AQUACULTURE OF THE BIG-BELLIED SEAHORSE *HIPPOCAMPUS ABDOMINALIS* LESSON 1827 (TELEOSTEI: SYNGNATHIDAE)

By

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Photo: Chris Woods

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ABSTRACT

Seahorses (Teleostei: Syngnathidae) are subjects of worldwide demand for medicinal use, as curios, and as live ornamental aquarium fish. Aquaculture has the potential to replace or at least supplement potentially unsustainable wild exploitation as the supply source of seahorses. The primary aim of the research within this thesis was to determine techniques for improving the technical and economic feasibility for commercially culturing the big-bellied seahorse *Hippocampus abdominalis* in New Zealand.

In a preliminary investigation, the breeding of wild *H. abdominalis* in captivity and rearing of juveniles was examined, as difficulties have been encountered with these in historical attempts at culturing *H. abdominalis*. Breeding was found to be facilitated by providing tanks with a water height of 1 m. This depth of water allowed females to transfer their eggs to male seahorses during the vertical rising stage of mating. Growth rates of progeny to sexual maturity were reasonable with seahorses reaching an average 11 cm in standard length (SL) at one year of age, but high juvenile mortality was observed in the first few months of age, with an average 10.6% of juveniles surviving to one year. Further on-growing of these first generation progeny to seven years of age (average of 27 cm SL for both sexes) demonstrated the robustness of the species in captivity and potential to supply large seahorses to the medicinal trade where large size is desirable.

To improve juvenile survival and growth, the effects of initial tank colour, lighting arrangement and stocking density on early juveniles were tested. Juveniles at one week of age were found to have higher attack rate and capture success on *Artemia* nauplii in clear jars than those contained in white- or black-wrapped jars, but this effect of tank colour had less affect on one month-old juveniles. Juveniles were also found to suffer fewer incidences of air bubble ingestion in side-illuminated tanks due to positively phototactic prey (*Artemia*) being drawn away from the water surface. The rearing of juveniles from birth to two months of age in glass aquaria with side-illumination and tank surfaces blacked-out above the waterline resulted in survival rates of >80% due to increased feeding efficiency and reduced risk of air bubble ingestion. Juvenile growth and survival at stocking densities of 1, 2 and 5 juveniles l^{-1} demonstrated that increasing stocking density resulted in reduced growth and survival, due to the greater occurrence of juveniles grasping and wrestling each other with their prehensile tails.

Producing live foods for fish is a significant cost in finfish culture. This has led to concerted efforts to develop appropriate artificial or inert diets to reduce culture costs. To determine whether juvenile seahorses could be weaned from live food to inert diets, two inert diets (Golden Pearls and frozen copepods) were tested. It was demonstrated that one and two month-old juvenile *H. abdominalis* could ingest and survive on these inert foods. Co-feeding the inert diets with live *Artemia* improved feeding on the inert foods. However, growth and survival rates of juveniles on the inert diets were inferior to those fed only on live enriched *Artemia*.

Cultured live foods such as *Artemia* are often enriched with various enrichment media to boost their nutritional value. However, enrichment media can vary in their nutritional value relative to the final target organisms they are being fed to, as well as their relative cost-effectiveness. Therefore, the effect of different *Artemia* enrichments on the growth and survival of *H. abdominalis* and their relative cost-effectiveness was tested using three commercial enrichment products (Super Selco®, DHA Protein Selco® and Algamac-3050®) and a low-cost *Artemia* on-growing diet (EPABSF/*Spirulina platensis*). On a cost/benefit basis, EPABSF/*S. platensis* worked out to be the most cost-effective for *H. abdominalis*, with comparable growth rates to seahorses fed *Artemia* enriched with DHA Protein Selco® and Algamac-3050®. Juvenile growth rates were poorest on *Artemia* enriched with Super Selco®.

Feeding seahorses frozen mysid shrimp may help reduce culture costs and also increase cultured seahorse marketability to the aquarium trade, but their efficacy in seahorse culture is largely untested. Frozen mysids (*Amblyops kempi*) were shown to be an acceptable alternative to live enriched *Artemia* for *H. abdominalis*, providing comparable rates of seahorse growth and survival. When daily rations of frozen mysids at 5%, 10%, 15% and 20% wet body weight (wbw) were tested there was no growth advantage to feeding seahorses more than 5% wbw per day in terms of increase in seahorse length. There was a wet weight gain and Condition Factor (CF) advantage associated with increasing feed ration >10%. Feed conversion ratios (FCR) became less efficient as feed ration increased based on the total amount of mysids offered to seahorses, with increasing food wastage. However, when actual mysid consumption was taken into account there were no significant differences in FCR between rations.

The natural diet and male reproductive output of *H. abdominalis* in Wellington Harbour was examined for use as aquaculture benchmarks. Natural diet consisted mainly of epibenthic and epifaunal crustaceans (e.g. amphipods, mysid shrimp and caridean shrimp). There were no sex-related differences in diet although there were some size-related differences with smaller seahorses consuming more amphipods. Some of the prey species eaten by wild *H. abdominalis* may show potential as cultured foods. Wild males produced an average of 271 juveniles per brood, with brood size increasing with parent male size. Comparison of wild reproductive output data with those of cultured male *H. abdominalis* revealed that cultured male output was approximately 27% lower than that of wild males. However, there were no differences in the quality (size and weight) of the juveniles produced by wild and cultured male *H. abdominalis*. It is suggested that cultured female reproductive output is the primary determinant in lower cultured male seahorse reproductive output.

The research within this thesis has contributed to improving the technical and economic feasibility for commercially culturing the big-bellied seahorse *Hippocampus abdominalis*.

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benefited no-one (including the seahorses). Greg and Mic, you have responsibly proven that commercial seahorse aquaculture is a viable activity.

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This thesis is dedicated to my family

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GENERAL INTRODUCTION

1.1 Taxonomic classification

Seahorses are bony fishes (Teleosts) belonging to the family Syngnathidae (Lourie *et al.*, 1999). The family Syngnathidae also includes pipefishes, pipehorses and seadragons. The primary taxonomic groupings within this family reflect the location and development of the male brood pouch, head/body axis, development of the caudal fin, and prehensile ability of the tail (Herald, 1959; Lourie *et al.*, 1999; Wilson *et al.*, 2001; Kuiter, 2003) (Fig. 1.1). Together, seahorses and pygmy pipehorses comprise the subfamily Hippocampinae, characterized by a fully enclosed brood pouch with a small opening for the incubation of eggs, an absent or vestigial caudal fin, and a prehensile tail. Pipefish form the subfamily Syngnathinae, with the head in line with the body, a small caudal fin often present, eggs incubated in a pouch formed by simple or overlapping skin membranes under the trunk or tail. The tail is not normally prehensile. There is also a group of pipefish which form the subfamily Doryrhampinae, characterized by mostly exposed broods and a large flag-like caudal fin. Seadragons and pipehorses comprise the subfamily Solegnathinae, in which the head is held at a slight angle to the body, the tail is more or less prehensile, the caudal fin is absent, and incubated eggs are exposed under the tail or trunk section (Kuiter, 2003).

All seahorses belong to the one genus, *Hippocampus*. Historically, 110–120 seahorse species names exist in literature and museum collections, but many of these appear to be compromised by misidentification, synonymy, and even misspelling on labels (Lourie *et al.*, 1999; Kuiter, 2003). Recent taxonomic revisions of seahorses have seen a reduction in the number of species described. Kuiter (2003) recognized approximately 70 seahorse species, whilst Lourie *et al.* (1999) and Lourie *et al.* (2004) recognize 32–33 seahorse species. The latter taxonomic guides have been adopted by the United Nations Convention on International Trade in Endangered Species (CITES) and the World Conservation Union (IUCN) as their official conservative taxonomic references. In the absence of a reliable seahorse fossil record, recent genetic analyses suggest that seahorse evolution is pre-Tethyan (>20 million years ago) with little phylogeographic variation amongst seahorse species (Casey *et al.*, 2004).

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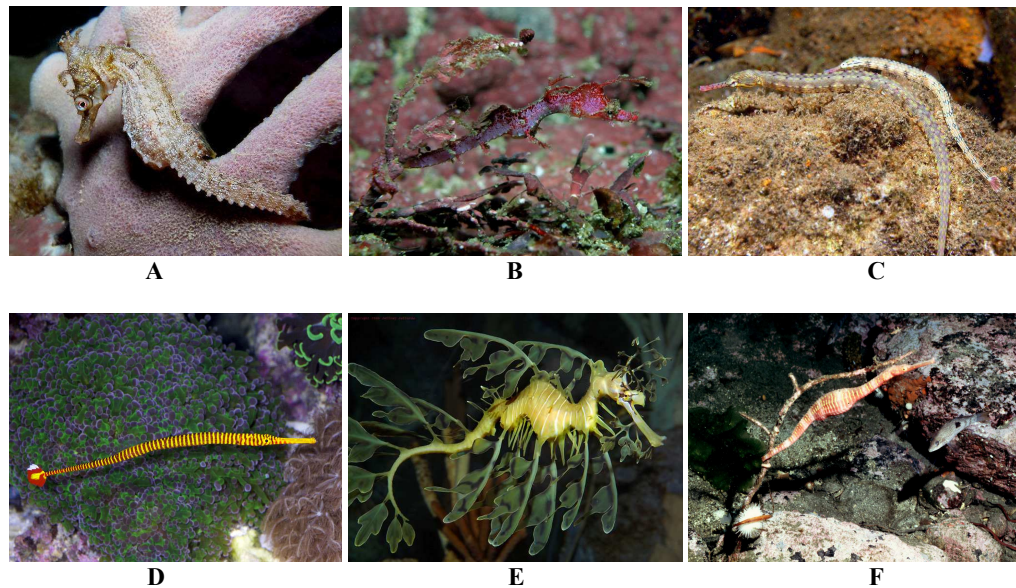
Figure 1.1 Examples of subfamilies of Syngnathidae: (A) Hippocampinae – White's seahorse (*Hippocampus whitei*, source: Dave Harasti), (B) Hippocampinae – Sydney pygmy pipehorse (*Idiotropiscis lumnitzeri*, source: Dave Harasti), (C) Syngnathinae – Reef-top pipefish *Corythoichthys haematopterus*, source: Dave Harasti), (D) Doryrhampinae – Yellow banded flag tail pipefish (*Dunckerocampus pessuliferus*, source: Greg Rothschild), (E) Solegnathinae – Leafy Seadragon (*Phycodurus eques*, source: Jeffery Jeffords), (F) Solegnathinae – Spiny Pipehorse (*Solegnathus spinosissimus*, source: Karen Miller).

1.2 Distribution and habitat

Seahorses are found in both temperate and tropical shallow coastal waters (<150 m depth), with a latitudinal distribution from about 50° north to 50° south and greatest species diversity in the Indo-Pacific (Lourie *et al.*, 1999; Perante *et al.*, 2002; Lourie *et al.*, 2004). Most seahorse species are restricted to seawater, but certain species such as *H. abdominalis*, *H. capensis* and *H. kuda* may also be found in fluctuating salinity habitats such as estuaries and lagoons (Bell *et al.*, 2003; Choo & Liew, 2003; Martin-Smith & Vincent, 2005).

Seahorses are found amongst cnidarians, corals, macroalgae, mangrove roots, octocorals, seagrasses, sponges, and tunicates, where they anchor themselves to the substratum with their prehensile tails, as well as on open sandy and muddy bottoms or in rocky crevices (Choo & Liew, 2003; Dias & Rosa, 2003; Kendrick & Hyndes, 2003; Lourie *et al.*, 2004; Curtis & Vincent, 2005, 2006; Lourie *et al.*, 2005; Martin-Smith & Vincent, 2005). Overall, the most commonly reported habitat is seagrass and mangroves the least reported (Foster & Vincent, 2004). Artificial structures such as jetties and

mussel farms also provide suitable habitat for seahorses (Kuiter, 2003). Within suitable habitats, some seahorse species appear to exhibit preferences for different substratum (Dias & Rosa, 2003), and some species may even change habitat and depth seasonally and as they grow (Foster & Vincent, 2004). For example, Curtis & Vincent (2005) observed distribution and habitat use by *H. guttulatus* and *H. hippocampus* in the Ria Formosa lagoon in Portugal. They found that *H. guttulatus* abundance was positively related with complex habitats whilst *H. hippocampus* used more open and less specios habitats. Both species preferred grasping holdfasts over barren surfaces but *H. hippocampus* avoided fauna and flora that formed large colonies or tracts of dense vegetation. Curtis & Vincent (2005) related these habitat preferences to differences in morphology and foraging strategies between the two species.

1.3 Biology

Seahorses are one of the most easily identifiable of all types of fishes by virtue of their unusual morphology. The equine-like head of the seahorse has a tubular snout with a terminal, toothless mouth. The covered gills are squat grape-like structures with the exhalant opening reduced to a small pore. The body of the seahorse has a leathery armoured appearance. This results from an external covering of bony plates arranged in a series of rings (Fig. 1.2). Where the plates intersect, the skin is raised into small tubercles or spines, whose degree of development varies with species and sometimes age of the seahorse. Tendril-like skin growths, or filaments, sometimes occur on the upper surfaces of the head and neck (Lourie *et al.*, 1999).

Figure 1.2 X-ray image of a juvenile male seahorse, *Hippocampus abdominalis*, showing characteristic skeletal structure (source: John Baehler/Alan Blacklock, NIWA)

Seahorses use their fin-less, strong prehensile tail to anchor themselves to various substrates (Hale, 1996). They do not often swim, but when they do, they usually remain upright, with forward inclination when swimming speed increases (Blake, 1976). The body form and fin development in seahorses favours maneuverability in complex habitats over speed in open water. Rapid undulations of the large dorsal fin on the back provide the main propulsive force, while the smaller pectoral fins behind the gills provide steering control. They lack pelvic and caudal fins, and have a reduced anal fin which has a minor role in ascending propulsion (Blake, 1976; Lourie *et al.*, 1999).

Most seahorse species studied to date are active during the day, appear to exhibit low mobility, and appear to maintain individual home ranges, at least during the breeding season, without territorial defense (Perante *et al.*, 2002; Foster & Vincent, 2004; Vincent *et al.*, 2005; Curtis & Vincent, 2006). For example, *H. comes* ranged only 1m^2 on coral reefs at night (Perante *et al.*, 2002) whilst *H. kuda* ranged up to 35 m^2 over eelgrass beds (C.K. Choo, unpubl. data cited in Foster & Vincent, 2004). Sex differences in home ranges have been observed for some species such as *H. whitei*, where females have larger home ranges (Foster & Vincent, 2004). It has been postulated that sex differences in home range, if they exist, may arise due to physical and energetic constraints associated with male pregnancy (Vincent *et al.*, 2005).

Seahorses often blend-in very well colour-wise with their environment, and usually exhibit drab colouration. Pigment cells in their skin enable them to alter their colour, which, depending on species and habitat, can vary from drab grey and brown, white or black, to bright red, yellow and even purple (Vincent, 1996; Lourie *et al.*, 1999; Foster & Vincent, 2004). Superimposed on their base colour, seahorses may also display a wide variety of darker banding and blotching. Colour changes can also serve as a means of communication amongst seahorses, particularly during courtship and reproduction when colouration can become its brightest and colour changes more rapid (Vincent & Sadler, 1995; Masonjones & Lewis, 1996; Lourie *et al.*, 1999; Moreau & Vincent, 2004).

Seahorses are ambush predators which predominantly rely on stealth and camouflage to approach their prey (James & Heck, 1994; Flynn & Ritz, 1999; Lourie *et al.*, 1999; Foster & Vincent, 2004). Feeding typically occurs during diurnal or crepuscular hours, but can also occur at night in some nocturnally active species (James & Heck, 1994;

Foster & Vincent, 2004; Felício *et al.*, 2006). Prey is captured with a rapid flick of the seahorses' head and simultaneous buccal cavity expansion, which creates a strong inhalant current (Bergert & Wainwright, 1997). Typically, prey is consumed whole without mastication, although some seahorse species may break larger prey into smaller pieces by repeated feeding strikes before ingestion (Woods, 2002; Felício *et al.*, 2006). Seahorses are typically carnivorous, feeding upon a wide range of epifaunal and planktonic prey, particularly crustaceans such as copepods, amphipods, isopods, and caridean, euphausid and mysid shrimps (Reid 1954; Lovett 1969; Tipton & Bell 1988; Do *et al.*, 1998; Texeira & Musick 2001; d'Entremont, 2002; Kendrick & Hyndes, 2005; Felício *et al.*, 2006). Ontogenetic differences in diet have been observed with smaller seahorses selecting smaller prey items (Kanou & Kohno, 2001; Texeira & Musick, 2001) and prey preferences may be exhibited (Felício *et al.*, 2006).

Seahorses range in size from the diminutive *H. denise* (<3 cm length) to the large *H. abdominalis* (up to 35 cm length), with males and females of most species reaching approximately the same size (Foster & Vincent, 2004). However, males usually have a relatively longer tail, while females have a relatively longer body trunk (Foster & Vincent, 2004). Longer tails may enable males to support a large caudal brood pouch whilst still grasping a holdfast, or may give males an advantage in tail-wrestling that may occur during mating competition (Vincent, 1990).

Sexual differentiation in most species becomes obvious with the development of the brood pouch in male seahorses although this brood pouch development may not necessarily correlate with first development of mature testes in males (Cai *et al.*, 1984; Thangaraj *et al.*, 2006). Determination of sexual maturation in females is not as externally visible as in males. Methods for determining onset of female sexual maturity include size at which ovaries first appear (Kanou & Kohno, 2001) or the size of the smallest female to release her eggs (Cai *et al.*, 1984). Foster & Vincent (2004) suggested that size at first maturity should be defined as size when 50% of the males in a population first bred, although they recognized that conservation concern and pragmatism may dictate that the presence of a fully developed brood pouch will still be a key index of sexual maturity. Size, rather than age serves as a better indicator of first maturity than age in seahorses (Foster & Vincent, 2004). As with other marine teleosts, size at first maturation in seahorses is positively related with maximum size for each species (Foster & Vincent, 2004). For example, maximum recorded adult height (cm)

and height at first maturity (cm) for *H. barbouri* are 15 cm and 8 cm respectively, whilst for *H. fisheri* they are 8 cm and 5 cm respectively (Foster & Vincent, 2005).

The timing of the breeding season varies with location and may be influenced by environmental factors such as photoperiod and temperature. The duration of the breeding season is typically longer in tropical than temperate water species with year-round breeding occurring in some species (Foster & Vincent, 2004). For example, pregnant male *H. comes* were found year-round in the Philippines where water temperatures were relatively constant (Perante *et al.*, 2002). Most seahorse species mate monogamously, at least within breeding seasons, with the male accepting eggs from only one female (Foster & Vincent, 2004). Seahorses studied to date maintain conventional sex roles with males competing more intensely for mates (Vincent *et al.*, 1992; Vincent, 1994b; Foster & Vincent, 2004; but see Wilson & Martin-Smith *in press*).

Members of the family Syngnathidae are characterized by particularly pronounced adaptations for male parental care, with the female depositing her eggs into/onto a specialized incubation area that the male possesses following an often elaborate courtship and mating ritual (Vincent, 1995a; Vincent & Sadler, 1995; Lourie *et al.*, 1999; Wilson *et al.*, 2001). In a molecular phylogenetic study of syngnathids, Wilson *et al.* (2001) found evidence that the rapid diversification of male pregnancy in the Syngnathidae and increasing complexity of brood pouch structure indicate that highly developed male parental care has been closely associated with syngnathid radiation. In seahorses, the male incubating/brooding structure is the most developed of the Syngnathidae, forming a well-vascularised enclosed pouch with a muscular opening (Lourie *et al.*, 1999; Wilson *et al.*, 2001; Carcupino *et al.*, 2002). Fertilisation of the eggs appears to occur just prior to their deposition into the males' pouch (Van Look *et al.*, 2007). Once the female's fertilized eggs are deposited in the brood pouch, it is sealed and the eggs are then isolated from the external environment. Here, the developing young are kept by the male until they are ready for birth and are released from the brood pouch. It is perhaps this paternal care of the developing young that has endeared the seahorse to many people.

Within the brood pouch, the extensive capillary network allows for oxygenation and removal of waste products by diffusion, and the osmolarity of the pouch is gradually

increased to that of seawater during the course of the pregnancy (Linton & Soloff, 1964). Maternally derived nutrients (Boisseau, 1967) along with male-derived inorganic compounds (e.g. calcium, Linton & Soloff, 1964) and possibly anti-bacterial compounds (Melamed *et al.*, 2005) are also provided to the developing young. However, the definitive role of the male seahorse's brood pouch still remains to be determined.

Male seahorses typically go through several pregnancies in a single breeding season and the duration of brooding by male seahorse varies between approximately 9–45 days (Foster & Vincent, 2004). Brood size in seahorses varies markedly, ranging from one to five per brood for *H. zosterae*, up to 2000 in *H. ingens*, although males of most species produce 100–300 juveniles per brood (Foster & Vincent, 2004). Juvenile size at birth is also variable, ranging from 2 to 16 mm in length and appears to be positively related with increasing latitude (Foster & Vincent, 2004).

Upon birth the juveniles are fully formed, independent and capable of feeding. In most species, juveniles appear to be pelagic for several weeks feeding on planktonic organisms, although this aspect of seahorse biology is poorly understood and the extent of juvenile dispersal by passive means is unknown (Foster & Vincent, 2004). Young seahorses are much more likely to disperse than adults (Foster & Vincent, 2004). However, the association between seahorses, both juveniles and adults, and floating seaweeds (e.g. *Sargassum* sp.) has also been noted in open-water for some seahorse species (e.g. *H. erectus* and *H. reidi*, Coston-Clements *et al.*, 1991), suggesting a possible large-scale "rafting" dispersal mechanism (Castro *et al.*, 2002; Teske *et al.*, 2005). Teske *et al.* (2005) examined mitochondrial control region (mtDNA CR) in the Indo-Pacific *H. kuda* complex and found that of six populations investigated all were characterized by ancestral monophyly and recent population expansions, suggesting they were founded by a few individuals and then rapidly increased in population size.

The natural life span, mortality rates, growth rates, and disease prevalence for different seahorse species are largely unknown in their natural environment. Inferred life spans range from 1 year in the very small species to an average 3–5 years for larger species (Foster & Vincent, 2004). From *in situ* data Curtis & Vincent (2006) calculated a life span of 4.3–5.5 years, annual survival rates of 29.4–32.2%, and instantaneous growth rate coefficient $K = 0.57$ for *H. guttulatus*.

Population densities for seahorses are generally low (ranging between 0–0.51 individuals m^{-2}) with patchy distribution, but can reach up to 10 individuals m^{-2} in some seagrass patches (Foster & Vincent, 2004). Sex ratios within populations may be equal (Curtis & Vincent, 2006) or unequal in either sexes favour (Texeira & Musick, 2001; Bell *et al.*, 2003; Martin-Smith & Vincent, 2005).

1.4 International exploitation of wild seahorses

In 1996, it was estimated that at least 20 million seahorses were traded internationally, primarily to be used in traditional Chinese medicine (TCM) and its derivatives: Korean *hanyak*, Japanese *kanpo* and Indonesian *jamu* medicine (Vincent, 1996). Virtually all these seahorses were collected from the wild. Principal exporting countries at that time were India, the Philippines, Thailand and Vietnam, while principal importing countries were China, Hong Kong, and Taiwan (Vincent, 1996). Seahorse species predominantly used in TCM are species such as *H. histrix*, *H. kelloggi*, *H. kuda*, *H. spinosissimus* and *H. trimaculatus* (Vincent, 1996; Lourie *et al.*, 2004). Seahorses for supply to the medicinal trade may be caught as incidental bycatch during commercial fishing such as shrimp-trawling, or as part of targeted fisheries using techniques such as hand-collection in artisanal fisheries in India and the Philippines (Maricharmy *et al.*, 1993; Vincent, 1996; Martin-Smith *et al.*, 2004; Baum & Vincent, 2005; Giles *et al.*, 2005; Salin *et al.*, 2005; Meeuwig *et al.*, 2006).

