.

3) Premature ovaries: with oocytes at all stages of development. Vitellogenic oocytes are surrounded by nutritive phagocytes detach from the ascinal wall and have ova accumulating in the lumen.

4) Mature ovaries: packed with ova. Nutritive phagocytes are either absent or form a thin mesh around the small oocytes.

5) Partially spawned ovaries: with loosely packed ova.

6) Spent ovaries: with unspawned relict ova and nutritive phagocytes either absent or forming a thin line around the periphery.

2.4 Water quality testing

Water quality was monitored during each of the experiments. Dissolved oxygen, pH, and ammonia were measured weekly using a WTW (CellOx 325) probe, a WTW pH electrode (SenTix 81) probe connected to a WTW Multi (340i) water quality meter, and a Palintest ammonia comparator kit (Palintest UK, Tyne & Wear, United Kingdom), respectively (all WTW equipment manufactured by Werkstatten GmbH & Co., Weilheim, Germany).

2.5 Data collection

2.5.1 Gonad index

In the current study, gonad index (GI) is measured as the percentage of the total wet weight (greenweight) of the urchin that is made up by the unblotted wet weight of the gonads. Some previous studies on *E. chloroticus* have used a GI based on the percentage of the total opened (cracked in half and left to drain) drained weight of the urchin that is made up of blotted weight of the gonad (Barker et al., 1998; Buisson, 2001; Fell, 2002). GI calculated from the drained weight is normally approximately twice the value of the GI calculated from the wet weight. There is a very strong correlation between Wet Weight GI values and Drained Weight GI values with a sample of urchins taken from the present study (n = 2200 individual urchins) showing a significant correlation (ANOVA: $F = 34894.4$, $df = 1, 2200$, $P < 0.0001$) (Fig. 2.2). The GI results referred to in the present study are all presented as Wet Weight GI as this is the most common technique used in previous publications making the results easier to compare with other studies. This method of calculating GI is also more applicable to the kina fishing industry as previous study has shown that there is only a 1.25% average difference between the technique used to calculate GI in this study and the technique used to calculate GI in a commercial kina processing factory (James, 2006c). In a commercial factory the GI is calculated as the weight of roe (determined from the total number of pottles (small pots) of roe, minus the weight of the pottles and kina liquor in each pottle) as a percentage of the total greenweight of kina processed. This makes the results both easy to understand and to apply in terms of economic modelling for kina roe enhancement ventures.

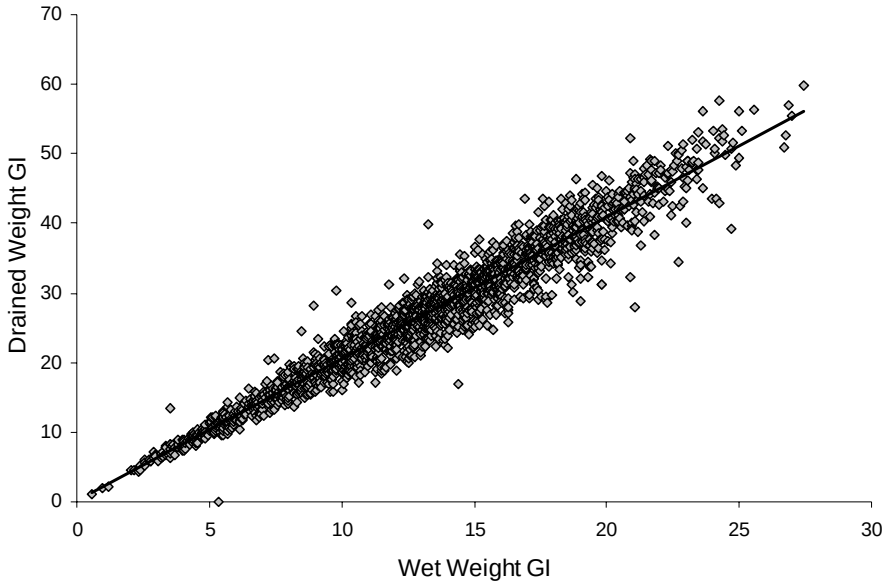


Figure 2.2 The relationship between Drained Weight (gonad index) values and Wet Weight (gonad index) values for a sample of urchins taken from the present study, Y = 2.0435 X - 0.0023; r^2 = 0.969; *n* = 2200.

2.5.2 Analysis of Gonad Index

Gonad indices are indicators of gonad development that are independent of urchin size (Fell, 2002) and are used to compare gonad size of individuals within or between populations (Brewin, 1994). Gonor (1972) summarised the assumptions of using a gonad index and concluded that the most important assumption is that the mean gonad index of a population at any point in time is independent of their body size. If this is not the case then differences in gonad index may simply reflect differences in the sizes of the urchins, rather than in their gonad development. There have been a number of previous studies showing that the gonad sizes of *E. chloroticus* from limited size ranges do not vary as a function of urchin size (Brewin, 1994; Buisson, 2001; Fell, 2002). A plot of test diameter versus gonad index from the initial wild urchin sample is an appropriate check for the effect of body size on gonad index (Brewin, 1994) and has been included for each of the experimental chapters.

2.5.3 Gonad colour

The colour of sea urchin gonad has traditionally been measured using some form of 'match-by-eye' technique. However, there are inherent difficulties with this subjective technique and Robinson et al. (2002) described a more accurate technique for breaking colour down into three components in a three-dimensional measurement system similar to that developed by the Commission Internationele de l'Eclairage in 1976 (Robinson et al., 2002). This technique called 'CIE (L*a*b*) 1976' uses a spectrophotometer to take measurements on the *X* axis or hue (a*) which extends from green on the negative side to red on the positive side and the *Y* axis or chroma (b*) which extends from blue on the negative side to yellow on the positive side. These two axes will define any colour. The intensity or lightness of the colour is determined by the *Z* axis (L*), which extends from black on the negative side to white on the positive. The numerical values derived from this technique provide a true objective measure of the variance within a sample and therefore can be statistically evaluated (Robinson et al., 2002). This technique was used in Chapters 3-7 in the current study. In Chapter 3 the more traditional technique of matching roe colour to a colour card was also used (See Fig. 2.3).

The colour of the urchin gonads was assessed with either a ColorTec-PCM^{TM} (ColorTec, Clinton, New Jersey, USA) 12mm 45/0 colour meter or a Minolta CM2500D spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan) (2 pulsed xenon lamps as illumination source, diffuse illumination with 8° viewing area, with spectral component excluded for gonad measurements). The colour meter/spectrophotometer was placed inside a custom made waterproof plastic cover with a clear glass cover over the aperture. A number of gonads from an individual urchin (enough to completely fill to overflowing)

were placed in a shallow (5 mm deep) plastic tray (40 mm x 40 mm square). The spectrophotometer aperture was then firmly pressed down on the plastic tray so that the glass covered viewing area of the spectrophotometer was filled by the gonad sample. Three replicate measurements per sample were taken and averaged to give a single measurement for the lightness (L*), the degree of redness (a*), and the degree of yellowness (b*) of a sample. The L*, a*, and b* readings for the 'acceptable roe colours' for sale in Japan (as defined by Japanese representatives of the Japanese Seafood company Nippon Suisan Kaisha Ltd) are listed in Table 2.3.

Table 2.3 The lightness (L*), redness (a*), and yellowness (b*) readings for the 'acceptable roe colours' for sale in Japan as defined by Japanese representatives of the Japanese Seafood company Nippon Suisan Kaisha Ltd.

Maine colour chart reference number	L* reading	a* reading	b* reading
104	87.15	-5.17	43.86
105	84.01	-4.26	56.03
106	77.76	6.20	48.44
107	82.17	-2.11	67.97
108	76.86	4.28	61.03
109	69.19	15.69	36.46
110	71.57	13.19	49.85
111	69.20	17.92	40.22
112	63.95	17.31	34.77

Figure 2.3 The Maine colour chart commonly used to match the colour of urchin roe in order to grade their colour quality.

2.6 General

All kina collections were made under the NIWA Special Permit (Number 318). Ethical approval is not required for experiments on sea urchins. All kina collections were made by either snorkelling or SCUBA diving from one of two vessels. A commercial kina fishing boat ('L') and a fishing charter boat ('Pamir').

Chapters 3-7 are included here in full as they have been published, or submitted, to the peer reviewed journal 'Aquaculture' (minor changes have been made to fit the thesis format). This has resulted in some repetition in the 'Introduction' and 'Materials and methods' sections of each Chapter. Chapter 8 consists of a discussion of these individual papers and provides a platform for integrating the entire research project.

Chapter Three

A comparison of roe enhancement of the sea urchin *Evechinus chloroticus* in sea-based and land-based cages

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[Published in: Aquaculture 253 (2006) 290-300]

Received 12 October 2004, received in revised form 28 March 2005; accepted 31 March 2005

3.1 Abstract

A 12-week experiment was conducted to compare the efficacy of enhancing the roe of wild caught *Evechinus chloroticus* held in tanks on land and in sea-cages. The sea-cages were suspended from a mussel longline at depths of 3 and 6 m and identical cages were placed in land-based tanks supplied with flow-through seawater. Both land and sea-based systems had high and low density treatments. The urchins were fed an artificial diet *ad libitum* throughout the experiment. An initial and a final census were conducted to measure urchin test diameter, wet weight, gonad index and roe colour. The results showed urchin roe quantity (GI) and colour quality can be enhanced in 12 weeks by feeding the NIWA artificial diet in land-based tanks or sea-cages, with high survival.

There were no significant differences between the enhancement of roe quantity (GI) or colour quality in urchins held in land-based tanks or in sea-cages. There were also no differences between urchins held in sea-cages at 3 m and at 6 m, or between urchins held at low and high density.

Keywords: Evechinus chloroticus, Sea- and land-based, Cage culture, Gonad indices.

3.2 Introduction

The sea urchin, *E. chloroticus*, is commonly known in New Zealand by its Maori name 'kina'. The species has been commercially fished in New Zealand since 1986 and the urchin roe has been mainly sold on the domestic market. There have been a number of attempts to export urchin roe to overseas markets such as the Japan, but only a small amount has been exported due to the roe having a bitter taste (McShane et al., 1994) poor colour, and inconsistent or low yields (P. Herbert, Sea Urchin New Zealand, pers comm.). Consequently, the fishery has not expanded in New Zealand to the same degree that has occurred in other countries (Andrew et al., 2002). Currently, fishing urchins in New Zealand is economically marginal and requires significant local knowledge and resources because of the variable quality and wide distribution of urchins that have good quality roe (James et al., 2004).

Growing worldwide interest in the enhancement of sea urchin roe from wild caught urchins has created intense interest in New Zealand. If it is possible to consistently

improve the quality and quantity of roe from wild urchins, this would present the opportunity to export enhanced roe into lucrative international markets. However, markets such as Japan require roe of a suitable quality and reliable techniques for roe enhancement it will be necessary to achieve this.

There has been extensive international research into the factors that effect sea urchin roe enhancement (Pearse and Cameron, 1991; Klinger et al., 1997; Robinson and Colborne, 1997; Walker and Lesser, 1997; Lesser and Walker, 1998; Lawrence et al., 2001). Key factors that have been identified are the availability of an effective artificial diet, the reproductive condition of the animals, and the availability of suitable holding systems. Most previous research has focused on land-based experimental scale trials, but more recently there has been increasing worldwide interest in sea-based urchin roe enhancement. In Canada (New Brunswick) an experiment to test the feasibility and efficacy of enhancing the roe of *Strongylocentrotus droebachiensis* held in hanging sea-cages was successful (Robinson and Colborne, 1997). In Newfoundland, a roe enhancement ranching trial, holding the urchins in underwater corrals on the seafloor, was undertaken with some success (Bridger, et al., 1998). In Scotland, a series of experiments investigating the potential for polyculture of the urchin *Psammechinus miliaris* with salmon cages (Kelly, et al., 1998) showed that urchins held in lantern nets had greater roe production than those held in pearl nets because the salmon feed pellets were more available through the larger mesh of the lantern nets. An experiment holding *S. droebachiensis* in small sea-cages in Maine showed it was feasible to enhance the gonads of 'post-spawned' urchins using a variety of natural algae as feed (Vadas, et al., 1999). Growth trials of *Loxechinus albus* fed natural algae have also been carried out in

lantern nets in Chile with some success (Mendes and Becarra, 2004). In Norway, sea-cage technology has been developed for roe enhancement of *S. droebachiensis* with the urchins held at stocking densities of 35 kg m^{-2} in stacked baskets suspended beneath a supporting structure (Anon, 2000; Aas, 2004).

There have been a number of studies on the New Zealand sea urchin *E. chloroticus* testing the efficacy of natural diets and artificial diets on roe enhancement in land-based holding systems (Barker et al., 1998; Buisson, 2001; James et al., 2004). In addition, a study by Fell (2002) investigated the effects of holding kina in sea-cages and feeding a combination of artificial and natural diets, with promising results. Although both land and sea-based holding systems have shown potential for increasing the roe production of kina, there has only been a single comparative study on land-based versus sea-based holding systems for roe enhancement of urchins (Cuthbert and Hooper, 1995). In order to develop a viable kina roe enhancement industry in New Zealand the most economic and effective holding systems must be established. The advantages of land-based systems include greater control of environmental parameters, such as light levels and photoperiod, and easy access to urchins for cleaning and feeding; the disadvantages include the high set-up and running costs. Alternatively, sea-based systems offer relatively low set-up and maintenance costs, particularly when they can utilize existing mussel farm sites. The disadvantages of sea-based systems include difficult access to commonly remote farming sites, and issues related to marine compliance and exposure to environmental factors, such as storms and currents. A study into the economic feasibility of sea urchin gonad enhancement of *S. droebachiensis*, using both land-based and sea bottom corral culture in NewFoundland, found significant enhancement potential with bottom culture appearing

to be more viable, but higher risk, than land-based culture (Burke, 1997). Similarly, in New Zealand sea-cage culture of lobsters has been shown to be more economically viable than land-based culture due to the significant reduction in infrastructure costs (Jeffs and Hooker, 2000). Urchin roe enhancement projects in New Zealand may be faced with a choice of land vs. sea-based holding systems and this study investigates whether there are any significant advantages in either system in terms of roe production and quality.

In previous sea urchin roe enhancement trials in New Zealand the efficacy of the experimental treatments has been measured by the increase in the Gonad Index (GI) of the urchin and by the colour of the urchin roe at the conclusion of the trial (Barker et al., 1998). GI is relatively simple to measure but the colour of the roe is traditionally measured using some form of 'match-by-eye' technique. There are inherent difficulties with this subjective technique and a more accurate technique is to use a spectrophotometer to take measurements using the international standard CIELAB system of colour measurement (Robinson et al., 2002). This system allows an accurate comparison and statistical analysis of colour measurements and was used in addition to the more traditional technique of matching roe colour to the 'Maine' sea urchin colour chart.

3.3 Materials and methods

3.3.1 Urchin collection and maintenance

Sea urchins were sourced from urchin barrens of the north end of Koropuki Island, one of the Mercury Islands, off the north-east coast of the North Island of New Zealand (36^{o}39.400'S 175^{o}50.519' E) on 15 September 2003. The urchins were transported in the

collection bags by boat and then in a trailer and were covered with damp sacking and a wind proof cover during the journey. On arrival at the NIWA Aquaculture facility at Mahanga Bay, Wellington, the urchins were placed in plastic crates (605 mm long x 420 mm wide x 316 mm high, internal surface area = 1.116 m^2) (Fig. 3.1).

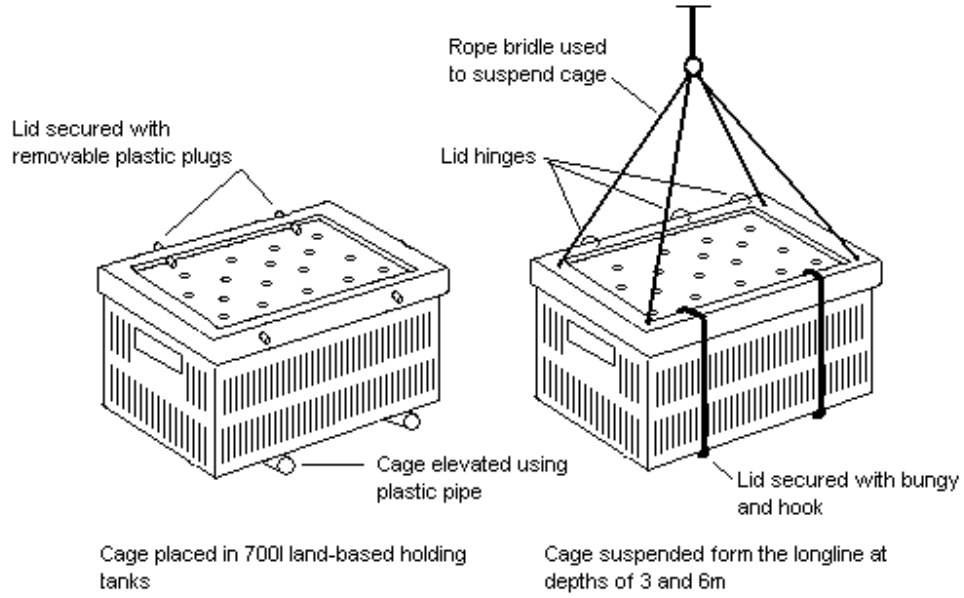

Figure 3.1 Construction of the sea urchin cages placed in 700 l land-based holding tanks and suspended at depths of 3 and 6 m from a longline.

The urchins were randomly allocated to 18 plastic crates (hereafter referred to as sea-cages), nine with 25 urchins (22.4 urchins m^{-2} = low density) and nine with 35 urchins (31.3 urchins m^{-2} = high density). Six of the cages (3 high density and 3 low density) were placed in 6 x 700 l plastic 'Mega bins' situated inside the NIWA Aquaculture facility. These cages were supplied with 4.8 l/min of 10-μm filtered seawater at ambient temperature and a controlled light/dark photoperiod of 12L:12D. The mean (± 1 S.E.)

oxygen saturation measured in the tanks during the experiment was 96.0 ± 0.6%. The remaining 12 cages (6 high density and 6 low density) were placed randomly along a mussel longline situated approximately 100 m offshore in Mahanga Bay. Cages were suspended at either 3 or 6 m with three replicates of low and high density treatment at each depth. The average depth of water under the longline was 8 m at a mid tide.

3.3.2 Seawater parameters

Stowaway Tidbit™ temperature loggers placed in the land-based tanks, and suspended on the longline at a depths of 3 and 6 m, recorded the ambient seawater temperatures once every hour for the duration of the experiment. Because of the large number of temperature recordings an AFC autocorrelation analysis was used to show the period (70 hrs) over which the data correlation (lag time) became significant. One-way ANOVA was used to test for differences in the 70 h mean seawater temperatures between land-based, sea-based 3 m and sea-based 6 m treatments.

The ambient photosynthetic irradiance (µmoles photons m^{-2} s^{-1}) was recorded using two Odyssey cosine sensors suspended from the mussel longline at depths of 3 m and 6 m for a 7 day period. One way ANOVA was used to test for differences in light levels at the two depths at the 12.30 pm reading for each day that the loggers were suspended on the longline (n = 6).

3.3.3 Experimental protocol

The trial commenced on 18 September 2003 and ran for a total of 12 weeks, with a census conducted at week 12 to assess the effect of experimental treatments on a range of urchin variables. The NIWA artificial sea urchin roe enhancement diet (for proximate analysis see James et al., 2004) utilized in this experiment has been shown to be an effective diet for *E. chloroticus* (James et al., 2004; Woods and James, 2004). The diet was made at the NIWA Aquaculture facility shortly before the trial and frozen at –20^{o}C. For feeding it was removed from the freezer, defrosted and cut into cubes. The feeding rate from the beginning of the trial to the first census at week 7 was 1.0% of mean urchin body weight per day, but this was increased to 1.5% per day from week 8 through to week 12 of the trial. The urchins were fed three times per week and uneaten food was removed from the cages at the same time. The cages suspended from the longline were hauled to the surface for feeding; those in the land-based tanks were opened for feeding.

An initial census of the condition of the urchins (on 16 September 2003) and a final census (after 12 weeks) measured test diameter (mm), total wet weight (g), gonad wet weight (g) and gonad colour (objective, *L*, a*, b**).

Gonad colour was objectively assessed using a Minolta C-508 Spectrophotometer with spectral component excluded, using the international standard *L*, a*, b** form of colour measurement. A secondary measure of colour assessment was made by matching urchin gonads against the 'Maine colour chart' as used in the USA. The colours considered acceptable from the chart for the purpose of this trial are 104-112.

GI was calculated from the wet weights (greenweight) of individual urchins as follows:

GI (%) = Gonad wet weight / Total wet weight x 100

A graph comparing the test diameter against the gonad index of the wild urchins collected at the beginning of the experiment (Fig 3.2) shows no significant relationship between test diameter gonad index (ANOVA: F = 0.52, *df* = 1,58, *P* = 0.472).

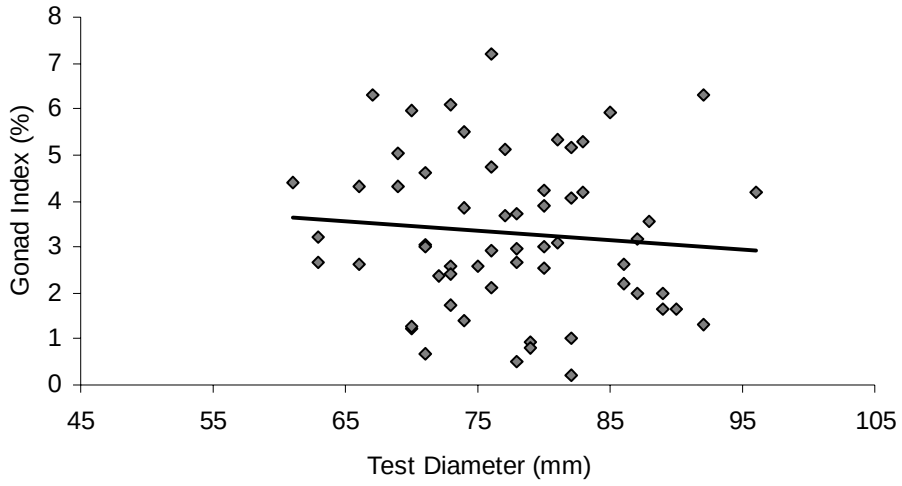

Figure 3.2 The relationship between Gonad Index values and Test Diameter for urchins collected from the initial wild sample, Y = -0.020X + 4.866; r^2 = 0.009; *n* = 60.

A measure of the reproductive stage, from histology samples collected from the gonads of 15 individual urchins, was included in the initial census only. Histology samples were stained using standard Harris's haematoxylin stain, counterstained with Eosin (Bancroft and Stevens, 1990). The sex ratio and reproductive condition of the

initial urchin sample was assessed using the six reproductive stages described in Walker (1982) and Byrne (1990).

3.3.4 Statistical analysis

Mean values for urchin test diameter, wet weight, gonad index and colour were calculated using 20 urchins sampled from each replicate cage (n = 3 per treatment). For the initial census the wild urchins were randomly placed in groups of 20 to obtain three mean values. For the various gonad characteristics, one-way ANOVAs were used to calculate significant differences between initial and final samples, and amongst treatments, at the conclusion of the experiment. Two-way ANOVAs were used to examine the combined effects of cage position (land-based vs. sea-based 3 m vs. sea-based 6 m) and urchin density. Where significant P-values were generated a Tukey-Kramer post-hoc comparison tests was used to evaluate pair-wise means. A Modified-Levene test was used to verify equal variance.

3.3.5 Cage fouling

A comparison of the amount and type of fouling present on the cages held on the mussel longline was made at the conclusion of the experiment. A 370 x 550 mm area on the upper, outer surface of the lid of each cage was sampled and all the fouling algal species that were present were identified to species level where possible. The fouling organisms were collected, washed to remove any sedimentation, and then placed in a pre-dried and weighed plastic pottle and oven dried at 60^{o}C for 24 h to determine the dry weights of

fouling algae. There was no fouling on the cages held in the inside tanks and these were not sampled. A one-way ANOVA was used to test for statistical differences between the dry weights of fouling present on the sea-based treatments.

3.4 Results

3.4.1 Initial census

The baseline morphological variables, gonad colour, and gonad reproductive stage of the urchins at the initial census are listed in Table 3.1.

Table 3.1 Mean (± 1 S.E.) initial morphological and gonad colour variables of initial wild urchin sample.

	Initial wild urchin
Urchin test diameter (mm)	77.5 ± 1.0
Urchin wet weight (g)	189.8 ± 7.9
Gonad Index (%)	3.29 ± 0.22
Gonad lightness (*L**)	32.03 ± 1.05
Gonad redness (*a**)	10.36 ± 1.78
Gonad yellowness (*b**)	20.39 ± 1.15
Sex	60% female / 40% male
Reproductive stage	Stage 1/2 (recovery/growing)

3.4.2 Seawater temperatures

The ambient seawater temperatures increased during the trial, reflecting the seasonal changes during this period (Fig. 3.3). The cages held in land-based tanks experienced the highest mean temperature, followed by those suspended from the mussel longline at 3 m depth, with the cages suspended at 6 m depth experiencing the lowest mean seawater temperatures (Table 3.2). There were no significant differences in the mean ambient temperatures of the different treatments calculated at any of the 70 h time points (ANOVA: F = 0.44, *df* = 2, 82, *P* = 0.647).

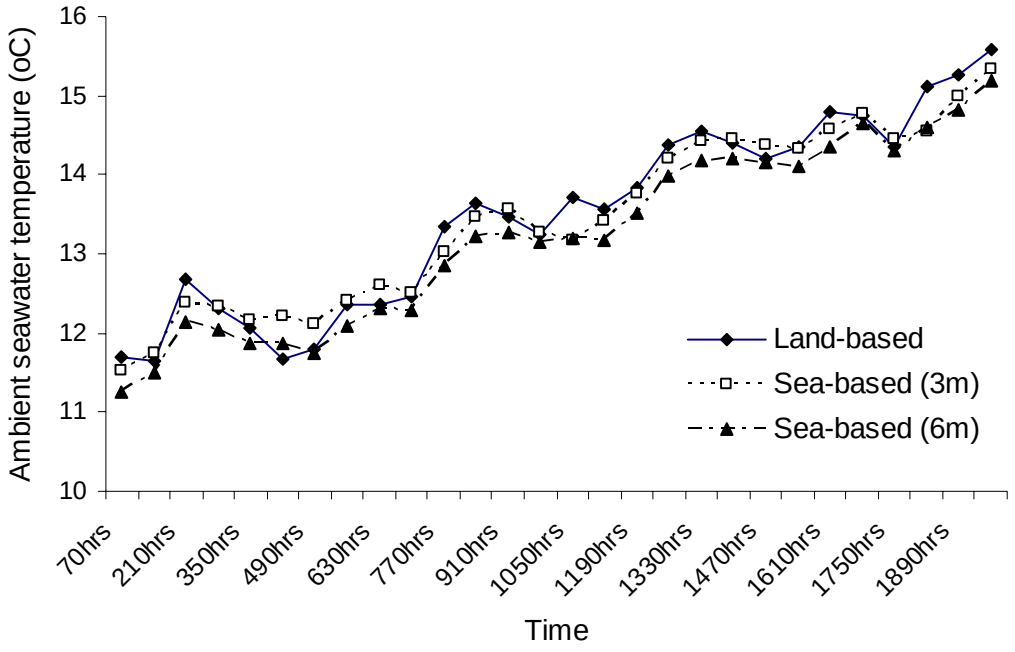

Figure 3.3 Ambient seawater temperatures recorded at 70 h intervals during the 12 week sea urchin roe enhancement trial.

Table 3.2 The maximum, minimum and mean (± 1 S.E.) ambient seawater temperatures recorded during the sea urchin roe enhancement trial at Mahanga Bay.

Position of cages	Seawater temperature (^{o}C)		
	Maximum	Minimum	Mean
Land-based tanks with ambient water	16.4	10.9	13.53 (± 0.03)
Suspended on mussel longline at 3 m depth	15.5	11.3	13.48 (± 0.03)
Suspended on mussel longline at 6 m depth	15.3	11.1	13.26 (± 0.03)

Table 3.3 The mean percentage survival (± S.E.) for the urchins held in the cages in land-based tanks, and suspended at a depth of 3 m and 6 m on the longline in Mahanga Bay, at high and low densities after the 12 week trial.

	% Survival (± 1 S.E.)
Land-based tanks / low density	88.00 (± 4.00)
Land-based tanks / high density	82.86 (± 4.36)
Sea-based / 3 m depth / low density	84.00 (± 4.00)
Sea-based / 3 m depth / high density	85.71 (± 4.36)
Sea-based / 6 m depth / low density	96.00 (± 2.31)
Sea-based / 6 m depth / high density	81.90 (± 12.49)

3.4.3 Urchin survival

Urchin survival ranged from 82-96% (Table 3.3) with the highest mortality occurring during the first 3 weeks of the trial. There were no significant differences between the treatment means (ANOVA: F = 0.69, df = 5, 18, P = 0.638).

3.4.4 Cage fouling and light levels

The level of fouling (dry weight) was significantly higher on the cages suspended at 3 m depth than on the cages suspended at 6m depth (Student *t*-test: $T = 2.57$, $P < 0.05$). The average dry weight of fouling per cage at 3 m was 6.53 g (± 0.16) and at 6 m was 1.20 g (± 0.72). The number of fouling species found growing on the cages was also slightly greater in the shallow cages (Table 3.4).

The light levels recorded at a depth of 3 m were significantly higher than those recorded at a depth of 6 m (Student *t*-test: $T = 1.97$, $P < 0.05$) (Fig. 3.4).

Table 3.4 The number and type of fouling species found on the seacages suspended at two depths from the longline at Mahanga Bay for 12 weeks (species marked with * denotes an introduced species).

Fouling species on cages at 3 m	Fouling species on cages at 6 m
Ulva spp.	*Valeriemaya* sp.
Enteromorpha sp.	*Cutleria multifida**
Scytosiphon lomentaria	colonial diatoms (unidentified)
*Cutleria multifida**	*Callithamnion* sp.
*Polysiphonia brodiaei**	

3.4.5 Urchin test diameter and wet weight

There were no significant differences between the average test diameter (ANOVA: F = 0.81, *df* = 6, 21, $P = 0.577$) or the average wet weight (ANOVA: F = 1.54, *df* = 6, 21, P =

0.235) of the urchins in the initial sample and the urchins from any of the experimental treatments after the 12-week trial.

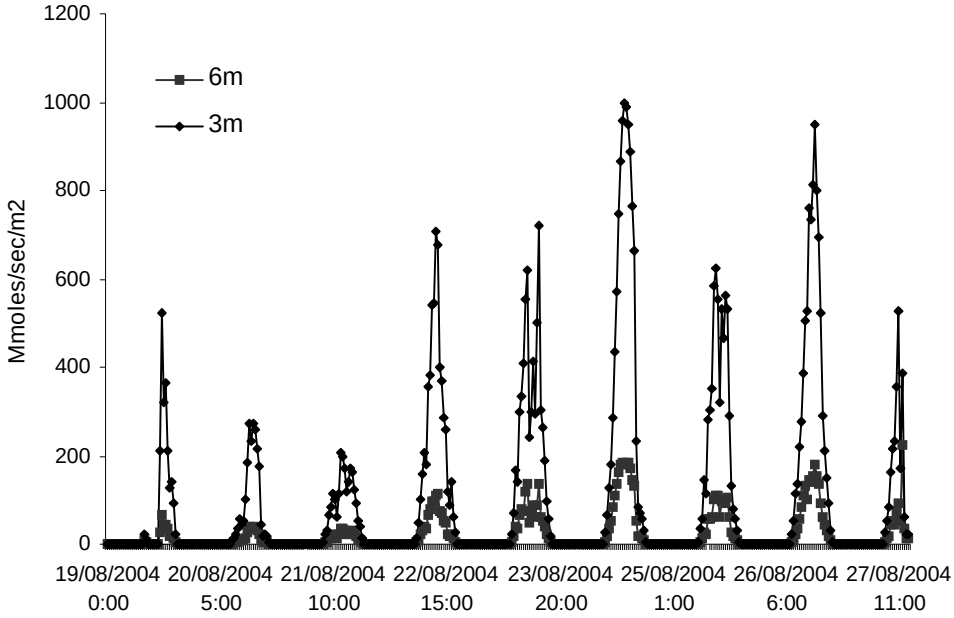

Figure 3.4 The photosynthetic irradiance levels (µmoles photons m^{-2} s^{-1}) recorded by photo sensors suspended at depths of 3 m and 6 m for 7 days on the longline in Mahanga Bay.

3.4.6 Urchin gonad index (GI)

Mean GIs of the urchins from the initial sample were significantly lower than for the urchins from any of the experimental treatments after 12 weeks (ANOVA: F = 24.65, *df* = 6, 21, $P < 0.001$). The urchins held in the sea-cages at low density at a depth of 6 m had the highest GI (12.69% ± 1.02) and the urchins held in land-based tanks had the lowest GI measurements, after the 12 weeks, with those held at low density (11.01% ± 0.49) having a slightly higher GI than those held at high density (10.78% ± 0.97) (Fig. 3.5). A

two-way ANOVA, examining the effects of cage position and urchin density showed no significant main effects (Cage position: F = 2.11, *df* = 2, 18, *P* = 0.16; Urchin density F = 0.82, *df* =1, 18, *P* = 0.38) or interactions (F = 0.08, *df* = 2, 18, *P* = 0.92).

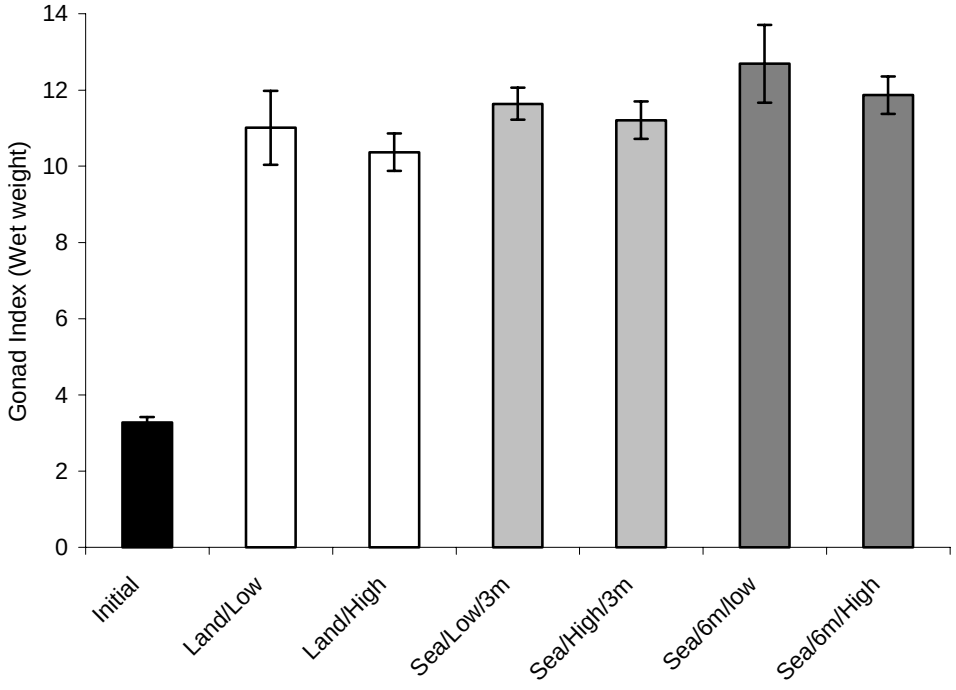

Figure 3.5 The mean gonad index (± 1 S.E.) of urchins from the initial urchin sample and from urchins held in land-based tanks at high and low density, and in sea-cages at low and high density at 3 and 6 m depth, for 12 weeks (*n* = 420).

3.4.7 Urchin gonad colour

The mean gonad lightness (*L**) of the urchins increased significantly from the initial mean of *L** = 32.02, in all of the experimental treatments during the 12 week experimental period (ANOVA: F = 9.85, *df* = 6, 21, *P* < 0.001). The urchins held in the land-based/low density treatment had significantly lighter gonads than all other treatments except the land-based high density treatment. There were no differences in the

lightness between the latter treatment and any other experimental treatments after 12 weeks (Fig. 3.6).

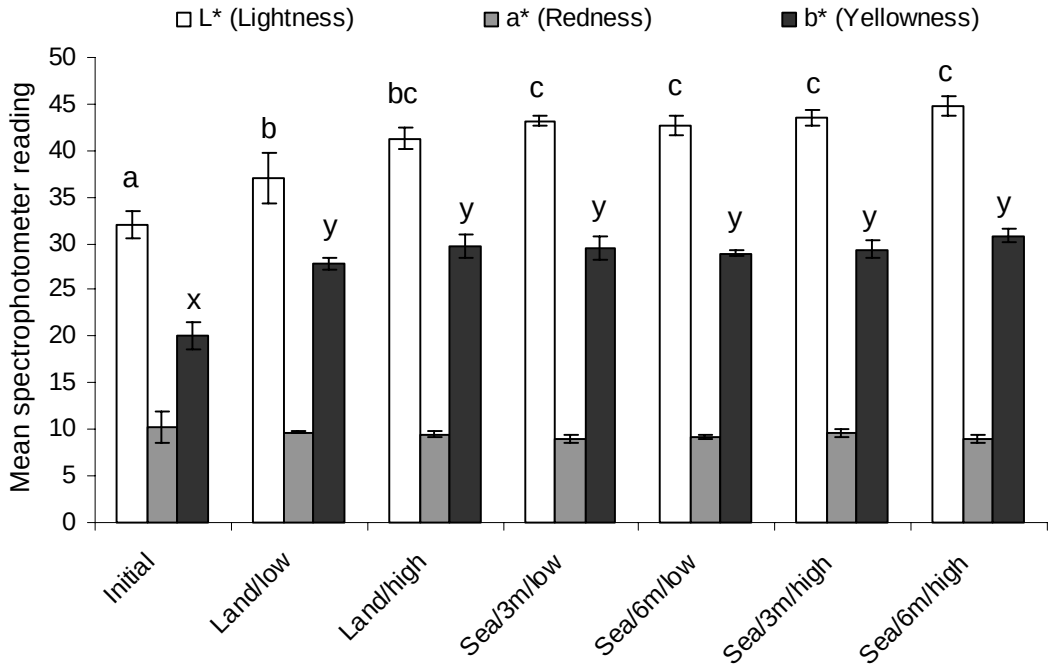

Figure 3.6 The mean lightness (*L), redness (*a**) and yellowness (*b**) (± 1 S.E.) readings of urchins from the initial sample and from urchins held in land-based tanks at high and low density, and in sea-cages at low and high density at 3 and 6 m depth, for 12 weeks. Different letters above the bars indicate a significant difference between treatments.**

The mean redness (*a**) of the urchin gonads remained at a similar level to the mean initial redness (*a** = 10.36) after the 12 week experimental period. There were no significant differences between the urchins held in any of the experimental treatments (ANOVA: F = 0.37, *df* = 6, 21, *P* = 0.866) (Fig. 3.6).

The mean yellowness (*b**) of the urchin gonads increased significantly during the 12 week experimental period (ANOVA: F = 12.93, *df* = 6, 21, *P* = 0.001) in all of the experimental treatments from an initial mean of *b** = 20.39, but there was no significant

difference between the mean gonad yellowness of the urchins held in the experimental treatments after 12 weeks (Fig. 3.6).

Table 3.5 The percentage of urchin gonad that is of an acceptable colour, using the Maine colour chart.

	% acceptable coloured gonad (i.e. colours 104-112) from Maine colour chart
Initial wild urchin sample	26.6
Land-based tanks / low density	58.3
Land-based tanks / high density	51.6
Sea-based / 3 m depth / low density	65.5
Sea-based / 3 m depth / high density	66.6
Sea-based / 6 m depth / low density	74.6
Sea-based / 6 m depth / high density	66.1

Both the Maine colour chart and the spectrophotometer readings showed a higher percentage of urchins with acceptable coloured roe at the end of the experiment than in the initial wild sample. Urchins held in sea-cages at 6 m and at low density had the highest percentage of acceptable gonad after the 12-week experimental period (Table 3.5).

3.5 Discussion

3.5.1 Seawater temperature

The gradual increase in the ambient seawater temperature experienced during the experiment in all the experimental treatments is a reflection of natural seasonal changes between winter and summer. There were no significant differences between the ambient seawater temperatures inside the facility and at depths of 3 or 6 m in Mahanga Bay, indicating that the effects of pumping and storage of seawater for the land-based facility had no significant effect.

3.5.2 Urchin survival

In previous studies on *E. chloroticus* survival rates have shown the species to be robust in terms of its ability to survive capture, transport and holding in both land- and sea-based systems for up to 3 months. Barker et al. (1998) had survival rates of 95% and 96% in two separate land-based experiments and Fell (2002) had survival rates ranging between 93.3% and 99.5% in sea-cage experiments. In the later experiment it was noted that urchins may be susceptible to delayed mortality, caused by thermal shock when being transported from one site to another, and that mortalities may occur a number of weeks after translocation (Fell, 2002). The urchins used in this trial were transported by boat from the Mercury Islands to Whitianga, and by road from Whitianga to Wellington (approx 11 h). In a previous study (James et al., 2004) urchins sourced from the same area and transported using the same techniques for a land-based roe enhancement trial

had a relatively high survival rate (94.4%) over 10 weeks. The survival of the urchins in this trial was lower, ranging between 81.9% and 96.0% (average = 86.4 ± 5.3%). There was little difference in survival between the experimental treatments and most of the urchin mortality occurred in the first three weeks of the experiment. This indicates that the lower survival rate was due to the quality or condition of the urchins after transportation from Whitianga to Wellington rather than to the experimental conditions in which the urchins were held. The lower ambient seawater temperatures experienced in Wellington compared to those at Mercury Islands may also have had some delayed effects on the urchins post transport that resulted in the mortalities in the first few weeks of the trial.

3.5.3 Urchin test diameter, wet weight and reproductive stage

There was no increase in kina test size or urchin wet weight over the 12-week trial period. However, there was a significant increase in the GI of the kina in all the experimental treatments, compared to the GI of the wild urchins at the beginning of the experiment. The histology of the urchins collected at the beginning of the experiment showed that the gonads were in the recovery/growing stage when nutritive cells are building up at the end of the dormant stage after spawning in late summer/autumn (Dix, 1970c; Pearse and Cameron, 1991; Buisson, 2001). Previous studies on *E. chloroticus* have consistently shown significant increases in GI in urchins fed the NIWA artificial diet, regardless of the reproductive stage of the urchins (James et al., 2004; James, unpublished data). This experiment was conducted during a relatively favourable time of the year in terms of natural gonad development (late recovery/growing stage) and the urchins held in the

experimental treatments had percentage increases in GI ranging from 230% (land-based/high density) to 281% (sea-based/6 m/low density).

3.5.4 Urchin gonad index - density and cage position (land-based vs. sea-based 3 m vs. sea-based 6 m)

In a previous study by Fell (2002), *E. chloroticus* held in sea-cages at a maximum density of 19.7 urchins m^{-2} of internal surface area showed good gonad growth in spring and summer but very little gonad growth in mid to late winter. Final GI values in this study ranged between approximately 19 and 23% (these percentages are calculated using the drained weight of the sea urchins). In this experiment there was a trend for urchins held at a density of 22.4 urchins m^{-2} to have consistently higher GI after 12 weeks than urchins held at a density of 31.3 urchins m^{-2}, whether sea-based at 3 or 6 m depth or land-based, but the differences between the high and low density were not significant. The pattern of higher GI in urchins held at the lower density indicates that density may be having an effect but it is not significant at these densities and that urchins can be held at up to 31.3 urchins m^{-2} without having a significant adverse effect on the increase in GI.

Holding urchins in identical cage systems suspended from a mussel longline and in land-based tanks had no significant effect on roe productivity, in terms of increase in GI, over a 12-week period.

3.5.5 Cage fouling and photosynthetic irradiance

The measurements of algal fouling taken at the conclusion of the experiment were made on the exterior surface of the cages and it was assumed that similar species would have been present and available within the cages for urchins to graze. There was evidence of sea urchin grazing marks inside the cages and the complete lack of fouling on the inside surfaces suggested that the urchins had grazed fouling organisms that were present. Although there was little difference between the number of species present on the 3 and 6 m sea-cages (3 and 5 species respectively), the dry weight of the algae that settled on the 3 m cages over the 12 week experimental period was significantly higher, correlating with the significantly greater ambient photosynthetic irradiance levels recorded at 3 m than at 6 m. Even on the 3 m cages the quantity of fouling is very small relative to the amount of feed fed the urchins and it does not appear to have had any effect on the GI of the urchins held in the experimental treatments.

3.5.6 Urchin gonad colour

There was an increase in colour quality in the roe during the experiment, regardless of the cage position or urchin density. Previous studies have shown that higher levels of natural algae in urchin diet improve the colour of urchin roe (Barker et al., 1998). In this study the type and quantity of algal fouling on the sea-cages does not seem to have influenced the roe colour quality, either because the urchins were not feeding on the algae, or because the algae that were present were not suitable species or were not present in sufficient quantities. The improved colour quality of the urchin roe reflects the efficacy of the artificial diet used in the experiment.