In TCM, dried seahorses (Fig. 1.3) have been used for over 600 years, and in combination with other animal and plant extracts are credited with having a role in increasing energy flows within the body, decreasing cholesterol levels and reducing the risk of arteriosclerosis. They are also reputed to have a curative role in ailments such as asthma, arthritis; goiter, impotence, kidney disorders and various skin afflictions (Vincent, 1996). In Brazil, whole seahorses (*H. reidi*) are an important medicinal resource used to treat asthma and gastritis (Alves & Rosa, 2006). Until at least the eighteenth century, seahorses were also utilized for their medicinal properties in many western countries, with recorded applications back to 342 B.C., where they were reputed to have medicinal properties with regards to baldness, leprosy, urine retention and rabies (Bellomy, 1969; Vincent, 1996; Lourie *et al.*, 1999).

The monetary value of seahorses in the medicinal trade is extremely variable, with market determinants such as seahorse size, colour, condition and state (e.g. dried in good condition vs. in poor condition, whole vs. in pieces), history of usage, rarity, and the commercial effectiveness with which they are marketed all combining to dictate a large range in market value within and between countries. For example, in Shanghai, China 1997, retail prices for seahorses varied between US$302–846 kg^{-1} on a dry weight basis (E. Zhang, p. 52 in Moreau *et al.*, 2000), whilst in India 1998 the retail price varied between US$40–88 kg^{-1} on a dry weight basis (A. Lipton, p. 76 in Moreau *et al.*, 2000). Whole large seahorses generally achieve the highest prices. Inferior and very small seahorses are usually used in ground form in lower value patented medicines (Vincent, 1996).

Figure 1.3 Examples of seahorses in medicinal usage in Singapore: (A) TCM shop, (B) large dried seahorses on display in TCM shop window, (C) box of dried seahorses for sale in TCM shop, (D) Patented Seahorse "Genital Tonic Pills" (source: Chris Woods).

To date, evidence regarding the efficacy of the medicinal properties of seahorses and related syngnathids in published scientific literature has been scant in western literature. According to Vincent (1996), the Chinese generally regard historical use of TCM as testimony to a product's efficacy, and clinical trials are rare, but there have been

publications relating to medicinal properties such as anti-ageing effects (Yu *et al.*, 1995), arthritis (Shi *et al.*, 2006) and improved immune responses (Qu *et al.*, 1991; Zhang *et al.*, 2003).

A smaller international trade in live seahorses for the aquarium trade was also estimated at several hundred thousand seahorses per year in 1996, sourced from countries such as Brazil, Indonesia and the Philippines, and primarily for importation into the USA and Europe (Vincent, 1996; Larkin *et al.*, 2001; Baum & Vincent, 2005). Seahorses for supply to the aquarium trade may be caught as incidental bycatch during commercial fishing (Baum *et al.*, 2003; Baum & Vincent, 2005; Giles *et al.*, 2006), or as part of targeted fisheries such as by hand-collection in artisanal fisheries in the Philippines (Vincent, 1996; Martin-Smith *et al.*, 2004; Baum & Vincent, 2005). Historically this aquarium trade in wild-collected seahorses predominantly involved species such as *H. histrix*, *H. kuda*, *H. erectus* and *H. zosterae*.

Within the live aquarium market, desirable seahorse attributes are different to those of the medicinal market, as primary physical attributes that command higher prices in this trade are attributes such as colouration and body patterning, body shape, body condition, and body ornamentation. For example, in the Philippines aquarium trade, "yellow" seahorses were usually more valuable than "black" seahorses (Vincent, 1996). In the aquarium trade, size and a history of usage are not as important as criteria as they are in the medicinal trade. Prices for live seahorses within the aquarium trade vary markedly between and within countries. For example, in 2002 wild-caught putative *H. kuda* were being retailed for US$3.85–5.75 per seahorse in Indonesia (Arif Husen, Piranti Aquatica, pers. comm) while in the USA they were commonly being retailed for between US$9–39.

There are also a large number of dead seahorses sold as a diverse range of curios (Vincent, 1996; Baum & Vincent, 2005; Grey *et al.*, 2005; McPherson & Vincent, 2005), from key-rings and jewellery, to paperweights and dried whole animals. According to Grey *et al.* (2005), a mean of 64 738 ± SD 97 398 individual seahorses were reportedly imported into the USA for curios each year, between the years 1997 to 2001, with an average individual seahorse value of US$0.16 ± SD 0.095. As in the medicinal and live aquarium trades virtually all the seahorses sold in the curio trade were collected from the wild.

Since the first attempts at quantifying the international seahorse trade, in the early to mid 1990s (Vincent 1996), the available evidence indicates the seahorse trade has increased. Between the early to mid 1990s to 2000, it was estimated that the number of countries involved in the seahorse trade had increased from 32 to 80, with many new source countries in Africa and Latin America, and that the Asian trade had conservatively increased from 45 tonnes to 50 tonnes of dried seahorses (Lourie *et al.*, 2004).

1.5 Concerns regarding seahorses and their exploitation

By virtue of their life history characteristics seahorses are regarded as being particularly vulnerable to direct over-exploitation, indirect fishing pressure through non-selective fishing gear or other disruptions such as habitat loss/degradation and environmental pollution around their coastal habitats (Lourie *et al.*, 1999; Bell *et al.*, 2003; Foster & Vincent, 2004; Baum & Vincent, 2005; Martin-Smith & Vincent 2005, 2006). Seahorses are generally characterized by sparse distribution, low mobility, and small home ranges therefore they might be slow to recolonize affected areas. Seahorses exhibit lengthy parental care so survival of young *in marsupio* is reliant upon the survival of the male. In turn, male seahorse reproductive output is limited by the relatively low fecundity of female seahorses compared to many other fish species (Foster & Vincent, 2004), which adds to the population vulnerability of seahorses. In most cases, seahorses exhibit mate fidelity so removal of a mate may impede finding a new mate (Foster & Vincent, 2004). Differences in these life history characteristics between seahorse species are likely to affect their relative vulnerability.

Seahorses are regarded as being directly threatened by human exploitation for the medicinal, live aquarium and curio trades (Vincent, 1996; Baum & Vincent, 2005). Demand for seahorses increased significantly during the 1980s, prompted largely by China's economic restructuring (Vincent, 1996; Lourie *et al.*, 1999). The use of prepackaged medicines also appears to have increased over recent years in countries such as China; large seahorses are too valuable to be used in pills, so patent medicines are creating greater demand for smaller seahorses which are processed into pill-form (Vincent, 1996), creating the risk of seahorse population instability as a greater proportion of the sexually reproductive population are removed. Some fishers and traders in key areas of exploitation in Southeast Asia and India reported declines in

seahorse catches of 15–50% between 1990 and 1995 as demand for seahorses increased (Vincent, 1996).

Concern focuses not only on the exploited seahorses themselves, but also on the welfare of those that exploit them. The seahorse fishery is economically important to thousands of people, ranging from the seahorse collectors and their families and communities, aquarium exporters/retailers, marine product exporters/retailers and shell-craft dealers, to differing levels of middle operators (Vincent, 1996; McPherson & Vincent, 2005; Salin *et al.*, 2005; Giles *et al.*, 2006).

Of 33 seahorse species, the World Conservation Union (IUCN) lists 25 species as Data Deficient, 7 as Vulnerable and 1 as Endangered on its Red List (IUCN, 2003: http://www.redlist.org, accessed 18/09/06). The overall aim of the Red List is to convey the urgency and scale of conservation problems, acting as a catalyst for conservation action. It has no direct legislative or legal implications for exploitation or trade.

Concern over the sustainability of seahorse exploitation led to the recent listing of all seahorse species on CITES Appendix II on 15 May 2004 (http://www.cites.org, AC19 Doc. 16.1, accessed 23/05/05). Listing of the whole *Hippocampus* genus, rather than individual species for which there was evidence of populations being threatened, was deemed necessary due to the look-alike nature of some seahorse species, which could have led to difficulties in trade-identification of different seahorse species (Lourie *et al.*, 2004).

Appendix II-listed species are those for which the wild populations are threatened, or might become threatened, by international trade. The Appendix II listing does not prohibit regulated fishing and trade, but it does require appropriate regulatory controls (e.g. regular export/import monitoring) and Non-detrimental Impact Findings (NDF) to be made to facilitate exploitation between Parties (countries) that have signed the voluntary international CITES agreement. Although CITES is legally binding on the Parties for international trade it does not take the place of national laws.

Subsequent to the Appendix II-listing in 2002, the CITES Animals Committee confirmed in 2004 that a minimum size limit would offer a means for Parties to make interim NDF's for all seahorses taken from the wild and being traded internationally.

The size limit — a height of 10 centimeters — falls between size at maturity and maximum size for most seahorse species, particularly the species at which the CITES listing was primarily directed (e.g. *H. barbouri*, *H. erectus*, *H. ingens*, *H. reidi* and *H. spinosissimus*) (Foster & Vincent, 2005). This size restriction reflects a trade-off between ensuring persistence of wild seahorse populations and the need for continued trade, and allows continued exports in all but one currently traded species (the dwarf seahorse *H. zosterae*, with a maximum recorded adult height of 2.5 cm, Lourie *et al.*, 2004).

1.6 Seahorses in New Zealand

New Zealand has a single marine seahorse species, the big-bellied sea horse (*H. abdominalis* Lesson 1827), also referred to as the large-belly seahorse or pot-bellied seahorse (*Hinamoki*, *Kiore moana*, *Kiore-tawhiti*, *Kiore waitai*, or *Manaia* in Māori; Strickland, 1990) (Parrott, 1960; Paulin & Roberts, 1992; Francis, 1998; Lourie *et al.*, 1999; Kuiter, 2003; Lourie *et al.*, 2004) (Fig. 1.4). This species also occurs in the seawaters of south-eastern Australia (Lourie *et al.*, 1999; Lourie *et al.*, 2004). As its common names denote, this species has a pronounced abdomen relative to other species, and is one of the largest of all seahorses, reaching up to 35 cm in length (Francis, 1998) (Fig. 1.4).

A   B

Figure 1.4 Adult female (A) and male (B) *Hippocampus abdominalis* (source: Chris Woods).

This species is highly variable in morphological characteristics such as snout length, tail length, degree of abdomen development, and colouration (see Fig. 1.5). Kuiter (2003) differentiated *H. abdominalis* into three species: the Australian pot-belly seahorse *H. bleekeri* (Australia-only), the eastern pot-belly seahorse *H. cf. abdominalis* (Australia-only), and the New Zealand pot-belly seahorse *H. abdominalis* (NZ-only) based on

apparent differences in head size, snout length and degree of "spotting" in body colouration. However, this differentiation has not been validated with supporting meristic or genetic identification, and is not supported by other seahorse taxonomic guides (see Lourie *et al.*, 1999; Lourie *et al.*, 2004), or legislative authorities (e.g. CITES). For example, in a preliminary genetic comparison using mitochondrial DNA from the cytochrome *b* gene, six allozyme loci and morphometrics, Armstrong (2001) found no evidence of more than one species in samples from New Zealand and southeastern Australia. In their examination of the cytochrome *b* gene from 22 seahorse species, Casey *et al.* (2004) found *H. abdominalis* was one of the few species to show any phylogeographic genetic variation from the other species, suggesting its early evolutionary divergence from other Indo-Pacific seahorses. Phylogenetic research suggests that *H. abdominalis* shares its closest phylogenetic link with the much smaller *H. breviceps* in Australia (Jones *et al.*, 2003; Casey *et al.*, 2004).

It may be possible that geographically different seahorse populations evolve different morphological traits if they originate from a few founding individuals (Teske *et al.*, 2005). This has been observed in the widespread *H. kuda* complex which exhibits quite marked localized morphological variations and where localized morphological evolutions occur (Teske *et al.*, 2005).This may also be the case for *H. abdominalis* with its pelagic juveniles and observed "rafting" behaviour, and may help to explain confusion over its taxonomy. Pelagic juvenile *H. abdominalis* up to 8 cm in length have been found in near-surface plankton tows kilometres from shore over deep water (e.g. >400m) (Hickford, 2000; Woods, unpubl. data), and adult *H. abdominalis* have been captured near-shore associated with floating seaweed and debris (Kingsford & Choat, 1985). This suggests a possible large-scale dispersal mechanism for this species as suspected for other seahorse species. Further taxonomic resolution is required for this morphologically variable species of seahorse. In this thesis I employ the classification of *H. abdominalis* as a single species following Lourie *et al.* (1999) and Lourie *et al.* (2004).
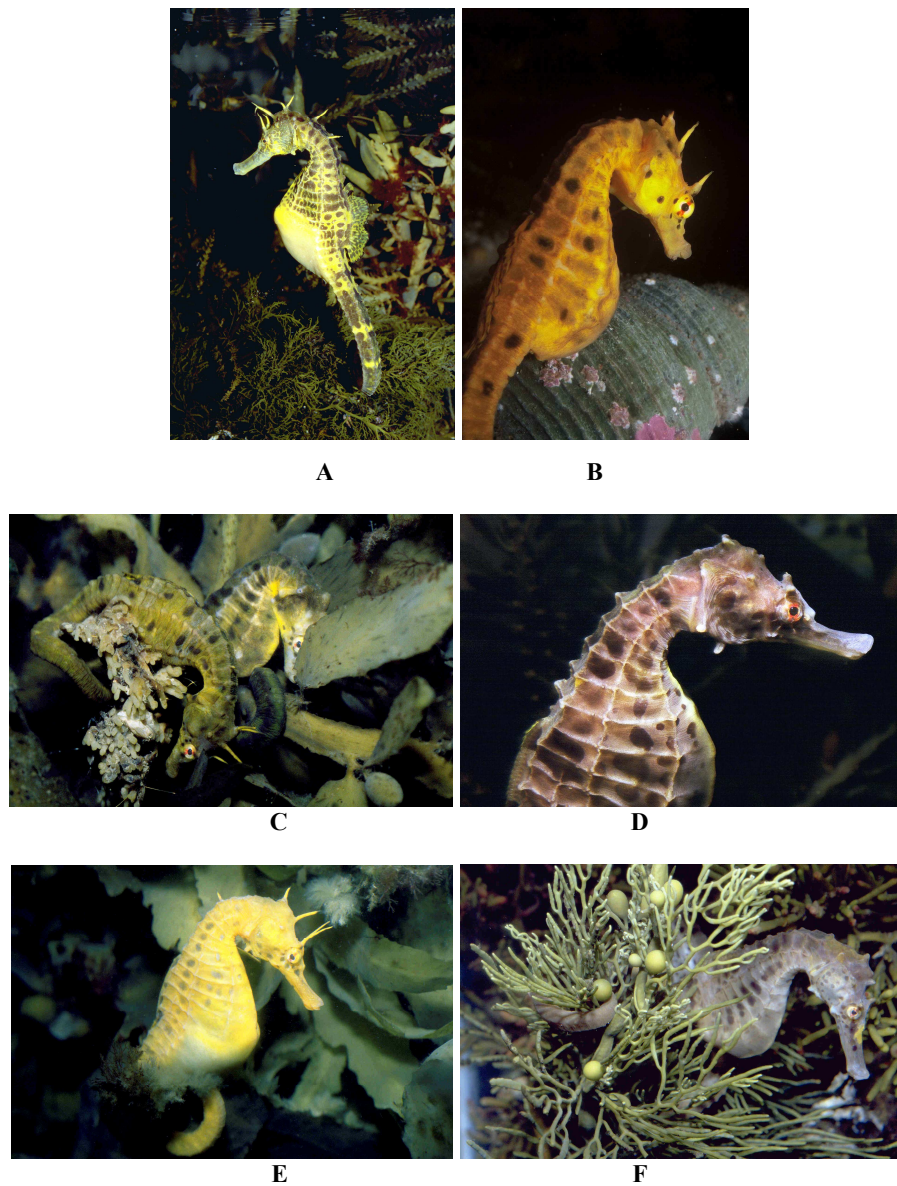
Figure 1.5 Examples of morphological and colour variation amongst individual *Hippocampus abdominalis* from Wellington Harbour, New Zealand: (A) long snout, body and tail with strong bright yellow colouration and extensive blotching, dermal cirri on head and neck, (B) short snout and tail with gold/orange colouration and some dermal cirri on head, (C) as in (B) but dull colouration of grey, black and yellow, (D) medium snout length, dull grey and black colouration, no dermal cirri, (E) medium snout length, bright light gold colouration with minimal blotching, and dermal cirri on head and neck, (F) long thick snout, dull grey colouration, no dermal cirri (source: Chris Woods).

Big-bellied seahorses are widespread around New Zealand, from the Three Kings Islands in the north to the Snares Islands in the south, and the Chatham Islands to the east (Scrimgeour, 1986; Paulin & Roberts, 1992). Depth range varies considerably from intertidal down to 100 m (Amaoka *et al.*, 1990; Paulin & Roberts, 1992; Francis, 1998; Lourie *et al.*, 1999; Stevenson & Beentjes, 2001). Habitat varies from intertidal rock

pools, to more commonly amongst shallow macroalgal stands, submerged rocky outcrops, to exposed open sea floor and some estuaries (Francis, 1998; Woods, 2003a; Martin-Smith & Vincent, 2005). Artificial structures are also commonly utilized by *H. abdominalis*, from salmon cage netting and mussel spat-catching ropes and long-lines (Morrisey *et al.*, 2006; A. Pannell, Marlborough Mussel Co. Ltd, pers. comm.), to lobster pot lines and large wooden and concrete wharf pilings (Woods, pers. obs). It is not definitively known whether they occupy home ranges or are free-ranging, although some evidence suggests certain populations may exhibit site fidelity (Van Dijken, 2001). Unlike most seahorse species, *H. abdominalis* is a relatively strong swimmer and has been known to swim over hundreds of meters in the course of a day (Vincent, 1990).

The World Conservation Union (IUCN) currently lists *H. abdominalis* on its Red List of threatened species (http://www.redlist.org), with a Red List rating of Data Deficient (DD) (Woods *et al.*, 2006). This evaluation of DD means that there are insufficient data available to quantify whether this species is at risk of serious population decline or extinction.

Despite being a large seahorse, and reasonably widespread around New Zealand, there is very little known about this seahorse in its natural habitat. Little historical data exists for population structure, growth rates, longevity or abundance in New Zealand, although Van Dijken (2001) conducted a small *in situ* study on *H. abdominalis* in northern New Zealand investigating site fidelity and inferred growth rates. He found growth rates of up to 2.8 mm per month for 16 cm length seahorses, an inferred time span of 10-11 years to reach 25 cm in length and some site fidelity exhibited. Graham (1939) conducted some *ex situ* observations on breeding in *H. abdominalis* and found males to brood during summer. What little information that exists from research trawl collections in New Zealand suggests that the species may be widespread if scarce on soft bottoms (e.g. Stevenson & Beentjes, 2001), although such trawls are usually conducted away from habitats such as macroalgal stands, where seahorse biomass is likely to be highest.

In Tasmania, Australia, *H. abdominalis* is usually found singly, with population densities ranging from 12–111 ha^{-1} in areas of suitable habitat with no significant association between males and females but with a female-biased sex ratio in adults (Martin-Smith & Vincent, 2005). Wilson & Martin-Smith (*in press*) observed that in

both high and low density populations *H. abdominalis* exhibited *in situ* promiscuous courtship behaviour, but genetic assessment of male broods indicated that males mated monogamously. Lovett (1969) examined the general *in situ* and *ex situ* biology of *H. abdominalis* in Tasmania, Australia. She found their diet to consist mainly of crustaceans such as amphipods, mysids and caridean shrimp. Lovett (1969) estimated seahorses 9.1–11 cm in length to be 1 year old whilst those 22+ cm in length were 4+ years old, with breeding occurring during spring/summer.

Predators of *H. abdominalis* in New Zealand include fishes such as skates (*Dipturus* spp.), red cod (*Pseudophycis bachus*), trumpeter (*Latris lineata*), blue cod (*Parapercis colias*), ling (*Genypterus blacodes*), sea perch (*Helicolenus percoides*) (Graham, 1974), and banded wrasse (*Notolabrus fucicola*) (Denny & Schiel, 2001). Other predators include crustaceans such as rock lobster (*Jasus edwardsii*), masking crabs (*Notomithrax* spp.), and little shags (*Phalacrocorax melanoleucos*) (Woods, pers. obs).

In New Zealand, seahorses cannot be targeted for commercial fishing, but if caught as incidental bycatch during commercial fishing (e.g. dredging, set-netting and various trapping/potting methods), they may be legally sold to Licensed Fish Receivers (LFR) for local use or export (Woods, 2000). Data from the Ministry of Fisheries databases Catch Effort and Landing Returns (CELR) (Table 1.1) show that the total for estimated catch is 240 kg and 1625 kg for landed catch for the fishing years 1989–1990 to 2004–2005. It should be noted that because of the difference between the ways in which fishing year is recorded for estimated and landed catch data, estimated catch data appears one fishing year ahead of landed catch data (e.g. 170 kg for 1992–1993 estimated matches with 687 kg for 1993–1994 landed). There are marked disparities between estimated and landed catches, which may indicate either that estimated catch data are seriously underestimated or that landed catch data are incorrect due to reasons such as misidentification, species miscoding or data entry error, or a combination of these. Detailed examination of CELR data reveals that the single largest catch of seahorses reported by one fisher was 60 kg in the Nelson/Marlborough dredge fishery. This is the exception in terms of catch size, as the majority of CELRs reported seahorse catches of <1 kg. Recorded areas of catch are mainly on the eastern side of New Zealand and central New Zealand.

Seahorses are not yet part of the New Zealand fisheries Quota Management System (QMS) regulating total amount of commercial catch. As a non-QMS species, the recording of accurate export data for *H. abdominalis* has historically not taken place. Historically, dried seahorses were either included in the export category 0305.59.00 ("Other fish, whether or not salted but not smoked"), or 0301.10.00 ("Ornamental fish"). Therefore, reconciliation of exports with estimated and landed catches is not possible. However, with the CITES Appendix II-listing, any exports of bycatch seahorses now have to be certified, providing a mechanism for export monitoring. There are no catch limits for the amateur-take of seahorses in New Zealand in terms of numbers or sizes.

Hippocampus abdominalis is listed on the Ministry of Fisheries list of fish that can be farmed under the Freshwater Fish Farming Regulations 1983 (relates to all land-based fish-farming regardless of whether freshwater or saltwater species are farmed), gazetted in March 2004 (http://www.mfish.govt.nz, accessed 25/05/05). Export of cultured *H. abdominalis* from New Zealand must be approved and certified by the relevant authority (Department of Conservation). Cultured *H. abdominalis* are not subject to the CITES minimum height restriction.

Table 1.1 Estimated catch summary (kg) and landings (kg) of seahorses as reported by Catch Effort Landing Return (CELR), fishing years 1989-1990 to 2004-2005 (source: New Zealand Ministry of Fisheries).

Fishing year	Estimated catch (kg)	Landed (kg)
1989-1990	5	0
1990-1991	0	126
1991-1992	0	48
1992-1993	170	0
1993-1994	0	687
1994-1995	0	0
1995-1996	0	0
1996-1997	0	0
1997-1998	0	0
1998-1999	0	30
1999-2000	1	18
2000-2001	62	21
2001-2002	2	569
2002-2003	0	42
2003-2004	0	34
2004-2005	0	50
TOTAL	**240**	**1625**

1.7 Seahorse aquaculture and background to the thesis

In the mid 1990s there was a sudden awareness in many western countries of the large and sometimes valuable trade in seahorses and the related concerns regarding the exploitation of seahorse wild stocks on which this was based. This awareness mainly arose following the publications on the international trade in seahorses by Vincent (1994a, 1995b, 1996), Moore (1995), Paulin (1995), Prein (1995), Blair (1997), and Moreau (1997). These publications, and the many popular media articles that they spawned, resulted in widespread interest in a wide variety of countries (including New Zealand) in the potential for seahorse aquaculture. This interest was driven as a purely commercial activity, as a possible means for alleviating pressure on exploited wild stocks by providing an alternate source of seahorses, or by providing seed stock for replenishing depleted wild stocks. The citing in some of these articles of very high medicinal market prices (e.g. NZ$1,000–2,000 kg^{-1} of dried seahorses) further excited potential aquaculturists motivated by the potential economic benefits to seahorse aquaculture.

Prior to this western interest in seahorse aquaculture, research- and commercially-driven seahorse aquaculture had been trialed and conducted in a range of Asian countries where seahorses were a historical resource or product. For example, seahorse aquaculture had been trialed in several different provinces in China, from the 1950s to early 1990s (Qiu, 1991; Liang, 1992; Vincent, 1996). These trials apparently met with variable success, with some achieving survival rates of 50–60% at times with multiple captive generations. However, problems with high juvenile mortality, disease and nutrition, combined with China's economic restructuring in the 1980s resulted in the cessation of most of these commercial attempts (Vincent, 1996). As an example, the Zhejiang Mariculture Research Institute (ZMRI) successfully overcame their main cause of mortality in older juvenile *H. trimaculatus*, "overwintering" or very cold temperatures in winter (Qiu, 1991), by heating outdoor seahorse culture ponds; they could then on-grow juveniles to maturity and consequently produce juveniles from this broodstock, with a production of 10 000 seahorses. However, the low cost of wild-caught dried seahorses available in local markets meant that culturing *H. trimaculatus* was not economically viable (Qiu, ZMRI, pers. comm.). Despite such impediments, the aquaculture of seahorses in China has continued (Hong & Zhang, 2003).

In Thailand, seahorse aquaculture trials had been initiated in 1988 at the Bangsaen Institute of Marine Sciences (BIMS, Burapha University) with the aim of releasing the cultured seahorses back into the wild to replenish heavily depleted wild stocks (Prein, 1995). Reasonable survival rates (e.g. 60% at 75 days) were achieved from wild-caught pregnant males but there were problems with captive breeding and diseases (Vincent, 1996). This project continued to develop seahorse aquaculture in Thailand.

In the Philippines, the Department of Agriculture embarked on trialing seahorse aquaculture in 1988 with juvenile grow-out in floating cages, but was not successful due to high juvenile mortality and reliance on capturing pregnant male seahorses (Vincent, 1996). Seahorse aquaculture in the Philippines continued to be developed by the Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC).

In Vietnam, the Institute of Oceanography initiated captive breeding trials around 1991 with steady improvements in success (Vincent, 1996), and continued to develop seahorse aquaculture in Vietnam (Job *et al.*, 2002). Another small seahorse aquaculture

project was also started in 1996 at the Shrimp-Artemia R&D Institute (SARDI) of the Can Tho University. In Indonesia, the Seafarming Development Centre in Sumatra was involved in seahorse aquaculture (Vincent, 1996).

In Europe and North America, seahorse breeding and rearing appears to have been restricted to public aquaria for their own captive breeding programme and display (e.g. Birch Aquarium at Scripps, California, USA), although the Tropical Marine Centre (TMC) in England was investigating the commercial aquaculture potential for supplying the UK domestic aquarium market (Vincent, 1996).

The potential for culturing *H. abdominalis* in New Zealand to supply the TCM trade was first publicly raised by Gilbertson (1987) as a conceptual marketing exercise, and a year later by Hayden (1988) in a draft discussion document compiled for the Ministry of Agriculture, Forestry and Fisheries (MAFFish) evaluating species with aquaculture potential. According to Vincent (1996), a few attempts at the commercial culture of *H. abdominalis* in New Zealand were made in the mid 1980s to mid 1990s. These attempts were made to supply dried seahorses to the TCM trade and live seahorses to the aquarium trade in North America, but failed due to problems with poor breeding and high juvenile mortality. They were consequently abandoned with no resolution of the culture problems encountered.

In the mid 1990s, there were no known economically viable commercial seahorse aquaculture ventures operating for *H. abdominalis*. Research was being conducted on aquaculture techniques for *H. abdominalis* at the University of Tasmania in Australia, from which some popular articles were published (Forteath, 1995, 1997; Sobolewski, 1997). However, with the development of this research into a commercial seahorse aquaculture company (Seahorse Aquaculture Pty Ltd) detailed research findings became proprietary, and therefore not accessible to potential competitors. This also extended to certain associated student research (e.g. Filleul (1996) on culturing juvenile *H. abdominalis*).

In 1997, with the large public interest in seahorse aquaculture in New Zealand, the National Institute of Water and Atmospheric Research (NIWA) decided to initiate a preliminary assessment into the aquaculture potential of *H. abdominalis*. At the time at which this investigation was initiated, most of the published material available on

seahorses was either related to amateur aquarium keeping of small numbers of seahorses (Herald & Rakowicz, 1951; Straughan, 1961; Giwojna, 1990; Shute & Tullock, 1993; Scarratt, 1995), the international seahorse trades and related conservation concerns (Maricharmy *et al.*, 1993; Vincent 1996), or interesting behavioural, phylogenetic, physiological, and general biological/ecological aspects of seahorses (Gill, 1905; Breder, 1940; Jacob & Rajendran, 1948; Peters, 1951; Fish, 1953; Whitley & Allan, 1958; Strawn, 1958; Herald, 1959; Bellomy, 1969; Blake, 1976; Vincent, 1990; Matlock, 1992; Vincent *et al.*, 1992; James & Heck, 1994; Bergert & Wainwright, 1997; Ritz *et al.*, 1997). There was a very limited amount of available information relevant to commercial seahorse aquaculture, the majority of which required translation for English-speakers (Correa *et al.*, 1989; Qiu, 1991; Liang, 1992; Mi, 1993), although there were a few more accessible publications (Chen, 1990; Lockyear *et al.*, 1997).

The primary aim of the present study is to determine whether wild-caught adult *H. abdominalis* can breed in captivity, and whether any subsequent progeny could be successfully reared to maturity to form the basis of a captive broodstock. If this is achievable, then aquaculture techniques for *H. abdominalis* can be further researched in order to develop this species into a commercial aquaculture species for New Zealand for supply to either the medicinal or live aquarium trades. The project spanned the period 1997–2004.

The central null hypothesis of the thesis is that: H_0 "*H. abdominalis* cannot be cultured in captivity, with low juvenile survival and growth to maturity". The central alternative hypothesis is that: H_1 "*H. abdominalis* can be cultured in captivity, with high juvenile survival and growth to maturity". Specifically, the following objectives addressed are:

1) Conduct a preliminary assessment of seahorse breeding, juvenile rearing and growth rates
2) Determine techniques for improving early juvenile seahorse survival
3) Determine whether juvenile seahorses can be weaned on to artificial and frozen foods to reduce feeding costs associated with live foods
4) Determine the effect of live food (*Artemia*) enrichment on seahorse growth and survival

5) Determine whether frozen mysids can be used as a food source to reduce the requirement for live foods, and the appropriate daily ration for feeding seahorses with frozen mysids

6) Determine natural seahorse diet and male reproductive output for use as aquaculture benchmarks

The findings of these specific objectives will be contextualized in the general discussion (Chapter 7) in relation to developments in seahorse aquaculture research and commercial seahorse aquaculture. The last decade has seen a dramatic increase in the amount of research directed into seahorse aquaculture which has been published (e.g. Lockyear *et al.*, 1997; Wilson & Vincent, 1998; Payne & Rippingale, 2000; Woods, 2000a, b, 2003b, c, d, 2005a; Chang & Southgate, 2001; Job *et al.*, 2002; Gardner, 2003; Hilomen-Garcia *et al.*, 2003; Payne, 2003; Shapawi & Purser, 2003; Woods & Valentino, 2003; Wong & Benzie, 2003; Job *et al.*, 2006; Lin *et al.*, 2006; Sheng *et al.*, 2006; Wilson *et al.*, 2006; Martinez-Cardenas & Purser, 2007). Such research has significantly advanced our understanding of seahorse culture requirements and has enabled commercial seahorse aquaculture to become a viable commercial activity.

CHAPTER 2

PRELIMINARY ASSESSMENT OF THE CULTURE POTENTIAL OF
HIPPOCAMPUS ABDOMINALIS

2.1 Introduction

2.1.1 Potential for commercial seahorse aquaculture in New Zealand

Interest in seahorse aquaculture in New Zealand has primarily focused on the potential for culturing the naturally occurring big-bellied seahorse *H. abdominalis* as a commercial commodity for the medicinal, aquarium and curio trades. We do not historically, or currently, have sufficient quality data to determine if *H. abdominalis* is at risk in New Zealand from commercial bycatch and exploitation, either on a commercial or amateur level, or if wild stocks have declined. Hence, the concept of culturing *H. abdominalis* for stock replenishment/enhancement is not a primary factor motivating the development of culture techniques for this species.

Due to the general declining availability of large seahorses for TCM, aquaculture offers the potential to supply medicinal traders with a reliable supply of high quality seahorses of a size at which premium prices may be negotiated. *Hippocampus abdominalis* has been utilized in the medicinal trade on a very small scale as an exported bycatch (Vincent, 1996; Martin-Smith & Vincent, 2006). Various fishing companies with strong Asian connections (e.g. Fig. 2.1) have historically advertised for bycatch *H. abdominalis* in New Zealand in conjunction with other high value fish/fish-derived products such as shark fins, ling swimbladders, and spiny pipehorses (*Solegnathus spinosissimus*, referred to as *Hai long* in TCM), with the main impediment to large volume exporting appearing to be the relatively small amounts of seahorses caught as bycatch (Addy Chee, Sebanto Holdings Ltd, pers. comm.).

Figure 2.1 Advertisements for seahorses caught as bycatch in a fishing industry publication (source: (A) *Seafood New Zealand* **10(2), 2002 and (B)** *Seafood New Zealand* **14(7), 2006)**

Being a large and relatively smooth-skinned seahorse species, *H. abdominalis* has strong potential to be a high value product in the TCM trade, particularly in China and Hong Kong where such attributes are valued (Vincent, 1996). However, there are still market acceptability issues such as history of usage and proven/believed medical efficacy, and desired colouration, which may strongly affect their medicinal marketability. For example, the common belief that "wild is better than cultured" in terms of medical efficacy in TCM must be considered, although such factors may vary in their strength in relation to variables such as generational viewpoints (i.e. young vs. old) and wild product availability (Thomas Fok, Hong Kong Chinese Medicine Merchants Association Ltd, pers. comm.).

TCM pricings for *H. abdominalis* are difficult to obtain due to the small number of animals traded, and lack of detailed export documentation. However, wholesale prices offered by TCM traders and seafood export companies with strong Asian connections for *H. abdominalis* in New Zealand between 1997 and 2004 ranged from NZ$200–500 kg^{-1} dry weight (Woods, unpubl. data). Assuming a "normal" retail mark-up of 2- to 4-fold, these wholesale prices compare well with some of the retail prices given for seahorses in the TCM trade.

There has been a small domestic trade in wild-caught *H. abdominalis* in New Zealand as a live aquarium fish. For example, in Wellington in the early 1990s wild-caught *H. abdominalis* were being retailed for NZ$15–20 each. However, once Fisheries inspectors became aware of this illegal trade in wild-caught seahorses it was promptly stopped. There has not officially been any export of wild-caught live *H. abdominalis* for the aquarium trade. There is a ready market for *H. abdominalis* in the international aquarium market, where it is regarded as rather an uncommon and unusual species (both of which can result in an "exotic" appeal) as well as a relatively hardy tank specimen (Abbott, 2003; Kuiter, 2004a). However, as it is a temperate species its popularity and marketability are likely to restricted in comparison to tropical seahorse species, as most marine aquarium hobbyists are geared to keeping tropical species and setting up a separate temperate tank, which may require an expensive cooling system in warmer climates may not be attractive (Indiviglio, 2002; Abbott, 2003).

Aquaculture offers the potential to legally supply *H. abdominalis* to a domestic New Zealand aquarium market with no competing wild-caught supply. Moreover, there is potential to supply the much larger international export aquarium market in which recent conservation concerns and legislation changes, such as the CITES Appendix II-listing of seahorses, are potentially affecting both customer perception and reliability of wild-caught supplies. Aquaculture also offers the advantage of the reliable supply of healthy seahorses conditioned to a captive environment (Giwojna & Giwojna, 1999a; O'Sullivan 2003; Knop, 2004; Willis & O'Sullivan, 2005). This is an important consideration in customer perception of seahorses as an acceptable aquarium species and return business for the retailer (Yap, 2005). Wild-caught ornamental fish species may reach the aquarium retailer in poor condition with an increased risk of disease and mortality due to physical damage during collection and holding (e.g. skin abrasion during collection or attacks from incompatible aquarium species), poor aquarium management at intermediate holding facilities, disease, variable holding periods (whilst the dealer accumulates sufficient stock for shipment), and a poor diet or no feeding at all before arrival at the retail shop (Vincent, 1996; Chan & Sadovy, 1998; Giwojna & Giwojna 1999a; Bunting *et al.*, 2001).

As the first commercial attempts at culturing *H. abdominalis* in New Zealand proved, technical issues exist related to poor breeding success and high juvenile mortality (Vincent, 1996). These technical issues were almost certainly exacerbated by the

general lack of relevant literature on seahorse aquaculture at the time. These commercial attempts at culture did not resolve these technical issues.

2.1.2 Life history traits relevant to seahorse aquaculture

In terms of reproduction, seahorses are characterized by adaptations for male parental care, with the female depositing her eggs into a specialized brood pouch that the male possesses following an often elaborate courtship and mating ritual (Lourie *et al.*, 1999; Wilson *et al.*, 2001; Foster & Vincent, 2004; Dzyuba *et al.*, 2006). These courtship displays usually involve a series of ritualized stages of physical displays prior to egg transfer from female to male (Vincent & Sadler, 1995, Masonjones & Lewis, 1996; Giwojna & Giwojna 1999a; Kvarnemo & Simmons, 2004; Moreau & Vincent, 2004).

Behaviours typically exhibited during courtship include various phases of colour-changing, posturing, head-pointing or flicking, and swimming together (Vincent & Sadler, 1995; Masonjones & Lewis, 1996; Lourie *et al.*, 1999; Masonjones, 2001). Such courtship may last up to 9 h in duration (*H. whitei*, Vincent & Sadler, 1995) and typically begins in the morning (Masonjones & Lewis, 1996). For example, Masonjones & Lewis (1996) described four discrete phases of courtship in *H. zosterae*. Phase one courtship occurs 1–2 days before mating and is characterized by colour-brightening, reciprocal quivering, pectoral fins extended and erect body posture. Courtship phases two to four occur on the day of copulation. Phase two involves colour-brightening, female head-pointing and male body-quivering and pumping of the brood pouch. Phase three involves colour-brightening, pumping of the brood pouch and male head-pointing in response to female head-pointing. Phase four is when mating occurs. Mating occurs when male and female both rise vertically in the water column and she transfers her mature eggs into his dilated and open pouch in a copulatory mid-water embrace (Masonjones & Lewis, 1996). Graham (1939) observed *ex situ* breeding behaviour in a small number of *H. abdominalis*. He observed limited swimming and head-jerking courtship behaviours, but that actual egg transfer took place in one instance without a vertical rising component at intervals of five minutes with at least 13 breeding mating "embraces".

It is thought that females may require courtship over several days in order to evaluate a male's availability/desirability as a mate before hydrating and transferring a clutch of eggs (Masonjones & Lewis, 2000). In *H. zosterae*, females invest a substantial amount

of energy in each egg and egg clutches represent between 8 and 29% of a female's body weight (Masonjones, 1997 *in* Masonjones & Lewis, 2000). Therefore, the loss of a clutch to an unavailable or unsuitable male represents a significant reproductive cost.

The female seahorse hydrates part of her post-vitellogenic clutch at mating and is thought to deposit all such eggs into the male's pouch (i.e. fractional spawning) (Foster & Vincent, 2004). The male seahorse is generally thought to only accept eggs from one female per mating (Foster & Vincent, 2004). However, examples of male seahorses accepting eggs from multiple females and females distributing their eggs amongst different males have been observed *ex situ* (Giwojna & Giwojna, 1999a; Kuiter, 2004b). Direct measurements of egg numbers transferred are rare, although recorded female clutch sizes range from 5 (*H. zosterae*, Vincent, 1990) to 1300+ eggs (*H. erectus*, Texeira & Musick, 2001) and may be related to female size (Texeira & Musick, 2001). Overall, interspecific differences in egg sizes are positively related more with increasing latitude than with female size (Foster & Vincent, 2004).

The duration of the breeding season is typically longer in tropical than temperate water species with year-round breeding occurring in some species (Foster & Vincent, 2004). For example, year-round breeding occurs in the tropical *H. kuda* and *H. bargibanti* but from March to October in the higher latitude *H. guttulatus* (Foster & Vincent, 2004). Poortenaar *et al.* (2004) examined the *in situ* reproductive cycle in female *H. abdominalis* and found reproductive females with vitellogenic eggs to be present year-round, but with a peak in GSI in spring/summer. Graham (1939) observed male *H. abdominalis* to be pregnant during summer. In Tasmania, Australia, Lovett (1969) observed male *H. abdominalis* to be pregnant over spring/summer and females to have their highest GSI at the end of winter/beginning of spring.

The exact point of sperm–egg interaction in seahorses is not clear. Traditionally, it has been assumed that males fertilized the eggs once they were sealed inside their pouch (e.g. Boisseau, 1967; Kvarnemo & Simmons, 2004). Kvarnemo & Simmons, 2004 examined testes size in a range of syngnathids both with, and without pouches. They found that syngnathids have a relatively low testes investment compared to other teleosts. They also found that this low testes investment trend occurred in syngnathids both with, and without pouches. Recently, Van Look *et al.* (2007) examined *ex situ* breeding in *H. kuda* to determine the point of sperm–egg interaction. They found that

although males produced few spermatozoa, the relative sperm:egg ratio was relatively low at <2.5:1 compared with other teleosts. They inferred from their sperm sampling that the spermatozoa, which remain viable in seawater, are ejaculated from the male into a mixture of ovarian fluid and eggs, while the male and female are in close contact. Thereafter, this mixture enters the brood pouch of the male, i.e. physiologically "external" fertilization probably occurs within a physically "internal" environment. This mating and fertilization pattern would generally appear to preclude sperm competition from other males (Van Look *et al.*, 2007).

Information on fertilization success is poor. Lin *et al.* (2006) found *ex situ* temperature-dependent fertilization rates in *H. kuda* varying from 70% at 18°C, up to 92.4% at 28°C, and down to 54.3% at 32°C, with commensurate hatching rates of 78.9%, 94.7% and 50.7% respectively. Texeira & Musick found *in situ* undeveloped rate of eggs transferred from female to male *H. erectus* of 2–33%. However, the presence of lipid cells in the pouch epithelium of *H. brevirostris* suggests egg yolk can be re-absorbed in seahorses (Rauther, 1925) which can complicate such investigations.

In the sealed brood pouch of the male, the developing young are nurtured and protected from direct predation. The folded internal surface of the male's brood pouch appears to consist of swollen epidermal cells with an extensive capillary network in the dermis and the narrow ends of the roughly pear-shaped eggs are embedded in pit-like depressions in the pouch epithelium (Lovett, 1969; Carcupino *et al.*, 2002; Dzyuba *et al.*, 2006; Laksanawimol *et al.*, 2006). The internal pouch epithelium also shows cellular specialization, such as modified flame cone cells which typically have a role in metabolic pathways related to both Kreb's cycle and the pentose shunt (Carcupino *et al.*, 2002). Within the males' brood pouch, developing young are serviced with gas exchange, osmoregulation, waste removal and certain mineral and nutrient transfer during brooding (Linton & Soloff, 1964; Masonjones, 2001). Male seahorses do not appear to construct a specialized embryo matrix to support the embryos as some other syngnathids do, which is ejected at birth along with the young (Ripley & Foran, 2006). However, the brood pouch of male seahorses does undergo morphological and cellular changes during pregnancy (Laksanawimol *et al.*, 2006) and pregnant male seahorses do incur a metabolic cost during pregnancy. For example, Masonjones (2001) recorded metabolic rate increases of 10–52% over pre-gravid levels in pregnant male *H. zosterae*.

In the majority of seahorse species studied to date, monogamy is the general rule, at least within breeding seasons. Male seahorses generally accept eggs from only one female and monogamous pair-bonds appear to be reinforced by ritualized daily greetings between male and female before, during, and after brooding (Foster & Vincent, 2004), although males may switch partners between broods (*H. subelongatus*, Kvarnemo *et al.*, 2000). Seahorses may experience increased reproductive efficiency (i.e. larger broods and less time spent on courtship) (Vincent, 1990) and shortened inter-brood periods (Kvarnemo *et al.*, 2000) as a result of monogamy.

Seahorses generally maintain conventional sex roles with males competing more intensely for mates than females (Vincent *et al.*, 1992; Vincent, 1994b; Foster & Vincent, 2004). However, Wilson & Martin-Smith (*in press*) observed promiscuous courtship behaviour and sex-role reversal in high density, female-biased populations of *H. abdominalis* in Tasmania, Australia, although all broods from males sampled proved to be genetically monogamous. Mate selection in seahorses appears to be size-assortative, with breeding pairs comprising similar-sized males and females (Vincent & Sadler, 1995; Jones *et al.*, 2003; Vincent & Giles, 2003).

The duration of pregnancy in seahorses varies between approximately 9–45 days, depending on species and water temperature (Foster & Vincent, 2004) with time for gestation decreasing with increasing temperature. At the end of pregnancy, males usually go into labour at night, actively forcing the juveniles from the pouch (Vincent, 1990; Moreau & Vincent, 2004). Upon birth, juveniles are fully formed, independent, and capable of feeding. It is at this stage, and in the following few months of life that the juveniles are most vulnerable and where large scale mortalities commonly occur in the captive environment (Herald & Rakowicz, 1951; Bellomy, 1969; Vincent & Clifton-Hadley, 1989; Scarratt, 1995; Forteath, 1995; Vincent, 1995c, 1996; Garrick-Maidment, 1997). Appropriate nutritional, physical and environmental parameters must be determined for juveniles to ensure that as many as possible survive, and their growth be maximized to reduce the time required until they reach a marketable size.

Successful seahorse breeding is a necessary prerequisite if commercial seahorse aquaculture is to be pursued, as this is a major determinant in juvenile supply. A number of authors have identified variables that can affect the breeding success of seahorses such as broodstock nutrition, excessive aeration and water turbulence, photoperiod,

water temperature, stocking density, tank dimensions, and water chemistry (Sobolewski, 1997; Wilson & Vincent, 1998; Giwojna & Giwojna 1999a, b; Brittain, 2002; Lin *et al.*, 2006), within which there may be species-specific differences.

For the commercial aquaculture potential of *H. abdominalis* to be explored, a starting point of investigation is required, from which both problematic and promising issues may consequently be identified, and then further investigated. To this end, a small scale attempt at maintaining and breeding wild *H. abdominalis* in a hatchery was conducted to examine captive breeding and juvenile rearing; topics which were identified as problematical in earlier commercial aquaculture attempts (Vincent, 1996). Specific objectives were:

1) Observe and describe mating behaviour and reproduction of adult seahorses in captivity.

2) Rear offspring produced from these broodstock for a period of one year and record their growth and survival.

In doing this, the aim was to obtain an indication as to the possibility of culturing this species commercially, and highlight any potential technical difficulties requiring further attention. This chapter is based on data from Woods (2000a). In addition, a number of these first captive generation seahorses were then cultured for a further six years in order to obtain longer term growth and survival data.