3.6 Conclusions

Urchin roe quantity (GI) and quality (colour) can be enhanced in 12 weeks by feeding the NIWA artificial diet in land-based tanks or sea-cages, with high survival. There were no significant differences between the enhancement of roe quantity (GI) or quality (colour) in urchins held in land-based tanks or in sea-cages. There were also no differences between urchins held in sea-cages at 3 m and at 6 m, or between urchins held at low and high density.

Chapter Four

The effects of wave and feeding disturbance on roe enhancement of *Evechinus chloroticus* held in sea-cages

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[Published in: Aquaculture 252 (2006) 361-371]

Received 14 April 2005, received in revised form 22 July 2005; accepted 29 July 2005

4.1 Abstract

An experiment was conducted over a 10-week period to test the effects of wave and feeding disturbance on the gonad index (GI) and gonad colour of sea urchins fed an artificial diet. The sea urchins were held in sea-cages at a depth of 6 m. Eight cages were suspended from a surface line (wave disturbed) and eight were subsurface buoyed from a bottom line (not wave disturbed). These two treatments were further divided into four replicates that were fed and cleaned in situ underwater (no feed disturbance) and four replicates that were removed from the water three times per week for feeding and cleaning (feed disturbance). Increased water movement, probably caused by the vertical

motion in the wave disturbed cages resulted in a higher GI in these urchins compared to urchins in cages that were sub surface buoyed and did not experience any vertical movement. Feeding disturbance had no effect on the GI values or colour quality of the urchin gonads, regardless of whether the urchins were held in sea-cages that were wave disturbed or not wave disturbed.

Keywords: Evechinus chloroticus, Sea cages, Wave disturbed, Feeding.

4.2 Introduction

There has been extensive international research into the factors that effect sea urchin roe enhancement (Pearse and Cameron, 1991; Klinger et al., 1997; Robinson and Colborne, 1997; Walker and Lesser, 1997; Lesser and Walker, 1998; Lawrence et al., 2001a). The key factors that have been identified are the availability of an effective artificial diet, the reproductive condition of the urchins and the availability of suitable holding systems (Pearse and Cameron, 1991; Lesser and Walker, 1998; Lawrence et al., 2001). There has been increasing worldwide interest in sea-based urchin roe enhancement with promising results (Robinson and Colborne, 1997; Bridger, et al., 1998; Kelly, et al. 1998; Vadas et al., 1999; Mendes and Becarra, 2004; Aas, 2004).

In New Zealand the sea urchin (kina) *Evechinus chloroticus* (Val.) has been commercially fished since 1986 and the urchin roe has almost entirely been sold on the domestic market. There have been a number of attempts to export urchin roe to overseas markets such as Japan, but only a small amount has been exported due to the roe having a

bitter taste (McShane et al., 1994) poor colour, and inconsistent or low yields (P. Herbert, Sea urchin New Zealand, pers comm.). Consequently, the fishery has not expanded in New Zealand to the same degree that has occurred in other countries (Andrew, et al., 2002). Currently, fishing urchins in New Zealand is marginally economic and requires significant local knowledge and resources because of the variable quality and wide distribution of urchins that do have good quality roe (James et al., 2004). Growing worldwide interest in the enhancement of sea urchin roe from wild caught urchins has created intense interest in New Zealand. If it is possible to consistently improve the quality and quantity of roe from wild urchins this would present the opportunity to export enhanced roe into lucrative international markets. There have been a number of studies on the sea urchin *E. chloroticus* in New Zealand testing the efficacy of natural diets and artificial diets on roe enhancement in land-based holding systems (Barker et al., 1998; Buisson, 2001; James et al., 2004). A study by Fell (2002) investigated the effects of holding kina in sea-cages and feeding a combination of artificial and natural diets. The final GI values in this study ranged between approximately 19 and 23% (these percentages are calculated using the drained weight of the sea urchins). A more recent study comparing the efficacy of land- versus sea-based holding systems showed little difference in the GI or the colour of sea urchin roe held in either system (James, 2006a).

There are a number of other factors that affect urchin roe enhancement such as temperature and photoperiod (Spirlet et al., 2000, Buisson, 2001, Garrido and Barber, 2001; Siikavuopio et al., 2006; Hofer and Watts, 2004). Factors that have been less studied are disturbance effects such as wave disturbance, and feeding and cleaning disturbance. Urchins held in sea-cages may need to be removed from the water on a

regular basis to replenish feed supplies, remove uneaten feed and clean the cage but there have been no studies to determine the effects of removing urchins from the water for feeding. Similarly, there have been no studies on the effects that environmental conditions such as vertical wave movement may have on urchins held in a sea-cage system.

The aim of this study was to test the effects of both feeding and wave disturbance on the roe enhancement of sea urchins held in sea-cages by measuring roe production, survival and roe colour quality.

4.3 Materials and methods

4.3.1. Urchin collection

Sea urchins were sourced from a 6-10 m rocky site at Port Gore in the outer Marlborough Sounds (41^{o}02'S 174^{o}14'E) in the north of the South Island of New Zealand on Friday 26 March 2004. The urchins were collected on snorkel and were placed in mesh bags (approximately 60 urchins, 17kg per bag) before being hauled onboard the fishing vessel. Once onboard the vessel the urchins were placed into 16 ventilated, free draining plastic crates which were covered with felt sacking and kept damp with sprayed seawater for the 2 h journey to Wellington Harbour. A second wild control sample was collected from the same site in Port Gore on Tuesday 20 July 2004 and the urchins were caught and transferred in the same way as the initial wild sample.

4.3.2. Experimental methods

On arrival at Mahanga Bay in Wellington Harbour the density of the sea urchins in each crate (hereafter referred to as sea-cages or cages) was reduced to 35 urchins per cage (31.3 urchins m^{-2} internal surface area). Eight cages were suspended at a depth of 6 m below a mussel long-line in Mahanga Bay (wave disturbed). The other eight cages with a buoy attached to the top bridle, were attached to a backbone rope on the seafloor adjacent to the mussel long-line and suspended 2 m above the seafloor. These cages were also 6 m below the water surface at mid tide (no wave disturbance). For each set of eight cages, four were permanently attached using shark clips and four were attached to the backbone with stainless steel carabineers that could be easily detached and sent to the surface attached to an extension rope for feeding and cleaning (Fig. 4.1).

The experimental site at Mahanga Bay ($41^{o}18'$ S, $174^{o}50'$ E) is subject to almost constant wave action, typically of 0.1-0.5 m amplitude at the surface. Calm days and days with waves greater than 0.75 m are rare (Hickman et al., 1999).

The trial commenced on 31 March 2004 and ran for a total of 10 weeks. NIWA artificial sea urchin roe enhancement diet (see James et al., 2004 for proximate analysis) was produced prior to the trial and frozen at -20^{o}C. The diet was defrosted, cut into cubes and fed to all of the urchins three times per week during the trial at a rate of 1.5% mean urchin body weight per day. Uneaten food was removed from each cage at each feed except for food that was not easily accessible to the cleaner, which was left in the cages. Any dead urchins were removed during feeding and cleaning, and the tank and date were recorded.

In order to feed the sea urchins in the suspended and sub-surface buoyed treatments without feeding disturbance the artificial feed was delivered and removed by divers. In treatments with feeding disturbance the cages were brought to the surface and hauled onto a boat where the lid could be lifted and the old feed removed and replaced with fresh feed.

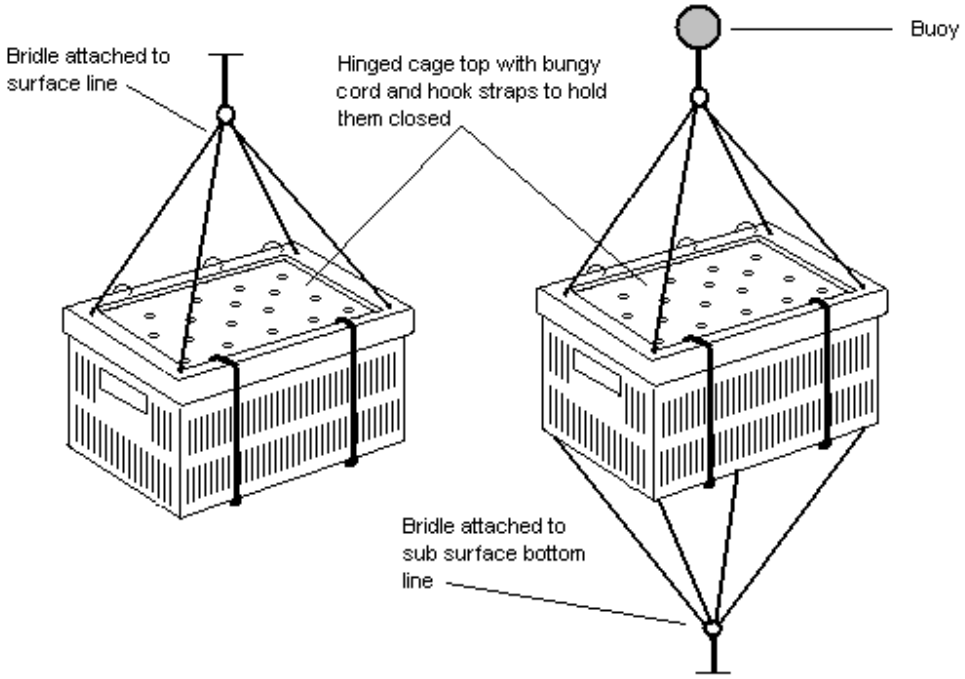

Figure 4.1 The sea-cage system used to hold sea urchins in during the experiment. The cages suspended from the surface line were 6 m below the surface and those attached to the sub surface bottom line and suspended 2 m above the seafloor and 6 m below the water surface at mid tide.

4.3.3 Seawater parameters

The ambient seawater temperature was recorded once every hour for the duration of the experiment using stowaway Tidbit™ temperature loggers suspended at 6 m depth from the mussel long-line, or the seafloor backbone.

To test for differences in water movement, 48 plaster rectangular cubes were constructed from dental plaster, dried in a 60^{o}C oven for 24 h, weighed to two decimal places and placed in the experimental cages. Two plaster cubes (approx 50 mm x 40 mm x 40 mm) were suspended below the lid using cable ties in each experimental cage. An additional 8 plaster cubes were suspended in the same way as the temperature loggers on separate lines at a depth of 6 m, 4 from the surface long-line and 4 from the seafloor longline. As a control, 8 plaster cubes were also placed in a container of seawater for a similar period. The plaster cubes remained in these positions for seven days in week 9 of the experiment and were then removed from the water and placed back in the 60^{o}C oven for 24 h, before being weighed to two decimal places. The weight loss of the plaster cubes was calculated by subtracting the final from the initial dry weights.

4.3.4 Urchin condition assessment

An initial examination of the condition of the urchins was carried out on 29 March 2004. A census was conducted at weeks 7 and 10 (15 urchins were randomly selected from the cages at each census) to assess the effect of the following experimental treatments:

1. Suspended from a longline and fed without the cage being removed from the water (wave disturbed/no feed disturbance).
2. Suspended from a longline and fed by removing the cage from the water (wave disturbed/feed disturbed).
3. Sub-surface buoyed and fed without the cage being removed from the water (no wave disturbance/no feed disturbance).

4. Sub-surface buoyed and fed by removing the cage from the water (no wave disturbance/feed disturbance).

Specific sea urchin variables measured and analysed at the initial census, again after 7 and 10 weeks and from the final wild sample were; test diameter (mm), total wet weight (g), gonad (roe) weight (g), gonad index and gonad colour (*L**, *a**, *b**). A measure of the reproductive stage of the urchins (from a histological sample) was included in the initial wild, 10 week and final wild census.

Gonad index (GI) was calculated as:

G I = Gonad wet weight / Total wet weight x 100

A graph comparing the test diameter against the gonad index of the wild urchins collected at the beginning of the experiment (Fig 4.2) shows no significant relationship between test diameter gonad index (ANOVA: F = 1.75, *df* = 1,58, *P* = 0.256).

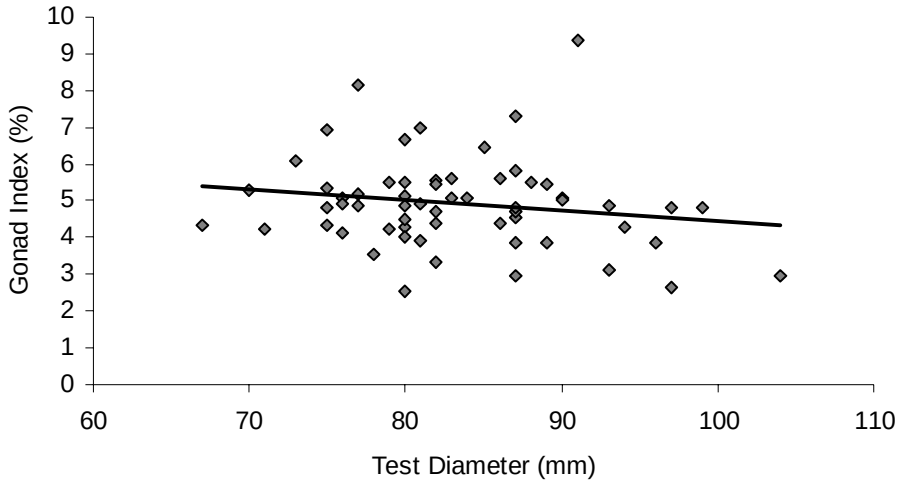

Figure 4.2 The relationship between Gonad Index values and Test Diameter for urchins collected from the initial wild sample, Y = 0.029 X + 7.291; r^2 = 0.029; *n* = 60.

Gonad (roe) colour was objectively assessed using a ColorTec-PCM^{TM} (ColorTec, Clinton, New Jersey, USA) 12 mm 45/0 colour meter using the international standard CIELAB (or *L**, *a**, *b**) system of colour measurement.

Histological samples were collected and stained using standard Harris's haematoxylin stain, counterstained with Eosin (Bancroft and Stevens, 1990). The reproductive condition of the initial urchin sample was assessed using the six reproductive stages described in Byrne (1990), Walker (1982) and Buisson (2001).

4.3.5 Statistical analysis

The mean GI of the initial and final wild urchin samples, and the 7- and 10-week experimental samples (individually for each of the experimental treatments), respectively,

were compared using a Student *t*-test. To compare the urchin test diameter, wet weight, gonad index (GI) and colour between the initial wild, final wild and experimental 10-week samples, mean values were calculated from 15 urchins sampled from each replicate cage (n.b. replicates for the initial and final wild census were obtained by randomly placing individuals in groups of 15 to obtain four mean values). A Modified-Levene test was used to verify equal variance and the data were transformed appropriately prior to analysis (if required). For gonad characteristics, mortality and plaster cube dry weight loss, one-way ANOVA were used to analyse the mean values for significant differences between treatments. Where significant differences were detected Tukey-Kramer post-hoc comparison tests were used to evaluate between which treatments the differences occurred.

4.4 Results

4.4.1. Seawater temperatures

There were no significant differences in the mean ambient temperatures recorded between the cages suspended at a depth of 6 m below a mussel longline (13.07 ^{o}C ± 1.54), and the cages that were sub surface buoyed and suspended 6 m above the seafloor and 6 m below the water surface at mid tide (13.09^{o}C ± 1.54) (ANOVA: F = 1.22, *df* = 1, 3174, *P* = 0.269). Ambient seawater temperatures decreased during the trial reflecting the seasonal changes during this period (Fig. 4.3).

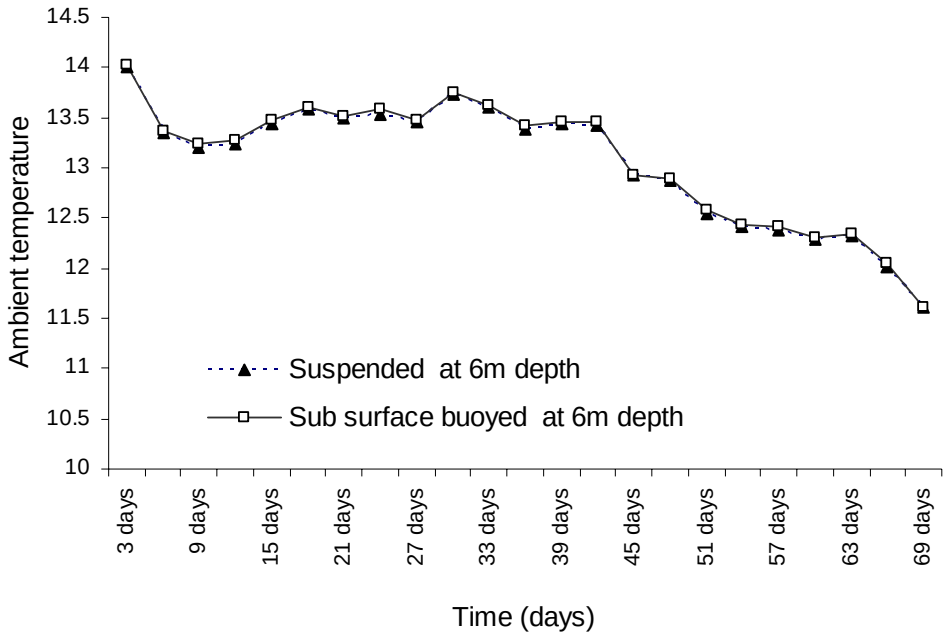

Figure 4.3 Mean daily ambient seawater temperatures (^{o}C) recorded every three days during the 10-week sea urchin roe enhancement trial by temperature loggers suspended at 6 m depth from the mussel longline and attached to a line below a sub surface buoy at a depth of 6 m alongside the mussel longline.

4.4.2. Water Movement

Plaster cubes suspended from a surface longline (exposed to wave disturbance and not caged) at a depth of 6 m had significantly higher dry weight loss than those that were suspended above a stationery seafloor longline at a similar depth. These in turn had significantly greater weight loss than the plaster cubes placed in still water for 7 days (ANOVA: F = 362.9, *df* = 2, 16, *P* < 0.001).

The plaster cubes placed in the cages suspended from a surface longline at a depth of 6 m for 7 days had a significantly greater weight loss than those placed in cages that were suspended above a seafloor longline at a similar depth (ANOVA: F = 33.9, *df* = 3, 32, *P* < 0.001) (Fig. 4.4). A Tukey-Kramer post hoc test showed that there was no significant difference between the weight loss of the plaster cubes in the two suspended treatments ('wave disturbance only' and 'wave and feed disturbance'), or between the two sub surface buoyed treatments ('no disturbance' and 'feed disturbance only').

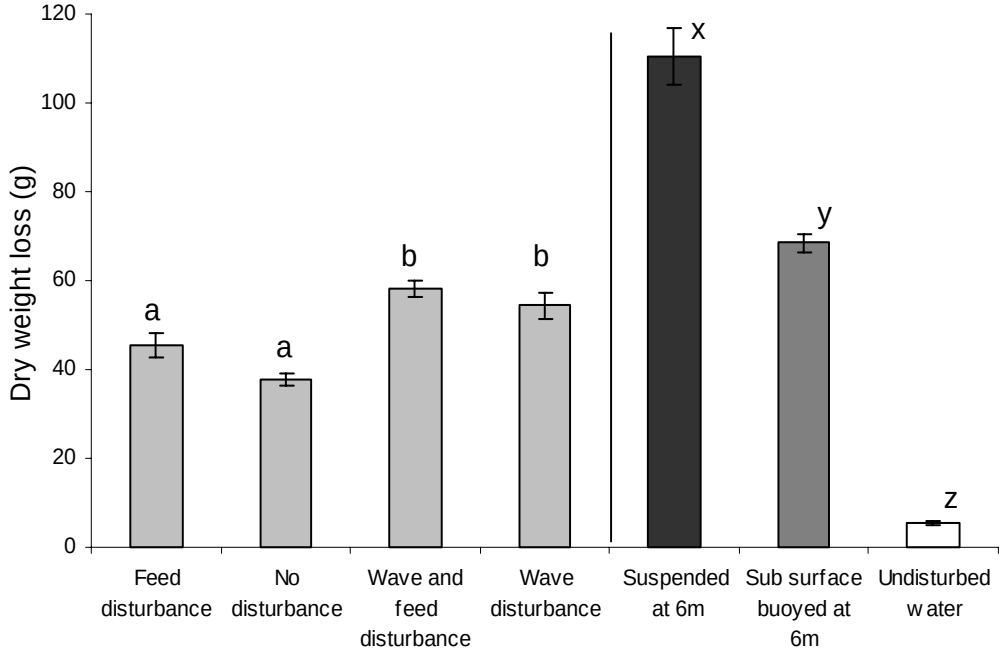

Figure 4.4 The dry weight loss (± 1 SE) over 7 days of plaster cubes placed in the experimental cages exposed to the four experimental treatments (grey bars) (*n* = 8 per treatment), suspended from the longline at a depth of 6m (black bar) (*n* = 8), suspended 2 m above the sea floor and 6 m below the water surface (dark grey bar) (*n* = 8), and placed in a bucket of undisturbed seawater (white bar) (*n* = 8). The vertical black line separates the plaster cubes situated inside and outside the experimental cages (*n* = 4).

4.4.3. Urchin reproductive stage

The histological samples from the initial wild urchin sample (n = 15) showed that 25% were male and 75% were female and that all of the urchins sampled were in the 'spent' stage, where the ovary contains unspawned relict ova and the testis has thin ascinal walls largely devoid of contents (Byrne, 1990).

The histological samples from the urchins held in each of the experimental treatments for 10 weeks (n = 15 for each treatment) showed that 60% were male and 40% were female (except the 'no disturbance' treatment which had 54% male and 46% female). All of the female urchins sampled were in the 'recovery' stage, where the ovary contains primary oocytes and clusters of early oocytes along the acinal wall surrounded by a meshwork of nutritive phagocytes. All of the males sampled were also in the 'recovery' stage where the ascinal walls of the testis are lined with a thin layer of spermatogonia and primary spermatocytes, and the ascinus is full with a meshwork of nutritive phagocytes (Byrne, 1990). The exceptions were urchins held in the 'no disturbance' treatment where 33% of the urchins held in this treatment remained in the previous 'spent' stage at the conclusion of the experiment.

The histological samples from the final wild urchin sample (n = 15) showed that 60% were male and 40% were female. Both the males and females respectively were at the 'growing' stage where the ovary contains primary oocytes that are increasing in size but are still attached to the ascinal wall. The testis has a thickening layer of spermatogonia and primary spermatocytes on the ascinal wall with columns of spermatocytes projecting centrally. The males and females at this stage are becoming increasingly packed with

nutritive phagocytes (Byrne, 1990). The exceptions were 2 males that were still in the previous stage.

4.4.4 Urchin survival

Mortality occurred predominantly in the first 3 weeks of the trial. After week 3, mortalities were uncommon and overall survival during the experiment was high amongst all treatments after 10 weeks (Wave disturbance only = 99.46% ± 0.75; Wave and feed disturbance = 98.98% ± 1.41; Feed disturbance only = 99.82% ± 0.25; No disturbance = 99.82% ± 0.25; means ± 1 S.E.). There were no significant differences in survival between the experimental treatments (ANOVA: F = 0.55, *df* = 3, 16, *P* = 0.65).

4.4.5 Urchin test diameter and wet weight

There were no significant differences between the average test diameter (ANOVA: F = 0.38, *df* = 5, 24, *P* = 0.85) or total wet weight (ANOVA: F = 0.61, *df* = 5, 24, *P* = 0.69) of the urchins in the initial sample (average test diameter = 83.25 ± 0.96 mm; wet weight = 240.7 ± 7.7 g), the experimental treatments after 10 weeks (average test diameter = 82.04 ± 0.44 mm; wet weight = 240.3 ± 3.3 g), or the urchins in the final wild sample (average test diameter = 82.16 ± 0.93 mm; wet weight = 245.5 ± 6.6 g).

4.4.6 Urchin Gonad Index

The mean gonad index (GI) of the final wild urchin sample was significantly larger than that of the initial wild urchin sample (Student *t*-test: $T = 2.42$, $P < 0.05$). The GI values from the urchins held in each of the experimental treatments after 7 (ANOVA: F = 27.13, $df = 4, 20$, $P < 0.001$) and 10 weeks (ANOVA: F = 63.48, $df = 5, 24$, $P < 0.001$) were in turn significantly larger than the GI of the initial and final wild urchin samples (Fig. 4.5) After 7 weeks the 'Wave and feed disturbed' and 'Wave disturbed' treatments had significantly larger GI values than the 'No disturbance' treatment (ANOVA: F = 4.56, $df = 3, 16$, $P = 0.02$). There was no significant difference between the GI of the 'Feed disturbed' and any other treatment. After 10 weeks the 'Wave and feed disturbed' and 'Wave disturbed' treatments had significantly larger GIs than the 'No disturbance' and 'Feed disturbance' treatments (ANOVA: F = 12.86, $df = 3, 16$, $P < 0.001$). A Tukey-Kramer post hoc test showed no differences within the suspended ('no disturbance' and 'feed disturbance') treatments, or within the sub surface buoyed ('wave disturbance' and 'feed and wave disturbance') treatments. The GI values for urchins held in each of the experimental treatments were significantly larger at 10 weeks than at 7 weeks (Student *t*-test: $T = 2.35$, $P < 0.05$ for each treatment) (Fig. 4.5). There was a significant correlation ($r^2 = 0.53$) (ANOVA: F = 16.08, $df = 1, 16$, $P = 0.001$) between urchin GI (regardless of treatment) and weight loss in the plaster cubes (resulting from increased water movement) in urchins sampled at the conclusion of the trial (Fig. 4.6).

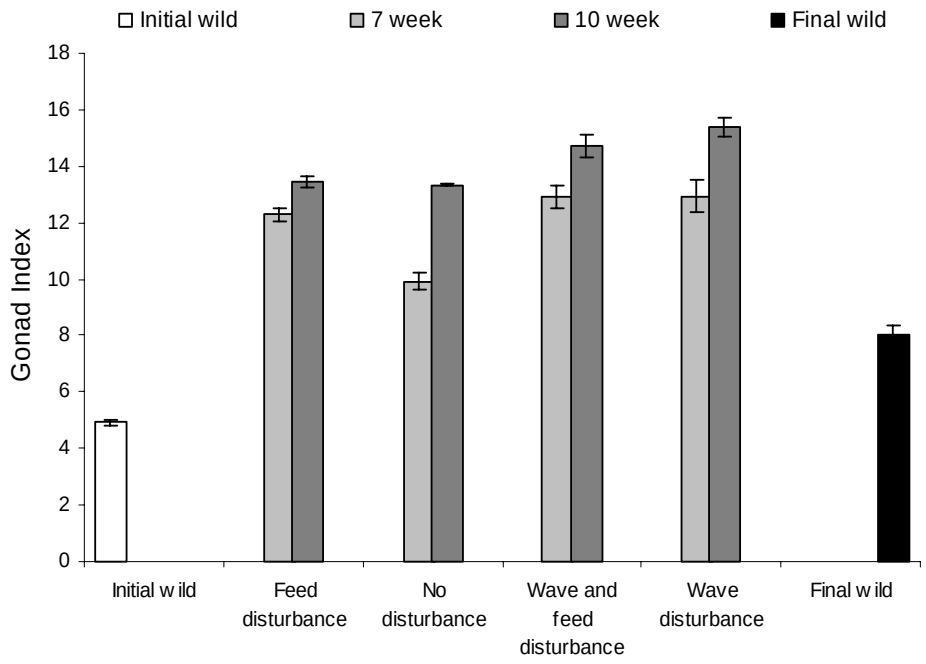

Figure 4.5 The mean gonad index (± 1 S.E.) of the initial wild sample, urchins exposed to the experimental treatments for 7 and 10 weeks, and the final wild sample (*n* = 4).

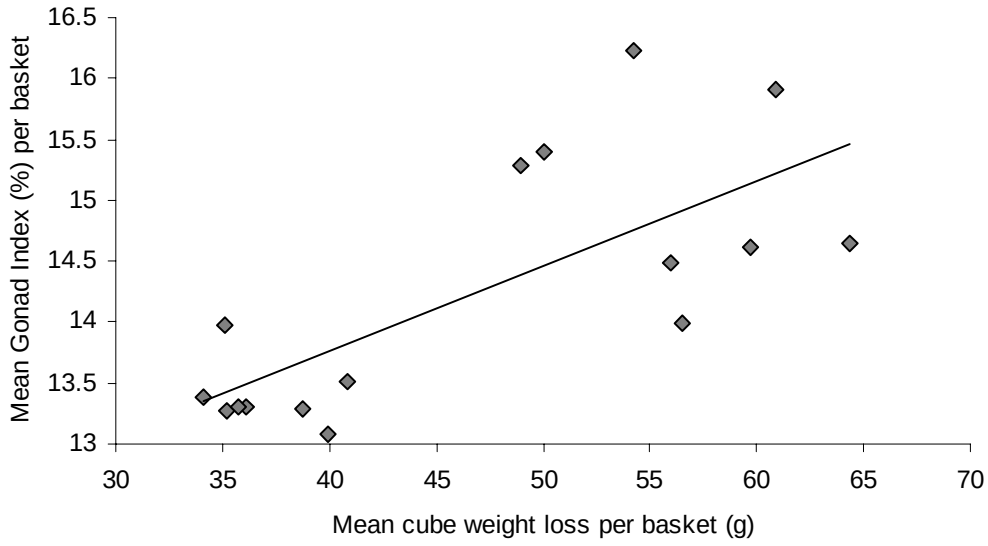

Figure 4.6 The relationship between the GI of the urchins exposed to the one of the four experimental treatments for 10 weeks and weight loss in the plaster cubes situated in the cages for a 7-day period during the experiment $Y = 0.0699X + 10.968$; $r^2 = 0.535$; $n = 16$.

4.4.7 Urchin gonad colour

The lightness reading of the final wild sample was significantly lower than the reading for the gonads from urchins exposed to the 'wave and feed disturbance' treatment (ANOVA: F = 3.36, *df* = 5, 24, *P* < 0.025). A Tukey Kramer post hoc test showed no other differences in the lightness (*L**) of the gonads during the experiment, i.e. between the readings from the initial wild sample and the 10 week samples (Fig. 4.7a).

The redness (*a**) of the initial wild sample was significantly higher than in all the experimental treatments, and the redness of the final wild sample was significantly higher than in the initial sample (ANOVA: F = 21.06, *df* = 5, 24, *P* < 0.001) (Fig. 4.7b). There were no significant differences between the experimental treatments.

The final wild sample had a significantly lower yellowness (ANOVA: F = 6.27, *df* = 5, 24, *P* < 0.002) than the initial wild sample and the experimental treatments (Fig. 4.7c).

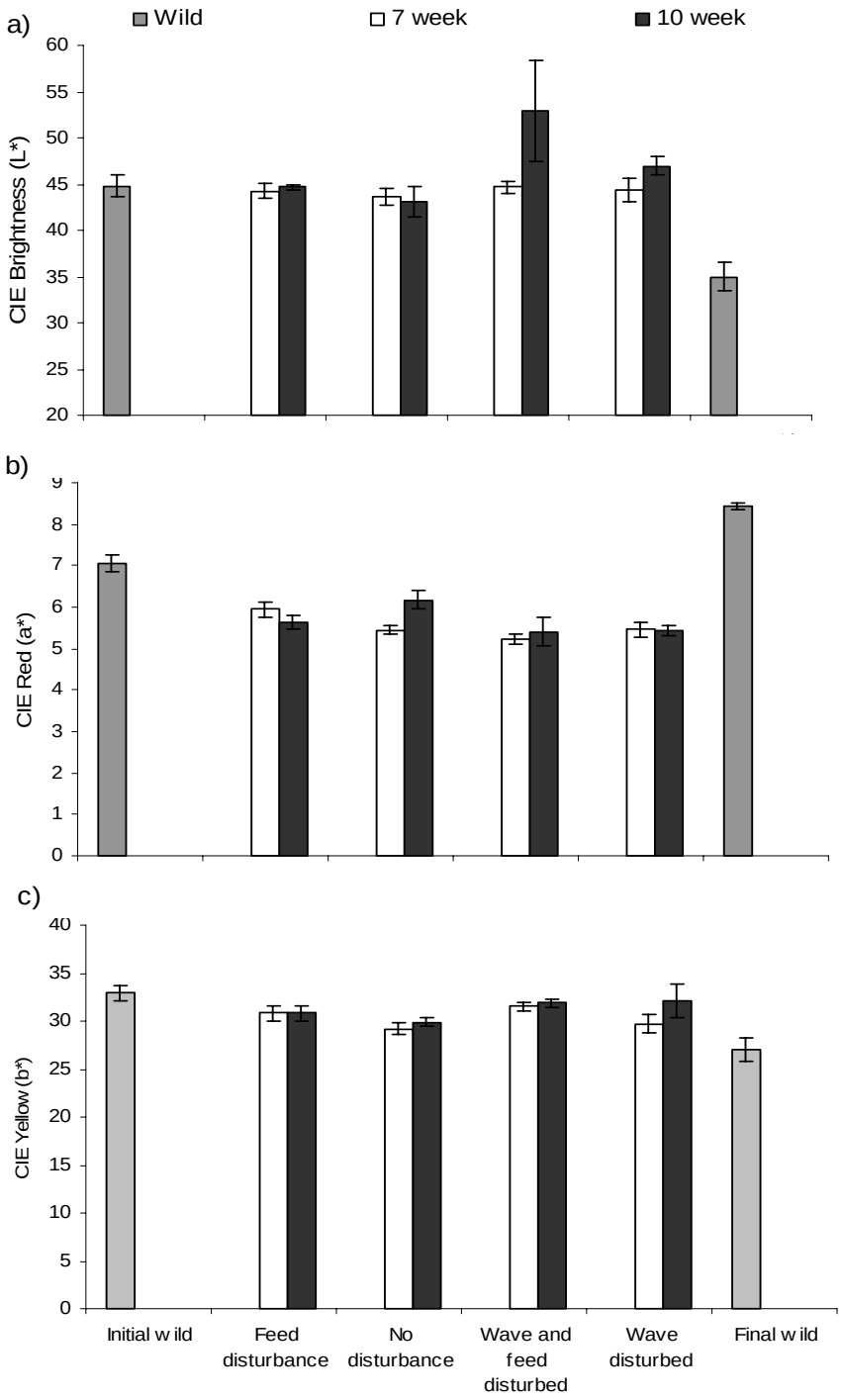

Figure 4.7 Mean gonad colour (± 1 SE) of urchins in the initial and final wild samples and exposed to the four treatments for 7 and 10 weeks, a) lightness (*L), b) redness (*a**) and c) yellowness (*b**).**

4.5 Discussion

4.5.1 Wave disturbance effects

Water movement, measured by the reduction in size of plaster cubes after a 7-day period, was significantly lower within the sea-cages when compared to the water flow outside the cages, and there were significant differences between the suspended and sub surface buoyed cages in the amount of water movement experienced within the cages. Significantly higher water movement in the suspended cages is likely to result from vertical movement of the cages caused by wave action at the water surface. Hickman et al. (1999) observed this phenomenon in a previous study conducted on the New Zealand dredge oyster *Tiostrea lutaria*, where wave action was used to increase water movement in suspended cages. In the current experiment, water movement appears to have significantly affected GI, with urchins held in sea-cages suspended from the surface and exposed to greater water movement, having a significantly higher GI after 10 weeks than those that were sub surface buoyed at a similar depth. This is supported by the correlation between increasing urchin GI and increased weight loss in the plaster cubes. There is anecdotal evidence from fishers that in New Zealand urchins prefer areas of high current and subsequent increases in the water movement past urchins. Urchins exposed to high current with a generous food supply have higher GIs and better gonad colour than urchins found in areas of low current (P. Herbert, Sea Urchin New Zealand, pers comm.). Barker (2007) notes that adult *E. chloroticus* are normally found in areas with moderate currents and wave action, but there is little other mention in the literature to a preference for a moderate to high current environment, or, that the gonad condition is better in areas of

higher current. The results from this study indicate that water movement has a significant effect on increasing the GI of urchins held in sea-cages. Further research is required to identify optimal water flows through holding cages as too much water movement could be detrimental to the urchins and too quiescent a site could be equally detrimental. However, the latter could be improved by the choice of a cage design that created additional water flow through the cages using the action of tide, wave action, or both. The test epithelium of sea urchins is minimally ciliated and urchins have no mechanism to pump water, or actively move water across their outer surface (Barnes, 1974). Increased water movement may increase the oxygen availability to and the waste removal from the urchins.

There were no significant differences in water movement between the sub surface buoyed treatments that were fed and cleaned underwater and those that were removed from the water for feeding and cleaning. This indicates that the periodic increase in water movement caused by removing the cages from the water was not sufficient to create a positive effect on the urchin GI values.

4.5.2 Feed disturbance effects

Feeding disturbance had no significant effect on urchin GI or colour in this study. Urchins exposed to regular (three times per week) removal from the water and consequent exposure to air for cleaning and feeding had GIs that were not significantly different from those that remained underwater and were fed and cleaned in situ. This result was the same whether the urchins were held in suspended or subsurface buoyed sea-cages. These results have implications for sea-cage design as they show that the

removal of sea-cages from the water for feeding and cleaning, at the rates used in this study, have no detrimental effect on GI, colour or mortality of captive urchins.

4.5.3 Seawater temperature

The gradual decrease in ambient seawater temperature experienced during the experiment is a reflection of natural seasonal changes between autumn and winter. In this trial there were no differences between the water temperatures experienced in the two experimental treatments and this indicates that differences in GI and gonad colour between experimental treatments were not related to differences in water temperature.

4.5.4 Gonad colour

The experimental treatments appeared to have very little effect on gonad colour. The only significant difference observed was a reduction in the redness of the experimental urchins compared to the wild samples.

4.5.5 Urchin survival

Previous studies on *E. chloroticus* have shown the species to be robust for holding in captivity. Barker et al. (1998) had survival rates of 95% and 96% in two separate land-based experiments. Fell (2003) recorded between 93.3% and 99.5% survival in sea-cage experiments. James et al. (2004) recorded survival of 81.9-96.0% in a comparative study between land and sea-based holding systems. The urchins used in this trial were transported by boat from the outer Marlborough Sounds directly to Wellington Harbour

(approx. 2 h), where ambient seawater temperatures at the two sites were similar, and the urchins were transferred directly into sea-cages from the transport boat to reduce handling. Minimising thermal shock, transport times and handling resulted in low mortality rates (98.9–99.8% survival) in this trial, and should be taken into consideration in any commercial urchin roe enhancement venture.

4.5.6 Urchin test diameter, wet weight and reproductive stage

No increase in test size or wet weight was observed over the 10-week trial period. However, there was a significant increase in the GI of the urchins in all the experimental treatments, compared to the GI of wild urchins at the beginning of the experiment. The histology results of the urchin gonads at the beginning of the experiment showed that they were in the 'spent' stage following spawning in late summer (Byrne, 1990; Walker and Lesser, 1998; Buisson, 2001). At the conclusion of the trial the experimental urchins were in the 'recovery' stage and those collected from the wild were in the following reproductive stage which is the 'growing' stage. This is despite the GI of the experimental urchins being significantly larger than those of the wild urchins collected at the conclusion of the experiment. The final wild sample was collected 30 days after the experiment ended due to logistical reasons and this is likely to account for the differences in the histological stage between the experimental and wild urchins at the conclusion of the experiment.

4.6 Conclusions

The results of this study indicate that some or moderate wave disturbance can significantly increase the GI of urchins held in sea-cages and the likely cause of this is increased water movement through the sea-cages. Urchins held in sea-cages without wave induced vertical movement experienced lower water flows and had significantly lower GIs. The disturbance effects of removing sea-cages from the water and exposing the urchins to air three times per week did not have any adverse effect on the GI, and little effect on the colour of the gonads of urchins held either in suspended cages or sub surface buoyed cages.

Chapter Five

Long term roe enhancement of *Evechinus chloroticus*

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[Accepted with minor changes in 'Aquaculture' on 19 Oct 2007]

5.1 Abstract

The effects of roe enhancement on gonad growth and colour, survival and reproductive development in *Evechinus chloroticus* were tested in a 27-week experiment. Urchins were collected on 28 July 2006 and held at 14^{o}C and 12:12 h light/dark in groups of 18 urchins (mean test diameter = 92.6 ± 0.5 mm ; mean wet weight = 316.5 ± 5.5 g) per basket and fed *ad libitum* a formulated moist feed. The urchins were sampled at the beginning of the experiment and every three weeks after, for 27 weeks.

The maximum GI value of roe enhanced *E. chloroticus* is likely to be reached within 12 weeks and the most economic roe enhancement period is likely to be 9 weeks in terms of optimising returns for minimal holding costs. There were no effects on survival from long term roe enhancement and changes in lightness of the gonads of urchins held in the trial occurred as a result of the urchins feeding on the artificial diet

rather than being exposed to long term enhancement. The experimental conditions effected the reproductive development of *E. chloroticus* during the experiment. Although the gonads of the experimental urchins progressed through the reproductive stages at a similar rate to those taken from the wild source population at the same times, the former were less advanced after 12 weeks and more advanced after 27 weeks. The 27-week result is possibly due to the early initiation of the photoperiod cue for gametogenesis during the experiment. The experimental results indicate that the cue for gametogenesis occurs in the wild population in mid September at the latest. The results from this study support the hypothesis that food availability is the strongest driver of gonad enhancement (increase in gonad size) in *E. chloroticus* but that other environmental factors have an influence on both gonad size and the gametogenic cycle of this species.

Keywords: Evechinus chloroticus, Long-term enhancement.

5.2 Introduction

In New Zealand there is substantial interest in roe enhancement of *Evechinus chloroticus* (Valenciennes) (commonly known by its Maori name 'kina') to provide consistency of supply and increased returns for the domestic kina roe market. There is also interest in the potential of roe enhancement to produce good quality roe that is suitable for export to lucrative markets such as Japan. A number of short term (10-12 weeks) roe enhancement

experiments have been carried out on this species (Barker et al., 1998; Buisson, 2001; Fell, 2002; James et al., 2004; James, 2006a, 2006b and 2006c).

Similarly, there have been many roe enhancement experiments carried out on a range of other sea urchin species (examples include; Kelly et al., 1998; McBride et al., 1998; Walker and Lesser 1998; Watts et al., 1998; Spirlet et al., 2000; Garrido and Barber, 2001; Pearce and Dagget, 2002; Robinson et al., 2002; Lawrence et al., 2003; Pearce et al., 2004; Shpigel et al., 2004; Siikavuopio et al., 2004a; Siikavuopio et al., 2004a; Siikavuopio et al., 2006). See Table 1.1 for relevant urchin species from each study. Most of these have been run for 10-16 weeks, during which time a range of parameters is measured at the beginning and conclusion of the experiment and occasionally on one or more occasions during the experiment. There are a limited number of published papers describing experiments where urchins have been held for longer periods (6-7 months) (Cuthbert and Hooper, 1995; Fernandez and Pergent, 1998; Grosjean et al., 1998; Hagen, 1998; Walker and Lesser, 1998; Kelly, 2001) but only Cuthbert and Hooper (1995), and the latter two, sampled a range of parameters at regular intervals.

There have been no studies investigating the maximum amount of gonad that *E. chloroticus* is capable of producing if enhanced for periods longer than 10-12 weeks, and the long term effects of roe enhancement on gonad development and the gametogenic cycle of this species are both unknown.

There have been a number of studies that have described the seasonality of the reproductive cycle in a variety of sea urchin species from wild populations (Kelly, 2000; Kelly, 2001), including *E. chloroticus* (Walker, 1982; Brewin et al., 2000; Lamare, et al.,

2002). As Kelly (2001) notes, most of these have focused on the collection of individuals from populations over one or more seasons. The synchronicity of the reproductive cycle between wild populations of sea urchin species, combined with monitoring of the environmental conditions that occur naturally during these cycles, has led to the observation that the gametogenic cycle in sea urchins is generally controlled by a range of exogenous factors such as temperature, food availability and photoperiod (Byrne, 1998; Walker and Lesser, 1998; Kelly, 2001). For *E. chloroticus*, the role that these exogenous factors have on the gametogenic cycle is still unclear. Brewin (1994) suggested that photoperiod may be an important cue for regulating gametogenesis in *E. chloroticus* because holding urchins in constant darkness inhibited the onset of gametogenesis. Buisson (2001) held *E. chloroticus* 6 months out-of-phase with natural photoperiods and noted that after three months of manipulation the gonads appeared to be in very similar reproductive condition to wild urchins but with a much higher abundance of nutritive phagocyte cells. Buisson (2001) stated that longer experimental periods were required to evaluate the effects of photoperiod on gametogenesis. James et al. (2007) observed that the gametogenic cycle of *E. chloroticus* held at constant temperature in four roe enhancement experiments conducted over a 12-month period remained relatively synchronous with that of the wild source population. The results from the latter two studies indicate that exogenous factors such as photoperiod and temperature may not be as important as a cue for the gametogenic cycle of *E. chloroticus* as they are for other sea urchin species.

In this experiment, wild *E. chloroticus* were collected and held for 27 weeks in land-based tanks, at constant temperature and photoperiod and fed the NIWA artificial

roe enhancement diet (James et al., 2004). The urchins were sampled at three week intervals throughout the experiment to investigate changes in GI values, survival, gonad colour and reproductive stage that occurred over a 27-week period. The long term experiment provided the opportunity to calculate the optimal roe enhancement period for *E. chloroticus*, and to monitor changes in the gametogenic (or reproductive) cycle while environmental cues (temperature and photoperiod) were held constant.