2.2 Materials and Methods

2.2.1 Courtship and breeding

Twelve adult seahorses (*n* = 5 males, *n* = 7 females) were collected by snorkeling amongst macroalgal stands *(Macrocystis pyrifera, Carpophyllum flexuosum, C. maschalocarpum, Sargassum sinclairii)* in Wellington Harbour (174°50' S, 41°17.50' E) from February 1997 to July 1997. These adults were separated by at least 20 m (sometimes by as much as 1 km) from each other, but it was not known whether there were any pre-existing pair-bonds between them. Standard length (SL) of adult seahorses ranged from 13 to 26 cm, and these were maintained at NIWA's Mahanga Bay hatchery in Wellington. Length of adult seahorses was measured as the sum of distance from the tip of the snout to the mid-point of the cleithral ring, from there to the lateral mid-point

of the last trunk ring, and from there down to the tip of the tail when extended (as in Lourie *et al.*, 1999) following anaesthesia with AQUI-S^{TM}. For measuring large adults, anaesthesia was deemed necessary due to their strength in resisting the straightening of their tails; all seahorses were kept submerged in seawater during handling.

The adults were initially held in aerated 75-l round white polyethylene tanks (42 cm high x 57 cm wide) supplied with flow-through ambient seawater, filtered to 20 μm, at a flow rate of 1 l min^{-1} maintained on a 10 h light:14 h dark photoperiod (2 x 55W cool white fluorescent tubes). These tanks had a central upstand overflow and attachment substratum of 20 mm polyethylene mesh and macroalgae. However, courtship behaviour appeared to be significantly impeded by the low water depth in these tanks. Following reports that vertical tank heights >90cm appear to best facilitate egg transfer in *H. abdominalis* (Sobolewski, 1997), in August 1997 all the seahorses were transferred to a 500-l upright fibreglass cylindrical tank for breeding (Fig. 2.2), and photoperiod increased from 10 h light:14 h dark (approximate winter photoperiod) by one hour of light per week until a photoperiod of 15 h light:9 h dark was reached (approximate summer photoperiod). This summer photoperiod was selected as breeding in wild *H. abdominalis* has historically been observed during spring/summer (Graham, 1939; Lovett, 1969; Francis, 1996) and the gradual increase in photoperiod was commensurate with ambient seawater temperature increasing over the study period. When a total of 12 pregnancies had been achieved – which took approximately 27 weeks – the photoperiod was decreased by one hour of light per week until a photoperiod of 10 h light:14 h dark was reached and courtship behaviour stopped, so that effort could be concentrated on raising the juveniles that were produced. This decrease in photoperiod was commensurate with ambient seawater decreasing at the onset of autumn.
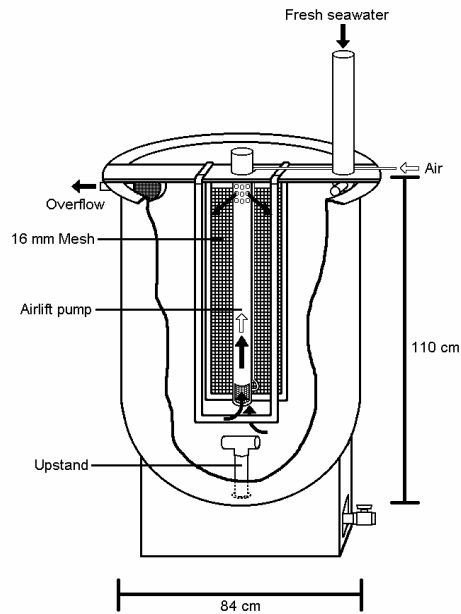
Figure 2.2 Design of 500-l courtship and breeding tank used for *Hippocampus abdominalis*. Water movement is indicated by filled arrows and air movement by open arrows (source: Chris Woods).

The courtship and breeding tank was supplied with flow-through ambient seawater filtered to 20 μm at a flow rate of 2 l min^{-1}. Aeration and water circulation were provided by an air-lift pump running up the centre of the tank. A PVC pipe and 16 mm polyethylene mesh framework surrounding the air-lift pump provided substratum for the seahorses to attach to. An upstand at the bottom of the tank with plastic aquarium plants attached to it also provided attachment substratum, as well as ensuring a certain level of reserve water should accidental draining of the tank occur.

The broodstock were predominantly fed decapsulated brine shrimp (*Artemia salina*, Salt Creek brand), 2–9 mm long, cultured at 25–28°C and enriched on a microalgal diet of *Chroomonas salina* (Culture Strain-174), *Isochrysis* sp. (Tahitian strain), and *Pavlova lutheri* (CS-182), at a mean ± 1 SE rate of 573.3 ± 81.7 brine shrimp seahorse^{-1} day^{-1}. Supplemental feeds of amphipods (*Gammarus* sp., O*rchestia chilensis*), glass shrimp (*Palaemon affinis*), mosquito larvae (*Opifex fuscus*), and white worms (*Enchytrea* sp.) were also added when available. These feeds were used as they are all types of food that seahorse researchers, seahorse culturists and aquarium hobbyists commonly use to feed to seahorses. The feeding regime was selected because it was sustainable from a supply viewpoint, practicable and approximated that published for other seahorse species in

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captivity at the time of the study (e.g. Herald & Rakowicz, 1951; Giwojna, 1990). The bottom of the courtship tank was siphoned daily and the whole tank drained and cleaned weekly.

All the adults (both male and female, unless brooding males were absent during the practice of pregnancy separation) were observed daily for courtship behaviours, as well as general behaviour and condition. Each adult was readily identifiable due to its relative size, sex, colouration, morphological, and behavioural characteristics. Focal sampling was used at random times during the day to observe individuals for a period of one hour. Observation was carried out from an elevated position 1 m away from the courtship tank, with observation starting 15 min after the observer was in place in an attempt not to disturb or influence seahorse behaviour. Continuous recordings of that individual's behaviour and its interactions with other adults were made during this time. This enabled courting pairs to be noted and date of the commencement of brooding recorded in most cases. Unreciprocated courtship was defined as the non-reciprocation of courtship behaviour within 10 min of a courtship attempt being made by a member of the opposite sex. Reciprocated courtship was defined as the reciprocation of courtship behaviour within 10 min of a courtship attempt being made by a member of the opposite sex. If reciprocated courtship was still occurring after one hour of observation, the observation was continued until either mating occurred or courtship stopped (defined as >10 minutes after cessation of courtship behaviour).

Where mating was not observed but did occur, the commencement of brooding was recorded as the onset of obvious brooding behaviour, as characterized by specific behaviours, colouration, and pouch distension (see results section). One week after brooding commenced, males were separated from the other adults and placed back in 75-l round white polyethylene plastic tanks to prevent cannibalism of the juveniles by other adults upon their release. This 'waiting period' of one week was used because males could sometimes cease courtship, become reticent and dull their colouration (several of the physical indicators of brooding) for several days before recommencing courtship activity. Brood duration was recorded, as was the number of young released by each male.

Using mean daily temperatures for the period of brooding by each male seahorse, a linear relationship between temperature and the reciprocal of brood duration (1/days)

was able to be calculated. Extrapolation of the regression line back to the x-axis indicates a "biological zero point" (i.e. the theoretical temperature at which development ceases (Seki & Kan-no, 1977; Uki & Kikuchi, 1984)). This zero point allows the calculation of effective accumulated temperature (EAT) in degree-days for each brood which can be used to predict brood duration at given temperatures. EAT = (mean temperature during each brood – BZP) x brood duration (days) for each brood.

Water parameters (mean $\pm$ 1 SE) during the courtship and brooding period were as follows: temperature 14.59 ± 0.17°C (range 10.6–19.5°C), dissolved oxygen 8.97 ± 0.13 mg l^{-1} (range 8.7–9.1 mg l^{-1}), salinity 34.56 ± 0.08 ppt (range 32.7–35.4 ppt), and pH 8.07 ± 0.01 (range pH 7.93–8.19).

2.2.2 Rearing of juveniles

Once released from the male's pouch, the juveniles were transferred in seawater, without exposure to air, to 75-l round white polyethylene tanks (Fig. 2.3). Each brood was kept in isolation in its own separate 75-l tank. Attachment substratum was provided by 16 mm polyethylene mesh lining the inside of the tank wall and surrounding the upstand, as well as clumps of shade cloth strands (1 mm diameter), anchored to small concrete weights. Immediately after release, three juveniles from each brood were randomly selected and their SL and wet weights (WW) (after quick blotting on paper towels) measured following anaesthesia on a Mettler microgram balance. These juveniles were then returned to the tank. These measurements were repeated weekly on three randomly selected juveniles for each different brood for a period of one year.

Figure 2.3 75-l tanks used for rearing juvenile seahorses (source: Chris Woods).

Juveniles were first fed 24 h old decapsulated *Artemia franciscana* nauplii (INVE AF Grade) (mean ± 1 SE length 0.43 ± 0.01 mm) (instar II) at an approximate concentration of 1000 brine shrimp seahorse^{-1} day^{-1} for the first week. Following this they were fed progressively larger *A. salina* (Salt Creek brand) nauplii enriched for 24 hours on a microalgal diet of *C. salina*, *Isochrysis* sp., and *P. lutheri* (Table 1), at approximate concentrations of 1000 brine shrimp seahorse^{-1} day^{-1}. As with the feeding regime for the adults, the feed and feeding regime used were sustainable from a supply viewpoint, practicable and approximated that published for other seahorse species in captivity at the time of the study based on published material. As many uneaten *Artemia* as possible were flushed to waste from each tank before new enriched *Artemia* were fed to the juveniles each day. Progression onto the next larger size of *Artemia* only occurred once the ability of the juveniles to feed on these was confirmed by observing a trial feeding.

Table 1 Approximate feeding schedule for juvenile *Hippocampus abdominalis*. Mean ± 1 SE length of *Artemia* nauplii.

Age of juveniles (weeks)	*Artemia* length (mm)
0-2	0.43 ± 0.01
2-4	0.58 ± 0.02
4-6	0.83 ± 0.01
6-8	1.03 ± 0.08
8-10	1.27 ± 0.08
10-12	1.86 ± 0.09
12-14	2.1 ± 0.05
14	2.38 ± 0.07

From 14 weeks of age onwards, juveniles were fed the same size range and number of adult brine shrimp as the adult seahorses, and from 8 months of age onwards they were also fed glass shrimp (5–10 mm in length) and *Gammarus* sp. amphipods (2–8 mm in length) at the rate of 5 seahorse^{-1} week^{-1}. The ability of the juveniles to feed on these larger prey items was confirmed via a trial feeding and the quantity fed was dictated by the average number of glass shrimp and amphipods that could be reliably collected in a one hour period every week. Tanks were siphoned daily and any dead juveniles removed and recorded.

Daily water parameters (mean ± 1 SE) during the one year period were as follows: temperature 15.72 ± 0.17°C (range 10.9–19.4°C), dissolved oxygen 8.58 ± 0.33 mg l^{-1} (range 7.1–10.6 mg l^{-1}), salinity 34.67 ± 0.04 ppt (range 32.5–35.4 ppt), and pH 8.19 ± 0.02 (range pH 8.09–8.27). These values are an approximate reflection of the wild

conditions as an ambient flow-through seawater system was used with a low biomass in each tank.

2.2.3 Long term growth and survival

At the conclusion of one year, a random sample of 60 of the surviving seahorses ($n = 30$ males, $n = 30$ females) from mixed broods were transferred to another 500-l fiberglass tank identical in setup to the adult courtship tank with flow-through ambient seawater filtered to 20 μm at a flow rate of 2 l min^{-1}. Here they were kept for a further six years of captivity in order to obtain some longer term captive growth and survival data. Seahorses were fed daily with *A. salina* (mean ± 1 SE length 3.1 ± 0.04 mm) enriched with Algamac-2000/3050$^{®}$ and *Spirulina platensis* (blue-green microalga) (95:5% mix), wild 2–6 mm amphipods (*O. chilensis*) and wild 3–10 mm glass shrimp (*P. affinis*).

Seahorses were checked daily for mortalities. The bottom of the tank was siphoned daily and the whole tank drained and cleaned every two weeks. Mean ± 1 SE daily feeding rates were 251 ± 46.2 *A. salina* seahorse^{-1} day^{-1} and approximately 5 amphipods and 5 shrimp seahorse^{-1} day^{-1}. Yearly measurements of seahorse SL and WW were conducted on a sample of 10 male and 10 female seahorses selected randomly. Daily water parameters (mean ± 1 SE) during the six year period were as follows: temperature 14.45 ± 0.27°C (range 9.3–19.9°C), dissolved oxygen 7.92 ± 0.02 mg l^{-1} (range 7.2–9 mg L^{-1}), salinity 34.2 ± 0.03 ppt (range 31.3–35.4 ppt), and pH 8.19 ± 0.04 (range pH 8.0–8.3).

2.2.4 Statistical analyses

Data were analysed with STATISTICA 6.1 (Statsoft, Tulsa, Oklahoma, USA) and NCSS 2004 (Hintze, J., Number Cruncher Statistical Systems, Kaysville, Utah, USA). Data were tested for normality (Shapiro-Wilk's W test) and homogeneity of variances (Levene's test). Data were transformed appropriately before analysis to ensure data normality and homogeneity of variances. Possible inflection points in juvenile growth (length and weight) were tested for. All data are presented as mean ± 1 SE.

2.3 Results

2.3.1 Courtship and breeding

Courtship behaviours were observed once the photoperiod increased beyond 11 h light:13 h dark (17/08/1997), and ceased when the photoperiod was decreased below 11 h light:13 h dark (13/3/1998) following the twelfth mating. From a total of 56 h of focal observations the following were achieved: 64 unreciprocated courtship attempts by males, 31 reciprocated courtship attempts by males, 11 unsuccessful attempted matings without egg transfer, and 10 successful matings with egg transfer. These 10 matings involved certain males and females mating repeatedly over time (see Table 2.2). In all cases males appeared to initiate courtship, although it is possible that females may have exhibited subtle signals that were not perceptible to the observer.

Courtship initiation involved a series of colour changes and postural displays. Males dilated the opening of the pouch and inflated the pouch to balloon-like proportions with water by swimming forwards, or by pushing their body forwards in a pumping action, then closing the pouch opening. At the same time they would lighten their pouch in colour to white or light yellow. Males also brightened their overall body colouration during courtship, typically intensifying the colour yellow. A male would repeatedly approach his selected female with his head tucked down, and dorsal and pectoral fins rapidly fluttering (Fig. 2.4A). If the female was not receptive she would ignore the male, who would then look for another potential mate. If no females were receptive, the male would stop displaying and deflate his pouch by dilating the pouch opening and bending forwards, expelling the water inside (Fig. 2.4B).

If a female was receptive to a courting male, she would reciprocate with her own colour changes and head tucking, typically intensifying the lighter colours such as yellow and white, highlighting the contrast between these colours and their overall darker blotching and banding. A series of short bursts of swimming together in tandem then ensued, sometimes with tails entwined, or with the female tightly rolling her tail up. After coming to rest, the male would attempt to get the female to swim towards the water surface with him by repeatedly pointing his snout upwards. If the female responded by also pointing her snout upwards then the final stage of courtship followed. This involved both male and female swimming directly upwards towards the water surface with both their heads pointing upwards and tails pointing straight down. If they reached the water surface, one or both seahorses could often be seen and heard to snap their

heads. This final stage of courtship was observed in 21 of the 31 observed reciprocated courtships.
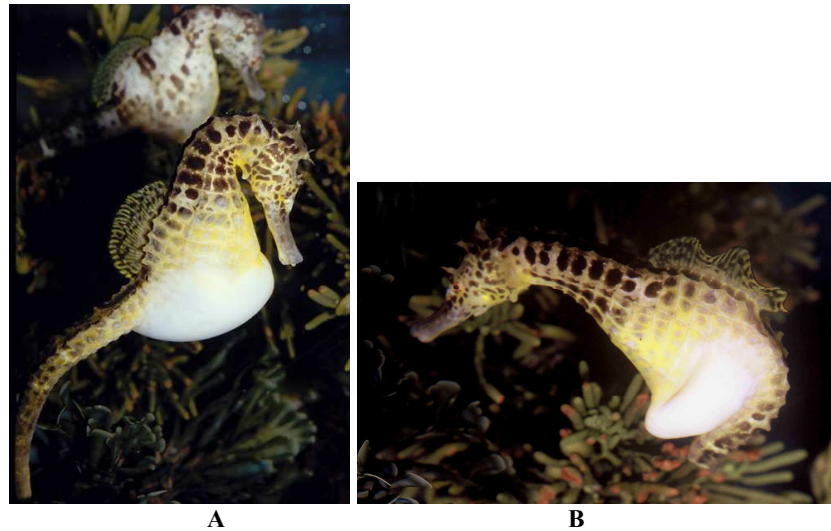
Figure 2.4 Male courtship: (A) Male with inflated brood pouch (front) courting reciprocating female (back), (B) Male deflating brood pouch following unsuccessful courtship (source: Chris Woods).

To transfer her eggs to the male, the female would face the male, slightly above him. Pressing the base of her abdomen against the male's pouch she would squirt her eggs through the opening in the front of his pouch which was dilated to approximately 8–12 mm in diameter. In seven of the 10 observed matings, the transfer of eggs was completed in one episode, in two matings two separate episodes of egg transfer were observed, and in one observed mating involving a female with an extremely swollen abdomen it took six episodes of egg transfer before mating was concluded.

Following successful egg transfer, the male would repeatedly arch and contort his body in an attempt to evenly distribute the eggs within his pouch; at the same time his colouration would begin to dull. Males then became relatively reclusive, spending more time near the bottom of the tank. Females would also dull their colouration and their abdomens would appear visibly shrunken following egg transfer.

The captive broodstock did not appear to be rigidly monogamous. Both males and females were observed mating with different partners, although some pairings were repeated (Table 2.2). In the 10 observed matings, males (SL = 18.09 ± 0.75) mated with females (SL = 20.49 ± 0.93) approximately the same size or larger than themselves

(paired Student's *t*-test, $t_9 = 3.02$, $P<0.05$). For those seahorses that mated more than once, the mean ± interval between matings was 10.4 ± 5.7 days (range 0–50 days) for males and 34.5 ± 12.2 (range 9–64 days) for females.

Table 2.2 Mating pairs of captive *Hippocampus abdominalis*, number of juveniles per brood, date of conception, and brood duration (days). Standard Length (SL cm) of the adults is given in brackets. Where the mother of a brood was not observed this is indicated by a question mark. * Denotes a brood where abnormal newborn juveniles were observed. M = Male, F = Female.

Brood	Father	Mother	# of juveniles	Conception date	Brood duration (d)
1	M1 (15.8)	?	721	24/8/1997	69
2	M1 (16)	F1 (18.9)	214	11/11/1997	34
3	M2 (21.5)	F2 (23.2)	395	4/12/1997	35
4	M3 (16.8)	F3 (19)	86	1/12/1997	24
5*	M4 (15.5)	?	422	8/12/1997	34
6	M1 (16)	F4 (23)	359	19/12/1997	38
7	M3 (16.8)	F5 (16.4)	149	31/12/1997	27
8	M2 (21.5)	F2 (23.2)	253	17/1/1998	30
9	M3 (16.8)	F2 (23.2)	356	26/1/1998	33
10	M2 (21.5)	F2 (23.2)	53	16/2/1998	24
11	M1 (17)	F5 (16.4)	128	5/2/1998	28
12	M5 (17)	F6 (18.4)	96	13/3/1998	32

Competition between multiple males courting the same female was observed. This occurred at the initial stage of courtship when male and female were "promenading" around the tank and involved males trying to cut inside each other to get alongside the female (Fig. 2.5). In this situation, the transition to the final vertical rise and egg transfer was prevented.

Figure 2.5 Competition between multiple males (yellow male second from left and male bottom right) for one female (centre) (source: Chris Woods).

As brooding progressed, the relative bulging of the males' pouch increased with the development of the enclosed juveniles. The larger the number of juveniles brooded the more the pouch appeared to bulge. Near the day of parturition, the outer surface of the

pouch appeared silvery white, probably due to skin-stretching, with a distinct darkness inside, probably due to the mass of juveniles inside (Fig. 2.6).

Figure 2.6 Brooding male close to parturition (source: Chris Woods).

Brooding by males culminated in the release of the juveniles during the dark portion of the photoperiod. Mean ± 1 SE number of days for each male to brood was 34 ± 3.4 (range 24–69 days). Using mean daily temperatures for the period of brooding by each male seahorse, a linear relationship between temperature and the reciprocal of brood duration (1/days) was calculated (Fig. 2.7). Extrapolation of the regression line back to the x-axis indicates a "biological zero point" of 6.52°C. From this, an effective mean accumulated temperature (EAT) of EAT = 331.02 ± 13.21 degree-days was calculated. However, due to the fluctuating ambient water temperatures and consequent use of mean daily temperatures, this EAT should be treated only as a general approximation.
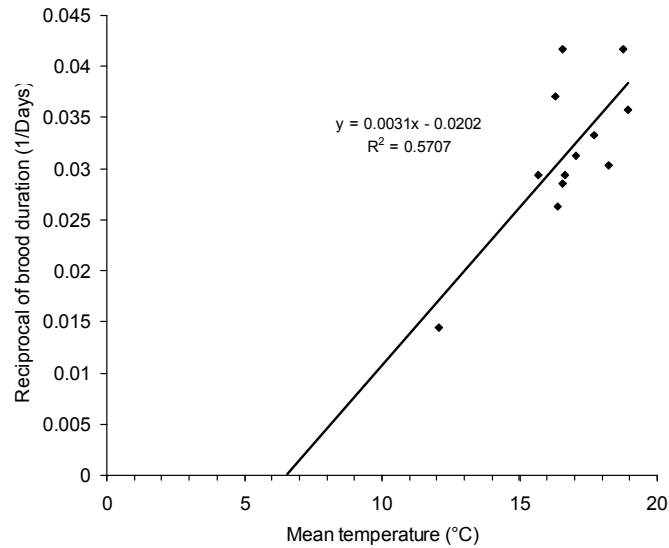
Figure 2.7 Relationship between mean daily temperature (°C) and reciprocal of brood duration (1/days) in captive *Hippocampus abdominalis* broods (*n* = 12) with extrapolated biological zero point (6.52°C).

Because of the non-independence of some broods due to repeat pairings, overall statistical analyses of brood data in relation to variables such as parent male and female size or number of juveniles per brood are problematic. Therefore, analyses were conducted on only 8 broods that were independent of repeat-pairings (broods 2, 3, 4, 6, 7, 9, 11 and 12 in Table 2.2); where repeat-pairings did occur, only one brood from these was randomly selected for analysis. There was no significant relationship between brood size and parent male size (r^2 = 0.17, $P>0.05$) but brood size was significantly related to parent female size (r^2 = 0.80, $P<0.01$), i.e. larger females produce larger broods and are the main determinant of brood size rather than males (Fig. 2.8).
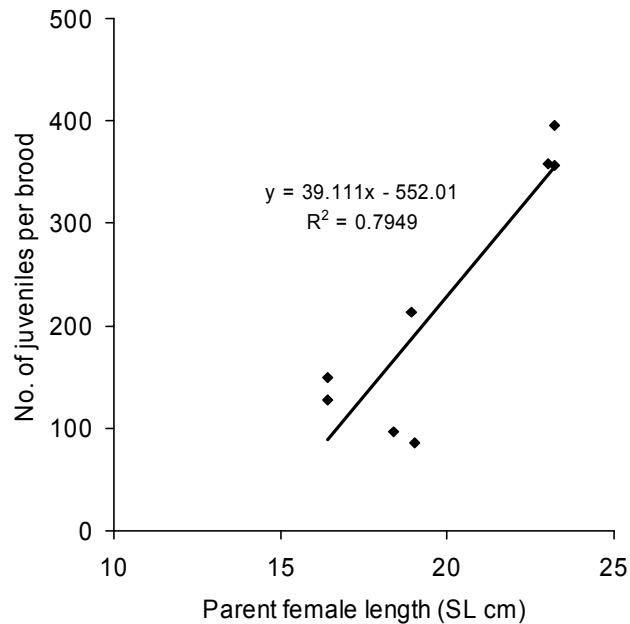
Figure 2.8 Relationship between parent female size (SL cm) and number of juveniles produced by male *Hippocampus abdominalis* in independent broods (*n* = 8).

To give birth to the juveniles, the males would dilate the opening to their pouches and repeatedly bend forward, compressing the pouch in an attempt to expel the juveniles. In some instances the colour of the male's pouch immediately preceding, and during, release of the juveniles, would alternately flash between light and dark. Released either singly or in small groups, the majority of juveniles were released within 1–2 h, although in one case a total of 3 days were required for full release.

Following the release of juveniles, males were observed to resume courtship as early as the same day they released their juveniles when placed back in the courtship tank. Weight gain/loss during courtship and pregnancy for male and female seahorses was not recorded.

2.3.2 Rearing of juveniles

Twelve broods of juveniles were produced between November 1997 and March 1998. The mean number of juveniles per brood was 269.3 ± 55.5 (range 53–721). Mean SL of juveniles when first released was 15.7 ± 0.5 mm. Mean wet weight of juveniles when first released was 0.008 ± 0.0001 g. Analysis of juvenile length and wet weight in relation to brood size from the 8 broods that were independent of repeat-pairings (see

section 2.3.1 above), revealed no significant relationship between juvenile length and brood size ($r^2 = 0.01$, $P>0.05$). However, there was a significant relationship between juvenile wet weight and brood size ($r^2 = 0.75$, $P<0.05$), i.e. larger broods produce juveniles of lower wet weight (Fig. 2.9).
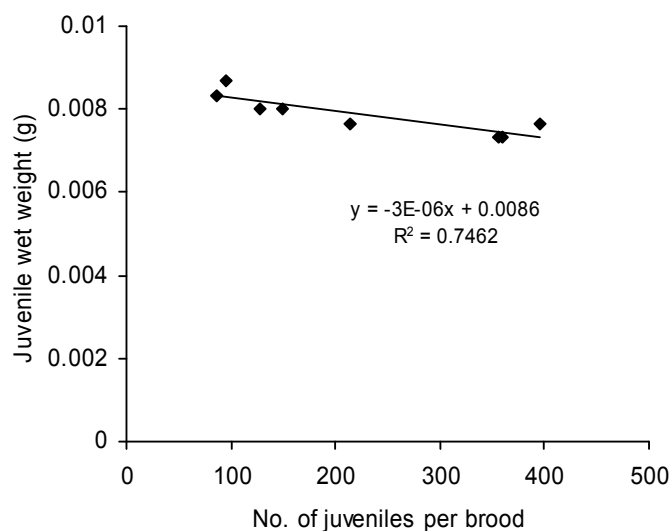
Figure 2.9 Relationship between mean juvenile wet weight (g) and number of juveniles per brood produced by male *Hippocampus abdominalis* ($n = 12$).

Upon their release, juveniles were independent and capable of capturing *Artemia* nauplii. Although like adults in shape, they were much slimmer and their heads and dorsal fins appeared proportionately larger (Fig. 2.10). For the first 2–4 weeks the juveniles congregated near the water surface, either free-swimming, or attached to the shade cloth strands. Once they reached approximately 25 mm SL and 0.4 g in wet weight, juveniles started to move away from the water surface, spending less time swimming and more time holding onto the available substratum.

Figure 2.10 One day-old juvenile *Hippocampus abdominalis* (Standard Length = 16 mm) (source: Chris Woods).

2.3.2.1 Growth of juveniles

After one year of growth the juveniles had attained a SL of 110.7 ± 1.4 mm (Fig. 2.11A) per brood, and wet weight of 3.07 ± 0.23 g (Fig. 2.11B) per brood (these means are taken after one year of growth for each brood). Although broods were born at different times there was no significant relationship between either overall mean length or weight increase for each brood and mean seawater temperature each brood was exposed to during its one year of culture ($r^2 = 0.24$ and $r^2 = 0.26$, $P>0.05$ respectively). At one year of age the majority of juveniles were reproductively mature as demonstrated by courtship and breeding, and the development of brood pouches in males. The early juveniles in the last two broods suffered from exposure to a concentrated bloom (up to 33×10^{-6} cells l^{-1}) of the toxic dinoflagellate *Gymnodinium brevisulcatum* 4 weeks after release. This proved fatal with complete mortality within the following 2 weeks. Consequently, the data from these last two broods was not included with the other broods after 4 weeks for growth or survival data.

A possible inflection point occurred at 74.3 mm SL, between 20–21 weeks of age, at which point the growth rate in terms of seahorse length appeared to slow. Similarly, the pattern of weight gain exhibits a possible inflection point occurring at 1.26 g, between 26–27 weeks of age, at which point the growth rate in terms of seahorse weight appeared to slightly increase.

This slightly converse relationship between length increase and weight gain can be explained by the changing body shape of the juveniles as they matured. Following growth past 80 mm SL, the body shape of juveniles began to change, with a marked deepening of the abdomen and general thickening of the whole body. In males this also included the development of the fleshy brood pouch.

Both within and between broods there could be a large variation in body shapes. For example, even similar sized juveniles within the same brood could have long snouts (snout length approximately 1/2 of head length) while others had short snouts (snout length approximately 1/3 of head length), some had slender bodies (lateral trunk width less than 1/2 trunk height) while others had deep bodies (lateral trunk width equal to, or slightly greater than 1/2 trunk height), and some juveniles grew head and neck filaments while others did not. Body colouration was also highly variable.

Sexual differentiation based on the appearance of the brood pouch in males was generally possible once the juveniles reached approximately 80 mm SL, which could be from 4 months of age onwards. Sex differentiation based on other characters such as size, shape and colour at this stage was generally not possible. However, by one year of age and the attainment of sexual maturity, the base colour of males tended towards brown/yellow/grey, whilst female base colour spanned a range from white to grey to brown and yellow. Also, at this age females started to develop a much rounder and more pronounced abdomen than males. The sex ratio of surviving female to male juveniles after one year of age was 1.8:1. Because the brood pouch in males only started developing once they reached 80 mm SL, the visual determination of the sex ratio at birth was not possible.
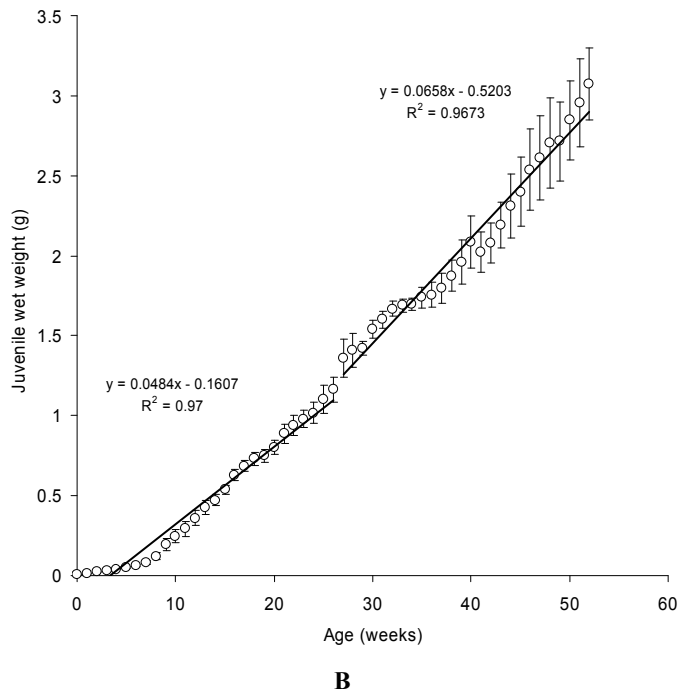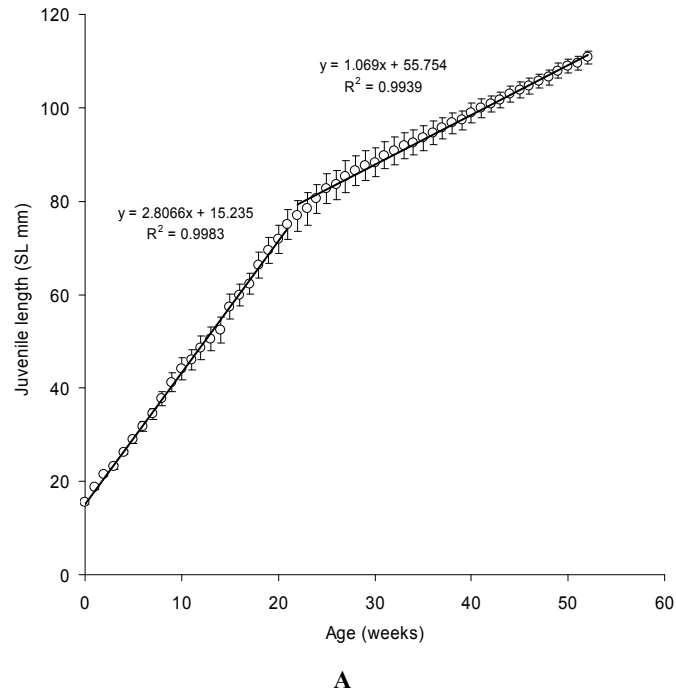
Figure 2.11 (A) Standard length (SL mm), and, (B) wet weight (g) of juvenile *Hippocampus abdominalis* versus time (*n* = 10 broods with *n* = 3 juveniles measured per brood per week).

2.3.2.2 Survival of juveniles

Mortality was greatest during the first 2 months of life (Fig. 2.12), with percent survival per brood at 1 month = $36.2 \pm 6.8\%$, and percent survival per brood at 2 months = $21.5 \pm 4.3\%$. From this stage onwards the rate of mortality was markedly reduced. At the end of 1 year the percent survival rate per brood was $10.6 \pm 3.5\%$.
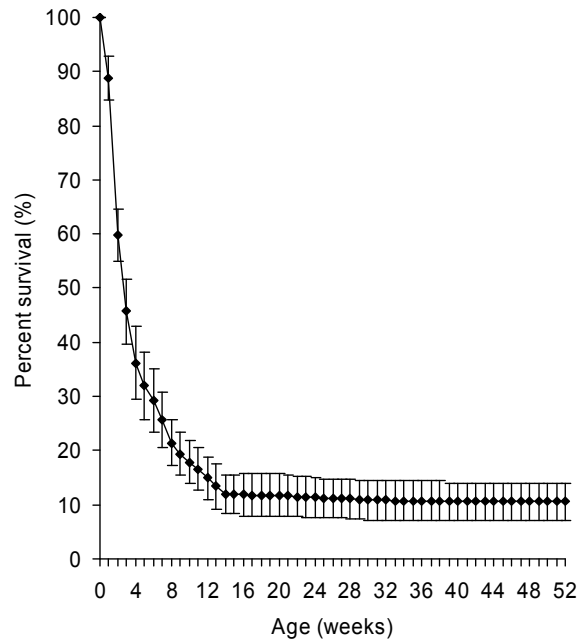
Figure 2.12 Percent survival of juvenile *Hippocampus abdominalis* per brood (*n* = 10).

Of the young seahorses which died, the majority (61%) appeared thin, with few brine shrimp nauplii in their guts although *Artemia* densities around them were reasonably high. Of the remaining deaths, 32% of dead juveniles had large air bubbles in their gut, and 7% had hyperinflated swimbladders. These juveniles appeared to suffer buoyancy control problems and difficulty in feeding prior to death.

2.3.2.3 Abnormalities at birth

In one brood (brood size of 422 juveniles for a 15.5 cm male, see Table 2.2), 14 juveniles were born which were underdeveloped with stumpy tails and snouts. These juveniles could not attach, swim or feed properly and died within the first week. These juveniles were not included in the growth or survival data. No other abnormal juveniles were observed in any other brood.

2.3.3 Long term growth and survival

Male and female F1 seahorses progressively increased in SL over the next six years (one-way ANOVA, $F_{6,139} = 958.9$, $P<0.001$), with SL in each year significantly greater than the preceding one (Tukey HSD, $P<0.001$), to reach a final SL of 26.9 ± 0.2 cm (Fig. 2.13). There were no significant differences in SL between males and females in any year (Student's t-test, $P>0.05$).
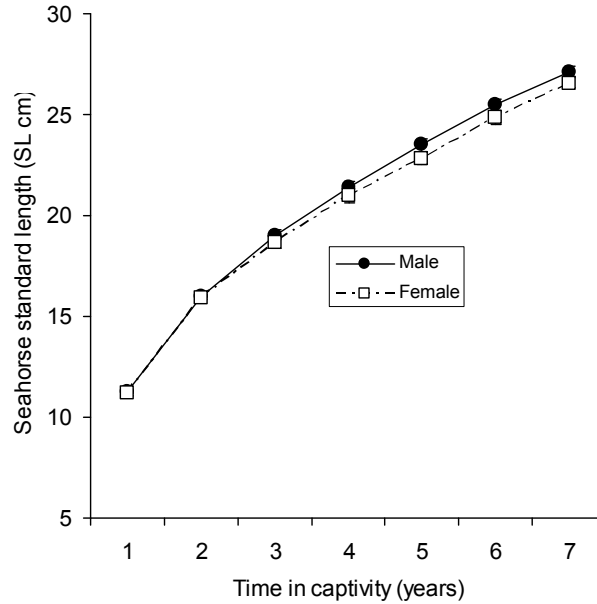
Figure 2.13 Standard length (SL cm) of F1 male and female *Hippocampus abdominalis* reared a further six years.

Male and female F1 seahorses progressively increased in WW over the next six years (one-way ANOVA, $F_{6,139} = 3300.9$, $P<0.001$), with WW in each year significantly greater than the preceding one (Tukey HSD, $P<0.001$), to reach a final combined WW of 38 ± 1.3 g (Fig. 2.14). There were significant differences in WW between males and females, with males first becoming significantly heavier than females at Year 5 (Student's t-test, $t_{1,18} = 2.22$, $P<0.05$) through to Year 7 of age (Student's t-test, $t_{1,18} = 2.4$, $P<0.05$), when the WW of males was 40.8 ± 1.3 g and WW of females was 35.2 ± 1.9 g.
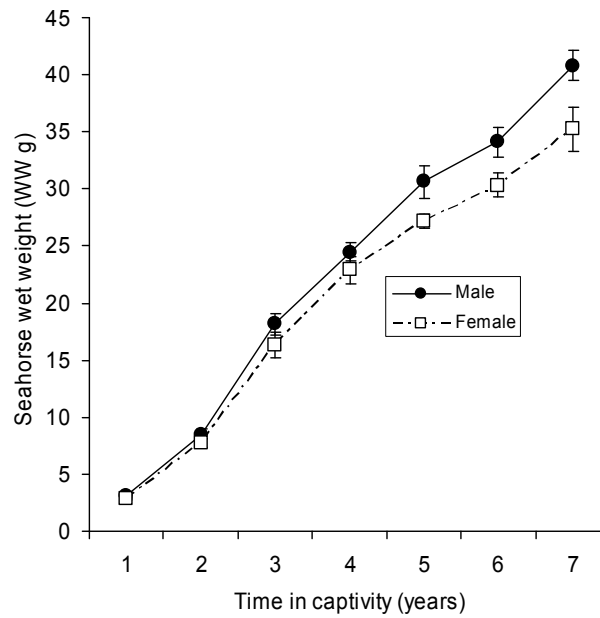
Figure 2.14 Wet weight (WW g) of F1 male and female *Hippocampus abdominalis* reared for a further six years.

Survival of captive seahorses was high, with 55 (28 male, 27 female) of the original 60 (91.7%) one year-old seahorses surviving a further six years in captivity.

2.4 Discussion

In this preliminary investigation, wild *H. abdominalis* were successfully kept in a captive environment under a simulated summer photoperiod. They bred and produced offspring, some of which were successfully reared past one year of age, attaining reproductive maturity. However, the observations and data derived from this preliminary study should be regarded as indicative only for this species as they were derived from a small sample size of wild seahorses and are therefore vulnerable to a number of potential biases.

At the size of 11 cm SL (one year of age) the captive seahorses produced from the original broodstock were of a suitable size for sale to the aquarium market, where attributes such as colour and body shape/ornamentation are of greater importance than outright size (G. Leveridge, Seahorse New Zealand, pers. comm.). At 11 cm SL, seahorses this size are likely to be too small to fetch a sufficient price in the medicinal

trade to make an aquaculture venture based on supplying solely to this trade in a country such as New Zealand, where materials and labour costs are relatively high, economically feasible. To profitably supply the medicinal trade, *H. abdominalis* needs to be cultured to a larger size.

However, what is regarded as a large seahorse size will vary according to what is available at the time from competing wild fisheries and other aquaculture sources. According to Lourie *et al.* (2004) seahorse species typically used in TCM are variable in their maximum recorded heights (HT_{max}) (see Table 2.3) (HT to SL conversion factors vary according to species). Assuming these species could be consistently sourced and traded at their maximum size, *H. abdominalis* would have to be produced to a size >17–19 cm HT (= 19.7–22 cm SL using a conversion factor of 0.865 (see chapter 7)) to be potentially the largest amongst these traded species. This is with the exception of *H. kelloggi* and *H. ingens* whose HT_{max} would probably be difficult to surpass aquaculturally whilst being economically viable. The sample of captive one year-old offspring reared for a further six years exhibited reasonable growth rates, achieving ~20 cm SL between years 3–5 and excellent survival. To investigate whether it is worth pursuing the on-growing of *H. abdominalis* to 20–22 cm SL for the medicinal trade, economic analyses and detailed market research should be performed.

The age and growth rates of wild *H. abdominalis* are generally not well known. *In situ* growth rates for *H. abdominalis* from warmer waters in northern New Zealand were found to be relatively high for small seahorses (up to 2.8 mm per month for 16 cm length seahorses), but slowed with increasing seahorse size (Van Dijken, 2001). Based on von Bertalanffy growth curves, Van Dijken (2001) estimated that it may take 10–11 years for *H. abdominalis* to reach 25 cm in length. However, Van Dijken qualified this calculation as an over-estimate biased by small sample size and incorrect initial birth size. Based on operculum ageing and length/weight relationships in wild animals, Lovett (1969) estimated that for *H. abdominalis* in Tasmania, seahorses from 9.1–11 cm in length were 1 year old; those 9.1–16.1 cm were 1–2 years old; those 14.4–19 cm were 2–3 years old; those 18–22 cm were 3–4 years old; and finally, those over 22 cm in length were 4+ years old. The long term growth data obtained in this investigation are comparable to Lovett's estimations, and appear to contradict Van Dijken's calculations. In this study, seahorses reached 25 cm in length in six years rather the 10–11 calculated by Van Dijken (2001). Extrapolation of the long term growth data from this study

indicate that these captive *H. abdominalis* would appear to take 8–9 years to reach 30 cm SL and 10–11 years to reach their possible maximum 35 cm SL.

Table 2.3 Maximum recorded heights (HT$_{max}$ cm) for seahorses common in trade for TCM – source: Lourie *et al.*, 2004), with *Hippocampus abdominalis* (in bold type) included for size comparison (calculated HT$_{max}$).

Species	HT$_{max}$ cm
H. camelopardalis	10.0
H. borboniensis	14.0
H. fuscus	14.4
H. barbouri	15.0
H. histrix	17.0
H. kuda	17.0
H. trimaculatus	17.0
H. spinosissimus	17.2
H. reidi	17.5
H. comes	18.7
H. erectus	19.0
H. kelloggi	28.0
H. abdominalis	**30.3**
H. ingens	31.0

Caution should be exercised in comparing this long term captive growth data to wild *H. abdominalis*, as it may in fact be slower than occurs in wild seahorses. Although the captive seahorses experienced a reasonably "normal" ambient seawater environment, they were kept at greater densities than they would probably encounter in the wild. Captive seahorses were fed a restricted range of prey organisms at a feeding level determined by the researcher. In their natural environment, seahorses are able to selectively target their prey from within a wider range of organisms, and provided food supply is ample, feed to their desired satiation level. Additionally, there is the consideration of the effect that heritable characteristics have on growth rates. Seahorses with different genetic traits may exhibit different growth patterns in terms of maximum size and rapidity of growth rate; as the broodstock used in this investigation were collected solely from Wellington Harbour, and their number relatively small, their genotypes and related growth characteristics may be different to other populations of seahorses. It is highly likely that with greater food abundance, greater variety in prey organisms offered (with a wider associated nutritional composition), and manipulation of environmental variables, that growth rates of captive *H. abdominalis* can be significantly improved. For example, James & Woods (2000) have found that maintaining sea water temperature at 18 and 21°C resulted in significantly greater increase in length for large juvenile *H. abdominalis* compared with sea water

temperatures of 12 and 15°C, whilst Tung (2005) found 20°C seawater promoted greater growth and survival in 0–14 day old *H. abdominalis* compared to 15 and 10°C seawater in combination with a 14 h light:10 h dark photoperiod.

Comparative captive growth data for *H. abdominalis* exists mostly for early juveniles, rather than long term growth, but these data do show the growth effects that certain culture variables can have. For example, Tung (2005) investigated the early growth and survival of *H. abdominalis* under varying photoperiod, temperature and prey density regimes and found highest growth at 37.7 mm SL after 3 weeks in culture for juveniles held at a 14L:10D photoperiod and an *Artemia* density of 1000 nauplii seahorse^{-1} d^{-1}. Shapawi & Purser (2003) found that length increase over 28-day periods in 4-week old *H. abdominalis* varied from 10.5 to 21.4 mm depending on enrichment used to enrich their prey *Artemia*. Filleul (1996) also examined the influence of *Artemia* enrichment in 12-day old juvenile *H. abdominalis* over a 28-day period and found that length increase varied between 20.7 to 45.4 mm depending upon the *Artemia* enrichment treatment.

The growth rate of juvenile *H. abdominalis* is slow in comparison to tropical seahorse species, which currently dominate both aquarium and medicinal trades, although differences in final adult size can make comparisons difficult. For example, Job *et al.* (2002) achieved a SL of 11 cm in *H. kuda* at around 85 days after birth. Correa *et al.* (1989) cultured *H. erectus* and found sexual maturity could be achieved in seahorses after approximately 3 months at a length of 10 cm. Chen (1990) stated that at 3 months of age the seahorses *H. trimaculatus*, *H. kuda*, and *H. japonicus* attain lengths of 11, 9, and 5.5 cm respectively in culture. Based on their rapidity of growth, tropical seahorse species have a distinct aquaculture advantage over the generally slower-growing temperate seahorse species. However, the marketing difference between *H. abdominalis* and tropical seahorse species for the aquarium trade lie in it being a temperate species with a distinctive body shape, and for the medicinal trade in its very large adult size.

Intersexual differences in growth rate may lead to sex differences in profitability in aquaculture (Björnsson, 1994). In male *H. abdominalis*, the development of the fleshy brood pouch with sexual maturity eventually results in males being heavier than females of approximately the same total length (Vincent, 1990). This was significantly evident at Year 5 with the F1 seahorses in this investigation. So, if seahorses are marketed on weight alone (i.e. in the medicinal trade) then males may be more desirable to farm than

females, although other desirable attributes may overrule this advantage in weight. If seahorses are to be marketed on their length, then male and female *H. abdominalis* should be equally valuable.

The courtship behaviours observed in captive *H. abdominalis* were very similar to those displayed in other *Hippocampus* spp. (Masonjones & Lewis, 1996; Garrick-Maidment, 1997; Giwojna & Giwojna, 1999a), beginning with colour and postural displays, and culminating with a vertical ascent in the water column for egg transfer. Vertical tank height with a reasonably low stocking density was critical to successful culmination of the courtship ritual with egg transfer, due to the final vertical swimming component of the courtship ritual. For seahorses in general, there appears to be a logical increase in the tank size required for successful breeding of larger species (Sobolewski, 1997; Giwojna & Giwojna, 1999b).

Reproduction successfully occurred at a stocking density of 1 adult seahorse 42 l^{-1} in the 500-l breeding tanks used. There is a paucity of information relating to appropriate broodstock stocking densities for other seahorse species. Qiu (1991) used a stocking density of 1 seahorse 20 l^{-1} for 11 cm SL broodstock *H. trimaculatus* in outdoor ponds, while Sobolewski (1999) and Forteath (2000) suggested a possible broodstock density for *H. abdominalis* of 1 seahorse 10–20 l^{-1} dependent upon adequate food and water quality.