5.3 Materials and methods

5.3.1 Collection site

Sea urchins were collected from a rocky site at Port Gore in the outer Marlborough Sounds (41^{o}01.173'S 174^{o}16.105'E) in the north of the South Island of New Zealand (Fig. 5.1) on three separate occasions; 28 July 2006, 31 October 2006 and 6 February 2007. The site consisted of a rocky reef with macroalgal species dominant to a depth of approximately 8-10 m and below this the seafloor consisted of bare rock and sandy/gravel. There were very large, bare rocks present throughout the site. The sea urchins at this site were present at depths between 8-10 m.

5.3.2 Urchin collection

Urchins were collected (mean test diameter = 92.6 ± 0.5 mm ; mean wet weight = 316.5 ± 5.5 g) using SCUBA and were placed in mesh bags (approximately 60 urchins per bag) before being hauled onboard the fishing vessel. Once onboard the vessel the urchins were placed in plastic crates, covered with heavy cloth and kept damp with sprayed seawater

for the return journey to Mana Harbour which is a one hour drive from Wellington. The urchins were then transferred by road to the Mahanga Bay Research Facility, Wellington. Collections were made at the beginning of the trial, after 12 weeks and at the conclusion of the 27-week experiment.

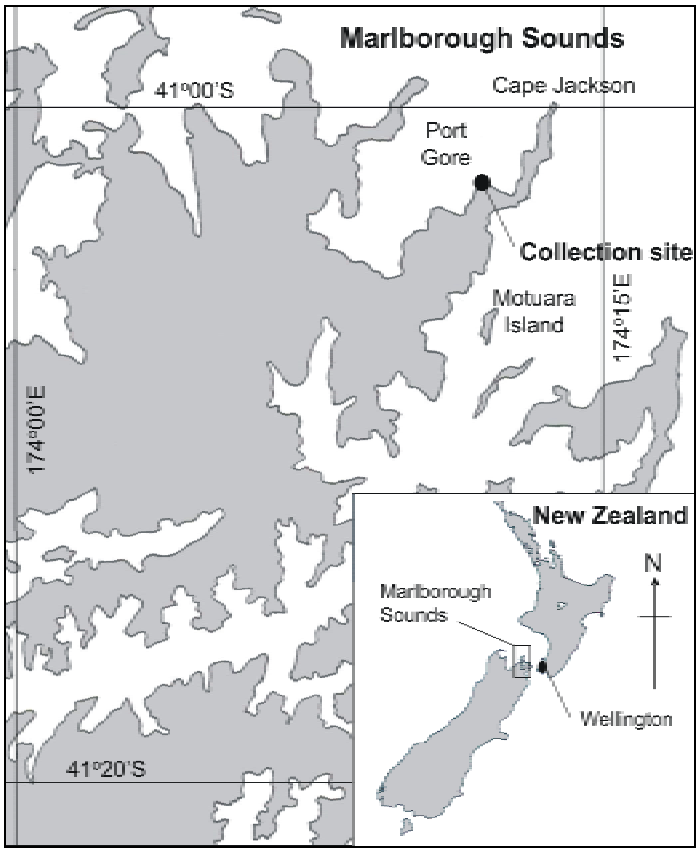

Figure 5.1 The location of the collection site in the Marlborough Sounds at the top of the South Island of New Zealand.

5.3.3 Holding systems

On arrival at Mahanga Bay in Wellington Harbour the urchins were transferred from the mesh bags and randomly allocated to thirty six 570 mm long x 370 mm wide x 230 mm

deep (0.048 m^3) dark blue plastic holding baskets. The bottom and sides of each basket consisted of a series of 10 mm slots to allow water movement through the basket whilst retaining the approximately 20 mm square feed cubes. The urchins were held at a density of 18 urchins per basket (27.9 urchins m^{-2}, or 8.8 kg m^{-2} internal surface area). Each basket was placed in one of 36 black polyethylene tanks (640 mm long x 440 mm wide x 300 mm deep). Each tank was fitted with an airstone that delivered air directly under the middle of the basket. The thirty six tanks were divided into three systems (rows of 12 tanks). Each system had a 225 l reservoir tank and the 12 tanks were supplied with a constant supply of 12.6 l/min, 10μm-filtered seawater from the reservoir tank. The water was returned to the reservoir tank from the tanks outlets. Each of the three systems had 20 l/min of ambient 10-μm filtered seawater supplied to the reservoir tank which equated to an exchange rate of 8.9% per minute. Each system had a 6 kw (heating) reverse cycle heat pump with a stainless steel and polyethylene heat exchanger installed between the reservoir tank and the outlet manifold that regulated the seawater temperature in the system to a constant 14.0^{o}C. The systems utilised Onga 142 1500 w pumps to ensure adequate flow through the heat exchangers (Fig. 5.2).

The experimental area was lit using fluorescent lighting and the ambient light at the water surface was measured using an Odyssey cosine sensor (Dataflow Systems Pty Ltd, Christchurch, New Zealand). The lights were connected to an automatic timer that maintained a 12 h L:12 h D photoperiod throughout the experiment. A stowaway Tidbit™ temperature logger (Onset Computer Corporation, Massachusetts, USA) was placed in one tank from each of the three systems. Each logger recorded the ambient seawater temperature once every hour for the duration of the experiment. Dissolved oxygen, pH,

and ammonia were measured weekly in each experimental tank and in the sump tanks of each system.

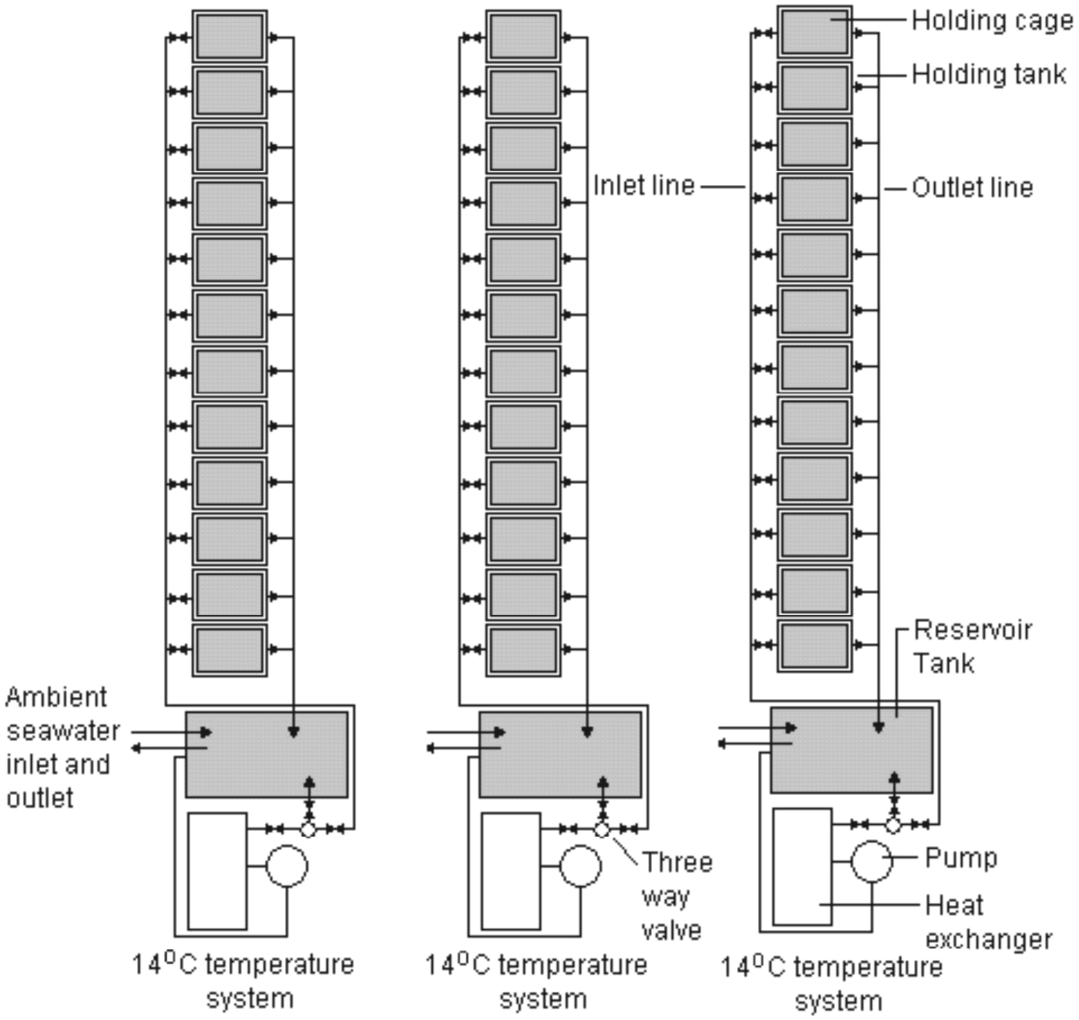

Figure 5.2 A schematic diagram of the holding system used to maintain the water flows and temperatures in the three experimental systems and 36 experimental tanks.

5.3.4 Urchin Husbandry

On their arrival at the laboratory the urchins were held in ambient water temperatures (approximately 12.0^{o}C) and photoperiods (10.5 h /13.5 h light/dark) which were approximately equivalent to those at the collection site in the Marlborough Sounds. The urchins were then acclimated at a rate of 1^{o}C (temperature), and 1 h (photoperiod) per day until all of the replicate baskets had reached the experimental temperature (14^{o}C) and photoperiod (12:12 L/D).

The NIWA artificial sea urchin roe enhancement diet was fed three times per week to all of the urchins throughout the experimental period. Any uneaten food from previous feeds was removed from each basket during feeding but food that was not easily accessible to the cleaner was left in the baskets. The systems were cleaned each week by removing each basket from its tank and placing it in a spare tank containing seawater at the correct temperature (14^{o}C). Then each basket containing the urchins was sprayed clean with seawater of the appropriate temperature and the empty holding tank was cleaned with detergent and chlorine before being rinsed, refilled and the basket placed back into the tank. This was repeated until all remaining tanks in each of the three systems had been cleaned.

5.3.5 Experimental methods

The experiment started on 3 August 2006, with 36 replicate baskets of urchins. An initial census of the condition of the urchins was carried out at the beginning of the experiment and subsequently, every three weeks when four replicate baskets were randomly selected.

In the experiment, nine treatments were established with time being the variable between each treatment (i.e. Treatment One – 'Week 0' to Treatment 9 – 'Week 27').

5.3.6 Data collection

Urchin condition, or gonad index (GI), was recorded at the start of the experiment and every three weeks after that, based on random samples of 15 urchins from each replicate basket (n = 4), and 60 wild urchins from the initial wild population (referred to henceforth as 'wild'). The wild population was also sampled after 12 weeks and at the conclusion of the experiment after 27 weeks. Variables measured for each urchin were test diameter (D, mm), total wet weight (W, g), and gonad wet (unblotted) weight (G, g). These data were used to calculate GI (= 100 × G / W). A measure of increase in gonad index from the previous census (X) for each urchin expressed as the difference between the mean GI at the first census ($\overline{GI}^1$) and at the next census ($\overline{GI}^2$) (i.e., X = ($\overline{GI}^2$) - ($\overline{GI}^1$) in units of kilo of roe / tonne of wet weight urchins).

A graph comparing the test diameter against the gonad index of the wild urchins collected at the beginning of the experiment (Fig 5.3) shows no significant relationship between test diameter gonad index (ANOVA: F = 0.52, df = 1,58, P = 0.110).

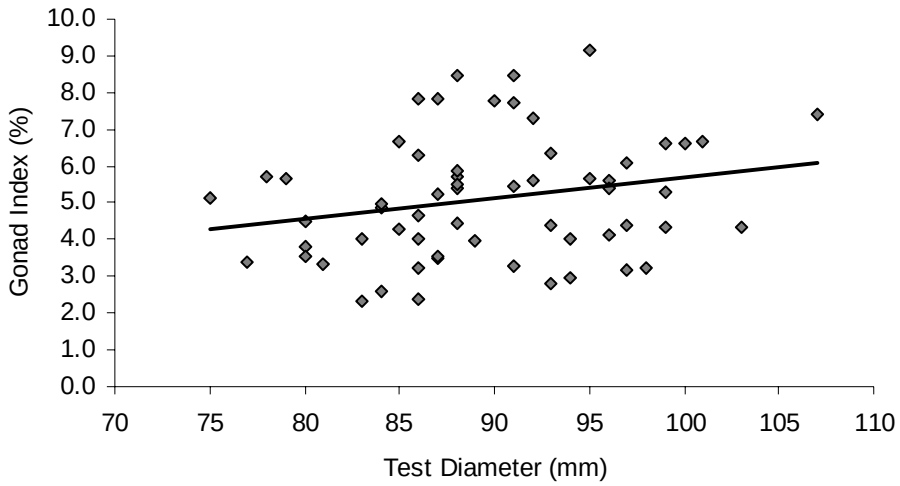

Figure 5.3 The relationship between Gonad Index values and Test Diameter for urchins collected from the initial wild sample, Y = 0.0565 X + 0.0507; r^2 = 0.054; *n* = 60.

The gonad colour and reproductive stage of a random sample of 15 urchin gonads from each treatment, and from the initial and final wild populations of urchins, was recorded. The colour of each urchin's gonads was objectively assessed with a Minolta CM2500D Spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan) (2 pulsed xenon lamps as illumination source, diffuse illumination with 8° viewing area, with spectral component excluded for gonad measurements) with three replicate measurements per sample averaged to give a single measurement, using the international standard CIELAB (or L*a*b) system of colour measurement. The CIELAB system measures the lightness (L*), the degree of redness or greenness (a*), and the degree of yellowness or blueness (b*) (Robinson et al., 2002).

The reproductive stage of the urchin gonads was determined using histological analysis. Histological samples were collected and stained using standard Harris's

haematoxylin stain, and then counterstained with eosin (Bancroft and Stevens, 1990). The reproductive condition of the initial urchin sample was assessed using the six reproductive stages described in Byrne (1990).

5.3.7 Statistical analysis

One-way analysis of variance was used to compare differences in urchin test diameter, wet weight, mortality, GI, increase in GI, and L*, a*, b* colour readings. Student *t* tests were used to compare the test diameter, wet weight, GI and L*, a*, b* colour readings of the wild and experimental samples at weeks 12 and 27. The wet weight data and b* were $\log^2$ transformed, the GI data square root transformed and the survival data arcsine square root transformed prior to analysis. A Modified-Levene test was used to test for homogeneity of variances. Where significant differences were detected, Tukey-Kramer post-hoc comparison tests were used to identify treatments which varied significantly from each other. A Chi-square test of homogeneity was used to test for differences between the reproductive stages of male and female urchins from each three week sample, urchins from each of the three week sample compared with the following three week sample (e.g. week 0 and week 3) and to test between wild and experimental urchins at 12 and 27 weeks. A Kruskal Wallis non parametric test was used to test for differences between the reproductive stages of urchins from all of the three week samples and a Bonnferroni multiple comparison test was used to identify between which samples the differences occurred. All statistical analyses were conducted using NCSS 2000 (Number Crunching Statistical Systems, Kaysville, Utah, USA). Errors and confidence intervals are expressed as ± one standard error.

5.4 Results

5.4.1 Ambient and experimental seawater temperatures, water quality and light level

Ambient seawater temperatures were measured at the urchin collection site in Port Gore for two years prior to the experiment during the experimental period (August to February) (Fig. 5.4). The experiment began in winter and ran for 27 weeks, during which time ambient seawater temperatures at the collection site slowly increased (Fig. 5.4). The mean seawater temperature at the collection site in 2004-6 was 13.7^{o}C (maximum temperature = 16.8^{o}C; minimum temperature = 10.9^{o}C) and for the urchins held in the experiment at constant temperature it was 14.1^{o}C (maximum temperature = 15.6^{o}C; minimum temperature = 13.1^{o}C) (see Table 5.1 for values for each individual holding system).

The seawater quality (dissolved oxygen, ammonia and pH) remained comparable to the parameters kina would experience in the wild and within the acceptable limits (as defined by Siikavuopio, 2004a and 2004b; 2007a and 2007b for *Strongylocentrotus droebachiensis*) during the trial (Table 5.1) with no apparent effect over time as the density of urchins in the experimental systems decreased. The light level recorded at the water surface of the holding tanks was (1.26 ± 0.05) µmol photons m^{-2} s^{-1}.

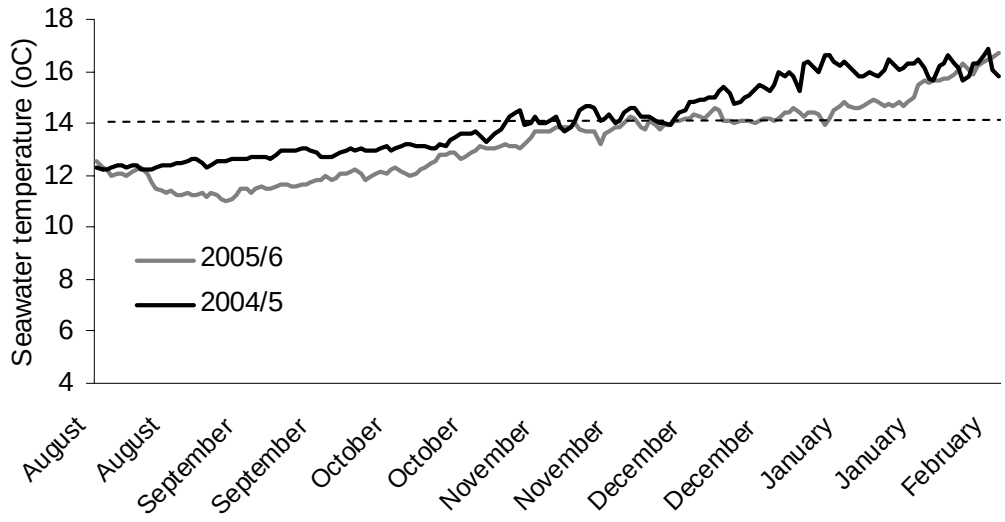

Figure 5.4 The ambient wild temperatures (^{o}C) recorded at the collection site for the wild population during the experimental period in 2004/5 and 2005/6. The dashed line shows the constant temperature (14^{o}C) the urchins were exposed to during the experimental period (see Table 5.1 for mean experimental temperature and standard error).

Table 5.1 The mean (± 1 SE) dissolved oxygen, pH, total ammonia (given as Total Available Nitrogen) levels (*n* = 27) and seawater temperature (*n* = 4536) recorded from each of the three systems during the 27 week experiment.

System	Percent dissolved Oxygen	pH levels	Dissolved ammonia (mg/l N)	Temperature (^{o}C)
System 1	93.4 (± 0.4)	8.24 (± 0.14)	0.35 (± 0.06)	14.09 (± < 0.01)
System 2	93.8 (± 0.4)	8.29 (± 0.09)	0.23 (± 0.03)	14.03 (± < 0.01)
System 3	94.2 (± 0.3)	8.28 (± 0.02)	0.23 (± 0.03)	14.23 (± < 0.01)

5.4.2 Urchin reproductive stage

5.4.2.1 Weeks 0-27 for experimental urchins

At the beginning of the experiment the male and female urchins were mostly at the growing stage. Over the following 27 weeks they progressed slowly and systematically through the reproductive stages until at 27 weeks the males were mostly at the spent stage, with some at the partially spent, and a few at the recovery stage, whilst the females were at the spent and recovery stages (Fig. 5.5). The female urchins were slightly ahead of the males in weeks 0-6 but there was little difference between the reproductive stages of the male and female urchins in weeks 9 and 12. By weeks 15 and 18 the females progressed slightly faster than males but there was little difference in weeks 21 and 24. At week 27 the female urchins had progressed further than the males (Fig. 5.5). Chi square analysis showed there were significant differences between the reproductive stages of males and females in all of the samples (n = 15) ($P < 0.01$) except for week 9 ($P = 0.88$), week 12 ($P = 0.14$) and week 24 ($P = 1.00$). The analysis also showed there were significant differences ($P < 0.01$ in all cases) between the reproductive stage of urchins (males and females combined) from each sample and the following three week sample (e.g. between weeks 0 and 3), meaning that the reproductive stage of the experimental urchins changed significantly every three weeks. A Kruskal-Wallis test showed there were significant differences between the reproductive stage of the urchins (df = 9, H = 134.4, $P < 0.001$) from the urchins sampled between weeks 0 and 27. A Bonnferroni multiple comparison test ($P < 0.05$) showed the reproductive stage of the urchins at weeks 0, 3, 6 and 9 were significantly different to those at weeks 12 and 15, which in turn were significantly different to those at weeks 18, 21, 24 and 27.

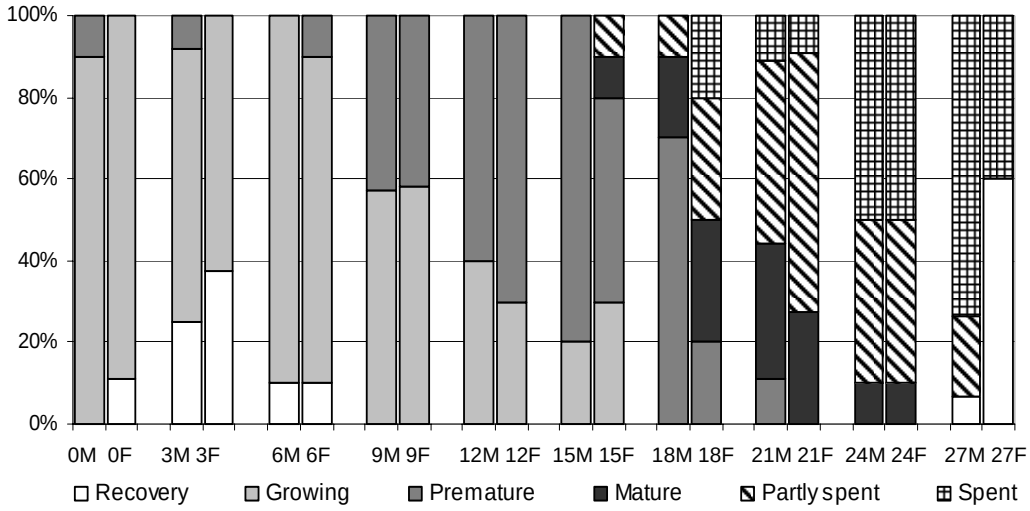

Figure 5.5 The proportion of individuals at the different reproductive stages (recovery to spent) for male (M) and female (F) urchins sampled at the initial census (Week 0), and every consecutive 3 weeks for 27 weeks (Week 3-27).

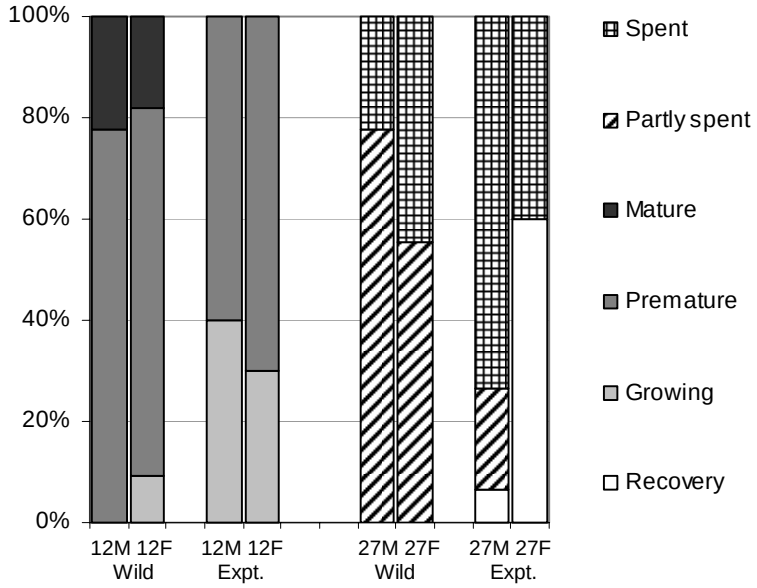

Figure 5.6 The proportion of individuals at the different reproductive stages (recovery to spent) for male (M) and female (F) urchins sampled at Week 12 (12) and Week 27 (27) from the wild population (Wild) and the experimental tanks (Expt.).

5.4.2.2 Comparison between experimental and wild urchins at weeks 12 and 27

A comparison of the reproductive stages of the wild and experimental urchins (males and females combined) at both 12 and 27 weeks showed that wild urchins were significantly more advanced than the experimental urchins ($P < 0.01$) at 12 weeks, but at week 27 the experimental urchins were significantly more advanced ($P < 0.01$) than the wild urchins (Fig. 5.6).

5.4.3 Urchin survival

Mean survival during the experiment was 88.0 ± 1.1% and ranged from 80.0-100.0% (Table 5.2). A one-way ANOVA showed there were significant differences in the survival of the urchins over time (One-way ANOVA: F = 2.71, df = 8, 36, $P = 0.025$) (n = 486) with the urchins sampled at week 12 having significantly higher survival than those sampled at weeks 15, 18 and 27 weeks (post-hoc Tukey test, $P < 0.05$) (Table 5.2). There were no other significant differences between the survival of urchins over time.

5.4.4 Test diameter and wet weight

A One-way ANOVA comparing the test diameter (One-way ANOVA: F = 6.24, df = 9, 600, $P < 0.001$) and wet weight (One-way ANOVA: F = 6.72, df = 9, 600, $P < 0.001$) of urchins collected from the wild population and sampled every three weeks from week 0-27 showed significant differences in test diameter and wet weight between the samples. The initial urchin test diameter was significantly smaller than from all of the experimental samples, except for those sampled at 18 and 24 weeks (post-hoc Tukey test, $P < 0.05$)

(Table 5.2). Excluding the initial sample, there were no significant differences in test diameter of urchins from the experimental samples except for those sampled at weeks 6 and 15 being significantly larger than those sampled at weeks 18 and 24 (post-hoc Tukey test, $P < 0.05$). The wet weight of the initial sample was significantly smaller than for the urchins sampled at weeks 3, 6, 9 and 15 weeks, and those sampled at week 24 were also significantly smaller than at weeks 3, 6 and 15. The urchins sampled at week 6 had a significantly higher wet weight than all other samples except for weeks 3, 9 and 15.

There were no significant differences in the test diameter and wet weight of urchins sampled from the source wild population at 12 and 27 weeks and those taken from the source population and held in the experiment for 12 and 27 weeks (Student t-test, $P < 0.05$ in all cases).

A one-way ANOVA showed significant differences in test diameter between the three wild samples at Weeks 0, 12 and 27 (One-way ANOVA: F = 9.93, df = 2, 180, $P < 0.001$), with urchins collected at 0 week having a significantly smaller test diameter than those collected at weeks 12 and 27; there were no difference between the latter (post-hoc Tukey test, $P < 0.05$) (Table 5.2). A one-way analysis showed significant differences in wet weight between the three wild samples (Weeks 0, 12 and 27) (One-way ANOVA: F = 9.93, df = 2, 180, $P < 0.05$), with urchins collected at week 12 being significantly heavier than those collected at week 0 (post-hoc Tukey test, $P < 0.05$) (Table 5.2).

Table 5.2 The mean (± 1 SE) percent survival, urchin test diameter (mm), wet weight (g), gonad weight (g) and percentage increase in gonad index from the previous census of urchins collected from the wild (0, 12 and 27 weeks) and held for varying periods (3-27 weeks) and fed the NIWA roe enhancement diet.

Week	% Survival	Test diameter (mm)	Wet weight (g)	Gonad index (%)	% increase in gonad index	Lightness (L*)	Redness (a*)	Yellowness (b*)
0 (wild)	-	89.7 (± 0.9)	294.9 (± 9.2)	5.1 (± 0.2)	-	39.7 (± 1.3)	13.1 (± 0.4)	26.0 (± 0.8)
3	86.7 (± 2.7)	94.0 (± 0.8)	344.8 (± 7.9)	7.1 (± 0.3)	39.4 (± 6.4)	39.3 (± 1.5)	12.4 (± 0.3)	29.3 (± 0.9)
6	88.3 (± 3.2)	96.3 (± 0.9)	367.1 (± 10.0)	9.8 (± 0.4)	37.5 (± 5.2)	40.9 (± 1.7)	12.7 (± 0.5)	27.9 (± 1.4)
9	88.3 (± 1.7)	93.8 (± 0.7)	330.8 (± 7.7)	11.8 (± 0.4)	20.0 (± 4.5)	39.2 (± 1.8)	12.0 (± 0.6)	26.9 (± 1.5)
12	96.7 (± 3.3)	94.0 (± 0.7)	327.7 (± 7.7)	14.1 (± 0.4)	19.9 (± 3.2)	48.8 (± 1.5)	11.9 (± 0.4)	29.4 (± 1.3)
15	85.0 (± 3.2)	96.1 (± 0.8)	348.4 (± 8.5)	13.3 (± 0.4)	-5.8 (± 2.9)	60.2 (± 2.4)	11.5 (± 0.8)	39.1 (± 1.9)
15 (wild)	-	95.1 (± 1.0)	338.4 (± 11.3)	9.0 (± 0.7)	-	45.3 (± 1.2)	13.3 (± 0.5)	31.8 (± 1.9)
18	85.0 (± 1.7)	92.5 (± 0.9)	329.2 (± 9.2)	13.9 (± 0.6)	4.3 (± 4.2)	46.6 (± 1.8)	12.1 (± 0.4)	26.3 (± 1.3)
21	88.3 (± 4.2)	93.9 (± 0.6)	322.6 (± 6.6)	13.8 (± 0.5)	-0.5 (± 3.6)	44.2 (± 1.8)	11.0 (± 0.5)	28.5 (± 1.8)
24	86.7 (± 2.7)	91.7 (± 0.7)	308.3 (± 7.4)	14.7 (± 0.5)	6.2 (± 3.9)	48.8 (± 1.3)	12.3 (± 0.5)	26.0 (± 1.1)
27	83.3 (± 3.3)	93.3 (± 0.7)	314.0 (± 6.7)	14.0 (± 0.5)	-3.9 (± 3.7)	47.4 (± 1.3)	12.3 (± 0.4)	24.6 (± 1.3)
27 (wild)	-	93.0 (± 0.8)	316.0 (± 6.9)	5.0 (± 0.2)	-	40.0 (± 2.0)	12.3 (± 0.6)	23.5 (± 1.7)

5.4.5 Gonad Index

A one-way ANOVA showed that there were significant differences in the GI of the experimental urchins sampled at 0-27 weeks (One-way ANOVA: F = 67.87, *df* = 9, 600, *P* < 0.001). There was a significant increase in GI between the urchins measured at weeks 0 and 3, weeks 3 and 6, weeks 6 and 9 and weeks 9 and 12 (post-hoc Tukey test, *P* < 0.05) (Table 5.2, Fig. 5.7). There were no significant differences in GI between urchins sampled at week 9 and week 15, and no differences between urchins sampled at weeks 12-27 (post-hoc Tukey test, *P* > 0.05) (Table 5.2, Fig. 5.7).

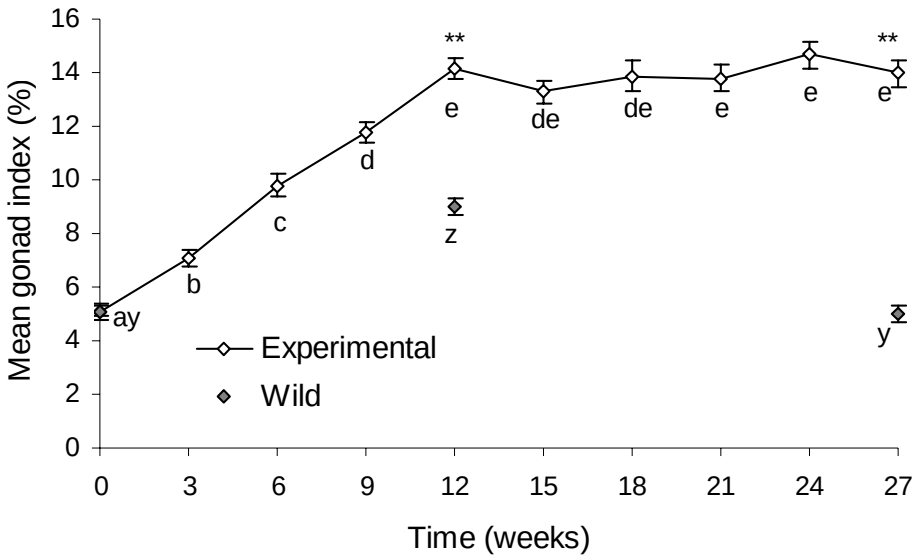

Figure 5.7 The mean gonad index (±1 SE) of wild urchins collected from Port Gore on three occasions and experimental urchins collected with the first wild collection on 3 August 2006 and sampled every three weeks for 27 weeks. Means which did not differ significantly (*P* > 0.05) within each series, according to post-hoc Tukey test are labelled with common letters (a-e for experimental and y-z for wild urchins). Differences between wild and experimental urchins sampled at the same time are denoted as ** (*P* < 0.05) and no asterix indicate no significant difference between the two.

5.4.7 Gonad colour

A one-way ANOVA showed significant differences in the lightness (L*) of the urchin gonads over time (ANOVA: F = 15.05, *df* = 9, 197, $P < 0.001$). Urchins sampled at weeks 0, 3, 6 and 9 had significantly lighter coloured gonads than those sampled in subsequent weeks (post-hoc Tukey test, $P < 0.05$). The gonads of urchins sampled in week 15 were significantly lighter coloured than in any other samples (post-hoc Tukey test, $P < 0.05$). One-way ANOVA analysis showed no significant differences in the redness (a*) of the urchin gonads over time (ANOVA: F = 1.53, *df* = 9, 197, $P = 0.142$). One-way ANOVA analysis showed significant differences in the yellowness (b*) of the urchin gonads over time (ANOVA: F = 8.69, *df* = 9, 197, $P < 0.001$). Post-hoc Tukey test ($P < 0.05$) showed that urchins from week 15 had a significantly higher yellowness value than did all other samples and that there were no differences between the values for the urchins taken from any other week (Table 5.2).

5.5 Discussion

The current study presents information on the long term effects of roe enhancement of *E. chloroticus*. Previous studies on this species have all focused on 7-12 week roe enhancement trials. In this study the maximum GI of 14.7% (equates approximately to 45.9g of roe) occurred at week 24, but there were no significant increases, or decreases, in the GI of the urchins sampled from weeks 12 to week 27. This indicates that 14.7% is the maximum GI that urchins collected from this population at this time year are capable of producing when held at a constant 14^{o}C and 12 h:12 h light/dark photoperiod. In another experiment (unpublished data) the effects of photoperiod and

temperature on roe enhancement of *E. chloroticus* collected from the same population a year earlier were recorded. A final GI of 16.7% was recorded in the experiment that was conducted at the same time of year, with urchins held at the same temperature and photoperiod for 10 weeks, but with a higher initial GI (initial GI = 8.4%). The urchins in the current experiment had an initial GI of 5.1%. The increase in GI between these two roe enhancement experiments is almost the same (an increase in GI of 8.3% and 9.0% respectively). This shows that the final GI values of urchins collected from a particular population and held for roe enhancement can vary between years. However, the variation is most likely to be due to variation in the initial condition of the urchins and the amount of increase is likely to be similar. Regardless of the actual final GI value, the current study shows that the maximum GI is likely to be reached within the first 9-12 weeks of roe enhancement of wild caught urchins and will not change significantly if roe enhancement is continued for longer periods. In terms of the economic viability of roe enhancement of *E. chloroticus*, a nine week roe enhancement period would provide the greatest return for the least cost in terms of feed and holding costs.

The results from the current study indicate that there is little effect on urchin survival from prolonged roe enhancement. This is a similar result to that of Cuthbert and Hooper (1995) where *S. droebachiensis* was held for 25 weeks with no significant differences in mortality in the urchins held for varying lengths of time.

There were significant differences in terms of lightness and minor differences in terms of the redness (a*) or yellowness (b*) of the gonads as a result of roe enhancement. The latter two did not vary significantly with prolonged enhancement (other than in week 15) but the lightness (L*) of the roe increased significantly after 9 weeks of enhancement and remained higher until the conclusion of the experiment

(week 27). The reproductive stage of the urchins was at the transition stage between the growing and recovery stages after 9 weeks and it is possible that this could have influenced the lightness of the roe in the current experiment. However, this is unlikely as the current study shows that the other changes in the reproductive cycle of the urchins over the 27-week study had no influence on roe colour, despite the urchins going through the spawning stages during the experimental period. Robinson et al. (2002) showed that it is possible for artificial diets to significantly improve the colour of urchin roe (Robinson et al., 2002). James et al. (2004) tested the efficacy of three artificial diets for roe enhancement of *E. chloroticus* and showed that urchins fed the artificial diet used in the current experiment provided the most suitably coloured roe. The changes in colour (lighter gonads) that occurred in the first 9 weeks of this experiment, as well as in the previous study on *E. chloroticus* (James et al., 2004), suggest that the increasing lightness value of the gonads is likely to be due to the artificial diet used in the study and not long term enhancement. This is supported by the fact that further changes in gonad colour did not occur with roe enhancement for periods longer than 9 weeks. This result indicates that changes in the reproductive cycle are unlikely to effect roe colour regardless of the time of year that roe enhancement is undertaken.

Kelly (2001) investigated whether *Psammechinus miliaris* is a 'lengthening day' species, that is, it requires increasing photoperiods and temperatures to stimulate the onset of gametogenesis. The study held urchins in two photoperiods, ambient (or lengthening days) and fixed 7-h light (equivalent to the shortest day this species is exposed to in the wild), and two temperatures, ambient or never below 9^{o}C. The results showed that lengthening days was an important cue in both males and females of the species and that low temperatures were an important cue for completion of

vitellogenesis. Kelly (2001) notes that the urchins were held on a maintenance diet of macro-algae which provided adequate nutrition to complete gametogenesis but is unlikely to have been sufficient to promote proliferation of the nutritive phagocytes in the gonad. On a more nutritious diet *Psammechinus miliaris* would have had higher GI, partially masking some of the normal seasonal changes in gonad biomass, as has occurred in the current experiment. In the current experiment the urchins were kept at a constant 14^{o}C and 12 h :12 h light/dark photoperiod. Their progress, in terms of their reproductive cycle, was monitored and compared with that of urchins collected from the same wild population as the experimental urchins at the beginning, midway through and at the conclusion of the experiment. The comparison of urchins held in the experimental system for 12 and 27 weeks showed a significant difference (in terms of their reproductive stage) to those taken from the source population at 12 and 27 weeks, respectively. The wild urchins were more advanced at week 12 than the experimental urchins but this result was reversed after 27 weeks with the experimental urchins being more advanced in terms of their reproductive stage. This is despite the experimental urchins having a significantly higher GI value at both 12 and 27 weeks.

These results of the current experiment are difficult to interpret but are possibly due to the increase in both photoperiod and seawater temperature that the urchins were exposed to when they were collected from the wild and placed in the experimental holding system. The urchins were collected at the beginning of August when wild ambient temperatures are approximately 12.0^{o}C and the natural photoperiod is 10.5 h /13.5 h light/dark. They were then transferred into the holding systems with the seawater at a constant 14^{o}C and a photoperiod of 12 h:12 h light/dark. At the collection site the experimental photoperiod would naturally occur in approximately mid September and the experimental seawater temperature in

approximately mid November. The rapid change in seawater temperature (although acclimation of 1^{o}C and 1 h photoperiod per day was used) and photoperiod may have inhibited the gametogenic cycle in the first 12 weeks of the experiment. Towards the end of the experiment all of the urchins (wild and experimental) were at the spawning stage. The experimental urchins were more advanced than the wild urchins and had a percentage of urchins that were post spawned and in the recovery stage. Research from other sea urchin species indicates that the gametogenic trigger is likely to be changes in photoperiod (Walker et al., 1998; Lawrence et al., 2003). Barker (2007) suggested that gametogenesis occurs during mid-late winter and into spring (June to October) in *E. chloroticus.* The results of this study indicate that the gametogenic trigger for *E. chloroticus* is a day-length of 12 h L, or less, and occurs in the wild prior to mid September at this location. This may account for the experimental urchins being more advanced than their wild counterparts at the conclusion of the current experiment. The urchins in the experiment would have been exposed to the gametogenic trigger earlier than those in the wild. However, this does not explain the differences in reproductive stage at week 12.

There were no significant differences in the GI values of the experimental urchins from weeks 12 to 27, despite significant changes in the reproductive stages of the urchins between each 3 week sample from weeks 0 to 27. This is in contrast to a study by Kelly (2001) which found that GI values of juvenile *Psammechinus miliaris* fed an algal diet changed regularly over a 7-month period, gradually increasing over the first 6 months and then decreasing in the final month, coinciding with the gametogenic cycle of the urchins. In the current study the GI value of *E. chloroticus* fed an artificial diet was not affected by the gametogenic cycle, indicating that in this species feed availability is a more important driver of roe enhancement (in terms of

increase in GI value) than the reproductive cycle. This is likely to be a result of the gonads increasing in size due to an increase in the numbers of nutritive phagocyte cells (Barker, 2007; Buisson, 2001) until they reach a maximum size. The gonad size then remained relatively stable despite the urchin's reproductive cycle continuing, even when the urchins underwent a slow-trickle spawning in the experimental tanks. Again, this indicates that as reproductive cells are lost from the gonad they are immediately replaced with nutritive phagocyte cells and the GI value of the urchins remains constant.

There was no corresponding increase in the wet weight of the urchins with the increase in gonad weight. Nor was there a gradual increase in the test diameter of the urchins over the 27-week experimental period. This indicates that the urchins were allocating resources into storage of nutrients in the nutritive phagocyte cells rather than into somatic growth. The variation in wet weight and test diameter that was observed in the current experiment occurred haphazardly over the experimental period and is most likely due to the variations in the size of the initial wild collection and the random allocation of these urchins to the replicate baskets. The lack of increase in wet weight despite significant increases in gonad weight indicates that either the gonads are simply absorbing water in order for their size to increase, or, the density of the gonad is very similar to water and, therefore the total wet weight of the urchin is not increasing despite increases in the actual gonad weight. This is a similar result to a number of previous studies on *E. chloroticus* (James et al., 2004; James, 2006a and 2006b).

In summary, nine weeks appears to be the optimal roe enhancement period (in terms of reduced costs for maximum return) for urchins fed the artificial diet used in the current study. The results indicate that food availability is the strongest driver of

gonad enhancement but that other environmental factors have an influence on the gametogenic cycle of this species, particularly when roe enhancement takes place over extended periods.

Chapter Six

The effects of season, temperature and initial gonad condition on roe enhancement of the sea urchin *Evechinus chloroticus*.

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[Published in: Aquaculture 270 (2007) 115-131]

Received 4 Oct 2006, received in revised form 9 Mar 2007; accepted 12 Mar 2007

6.1 Abstract

A series of 10-week experiments was repeated, beginning during the austral autumn, winter, spring and summer to investigate the seasonal effects, independent of seasonal temperature variation, on roe development (enhancement) of *Evechinus chloroticus* over a 12-month period and whether this differed among urchins with different initial gonad index (GI) values. *E. chloroticus* collected from wild populations with high and low initial GI values were held in either ambient or constant (~ 14.7^{o}C) seawater temperatures. The study showed that, given a suitable roe enhancement diet, it is possible to significantly increase the GI of urchins throughout the year and that a

seasonal effect on both the final GI and increase in GI is primarily caused by seasonal variation in temperature. There are also significant effects from other factors, such as the initial condition of the urchins prior to the trial and the reproductive stage of the urchins, but these are not as significant as the effects of temperature. The results indicate that it would be possible to increase the productivity of sea urchins by capturing them in cooler water and enhancing them at a site with relatively warmer water. The initial condition (GI) of the urchins plays an important role in gonad production with urchins collected in low initial GI condition producing significantly larger increases in GI throughout each of the four seasons, than those with a high initial GI value. The urchins, collected from two neighbouring populations chosen on the basis of their having different GIs, developed through the stages of the reproductive cycle at a similar rate despite different final GI values and rates of increase in GI in both the temperature and population treatments, indicating that the differences in gonad production are related to environmental effects rather than the reproductive stage of the urchins. The exception to this occurred in the autumn samples where lower GI values and increase in GI were found during this season compared to in other seasons for the constant temperature treatment.

There is an overabundance of low GI urchins in the coastal waters of New Zealand and these urchins are relatively easily found and fished on snorkel compared to high GI urchins for which fishing pressure is much higher. It would appear to be more economic to fish and enhance low GI urchins in New Zealand due to their relative abundance, ease of access and higher productivity during enhancement.

Keywords: Seasonality, temperature, initial condition, *Evechinus chloroticus*.

6.2 Introduction

The sea urchin (kina) *Evechinus chloroticus* (Val.) is endemic to New Zealand, and has been commercially fished since 1986. However, attempts to export urchin roe to overseas markets such as Japan have met with little success, due to the roe having a bitter taste (McShane et al., 1996), poor colour, and inconsistent or low yields (P. Herbert, Sea Urchin New Zealand, pers comm.). Consequently almost all roe harvested is sold on the domestic market. The New Zealand fishery has therefore not expanded to the same degree as in other countries (Andrew et al., 2002) and is only marginally economic, requiring significant local knowledge and resources to target the best quality roe (James, 2006a and 2006b).