In seahorses, monogamy appears to be the rule rather than the exception. The majority of observed instances of monogamy have been in *in situ* studies, although *ex situ* monogamy has been observed in *H. zosterae* (Foster & Vincent, 2004). Where monogamy occurs it appears to be reinforced by ritualized daily greetings between male and female before, during, and after brooding (Vincent, 1995; Vincent & Sadler, 1995; Masonjones & Lewis, 1996), although there are exceptions (Moreau & Vincent, 2004; Wilson & Martin-Smith, *in press*). For example, Wilson & Martin-Smith (*in press*) have observed promiscuous courtship in high density female-biased wild populations of *H. abdominalis*, but with monogamous mating.

In this study, the broodstock obtained from the wild did not appear to be rigidly monogamous, although males were observed to only mate with one female per brood. Some males had sequential broods with the same females, while others had successive

broods to different females. This captive polygamy has also been observed in *H. abdominalis* in Australia (Forteath, 2000). However, because it is not known whether any of the broodstock used in this investigation were previously pair-bonded, what was observed in this investigation may be an artefact of an artificial environment rather than normal behaviour.

Competition, in the form of physical interference from other males was observed on several occasions to prevent mating in courting couples. Competition during courtship has also been observed for other seahorse species (Giwojna & Giwojna, 1999a), and the isolation of courting couples is recommended to ensure uninterrupted egg transfer. Keeping breeding tanks free of unnecessary clutter which may impede or prevent courtship and egg transfer is also recommended (Giwojna & Giwojna, 1999b).

The mean brood size of 269 produced in this investigation is similar to other reports for *H. abdominalis*. In New Zealand, Graham (1974) reported up to 182 per brood, while in Australia, Forteath (1997) reported usual brood sizes ranging from a few juveniles in young males to several hundred in older, larger males. The largest brood of 721 that was produced in this investigation is large for *H. abdominalis*, but the largest reported to date for *H. abdominalis* is 1113 (Forteath, 1999). A study by Vincent & Giles (2003) on *H. whitei* showed that female size, rather than male size, was the key determinant in the number of young released by the male, probably as a function of females producing more eggs in size-assortative pairings. In this study, male *H. abdominalis* appeared to mate with females of the same size, or slightly larger than themselves. However, brood size was positively related with female size, not male size, indicating that females are the key determinant in the number of young released by the male *H. abdominalis*. A negative relationship was observed between the number of juveniles per brood and their wet weight at birth in *H. abdominalis*. Observations by Dzyuba *et al.* (2006) in brooding *H. kuda* suggest that embryos within the brood pouch where few embryos are present may attach to functionally advantageous pouch sites and thus gain greater physiological support during gestation. If greater numbers of embryos are present, this may result in some resource limitation for some embryos and could explain the negative relationship in juvenile wet weight with increasing brood size observed in this study. Caution must be exercised with these findings for *H. abdominalis* as they were derived from a relatively small sample size in an artificial environment.

At birth, the size of other seahorse species juveniles varies markedly, ranging from 2 mm in *H. bargibanti* to around 11 mm in length for species such as *H. erectus* and *H. guttulatus*, and is related to initial egg size rather than adult size, with larger eggs generally producing larger juveniles (Foster & Vincent, 2004). For example, *H. trimaculatus* has an egg diameter of 0.8 mm and juvenile size of 6 mm, while *H. guttulatus* has an egg diameter of 2 mm and juvenile size of 11.8 mm. In this study, juvenile *H. abdominalis* were an average 15.6 mm SL. A small sample of two spilled eggs retrieved from the courtship tank confirmed the relatively large egg diameter (1.5–2 mm at the widest point) of *H. abdominalis*, in support of Foster & Vincent (2004).

An obvious aquaculture implication of the relatively large size at which juvenile *H. abdominalis* is born relates to the prey size that the juveniles can begin feeding upon. As observed in this study, newly born *H. abdominalis* could begin feeding immediately upon instar II *A. franciscana* nauplii. This potentially negates the requirement to invest labour and expense into providing smaller initial prey organisms such as rotifers for juvenile seahorses, which are sometimes required to rear other seahorse species, although this must be weighed against the potential benefits of providing such prey in terms of nutrition. Thompson (2002) investigated whether feeding both rotifers and enriched *A. franciscana* to newborn *H. abdominalis* was beneficial, but found no growth or survival benefit to this mixed feeding. In seahorse species that produce small juveniles with slim snouts, such as *H. kuda* and *H. subelongatus*, these juveniles may not be able to initially feed effectively on *Artemia* nauplii. These species may be restricted to smaller prey initially (such as rotifers and small copepods) to increase growth and survival rates (Ignatius *et al.*, 2000; Payne & Rippingale, 2000; Warland, 2002; Mai, 2004a; Sheng *et al.*, 2006). However, some seahorse species that produce small juveniles, such as *H. zosterae* with juveniles 8 mm in length, can feed effectively on *Artemia* nauplii (Abbott, 2003), as their snouts appear wider in diameter. A potential size advantage experienced by large seahorse juveniles upon birth could translate into potential increased survival, as fish mortality rates generally decrease with increasing body size (Houde, 1997), provided appropriate growth requirements are met.

Juvenile *H. abdominalis* appeared to be initially pelagic with young juveniles congregating near the water surface for the first 2–4 weeks. Lovett (1969) also reported captive juveniles swimming at the water surface for the first month, as well as 3–4 week old juveniles being caught in plankton trawls in the wild. This pelagic behaviour may

give juveniles access to zooplankton prey as well as act as a dispersal mechanism. An immediate implication for the aquaculture environment is that juveniles for at least the first month do not require tanks with any significant water depth (i.e. >30 cm). This means either a lower total hydraulic volume in the seawater system utilized, or an increased tank stocking density/plant space ratio as a greater number of smaller depth tanks may be stacked in place (in which juveniles utilize the majority of a lower water depth) rather than a smaller number of larger depth tanks (in which juveniles utilize the minority of a greater water depth).

Although juvenile *H. abdominalis* are initially pelagic, it is advisable to have substratum running from the water surface downwards so that pelagic juveniles can hitch onto this if they desire, rather than their siblings, otherwise 'balls' of juveniles may occur which can result in stress or even death as the juveniles wrestle against each other and are prevented from feeding. Such "balling" has frequently been observed in a number of seahorse species and is best avoided through the use of holdfast material (Bellomy, 1969; Giwojna, 1990).

The pattern of high mortality within the first few months as observed in this investigation has been a common occurrence in the captive rearing of seahorses (Herald & Rakowicz, 1951; Bellomy, 1969; Vincent & Clifton-Hadley, 1989; Scarratt, 1995; Forteath, 1995; Vincent, 1996; Garrick-Maidment, 1997), although the amount of mortality and the reasons for it can vary markedly. However, this pattern of high juvenile mortality is not determinate, as some researchers have been able to achieve relatively consistent high survival rates in the early juvenile stages. For example, survival rates of 98–100% have been achieved with *H. capensis* and *H. whitei* (N. Garrick-Maidment, The Seahorse Trust, pers. comm.) and survival rates of 90+% after one month have been achieved with *H. abdominalis* (A. Sobolewski, University of Tasmania, pers. comm.). Wilson & Vincent (1998) achieved 100% survival rate in partial broods of juveniles from wild-caught pregnant male *H. barbouri*, *H. fuscus*, and *H. kuda*, while Job *et al.* (2002) achieved 73% survival to market size (14 weeks of age, ~12 cm SL) with *H. kuda*. Such instances of high juvenile survival rates appear linked to good tank hygiene and prevention of disease, high quality and appropriate sized food, good broodstock condition, and appropriate environmental and physical conditions. If commercial aquaculture of *H. abdominalis* is to occur in New Zealand, then techniques

must be determined that ensure consistently high rates of survival from captive stock in which the life cycle has been successfully closed.

Suboptimal culture temperatures have been demonstrated to affect subsequent juvenile survival in the tropical seahorse *H. kuda* (Lin *et al.*, 2006). However, as the culture temperatures in this study were representative of the temperatures in which the wild broodstock breed (see Chapter 6), this is unlikely to have been the causative factor in the high mortalities observed in this study. That the majority of dead juveniles in this investigation appeared thin with few brine shrimp in their guts, even though brine shrimp were abundant and the juveniles were capable of ingesting the size of brine shrimp available, indicates a difficulty in prey capture. The white tank colour could have caused possible orientation confusion among early juvenile *H. abdominalis* and affected their ability to feed adequately on *Artemia* instar II nauplii. Larval marine fish which are positively phototactic may be attracted to reflective surfaces, resulting in "wall-banging" behaviour (Naas *et al.*, 1996). Such "wall-banging" behaviour was observed in early juvenile *H. abdominalis* kept in white tanks. Older juveniles did not appear to suffer from any such feeding problems, presumably as a result of the larger *Artemia* they were offered being more obvious, learned striking accuracy, or even simply the fact that they are not pelagic like early juveniles.

The occurrence of air bubbles within the gut appears responsible for some early juvenile mortality and has been observed in other attempts at raising seahorses in captivity (Herald & Rakowicz, 1951; Giwojna, 1990; Forteath, 1995). Ingestion of air bubbles by juveniles at the water surface, or in the water column as a result of aeration, as well as during exposure to air when being handled, have been suggested as possible causes for this affliction (Giwojna, 1990; Wolf, 1998; J. Purser, University of Tasmania, pers. comm.). The ingestion of air bubbles at the water surface when preying on brine shrimp was observed in this investigation, but not the ingestion of aeration bubbles in the water column. An additional factor to consider is the possibility that new-born juveniles experience problems in relation to initially inflating their swimbladders. It is believed that many physoclistic teleosts, such as seahorses (Peters, 1951) have to ingest air to initially inflate the swimbladder (Steen, 1970; Martin-Robichaud & Peterson, 1998). If juvenile *H. abdominalis* are confused by the white tank colouration, and if the pneumatic duct connecting the foregut and swimbladder closes before successful

inflation, ingestion of air by the juveniles in an attempt to fill their swimbladders could result in air bubbles trapped within the gut.

A small percentage of dead juveniles also possessed hyperinflated swim bladders. Hyperinflation of the swim bladder has been attributed to gas supersaturation (Weitkamp & Katz, 1980; Cornacchia & Colt, 1984), stress caused by such factors as inadequate water depth (Kolbeinshavin & Wallace, 1985), and excessive air ingestion (Nash *et al.*, 1977). It may hinder normal swimming, or in extreme cases result in floating fish and mortality. Dissolved oxygen saturation levels in the juvenile tanks were generally around 100%, but did occasionally reach up to 105% with ambient water fluctuations. However, as total gas and nitrogen saturation were not measured gas supersaturation cannot be confirmed as a causative agent of swimbladder hyperinflation. Swimbladder hyperinflation caused by stress associated by inadequate water depth is unlikely due to the initial pelagic behaviour of juvenile *H. abdominalis*.

In conclusion, it is possible to breed and rear *H. abdominalis* with reasonable growth rates using fairly standard hatchery techniques. However, the twin issues of improving juvenile survival rates and increasing growth rates require further research and development. The size that juveniles had reached after one year of growth is adequate for the aquarium trade as they are reproductively mature, yet with the potential to at least double in size. However, because larger-sized seahorses fetch the highest prices in the TM trade it may not be profitable enough to sell them at this size, and further on-growing and increasing of growth rates would be required.

CHAPTER 3

IMPROVING JUVENILE SEAHORSE SURVIVAL AND GROWTH

3.1 Introduction

Increased survival and growth rates are important elements in increasing production capacity in commercial aquaculture (Shang, 1981; Battaglene *et al.*, 1994; Huang & Chiu, 1997; Baskerville-Bridges & Kling, 2000). As production capacity greatly influences economic viability, it is desirable for any given commercial aquaculture operation to attain the greatest survival and growth rates biologically possible with the species it is culturing within operational constraints.

To maintain high growth rate and survival, fish must optimize their food intake by being effective predators (Gerking, 1994; Grecay & Targett, 1996; Østergaard *et al.*, 2005). This is particularly important in the early stages of life when tissue bulk and energy stores are often not present or well developed enough to sustain periods of sub-optimal feeding. In an aquaculture environment, the optimization of food intake can be strongly influenced by a variety of culture conditions such as tank colour, stocking density, feeding regime, temperature, water turbidity, prey density and size, and type and intensity of lighting (Duray et al., 1996; Grecay & Targett, 1996; Planas & Cunha, 1999; Mischke *et al.*, 2001; Brown *et al.*, 2002; Watanabe & Feeley, 2003; Peña *et al.*, 2004; Peña *et al.*, 2005).

For many fish species, the young must developmentally pass through a transition from endogenous to exogenous feeding (Blaxter, 1969; Kamler, 1992; Gerking, 1994). This switch from endogenous to exogenous derivation of food source is commonly referred to as "first feeding", and may still involve the utilization of remaining yolk supplies in addition to exogenous feeding (Gerking, 1994). First feeding in fish larvae is a vulnerable period during which larvae must develop appropriate functional organs and systems (e.g. digestive, swimbladder, nervous, visual, and skeletal systems) related to prey capture and either initiate feeding or face starvation (Blaxter, 1969; Kamler, 1992; Alves *et al.*, 1999; Peña *et al.*, 2004; Nan Chen *et al.*, 2006). High larval mortality rates during the first days of fish larval culture have been related to an ineffective feeding response at the beginning of exogenous feeding, a sub-optimal environment or food, or

inadequate visual system development (Kamler, 1992; Helvik & Karlsen, 1996; Planas & Cunha, 1999).

In seahorses, the young hatch from their eggs inside the parent male's pouch and develop into their juvenile form, passing the transition phase from endogenous to exogenous feeding within the safety of the parent male's pouch. There is no larval stage that is independent of the parent. When released from the pouch seahorse juveniles are well-developed morphologically with appropriate organ development such as an open and well differentiated digestive tract (Filleul, 1996), and are physically capable of active swimming and immediate exogenous feeding (Fig. 3.1). However, despite this developmental advantage at birth, one of the bottlenecks facing successful seahorse culture has been the low juvenile survival often encountered in the first few months of rearing (Herald & Rakowicz, 1951; Bellomy, 1969; Vincent & Clifton-Hadley, 1989; Scarratt, 1995; Forteath, 1995; Vincent, 1996; Garrick-Maidment, 1997; Julius, 2001), indicating this initial period as a critical period of vulnerability for seahorses. Possible causes of high initial juvenile mortalities in seahorses have been suggested as: disease (Vincent, 1996; Wilson & Vincent, 1998), swimbladder inflation problems or ingestion of air-bubbles causing loss of buoyancy control (Lawrence, 1998; J. Purser, University of Tasmania, pers. comm.), temperature (Qiu, 1991), salinity (Hilomen-Garcia *et al.*, 2003), and inappropriate or sub-optimal diets and the detection and ingestion of those diets (Correa *et al.*, 1988; Vincent, 1996; Wilson & Vincent, 1998; Chang & Southgate, 2001; Job *et al.*, 2002; Shapawi & Purser, 2003).

The preliminary study into the culture of *H. abdominalis* (Chapter 2) encountered high mortality within the first two months of the juveniles' release from their father's pouch, with a mean ± 1 SE survival per brood at 1 month of 32.5 ± 6.7%, and mean ± 1 SE survival per brood at 2 months of age of 18.5 ± 4.4%. After this, mortality rapidly dropped off, with a mean ± 1 SE of 10.6 ± 3.5% juveniles per brood surviving past 1 year of age to reach reproductive maturity. For the commercial culture of *H. abdominalis* to be economically viable, a dramatic improvement in early juvenile survival above this is required.
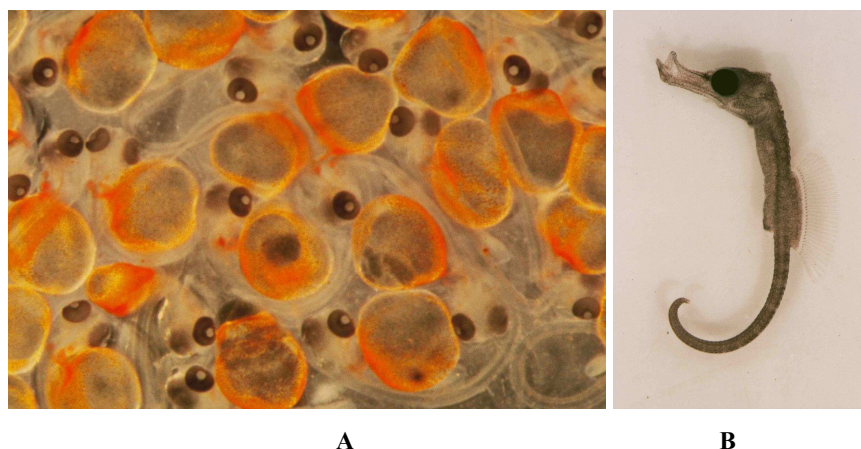
Figure 3.1 Developing embryos of *Hippocampus abdominalis* (A). Note the large maternally-derived yolk sac and pug-nose snouts of embryos which will not allow exogenous feeding) (SL = 9 mm). One day-old juvenile *H. abdominalis* just released from parent male's pouch (B). Note lack of yolk sac and well-developed snout and eyes for feeding (SL = 16 mm) (source: Chris Woods).

In many teleost fish, vision is the primary sense used in prey detection and capture (Blaxter, 1969, 1980; Hunter, 1981), although there is evidence that some fish use other non-visual senses to detect prey (Knutsen, 1992; Batty & Hoyt, 1995; Cox & Pankhurst, 2000). The characteristics of prey items (e.g. shape, colour, movement) when viewed against different backgrounds can affect the ability of fish to capture these prey (Dendrinos *et al.*, 1984; Hinshaw, 1985; Browman & Marcotte, 1987; Jakobsen *et al.*, 1987; Thetmeyer & Kils, 1995; Duray *et al.*, 1996; Naas *et al.*, 1996; Martin-Robichaud & Peterson, 1998; Downing & Litvak, 1999; Planas & Cunha, 1999; Utne-Palm, 1999). However, there may also be confounding effects such as prey stocking density, photoperiod, light intensity and even salinity (Watanabe & Feeley, 2003; Papoutsoglou *et al.*, 2005; Sheng *et al.*, 2006). Given that seahorses are well-developed and feed exogenously at birth, and have well-developed binocular vision which they use to ambush live prey (James & Heck, 1994; Flynn & Ritz, 1999; Lourie *et al.*, 1999; Foster & Vincent, 2004), the effect of environmental variables such as tank background colour and source of illumination as a possible causative factor in the high initial juvenile mortality of *H. abdominalis* as observed in the preliminary study requires investigation.

An important guiding principle in aquaculture is that a suitable density of fish should be stocked to maximize biomass production and minimize water usage, without compromising the health or quality of the fish (Shang, 1981). Understocking may result in underutilization of feed and space (as well as overbalancing the ratio of operating costs to revenue derived from production), whilst overstocking may result in

competition for food and space and in a decline in survival and growth rates (Shang, 1981; Sharma & Chakrabarti, 2003). According to Hickling (1962), the profit in most forms of fish culture lies in stocking at a density or rate well below the maximum standing crop, allowing and assisting the fish to grow up to or near the maximum standing crop in the shortest possible time, and then harvesting the increased weight. Maximum standing crop is defined as the maximum weight a fish stock can sustain without gaining or losing weight by consuming solely the food available (Hickling, 1962).

In finfish aquaculture, stocking density can not only affect fish growth, survival and physiological responses, but has also been shown to alter behavioural interactions (Wallace *et al.*, 1988; Haylor, 1991; Brown *et al.*, 1992; Christianssen *et al.*, 1992; Suresh & Lin, 1992; Jørgensen *et al.*, 1993; Björnsson, 1994; Hossain *et al.*, 1998; Papotsoglou *et al.*, 1998; Feldlite & Milstein, 1999; Irwin *et al.*, 1999; Baskerville-Bridges & Kling, 2000; Wang *et al.*, 2000; Ruane *et al.*, 2002; Iguchi *et al.*, 2003; Saillant *et al.*, 2003; Sharma & Chakrabarti, 2003). For example, Sharma & Chakrabati (2003) found that Catla (*Catla catla*) and rohu (*Labeo rohita*) showed higher survival and more efficient food conversion at stocking densities of 6667 and 8333 larvae m^{-3} compared with 10000 larvae m^{-3}, with higher ammonia, nitrite and phosphate and chemical oxygen demand at the higher stocking density. In comparison, Toko *et al.* (2007) found that with stocking densities of either 4, 6 or 8 fish m^{-3}, African catfish (*Clarias gariepinus*) attained greater growth, condition and feed conversion at the highest stocking density. They attributed this converse effect to decreasing levels of aggressive behaviours with increasing stocking density and the high tolerance of *C. gariepinus* to poor water quality conditions. Life stage may also influence what effect stocking density has. Bolasina *et al.* (2006) examined the effects of stocking densities of 10 or 50 swimming larvae l^{-1}, and 2 or 20 swimming/settling larvae l^{-1} in the Japanese flounder (*Paralichthys olivaceus*). They found that with the swimming larvae weight and length increases were greatest at the lower stocking density, but that with settled juveniles the weight and length increases were greatest at the higher stocking density. They attributed this growth increase in settled juveniles at higher density to the strengthening of social hierarchies with the dominant settled juveniles keeping the swimming larvae from settling, forcing the latter to expend more metabolic energy in doing so. Therefore, determining the effects of stocking density in species in commercial culture is important to optimize culture efficiency.

In this chapter, basic techniques for improving early (0–2 months) juvenile survival in *H. abdominalis* were investigated. Specifically, the influence of tank and lighting designs on feeding were examined to determine their effect on early juvenile survival and growth. Based on observations in Chapter 2, hypotheses to be tested were:

1) H_0: Juvenile seahorses are not phototactic; H_1: Juvenile seahorses are phototactic.

2) H_0: Tank background does not affect feeding efficiency; H_1: Tank background does affect feeding efficiency.

3) H_0: Prolonged access to the water surface in combination with top illumination and feeding on positively phototactic prey does not affect growth and survival; H_1: Prolonged access to the water surface in combination with top illumination and feeding on positively phototactic prey does affect growth and survival.

The effect of stocking density on growth and survival in five month-old juvenile *H. abdominalis* was also examined. The null hypothesis (H_0) is that stocking density does not affect juvenile seahorse growth and survival. The alternate hypothesis (H_1) is that stocking density does affect seahorse growth and survival with decreasing growth and survival the more densely seahorses are stocked. This chapter is based on data from Woods (2000b, 2003b).

3.2 Materials and methods

3.2.1 Initial juvenile survival

3.2.1.1 Source of juveniles for experiments

Thirty first-generation (F1) captive seahorses (10–15 cm Standard length (SL)) retained from the preliminary investigation were used as captive broodstock to produce juveniles for the experimental work between December 1998 and March 1999. These broodstock were kept in their respective brood groupings in round white 75-l polyethylene tanks supplied with flow-through seawater filtered to 20 μm at a flow rate of 1 l min^{-1} and maintained on a 14 h L:10 h D photoperiod (2 x 55W cool white fluorescent tubes, 4.2 μmol m^{-2} s^{-1} at the water surface). Individuals that exhibited courtship behaviour were separated and placed in a 1 m high 500-l (see Chapter 2) courtship tank with a member of the opposite sex from another brood who was also exhibiting courtship behaviour. If courtship was not observed within 3 days between this pair, they were returned to their

respective broods and another couple selected. If courtship was observed and egg transfer occurred, the female was returned to her brood tank and the pregnant male transferred to his own 75-l tank to await the release of his juveniles. Mean ± 1 SE water parameters for brooding males were as follows: temperature 17.1 ± 0.15°C (range 13–19.5°), dissolved oxygen 7.73 ± 0.12 mg l^{-1} (7.1–8.1 mg l^{-1}), salinity 34.59 ± 0.19 ppt (33.8–35.1 ppt), and pH 8.1 ± 0.05 (7.8–8.19).

Broodstock seahorses were predominantly fed *Artemia* 2–9 mm long cultured on a microalgal diet of *Chroomonas salina* and *Isochrysis* sp., at an approximate rate of 300 brine shrimp seahorse^{-1} day^{-1}. Supplemental feeds of glass shrimp, *Palaemon affinis* (3–10 mm in length), and *Gammarus* sp. amphipods (2–8 mm in length) at the rate of 5 seahorse^{-1} day^{-1} were also given.

Length (SL) and wet weight measurements of seahorses are presented as mean ± 1 SE.

3.2.1.2 Phototaxis in juveniles

Phototaxis in juveniles was tested using two horizontal Perspex cylinders (80 cm x 5 cm) illuminated with 2 x 55 W fluorescent white tubes (5.8 μmol m^{-2} s^{-1} at cylinder surface) and filled with seawater. The cylinders had removable caps at each end and half of each cylinder was blacked over with black electrical tape. The cut ends of each cylinder were painted black to prevent light transmittance along the cylinder walls.

Every week from birth to two months of age, three juveniles from two broods were randomly selected and then placed individually in the cylinders and left for 5 min. After this time whether they were in the light or dark section of each cylinder was noted. Cylinders were flipped 180° between testing each seahorse so that any bias arising from the position of light and blacked-out halves of the tubes in the room could be removed. Juveniles were only tested once for phototaxis, i.e. no repeat testing of juveniles at different ages.

3.2.1.3 Effect of background colour on feeding efficiency

To test the hypothesis that tank background colour affects feeding efficiency, 15 x one week-old juveniles (SL = 18.6 ± 0.34 mm) starved for 24 h were randomly selected from a single brood, and individually placed in seawater into 15 x 2-l transparent jars containing 20 μm filtered seawater. These were illuminated from above with 2 x 55 W

fluorescent white tubes (5.6 μmol m^{-2} s^{-1} at water surface) with a 14 h L:10 h D photoperiod.

Five jars were clear plastic, five jars were wrapped in white plastic and five were wrapped in black plastic. Attachment substratum for the juveniles was supplied by bottom-weighted clumps of shade cloth strands running from the jar bottom to the water surface. The surrounding background in the experimental area was black sheet plastic on three sides (approximately 20 cm away from the jars at the nearest point), white 75-l tanks with a wooden framework on the other side (70 cm away from the jars at the nearest point) and white ceiling (1.5 m away).

Juveniles were left in these jars for 30 min to acclimate to the jars. Fifty *Artemia* nauplii (mean ± 1 SE length = 0.57 ± 0.04 mm) were then released into each jar. After 1 min, the number of feeding strikes (attack rate) and the percentage of these strikes which resulted in prey capture (capture success) was recorded for each juvenile over a 1 min period.

This was repeated with another random selection of juveniles from the same brood when the juveniles had reached 1 month of age (SL = 30.8 ± 0.41 mm) with *Artemia* nauplii of mean length = 0.85 ± 0.02 mm.

3.2.1.4 Effect of access to the water surface and illumination source

To test the hypothesis that ingestion of air bubbles into the gut when juveniles are ingesting brine shrimp at the water surface in tanks illuminated from above affects survival, 30 juveniles from each of two broods (SL = 16 ± 0.1 mm) were collected within 14 h of release from their fathers and transferred in seawater to an aerated 100-l glass aquarium illuminated from above with 2 x 55 W fluorescent white tubes (5.6 μmol m^{-2} s^{-1} at water surface) with a 14 h L:10 h D photoperiod.

Five juveniles were randomly allocated to each of 6 x 2-l inverted transparent plastic jars (Fig. 3.2) within the aquarium. The lid of each jar (i.e. at the bottom) was pierced by a cable-tie with attached strands of shade cloth. The base of the jar (i.e. situated at the water surface) was removed in three of the jars. This allowed juveniles access to the water surface while in the other three jars the presence of the bottom prevented access to the water surface. A ring of polystyrene around each jar provided flotation and 35

mm diameter holes drilled in the sides of the jars screened with 1 mm mesh allowed brine shrimp nauplii into the jars and water to circulate, as well as preventing the juveniles from leaving their jars.

Juveniles were first fed 24 h-old *Artemia* nauplii (mean ± 1 SE length = 0.44 ± 0.01 mm) at an approximate concentration of 1000 *Artemia* seahorse^{-1} day^{-1} for the first week. Following this they were fed *Artemia* cultured on a microalgal diet of *Chroomonas salina* and *Isochrysis* sp. as follows: week 2–24 h-old *Artemia* (mean length = 0.57 ± 0.04 mm), week 4–24 h and 48 h-old *Artemia* (mean length = 0.85 ± 0.02 mm), also at a concentration of 1000 *Artemia* seahorse^{-1} day^{-1}. This feeding regime was based known feeding rates for juvenile seahorses in aquaria (e.g. Giwojna, 1990) and the ability to reliably supply the enriched *Artemia*. *Artemia* densities were determined based on averaged (5 x 5 ml samples) densities of *Artemia* in their culture vessel prior to harvesting in relation to the remaining number of seahorses in each jar.

Each day, a 30% water change of 20 μm filtered seawater was performed and jars inspected. Any mortalities observed were recorded, removed and not replaced. After 1 month, juveniles from each treatment were removed and their SL measured against a steel rule following anaesthesia with AQUIS®. Presence/absence of air bubbles in the gut and the externally visible condition of the swimbladder were recorded.
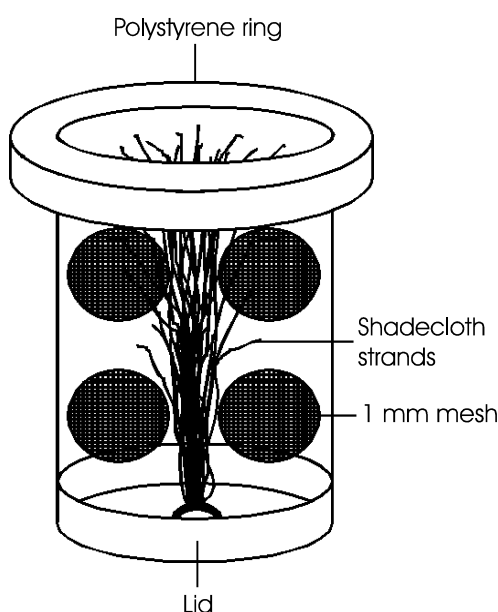
Figure 3.2 Two-litre jars used to test the effect of access to the water surface on juvenile survival with illumination from above, or from the side, and positively phototactic prey on *Hippocampus abdominalis* (source: Chris Woods).

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This experiment was then repeated two weeks later with another 30 juveniles from each of two broods (SL = 16.2 ± 0.2 mm) collected within 14 h of release from their fathers, but with illumination from the side, with 2 x 55 W fluorescent white tubes (5.6 μmol m^{-2} s^{-1} at tank side). The tank was blacked off from the waterline upwards to prevent illumination from above.

Mean ± 1 SE water parameters in the glass aquarium were as follows: temperature 16.0 ± 0.9°C (range 13.6–19.5°C), dissolved oxygen 7.5 ± 0.2 mg l^{-1} (7–8.4 mg l^{-1}), salinity 34.3 ± 0.2 ppt (33.4–34.7 ppt), and pH 7.94 ± 0.05 (7.8–8.14).

3.2.1.5 Survival in side-illuminated 60-l glass aquaria

Based on results obtained in the above experiments, whether entire broods of juveniles could be raised to two months of age with high survival, was tested using 4 x 60-l rectangular glass aquaria (60 cm x 36 cm x 26 cm). These tanks were aerated and supplied with 20 μm filtered seawater at a flow rate of 1 l min^{-1} and attachment substratum for the juveniles was provided by bottom-weighted clumps of shade cloth strands running from the tank bottom to the water surface. The tanks were blacked off from the waterline up, and illuminated from the side with 2 x 55 W fluorescent white tubes (4.3 μmol m^{-2} s^{-1} at tank side) with a 14 h L:10 h D photoperiod.

Within 14 h of release from the parent males, four broods of juveniles (mean ± 1 SE number of juveniles per brood = 79 ± 15.5) (SL = 16.2 ± 0.5 mm) were randomly mixed and transferred in seawater to these tanks to give an even number of 79 juveniles per 60-l glass aquaria. Juveniles from separate broods were randomly mixed to remove any potential brood-specific biases (e.g. differential growth rates).

Tanks were inspected daily and excess waste and faeces siphoned out. Any mortalities observed were recorded, removed and not replaced. Tanks were completely drained and cleaned every two weeks. After two months, juveniles were removed and their lengths measured against a steel rule and wet weights recorded following anaesthesia. Presence/absence of air bubbles in the gut and the externally visible condition of the swimbladder recorded.

3.2.1.6 Water parameters

With the exception of the 100-l glass aquarium (section 3.2.1.3), mean ± 1 SE water parameters in all the experimental treatments were as follows: temperature 14.1 ± 0.2°C (range 12.3–16.8°C), dissolved oxygen 8.3 ± 0.2 mg l^{-1} (7.8–8.7 mg l^{-1}), salinity 34.2 ± 0.1 ppt (33.4–34.7 ppt), and pH 8.15 ± 0.03 (8.04–8.22). There were no significant differences in water parameters between treatments or replicates within each separate experiment (two-way ANOVA, $P>0.05$).

3.2.2 Stocking density

Three stocking densities thought to be feasible for commercial culture of late juvenile *H. abdominalis* were tested: 1, 2, and 5 juveniles l^{-1}, with four replicate 9-l tanks per stocking density (i.e. 9, 18 and 45 seahorses per tank). Two hundred and eighty-eight captive-bred F2 five month-old juvenile *H. abdominalis* (SL = 72 ± 0.3 mm, mean wet weight = 0.51 ± 0.01 g, Condition Factor = 0.16 ± 0.002) were randomly selected from a captive population and randomly allocated to one of the three treatments.

Condition Factor is commonly referred to as Fulton's K condition factor, where CF (or K) = 100 (W/L^3). This is a commonly used technique for deriving a weight-to-length index as a simple indicator of fish health and/or growth, and assumes that heavier fish of a given length (larger CF value) are in better condition (e.g. Ricker, 1975; Lambert & Dutill, 1997; Booth & Hixon, 1999; Lloret & Planes, 2003; Hoey & McCormick, 2004; Froese, 2006). The exponent of ^{3} assumes isometric growth in fish, and may change depending upon the growth pattern of the species concerned, making comparisons between species and between different sized individuals within species difficult (Bolger & Connolly, 1989; Chouinard & Swain, 2002; Lima *et al.*, 2002; Ribeiro *et al.*, 2004; Craig *et al.*, 2005; Peck *et al.*, 2005). Condition Factor in this investigation was calculated as CF = 10^{-6} (whole seahorse wet weight (g)/standard length (mm)$^{3.5}$). The exponent of $^{3.5}$ is derived from least-squares regression of log-transformed weight and length data from 1269 individual measurements of cultured *H. abdominalis* between 3 and 27 cm SL ($y = 3.4868x$ - 3.1962, $r^2 = 0.96$, $P<0.001$: Woods, unpublished data).

The juveniles were cultured from F1 captive broodstock at NIWA's Mahanga Bay hatchery, to the size required on a diet of enriched *Artemia*. SL was measured against a steel rule. Juvenile wet weight was measured on a Mettler P440 balance following quick blotting on a paper towel. There were no significant differences in either juvenile SL or

weight between stocking densities or replicates at the start of the experiment (two-way ANOVA, $P>0.05$).

Tanks used were 9-l transparent plastic flat-bottomed circular fish bowls surrounded by a dark blue background (Fig. 3.3). The blue background was adopted after discussions with several commercial seahorse culturists who observed increased predation by seahorses surrounded by this colour background. Ambient seawater filtered to 20 μm entered each tank down a central inflow line to the bottom of the tank where it then exited through a 360° spray nozzle (Plassay® microjet garden spray). This created a current out from the central inflow line and across the tank bottom, with a gradual weakening of the current up the tank sides and back down the central inflow line. Water flow through the tanks was approximately 0.25 l min^{-1}. Individual strands of separated black shadecloth (1 mm diameter) attached to the base of the seawater inflow line provided holdfasts for the seahorses.
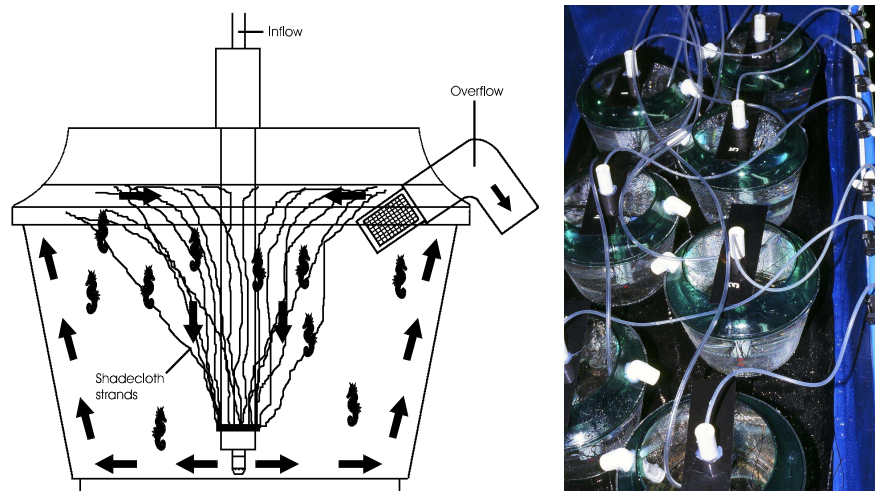
Figure 3.3 Nine-litre aquaria used to test the effect of stocking density in *Hippocampus abdominalis*. Water direction is indicated by filled arrows (source: Chris Woods).

A photoperiod of 12 h L:12 h D (as compared to the earlier photoperiods of 14 h L:10 h D, as reduced energy consumption within the NIWA facility was being sought) was provided by 2 x 58 W cool white fluorescent tubes on a timer above the tanks (6.8 μmol m^{-2} s^{-1} at water surface). Tanks were inspected daily and excess waste and faeces siphoned out. Tanks were completely drained and cleaned every two weeks. Juveniles were fed daily to excess with *Artemia* (mean length = 1.2 ± 0.04 mm) enriched with DC DHA Super Selco® (self-emulsified fish oil concentrate from INVE Aquaculture NV,

with enrichment at a rate of 0.6g DC DHA Super Selco$^®$ l^{-1} 24 h^{-1} 100 000 *Artemia*$^{-1}$) in three separate feeds (0900, 1200 and 1500-h), except for the weekend when only one feed was conducted daily.

Feeding to excess in this study was defined as feeding to such a level that there were always *Artemia* present in the tanks between 0900 and 1700-h (at least 10 *Artemia* per seahorse still remaining when the 1200 and 1500-h feeds were initiated), and that that level was sufficient to cause a marked reduction in attack response one hour after each feed (from >4 strikes min^{-1} to less than 1 strike min^{-1}). Unlike some fish, *H. abdominalis* may not cease attacking prey even when the digestive tract is full; seahorses may continue to attack, spitting out attacked prey and then attacking new prey with the same result (Woods, pers. obs.). Therefore, feeding to excess was used rather than feeding to satiation, and was proportional to stocking density. With regular daily siphoning of waste this feeding to excess did not appear to result in noticeable fouling of the tanks and any *Artemia* remaining were flushed from each tank each morning before new daily feeding was conducted.

If any mortalities occurred these were removed, recorded, and then replaced with identifiable juveniles of the same size from a single brood of juveniles whose tails had unusual kinks, in order that the original stocking densities were maintained. These replacement seahorses (*n* = 42) did not vary in any other attributes from the experimental seahorses apart from their kinked tails, which served to identify them as being different from the experimental animals, and were not included in final analyses.

On five separate days at random times the number of juveniles in each tank and treatment that were being grasped by a conspecific was recorded. These observations were used to determine whether tank conspecifics were interfering with each other.

The experiment was concluded after 60 days. Final juvenile length and wet weight were recorded. Mean Specific Growth Rate was calculated as SGR % (increase in body weight day^{-1}) = ((lnWf – lnWi)/t) x 100, where Wf = final wet weight, Wi = initial wet weight, and t = number of days. Condition Factor (CF) = (wet weight (g)/length (cm$^{3.5}$)) x 10^{-6}, was also calculated for individual juveniles.

Mean ± 1 SE water parameters during the experiment were as follows: temperature 14.9 ± 0.1°C (range 13.78–16.33°C), dissolved oxygen 7.9 ± 0.1 mg l^{-1} (range 7.8–8.4 mg l^{-1}), salinity 34.7 ± 0.03 ppt (range 33.76–34.9 ppt), and pH 8.1 ± 0.01 (range 8.06–8.24). These parameters did not vary among the tanks and there were no significant differences in water parameters between treatments of replicates (two-way ANOVA, $P>0.05$).

3.2.3 Statistical analyses

Data were analysed with STATISTICA 6.1 (Statsoft, Tulsa, Oklahoma, USA), SYSTAT 10.0 (SPSS Inc., Chicago, Illinois, USA), and NCSS 2004 (Hintze, J., Number Cruncher Statistical Systems, Kaysville, Utah, USA). Data were tested for normality (Shapiro-Wilk's W test) and homogeneity of variances (Levene's test), and transformed if necessary. Cochran's Q test (P at 0.05) was used to test for differences in phototaxis of juvenile seahorse. Non-parametric Kruskal-Wallis ANOVA with post-hoc Tukey HSD (P at 0.05) was used to test whether data met normality assumptions of parametric tests (e.g. percent survival and SGR). Differences in seahorse SL and wet weight were tested for using two-way ANOVA with post-hoc Tukey HSD (P at 0.05). For the CF data, an Analysis of Covariance (ANCOVA) was performed on log-transformed data with log-length as a covariate to treatment to test for any differences in data slope between stocking density treatments. This revealed no significant interaction effects (ANCOVA, $P>0.05$) and confirmed the assumption of homogeneity of slopes, so an ANOVA was performed for comparison of CF at a standardized log-transformed mean of 80.7 mm SL with post-hoc Scheffe's test (P at 0.05).

3.3 Results
3.3.1 Initial juvenile survival
3.3.1.1 Phototaxis in juveniles

Juvenile *H. abdominalis* were significantly positively phototactic from the day of first release to the end of the two month test period (Cochran's $Q_{1,48} = 36.75$, $P<0.001$) with 94% of juveniles present in the light portion of the test cylinders after the 5 min period. The alternate hypothesis (H$_1$) was accepted.

3.3.1.2 Effect of background colour on feeding efficiency

At one week of age, there was a significant difference in both the attack rate (Kruskal Wallis $H_{2,15} = 9.79$, $P<0.01$) (Tukey HSD, $P<0.05$), and capture success (Kruskal Wallis $H_{2,15} = 8.37$, $P<0.05$) (Tukey HSD, $P<0.05$) between juveniles contained in clear, white-wrapped, or black-wrapped jars (Fig. 3.4). Both attack rate and capture success were higher in clear jars than in white-wrapped or black-wrapped jars, and higher in black-wrapped jars than in white-wrapped jars.

At one month of age, there was no significant difference in attack rate among juveniles contained in clear, white-wrapped, or black-wrapped jars (Kruskal-Wallis ANOVA, $P>0.05$). However, there was a slight significant difference in capture success (Kruskal Wallis $H_{2,15} = 6.28$, $P<0.05$) (Tukey HSD, $P<0.05$), as capture success was higher in both clear and white-wrapped jars than in black-wrapped jars. The alternate hypothesis (H_1) was accepted.

A
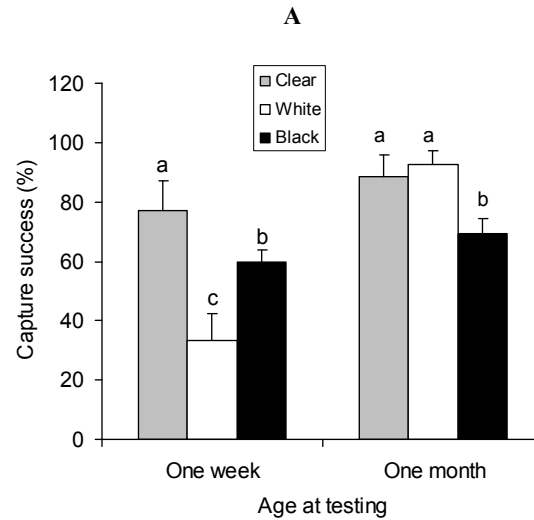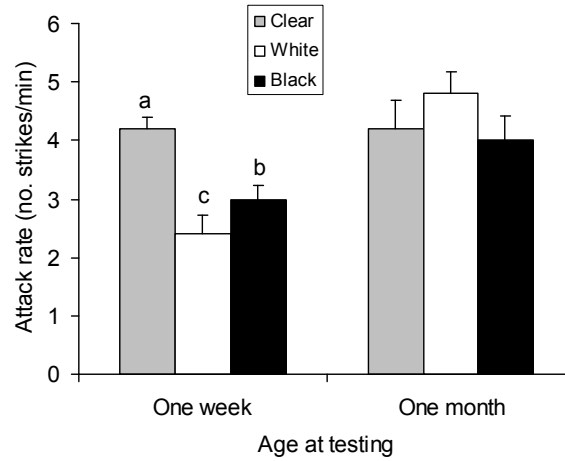
B

Figure 3.4 Attack rate (number of feeding strikes min^{-1}) (A) and capture success (percentage of feeding strikes that resulted in prey capture) (B) in juveniles seahorses (*Hippocampus abdominalis*) in jars with different coloured backgrounds at one week (top) and one month of age (bottom). Values are mean ± 1 SE. Within each age class, different letters denote significant differences between treatments (*P*<0.05). No letters denotes non-significance.

3.3.1.3 Effect of access to the water surface and illumination source

Two-way ANOVA revealed no significant size differences (either associated with treatments or their interaction terms) in juveniles in surviving juveniles after one month between the treatments, or in the occurrence of juveniles with hyperinflated swimbladders (ANOVA, $P>0.05$) (Table 3.1). After one month, mean overall juvenile SL was 30.9 ± 0.2 mm and mean occurrence of hyperinflated swimbladders was $5.8 \pm 1.9\%$.

In treatments with top-illumination ($F_{1,24} = 5.29$, $P<0.05$) and open access to the water surface ($F_{1,24} = 8.36$, $P<0.01$) percent survival (arcsine-square root transformed) was significantly lower (Table 3.1). Treatments with top-illumination had significantly lower survival compared with side-illumination (mean ± 1 SE $= 61.7 \pm 4.6\%$ vs. $73.3 \pm 3.8\%$) (Tukey HSD, $P<0.05$), and treatments with open access to the water surface also had significantly lower survival compared with no access to the water surface (mean ± 1 SE $= 60 \pm 4.3\%$ vs. $75 \pm 3.6\%$) (Tukey HSD, $P<0.05$). There was no significant interaction term between illumination source and access to the water surface (ANOVA, $P>0.05$).

In treatments with top-illumination ($F_{1,24} = 8.95$, $P<0.01$) and open access to the water surface ($F_{1,24} = 17.83$, $P<0.001$) the occurrence of ingested air bubbles between treatments was also higher. Treatments with top-illumination had significantly higher occurrence of air bubble ingestion compared with side-illumination (mean ± 1 SE $= 11.7 \pm 4.6\%$ vs. $1.7 \pm 1.7\%$) (Tukey HSD, $P<0.05$), and treatments with open access to the water surface also had significantly higher occurrence of air bubble ingestion compared with no access to the water surface (mean ± 1 SE $= 13.3 \pm 4.5\%$ vs. $0 \pm 0\%$) (Tukey HSD, $P<0.05$). There was a significant interaction term between illumination source and access to the water surface ($F_{1,24} = 8.95$, $P<0.01$). This interaction is caused because although juveniles with open access to the water surface had higher occurrence of air bubble ingestion overall, juveniles in top-illuminated treatments had much greater occurrence of air bubble ingestion than juveniles in treatments with side-illumination.

The alternate hypothesis (H_1) was cautiously accepted on the basis of survival of juveniles being lower, and occurrence of ingested air bubbles higher with top illumination and access to the water surface.

Table 3.1 Standard length (SL mm), percent survival (%), percentage with hyperinflated swimbladders, and percentage with air bubbles in the gut, of juvenile *Hippocampus abdominalis*. Juveniles were with and without access to the water surface in top- or side-illuminated aquaria after 1 month. Values are mean ± 1 SE.