The possibility of enhancing roe from wild urchins has attracted considerable attention in New Zealand, as the ability to consistently improve the quality and quantity of roe available for export would open up lucrative international markets. Key factors believed to affect roe enhancement are the availability of an effective artificial diet and the reproductive condition of the urchins (Pearse and Cameron, 1991; Lesser and Walker, 1998; Lawrence et al., 2001; Klinger et al., 1997; Robinson and Colborne, 1997; Walker and Lesser, 1998). Temperature and photoperiod are also important factors for roe enhancement (Pearse and Cameron, 1991; Spirlet et al., 2000; Buisson, 2001; Garrido and Barber, 2001; Hofer and Watts, 2004; Siikavuopio et al., 2006), but their effects have received only limited study for *E. chloroticus* (Barker et al., 1998; Buisson, 2001). Likewise, the specific effects of seasonality on roe enhancement (i.e., independent of seasonal temperature variations) are poorly understood. Gonad development in wild populations of *E. chloroticus* is subject to seasonal effects (Walker, 1982; McShane et al., 1996; Barker et al., 1998; Brewin et

al., 2000; Lamare et al., 2002), but the importance of these effects for roe enhancement is unclear. Relatively few studies have repeated roe enhancement experiments more than once throughout the 12-month reproductive cycle, for *E. chloroticus* (Barker et al., 1998; Buisson, 2001; Fell, 2002) or for other urchin species (Spirlet et al., 2000; Garrido and Barber, 2001; Shpigel et al., 2004a; Siikavuopio et al., 2006), and none have attempted to differentiate between the effects of season and temperature.

The importance of initial gonad index (GI) for roe enhancement of wild urchins is also unclear. In New Zealand, and elsewhere in the world, large congregations of sea urchins in poor condition (i.e. having a low GI) are common (Andrew, 2003; James et al., 2004; S. Siikavuopio, Fiskeriforskning, pers comm.). For commercial roe enhancement it would be significantly cheaper to harvest these urchins (which are easily located, abundant, and have not previously been fished), rather than sourcing high GI urchins. However, their potential for roe enhancement relative to stocks of good quality (high GI) urchins is unknown. Because feed availability is one of the key environmental factors affecting GI it is difficult to find two distinct samples of urchins of similar age and size with significantly different GI values from a single population. The two sites selected for use in this experiment had been observed over several years by experienced local urchin fisherman and represent populations of urchins (that are likely to be of similar age) that had grown for a number of years in two distinct environments on either side of a small peninsular of land (P. Herbert, Sea Urchin New Zealand, pers comm.). The western side of the peninsula is completely bare of algae with very weak currents (there is a safe anchorage for boats on this side of the peninsular). The urchins found here are generally smaller and have low GI values. In contrast, on the eastern side of the peninsular there is a large biomass of brown algae

present and stronger currents. The urchins are larger with higher GI values (P. Herbert, Sea Urchin New Zealand, pers comm.). Although the distance between the two sites in a straight line is only approximately 50 m, it is ~500 m to travel from one side of the peninsular to the other following the coastline. Keesing (2001) suggests that most sea urchin species show fidelity to individual crevices and shelters and even those that do not, such as *Heliocidaris erythrogramma*, show little diel movement. A study by Sanderson et al. (1996) showed minimal movement in urchins (*H. erythrogramma*) in a cleared 100 m^2 area over a one year period. The longest recorded migration of *E. chloroticus* is 4.8 m (Dix, 1970a) and although they are capable of more extensive movement it is highly unlikely there would be any mixing of urchins from the two study sites in the current study.

The aim of this study was to investigate seasonal effects on roe enhancement of *E. chloroticus* over a 12-month period by enhancing the roe of wild caught urchins over four 10-week periods, at both ambient and constant temperatures, through each of the seasons (austral autumn, winter, spring and summer). It should be noted here that seasonal effects refer to the time of year that the urchin is collected and held, and does not refer to inter annual seasonal effects. The experiment was repeated with urchins both in good (high GI) and poor (low GI) initial condition to identify the effect of initial gonad index on roe enhancement. The relevance of the study in terms of commercial roe enhancement of sea urchins is discussed.

6.3 Methods

6.3.1 Experimental sites

Sea urchins were sourced from two sites at Motuara Island in the outer Marlborough Sounds in the north of the South Island of New Zealand. Both sites are situated at the

southern end of the island where a point of land extends southeast (Fig. 6.1) separated by a shoreline distance of ~500 m. The first site (41°05.97'S 174°16.62' E) is on the eastern side of the point and had a steeply shelving profile with heavy beds of *Macrocystis pyrifera* and associated algal species to a depth of approximately 8 m. Below this, the seafloor consists of bare rock and sand/gravel. Urchins at this site are present at depths of between 6 and 10 m. Anecdotal evidence indicates that the site is exposed to strong tidal currents (P. Herbert, Sea Urchin New Zealand, pers comm.). Urchins collected from this site are referred to as the high GI population. The second site (41°06.00'S 174°16.52' E) is situated on the western side of the point and consists of bare rock barrens. It has a gently sloping profile and is devoid of algal cover. Urchins at this site are present in depths ranging between 1 and 6 m. Urchins collected from this site generally had a lower GI than those from the eastern site, and are referred to as the low GI population. Water temperature was recorded hourly at the high GI site by a Stowaway Tidbit™ logger (Onset Computer Corporation, Massachusetts, USA) attached to a sub surface buoy at a depth of 6 m. Due to the loss of loggers the temperatures were only collected at the site between 19 July 2004 and 3 February 2005.

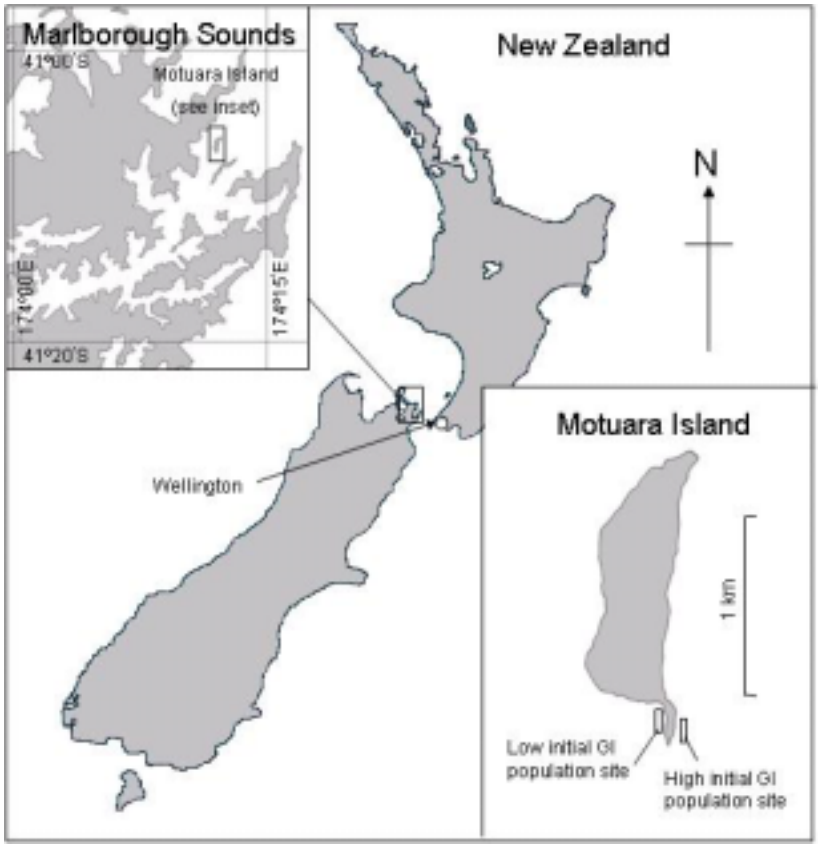

Figure 6.1 The location of Motuara Island in the Marlborough Sounds at the top of the South Island in New Zealand and the location of the two collection sites at the southern tip of Motuara Island.

6.3.2 Urchin collection

Urchins were collected (on 28 March 2004, 23 July 2004, 1 October 2004 and 4 February 2005) on snorkel and placed in mesh bags (approximately 60 urchins per bag) before being hauled onboard a fishing vessel (high initial GI population: mean test diameter 95.1 mm ± 0.5, mean wet weight 253.8 g ± 5.6; Low initial GI population: mean test diameter 71.9 mm ± 0.4, mean wet weight 159.8 g ± 2.6; see Table 6.1 for mean size and range of seasonal collections). Once onboard the bags were covered with felt sacking and kept damp with sprayed seawater for the four hour sea and land return journey to Mahanga Bay Research Facility maintained by the

National Institute of Water & Atmospheric Research Ltd. (NIWA), Wellington. Collections were made at the start and end of each trial.

Table 6.1 The mean (± 1 S.E), maximum, minimum test diameter (mm) and wet weight (g) for wild urchins collected at the start of the experiments from the high (*n* = 60) and low initial (*n* = 60) gonad index populations, and for each season (*n* = 60 per treatment) (autumn – summer).

Season	High GI initial test diameter	Low GI initial test diameter	High GI initial wet weight	Low GI initial wet weight
Autumn				
Mean	95.1 (± 1.2)	70.0 (± 0.8)	358.9 (± 11.3)	154.0 (± 11.3)
Minimum	72.0	59.0	173.0	97.0
Maximum	116.0	91.0	589.0	312.0
Winter				
Mean	98.3 (± 1.2)	71.5 (± 0.9)	376.0 (± 11.8)	158.7 (± 6.0)
Minimum	75.0	55.0	180.9	79.1
Maximum	125.0	91.0	643.9	318.4
Spring				
Mean	95.6 (± 1.3)	76.2 (± 0.9)	350.9 (± 13.3)	240.5 (± 5.6)
Minimum	79.0	65.0	215.0	115.0
Maximum	112.0	91.0	579.0	295.0
Summer				
Mean	89.5 (± 1.7)	69.7 (± 0.9)	306.3 (± 9.0)	145.4 (± 5.6)
Minimum	71.0	54.0	176.0	73.0
Maximum	110.0	88.0	492.0	288.0

6.3.3 Holding systems

On arrival at Mahanga Bay the urchins were transferred to one of 12 experimental holding cages (the bottom of each cage consisted of plastic mesh with 10 mm square holes to retain the approximately 20 mm square feed cubes), each 985 mm long × 495 mm wide × 320 mm high, with a 31l internal capacity and 1.88 m^2 internal surface area. Forty five urchins were placed in each cage at a density of 23.9 urchins m^{-2} of internal surface area. An airstone supplying a constant flow of air was placed directly below each cage, which was then placed in one of two tank systems (Fig. 6.2). Both systems consisted of four 700 l holding tanks and a 225 l reservoir tank supplied with 20 l/min of ambient 10 μm-filtered seawater. Three of the four holding tanks in each system received a constant flow (12.6 l/min) of seawater pumped from the reservoir tank and supplied through a four-outlet manifold. This water was then returned to the reservoir tank through bottom drains installed in each tank. One tank in each system remained empty, and was used to aid cleaning once per week as described below.

Contrasting temperature regimes were established in the two tank systems by operating one system at ambient seawater temperature (denoted AMB), and the other at a nominally constant temperature of 14.7°C (denoted CONST). This constant temperature was the best estimate of the annual average temperature at the collection site taken from previous temperature collections in the outer Marlborough Sounds (unpublished data). This was achieved by installing a 6 kw (heating) reverse cycle heat pump with a stainless steel and polyethylene heat exchanger between the reservoir tank and the outlet manifold. Stowaway Tidbit™ loggers in sealed containers, placed into one tank from each of the AMB and CONST holding systems, recorded water temperatures hourly for the duration of the study. All tanks were exposed to a controlled light/dark photoperiod of 12 h L : 12 h D. Ambient

photosynthetic irradiance (µmol photons m^{-2} s^{-1}) was recorded using an Odyssey cosine sensor (Dataflow Systems Pty Ltd, Christchurch, New Zealand) placed inside a cage for a 72-hour period. Dissolved oxygen, pH, and ammonia were measured weekly in each experimental tank and in the sump tanks of each system using a WTW (CellOx 325) probe and a WTW pH Electrode (SenTix 81) probe connected to a WTW Multi (340i) water quality meter (all WTW equipment manufactured by Werkstatten GmbH & Co., Weilheim, Germany) and a Palintest ammonia comparator kit (Palintest UK, Tyne & Wear, United Kingdom) respectively.

6.3.4 Husbandry

All urchins were fed NIWA's artificial sea urchin roe enhancement diet (see James et al., 2004 for details) at a rate of 1.5% mean body weight per day three times per week throughout the experimental period. Uneaten food was removed from each basket at each feeding, unless it was not easily accessible in which case it was left. The tank systems were cleaned each week by transferring the baskets from one of the tanks into the clean empty tank in each system. The dirty tank was then emptied, cleaned, and refilled, and this cycle was repeated until all three full tanks in each system had been cleaned. During cleaning, any dead urchins were removed from the cages and were not replaced. Urchin mortality was recorded throughout each experiment.

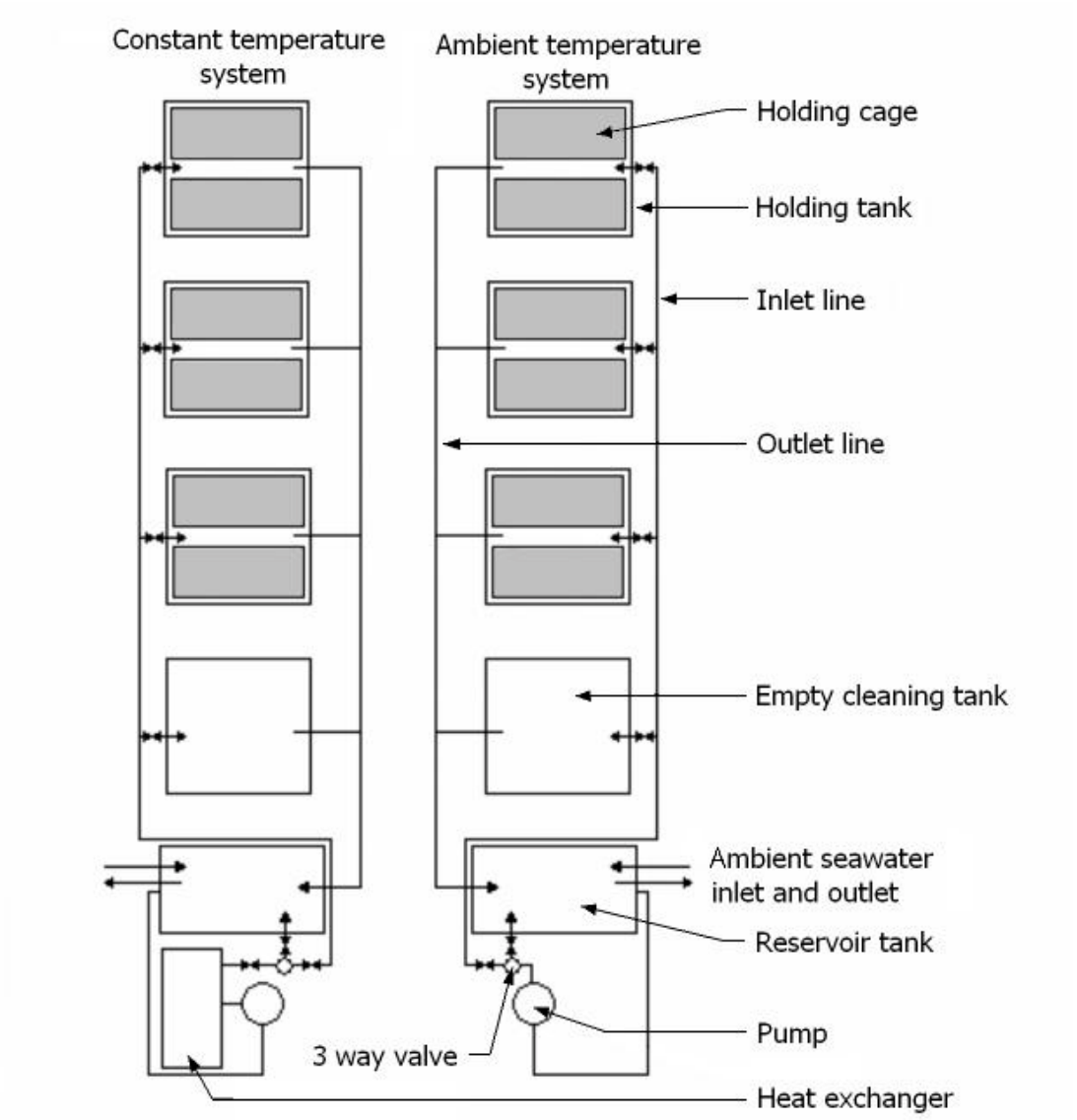

Figure 6.2 A schematic diagram of the holding system used to maintain the water flows and temperatures in the experimental tanks.

6.3.5 Experimental design

Ten to twelve weeks has been shown to be a suitable roe enhancement period for *E. chloroticus* (Barker et al., 1998; Buisson, 2001; Fell, 2002; James et al. 2004, James 2006a and 2006b). In this study, four 10-week experiments were carried out between 31 March 2004 and 26 April 2005 to determine the effects on roe enhancement of

initial GI (high vs. low), water temperature (AMB vs. CONST), and season. In each experiment, four treatments were established by holding urchins from the high and low GI populations in both the AMB and CONST regimes, with three replicate cages per treatment. The four treatments were thus:

1. high GI population × ambient temperature
2. low GI population × ambient temperature
3. high GI population × constant temperature
4. low GI population × constant temperature

The replicates were assigned in pairs so that each tank held one replicate of both population treatments. To assess the effect of season independently of temperature, each experiment was repeated four times, starting on 4 April 2004 (austral autumn), 25 July 2004 (winter), 5 November 2004 (spring), and 6 February 2005 (summer). This created a total of 16 experimental groups, comprising 2 populations × 2 temperature regimes × 4 seasons.

6.3.6 Data collection

Urchin condition (GI) was recorded at the start and end of each experiment, based on random samples of 20 urchins from each replicate cage, and 60 wild urchins from both the high and low GI populations on Motuara Island (referred to henceforth as ‘wild’). Variables measured for each urchin were test diameter (D, mm), total wet weight (W, g), and gonad wet (unblotted) weight (G, g). These data were used to calculate GI (= 100 × G / W) and an index of increase in gonad index (Y) for each urchin expressed as the difference between its GI at the end of the experiment and the

mean GI ($\overline{GI}$) for the corresponding wild population at the start of the experiment (i.e., $Y = GI_{final} - \overline{GI}_{initial}$ in units of kilo of roe / tonne of wet weight urchins).

A graph comparing the test diameter against the gonad index of the wild urchins collected at the beginning of each experiment (Fig 6.3, 6.4, 6.5 and 6.6) shows a significant relationship between test diameter gonad index in autumn (ANOVA: F = 43.00, *df* = 1,118, *P* < 0.001), winter (ANOVA: F = 36.25, *df* = 1,118, *P* < 0.001), spring (ANOVA: F = 22.54, *df* = 1,118, *P* < 0.001) and summer (ANOVA: F = 16.57, *df* = 1,118, *P* < 0.001).

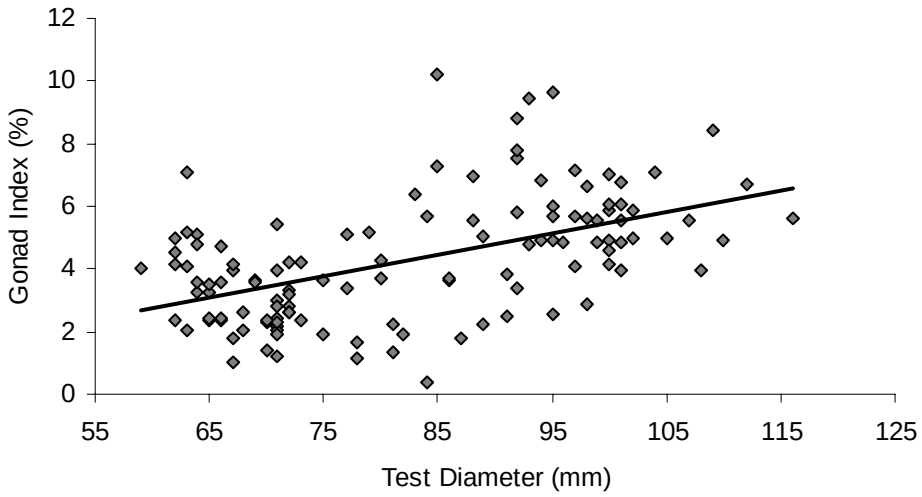

Figure 6.3 The relationship between Gonad Index values and Test Diameter for urchins collected from the initial wild sample in autumn, Y = 0.068 X – 1.389; r^2 = 0.267; *n* = 120.

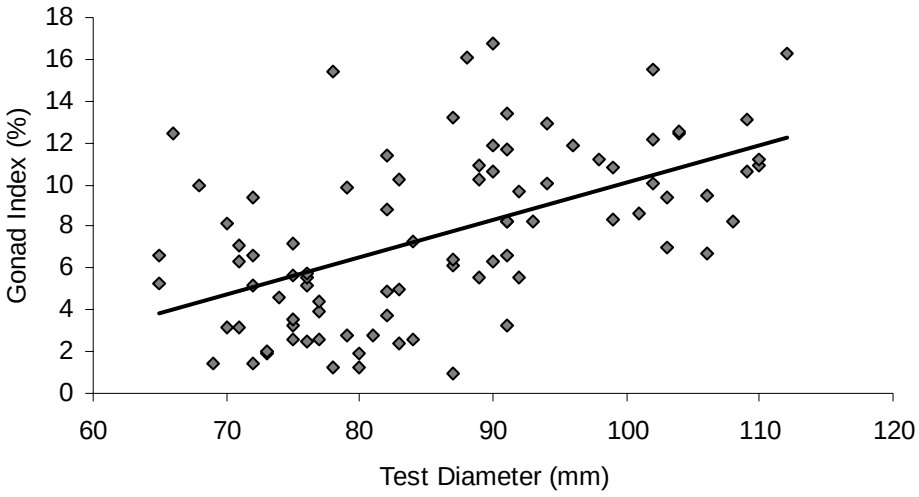

Figure 6.4 The relationship between Gonad Index values and Test Diameter for urchins collected from the initial wild sample in winter, Y = 0.178 X – 7.746; r^2 = 0.296; *n* = 120.

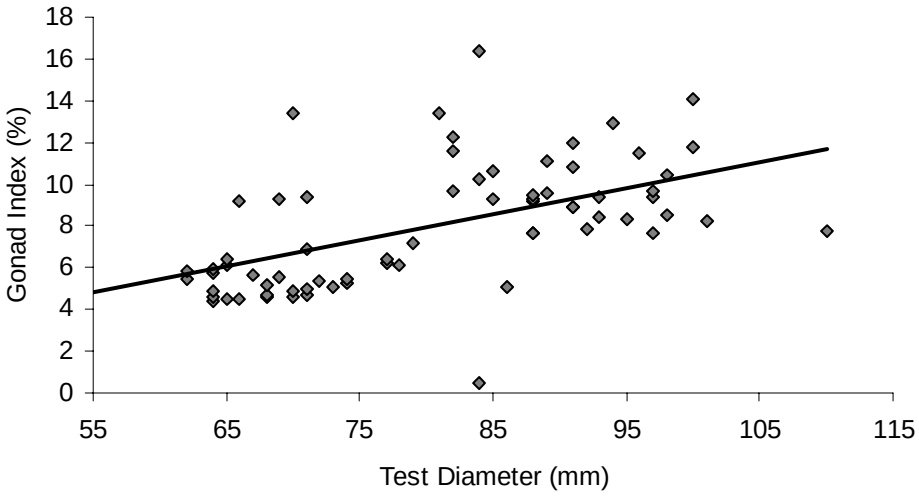

Figure 6.5 The relationship between Gonad Index values and Test Diameter for urchins collected from the initial wild sample in spring, Y = 0.1233 X – 1.9325; r^2 = 0.284; *n* = 120.

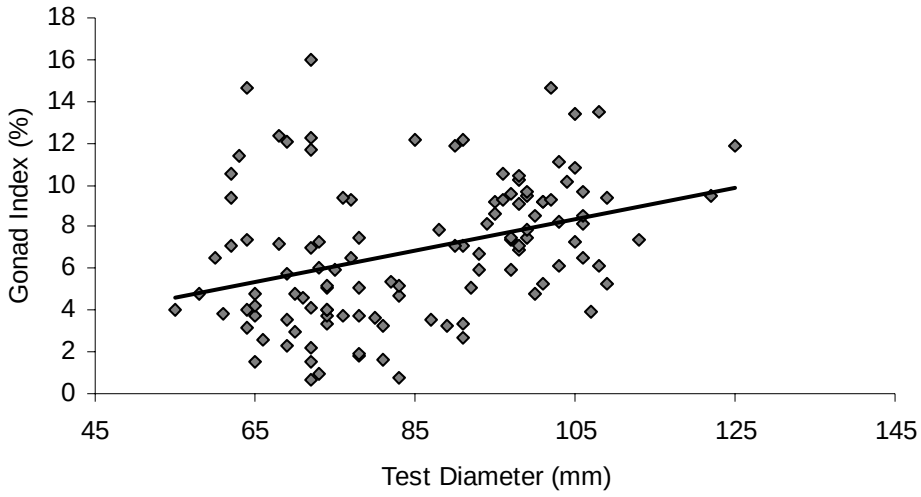

Figure 6.6 The relationship between Gonad Index values and Test Diameter for urchins collected from the initial wild sample in summer, Y = 0.075 X – 1.497; r^2 = 0.126; *n* = 120.

The reproductive stage of a random sample of 15 urchin gonads from each treatment, and from the initial and final wild populations, was determined using histological analysis. Histological samples were collected, fixed and stained using standard Harris's haematoxylin stain, counterstained with eosin (Bancroft and Stevens, 1990). The sex was determined for each sample and reproductive condition assessed using a six stage scale ranging from Stage 1 (recovery) to Stage 6 (spent), as described by Walker (1982) and Byrne (1990).

6.3.7 Statistical analysis

Three-way analysis of variance was used to compare mortality, D, W, GI, and Y, between populations, temperature treatments, and seasons, taking into account all possible two- and three-way interactions. These analyses allowed the description of

the main effects of each factor and to identify interactions of potential importance. Similar analyses were used to compare seasonal differences in GI between the wild populations. A two-way ANOVA was used to compare differences in the test diameter and wet weights of the wild urchins collected at the beginning of each trial. Gonad indices for the pooled data-set exhibited slight negative skewness, due to the effect of approximately 20 individuals (throughout both populations and temperature treatments) which had unusually low GIs, but were otherwise approximately normally distributed. Arcsine square root transformation of D, W, GI and Y increased rather than decreased skewness, so all analyses for these parameters were performed using untransformed data. Mortality rates were converted to percentages and arcsine square root transformed. Homogeneity of variances was tested using Modified Levene's test. Bonferroni post-hoc comparison tests were used to identify the treatments which differed significantly.

Direct comparisons of GI provide an intuitively simple way to contrast reproductive output between populations and treatments, but are potentially confounded by non-isometric growth across the full body weight range of interest (Packard and Boardman, 1999). In such cases, analysis of covariance (ANCOVA) provides a more robust way to compare indices (such as GI) based on ratios, particularly in the presence of extensive variation in body size (Packard and Boardman, 1999; see also Unwin et al., 2004). However, attempts to analyse the data within a single ANCOVA model failed, because of significant second and third order interactions between the covariate (W) and one or more of population, temperature regime, and season. Linear regression of GW on W for all sixteen groups suggested that these interactions were due mainly to four groups (two from each population) containing several large individuals with unusually small gonads for their body mass,

depressing the slopes of their individual regression lines and generating y-intercepts significantly greater than zero. Data for the remaining groups were more uniform in slope, with y-intercepts within two standard deviations of zero, a necessary condition for isometry (Packard and Boardman, 1999). Therefore, it was assumed that the analysis of GI was not seriously compromised by lack of isometry, but that – where possible – these analyses should be backed up using single factor ANCOVA of GW on W for specific subsets of the data, chosen so as to ensure isometry and homogeneity of slopes. Twelve ANCOVA models were examined for this phase of the analysis: one for each combination of population × temperature treatment, with season as a factor (omitting seasons which violated homogeneity of slopes); and one for each combination of population × season, with temperature treatment as a factor. These analyses were used to estimate mean GW for each factor of interest, standardised to a common W.

Statistical analyses were conducted using SYSTAT 10 (Wilkinson, 2000) and NCSS 2000 (Number Crunching Statistical Systems, Kaysville, Utah, USA). Statistical tests were considered significant if $P < 0.05$, and highly significant if $P < 0.001$. Errors and confidence intervals are expressed as ± one standard error.

6.4 Results

6.4.1 Seawater temperatures and water quality

Seawater temperatures in the experimental baskets varied among seasons and treatments throughout the study (Table 6.2, Fig. 6.7). Temperatures in the AMB

treatments were lowest during winter (mean 11.3 ± 0.1°C) and highest during summer (mean 18.2 ± 0.2°C), whereas those in the CONST treatments were above AMB (mean 14.7 ± <0.1°C) in winter, and below AMB (mean 15.3 ± 0.1°C) in summer. Spring temperatures in the CONST temperature treatment were similar to ambient temperatures for the wild populations where the urchins were collected. The AMB experimental regime was similar to the ambient temperatures experienced by the wild populations in winter (mean difference 0.29°C) but higher in spring (mean difference 2.12°C), probably because of the heating effect of water reticulation in the holding systems. Although there were some temperature fluctuations in the CONST temperature treatment these were relatively minor compared to the seasonal variation in the experimental AMB temperatures where the summer high and winter low were more extreme than in the CONST temperature treatment. The autumn CONST temperature (13.22 ± 0.02^{o}C) had a slightly lower mean than any other season (winter: 14.69 ± 0.02 ^{o}C; spring: 14.73 ± 0.04^{o}C, summer; 15.27 ± 0.06 ^{o}C).

Seawater quality (dissolved oxygen, ammonia and pH) remained within acceptable limits during all experiments (Table 6.2). Mean ambient photosynthetic irradiance recorded in the holding cages during the light periods was 0.39 µmol photons m^{-2} s^{-1}.

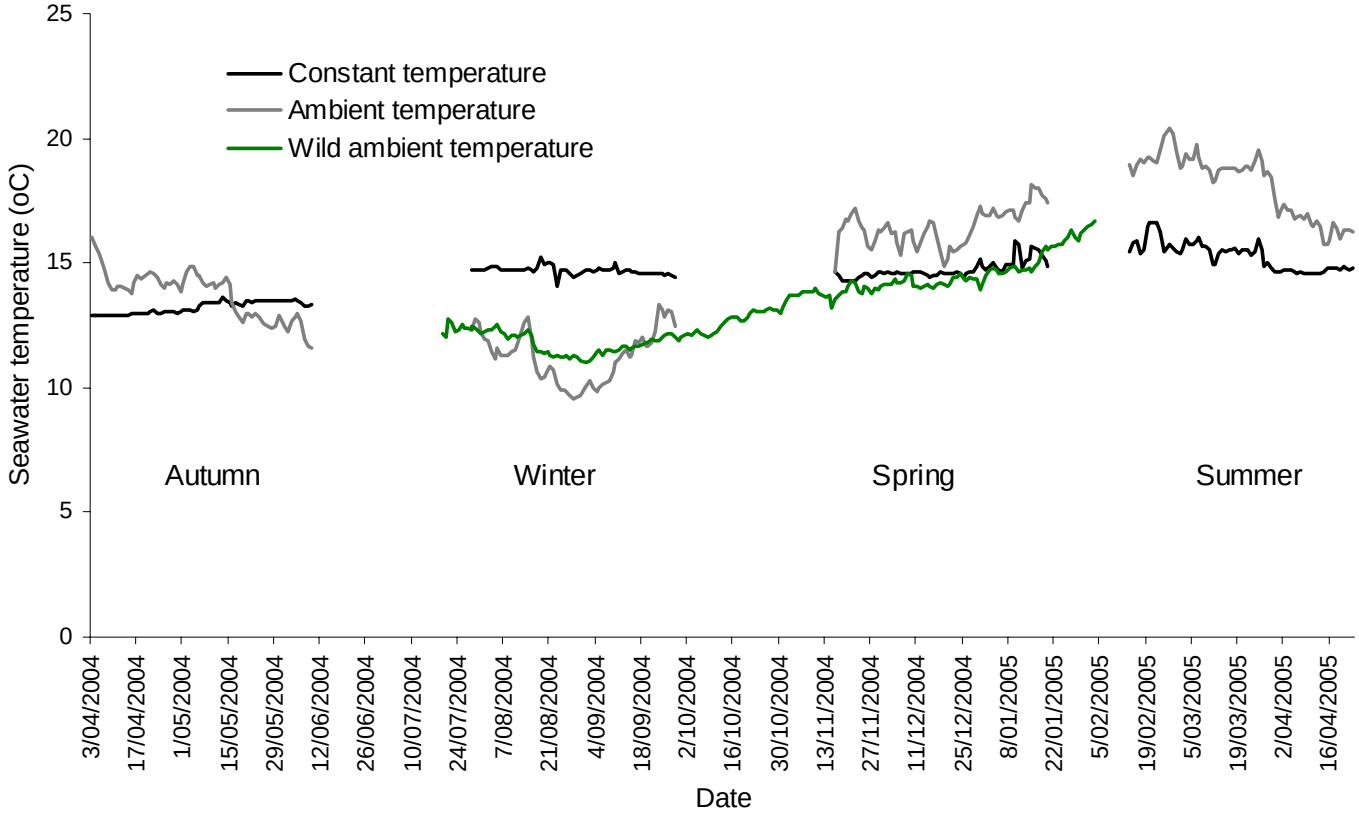

Figure 6.7 The temperatures (^{o}C) recorded in the experimental tanks during the enhancement trials and in the wild during the experimental period.

Table 6.2 The mean (± 1 S.E.), maximum, minimum daily temperatures for each of the experimental treatments and for each season (note the wild treatment was only recorded for the winter and spring periods). The mean (± 1 S.E.), maximum, minimum dissolved oxygen, pH and ammonia levels recorded in the experimental tanks during each of the four experimental periods.

Season	Temperature treatment Constant (^{o}C)	Ambient (^{o}C)	Wild (^{o}C)
Autumn	(*n* = 70)	(*n* = 70)	
Mean	13.22 (± 0.02)	13.72 (± 0.11)	Not available
Maximum	13.62	16.01	
Minimum	12.90	11.60	
Winter	(*n* = 70)	(*n* = 70)	(*n* = 70)
Mean	14.69 (± 0.02)	11.28 (± 0.13)	11.57 (± 0.05)
Maximum	15.20	13.30	12.50
Minimum	14.07	9.57	10.98
Spring	(*n* = 70)	(*n* = 70)	(*n* = 70)
Mean	14.73 (± 0.04)	16.46 (± 0.09)	14.34 (± 0.05)
Maximum	15.88	18.11	15.68
Minimum	14.25	14.67	13.56
Summer	(*n* = 70)	(*n* = 70)	
Mean	15.27 (± 0.06)	18.15 (± 0.15)	Not available
Maximum	16.63	20.38	
Minimum	14.57	15.70	
Season	Percent dissolved oxygen in expt. tanks	Dissolved pH levels in expt. tanks	Dissolved ammonia (mg N/l) in expt. tanks
Autumn	(*n* = 60)	(*n* = 12)	(*n* = 12)
Mean	96.1 (± 0.29)	8.04 (± 0.01)	0.7 (± 0.02)
Maximum	101.1	8.1	0.9
Minimum	88.4	7.9	0.4
Winter	(*n* = 60)	(*n* = 12)	(*n* = 12)
Mean	97.5 (± 0.43)	8.04 (± 0.01)	0.4 (± 0.05)
Maximum	101.9	8.1	0.6
Minimum	86.6	7.9	0.2
Spring	(*n* = 60)	(*n* = 12)	(*n* = 12)
Mean	95.3 (± 0.26)	8.05 (± 0.01)	0.4 (± 0.01)
Maximum	89.6	8.1	0.5
Minimum	14.25	7.9	0.2
Summer	(*n* = 60)	(*n* = 12)	(*n* = 12)
Mean	97.4 (± 0.78)	8.02 (± 0.03)	0.4 (± < 0.00)
Maximum	101.7	8.2	0.4
Minimum	92.0	7.9	0.4

6.4.2 Urchin reproductive stage

6.4.2.1 High initial GI population

During autumn the reproductive stage of the male urchins advanced between the initial wild sample (spent and recovery stages) to the final experimental sample (recovery and growing stages). The change in reproductive stage in female urchins between the initial wild sample and the final experimental sample in autumn was smaller with the former at the recovery stage, and the later at the recovery and growing stage. During winter there was an advance in the reproductive stage of the male and female urchins from the initial wild sample (recovery and growing stages) to the final experimental sample (growing and premature stages). During spring there was an advance in the reproductive stage of the urchins from the initial wild sample (mostly premature stage) to the final experimental sample (mostly mature stage for males and a rapid and marked advance to mostly partly spawned for females). During summer the reproductive stage of the male high initial urchins did not change markedly (there was a higher percentage of partly spent to completely spent) from the initial wild sample to the final experimental sample but the females advanced from the partly spawned stage to the spent stage (Fig. 6.8a).

6.4.2.2 Low initial GI population

During autumn and winter the reproductive stage of the low initial urchin population did not change markedly from the initial wild sample to the final experimental sample for male or female. The urchins sampled in autumn were mostly at the recovery stage (the females from the final experimental sample were slightly more advanced into the growing stage) and in winter were mostly at the growing stage (the males from the final experimental sample were slightly more advanced than the initial sample).

During spring there was a significant advance in the reproductive stage from the initial wild sample (mostly at the premature stage for females and at the growing and premature stage for males) to the final experimental sample (mostly mature and partly spawned stages for female and mostly premature for male with some mature and some partly spent). During summer there was a marked advance in the reproductive stage of the female urchins from the initial wild sample (mostly partly spawned stage) to the final experimental sample (mostly spent stage). In contrast the male urchins from both samples in summer were at the spent stage (Fig. 6.8b).

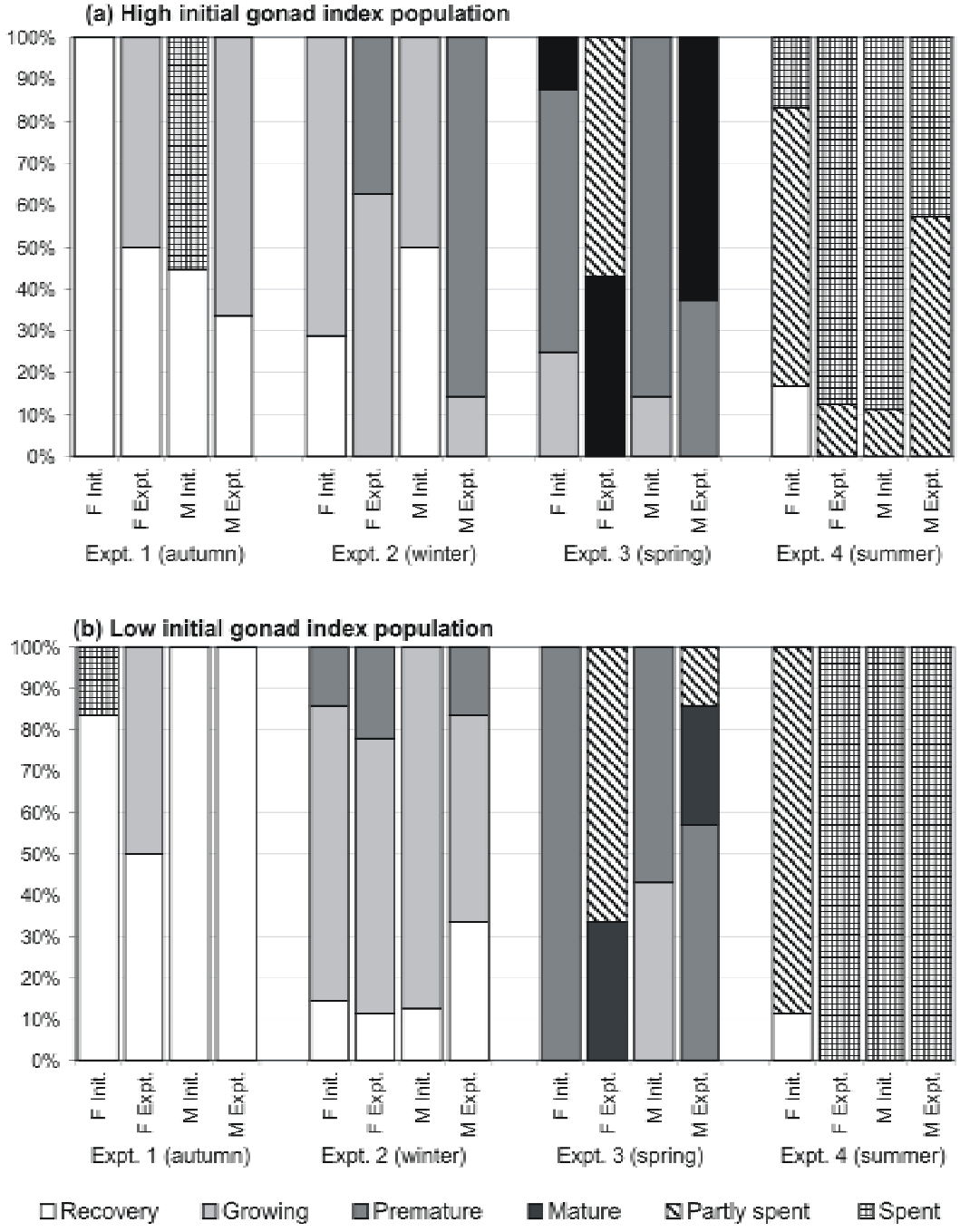

Figure 6.8 The proportion of female (F) and male (M) sea urchins (*n* = 15) sampled at the beginning of each experiment (i.e. wild urchins) (Init.) and at the conclusion of each experiment (Expt.), for each of the four trials (1-4) from each reproductive stage (recovery to spent) for (a) the High initial GI population and (b) the Low initial GI population.

6.4.2.3 High and low initial GI population comparison

The two populations showed a similar pattern of development through each of the seasons. The reproductive stage of the urchins from the final experimental samples from both the high and low populations progressed from the recovery stage in autumn to the spent stage in summer. The males from the high initial GI population were slightly more advanced in autumn but there was no difference in the stages of the females. During winter and spring there were only slight differences between males or females between the two populations. During summer the males from the low initial GI population were slightly more advanced than those from the high initial GI population but the differences between females from the two populations were slight (Fig. 6.8 a and b).

6.4.3 Urchin survival

Mean survival ranged from 92-99 % during the experiments (Table 6.3). A three-way ANOVA showed no significant interactions between season x temperature, season x population, population x temperature or between population x temperature x season (Table 6.4). Pooled means showed that urchin survivorship was significantly higher for the low initial GI population (99.3 ± 0.3%) than the high initial GI population (92.9 ± 0.8%), irrespective of temperature treatment or season (Table 6.4).

Table 6.3 The mean (±1 S.E.) percent survival of urchins held in each of the experimental treatments (*n* = 135 per treatment) and for each season.

Season	High initial GI Constant (^{o}C)	Low initial GI Constant (^{o}C)	High initial GI Ambient (^{o}C)	Low initial GI Ambient (^{o}C)
Autumn	94.8 (±0.7)	99.3 (±0.7)	94.8 (±0.7)	99.3 (±0.7)
Winter	95.6 (±2.6)	100.0 (±0.0)	94.8 (±2.0)	100.0 (±0.0)
Spring	90.0 (±2.9)	99.2 (±0.8)	90.8 (±4.6)	100.0 (±0.0)
Summer	90.3 (±1.5)	99.2 (±0.7)	92.6 (±1.5)	97.0 (±2.0)

Table 6.4 Significance tests and related statistics (degrees of freedom, mean square, F ratio, *P* value) for the ANOVA analysis of urchin mortality.

Effect	*DF*	Mean square	F-ratio	*P*
population	1	1.022626	59.46	< 0.0001
temperature	1	1.298529E-04	0.01	0.931
season	3	3.638096E-02	2.12	0.117
season × population	3	6.641833E-03	0.39	0.117
season × temperature	3	8.871669E-03	0.52	0.674
population × temperature	1	2.778783E-03	0.16	0.690
season × population × temperature	3	8.373908E-03	0.49	0.694
Error	32	0.0171975		

6.4.4 Test diameter and wet weight

6.4.4.1 Wild urchins

A two way ANOVA showed a significant interaction between season and population (Test diameter: F = 5.06, *P* = 0.001; two-way ANOVA, *df* = 3, 442 and Wet Weight:

F = 5.01, *P* = 0.002; two-way ANOVA, *df* = 3, 441). There was a significant difference between the test diameter and wet weight of the high and low initial GI populations due to the effects of season (Test diameter: F = 11.34, *P* = 0.001; two-way ANOVA, *df* = 3, 442 and Wet Weight: F = 9.91, *P* = 0.002; two-way ANOVA, *df* = 3, 441) and population (Test diameter: F = 951.00, *P* = 0.001; two-way ANOVA, *df* = 3, 442 and Wet Weight: F = 843.1, *P* = 0.002; two-way ANOVA, *df* = 3, 441). The test diameter and wet weight were significantly larger in the high initial GI population regardless of season (post-hoc Bonferroni tests, *P* < 0.001). The test diameters of urchins collected in summer were significantly smaller than those collected in all other seasons and there was no significant difference between the test diameter of urchins collected in autumn, winter or spring (post-hoc Bonferroni tests, *P* < 0.001). The wet weight of urchins collected in summer were significantly lower than those collected in all other seasons and there were no significant differences among wet weights of urchins collected in autumn, winter or spring (post-hoc Bonferroni tests, *P* < 0.001).

6.4.4.2 Experimental urchins

A three-way ANOVA showed no significant interactions between season, temperature or population for urchin test diameter or wet weight. The high initial GI population had significantly higher test diameters and wet weights than those from the low initial GI population (Table 6.5 and 6.6) which is a reflection of their initial wild test diameter and wet weight values. There were also significant differences in the test diameters and wet weights of the experimental urchins between seasons with the urchins having significantly higher values for both test diameter and wet weight in

autumn and spring compared to winter and summer regardless of population (Table 6.5 and 6.6).

Table 6.5 Mean (± 1 S.E) test diameter and wet weight of the experimental urchins from the High and Low initial gonad index populations held in constant (14.7°C) or ambient temperatures for 10 weeks during each of the four seasons. Different letter notations indicate significant differences ($P < 0.05$) between seasons (x, y), populations and temperature treatments (a, b) ($n = 60$ per treatment).

Season and Treatment	Test diameter (mm)	Wet Weight (g)
Autumn	x	x
High/Constant	94.9 (± 1.9) a	330.8 (± 4.1) a
Low/Constant	73.8 (± 0.8) b	178.1 (± 4.6) b
High/Ambient	95.9 (± 0.6) a	362.3 (± 10.6) a
Low/Ambient	74.0 (± 0.3) b	179.7 (± 1.2) b
Winter	y	y
High/Constant	89.4 (± 0.7) a	292.9 (± 6.7) a
Low/Constant	71.9 (± 1.7) b	157.9 (± 9.5) b
High/Ambient	91.1 (± 1.3) a	304.9 (± 10.2) a
Low/Ambient	72.4 (± 1.2) b	165.3 (± 7.1) b
Spring	x	x
High/Constant	94.4 (± 1.9) a	339.5 (± 18.4) a
Low/Constant	77.2 (± 0.3) b	191.8 (± 1.7) b
High/Ambient	94.2 (± 0.7) a	332.5 (± 4.3) a
Low/Ambient	74.5 (± 1.9) b	186.6 (± 3.1) b
Summer	y	y
High/Constant	91.7 (± 1.2) a	316.5 (± 12.1) a
Low/Constant	72.3 (± 0.9) b	166.5 (± 6.2) b
High/Ambient	93.3 (± 0.5) b	332.1 (± 1.3) a
Low/Ambient	71.9 (± 1.8) a	163.2 (± 8.6) b

Table 6.6 Significance tests and related statistics (degrees of freedom, mean square, F ratio, *P* value) for the ANOVA analysis of urchin test diameter (mm) and wet weight (g) measured at the conclusion of each experiment.

Effect	*DF*	Mean square	F-ratio	*P*
Test diameter				
population	1	88793.4	1486.2	< 0.0001
temperature	1	66.4	1.11	0.292
season	3	897.0	15.01	< 0.0001
season × population	3	412.9	2.30	0.075
season × temperature	3	49.3	0.83	0.479
population × temperature	1	142.1	2.38	0.123
season × population × temperature	3	3.6	0.06	0.980
Error	937	59.7		
Wet Weight				
population	1	5551199	1506.92	< 0.0001
temperature	1	9785.3	2.66	0.103
season	3	60445.5	16.41	< 0.0001
season × population	3	10826.8	2.94	0.032
season × temperature	3	5062.6	1.37	0.367
population × temperature	1	9330.9	2.53	0.112
season × population × temperature	3	3110.1	0.84	0.469
Error	937	3683.8		

6.4.5 Gonad Index and Gonad Weight

Variation in GI during the experimental study was almost totally dominated by seasonal effects, both when considered as a single factor and via interactions between

season and population, and season and temperature regime. Population and temperature regime effects were limited to these two interactions, together with a much weaker interaction between population and temperature (Table 6.7).

Table 6.7 Significance tests and related statistics (degrees of freedom, mean square, F ratio, *P* value) for the ANOVA analysis of final gonad index.

Effect	*DF*	Mean square	F-ratio	*P*
population	1	31.67	2.13	0.145
temperature	1	36.85	2.47	0.116
season	3	551.51	37.04	< 0.0001
season × population	3	320.73	21.54	< 0.0001
season × temperature	3	288.81	19.40	< 0.0001
season × population × temperature	3	17.92	1.20	0.307
Error	937	14.89		

Inspection of pooled means for both populations and treatments suggested a steady increase in mean final GI during the course of the trials, from 12.5 ± 0.5% in autumn to 15.8 ± 0.5% in summer (Fig. 6.9a). Final GIs in spring and summer were higher than those in autumn and winter (post-hoc Bonferroni tests, $P < 0.0001$), with the largest increase in GI occurring from winter to spring. However, gonad development followed markedly different seasonal trajectories in the two populations (Fig. 6.9b), with maximum GI recorded in spring in the high GI population, and summer in the low GI population. The differences between the two populations are consistent with a delay of roughly three months in the low GI population, for which seasonal mean GIs in winter, spring, and summer were similar to those for the high GI group from the previous season.

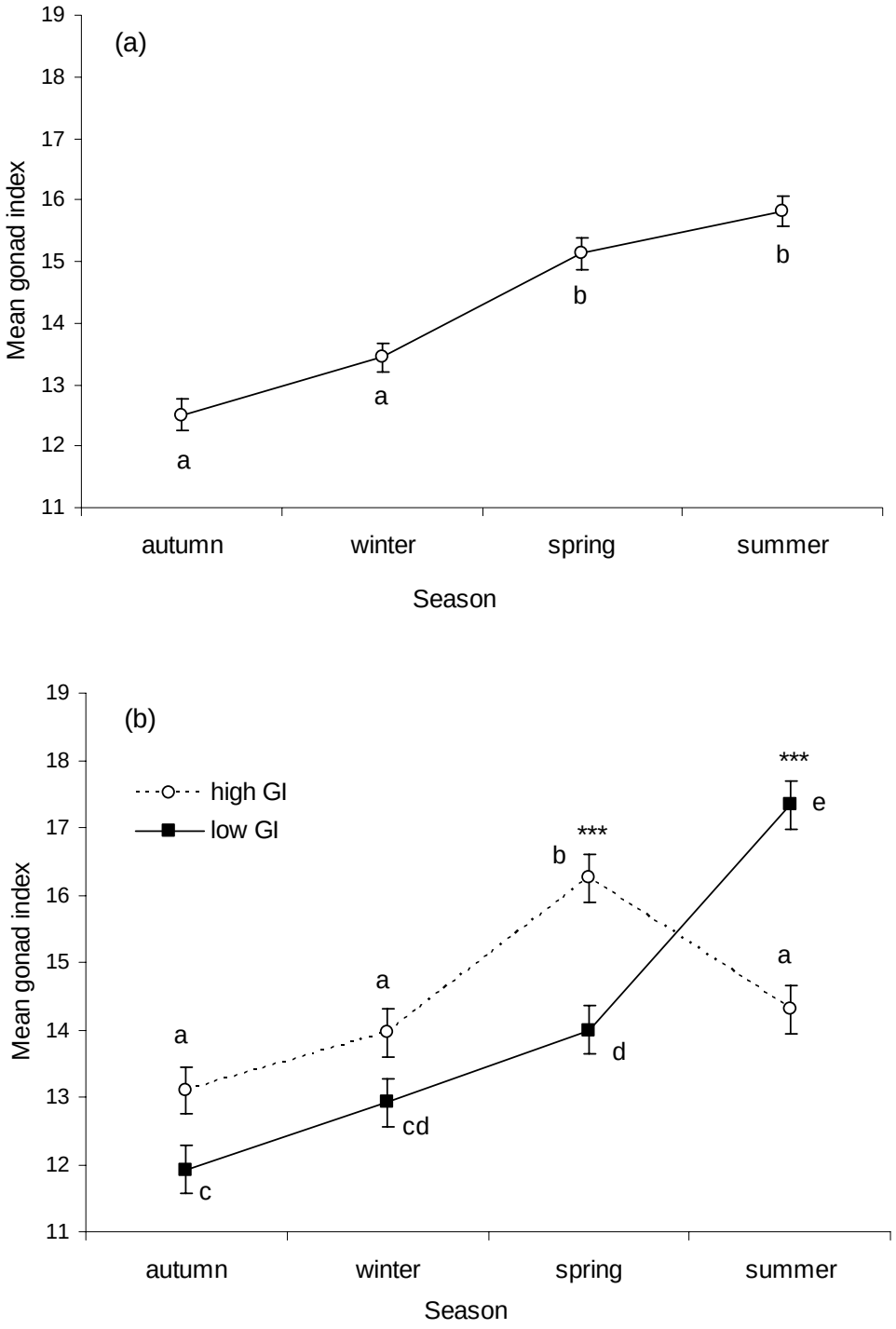

Figure 6.9 Differences in final mean gonad index (± 1 S.E.) for the effects/interactions in the ANOVA model summarised in Table 7. Successive graphs show means for (a) season, (b) season × population and (c) season × temperature treatment. Means which did not differ significantly (*P* > 0.05) within each series, according to post-hoc Bonferroni tests, are labelled with common letters. In (b) and (c), significant differences (all *P* < 0.001) between the two series within each season are denoted *.**

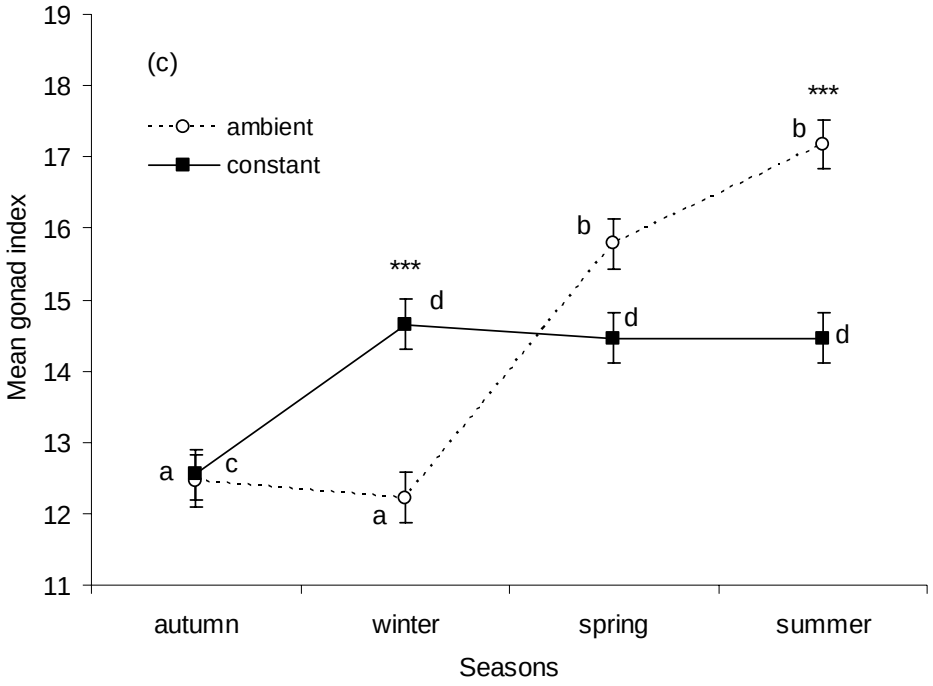

Figure 6.9 Continued. Differences in final mean gonad index (± 1 S.E.) for the effects/interactions in the ANOVA model summarised in Table 7. Successive graphs show means for (a) season, (b) season × population and (c) season × temperature treatment. Means which did not differ significantly (*P* > 0.05) within each series, according to post-hoc Bonferroni tests, are labelled with common letters. In (b) and (c), significant differences (all *P* < 0.001) between the two series within each season are denoted *.**

Gonad index trajectories also differed between the two experimental temperature regimes (Fig. 6.9c). Urchins subject to the ambient temperature (AMB) regime underwent little if any change in gonad index during autumn and winter, but GIs increased markedly in spring, and again in summer. By contrast, for urchins subject to the constant temperature (CONST) regime, mean final GI increased significantly from autumn to winter, but remained essentially constant thereafter.

ANCOVA models of variations in mean gonad weight standardised to a common mean weight for individual treatments were consistent with the above

results, but also highlighted additional differences between seasons and treatments for each population. For the high GI population, mean gonad weight under CONST was 100.7 % of mean GI under AMB in autumn, compared to 129.4% in winter, 99.6% in spring, and 82.3% in summer. Corresponding figures for the low GI population were 99.3%, 110.8%, 83.2%, and 84.9%. Both sets of results reinforce the conclusions from the GI analyses (Fig. 9c), confirming the general tendency for reproductive output under CONST to be enhanced relative to AMB in winter, but suppressed in summer, in both populations. The strongest effects were associated with the high GI population in winter, for which mean gonad weight at weight = 299 g was 37.0 ± 2.9 g under AMB, compared with 47.8 ± 2.9 g under CONST. When compared across seasons for each temperature regime × population, mean GW for winter, spring, and summer as a percentage of mean GW in autumn was 96.5%, 125.8%, and 119.0% for the high GI × AMB group; 123.4%, 123.5%, and not estimated for the high GI × CONST group; not estimated, 131.9%, and 155.3% for the low GI × AMB group; and 110.7%, 110.9%, and 132.4% for the low GI × CONST group. The largest percentage increase over the duration of the study was for the low GI × AMB group, in which mean gonad weight at weight = 177 g was 20.7 ± 1.9 g in autumn compared to 32.1 ± 1.9 g the following summer. A smaller but still highly significant increase occurred in the low GI × CONST group, from 20.4 ± 1.7 g in autumn to 27.0 ± 1.7 g in summer at weight = 174 g. Given that mean weight differed by only 3 g between the two low GI groups, this result provides clear evidence that the AMB regime was significantly more effective than the CONST regime in enhancing reproductive output in the low GI population.

Final GIs for urchins in the experimental study (typically 12-17; Fig. 6.9) were consistently higher (Student t-test, $P < 0.001$ in all cases) than those for their wild

counterparts, which ranged among seasons from 5.6 ± 0.7% to 10.1 ± 0.8% for the high GI population, and 3.0 ± 0.7% to 5.3 ± 0.7% for the low GI population (Fig. 6.10). Seasonal variation in GI also differed markedly between the two wild populations (ANOVA, P = 0.0001). For the high GI population, gonad development in the wild (Fig. 6.10) was similar to that observed in the experimental study (Fig. 6.9b), rising to a well defined peak in mid November (spring) and declining thereafter. However, in the low GI population gonad development in the wild increased from autumn to winter but changed little if at all thereafter (Fig. 6.10), in sharp contrast to the pattern observed in the laboratory (Fig. 6.9b) where there was a significant increase in GI in summer.

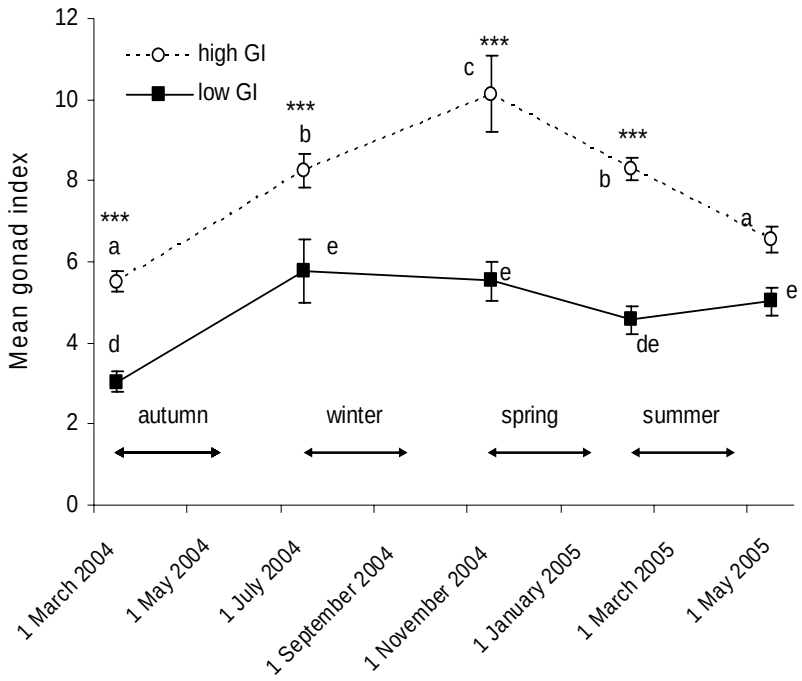

Figure 6.10 Differences in gonad index (mean ± 1 SE) for the High initial GI and Low initial GI populations of wild kina collected from Motuara Island on five occasions from 27 March 2004 to 5 May 2005. Means which did not differ significantly (p > 0.05) within both series, according to post-hoc Bonferroni tests, are labelled with common letters. Significant differences between the two populations within each season are denoted *.**

6.4.6 Increase in gonad index

The increase in gonad index (Y) was strongly influenced by both season and population (i.e. initial GI), and interactions between season and both population and temperature ($P < 0.0001$ in all cases, Table 6.8). Increases in GI rose from 57.0 ± 2.5 kg/tonne in autumn and winter to 99.9 ± 2.5 kg/tonne in spring (Fig. 6.11a), but the effect of season was greater in the low GI population than the high GI population, with increase in GI increasing steadily over the course of the study and peaking at 123.0 ± 3.6 kg/tonne in summer (Fig. 6.11b). In the high GI population, by contrast, the increase in GI rose markedly from autumn/winter to spring/summer, but showed little change otherwise (Fig. 6.11b). Seasonal changes were also influenced by temperature regime, being relatively muted in urchins held at constant temperature but much more evident in urchins held at ambient temperature, with a rapid increase from winter to summer and some evidence of a seasonal minimum in winter (Fig. 6.11c).

Table 6.8 Significance tests and related statistics (degrees of freedom, mean square, F ratio, *P* value) for the ANOVA model used to analyse variation in increase in GI.

Effect	*df*	Mean square	F-ratio	*P*
population	1	2073.25	139.23	< 0.0001
temperature	1	36.85	2.47	0.116
season	3	1143.06	76.76	< 0.0001
season × population	3	130.75	8.78	< 0.0001
season × temperature	3	288.81	19.40	< 0.0001
population × temperature	1	81.55	5.48	0.020
season × population × temperature	3	17.92	1.20	0.307
Error	937	14.89		

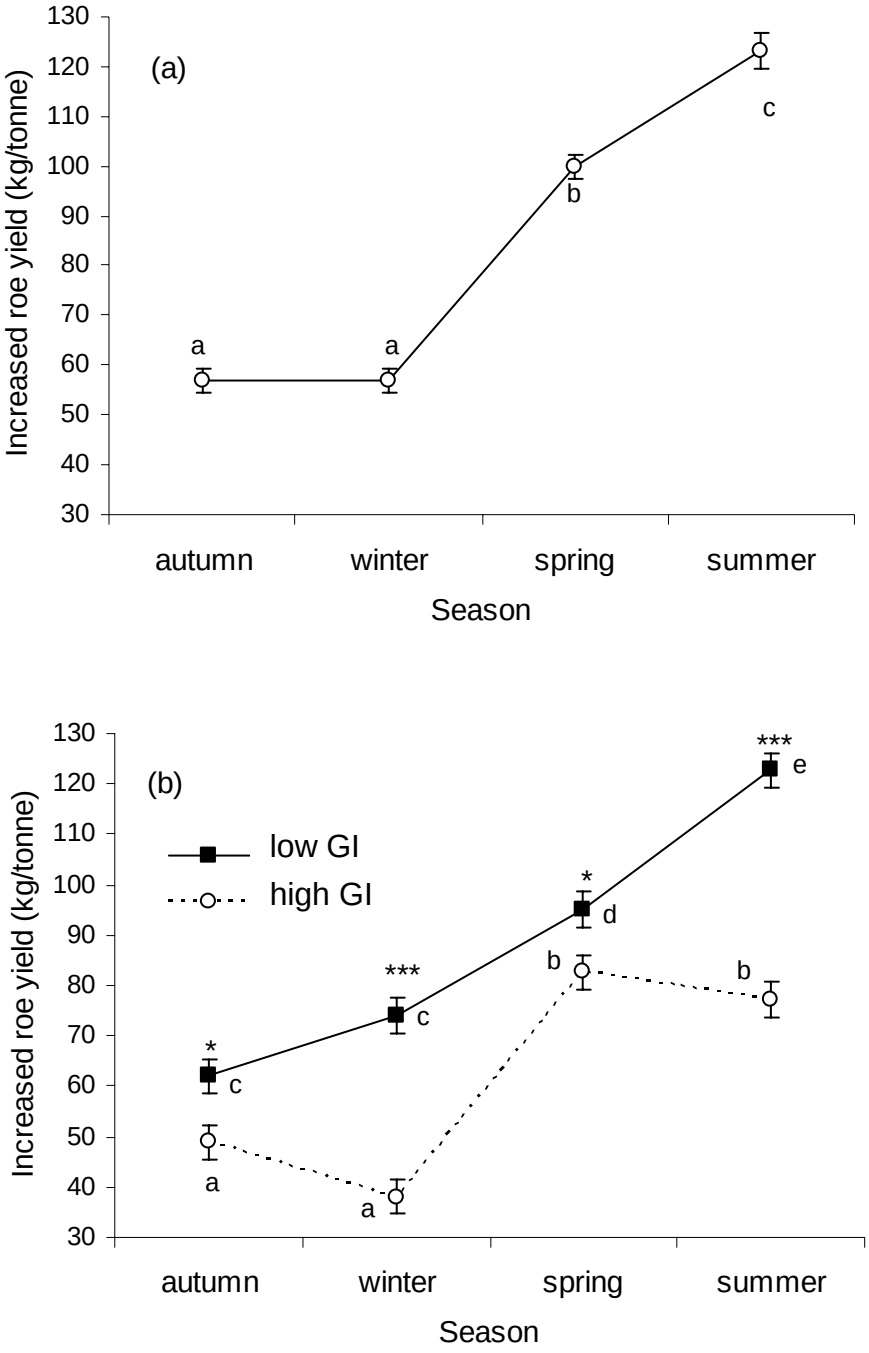

Figure 6.11 Differences in mean increased gonad yield (±1 S.E.) for the ANOVA model summarised in Table 6.8. Successive panels show means for (a) season, (b) season × population, and (c) season × temperature treatment. Means which did not differ significantly ($P > 0.05$) within each series, according to post-hoc Bonferroni tests, are labelled with common letters. In (b) and (c), significant differences between the two series within each season are denoted * ($P < 0.05$) or * ($P < 0.001$).**

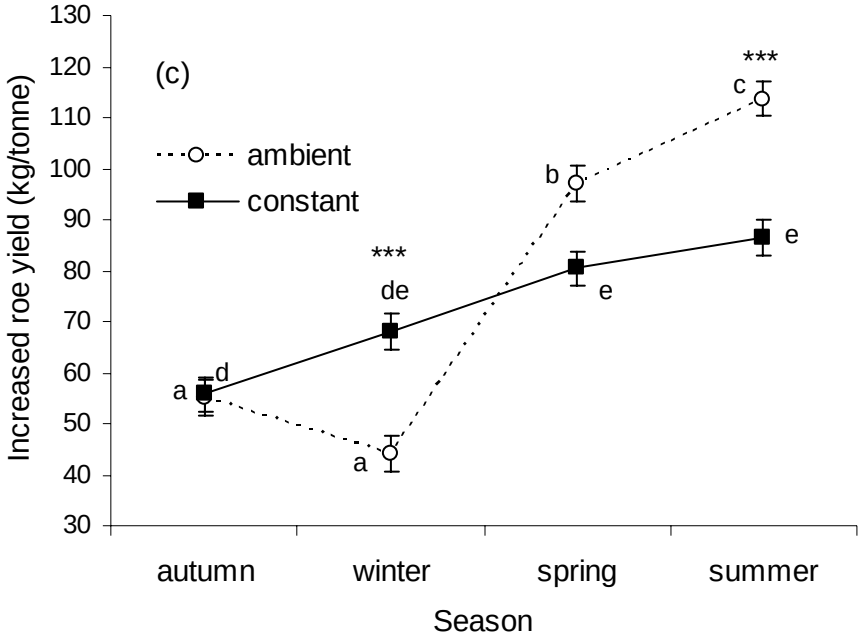

Figure 6.11 Continued. Differences in mean increased gonad yield (±1 S.E.) for the ANOVA model summarised in Table 6.8. Successive panels show means for (a) season, (b) season × population, and (c) season × temperature treatment. Means which did not differ significantly ($P > 0.05$) within each series, according to post-hoc Bonferroni tests, are labelled with common letters. In (b) and (c), significant differences between the two series within each season are denoted * ($P < 0.05$) or * ($P < 0.001$).**

6.5 Discussion

It is well documented that ambient seawater temperatures have a significant effect on the reproductive cycle of wild urchins in a number of species (Pearse and Cameron, 1991; Brockington and Clarke, 2001; Garrido and Barber, 2001; Siikavuopio et al., 2006), including *E. chloroticus* (Walker, 1982; Buisson, 2001; Lamare et al., 2002) and that it is possible to manipulate the reproductive cycle in some echinoid species with changes in temperature (Pearse and Cameron, 1991). There have been very few long term studies investigating the effects of season independent of seasonal

temperature variations on urchin roe enhancement. Garrido and Barber (2001) investigated the effects of food ration and temperature on gonad growth of *S. droebachiensis* in winter and summer and found that food availability was the most important factor regulating the relative size of gonads throughout the year for this species. Shpigel et al. (2004a) repeated experiments studying the effects of photoperiod and temperature on the reproduction of *Paracentrotus lividus* in spring-summer and winter-spring and found that gonad growth and gametogenesis occurred throughout the year in this species at low water temperatures but that higher water temperatures resulted in a significantly lower GI in both spring-summer and winter-spring. Siikavuopio et al. (2006) held *S. droebachiensis* at a range of temperatures and found that there were differences in the feed intake, food conversion rates and GI values of *S. droebachiensis* held at different temperatures in summer compared to those held in winter and that optimal gonad growth was achieved by the urchins at higher temperatures in the summer than in winter.

Previous studies (Barker et al., 1998; Buisson, 2001; Fell, 2002) have repeated roe enhancement experiments on *E. chloroticus* more than once throughout a 12 month period and these have shown that seasonal environmental changes and their associated changes in ambient seawater temperature play a significant role in the gonad development of this species. However, as with other species there is no specific information on the effects of seasonal factors, independent of seasonal temperature changes and light regimes, on the roe development and enhancement of *E. chloroticus*. However, in a scenario where urchins may be brought to onshore holding facilities with temperature control for roe enhancement it is important to know whether potential yields are likely to vary seasonally.

This study showed season had a significant effect on gonad development with the GI of urchins being significantly higher in summer and spring than during autumn and winter (Fig 6.9a). There was also a gradual increase in the final GI of the experimental urchins throughout the seasons from autumn through to spring in the high GI population and through to summer in the low GI population. The latter reflects the normal reproductive cycle of *E. chloroticus* with spawning occurring in early autumn, followed by a dormant period in winter and a gradual buildup in GI until the urchin is once again in spawning condition in mid to late summer when ambient seawater temperatures are at their highest (Dix, 1970b; McShane et al., 1996; Barker, 2007; Lamare et al., 2002). This supports the findings of this study, and a number of previous studies (Barker et al., 1998; Siikavuopio et al., 2006), where there was a strong correlation between ambient water temperature (correlated to season), feeding rates and GI.

In this study the final GI values were significantly higher than the initial wild GI values for both temperature and population treatments showing that, although the degree of enhancement varies, it is possible to significantly enhance the roe of urchins in a 10-week period regardless of initial GI condition or holding temperature. The high initial wild population had a slow build up in GI value through autumn and winter to a peak in spring and a subsequent decrease in summer. This pattern was also reflected in the histological results with the urchins being in the recovery stage in autumn and the partly spawned or spent stage in summer. In contrast, the low initial wild population also had a slow build up in GI value through autumn and winter and into spring but then had a sharp and significant increase from spring to summer. However, the histological results for the low initial GI wild urchins were very similar to the high initial GI wild urchins, both being in the recovery stage in autumn and the

partly spawned or spent stage in summer. The significant increase in both the GI values and the increase in GI, particularly in the low initial GI population in summer, provides evidence that urchins are capable of rapid growth in gonad size regardless of the reproductive stage, or season when feed availability is not restricted. This would also indicate that the differences in GI values in the wild populations are not a reflection of differences in the reproductive cycle but instead are due primarily to the feed availability of the urchins in the wild.

The ambient seawater temperatures recorded in this study follow a normal seasonal pattern with temperatures decreasing through the autumn period to a winter low and then increasing through spring to a summer high. The latitudes of the collection sites (Site 1: 41^{o}05.972'S and Site 2: 41^{o}06.00'S) are similar to that of the experimental facility at Mahanga Bay (41^{o}17.56'S) (Fig. 6.1). The seawater temperatures are similar between these two sites and the AMB temperatures reflect the temperatures that the urchins would have been exposed to had they remained in the wild. The urchins held at constant temperature (~14^{o}C) throughout winter, spring and summer had no significant differences in GI but the urchins held at the CONST treatment in autumn had a significantly lower GI than those in each of the other seasons (Fig 6.9c). This may have been influenced by the slightly lower temperatures (average difference 1.70^{o}C) experienced in the CONST treatment during autumn as a result of the heat/chill units being unable to maintain constant temperature in the system but it is unlikely this alone would account for such a significant difference in GI values. In autumn the urchins in this study (both male and female) were mostly in the recovery stage with some at the growing stage. These results indicate that although temperature is the main driver of roe enhancement, during autumn, when the urchins are held at constant temperature and photoperiod with excess food, they are

not capable of converting available energy into gonad development to similar levels that are achieved under the same holding conditions during winter, spring or summer.

The relatively consistent final GI value during winter, spring and summer at constant temperature indicates that the strong seasonal differences apparent in urchins held at AMB temperatures are most probably due to seasonal differences in temperature rather than reproductive cycle, although reproductive cycle does appear to play a role in autumn. There were also no significant differences in the GI values of urchins held at either CONST or AMB temperatures in autumn and spring when the water temperatures in the two treatments were similar. However, when there was a difference in the water temperatures between the CONST and AMB treatments (in summer AMB was warmer than CONST and in winter AMB was cooler than CONST) there were corresponding significant differences in GI between the urchins held in the two temperature treatments supporting the theory that temperature is a greater influence on gonad development than any other seasonal effect.

A feature of growth in sea urchins is that those found in environments with high feed availability grow faster and subsequently larger than those found in environments where feed is limited. This has been shown for a number of species: *Centrostephanus rodgersii* (Andrew and Byrne, 2001), *Paracentrotus lividus* (Boudouresque and Verlaque, 2001), *Echinometra* sp. (McClanahan and Muthiga, 2001), *S. droebachiensis* (Scheibling and Hatcher, 2001) and *Strongylocentrotus franciscanus* and *Strongylocentrotus purpuratus* (Tegner, 2001). It is likely that the two groups of urchins used in the current study also show a difference in size as a consequence of feed availability with those from the western site being smaller than those from the eastern site. Although the urchins are possibly the same age (the populations have been observed there for several years and there is little or no

recruitment of juveniles at either site) the difference in size between the two groups could pose difficulties in terms of a comparison of GI values and increase in GI values. The only previous study on the effects of size on roe enhancement of *E. chloroticus* (Barker et al., 1998) was conducted on a range of relatively small individuals (30-40, 50-60, and 70-80 mm size classes). This study did show a size effect on roe enhancement but this is probably because to the size classes included small sexually immature urchins (sexual maturity in the species ranges from 35-75 mm depending on the location (Barker, 2007). In addition, the size range used only covered the lower spectrum of the total size range of the species, which can grow up to 190 mm. Pearce et al. (2004) also studied the effects of urchin size on roe enhancement in *S. droebachiensis*, comparing the effects of size across the full size range of the species (the study tested urchins ranging in size from 30.0-39.9, 40.0-49.9, 50.0-59.9 and 60.0-73.0 mm). The study found that urchin size had little effect on the urchin final GI values when compared across the middle two size ranges (40.0-49.9 and 50.0-59.9mm) in urchins fed both artificial diet and natural algae. The study also found that urchin size had a similar (i.e. little or no effect) effect in terms of the increase in GI of urchins fed natural algae and had even less effect on those fed an artificial diet where there was no significant difference between three of the four size classes of urchins tested (40.0-49.9; 50.0-59.9 and 60-73 mm). The results of these two studies indicate that although urchin size can have an effect on the GI value and increase in GI values of roe enhanced urchins it is unlikely to have a significant effect on urchins taken from the middle size range of the species as in the case of the present study. In terms of a statistical comparison of GI and increase in GI values. Packard and Boardman (1999) recommend the use of graphical analysis and analysis of co-

variance to normalise physiological data for variation in body size and these have been included in the present study.

The initial gonad condition (GI), or preconditioning, at the start of the enhancement period had a significant effect on both the final GI and the increase in GI. The results appear to support the observation that there is a 3-month delay in the development of the low initial GI population compared with high initial GI population, with the final GIs of the low GI population being similar to the final GIs of the high initial GI population from the previous trial. However, the histological results show that the reproductive stages of the two populations are relatively similar throughout the year so it would appear more likely that the differences in the increase in GI are due to a combination of feed availability, relatively high temperature and the initial GI condition of the urchins rather than the relative reproductive stage of the two populations. Maximum GI values are reached by the initial high population in spring prior to spawning events in summer but for the low initial GI population the maximum final GI was achieved in summer, despite the urchins being at the spent stage. Hill and Lawrence (2006) found that the urchin *Arbacia punctulata* had a stress tolerant life strategy which enabled it to cope better with two types of stress (high temperature and starvation) than the urchin species *Lytechinus variegatus* which has a competitive-ruderal (low stress) life strategy. Similarly, the significantly higher survival of the low initial GI population during this study indicates that this population has a higher tolerance to the stresses associated with capture and subsequent enhancement than the high initial GI population. The very low feed availability at the low initial GI population site would expose these urchins to a relatively high stress (i.e. low feed availability) environment which may in turn increase their stress tolerance and enable them to adapt better to the enhancement

environment where there is excess feed available. This may also account for the significantly higher increase in GI in the low initial population compared to the high initial population. In terms of aquaculture the higher survival of urchins that are taken from a higher stress environment (low initial GI in the present study) make them ideally suited for roe enhancement.

There are a number of implications for commercial roe enhancement of *E. chloroticus* from the current study. The results clearly show that it is possible to significantly increase the GI of captive sea urchins in a 10-week enhancement period regardless of the season, reproductive stage, temperature regime or initial condition of the urchins, but that these factors play a significant role in both the final GI values of the urchins and the increase in GI. There is a significant temperature effect on the increase in GI (AMB treatment) with higher increases in urchins held at higher temperatures. There is also a much weaker seasonal effect, likely to be related to the reproductive stage of the urchins (CONST treatment), with increases in GI being significantly higher in spring and summer (Fig. 6.9c). The results indicate that it would be more effective to increase the productivity of sea urchins by capturing them in cooler water and enhancing them onshore with temperature controlled facilities or at a land or sea-based site with relatively warmer water. The increase in GI results show that urchins from the low initial GI population had a significantly higher increase than the high GI population and significantly higher survival rates throughout the year. There is an overabundance of low GI urchins in the coastal waters of New Zealand and these urchins are relatively easily found and fished compared to high initial GI urchins for which fishing pressure is high. Therefore, for commercial enhancement it is likely to be more profitable to fish and enhance low GI urchins.

Chapter Seven

The effects of season, temperature and photoperiods on the gonad development of *Evechinus chloroticus*

P. James and P. Heath

[Accepted with minor changes in 'Aquaculture' on 19 Oct 2007]

7.1 Abstract

A series of experiments testing the effects of seasonality, temperature and photoperiod was carried out to investigate the effects of biotic (gametogenesis) and abiotic factors (temperature and photoperiod) on roe enhancement of *Evechinus chloroticus*. Urchins were collected and held at 10, 14 and 18^{o}C and in 6, 12 and 18 h light in three 10-week experiments during winter, summer and autumn. Groups of 20 urchins (mean test diameter = 85.9 ± 0.2 mm; mean wet weight = 264.4 ± 1.5 g) were held in baskets and fed, *ad libitum*, a formulated moist feed.

Seasonal effects on the final urchin gonad index (GI) of each experiment reflected the GI of the urchins at the beginning of each experiment. There were seasonal effects observed for the increase in GI values in the experimental urchins but these were restricted to a higher increase in GI in summer (10.7 and 12.5 kilo of roe / tonne of wet weight urchins in spring and winter respectively). Urchins held at 18^{o}C had significantly higher GI values and greater increases in GI than those held at 14^{o}C, except in summer when there was no significant difference between the final GI

values of these two temperature treatments. Urchins held at 14^{o}C had significantly greater gonad growth and increase in GI than those held at 10^{o}C in each of the three experiments. In terms of the economics of roe enhancement, the benefits of increases in GI must be weighed against any increase in holding temperatures in land-based holding systems, or in transporting urchins to sites with warmer ambient temperatures in sea-based holding systems. Photoperiod appeared to have no effect on gonad growth except in summer when the short day treatment (6 h L) had significantly lower GI values and a significantly lower increase in GI than in the other treatments (12 or 18 h L). Season had a limited effect on gonad colour but temperature and photoperiod had no effect. None of the experimental factors had a significant effect on urchin survival. The reproductive stage of the experimental urchins advanced with increasing temperature. Increasing day length appeared to have little effect on gonad growth, increases in GI or the progress through the reproductive stages of the experimental urchins. The exception to this was in summer when the GI was significantly lower in the 6 h L treatment. Seasonal changes in the reproductive cycle of *E. chloroticus* do not appear to have a significant effect on gonad growth other than during periods when there are a high percentage of urchins in the 'spent' stage (summer).

Keywords: Seasonality, temperature, photoperiod, *Evechinus chloroticus*.

7.2 Introduction

In New Zealand the domestic market for the sea urchin *Evechinus chloroticus* (Val.) has developed significantly over the past few years. Sea urchin roe is now commonly sold in supermarket chains and a range of other fish retail outlets, increasing the demand for consistent quantities of suitable quality sea urchin roe. This demand, and a surge of interest in the fishery, has meant that the value of sea urchin quota has increased markedly, although surprisingly the domestic retail value of sea urchin roe has remained relatively constant. Fishing consistent quantities of sea urchin with suitable quality roe to supply the domestic market has always been difficult in New Zealand due to inclement fishing conditions, poor roe colour, and inconsistent or low roe yields in various areas of the country (P. Herbert, Sea Urchin New Zealand, pers comm.; C. McManaway, NZ Sea Products Ltd, pers comm.; James et al., 2004; James, 2006a and 2006b). The worldwide interest in the enhancement of wild caught sea urchins in land- or sea-based facilities has been echoed in New Zealand, particularly in the far south of the South Island of New Zealand where the weather often disrupts the fishery and the fishing areas are very isolated, causing considerable disruption and loss of income to sea urchin fishers and processors in this area. It would be advantageous to be able to hold sea urchins in land- or sea-based holding facilities for extended periods of time (2-10 weeks) to maintain and/or improve the quantity and quality of the roe in order to provide consistency of supply to the domestic market. In addition, there are numerous sea urchins (individuals) in New Zealand that are easily accessible but have small and relatively poor roe quality and are uneconomic to fish (Barker, 2007; James et al., 2004; James et al., 2007). James et al. (2007) showed that sea urchins that have poor initial roe quality can perform better

in terms of roe enhancement than can sea urchins that have good initial quality. These poor quality sea urchins would be ideal for roe enhancement and it would be advantageous to use them, as currently they cannot be utilised in the New Zealand fishery.

There has been extensive international research into the factors that effect sea urchin roe enhancement (Pearse and Cameron, 1991; Klinger et al., 1997; Robinson and Colborne, 1997; Lesser and Walker, 1998; Walker and Lesser, 1998; Lawrence et al., 2001). The key factors that have been identified are the availability of an effective artificial diet, the reproductive condition of the urchins, temperature and photoperiod. However, factors such as temperature and photoperiod have species-specific effects and almost all of the research has been done on northern hemisphere sea urchin species (McBride et al., 1998; Walker and Lesser, 1998; Spirlet et al., 2000; Brockington and Clarke, 2001; Buisson, 2001; Garrido and Barber, 2001; Kelly, 2001; Shpigel et al., 2004; Siikavuopio et al., 2006). Brewin (1994) postulated that the cue for gametogenesis in *E. chloroticus* is increasing photoperiod and that manipulation of photoperiod may influence the gametogenic cycle when *E. chloroticus* is collected prior to being exposed to the lengthening day cue in the wild. The recommendations from Brewin's study were that further research should be undertaken to understand the effects of both photoperiod and temperature on gonad growth and gametogenesis of *E. chloroticus*. Buisson (2001) tested the effects of exposing *E. chloroticus* to photoperiods six months out of phase to ambient photoperiods. Unfortunately the results were compromised by the urchins being exposed to higher temperatures in the summer photoperiod treatment compared to those in the winter photoperiod treatment. The former had significantly higher GI values but there was little difference in the histology of the urchins exposed to the two

treatments and there were no conclusive results from the study. James et al. (2007) ran a series of 10-week experiments during the austral autumn, winter, spring and summer to investigate the seasonal effects, both dependent and independent of seasonal temperature variation, on roe development (enhancement) of *E. chloroticus* over a 12 month period. The study showed that there is a strong seasonal effect on both GI values and increase in GI, primarily caused by seasonal variation in temperature. These studies indicate that both temperature and photoperiod can have an effect on roe enhancement but that these effects have not been quantified for *E. chloroticus*. Nor are there any studies to show whether there is any interaction between these two factors and whether the effects are consistent with changes in season and reproductive condition.

The aim of the study was to clarify the individual and combined effects of varying temperatures and photoperiods on the survival, gonad index, colour and reproductive development of wild caught *E. chloroticus* held and fed artificial diets in land-based holding systems for 10-week periods during three seasons over one year. The results are also discussed in terms of potential roe enhancement of *E. chloroticus* in New Zealand.

7.3 Material and methods

7.3.1 Collection site

Sea urchins were sourced from a site at Port Gore in the outer Marlborough Sounds (41^{o}01.173'S 174^{o}16.105'E) in the north of the South Island of New Zealand (Fig. 7.1) at the beginning and end of each of the experiments which started on 12 August 2005 (spring), 9 December 2005 (summer) and 19 April 2006 (winter). The site

consisted of a rocky reef with macroalgal species dominant to a depth of approximately 6 m. Below this, the seafloor consisted of bare rock and sandy/gravel with very large, bare rocks present throughout the site. The sea urchins at this site were present at depths of between 6-10 m.

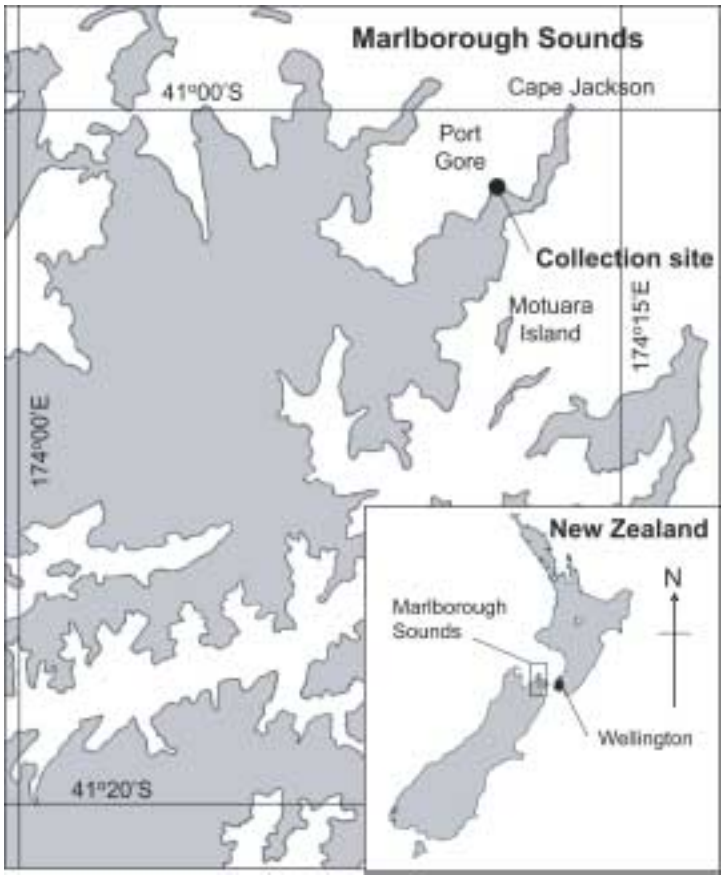

Figure 7.1 The location of the collection site in the Marlborough Sounds at the top of the South Island of New Zealand.

7.3.2 Urchin collection

The urchins were collected on snorkel and were placed in mesh bags (approximately 60 urchins per bag) before being brought onboard the fishing vessel. Once onboard the vessel, the bags were covered with felt sacking and kept damp with sprayed seawater for the transfer to the laboratory. The urchins were then transferred by road

to the Mahanga Bay Research Facility maintained by the National Institute of Water & Atmospheric Research Ltd. (NIWA), Wellington (a total journey of approximately 5 h). Collections were made prior to and at the conclusion of each of three experiments.

7.3.3 Holding systems

On arrival at Mahanga Bay in Wellington Harbour the urchins were transferred from the mesh bags and randomly allocated to 36 experimental dark blue plastic holding baskets (570 mm long x 370 mm wide x 230 mm deep) at a density of 20 urchins per basket (31 urchins m^{-2} internal surface area). Each basket was placed in one of 36 black polyethylene tanks (640 mm long x 440 mm wide x 300 mm deep). Each tank had an air-stone under the middle of the basket. The 36 tanks were divided into three systems (3 rows of 12 tanks) and each system had seawater delivered at a pre-determined temperature. Each system had a 225 l reservoir tank from which each of the 12 tanks was supplied with 12.6 l/min, 10 μm-filtered seawater via a pump drawing water from the reservoir tank. The water was returned to the reservoir tank from the tank's outlets via an outflow drain. Each of the three systems had 20 l/min supply of ambient 10-μm filtered seawater flowing into the reservoir tank. Each system had a 6 kw (heating) reverse cycle heat pump with a stainless steel and polyethylene heat exchanger installed between the reservoir tank and the outlet manifold that regulated the seawater temperature in the system to a nominal 10.0^{o}C, 14.0^{o}C and 18.0^{o}C respectively. The systems utilised Onga 142 1500w pumps to ensure adequate flow through the heat exchangers, into each of the 12 tanks in each of the three systems and back to the respective sumps (Fig. 7.2).

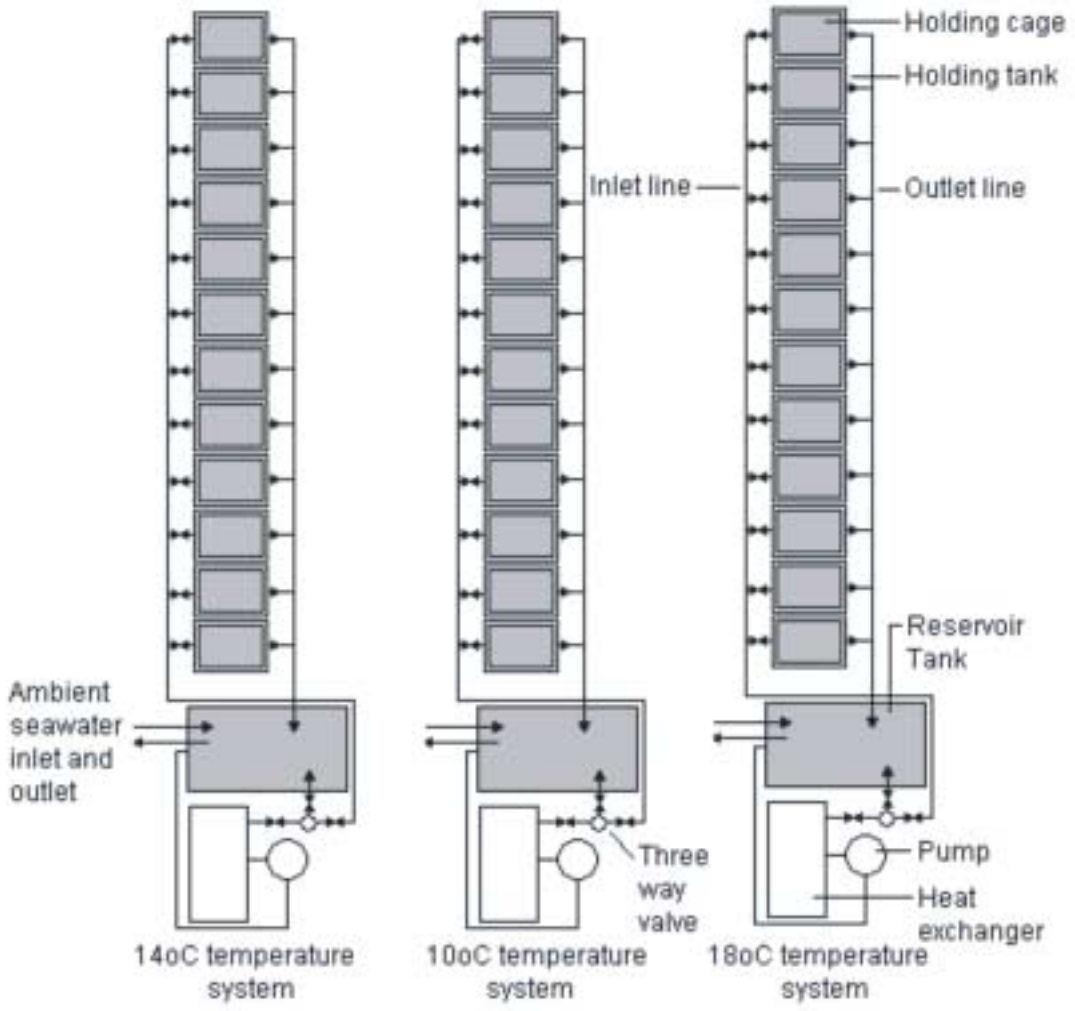

Figure 7.2 A schematic diagram of the holding system used to maintain the water flows and temperatures in the experimental tanks.

Each tank was fitted with a black polyethelene lid with a 50 mm lip that folded down around the tank to ensure that no light would enter the tank. Each lid was centrally fitted with a Oases Lunaqua 1 Lamp (Oase GmbH, Hörstel, Germany) with a 5 watt (12V) halogen bulb. Each light was connected to a switching control box that maintained the photoperiod at either 6 h light :18 h dark (6L:18D), 12 h light:12 h dark (12L:12D) or 18 h light:6 h dark (18L:6D). The 12 tanks in each of the three

systems were randomly divided into 4 replicates of each of the three photoperiod treatments.

A stowaway Tidbit™ temperature logger (Onset Computer Corporation, Massachusetts, USA) was placed in each of the three reservoir tanks. Each temperature logger recorded the ambient seawater temperature once every hour for the duration of the experiment. The ambient photosynthetic incident light level (µmol photons m^{-2} s^{-1}) from the lamps was recorded using a LI-COR (LI-192SB) underwater quantum sensor probe (Li-cor Ltd, Nebraska, USA) connected to a Kiethley 197 auto-ranging micro-ameter (Kiethley Instruments, Ohio, USA) placed directly against the lamp surface. The dissolved oxygen, pH and ammonia were tested once per week in each of the three holding systems and in the sump tanks of each system using a WTW (CellOx 325) probe and a WTW pH Electrode (SenTix 81) probe connected to a WTW Multi (340i) water quality meter (all WTW equipment manufactured by Werkstatten GmbH & Co., Weilheim, Germany) and a Palintest ammonia comparator kit (Palintest UK, Tyne & Wear, United Kingdom) respectively.

7.3.4 Urchin Husbandry

On their arrival at the laboratory the urchins were placed into baskets and held in water temperatures and photoperiods equivalent to those recorded at the collection site in the Marlborough Sounds. The urchins were then acclimated to the appropriate experimental temperature and photoperiod treatments at a rate of 1^{o}C (temperature), and 1 h (photoperiod) per day until all of the replicate baskets had reached their experimental temperatures and photoperiods.

The NIWA artificial sea urchin roe enhancement diet (James et al., 2004) was fed three times per week to all of the urchins throughout the experimental period at a rate of 1.0% mean urchin body weight per day (the urchins were still satiated at a feeding rate of 1.0%). Any uneaten food from previous feeds was removed from each basket during feeding but food that was not easily accessible was left in the baskets.

The systems were cleaned each week by removing each basket from its tank and placing it in a spare tank containing seawater at the correct temperature for the system that was being cleaned (i.e. 10, 14 or 18^{o}C). Each basket containing the urchins was sprayed clean with seawater of the appropriate temperature and the empty holding tank was cleaned with detergent and chlorine before being rinsed, refilled and the basket placed back into the tank. This was repeated until all 36 tanks in each of the three systems had been cleaned.

During cleaning any urchin mortalities were observed, recorded and removed from the experimental tanks but were not replaced.

7.3.5 Experimental methods

Ten to 12 weeks has been shown to be a suitable roe enhancement period for *E. chloroticus* (Barker et al., 1998; Buisson, 2001; Fell, 2002; James et al., 2004, James, 2006a and 2006b). In this study, three 10-week experiments were repeated on three occasions to determine the effects on roe enhancement of water temperature (10, 14 and 18^{o}C), photoperiod (6L:18D, 12L:12D and 18L:6D), and season.

In each experiment, nine treatments were established by holding urchins in one of three temperatures and three photoperiods, with four replicate cages per treatment. The nine treatments were thus:

1. $10^{o}C \times 6L:18D$
2. $10^{o}C \times 12L:12D$
3. $10^{o}C \times 18L:6D$
4. $14^{o}C \times 6L:18D$
5. $14^{o}C \times 12L:12D$
6. $14^{o}C \times 18L:6D$
7. $18^{o}C \times 6L:18D$
8. $18^{o}C \times 12L:12D$
9. $18^{o}C \times 18L:6D$

To assess the effect of season independently of temperature and photoperiod, each experiment was repeated three times, starting on 12 August 2005 (spring), 9 December 2005 (summer) and 19 April 2006 (winter). This created a total of 27 experimental groups, comprising 3 temperature regimes × 3 photoperiod regimes × 3 seasons.

7.3.6 Data collection

Urchin condition, or gonad index (GI), was recorded at the start and end of each experiment, based on a random sample of 60 urchins from the wild population at the beginning of the experiment. Fifteen experimental urchins from each replicate basket at the conclusion of the experiment, and 60 wild urchins collected from the initial wild population (referred to henceforth as ‘wild’) at the conclusion of the experiment. Variables measured for each urchin were test diameter (D, mm), total wet weight (W, g), and gonad wet (unblotted) weight (G, g). These data were used to calculate GI (=

100 × G / W) and an index of increase in GI (Y) for each urchin, expressed as the difference between its GI at the end of the experiment and the mean GI ($\overline{GI}$) for the corresponding wild population at the start of the experiment (i.e. $Y = GI_{final} - \overline{GI}_{initial}$ in units of kilo of roe / tonne of wet weight urchins).

A graph comparing the test diameter against the gonad index of the wild urchins collected at the beginning of each experiment (Fig 7.3, 7.4 and 7.5) show no significant relationship between test diameter gonad index in spring (ANOVA: F = 43.00, *df* = 1,118, *P* = 0.131), summer (ANOVA: F = 36.25, *df* = 1,118, *P* = 0.919), and winter (ANOVA: F = 16.57, *df* = 1,118, *P* = 0.105).

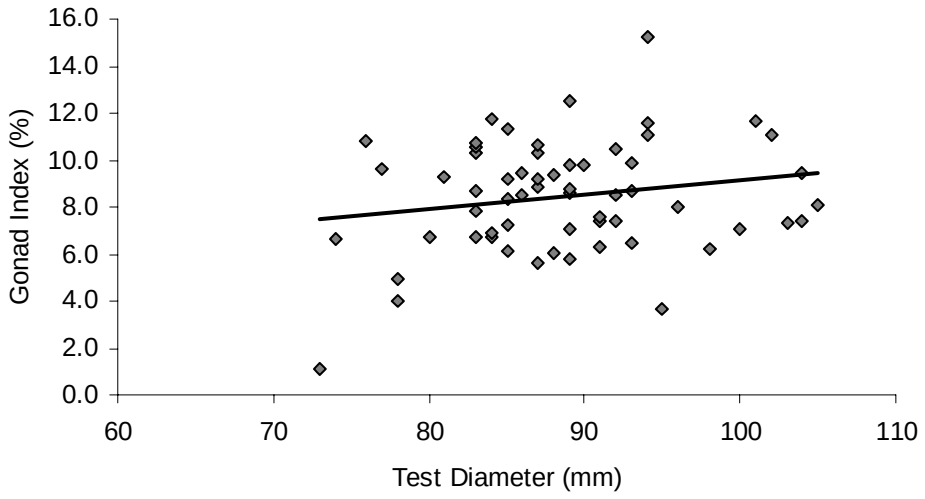

Figure 7.3 The relationship between Gonad Index values and Test Diameter for urchins collected from the initial wild sample in spring, Y = 0.063 X + 2.811; r^2 = 0.039; *n* = 60.

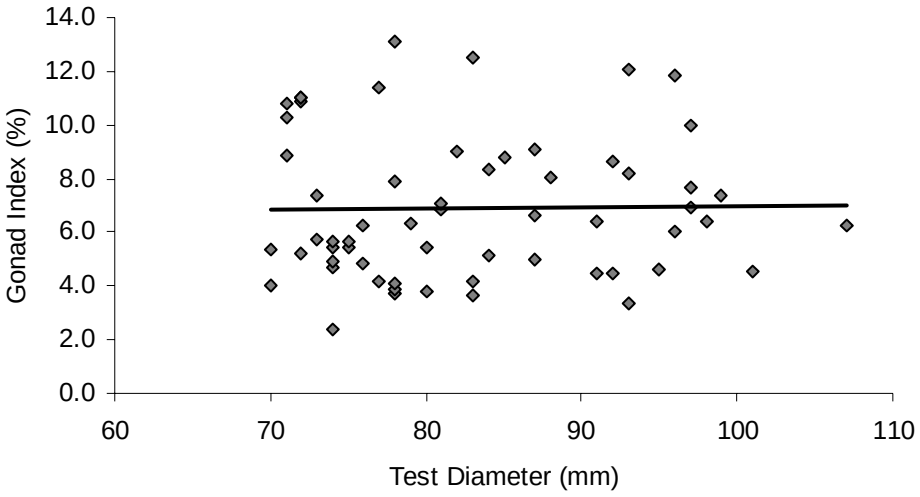

Figure 7.4 The relationship between Gonad Index values and Test Diameter for urchins collected from the initial wild sample in spring, Y = 0.004 X + 6.559; r^2 = 0.0002; *n* = 60.

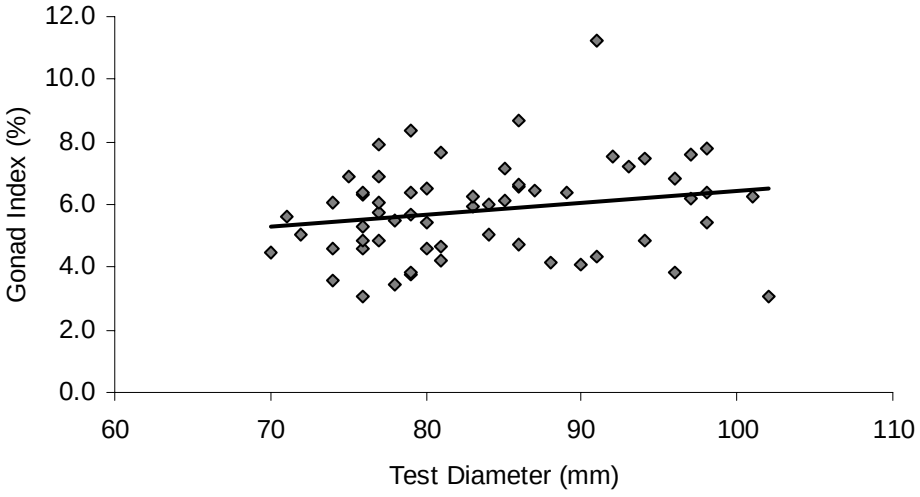

Figure 7.5 The relationship between Gonad Index values and Test Diameter for urchins collected from the initial wild sample in spring, Y = 0.038 X + 2.565; r^2 = 0.045; *n* = 60.

The colour of each urchin's gonads was objectively assessed with a Minolta CM2500D spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan) (2 pulsed xenon lamps as illumination source, diffuse illumination with 8° viewing area, with

spectral component excluded for gonad measurements) with three replicate measurements per sample averaged to give a single measurement, using the international standard CIELAB (or L*a*b) system of colour measurement. The CIELAB system measures the lightness (L*), the degree of redness or greenness (a*), and the degree of yellowness or blueness (b*).

The reproductive stage of a random sample of 15 urchin gonads from each treatment, and from the initial and final wild populations, was determined using histological analysis. Histological samples were collected, fixed and stained using standard Harris's haematoxylin stain, counterstained with eosin (Bancroft and Stevens, 1990). The reproductive condition of the initial urchin sample was assessed using the six reproductive stages described in Byrne (1990).

7.3.7 Food consumption (winter experiment only)

To measure the food consumption of the sea urchins, the normal food ration was measured into each of the 36 baskets and removed after 4 days in the third experiment. All of the food was then carefully removed after and placed in pre-weighed foil trays. This uneaten food was weighed before each tray was placed in a 60^{o}C drying over for 72 hours. The trays were then reweighed and the dry weight of uneaten food was calculated. The relationship between wet weight and dry weight of the urchin food was determined by drying food cubes. This gave a wet/dry ratio of -2.37 to convert from wet feed weight to dry feed weight for the feed initially placed into the baskets. The leach rates for the three experimental temperatures were measured and taken into consideration when calculating the consumption.

Measurement of food consumption was attempted over a 2-week period in the spring experiment and one week in the summer experiment. However, the results were compromised by the decomposition of the diets in the experimental tanks over these periods. In order to test the consumption of urchins held in groups there is a significant amount of disturbance and therefore only one attempt was made per experiment, with the measurement of consumption being successful in the third and final experiment.

7.3.8 Statistical analysis

Three-way analysis of variance was used to compare mortality, D, W, GI, and Y, between temperature, photoperiod treatments, and seasons, taking into account all possible two- and three-way interactions. These analyses allowed the description of the main effects of each factor and to identify interactions of potential importance. A two-way ANOVA was used to compare differences in the test diameters and urchin wet weights of the wild urchins collected at the beginning of each trial. One-way ANOVA was used to test for differences in GI within the temperature and photoperiod treatments for each experiment and Student t-test was used to test for differences between wild and experimental GI at the conclusion of each experiment. One-way ANOVA was used to test for differences in test diameter and wet weight of the initial wild collections for each experiment.

Gonad indices for the pooled data set exhibited slight negative skewness but were otherwise approximately normally distributed. Arcsine square root transformation of GI and Y increased rather than decreased skewness, so all analyses for these parameters were performed using untransformed data. Mortality rates were converted to percentages and arcsine square root transformed. Homogeneity of

variances was tested using Modified Levene's test. Tukey-Kramer post-hoc comparison tests were used to identify the experimental treatment means which differed significantly.

Statistical analyses were conducted using NCSS 2000 (Number Crunching Statistical Systems, Kaysville, Utah, USA). Statistical tests were considered significant if $P < 0.05$ and highly significant if $P < 0.001$. Errors and confidence intervals are expressed as ± one standard error.

7.4 Results

7.4.1 Seawater temperatures and quality

7.4.1.1 Ambient temperatures at the collection site

The ambient seawater temperatures at the collection site in Port Gore followed a natural seasonal pattern during the experimental period. The experiment began in winter (12 August 2005) when ambient seawater temperatures were low (12.2^{o}C) and slowly increased to a summer high of 17.2^{o}C after 201 days. After this the ambient seawater temperatures at the collection site decreased to 12.2^{o}C by the end of the experiments (321 days) (Fig. 7.6).

7.4.1.2 Ambient photoperiods at the collection site

The ambient photoperiods (sunrise to sunset) at the collections site in Port Gore varied with the seasons. In the spring experiment it ranged from 9:12 (hrs:mins) to 13:41, in the summer experiment from 15:01 to 13:28 and in the winter experiment from 10:47 to 9:13. The maximum day length at the site is 15:09 and the minimum is

9:13. The ambient photoperiods were taken from the sunrise and sunset tables for the 'Picton' site from the New Zealand Nautical Almanac 2005/06 Edition.

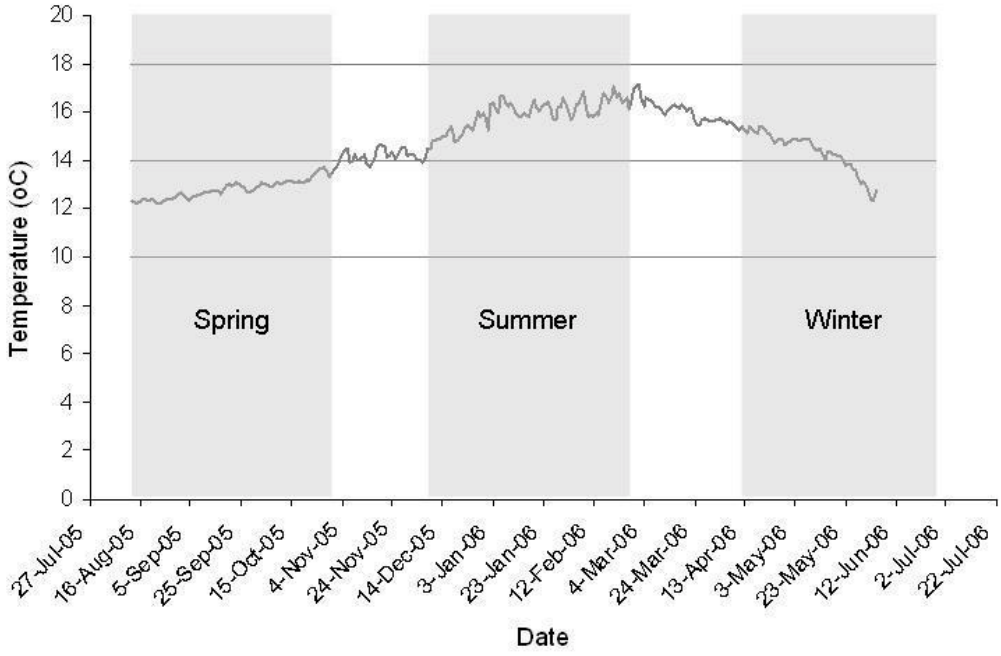

Figure 7.6 The ambient temperatures (^{o}C) recorded at the collection site for the wild population during the experimental period (grey line). The grey shaded areas indicate when the three experiments took place and the vertical lines show the three constant temperature treatments (10, 14 and 18^{o}C) the urchins were exposed to during the experimental periods.

7.4.1.3 Experimental temperatures, water quality and light level

The temperatures in each of the temperature treatments (10, 14 and 18^{o}C) remained relatively constant throughout each of the three experiments (Table 7.1).

The seawater quality (dissolved oxygen, ammonia and pH) remained within acceptable limits (Siikavuopio 2004a; 2004b; 2007a and 2007b) during each of the trials (Table 7.2). The incident light level recorded at the surface of the lamps in the

holding tanks at the beginning of the experiments (i.e. new lamps and clear covers) was 826.85 µmol photons m^{-2} s^{-1} and the average ambient photosynthetic irradiance recorded at the surface of the lamps in the holding tanks at the conclusion of the experiments was 564.00 ± 34.69 µmol photons m^{-2} s^{-1}.

Table 7.1 The mean (± 1 SE), maximum and minimum daily temperatures (^{o}C) for each of the holding systems, temperature treatments and for the wild population collection site, during the experimental periods (*n* = 70 for all treatments).

System		Wild collection site	System 1 10^{o}C	System 2 14^{o}C	System 3 18^{o}C
Spring	Mean	12.7 (± 0.03)	10.3 (± 0.07)	14.0 (± 0.04)	18.0 (± 0.06)
	Max.	13.2	12.2	14.9	18.4
	Min.	12.2	8.0	12.7	14.3
Summer	Mean	15.8 (± 0.06)	10.3 (± 0.05)	14.1 (± 0.06)	18.0 (± 0.21)
	Max.	16.8	11.4	16.4	18.4
	Min.	14.7	9.2	13.1	15.9
Winter	Mean	14.6 (± 0.10)	10.3 (± 0.01)	14.2 (± 0.07)	18.01 (± 0.03)
	Max.	15.7	10.6	17.9	18.5
	Min.	12.2	10.3	13.6	17.4

7.4.2 Urchin reproductive stage

The reproductive stage of the urchins, based on the staging and classification of the gonad reproductive state described by Byrne (1990), varied between the three seasons and between temperature treatments within each experiment.

7.4.2.1 Temperature treatments

At the beginning of the spring experiment the wild urchins were at the growing and premature stages. At the conclusion of the experiment the wild urchins were at the premature and mature stages. At the conclusion of the experiment the experimental urchins held at 10^{o}C were still at the growing and premature stages while those held at 14^{o}C were at the premature stage and those held at 18^{o}C were ahead of the wild urchins at the premature and mature stages, with spawning having begun in a small number of the male urchins (Fig. 7.7).

At the beginning of summer experiment the wild urchins were at the premature and mature stages, with the males having partially spawned. At the conclusion of the experiment the wild urchins were at the partially spent and spent stages. The experimental urchins held at 10^{o}C were at the mature, partially spent and spent stages. Those held at 14^{o}C were at the partially spent and spent stage with some of the males still being at the mature stage. Those held at 18^{o}C were at a similar stage to the wild urchins at the partially spent and spent stages (Fig. 7.7).

Table 7.2 The mean (± 1 SE), maximum, minimum dissolved oxygen, pH and total ammonia (measured as total available nitrogen) levels recorded in the experimental tanks during each of the three experimental periods.

	System & temp.		Percent dissolved Oxygen (mg/l) (n = 80)	pH levels (n = 13)	Dissolved ammonia (mg/l N) (n = 13)
Spring	System 1	Mean	94.8 (± 0.2)	8.02 (± 0.04)	0.31 (± 0.10)
	10°C	Max.	101.1	8.07	0.40
		Min.	88.4)	7.98	0.20
	System 2	Mean	93.4 (± 0.2)	7.95 (± 0.10)	0.25 (± 0.21)
	14°C	Max.	97.9	8.06	0.40
		Min.	92.0	7.85	0.10
	System 3	Mean	93.3 (± 1.3)	7.98 (± 0.07)	0.33 (± 0.06)
	18°C	Max.	104.1	8.05	0.41
		Min.	88.1	7.91	0.20
Summer	System 1	Mean	95.4 (± 2.6)	8.01 (± 0.04)	0.30 (± 0.06)
	10°C	Max.	97.3	8.10	0.36
		Min.	92.6	7.94	0.24
	System 2	Mean	95.1 (± 0.20)	8.02 (± 0.10)	0.48 (± 0.01)
	14°C	Max.	97.3	8.07	0.49
		Min.	92.2	7.85	0.47
	System 3	Mean	93.3 (± 1.3)	7.98 (± 0.07)	0.33 (± 0.06)
	18°C	Max.	104.1	8.05	0.41
		Min.	88.1	7.91	0.20
Winter	System 1	Mean	95.5 (± 0.13)	8.17 (± 0.09)	0.22 (± 0.11)
	10°C	Max.	98.4	8.35	0.44
		Min.	91.4	8.02	0.07
	System 2	Mean	94.9 (± 0.43)	8.08 (± 0.11)	0.39 (± 0.15)
	14°C	Max.	101.3	8.24	0.48
		Min.	90.0	7.90	0.16
	System 3	Mean	93.3 (± 1.3)	7.98 (± 0.07)	0.33 (± 0.06)
	18°C	Max.	104.1	8.05	0.41
		Min.	88.1	7.91	0.20

Note: Dissolved Oxygen is expressed as mg/l at the given temperatures for each experiment as listed in Table 7.1

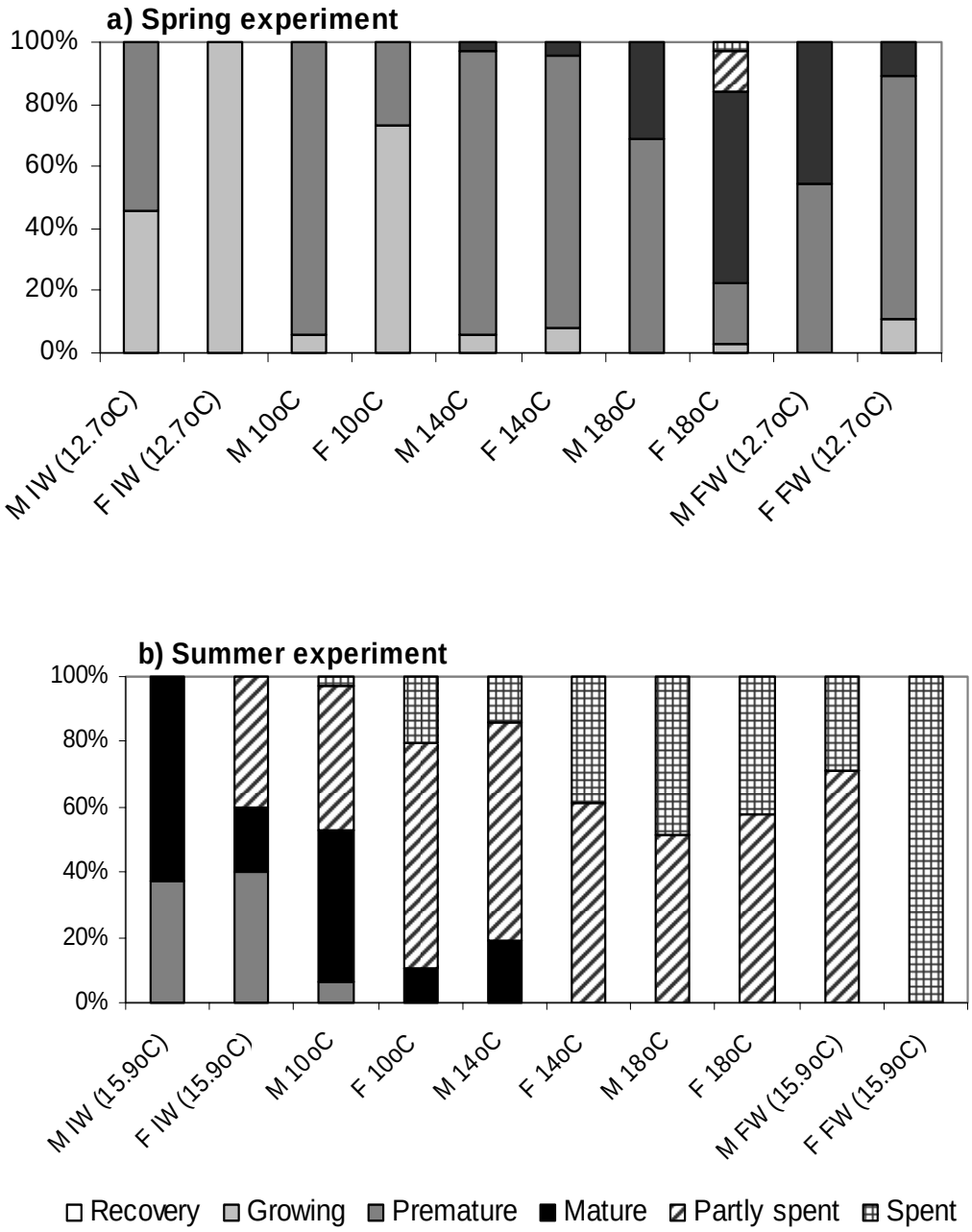

Figure 7.7 The proportion of individuals at the different reproductive stages (recovery to spent) for male (M) and female (F) urchins collected at the initial census - initial wild (IW), the conclusion of the experimental periods – final wild (FW) and for each experimental temperature for each of the three experimental periods a), b) and c). The temperature for each treatment is listed (i.e. 10, 14 and 18^{o}C) and the average temperature recorded from the wild collection site during the experimental period is given (in brackets) for the wild samples.

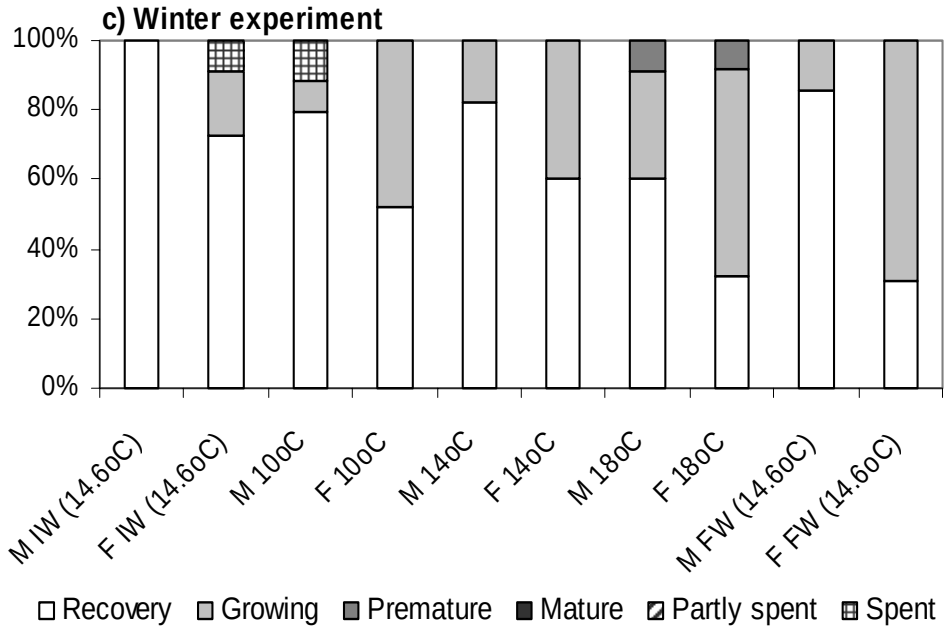

Figure 7.7 Continued. The proportion of individuals at the different reproductive stages (recovery to spent) for male (M) and female (F) urchins collected at the initial census - initial wild (IW), the conclusion of the experimental periods – final wild (FW) and for each experimental temperature for each of the three experimental periods a), b) and c). The temperature for each treatment is listed (i.e. 10, 14 and 18^{o}C) and the average temperature recorded from the wild collection site during the experimental period is given (in brackets) for the wild samples.

At the beginning of the winter experiment, the wild urchins were at the recovery stage with a small number of females being at the growing stage. At the conclusion of the experiment the wild urchins were at the recovery and growing stages. The experimental urchins held at 10^{o}C and 14^{o}C were also at the recovery and growing stages. Those held at 18^{o}C were also at the recovery and growing stages but a small percentage of urchins were at the premature stage (Fig 7.7).

7.4.2.2 Photoperiod treatments

The wild urchins were at the same reproductive stages as described for the temperature treatments at the beginning and conclusion of each experiment.

In the spring experiment, the experimental urchins held in 6h and 12 h L were mostly at the premature stage with a few urchins in the growing and mature stages. Those held in 18 h L were mostly at the premature and mature stages with some female urchins being in the partly spent stage (Fig. 7.8).

In the summer experiment the experimental urchins held in 6 h L were mostly at the partly spent stage with a few urchins in the mature and spent stages. The males held in 12 h L were at the same stages as those held in 6 h L but the females were mostly in the mature stage with a few urchins in the partly spent stage. The males held in 18 h L were widely spread across the reproductive stages with most urchins being either mature or partly spent with a few also being premature or spent. The females were mostly mature, or partly spent stages (Fig. 7.8).

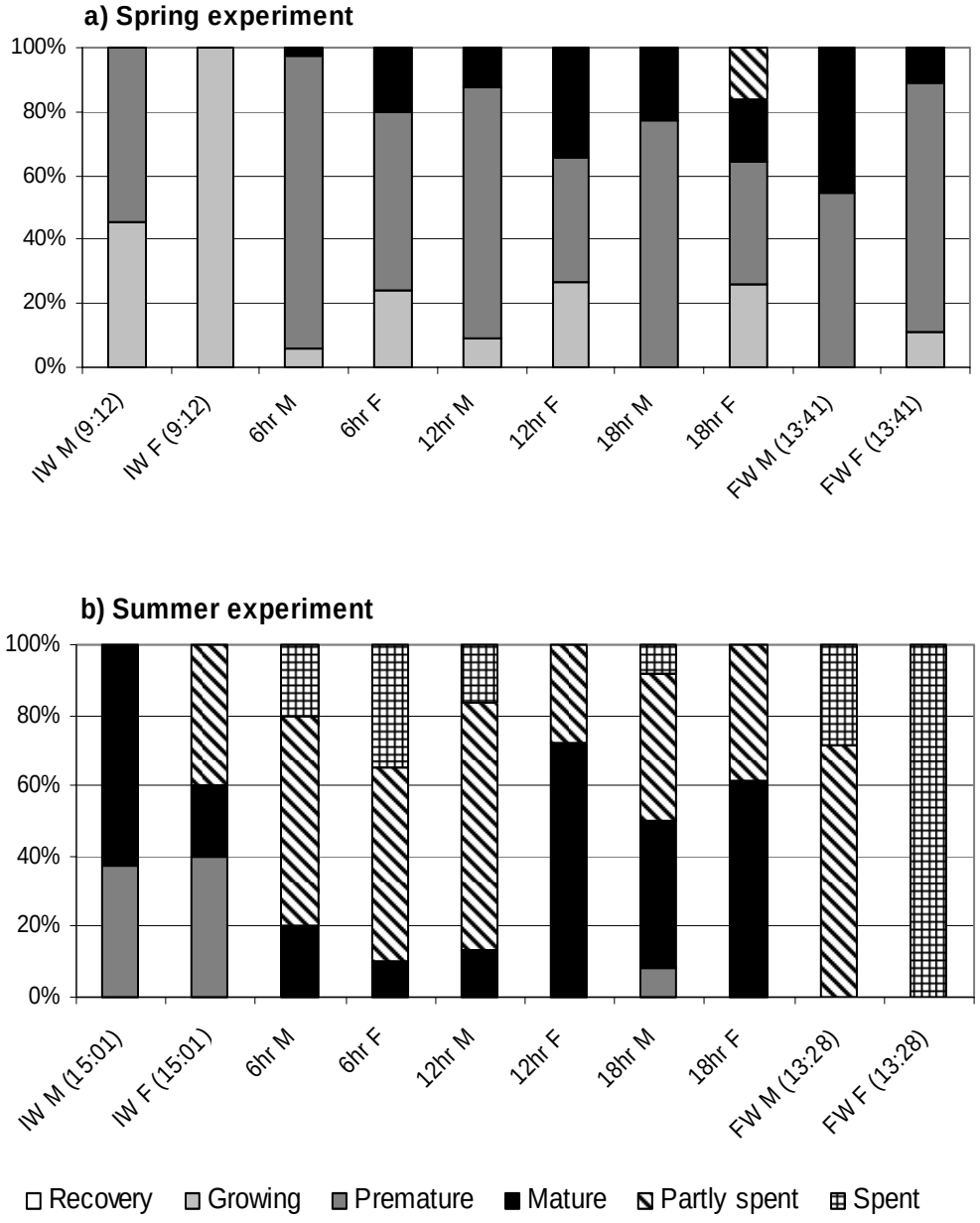

Figure 7.8 The proportion of individuals at the different reproductive stages (recovery to spent) for male (M) and female (F) urchins collected at the initial census - initial wild (IW), the conclusion of the experimental periods – final wild (FW) and for each experimental photoperiod for each of the three experimental periods a), b) and c). The numbers of hours of light for each treatment is listed (i.e. 6, 12 and 18 h) and the numbers of hours and minutes of light at the wild collection site at the time of the initial and final collections is given (in brackets) for the wild samples.

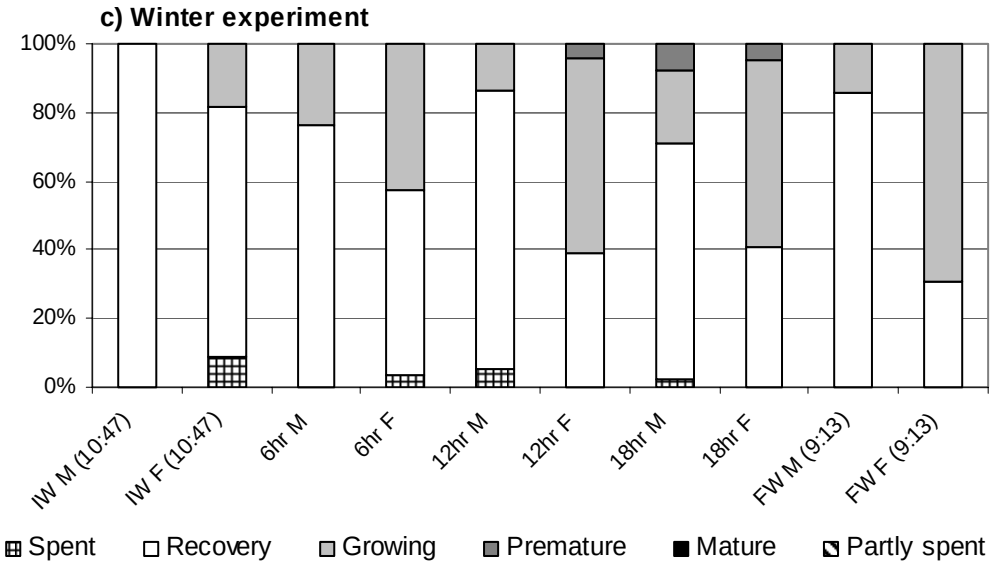

Figure 7.8 Continued. The proportion of individuals at the different reproductive stages (recovery to spent) for male (M) and female (F) urchins collected at the initial census - initial wild (IW), the conclusion of the experimental periods – final wild (FW) and for each experimental photoperiod for each of the three experimental periods a), b) and c). The numbers of hours of light for each treatment is listed (i.e. 6, 12 and 18 h) and the numbers of hours and minutes of light at the wild collection site at the time of the initial and final collections is given (in brackets) for the wild samples.

In the winter experiment the urchins held in the 6, 12 and 18 h L treatments were all at a similar reproductive stage with the males being mostly in recovery stage with a few urchins in the growing stage and the females being similar but having a higher percentage of urchins in the growing stage (Fig. 7.8).

7.4.3 Urchin survival

Urchin survival ranged from 94.3-99.6%. There was a significant difference in percent survival between the three experiments with the urchins in the spring experiment (98.5 ± 0.4%) having a significantly higher survival than those in the summer (95.7 ± 0.9%) and winter experiments (94.55 ± 0.8%). There was no

significant difference between the latter two experiments. There was a significant interaction between season x temperature, but no differences in survival between temperature treatments or photoperiod treatments and no significant interactions between season x photoperiod or season x temperature x photoperiod (Table 7.3).

7.4.4 Test diameter and wet weight

There were significant differences in the test diameter (One-way ANOVA: F = 8.1, df = 2, 180, $P < 0.001$) and wet weights (One-way ANOVA: F = 6.6, $df = 2, 180$, $P < 0.05$) of the initial wild samples from each of the three experiments. The urchins in spring had significantly larger test diameters (88.6 ± 0.9 mm) and wet weights (280.3 ± 9.3 mm) than those from the other two experiments (summer: 82.7 ± 1.2 mm and 236.8 ± 10.1 mm and winter: 83.7 ± 1.1 mm and 239.6 ± 8.7 mm).

Table 7.3 Significance tests and related statistics (degrees of freedom, mean square, F ratio and *P* values) for the ANOVA analysis of urchin survival.

Effect	*df*	Mean Square	F-ratio	*P*
temperature	2	1.94×10^{-2}	0.63	0.534
photoperiod	2	8.43×10^{-2}	2.73	0.072
season	2	0.31	10.03	0.0001
season x temperature	4	0.12	3.89	0.006
season x photoperiod	4	3.71×10^{-2}	1.21	0.314
temperature x photoperiod	4	2.26×10^{-2}	0.74	0.570
season x temperature x photoperiod	8	2.87×10^{-2}	0.93	0.494
Error	79	3.08×10^{-2}		

A comparison of results from each of the three experiments showed there was a significant difference in both the test diameter and wet weight of the urchins with the urchins in winter having a significantly larger test diameter (87.8 ± 0.3 mm) and wet weight (277.2 ± 2.8 g) than in spring (84.7 ± 0.3 mm and 258.7 ± 2.5 g) and summer (85.1 ± 0.3 mm and 258.7 ± 2.5 g respectively). There was no significant difference between the latter two experiments. There was a significant interaction between season x temperature but no differences in either test diameter or wet weight between temperature treatments or photoperiod treatments and no significant interactions between season x photoperiod or season x temperature x photoperiod (Table 7.4).

7.4.5 Urchin Gonad Index

7.4.5.1 Wild urchins

A two-way ANOVA showed that there were significant differences in the GI of the wild urchins between the three experiments (ANOVA: F = 29.54, df = 2, 360, P < 0.001) with the urchins collected in spring (9.06 ± 0.31) having a significantly higher GI than those collected in summer (6.32 ± 0.34) and winter (5.96 ± 0.19). There were no significant differences between the latter. There were no significant differences between the GI of the wild urchins samples at the beginning and end of all the experiments (ANOVA: F = 1.38, df = 1, 360, P = 0.241).

The final gonad indices for urchins taken from the wild population (6.0-9.1 %, Fig. 7.9) were consistently lower (P < 0.001 in all cases) than for urchins enhanced in each of the three respective experiments (10-19) (Fig. 7.9).

Table 7.4 Significance tests and related statistics (degrees of freedom, mean square, F ratio, *P* value) for the ANOVA analysis of urchin test diameter (mm) and wet weight (g) measured at the conclusion of each experiment.

Effect	*df*	Mean square	F-ratio	P
Test diameter				
temperature	2	44.17	0.90	0.406
photoperiod	2	50.05	1.02	0.359
season	2	1595.58	32.61	< 0.001
season × temperature	4	422.65	8.64	< 0.001
season × photoperiod	4	38.07	0.78	0.539
temperature × photoperiod	4	94.67	1.94	0.102
season × temperature × photoperiod	8	80.33	1.64	0.108
Error	79	48.92		
Wet Weight				
temperature	2	8725.15	2.34	0.096
photoperiod	2	2604.67	0.70	0.496
season	2	67301.48	18.08	< 0.001
season × temperature	4	15808.25	4.25	0.002
season × photoperiod	4	2246.92	0.60	0.660
temperature × photoperiod	4	5437.03	1.46	0.211
season × temperature × photoperiod	8	4752.82	1.28	0.251
Error	79	3722.07		

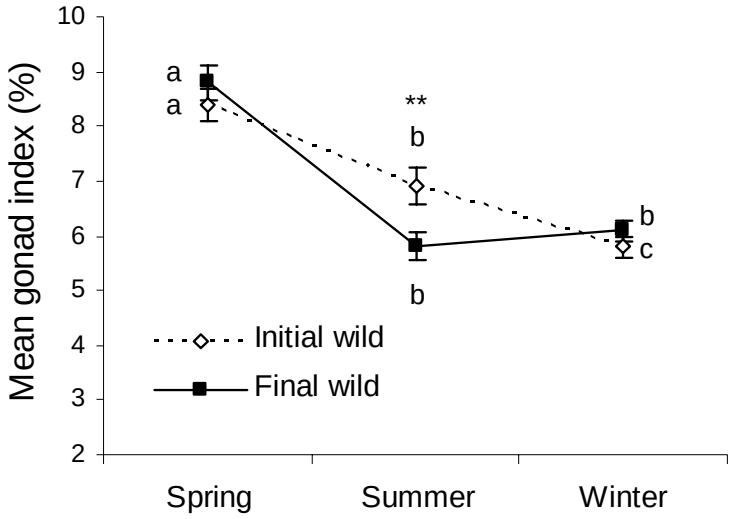

Figure 7.9 The initial and final mean GI (means ±1 SE) between the wild urchins collected from Port Gore on three occasions from 12 August to 19 April 2006. Means which did not differ significantly (*P* > 0.05) within each series, according to post-hoc Tukey test, are labelled with common letters. Differences between the two means within each experiment are denoted as ** (*P* < 0.05).

7.4.5.2 Experimental urchins

Variation in GI during the experimental study was due to temperature, photoperiod and seasonal effects. All interactions were significant other than between temperature and photoperiod ($P = 0.504$) (Table 7.5).

Table 7.5 Significance tests and related statistics (degrees of freedom, mean square, F ratio, *P* value) for the ANOVA analysis of GI.

Effect	*df*	Mean Square	F-ratio	*P*
temperature	2	4.73	306.75	<0.0001
photoperiod	2	0.06	4.01	0.018
season	2	1.31	85.36	<0.0001
season x temperature	4	0.11	7.76	<0.0001
season x photoperiod	4	0.05	3.04	0.016
temperature x photoperiod	4	0.01	0.83	0.504
season x temperature x photoperiod	8	3.75×10^{-2}	2.43	0.013
Error	1593	1.54×10^{-2}		

Inspection of pooled means across seasons for the temperature and photoperiod treatments showed a steady and significant decrease (post-hoc Tukey test, $P < 0.05$) in mean final GI between each of the experiments, from 16.5 ± 0.2 in spring to 14.5 ± 0.2 in summer and 13.6 ± 0.2 in winter (Fig. 7.10a). Similarly, there was a significant decline between the individual means for each of the three temperature and photoperiod treatments from a high in spring to a low in winter (Fig. 7.10b and c). The exception was the slight increase in GI in the 18^{o}C temperature treatment and the 6 h light photoperiod treatment between summer and winter (Fig. 7.10b and c).

There were significant differences in GI between each of the three temperature treatments in spring and winter (post-hoc Tukey test, $P < 0.05$). However, in summer there was no significant difference between the 14 and 18^{o}C temperature treatments

but a significant difference between these and the 10°C treatment (post-hoc Tukey test, $P < 0.05$) (Fig. 7.10b).

There were no significant differences in GI between any of the photoperiod treatments in spring or winter. In summer, the GI of the 6 h L treatment was significantly lower than for the other two (12 h and 18 h L) photoperiod treatments (One-way ANOVA: F = 73.3, df = 2, 540, $P < 0.001$), but there was no difference between the latter two treatments (post-hoc Tukey test, $P < 0.05$) (Fig 7.10c).

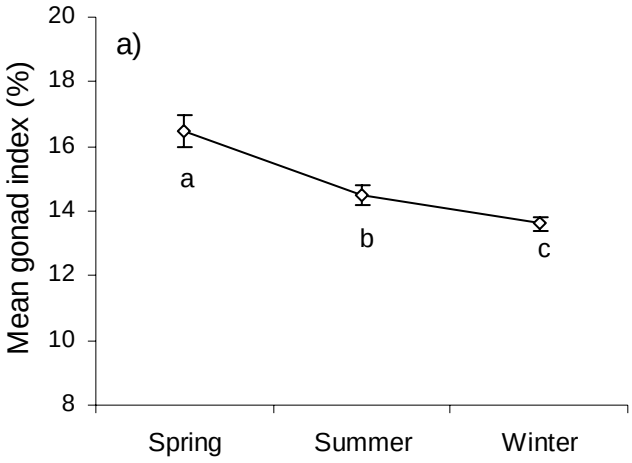

Figure 7.10 Mean final GI (±1 SE) showing means for a) time, b) time x temperature and c) time x photoperiod. Means which did not differ significantly ($P > 0.05$) within each series, according to post hoc Tukey test, are labelled with common letters. In b) and c) differences between each of the three series within each experiment is denoted as * ($P < 0.05$), if there are only differences between two of the three within each time this are denoted as ** ($P < 0.05$) and no stars indicate no significant difference between any of the three.**

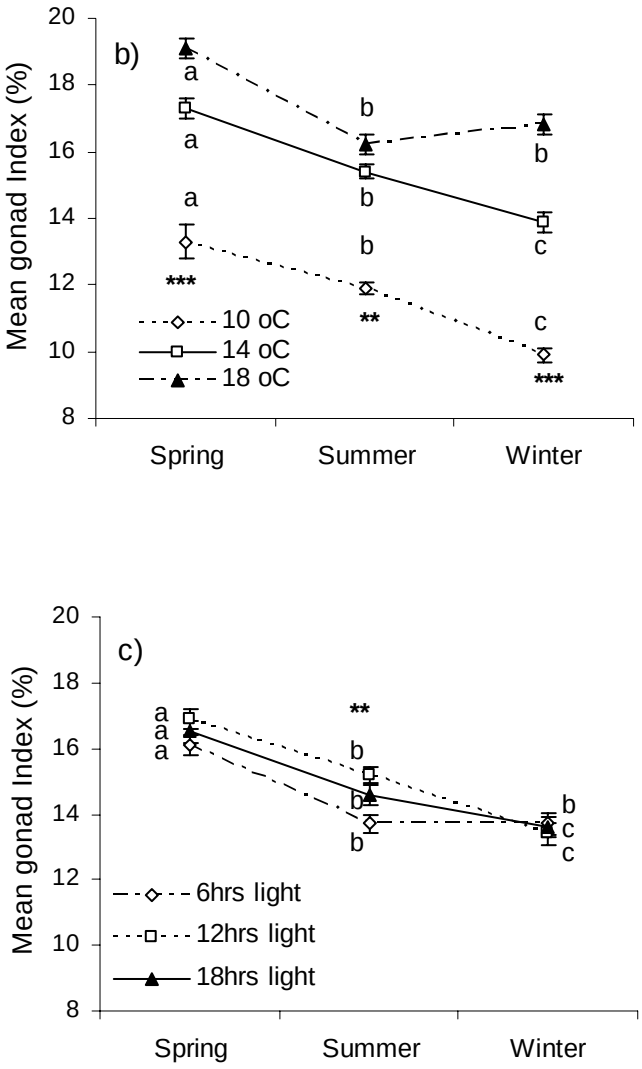

Figure 7.10 Continued. Mean final GI (±1 SE) showing means for a) time, b) time x temperature and c) time x photoperiod. Means which did not differ significantly (*P* > 0.05) within each series, according to post hoc Tukey test, are labelled with common letters. In b) and c) differences between each of the three series within each experiment is denoted as * (*P* < 0.05), if there are only differences between two of the three within each time this are denoted as ** (*P* < 0.05) and no stars indicate no significant difference between any of the three.**

7.4.6 Increase in GI

Increase in gonad index (Y) was strongly influenced by season, temperature and photoperiod and interactions between season and temperature, season and photoperiod and between season, temperature and photoperiod ($P < 0.05$ in all cases; Table 7.6). Increased yield rose from 76.9 ± 1.5 kg/tonne to 87.1 ± 1.5 kg/tonne in summer then dropped back to 74.6 ± 1.5 kg/tonne in winter (Fig. 7.11a). The effect of season was greater in the 10 and 14^{o}C temperature treatments (i.e. significantly higher in summer) than the 18^{o}C treatment where there was no significant differences between each season (One-way ANOVA: F = 0.5, df = 2, 540, P = 0.596). Similarly, the effect of season was greatest in the 12 and 18 h L photoperiod treatments (i.e. significantly higher in summer than the 6 h L treatment, where there were no significant differences between seasons (One-way ANOVA: F = 0.9, df = 2, 540, P = 0.374) (Fig. 7.11b and c).

One-way ANOVAs showed significant differences in the increase in GI between each of the three temperature treatments (10, 14 and 18^{o}C) in spring (One-way ANOVA: F = 7.2, df = 2, 540, $P < 0.001$), summer (One-way ANOVA: F = 80.24, df = 2, 540, $P < 0.001$) and winter (One-way ANOVA: F = 167.7, df = 2, 540, $P < 0.001$).

Table 7.6 Significance tests and related statistics (degrees of freedom, mean square, F ratio, *P* value) for the ANOVA analysis of increase in GI.

Effect	*df*	Mean Square	F-ratio	*P*
temperature	2	456750.3	379.74	<0.0001
photoperiod	2	6253.3	5.20	<0.005
season	2	24064.2	20.01	<0.0001
season x temperature	4	8508.3	7.07	<0.001
season x photoperiod	4	3632.6	3.02	0.017
temperature x photoperiod	4	743.0	0.62	0.649
season x temperature x photoperiod	8	3509.5	2.92	0.003
Error	1593	1202.8		

There were significant differences in summer between the 6 h L photoperiod treatment and the other two photoperiod treatments (12 and 18 h L) (One-way ANOVA: F = 7.2, *df* = 2, 540, $P < 0.001$) but no difference between the latter two treatments. There were no significant differences between any of the photoperiod treatments in spring (post-hoc Tukey test $P < 0.05$) or winter (post-hoc Tukey test $P < 0.05$).

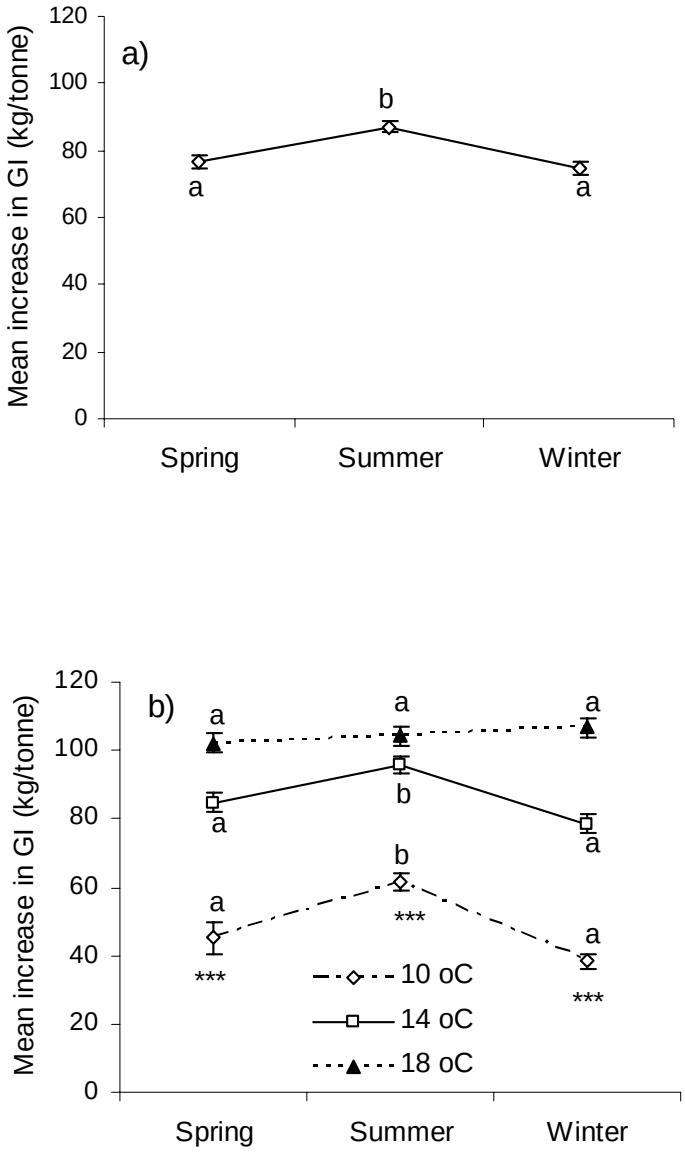

Figure 7.11 Mean increases in GI (±1 SE). Means which did not differ significantly (*P* > 0.05) within each series, according to post-hoc Tukey test, are labelled with common letters. In b) and c) differences between each of the three treatments within each experiment are denoted as * (*P* < 0.05), if there are only differences between two of the three this is denoted as ** (*P* < 0.05) and no asterix indicate no significant difference between any of the three treatments within each experiment.**

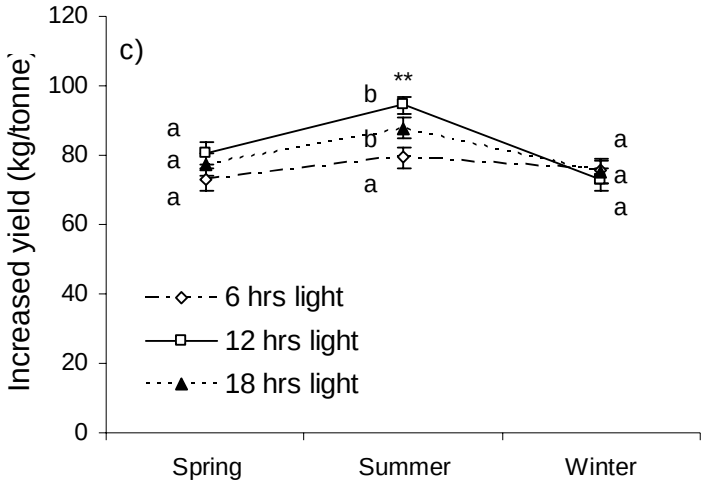

Figure 7.11 Continued. Mean increases in GI (±1 SE). Means which did not differ significantly (*P* > 0.05) within each series, according to post-hoc Tukey test, are labelled with common letters. In b) and c) differences between each of the three treatments within each experiment are denoted as * (*P* < 0.05), if there are only differences between two of the three this is denoted as ** (*P* < 0.05) and no asterix indicate no significant difference between any of the three treatments within each experiment.**

7.4.7 Gonad colour

There was a significant interaction between season and temperature but no other significant effects, or interactions between effects (Table 7.7).

A three–way ANOVA showed no significant differences in the lightness (L*) of the urchin gonads between season, temperature or photoperiod or any significant interactions between effects except for between season and temperature (Table 7.7).

There was a significant difference in the redness (a*) of the urchin gonads due to the effect of season with urchins in winter (11.8 ± 0.2) having a significantly lower value (less red) than in spring (13.5 ± 0.2) and summer (13.5 ± 0.2), but not for temperature or photoperiod (Table 7.7).

Table 7.7 Significance tests and related statistics (degrees of freedom, mean square, F ratio, *P* value) for the ANOVA model used to analyse variation in Lightness (L*), redness (a*) and yellowness (b*).

Effect	*df*	Mean square	F-ratio	*P*
Lightness (L)*				
temperature	2	927.58	1.26	0.283
photoperiod	2	1360.87	1.85	0.157
season	2	488.41	0.67	0.514
season × temperature	4	2047.58	2.79	0.025
season × photoperiod	4	1510.38	2.06	0.085
temperature × photoperiod	4	1348.71	1.84	0.120
season × temperature × photoperiod	8	1228.18	1.67	0.102
Error	513	734.42		
Redness (a)*				
temperature	2	12.70	0.72	0.488
photoperiod	2	4.48	0.25	0.776
Season	2	102.90	5.82	0.003
season × temperature	4	35.04	1.98	0.096
season × photoperiod	4	5.13	0.29	0.884
temperature × photoperiod	4	21.91	1.24	0.293
season × temperature × photoperiod	8	14.32	0.81	0.594
Error	513	17.69		
Yellowness (b)*				
temperature	2	558.13	3.60	0.027
photoperiod	2	187.18	1.21	0.299
season	2	2059.80	13.30	<0.0001
season × temperature	4	2133.12	13.77	<0.0001
season × photoperiod	4	113.99	0.74	0.567
temperature × photoperiod	4	145.06	0.94	0.442
season × temperature × photoperiod	8	63.71	0.41	0.914
Error	513	154.91		

There was a significant difference in the yellowness (b*) of the urchin gonads due to the effect of season, with urchins in summer (26.9 ± 0.6) having a significantly lower value than in spring (31.5 ± 0.4) and winter (31.8 ± 0.7). Urchins held at 18^{o}C (28.6 ± 0.5) had a significantly lower value than those held at 14^{o}C (31.8 ± 0.8) and 10^{o}C (31.5 ± 0.5). Photoperiod had no effect (Table 7.7).

Overall it appears that there was a seasonal effect on roe colouration but the experimental treatments had little or no significant effect on roe colouration other than temperature affecting the yellowness of the roe.

7.4.8 Food consumption

A two-way ANOVA showed significant differences between the food consumption (dry weight) of urchins in winter held in the three different temperature treatments (ANOVA: F = 278.79, *df* = 2, 36, *P* < 0.001). Urchins held at 18^{o}C consumed significantly greater quantities of feed (27.4 ± 1.1 g dry weight) than those held at 14^{o}C (22.4 ± 0.8g dry weight), and these in turn consumed greater quantities of food than those held at 10^{o}C (13.4 ± 0.6 g dry weight). There were no significant differences between the consumption rates of urchins held in the three photoperiod treatments (ANOVA: F = 0.05, *df* = 2, 36, *P* = 0.952). The results showed a significant linear increase in consumption in relation to increasing temperature (ANOVA: F = 122.6, *df* = 1, 36, *P* < 0.001) (Fig 7.12).

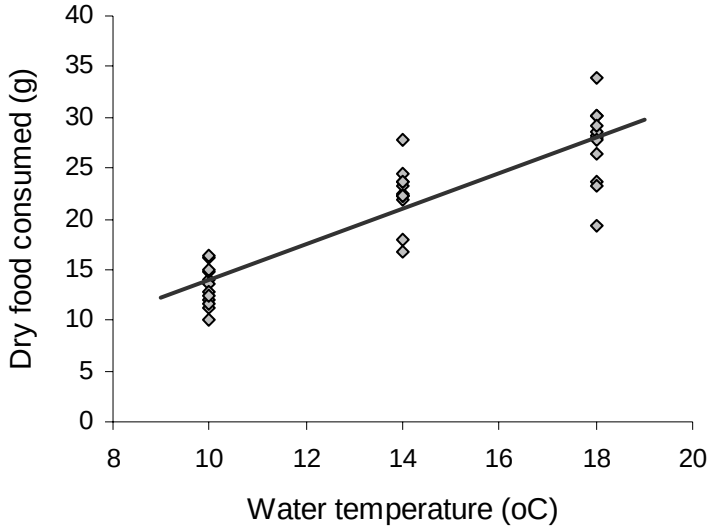

Fig 7.12 The relationship between the amount of food consumed over a 4 day period (dry weight (g), and seawater temperature (^{o}C) in the winter experiment, Y = 1.7514 X – 3.4752; r^2 = 0.7826; *n* = 36.

7.5 Discussion

7.5.1 GI and reproductive stage of wild vs. experimental urchins

The wild urchins collected at the Port Gore site in the Marlborough Sounds show a general pattern of GI growth and reproductive stage as previously described for urchins from this area (Dix, 1970c; Brewin, 1994; James et al., 2007). The GI values (although significantly larger) and reproductive stages of the experimental urchins reflect the pattern of the wild urchins. The highest wild and experimental GI values were recorded in spring when the urchins are at the premature stage. The GI values then decreased in summer when the urchins are in the mature and partially spent stage and decreased to a lesser extent in winter when the urchins are in the recovery and early growing stage. Conversely, the general pattern of the increase in GI of the experimental urchins does not follow the same pattern of the wild GI or reproductive

stages. The greatest increase in GI occurred in summer, when the urchins were not at their highest initial GI and were at the partly spent to spent stages. The increases in GI were no different in spring and winter despite the urchins in the later experiment being in the recovery and growing stage and having the lowest initial GI. A previous study on the effects of season on roe enhancement of *E. chloroticus* (James et al., 2007) also found that the largest increases in GI occurred in summer when the urchins were in the spent reproductive stage. Brewin (1994) observed that the increase in GI during roe enhancement experiments resulted from the ability of the nutritive phagocyte cells to rapidly expand within the gonad and this has also been observed for other urchin species (Walker and Lesser, 1998; Spirlet, 2000; Kelly, et al. 1998). This is most likely the cause of the increases in GI in the current study during summer, but why the increases are greater during this season and gametogenic stage is unclear.

7.5.2 Effect of temperature on GI and reproductive stage

There was a seasonal difference in the GI values of the urchins, with lower values in summer than in winter or spring, and this result is also reflected in the 10^{o}C and 14^{o}C temperature treatment results. However, there were no significant differences in the increase in GI of the urchins in the 18^{o}C treatment over the three experiments. This indicates that 18^{o}C is likely to be close to the optimal temperature for increasing the size of urchin gonad in *E. chloroticus* and that growth may decline at higher temperatures as found by Shpigel et al. (2004a) for *Paracentrotus lividus*. In terms of aquaculture of *E. chloroticus* it appears that optimal GI production for this species would be achieved by holding the urchins at 18^{o}C in winter and spring, but during summer they could be held at 14^{o}C to achieve similar levels of production. Further

research is required to understand the effects of holding temperatures higher than 18^{o}C.

Siikavuopio et al. (2006) observed that when food supply is unlimited, temperature is the most important factor that governs rate of growth in *Strongylocentrotus droebachiensis*. However, the effect of temperature can vary markedly between urchin species (Spirlet et al., 2000; Brockington and Clarke, 2001; Garrido and Barber, 2001; Shpigel et al., 2004). Shpigel et al. (2004a) found that higher water temperatures reduced gonad growth of *Paracentrotus lividus* in both spring-summer and winter-spring trials. The reason for the decrease in growth was the physiological cost to the urchins of water temperatures above 24^{o}C without any increase in feed intake to compensate. In contrast, the study by Siikavuopio et al. (2006) showed that optimal gonad growth was achieved for the cooler water species *S. droebachiensis* at higher temperatures in summer than in winter. The lower growth in winter was possibly due to the transition from nutritive to gametogenic cells during the winter reproductive stage. In the present study both temperature and season had a significant effect on roe production and development and there was a significant interaction between these two environmental effects. The urchins held at the highest temperature (18^{o}C) recorded the highest GI values and increase in GI in all three experiments except in summer where there was no difference between the 14 and 18^{o}C treatments. This exception in summer is likely to be due to the lower final GI value of urchins held in the 18^{o}C treatment in summer compared to those held in the 18^{o}C treatment in spring or winter. A possible cause may be the similar reproductive stages (approximately 50% partly spawned and 50% spawned) of urchins held at 14 and 18^{o}C in summer. These urchins may have reached their maximum potential GI for this reproductive stage in both the 14 and 18^{o}C treatments in summer. When

compared to the 10°C treatment in the summer experiment the female urchins are similar to the 14°C treatment but the male urchins are approximately 50% mature and 50% partly spawned. These results, together with no obvious spawning event indicate that the urchins are trickle spawning during the summer experiment. The urchins held at 14°C temperatures produced significantly greater GI values and increase in GI than those held at 10°C regardless of season. These results support the conclusions of James at al. (2007) who found that, although temperature is the primary driver of roe enhancement in *E. chloroticus*, the reproductive stage of urchins plays a smaller but significant role at certain times of the year.

7.5.3 Feed intake of experimental urchins

Spirlet et al. (2000) showed that feed intake for *P. lividus* was similar at 16°C and 24°C, but that gonad growth increased significantly at the higher temperature due to an increase in digestion efficiency and/or the nutrient conversion process. In contrast, Siikavuopio et al. (2006) showed a clear linear relationship between increased temperature and feed intake for *S. droebachiensis*. Brewin (1994) showed that there was some evidence to suggest that feeding rates and temperature are related for *E. chloroticus*. In the winter experiment in the current study, the increase in GI due to increasing temperature is likely to be due to an increase in consumption by urchins held at higher temperatures. This is supported by the linear increase in consumption with increasing temperature in the current experiment.

7.5.4 Effect of photoperiod on GI and reproductive stage

The photoperiod treatments in this study appeared to have little, or no, effect on the GI values, or the increase in GI of the experimental urchins. The exception is again in

summer when urchins exposed to the 6 h L treatment had lower GI values and increase in GI values compared with the other two photoperiod treatments in the same experiment. Buisson (2001) found that there was no effect on gonad production after switching *E. chloroticus* from winter to summer photoperiods for 12 week periods, although the study was confounded by higher temperatures in one of the photoperiod treatments. Similarly, Spirlet et al. (2000) and Shpigel et al. (2004a) found that photoperiod had less influence than temperature on the reproductive cycle and growth of *P. lividus* in culture. However, the study by Spirlet et al. (2000) did show that photoperiod had a significant effect at higher temperatures (24^{o}C). Interestingly, in the current study increasing day length appeared to advance the reproductive stage of *E. chloroticus* in spring, decrease it in summer and have no effect in autumn. In contrast, Brewin (1994) ran a series of roe enhancement experiments on *E. chloroticus* collected from the Marlborough Sounds and hypothesised that inhibition of gonad growth occurred in the experiment that commenced in October due to the lack of gametogenic stimulus, in this case the onset of longer days. In contrast, urchins collected after gametogenesis had been stimulated in the wild behaved similarly (in terms of the development of the reproductive cycles) in the experiments to those in the field population. Barker (2007) suggested that the timing of the gametogenic cue in *E. chloroticus* is similar across all populations and occurs during mid-late winter and into spring (June to October). In the current study the urchins were collected from the wild in spring (12 August) prior to the commencement of the experiment. The urchins were then exposed to shortened days (6 h L), and two longer day treatments (12 and 18 h L). The reproductive stages of the urchins in the spring experiment (Fig. 7.7a) do not appear to be inhibited by the absence of a longer day cue. Female urchins exposed to a 6 h L photoperiod had almost identical results (in

terms of the percentage of individuals in the various reproductive stages) to the female sample collected at the conclusion of the experiment from the wild population. Male urchins in the 6 h L treatments did appear to be slightly less advanced than their wild counterparts at the conclusion of the experiment but were still more advanced than the urchins from the initial wild sample. These results indicate that the lengthening day gametogenic cue occurs prior to August 12 for *E. chloroticus*.

It would be advantageous to be able to alter the gametogenic cycle of urchins held for roe enhancement as this would extend the period that the urchins are in the spent, or recovery stages, thus providing roe of suitable quality for the lucrative Asian markets (namely Japan). However, the results of this study indicate that in terms of roe enhancement it would appear that there is little or no advantage to be gained by altering the photoperiod of *E. chloroticus* held for 10-week periods with the exception being that very short photoperiods should be avoided during summer periods. However, altering the photoperiods of urchins held for longer periods than in the current study may still have some effect on gonad growth and development.

7.5.5 Combined effects of temperature and photoperiod on gametogenesis

Spirlet et al. (2000) suggested that temperature acted as an 'enhancer' of the gametogenic process in field populations of urchins but probably not as a trigger signal. The results of this study support these finding and indicate that there are relatively minor differences between the reproductive stages within photoperiod and temperature treatments, or when compared to the reproductive stages of wild urchins. The most notable effect is the trend for the reproductive stage of the urchins to be more advanced with higher temperatures. There was a seasonal effect from photoperiod and temperature on gonad growth in summer, with the urchins held at

18°C and 6 h L both having lower GI values. The 6 h L treatment and the 18°C treatments both had the highest proportion of spent gonads in summer (when males and females are combined) which may account for the lower GI values in these two treatments. This may indicate that it is only the 'spent' stage in the reproductive cycle that has an influence on the productivity of urchins and would account for the seasonal differences that were observed in the temperature and photoperiod treatments. However, the urchins in summer still had the highest increase in GI of the three experiments showing urchins are still capable of significant increases in GI when supplied with unlimited feed for periods of 10 weeks throughout the reproductive stages.

7.5.6 Effects of temperature and photoperiod on roe colour

The diet of urchins has been shown to be the principal factor affecting changes in urchin gonad colour during enhancement experiments (Robinson, et al., 2002; McBride et al., 2004; Shpigel et al., 2004). The only changes in colour (differences in the redness and yellowness of the gonads) observed in the current study occurred between seasons, as reported previously by McBride et al. (2004) for *Strongylocentrotus franciscanus*, but there appears to be limited effects on gonad colour of *E. chloroticus* from changes in either temperature (with the exception of changes in the redness of the roe as a result of different temperatures) or photoperiod within seasons. Changes in colour between seasons may be a response to changes in the reproductive condition of the urchins and the presence/absence of gametogenic cells.

7.5.7 Implications for roe enhancement of *Evechinus chloroticus*

In terms of future roe enhancement of *E. chloroticus* the current study has shown that the initial reproductive condition of urchins is responsible for the seasonal pattern in final GI values, but not the increase in GI. Holding temperature appears to be the most influential abiotic factor affecting both the GI values and increase in GI. In contrast, photoperiod appears to have very little influence on either. Temperature and photoperiod have limited effects on gonad colour and no significant effects on urchin survival. It seems unlikely that the small scale changes in the reproductive cycle, occurring as a consequence of varying temperature and/or photoperiod treatments during relatively short enhancement periods (10 weeks in this case), will have significant effects on sea urchin enhancement other than during periods when there are a high percentage of urchins in the 'spent' stage (summer).

In summary, there would appear to be no economic advantage in manipulating photoperiods or temperature to improve survivorship or roe colour (the minor differences in redness as a result of holding at different temperatures would be unlikely to change the value of the roe on the domestic, or international markets) of *E. chloroticus*. There would be no advantage (in terms of increase in GI) in manipulating photoperiods for roe enhancement. The benefits (increases in GI) from increasing holding temperatures for roe enhancement must be weighed against the costs of increasing holding temperatures in land-based holding systems, or in transporting urchins to sites with warmer ambient temperatures in sea-based holding systems.

Chapter Eight General Discussion

8.1. Introduction

The aim of this study was to describe, quantify and understand the effects of a range of holding systems and environmental factors on roe enhancement of *Evechinus chloroticus*. This thesis consists of a series of published, refereed journal papers and this chapter provides a platform for integrating the entire research project into a single discussion of relevant results.

8.1.1 Definition of roe enhancement

Roe enhancement requires the collection of mature wild urchins followed by holding and feeding, in either land-based or sea-based holding systems for 10-12 weeks, on formulated artificial diets to increase the size and quality of the roe. In order to understand the effects of environmental factors and/or holding systems on roe enhancement it is crucial to understand the mechanism by which roe develops normally in wild sea urchin populations. Roe enhancement refers to the increase in size and/or quality of the roe (gonad) over a relatively short period. This is not the same as the reproductive cycle (or gametogenesis), even though they are integrally linked to one another. In the past a number of authors have suggested that gametogenesis is one of the critical factors affecting roe enhancement (Lawrence, 2001; Walker et al., 2001). Gametogenesis does control the quality of sea urchin roe in terms of its firmness and the percentage content of reproductive cells, which have a

significant impact on the suitability of the roe for international export markets such as Japan (See Chapter 1, Section 1.5.4). This study focussed on the increase in size of the roe and its relationship to the reproductive stage of the urchin.

8.1.2. Roe enhancement in New Zealand

Previous research has shown that there is potential for a kina roe enhancement industry to be developed in New Zealand (Brewin, 1994; Barker et al., 1998; Buisson, 2001; Fell, 2002; James et al., 2004; James, 2006a and 2006b). There are two distinct objectives for a New Zealand roe enhancement industry. The first is to supply the domestic market (which is currently under-supplied) where the quantity of roe, and to a lesser extent the quality of the roe are important (see Section 1.5.2). Roe enhancement for the New Zealand market would provide a consistent product (consistency of supply is currently a significant issue for the wild fishery) and improve returns from the wild fishery. It would also allow utilisation of large numbers of poor quality kina that are currently uneconomic to fish. The market in New Zealand is unique in that the taste preference of local consumers is for roe in spawning condition when there are large numbers of male or female gametes present. However, roe is sold and eaten throughout the entire year in New Zealand and therefore throughout the entire reproductive cycle. Roe enhancement for the domestic market would aim to increase the quantity of roe all year round, regardless of the reproductive stage of the urchin, whilst maintaining the best possible roe quality.

The second objective for roe enhancement in New Zealand is to supply product for the Japanese market. Here the value of the roe is dictated by its size, taste, colour, shape, firmness and reproductive stage (Yokota et al., 2000). The Japanese market has been the focus of a number of previous studies, both worldwide (Vadas et

al, 2000; Lawrence et al., 2001; Pearce et al., 2004) and in New Zealand (Barker et al., 1998; Buisson, 2001; Fell, 2002; James et al., 2004). The best quality gonads for this market are generally found during the reproductive cycle when nutritive phagocytes (NP) have reached their maximum number and size prior to vitillogenesis (Walker et al. 2001; Lawrence et al., 1997; Lawrence et al., 2001; Yokota, 2000). For this reason it is important to be able to control the reproductive cycle, either by suppressing gametogenesis and preventing the development of reproductive cells, or by extending the period when NP are present in the gonad by accelerating their growth to enable maximum production of roe suitable for export.

In this chapter the results of the current study are utilised to discuss which of these two options (New Zealand vs. Japanese markets) would be most suitable for future roe enhancement endeavours in New Zealand.

8.1.3. Gametogenesis and roe enhancement

Regardless of the market that is being targeted it will be critical to understand the factors that control and stimulate gametogenesis, as well as the main drivers of increase in gonad size, and whether these factors and drivers can be manipulated, and to what extent.

Gametogenesis in echinoids has received considerable attention in the literature for a number of decades (reviewed in Pearse and Cameron, 1991; Yokota et al., 2000). It is described as “a single annual event that consists of an abrupt change in activity within the gonads from storage of nutrients in intergonadal somatic cells (nutritive phagocytes) to the rapid increase in numbers and/or size of germ cells” (Walker et al., 1998). The presence and function of the NP cells is an unusual feature of sea urchin gametogenesis and does not occur in other echinoids (other than sea

stars with lecithotrophic larvae) (Kasyanov, 2001). A number of studies have shown that increases in gonad size of sea urchins following spawning are due to the increase in both size and number of NP cells rather than reproductive cells (Pearse and Cameron, 1991; Barker et al., 1998; Buisson, 2001; Kelly, 2001).

The processes that control gonad size and gametogenesis are still not well understood despite their importance for aquaculture of sea urchins, including roe enhancement of *E. chloroticus*. Pearse and Cameron (1991) list age and size, nutrition, water temperature and light (photoperiod) as being the most important influences on gametogenesis of sea urchins.

8.2. Environmental effects on roe enhancement

8.2.1. Seasonality and environmental cues for gametogenesis

Seasonal cycles of feeding, growth and reproduction (gametogenesis) have been observed for a number of marine invertebrates (Brockington and Clarke, 2001) and a number of studies have documented seasonal changes in wild populations of a range of sea urchin species, including *E. chloroticus* (Byrne, 1990; Brewin, et al., 2000; Buisson, 2001; Lamare et al., 2002). Walker and Lesser (1998) suggested that the gametogenic cycle, the primary seasonal change that occurs in sea urchins, depends on the quality and quantity of food available and the ambient seawater temperature, and that the initiation of gametogenesis is often correlated to changing photoperiods. The only method of identifying the actual cause of seasonal changes for a particular sea urchin species is by isolating the environmental effects that the urchin is exposed to in order to determine which factors are affecting gonad growth and gametogenesis. Garrido and Barber (2001) exposed *S. droebachiensis* to low and high temperatures (3

and 12^{o}C) and to low and high feed rations. Urchins exposed to the high ration had significant increases in GI in both winter and summer experiments. The GI values of urchins exposed to the low feed ration did not change significantly in either season. The study also found that increasing temperature affected the rate of growth and the maturation of gametogenic cells at certain stages of the reproductive cycle. These results support the theory that feed availability is the primary driver of both gonad growth and gametogenesis, but that seawater temperature does play a role, particularly at certain times of the year.

The results of the current study suggest that this theory also applies to *E. chloroticus* with food availability being the main driver of seasonal changes in GI values, causing significant increases in GI values throughout the year regardless of reproductive stage, temperature or photoperiod. However, if seasonal changes in the gonad size and gametogenic cycle were solely driven by feed availability there would be little difference in GI values and reproductive stages between populations across New Zealand which had ample supplies of feed. Previous studies (Dic, 1970c; Brewin et al., 2000; Buisson, 2001) have shown this is not the case and so there are obviously other factors that also play a significant role in controlling seasonal changes in *E. chloroticus*.

8.2.2. The effects of temperature

In the current study *E. chloroticus* fed artificial food *ad libitum* and held at higher temperatures for 10-12 weeks produced larger increases in GI, regardless of the reproductive stage or photoperiod, than urchins held at lower temperatures. When held for 27 weeks at constant temperature and photoperiod, the gametogenic cycle of *E. chloroticus* was significantly different from urchins sampled from the wild source

population at both 12 and 27 weeks. This shows that temperature and/or photoperiod can also affect the gametogenic cycle.

Spirlet et al. (2000) showed that for *Paracentrotus lividus* temperature had a significant effect on gonad production, with increasing temperatures leading to an increase in either the digestion efficiency or the nutrient conversion process of the urchins. This is a similar result to that shown for *Lytechinus variegatus* where urchins were less efficient at processing food at 16^{o}C than at 23^{o}C (Klinger et al., 1986). The urchins used in the former study had been reared in a land-based recirculation system and had never been exposed to wild environmental conditions. Siikavuopio et al. (2006) also found that increasing temperature had a significant effect on the gonad growth of wild caught *S. droebachiensis* and that the highest gonad indices occurred at higher temperatures in summer than in winter. This result indicates that there is some seasonal response to temperature, depending on the environment the urchin is exposed to prior to collection. Unlike the gonad growth of *P. lividus*, Siikavuopio et al. (2006) found the increase in gonad growth of *S. droebachiensis* with increasing temperatures was directly related to an increase in food consumption and not an increase in feeding efficiency. The results of the current study show that this is also the case for *E. chloroticus*.

Because the current study included two series of experiments that were repeated throughout a 12-month period (Chapters 6 and 7) it is possible to compare the effects of temperature (and photoperiod in Chapter 7) on gonad growth throughout the seasons. The results from Chapter 7 show a clear pattern of increasing GI values, with increasing temperature regardless of season, with urchins held at 18^{o}C having significantly higher GI values than those held at 14^{o}C, and these in turn having significantly higher GI values than those held at 10^{o}C. The exception to this was in

summer when there was no significant difference between gonad growth in the 14^{o}C and 18^{o}C treatments. This could be due to the reproductive stage of the urchins during the summer experiment. The urchins were trickle spawning (i.e. had a high percentage of urchins in either the spawning or spent stages, see Chapter 7) in the experimental tanks and the 18^{o}C treatment had the highest percentage of urchins in the spent stage compared to the other temperature treatments in this experiment.

The recommendation on optimal holding temperatures for roe enhancement of *E. chloroticus* would be to hold kina in temperatures as close to 18^{o}C as possible throughout the year. However, in summer, kina could be held at ambient temperatures of 14^{o}C without any loss of productivity. In sea-cages this could be achieved by holding urchins at lower latitudes where ambient seawater temperatures are higher. In land-based holding systems a cost/benefit analysis would be required to calculate whether the increased returns from higher GI values would be worth the cost of having increased temperatures in holding systems and increased feed consumption.

In the current study there was a small but consistent trend towards the reproductive stage of kina held at higher temperatures being more advanced than that of kina held at cooler temperatures. This is a similar result to that described by Garrido and Barber (2001) and indicates that gametogenesis in wild *E. chloroticus* is driven by temperature as well as food availability. This is substantiated by the synchronicity of the reproductive stages of wild urchins with those of the experimental urchins that were exposed to the temperatures closest to ambient seawater temperatures in the wild (see Chapter 7). Surprisingly the gametogenic cycle in kina held in temperatures and photoperiods completely out of phase with ambient wild temperatures (and photoperiods) still remained relatively synchronous with their wild counterparts over 10-12 weeks, indicating that this time period is

insufficient for environmental changes to have a significant impact on roe enhancement.

8.2.3. The effects of photoperiod

Changes in photoperiod have been shown to influence the gametogenic cycle of a number of echinoids (McClintock and Watts, 1990; Pearse and Cameron, 1991, Walker and Lesser, 1998). One of the few studies to measure the effects of photoperiod on gonad growth, as well as gametogenesis, is a 12-week roe enhancement experiment conducted by Shpigel et al. (2004a). The study showed that it was possible to manipulate the gametogenic cycle of *Paracentrotus lividus* in a 12-week period by changing the natural photoperiod, and that short days accelerated and long days slowed the gametogenic process. It also showed that, at high temperatures, there was significantly increased gonad growth in urchins held at shorter photoperiods. This was the first experiment to show that manipulating photoperiod can affect gonad growth. In the current study photoperiod had a similar effect on gonad growth in the summer experiment, when urchins exposed to short days (6 h light) had significantly lower GI values than those exposed to either 12 or 18 h light treatments. Why this should occur during summer is unclear but it may be related to the fact that the largest differences between the experimental photoperiods and the ambient photoperiods occurred in the 6 h light treatment in summer. Other than this there were no significant differences in gonad growth between any of the treatments regardless of season. These results are in contrast to the only previous experiment testing the effects of photoperiod on gonad growth in *E. chloroticus*. Buisson (2001) found that *E. chloroticus* exposed to summer photoperiods in summer produced significantly larger gonads than those exposed to winter photoperiods during the same

period. However, the results were compromised by the summer treatment having significantly warmer temperatures during the experimental period which, as we have seen in the previous section, would have caused an increase in gonad growth, regardless of the photoperiod treatment.

Unlike the results from the temperature treatments there does not appear to be any obvious trend in the reproductive stages of the urchins held in different photoperiods. It appears unlikely that manipulation of photoperiod will significantly change either the gametogenic cycle, or the gonad value of *E. chloroticus* in a 10-12 week period. This is in contrast to the finding of Buisson (2001) who suggested that photoperiod may be an important factor affecting the gametogenic development of *E. chloroticus*. In Buisson's study there was significant variation in the experimental replicates and the author concluded that the effects of out-of-phase photoperiods on roe enhancement and gametogenic development were still unclear. For future development of roe enhancement in New Zealand, the lack of any significant effect on the gametogenic cycle in short term roe enhancement experiments would eliminate using the technique to extend selected gametogenic stages to make the roe more suitable for export, or for the domestic market.

There may still be potential for manipulation of the gametogenic cycle in *E. chloroticus* in longer term experiments. Walker and Lesser (1998) noted that it took 6 months for out-of-phase temperatures to have an effect on the reproductive cycle of *S. droebachiensis*. Pearse et al. (1986) showed that in 18-month experiments it was possible to change the gametogenic cycle to 6 months out-of-phase by subjecting the urchins to photoperiods that were also 6 months out-of-phase. Kelly (2001) showed that *Psammechinus miliaris* held at short day photoperiod for 7 months remained premature compared to urchins that were exposed to lengthening days that progressed

normally through the gametogenic cycle. Kelly (2001) concluded that changes in photoperiod were necessary for the progression of gametogenesis in this species. A study on the tropical species *Eucidaris tribuloides* held at two variable and two fixed photoperiods at a constant temperature (22^{o}C) for a 12-month period (McClintock and Watts, 1990) also showed that it is possible to manipulate the gametogenic cycle of this species by altering photoperiod. A technique used to standardise the gametogenic stages of *P. lividus* that were reared in a laboratory and had desynchronised reproductive stages (due to the lack of environmental cues) was to starve the urchins for 2 months prior to the roe enhancement experiment. This resulted in synchronisation of the reproductive stages at the spent stage. This technique has never been trialled on sea urchins collected from the wild and may be worth further research in order to manipulate the gametogenic cycle of *E. chloroticus.* However, on a cautionary note the longer the period required for roe enhancement the less economically viable it is likely to be. This should be taken into consideration before resources are expended testing the long term effects of manipulation of the gametogenic cycle of *E. chloroticus* by changing ambient photoperiods.

The conclusion from the current study is that manipulation of photoperiod is unlikely to significantly change either the gametogenic cycle, or the gonad value of *E. chloroticus* in short to medium (10-12 week) periods, but that manipulation for more extensive periods may result in changes in the gametogenic cycle.

8.3. Initial gonad condition and roe enhancement

8.3.1. Stress

Grime (1977) defined stress as an environmental condition that decreases production. There are a number of factors that can cause stress in wild or captive sea urchins.

These include low oxygen availability (Siikavuopio et al., 2007a), high nitrate and nitrite levels (Siikavuopio et al., 2004a and 2004b), high levels of carbon dioxide (Siikavuopio et al., 2007b), sudden temperature changes (Fell, 2002) and low feed availability (Spirlet et al., 2000). In wild sea urchins this can result in varying GI values within, or between, populations that consist of urchins exposed to different environmental conditions. The two groups of urchins sampled in Chapter 6 provide an excellent example of the results of long term feed limitation and the subsequent stress that occurs in wild urchins. Differences in the size of the roe (or GI value) of these urchins tend to persist throughout the reproductive cycle in wild populations, regardless of the reproductive stages of the urchins.

8.3.2. Gonad condition: a proxy for feed availability

Gonad condition is a generalised term used to describe the GI value of wild sea urchins. Researchers will often describe an urchin as being in good condition when it has a high GI value and poor condition when it has a low GI value. Sea urchin fishers, on the other hand, often use the term to describe both the GI value (high or low) and the reproductive condition of sea urchins. An urchin that is in spawning condition and leaking gametes, or in post spawning condition with a very small roe, is described as being in poor condition. Such urchins that are in imminent spawning condition are likely to spawn after they have been fished and are on their way to the processing factory. Alternatively they may release all their gametes into the storage pottles after processing, thus reducing their market value. This issue is further confused by the fact that fresh urchins in spawning condition (oozing gametes) are highly prized by local recreational kina fishers but, as already described, for commercial fishers they are very difficult to handle and process. For the purposes of this study the term

'condition' has been used to describe the GI value of the urchins. In Chapter 6, urchins were collected from two populations in very close proximity to one another (i.e. divided physically by a small headland) that were in poor condition (low GI) on one side and good condition (high GI) on the other side. The urchins that had not had a regular supply of food for an extended period are the ones in poor condition whilst those that had a good supply of food for an extended period are the ones in good condition. Both Cuthbert and Hooper (1995) and Vadas et al. (2000) recommended that roe enhancement trials using urchins with high and low yield (GI) should be undertaken to quantify the effects of roe enhancement on poor quality sea urchins. However, prior to the current study there have been no studies investigating the effects of roe enhancement of urchins of varying initial condition from a single larger population.

8.3.3. Effects of initial gonad condition on roe enhancement and gametogenesis

A recent study by Hill and Lawrence (2006) tested the effects of roe enhancement on two sea urchin species (*Arabacia punctulata* and *Lytechinus variegates*) which have different life strategies and stress levels. This was measured using the scheme devised in the 1970s by J.P. Grime in which habitats are categorized by the conditions they provide and the level of disturbance affecting them. *Arabacia punctulata* has a stress-tolerant life strategy and the authors hypothesised that it would be more tolerant to stress. *Lytechinus variegates* has a more competitive-ruderal strategy resulting from an environment where conditions are benign, resources are abundant and disturbance is common, and this species is less tolerant to stress. Hill and Lawrence (2006) exposed the two urchin species to high temperature and low food availability. The results showed that the stress tolerant species (*Arabacia punctulata*) had less change

in GI (i.e. was less affected by the stress of high temperatures). The study also showed that for these species the biotic stress of low feed availability is more important than abiotic stresses such as temperature on their energy budgets. In a roe enhancement experiment on *P. lividus* reared in the laboratory, researchers starved the urchins for two months prior to the experiment to synchronise the gametogenic stages of the experimental urchins. This also stressed the urchins, causing them to mobilise and restore NP cells, filling them with nutrients and rapidly increasing their size (Spirlet et al., 2000). In the results from Chapter 6, *E. chloroticus* taken from an area of high stress (resulting from very low feed availability) performed consistently, and significantly better at increasing their GI than did urchins taken from the same general population but in an area of low stress (resulting from high feed availability). This result supports the findings of Spirlet et al. (2000) and Hill and Lawrence (2006) that urchins exposed to stress prior to roe enhancement are capable of a more rapid and favourable response to high levels of food availability. The ability of sea urchins in poor condition (as a result of higher stress caused by low feed availability) has not previously been described for any other sea urchin species but may have very important ramifications for future roe enhancement in New Zealand, and elsewhere around the world.

Because of the very high numbers of kina barrens in New Zealand and the relatively low GI values of kina found within them (as a result of low feed availability and subsequent high stress), it would appear feasible to utilise the low GI kina found in barrens for roe enhancement, and continue utilising the high GI kina found elsewhere in the wild for the wild fishery. Over the past decade, increasing fishing pressure has meant that it has become increasingly difficult to harvest sufficient good quality (high GI) kina that are above the minimum economic cut-off point. This has

resulted in an increasing fishing effort without a corresponding increase in returns (P. Herbert, Sea Urchin New Zealand, pers comm.). This is particularly prevalent in areas such as SUR 7 (Fig. 1.11) where there has been a very active fishery for many years. Utilisation of kina found in kina barrens for roe enhancement could help to alleviate the fishing pressure on kina populations that are in good condition. The recommendation from this study is that any future roe enhancement industry in New Zealand should utilise the low GI urchins that are abundant and easily harvested, and will be highly productive when held in roe enhancement conditions. The high-stress environment within kina barrens, caused by low feed availability, appears to make these urchins particularly responsive (in terms of increase in GI) to roe enhancement.

It is difficult to predict the effect of fishing large numbers of easily accessible low GI urchins on the kina fishery in New Zealand with any certainty. However, the likely effect will be an increase in the catch per unit effort (CPUE) within the fishery without the need for any change to the total allowable commercial catch (TACC). This is contrast to the present situation where high GI urchins are becoming increasingly difficult to find, reducing the current CPUE.

It is highly unlikely that the kina found in barrens are contributing significantly to recruitment into the fishery as they are often found to have very low GI values and do not have a obvious spawning event (as was the case in the low GI population in Chapter 6). These urchins would not be capable of contributing large quantities of gametes for population recruitment. In contrast, if fishing pressure is reduced on high GI populations, they may in term be able to contribute greater numbers of viable gametes into future population recruitment. This is an area that requires further research in order to understand the implications of developing a roe enhancement industry in New Zealand on the existing kina fishery.

8.4 Holding conditions and husbandry

8.4.1 Collection and transportation of *E. chloroticus*

There are conflicting results on the ability of *E. chloroticus* to cope with capture and transportation stresses. Buisson (2001) reported that the species was "very robust" with few mortalities occurring as a consequence of collection, transportation or experimental conditions. In Buisson's study, urchins were transported at very low densities, in water, with sufficient surface area for all urchins to be attached to a surface. This method would not be suitable on a commercial scale due to the very large volumes of water and large surface area required to transport commercial quantities of sea urchin. In contrast, Fell (2002) stated that excessive handling and temperature shock resulted in a significant number of deaths of *E. chloroticus* held in roe enhancement experiments. Dale et al. (2005) showed that desiccation from exposure to air during handling had a significant effect on urchin (*S. droebachiensis*) mortality in subsequent roe enhancement trials. Increase in spine damage received by urchins prior to roe enhancement trials also led to increases in mortality during the trial (Dale et al., 2005). It would appear that collection and transport of the urchins are crucial factors in the survival of the urchins in subsequent roe enhancement experiments. In the present study the survival rates were very high (mean = 94.4 ± 1.0 %; min. = 80.0%; max. = 100.0%) throughout all the experiments, despite the sampling sites being approximately 6-8 h travelling time from the research facility. The high survival rates in the current study are likely to be due to the efficacy of the method of transportation, i.e. stacked in cages at very high densities, out of the water, covered from wind, rain and sunshine and regularly sprayed with cool seawater. Commercial scale trials carried out using urchins that underwent similar transportation times, also had very low levels of mortality (James, 2006c). The mean

survival from Chapter 3 (86.3 ± 6.2%) was lower than for all other experiments. This is possibly because the travel time from the collection site used in this experiment was considerably longer (approximately 11 h) than in all subsequent experiments, supporting the theory that travel time and desiccation are important factors controlling the subsequent survival of the urchins.

Previous high mortalities of *E. chloroticus* during transportation (Fell, 2002) show the susceptibility of the species to transportation in water, and this method should be avoided.

8.4.2 Survival of *E. chloroticus* during roe enhancement

One of the most important questions to ask of any aquaculture species is the ability of the species to survive a variety of holding conditions for extended periods of time. With kina roe enhancement, there are the additional stresses of capture and transport from the wild to the holding facilities.

There have been a large number of studies conducted on sea urchin roe enhancement (see Table 1.1) that have shown that with suitable holding conditions (sufficient dissolved oxygen, suitable pH levels and limited ammonia levels; Siikavuopio 2004a, 2004b, 2007a and 2007b) the mortalities of urchins held in land-based holding systems are relatively low (< 10%) and normally occur soon after the urchins have been collected at the beginning of the trials (Christiansen and Siikavuopio, 2007; Siikavuopio et al., 2007a and 2007b). Similarly, in previous sea-cage trials, mortality rates have been low (Robinson and Colborne, 1997; Buisson, 2001; James, 2006a and 2006b). However, in both land- and sea-based systems there are also examples where holding conditions have been unsuitable, resulting in high mortality, and where environmental conditions have affected the mortality rate in the

experimental urchins. Dagget et al. (2006) showed that tank design in a land-based system can have a significant effect on captive *S. droebachiensis.* They reported mortalities as high as 24.3% in a shallow washtub-type holding tank design, compared with 8.0% and 4.3% in alternative raceway type tanks in the same experiment. In a long term (25-week) sea-cage experiment, where one of four algal species was fed to *S. droebachiensis* held in either land-based aquaria or sea-cages placed on the seafloor, the latter had higher mortality and the type of algae used to feed the urchins also had a significant impact on urchin mortality. In the experiment urchins fed *Laminaria digitata* and *L. longicruris* had significantly less mortality than urchins fed *Ascophyllum nodosum*, *Alaria esculenta* or *Agarum clathratum* (Cuthbert and Hooper, 1995). An experiment looking at the effects of handling stress and exposure to air on captive *S. droebachiensis* (Dale et al., 2005) reported mortalities of up to 25% in urchins with prolonged exposure to air (24 h at 0.6^{o}C). Siikavuopio et al. (2007) showed that stocking density also has a significant effect on mortality, which increased with increasing density and reached catastrophic mortalities (80%) at densities of 16 kg m^{-2}.

The relatively low mortality in the experiments from the current study and the ability of the urchins to be transported extensive distances out of water, and survive, make the New Zealand species suitable for aquaculture. The holding systems and densities utilised in the current study were suitable to maintain high survival of *E. chloroticus* for the both the short term (10 to 12-week) and longer term (27-week) periods.

8.4.3. Sea- and land-based holding systems for roe enhancement of sea urchins

There is a range of factors related to holding systems that affect sea urchin roe enhancement and the current study has focussed on the effects of density, depth and water movement which have received very little attention in the literature. Previous research has mostly been done on *S. droebachiensis* which is the most commonly studied sea urchin in terms of roe enhancement (see Table 1.1). In this section the results of the current study are compared with those of previous studies and recommendations for optimal holding conditions are made. Finally, the economic viability of both land- and sea-based holding systems are evaluated for future roe enhancement ventures in New Zealand.

8.4.3.1. Stocking density

Studies on invertebrates such as lobsters (*Jasus edwardsii*) (Simon and James, 2007) and abalone (*Haliotis tuberculata*) (Mgaya and Mercer, 1995) held in land-based tank systems have shown that increasing densities are often correlated with decreasing growth rate (somatic and gonadal) and increasing mortality. Other than the current study, there have been two recent papers published which are the first to specifically investigate the effects of increasing density on roe enhancement of sea urchins. Christiansen and Siikavuopio (2007) measured the effects of holding *S. droebachiensis* individually or at increasing densities (2.5, 3.7 and 7.3 kg urchin m^{-2} internal surface area) for 56 days. They found no significant differences in gonad growth, feed intake or feed conversion ratio (FCR) for any of these treatments. However, in a subsequent study Siikavuopio et al. (2006b) tested the effects of holding two size classes of *S. droebachiensis* at stocking densities of 6, 12, 14 and 16 kg urchin m^{-2} internal surface area for 60 days. This study showed a clear correlation

between increasing density, increasing mortality and decreasing GI values. Interestingly, the results showed no change in feed intake between the various density treatments, indicating that the changes in GI values were not a result of a decrease in feed availability or consumption but were instead a consequence of the holding conditions experienced by the urchins at higher densities.

Fell (2002) held *E. chloroticus* in sea-cages at a stocking density of 19.7 urchins m^{-2} internal surface area and observed comparable gonad growth and survival to previous studies on the species. The results from Chapter 3 of the current study showed no significant differences in gonad growth or survival between *E. chloroticus* held at stocking densities of 22.4 and 31.3 urchins m^{-2} internal surface. To compare these results with the results of Siikavuopio et al. (2006b) it was necessary to convert the number of urchins m^{-2} of internal surface area (as used in the present study) into weight of urchin m^{-2} of internal surface area. This gave stocking densities of 4.32 and 5.92 kg urchins m^{-2} internal surface in the current study. Siikavuopio et al. (2006b) recommended an optimal stocking density of 6 kg urchin m^{-2} suggesting that the highest density tested in the current study may be close to the optimal density for *E. chloroticus* and higher densities may have an adverse effect on the captive urchins. Further experimental research, which tests higher stocking densities, is required to clarify this.

The factors that may be affecting urchins held at increasing density are oxygen depletion, waste accumulation in the holding system, territoriality and chemical interactions between individuals. As the urchin density increases these factors become more critical. It is clear that there is an upper limit for stocking densities of *S. droebachiensis* where urchins held above this point are exposed to increased stress and have reduced gonad growth and increased mortality. It is unclear whether this is

due to decreased oxygen availability, or increased waste accumulation in holding tanks. Echinoids excrete urea which is decomposed by ureolytic bacteria into ammonia (Siikavuopio, 2004b). Acute toxicity of ammonia is well documented as a major stressor of marine species and Siikavuopio (2004a and 2004b) showed that the gonad growth of *S. droebachiensis* was significantly reduced with increasing levels of both nitrite (concentrations above 0.5 mg $N\text{-}NO_2$ l^{-1}) and ammonia (concentrations above 0.016 mg l^{-1} of unionised ammonia). Siikavuopio et al. (2007a and 2007b) also studied the effects of hypoxia (low levels of dissolved oxygen) and carbon dioxide and found that decreased oxygen and increased carbon dioxide levels both reduced the gonad growth, feeding rates and feeding efficiencies of captive *S. droebachiensis*.

A preliminary experiment (Appendix 1) was undertaken during the current study to test whether holding urchins in tanks with the same quantity of inlet water either trickled or tipped into the tanks had an effect on gonad growth. Tipping created significantly more water flow in the tanks but did not appear to increase the oxygen level in the tanks. There was a significant difference in the size of the gonads between the two treatments after 10 weeks with the urchins held in tipper tanks having significantly higher GI values (Appendix 1). This indicates that increased water movement around individual sea urchins is facilitating better access to dissolved oxygen, and removal of waste products and metabolites from around the urchin. This in turn is producing a more suitable environment for captive sea urchins to utilise available food resources and to produce more roe. These results are supported by a recent study on paua (*Haliotis iris*) that has shown that increasing flow rates result in increased oxygen consumption, and increased somatic growth rates, in captive urchins in land-based holding systems (G. Moss, National Institute of Water and Atmospheric Research, pers comm.). In designing holding systems for roe enhancement of *E.*

chloroticus (and other sea urchin species) it is critical to have systems capable of delivering sufficient dissolved oxygen and removing unwanted waste products to all the individuals in the system. The latter is significantly more difficult, and expensive, to achieve than the former in a land-based holding system. As will be discussed in Section 8.4.3.3, both factors can be addressed in sea-based holding systems.

8.4.3.2. Disturbance

Other than the present study there is only one investigation into the effects of disturbance on captive sea urchins. Dale et al. (2005) measured the effects of handling (either rough or gentle) during harvest and transport and two levels of air exposure on *S. droebachiensis*. They showed that rough handling had a significant effect on mortality and on gonad growth. Gonad growth in the rough-handling treatment was still significantly greater than in the control urchins, but the degree of gonad growth was restricted as a result of significant spine damage from the rough handling. In addition, the desiccation of the urchins with prolonged exposure to air caused significant mortalities (25%), which occurred during the first four weeks of the experiment. This is a similar pattern (but at a much higher level) to the mortality described in Section 8.4.1 in the current study.

In the current study there were no significant effects from limited exposure of *E. chloroticus* to air when urchins were removed from the water to be fed for approximately 10 minutes, three times per week (see Section 4.5.2). This shows that although long periods of air exposure can have severely detrimental effects on sea urchins, they are tolerant of regular short duration removal from water without adverse effects. In light of the previous section (8.4.3.1), where removal of waste products has been shown to be important in a holding system, the removal of the

cages from the water for very short periods may assist in the removal of accumulated solids in the cages. The wave disturbance (or vertical movement) that urchins held in sea-cages suspended from a surface mussel longline are exposed to has been shown to have a positive effect on mortality and gonad growth (Chapter 4). This may again be related to the removal of accumulated solids and waste metabolites in the cages.

8.4.3.3. Water movement

There is strong anecdotal evidence from kina fishers in New Zealand that kina are generally in better condition, and often larger, at sites where there are strong currents (P. Herbert, Sea Urchin New Zealand; C. McManaway, NZ Sea Products, pers comm.). The benefits of increased currents may be twofold. Firstly the urchins are exposed to larger amounts of drift algae (Barker, 2007; Andrew, 2003) which increases food availability. Secondly, stronger currents increase the dissolved oxygen available to the urchins, and improve solid and metabolite waste removal.

In the aquaculture of any species the aim is to hold the urchins in optimal conditions for growth and survival. It appears that for kina, high feed availability and high water flow are both important factors in wild populations for producing large, good quality roe. During roe enhancement urchins are supplied with optimal levels of high quality food. However, the effects of water movement on captive sea urchins in either land- or sea-based holding systems has received virtually no attention in any previous studies. The only study that does mention the effects of water movement is Motniker et al. (1998) in which the effects of increasing the frequency of water exchanges, as well as varying salinities and densities, on roe enhancement of *S. droebachiensis* in a land-based system are described. The effects of water exchange were tested by exchanging the water within the holding tanks either once per hour or

once per day. The study showed that none of the experimental factors affected gonad growth but that the most critical of the experimental factors affecting mortality was the frequency of the water exchange in the tanks. Hahn (1989) lists water flow, along with the amount of dissolved oxygen present, concentrations of excreta, avoiding rapid changes in water temperature, and light levels, as the most important factors affecting the growth rates of abalone held in land-based tanks. Hahn (1989) also describes the accumulation of excreta as the single most important factor and suggests that sufficient water flow ameliorates the build up of excreta in holding tanks. This would support the theory from the current study that the accumulation of excreta and decreasing oxygen level in holding systems are the most important factors responsible for decreasing gonad growth in sea urchins held in short term roe enhancement trials. This explains the results in Chapter 4 showing that increasing the water movement past captive *E. chloroticus* had a significantly positive effect on gonad growth of the urchins over a relatively short period (10 weeks).

The anatomy of sea urchins makes them particularly susceptible to the effects of water movement. The gills that are used for gas exchange are situated externally and are simple, highly-branched out-pockets of the body wall lined with a ciliated epithelium (Barnes, 1974) (Fig. 1.3). Although sea urchins do have limited ability to pump seawater by altering the coelomic pressure within the peripharyngeal coelom (Fig. 1.6) and forcing fluid into and out of the gills, thus increasing gas exchange, they principally rely in the movement of water past their external surface for gas exchange. The primary circulatory medium is the coelomic fluid which contains coelomocytes that function to transport food and waste material. It is thought that the coelomocytes carry particulate waste to the gills, podia and axial glands (Fig. 1.3 and 1.4) for disposal but that there is no active soluble waste excretion. Instead this is also

controlled by the amount of water movement past the external surface of the urchin. This is likely to be the reason why urchins exposed to significantly higher water flows, as is the case in the experimental holding systems in Chapter 4, are capable of producing significantly larger GI values over a 10-week period compared to urchins in cages that have limited water flow. This has important implications for the design of both land- and sea-based holding systems where urchins are normally held at high densities, often in tank designs that do not encourage water flow throughout the tank (Dagget et al., 2006). In a land-based system it is critical to remove accumulated waste by providing sufficient water flow over the captive urchins. This may be done using tipper buckets (as previously described) which simply accumulate inflowing water and dump a large quantity into the holding tanks at regular intervals, ensuring water movement throughout the tank. Water movement can also be achieved using very high aeration within the tanks to move the water throughout the holding system. This has been successfully used in experimental and commercial scale land-based holding systems in New Zealand (James et al., 2004; James 2006c) (See Fig. 1.10a). Alternatively, in sea-based systems cages should be designed so that the urchins held in them are exposed to suitable water flows. The factors that must be taken into account in sea-cage design include the following:

- The direction of water flows and placement of vertical surfaces parallel to these flows.

- The outer mesh of the sea-cage must be large enough to allow water movement throughout the interior of the cage.

- The mesh on the bottom of the sea-cage must be small enough to retain food but large enough to allow water movement throughout the interior of the cage

during vertical movement of the cage (particularly when suspended from mussel lines).

- Handling and lifting of the cages should be kept to a minimum and open topped cages are ideal for easy access and feeding (regular 10 minute air exposure did not have a detrimental effect on gonad growth of captive urchins in the current study).

A sea-based holding system utilising these design criteria has been successfully tested for roe enhancement of *E. chloroticus* in New Zealand (See Fig 1.10b) (James, 2006c).

8.4.3.4. Land- vs. sea-based comparison and effect of cage depth

The results in Chapter 3 show that there are no significant differences in either gonad growth or mortality of *E. chloroticus* held at depths of 3 and 6 m below the water surface. A similar experiment on *S. droebachiensis* held in sea-cages placed on the sea-floor at 10 and 20 m depth showed no significant difference in the gonad growth of urchins when fed the optimal algal diet of *Laminaria digitata* and *L. longicruris* (Cuthbert and Hooper, 1995). Similarly, the results in Chapter 3 in the current study, and those of Cuthbert and Hooper (1995) show no significant difference in the gonad growth of sea urchins held in similar sea- and land-based holding systems for 10 weeks and 25 weeks respectively. Neither were there any differences in the mortality of the urchins in the current study where the holding cages were identical in the land- and sea-based holding systems. Cuthbert and Hooper (1995) reported higher mortality in sea-cages and more variable results in sea-cages than in aquaria but their experiment ran for 2.5 times longer than the present study and, unlike the present study, the design of the sea- and land-based holding systems was very different.

8.4.3.5. Economic implications

Worldwide, most roe enhancement trials and experiments have been undertaken in land-based facilities where it is relatively simple to control environmental factors and to maintain and feed the urchins (Table 1.1). However, the commercial reality of purchasing, establishing, running and maintaining land-based facilities can reduce the prospects for a venture to the point where it is no longer economically viable (Burke, 1997; Jeffs and Hooker, 2000; James, 2006c). There are also a number of hidden costs associated with sea-based roe enhancement, such as obtaining variations on existing marine farm licenses to enable kina to be held, and the current limitations on establishing new marine farms (Jeffs and Hooker, 2000). In addition, the often remote location of marine farms, the large distances involved in commuting between sites and the logistical requirements for boats, hauling equipment, and qualified and reliable staff also add to the expense of sea-based aquaculture and must be taken into consideration.

A comparison of commercial kina roe enhancement trials at a land-based paua farm at Te Kaha and sea-based mussel farms in the Marlborough Sounds showed that the cost of maintenance and feeding in a sea-based roe enhancement trial was only 17% of that in a land-based trial (James, 2006c). No significant differences in gonad growth or urchin mortality between land- and sea-based holding systems in the present study suggest that the lower set-up and maintenance costs of sea-based roe enhancement make this the more economically viable option. However, further economic modelling is required to calculate the overall cost/benefit of land-based vs. sea-based roe enhancement.

8.5 Implications for commercial roe enhancement in New Zealand

Because of the scale, the scientific nature and the limited dissemination of much post-graduate research, valuable information is often overlooked by commercial operators. This section is an attempt to succinctly summarise the results of this study that are applicable to the development of commercial roe enhancement in New Zealand.

8.5.1 General recommendations

- Future roe enhancement endeavours should focus, at least initially, on supplying the domestic market.
- Roe enhancement should aim to supply good quality kina roe to overcome current supply shortages caused by unfavourable weather conditions and remote fishing sites.
- Roe enhancement should aim to produce the maximum increases in GI in the shortest possible time period.
- Roe enhancement should aim to increase the quantity of roe of *E. chloroticus* all year round whilst maintaining the best possible quality.

8.5.2 Handling and land- and sea-based holding systems

- A maximum stocking density of 6 kg kina m^{-2} is suggested for *E. chloroticus*. Higher stocking densities may be possible without compromising gonad growth or kina mortality, but this requires further research.
- The handling and transportation protocols used in the current study are ideal for roe enhancement of *E. chloroticus,* for both future research and commercial collection of kina.

- Sea- and land-based holding systems should be designed so that the urchins are exposed to sufficient water flow to provide oxygen and remove metabolic wastes. Further research is required to define the optimal water flow rates at varying sea urchin densities.

- In land-based systems, high water flow can be achieved using high levels of aeration, or tipper buckets.

- There are no significant differences between gonad growth or urchin mortality in land- and sea-based holding systems, but the latter are significantly more cost effective to run and maintain. Further economic modelling is required to calculate the overall cost/benefit of land vs. sea-based systems.

8.5.3 Optimal environmental conditions for roe enhancement

- Roe enhancement ventures in New Zealand should utilise the low GI urchins that are abundant and easily fished, and are highly productive when held in roe enhancement conditions.
- The temperature of the seawater in urchin holding systems should be as close as possible to 18^{o}C for roe enhancement.
- In sea-cages this could be achieved by holding urchins further north where ambient seawater temperatures are higher.
- In land-based holding systems a cost/benefit analysis would be required to calculate whether increased returns from higher GI values would outweigh the cost of raising the water temperature and increasing the food consumption.
- Manipulating the photoperiod for the 10-12 week periods of roe enhancement is unlikely to be beneficial.

- Raising or lowering the water temperature to advance or inhibit the reproductive cycle of *E. chloroticus* causes only small differences over the 10-12 week roe enhancement periods. Significant changes in the reproductive cycle using temperature, and possibly photoperiod, may be possible if roe enhancement is carried out over longer periods.

8.6.Thesis conclusions and future research

8.6.1 Thesis conclusions

The most likely scenario for the establishment of a roe enhancement industry in New Zealand is one based on utilising the currently under exploited, low condition and plentiful kina found in kina barrens. These urchins are easily found, inexpensive to harvest, do not undermine the current fishery, and show good increases in GI from roe enhancement. Both land- and sea-based holding systems offer viable options for roe enhancement but sea-based systems have significantly lower maintenance and running costs. Roe enhancement in New Zealand should focus on maximising the increase in GI over as short a period as possible (9-12 weeks) to produce roe for the domestic market (Fig. 8.1).

This study has shown that in the short term (10-12 weeks) it is not possible to manipulate the gametogenic cycle of *E. chloroticus* to extend the season (January to May) (Fell, 2002) during which the roe would be suitable for export to overseas markets such as Japan (assuming it is possible to produce roe of high enough quality to be accepted in the Japanese market). Extending the season may be possible by holding urchins for much longer periods but this is unlikely to be economically viable

given the significant increase in costs from holding kina in either land- or sea-based systems for extended periods of time.

As with a number of other sea urchin species, feed availability is the major factor controlling the gametogenic cycle, and increases in GI values in both wild and captive *E. chloroticus*. However, there are a number of factors that have a significant effect on the amount of increase in GI and these should be taken into consideration when designing holding systems for kina and when planning site locations for future roe enhancement operations.

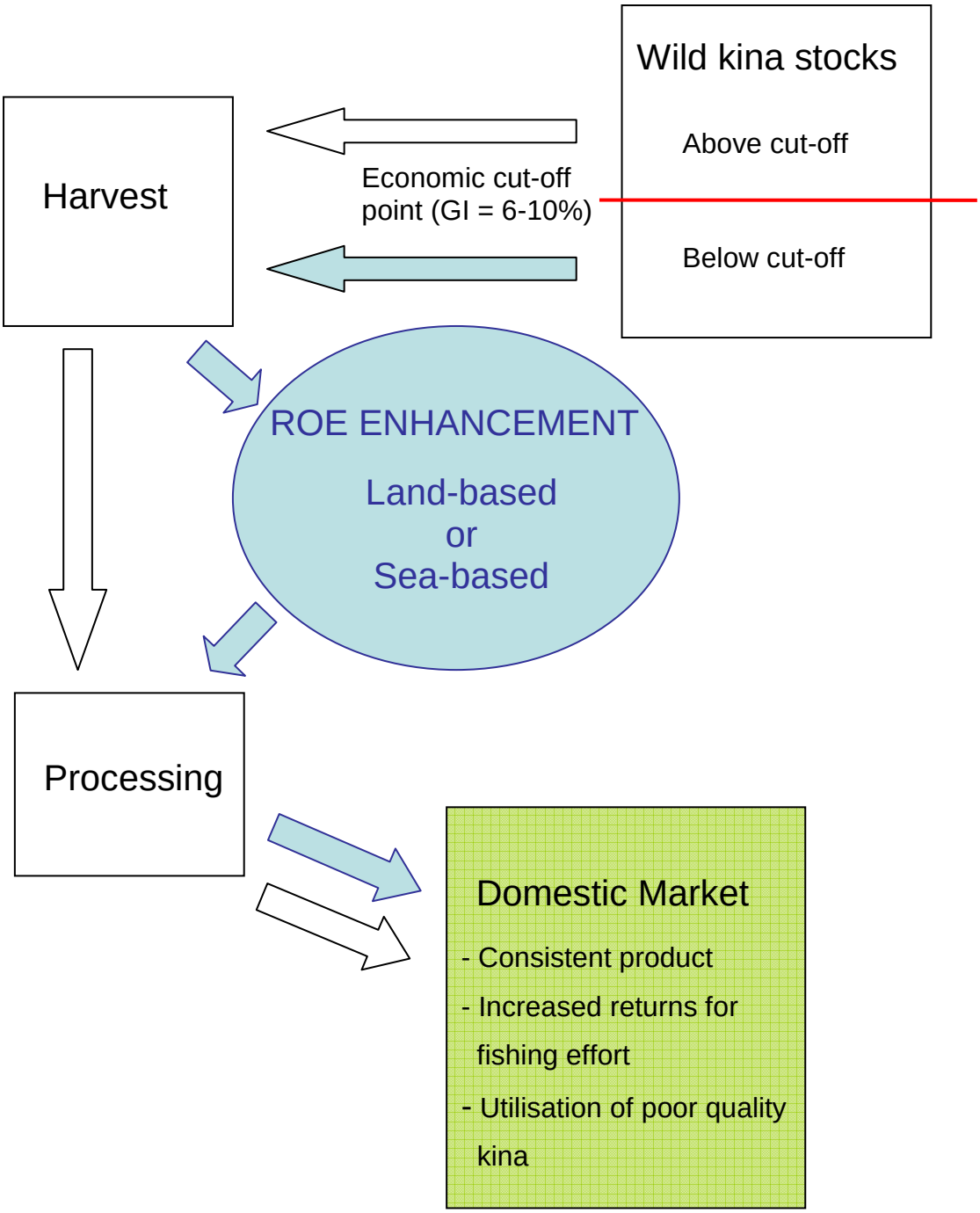

Figure 8.1 A schematic diagram showing the process that is most likely to be successful for future roe enhancement of *E. chloroticus* (shaded blue) ventures in New Zealand and to complement the existing fishery process (un-shaded). It utilizes low GI urchins for roe enhancement in sea-cage holding systems to supply consistent product for the domestic market (shaded green). This would increase returns from the existing fishing effort and utilize urchins that are currently uneconomic to harvest.

8.6.2 Future research

As with many studies this research has highlighted areas that require further research. These are as follows:

- To test stocking densities greater than 6 kg urchin m^{-2} cage internal surface area, and identify whether they negatively impact gonad growth of *E. chloroticus.*
- To identify whether 18^{o}C is optimal for roe enhancement of *E. chloroticus* or if higher temperatures increase gonad growth.
- To determine the optimal initial condition for roe enhancement by using urchins collected from a single population, but from numerous sites (within the restricted range of the population) where the kina are in a range of condition values rather than simply good (high GI) and bad (high GI).
- Investigate the micro habitats in both land-based and sea-based holding systems to investigate whether there are variations in habitat within the cages and whether they effect roe enhancement.
- Investigate the effects of environmental conditions on the taste quality of enhanced urchins.
- Investigate the effects on existing kina populations and the kina fishery of collecting large quantities of low GI kina for roe enhancement.

Appendix One Tipper vs. Trickle experiment

Introduction

The experiment in Chapter 4 clearly demonstrated that increases in water movement had a significant positive effect on gonad growth after 12 weeks of roe enhancement. An experiment was designed to test the hypothesis that it was the actual increased water movement and not just the increased availability of dissolved oxygen that accounted for this result. The following is a brief description of this small scale experiment.

Materials and methodology

The urchins for the experiment were collected using the same technique, at the same time, and from the same site as those used in the summer experiment in Chapter 6. The urchins were transported using the same techniques as described for the other experiments in this study. On arrival at the Mahanga Bay facility they were randomly placed into eight plastic holding baskets (see Chapter 5 for description) at a density of 20 urchins per basket. Each basket was placed in one of eight black polyethylene tanks fitted with an airstone that delivered air directly under the middle of the basket. The eight tanks were randomly allocated to four ‘trickle’ and four ‘tipper’ treatments. These treatments were as follows:

Trickle - 12 l/min. of ambient seawater trickled into the tank from a PVC pipe inlet

Tipper - 12 l/min. of ambient seawater trickled into a 10 litre tipper bucket designed to tip when the bucket contains 6 l of water. The bucket tips its entire contents into the middle of the tank then returns to the upright position and refills. Tipping occurs approximately every 30 seconds (Fig. 1).

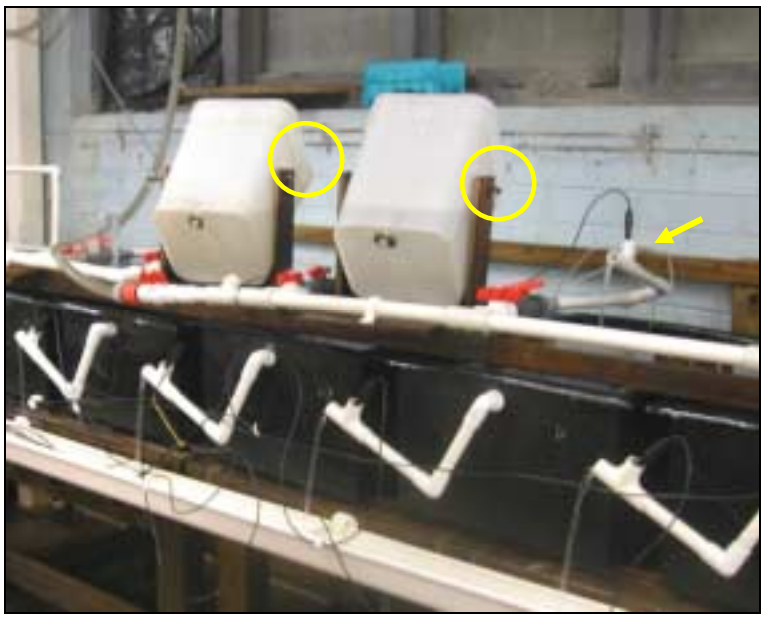

Figure 1 Experimental tanks including 2 tippers and 1 trickle (outlets of 4 tanks shown emptying into a drain). Note the offset hinge (circled) that allows the Tipper bucket to tip forward and empty its contents into the tank in one surge (furthermost two tanks). The tank on the right has a Trickle inlet (arrowed) that provides the same amount of water into the tank as into the Tipper bucket.

The urchins were fed the NIWA artificial diet *ad libitum* for 10 weeks and the variables listed in Section 2.5.1 were measured after 10 weeks.

The dissolved oxygen (DO) in each of the tank outflows was measured every 10 minutes (see General Methodology section for monitoring equipment that was used) for a 24 hr period prior to the urchins being placed in the tanks.

Plaster cubes (pre-weighed to constant weight) as described and used in Chapter 4 were attached to the side and bottom of the experimental baskets prior to urchins being placed in the baskets. The difference in the amount of water flow within each tank was measured by comparing the weight loss of the plaster cubes over two days. The cubes were removed after 2 days, dried to constant weight and reweighed.

Results

There was a significant difference in the dissolved oxygen levels of the seawater in the outlets of the 'Tipper' and 'Trickle' tanks (Fig. 2) (Student *t*-test, $T_{1, 60} = 2.16$, $P < 0.05$). However, the differences in the dissolved oxygen levels were unlikely to be significant on a biological level due to the very high values of dissolved oxygen in both treatments (average DO in trickle treatment = 93.6mg/l and for the tipper treatment = 94.1mg/l).

There was significantly greater weight loss, as a result of water movement in the Tipper tanks than the Trickle tanks regardless of the position of the plaster cubes (Fig. 3).

The urchins held in the Tipper tanks had a significantly higher GI (Student *t*-test, $T_{1, 60} = 2.16$, $P < 0.05$) than those held in the Trickle tanks (15.9% and 14.2% respectively) for 10 weeks (Fig. 4). Both experimental treatments had significantly higher GI values (One-way ANOVA: F = 163.7, $df = 3, 240$, $P < 0.001$) than the wild urchins collected at the beginning (initial) and conclusion (final) of the experiment.

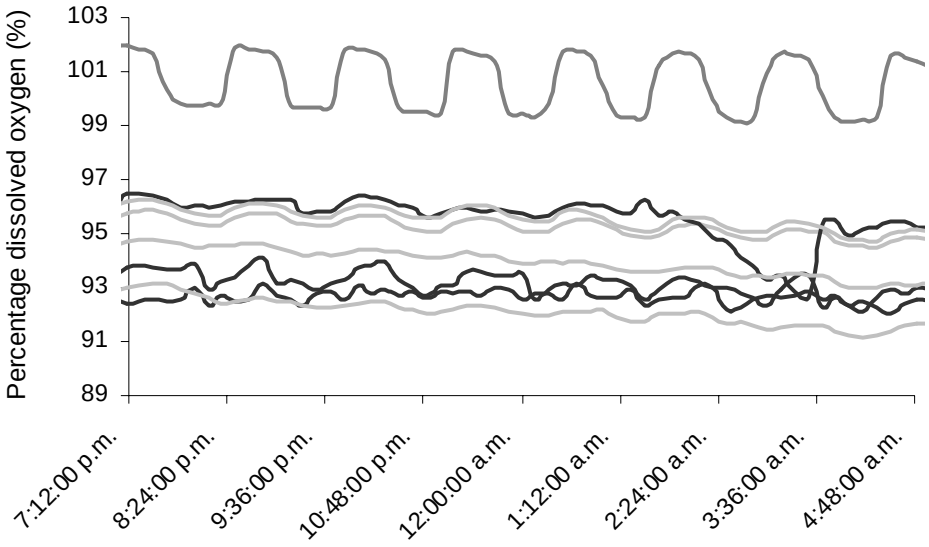

Figure 2. The comparative levels of dissolved oxygen in the 'Tipper' (black lines) and 'Trickle' (light grey lines) tanks measured every 10 minutes over a 24 h period. The dark grey line shows the dissolved oxygen levels in the seawater flowing into the tanks.

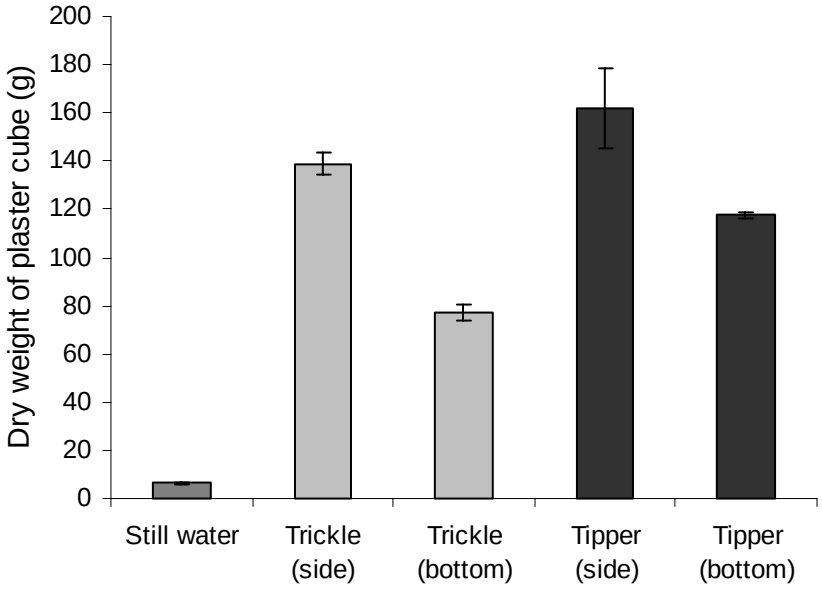

Figure 3. The dry weight loss of plaster cubes held in either 'Tipper' (black) or 'Trickle' (light grey) and attached to the side or bottom of the tanks over two days. A control treatment was placed in still water (dark grey) for the same period (*n* = 20).

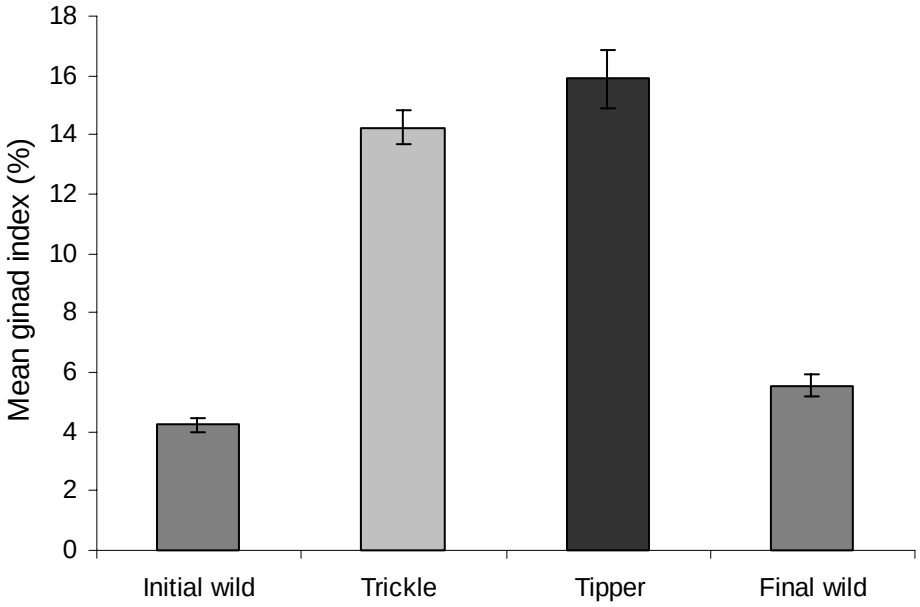

Figure 4. The mean gonad index (± 1 S.E.) of the urchins collected from the wild population at the beginning (initial) and conclusion (final) of the experiment and from the urchins held in the 'Trickle' and 'Tipper' tanks for 10 weeks.

Discussion

The experiment showed that *E. chloroticus* held in tanks with increased water movement have significantly greater gonad growth than those held in tanks with less water movement. This is not due simply to an increase in the dissolved oxygen levels from the higher water flows in the tipper tanks. Although this treatment had significantly higher dissolved oxygen levels than the trickle treatment, the dissolved oxygen in both treatments was at such a high level that the differences are unlikely to have any biological significance. It more likely results from a combination of better access to dissolved oxygen and better removal of waste metabolites from around the urchins, both caused by the higher water flows.

The implications of this study are discussed further in Chapter 8. Further research is recommended using a more reliable method of long term dissolved oxygen monitoring to verify the results of this experiment.

Appendix Two Clarification of involvement

Origins of concept:
Phil James was responsible for the NIWA kina research programme for past 5.5 years including future directions and long term planning. This includes reporting to FoRST, recommending future research, collaboration with commercial clients and publication and dissemination of results. Project budgeting and project management.
(overseen and advised by Phil Heath)

Planning on Paper, construction and installation of experimental systems:
Phil James had responsibility for all planning and construction of experimental systems, with input from senior technical officer, John Illingworth as required (e.g. 12 volt waterproof lighting system for photoperiod treatments), and other staff if required for construction phase (e.g. all diving collections required a minimum of three NIWA divers).

Daily routine, feeding and cleaning:
Phil James was responsible for approximately 80% of daily feeding, cleaning during experiments with a core group of staff working alongside to cover times when I was not available (note experiments ran for a total of 97 weeks)

Dissections and census:
Phil James was totally responsible (cracked every kina). Undertook all dissection with the aid of either one or two other NIWA staff (*n*=720 per census in larger experiments).

Histology:
The fixing and staining of all histology slides was contracted to Otago University at Wellington Hospital.

Reading histology:
Phil James was totally responsible for reading the results of the histology slides with assistance and advice from Graeme Moss and Chris Woods at NIWA.

Satistical anlaysis and publication:
Phil James was responsible for analysis of all data from Chapters 3, 4, 5 and 7 with assistance from Phil Heath and Doug McNaught as required. Phil was also responsible for analysis of Chapter 6 with verification of results by Martin Unwin (NIWA Christchurch). Phil was entirely responsible for the publication of all papers, including all correspondence with the journal ‘Aquaculture’.

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