Illumination	Vessel	SL (mm)	% survival	% hyperinflated	% air bubbles
Top	Open	30.7 ± 0.6	50 ± 4.5	6.7 ± 2.7	23.3 ± 9.5
Top	Closed	31.6 ± 0.4	73.3 ± 6.2	6.7 ± 2.7	0
Side	Open	30.6 ± 0.5	70 ± 4.5	3.3 ± 1.4	3.3 ± 1.4
Side	Closed	30.6 ± 0.4	76.7 ± 6.2	6.7 ± 2.7	0

3.3.1.4. Survival in side-illuminated 60-l glass aquaria

In side-illuminated glass aquaria, juvenile survival to two months of age was high, with mean survival per brood of 80.4 ± 4.0% (Fig. 3.5). Mean final SL and wet weight of juveniles were 43.0 ± 1.1 mm and 0.21 ± 0.02 g respectively. Mean frequency of occurrence per brood of hyperinflated swimbladders and ingested air bubbles was 8.0 ± 2.2% and 0.5 ± 0.3% respectively.
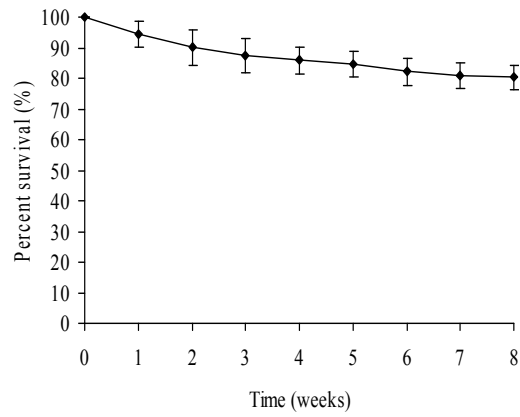
A
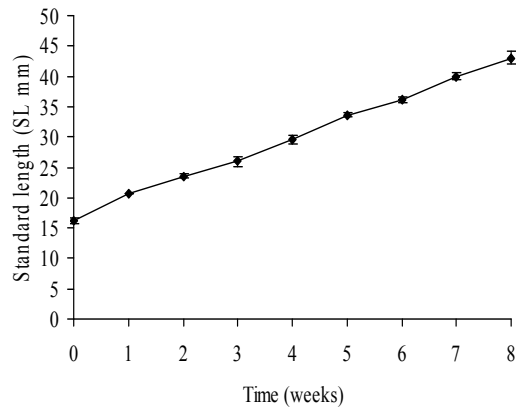
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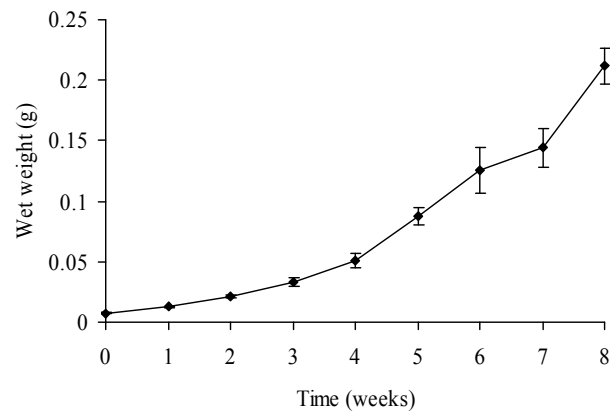
C

Figure 3.5 Percent survival (%) (A), standard length (SL mm) (B), and wet weight (g) (C) over a two-month period in 60-l side-illuminated glass aquaria in four broods of juvenile *Hippocampus abdominalis*. Values are mean ± 1 SE.

3.3.2. Stocking density

There was no significant difference in survival after 60 days between the stocking densities of 1 and 2 juveniles l^{-1}, with mean survival being 100% and 95.8 ± 2.7% respectively. However, survival in the 5 juveniles l^{-1} treatment (78.3 ± 3.7%) was significantly lower (Kruskal-Wallis ANOVA, $H_{2,12}$ = 9.12, $P<0.05$) (Tukey HSD, $P<0.05$).

After two months there was a significant difference in mean SL between treatments (ANOVA, $F_{2,245}$ = 18.45, $P<0.001$) (Table 3.2), with juveniles in the 1 juvenile l^{-1} treatment longer than those juveniles in the 2 and 5 juveniles l^{-1} treatments (Tukey HSD, $P<0.05$). There was also a significant difference in mean weights between treatments (ANOVA, $F_{2,245}$ = 35.86, $P<0.001$), with juveniles in the 1 juvenile l^{-1} treatment heavier than those in the 2 and juveniles 5 l^{-1} treatments, while juveniles in the 2 juveniles l^{-1} treatment were heavier than those in the 5 juveniles l^{-1} treatment (Tukey HSD, $P<0.05$). In terms of Condition Factor (CF), there was a significant difference between some of the treatments (ANOVA, $F_{2,245}$ = 5.8, $P<0.01$), with juveniles exhibiting higher CF with decreasing stocking density. At the standardized log-transformed mean of 78.15 mm SL, CF's were 0.185, 0.192, and 0.171 for the 1, 2, and 5 juveniles l^{-1} treatments respectively. However, because of the small sample size and associated errors with the 1 juvenile l^{-1} treatments, post-hoc tests only revealed a significant difference between the 2 juveniles l^{-1} treatments and 5 juveniles l^{-1} treatment (Scheffe's MCP, $P<0.01$). Comparison of the mean SGR also revealed a significant difference between the treatments (Kruskal-Wallis, $H_{2,12}$ = 9.85, $P<0.01$) with juveniles in the 1 juvenile l^{-1} treatment having a greater mean SGR than those in the 2 and 5 juveniles l^{-1} treatments; juveniles in the 2 juveniles l^{-1} treatment had a greater mean SGR than the 5 juveniles l^{-1} treatment (Tukey HSD, $P<0.05$). The alternate hypothesis (H_1) was accepted.

Table 3.2 Standard length (mm), wet weight (g), Condition Factor (CF), and Mean Specific Growth Rate (% day^{-1}) of juvenile *Hippocampus abdominalis* after two months at stocking densities of 1, 2 and 5 juveniles l^{-1}.Values are mean ± 1 SE. Column values with different superscripts are significantly different ($P<0.05$).

Density	Final length (mm)	Final weight (g)	Final CF	SGR (%)
1 juvenile L^{-1}	86.4 ± 1[a]	1.37 ± 0.19[a]	0.219 ± 0.008[a,b]	1.7 ± 0.12[a]
2 juveniles L^{-1}	81.8 ± 0.8[b]	1.14 ± 0.05[b]	0.224 ± 0.007[a]	1.34 ± 0.06[b]
5 juveniles L^{-1}	79.2 ± 0.5[b]	0.88 ± 0.03[c]	0.192 ± 0.003[b]	0.87 ± 0.02[c]

Observations on the number of juveniles in each tank and treatment that were being grasped by a conspecific revealed that the mean percentage of seahorses involved in grasping/wrestling with a conspecific was as follows: 1 juvenile l^{-1} (2.2 ± 1%), 2 juveniles l^{-1} (4.2 ± 0.7%), and 5 juveniles l^{-1} (10.9 ± 0.6%).

3.4 Discussion

3.4.1 Initial juvenile survival

The culture environment provided for finfish is of crucial importance to producing healthy fish with high growth rates and survival, particularly in the early stages of the fish's life when they are usually most vulnerable. Variables within the culture environment, such as tank colour and illumination source can have a marked influence on culture success, although these may vary according to fish species and life stage and may be confounded by a range of other culture variables (Chesney, 1989; Downing & Litvak, 1999; Papoutsoglou, 2001; Papoutsoglou *et al.*, 2005).

When the effect of tank background colour on feeding was tested in juvenile *H. abdominalis* at one week of age, both the attack rate on and capture success of *Artemia* nauplii were higher for juveniles in clear jars than those in white- or black-wrapped jars, and capture success was also higher in black-wrapped jars than white-wrapped. At one month of age capture success had increased in all jars but remained higher in clear and white-wrapped jars. This suggests that small *Artemia* nauplii are more visible for one week-old *H. abdominalis* in clear containers, but at one month the increased size of the *Artemia* may allow them to be increasingly visible against non-clear backgrounds. Increased feeding success as a result of increasing visual acuity or prior feeding experience (Clarke & Sutterlin, 1985; Ibrahim & Huntingford, 1992) could also be a factor in the improvement of feeding juvenile seahorses in non-clear tanks. These latter possibilities could be tested through examination of eye development in juveniles and prior exposure/no exposure feeding trials.

Martinez-Cardenas & Purser (2007) also tested the effect of tank background colour on feeding activity, growth and survival in juvenile *H. abdominalis*. They tested a variety of tank colours (clear, white, yellow, orange, red, blue, green, and black) with three, seven and 42 day-old juveniles with a similar tank set up to this experiment, but found no differences in feeding activity, growth or survival. The reasons for the different

results in the two studies are not clear although the different enrichments used in each study resulted in the *Artemia* having different coloured guts, which may have had some effect on feeding. For example, in this study the *Artemia* were cultured on a microalgal diet of *Chroomonas salina* and *Isochrysis* sp which resulted in them having dark-coloured guts (better contrast against a clear background), whilst Martinez-Cardenas & Purser (2007) enriched their *Artemia* with Super Selco® which resulted in them having pale-coloured guts.

Naas *et al.* (1996) considered black tanks superior to white tanks for rearing larval fish because they provided what could be considered to be close to a natural illumination. Black tanks also reduced "wall-trapping" (where fish larvae only stay in close proximity to the tank wall) due to visual confusion or attraction responses of the larvae to lighter colour tanks. In the preliminary study (Chapter 2), juvenile *H. abdominalis* exhibited a degree of attraction to the walls of the round white 75-l tanks they were originally maintained in, collecting at the tank wall, particularly when first born. Juvenile *H. abdominalis* are still positively phototactic at one month of age, but may be less confused by light-coloured tanks at this age. This is definitely the case for older (>three months) *H. abdominalis* which appear largely unaffected by white tanks; they do not orient to the walls near the surface of white tanks as newborn juveniles do (Woods, pers. obs.), instead they disperse throughout the tank and can accurately predate on moving prey.

Light intensity can modify the effect that tank colour has on feeding behaviour (Martin-Robichaud & Peterson, 1998), as well as influence overall feeding efficiency and consequently growth and survival (Batty, 1987; Battaglene *et al.*, 1994; Huse, 1994; Grecay & Targett, 1996; Downing & Litvak, 1999, 2001; Tamazouzt *et al.*, 2000; Watanabe & Feeley, 2003; Peña *et al.*, 2004; Imsland & Jonassen, 2005). Lighting intensity-effects may vary among species and types of fish depending on their visual acuity and the environment they typically inhabit; there appears to be a minimum light intensity or "visual threshold" for each species below which fish can no longer see and capture prey (Blaxter, 1969; Gerking, 1994; Watanabe & Feeley, 2003). For example, Huse (1994) related differences in optimal light intensity for feeding between larvae of cod (1 lux – 0.01 µmol m^{-2} s^{-1}), plaice (87 lux – 1.2 µmol m^{-2} s^{-1}), and turbot (860 lux – 11.6 µmol m^{-2} s^{-1}) with their vertical distribution in the water column. Watanabe & Feeley (2003) found growth of larval summer flounder (*Paralichthys dentatuts*) was

better at 50 lux (0.7 µmol m^{-2} s^{-1}) compared to 100–2000 lux (1.4–27.0 µmol m^{-2} s^{-1}), and related this to an ecological adaptation.

Seahorses typically inhabit relatively shallow habitats (Foster & Vincent, 2004) where light intensities may be relatively high, but appear able to feed effectively at a variety of light intensities. The illumination levels used in this investigation (4.3–5.6 µmol m^{-2} s^{-1}) are within the diurnal range of light levels encountered in *H. abdominalis'* complex natural macroalgal habitat in Wellington Harbour, New Zealand (Woods, unpubl. data from *in situ* measurements). This species has been noted as particularly active at dusk and dawn in the wild when light levels are low (Paulin & Roberts, 1992). Ouyang (2005) found that in cultured juvenile *H. abdominalis* swimming activity was highest in the first 6 hours of the photophase, although there could be a confounding effect with photophase duration. Ouyang (2005) examined locomotor activity and growth of juveniles at light levels of 1–45 µmol m^{-2} s^{-}1 and found no differences in either at these levels after 4 weeks of culture. Sheng *et al.* (2006) examined feeding in *H. trimaculatus* at different light intensities and found that feeding was highest at intensities of 1834 lux (24.8 µmol m^{-2} s^{-1}), 1014 lux (13.7 µmol m^{-2} s^{-1}) and 510 lux (6.9 µmol m^{-2} s^{-1}) in one, five and 10 day-old juveniles respectively. James and Heck (1994) demonstrated that the lined seahorse *H. erectus* is an effective crepuscular as well as diurnal predator, with no significant difference in prey capture between light intensities of 1.67 µmol m^{-2} s^{-1} and 76.28 µmol m^{-2} s^{-1}, but at a low light level of 0 µmol m^{-2} s^{-1}, prey capture was significantly reduced. Wong & Benzie (2003) found no differences in growth or survival in White's seahorse, *H. whitei*, kept at light intensities of 24 or 136 µmol m^{-2} s^{-}1.

In addition to utilising tank backgrounds and lighting intensities which enhance prey detection and capture, the manipulation of prey characteristics such as colour can also influence feeding success (Dendrinos *et al.*, 1984). Dendrinos *et al.* (1984) found that by using *Artemia sp.* stained with non-toxic food colouring (brilliant blue, pink, lemon yellow, red "c", and black) in glass tanks, that the feeding efficiency of Dover sole (*Solea solea*) was improved with all colours (black > red "C" > pink > blue > yellow > unstained). In this investigation, the use of *C. salina* and *Isochrysis* sp. microalgae to enrich *Artemia sp.* resulted in them possessing dark-coloured guts, which probably enhanced their contrast against clear backgrounds.

As a result of the potentially complex interplay between variables such as tank colour, stocking density, prey characteristics, fish species, illumination type, position and intensity, different experimental results and culture recommendations are often produced amongst different finfish species, and these can sometimes be contradictory (Papoutsoglou *et al.*, 2005). For example, Duray *et al.* (1996) found that grouper larvae (*Epinephulus suillus*) ingested more rotifers in tan tanks compared to black tanks, achieving a greater length in tan tanks. Tamazouzt *et al.* (2000) found greatest growth and survival of perch larvae (*Perce fluviatilis*) in light grey and white tanks and lowest growth in black tanks, whereas with another perciform fish *P. flavescens* Hinshaw (1985) found black tanks resulted in better growth and survival when compared with white tanks. Papoutsoglou *et al.* (2005) found growth in rainbow trout juveniles (*Oncorhynchus mykiss*) was reduced when juveniles were reared in black tanks compared to light blue and white tanks. Consequently, there is unlikely to be a "one rule" recommendation applicable for all finfish species in terms of what colour tank background and illumination method are best for enhancing fish growth and survival. These must be determined for each species in relation to its culture environment.

Illumination is an important factor affecting the distribution of pelagic fish larvae and their prey (Huse, 1994). Because *Artemia* are positively phototactic, top-illumination will result in *Artemia* congregating near the water surface in tanks where this is the strongest source of illumination. When preying on these, juvenile seahorses that have open access to the water surface risk air bubble ingestion. If ingested air bubbles are not expelled from the gut they can cause buoyancy problems and restrict feeding. This investigation demonstrated the effect that this can have on juvenile survival, as juvenile *H. abdominalis* prevented from accessing the water surface with top-illumination enjoyed higher survival than those with access to the water surface. Early juvenile *H. abdominalis* appear less able than older seahorses to get rid of ingested air bubbles from the buccal region prior to passage into the gut (Woods, pers. obs.). However, the results from the experiment examining the effects of lighting source and access to the water surface (section 3.2.1.4) should be treated with some caution. This experiment was carried out with treatments and their replicates within a central culture tank. Thus, the independence of the treatments and replicates is compromised to a degree. In addition, the micro-environments (e.g. temperature, dissolved oxygen) and prey distributions within replicate jars were not monitored. Therefore, potential differences within treatments and replicates that may have existed in relation to these cannot be quantified.

Access to the water surface immediately after release from the male's pouch is probably important for juvenile seahorses as they appear to initially inflate their swim bladder through the ingestion of air at the water surface. Non-inflation of the swimbladder in fish may result in dysfunctional buoyancy and atrophy of the swimbladder (Paperna, 1978; Chapman *et al.*, 1988; Battaglene & Talbot, 1990; Martin-Robichaud & Peterson, 1998). Lawrence (1998) attributed the main cause of mortality in juvenile seahorses (*H. angustus*) to poor swimbladder inflation as a result of oily surface films in tanks which prevent juveniles from initially inflating their swim bladders, a factor that has also been linked to poor swimbladder inflation and high mortality in other fish (Friedmann & Shutty, 1999).

Hyperinflation of the swimbladder has been attributed to gas supersaturation (Cornacchia & Colt, 1984), stress caused by such factors as inadequate water depth (Kolbeinshavin & Wallace, 1985), and excessive air ingestion (Nash *et al.*, 1977), and may hinder normal swimming, or in extreme cases result in floating fish and increased mortality (Weitkamp & Katz, 1980). Although only a small percentage of juvenile *H. abdominalis* were affected by hyperinflated swimbladders, the occurrence of hyperinflation in juveniles which were prevented from accessing the water surface in the top- vs. side -illuminated glass aquarium experiment indicates that hyperinflation is not the result of excessive air ingestion. In Tasmania, the use of dip nets to extract newborn juveniles from tanks and exposing them to air for tank transfer, as well as top-illumination in tanks result in higher incidence of hyperinflation (J. Purser, University of Tasmania, pers. comm.).

Because prey detection and capture were significantly higher for early juvenile *H. abdominalis* in clear vessels, and side-illumination decreased air bubble ingestion, a simple side-illuminated glass aquarium arrangement was tested to see if this could produce high juvenile survival in entire broods through the first two months of life when juveniles appear most vulnerable. This aquarium arrangement produced high juvenile survival as well as reasonable growth in four broods. This was a marked improvement on the typical survival rate in the preliminary investigation (Chapter 2) with the same feeding regime, similar water conditions, but using top-illuminated white 75-l tanks. Side illumination of tanks has also produced better survival rates and less swimbladder hyperinflation in newborn *H. abdominalis* in Tasmania (J. Purser, University of Tasmania, pers. comm.). Juvenile rearing tanks similar to the side-illuminated glass

aquaria used for this investigation are recommended by one commercial seahorse aquaculturist in Australia for rearing newborn *H. abdominalis* based on the results from this investigation in combination with their own experience at improving initial survival rates (Warland, 2002). Juvenile rearing tanks blacked-off to the waterline were also used by The Seahorse Farm in Napier for its large-scale rearing of juvenile *H. abdominalis* based on the research in this chapter and have confirmed the effectiveness in reducing early juvenile mortalities.

After initial rearing in side-illuminated glass aquaria for two months, juveniles were transferred into top-illuminated white 75-l tanks. Mortalities in these tanks from this age onwards were negligible (e.g. average survival of 78% per brood at 6 months) and juveniles were observed to be able to prey effectively on *Artemia* and gammarid amphipods, indicating that the critical period of influence of tank colour/prey distribution and associated problems with predation near the water surface had been passed. This is of importance to commercial scale culture because as the juvenile seahorses grow in size and require larger tanks, large clear tanks made of acrylic or glass can be more expensive and harder to source than non-clear tanks made from materials such as polyethylene plastic, and concrete, and where illumination is typically ceiling-mounted.

These experiments have demonstrated that initial juvenile survival in cultured *H. abdominalis* can be increased through the use of simple tank characteristics that enhance prey capture, as well as reducing air bubble ingestion during feeding. Relatively high early juvenile survival can be produced using clear, side-illuminated aquaria during the first two months of life, which is the most vulnerable period for cultured juvenile *H. abdominalis*. However, there will still be a range of other abiotic and biotic factors that will influence initial juvenile seahorse survival and growth that need to be investigated in order to determine optimal rearing techniques for *H. abdominalis*. For example, Hilomen-Garcia *et al.* (2003) demonstrated that in the resilient seahorse *H. kuda* (which can be found in estuarine habitats), nine week-old juveniles transferred to tanks of variable salinity had highest survival at salinities of 15–20 ppt, whereas Ignatius *et al.* (2000) and Payne & Rippingale (2000) noted differential growth and survival rates between young seahorse juveniles fed *Artemia*, copepods and rotifers. In juvenile *H. abdominalis* from birth to 14 days old, Tung (2005) found that an increased photoperiod

of 14 h L:10 h D (as used in this investigation) compared with 7 h L:17 h D resulted in greater juvenile growth and survival, and attributed this to increased visibility of food.

3.4.2 Stocking Density

In commercial finfish aquaculture, potentially lower production costs per fish may be obtained at higher stocking densities, provided growth, survival, and fish health are not compromised at higher densities (Shang, 1981; Wallace *et al.*, 1988; Wallat *et al.*, 2004). Intensive stocking is particularly pertinent to aquaculture operations where resources such as land area or water are restricted (Suresh & Lin, 1992). Optimal stocking density varies amongst species (Wallace *et al.*, 1988; Bjørnsson, 1994; Huang & Chiu, 1997; Papoutsoglou *et al.*, 1998), and exogenous factors such as water temperature, water quality, light, and feed quality/quantity can affect the result that different stocking densities have within a given fish species (Berg *et al.*, 1996; Hossain *et al.*, 1998; Baskerville-Bridges & Kling, 2000; Boujard *et al.*, 2002; Sharma & Chakrabarti, 2003; Foss *et al.*, 2006).

Optimal stocking densities for seahorses that are relevant to commercial aquaculture remain largely undetermined. However, with the burgeoning interest in seahorse aquaculture, information on stocking densities utilised for different seahorse species is gradually becoming available. For example, Wong & Benzie (2003) tested stocking densities of 0.5 and 1 juvenile l^{-1} in the seahorse *H. whitei*, from 38–61 mm in length, and found no difference between these densities in terms of seahorse growth or survival. For *H. erectus*, Correa *et al.* (1989) used stocking densities of 6 juveniles l^{-1} for juveniles up to 35 mm in length in floating cages (97% survival), reducing to 0.1 juvenile l^{-1} for these seahorses until they reached 150 mm in length in conico-cylindrical concrete tanks (70% survival). In an introduction to seahorse (*H. coronatus*, *H. histrix*, *H. kelloggi*, *H. kuda*, *H. japonicus*, and *H. trimaculatus*) culture in China, Chen (1990) recommended a stocking rate of 3 juveniles l^{-1} for newly born juveniles, reducing to 0.2-0.3 juveniles l^{-1} once the seahorses attain 6 cm in size.

In this investigation, stocking densities of 1, 2 and 5 juveniles l^{-1} were tested for juvenile *H. abdominalis* approximately 70 mm in length. It was found that with increasing stocking density, growth (in terms of length, weight, CF and mean SGR) was significantly reduced. Survival was also found to be significantly lower in the 5

juveniles l^{-1} treatment. The lower growth and higher mortality in the higher stocking density treatments used in this investigation with flow-through seawater are probably not the result of food competition. Nor are they likely to be the result of poor water quality. Both of these have been linked to stocking density growth effects in other fish species (Boujard *et al.*, 2002). In this study, feeding was to excess (allowing for differences in stocking density) and water parameters were within normal ranges and were not different between treatments. Rather, the physical impedance of feeding and expenditure of excess energy as the result of juveniles grasping and wrestling with each other may have been the principal factor that impacted upon both juvenile growth and survival.

Wrestling with conspecifics was particularly pronounced during feeding when juveniles would launch themselves from their resting holdfast material into the water column to feed; they would often then grasp hold of another seahorse if contact was made against that animal, while attempting to feed. Though not quantified, in the 5 juveniles l^{-1} stocking density, grasping and wrestling were commonly observed during feeding, with reduced incidence of occurrence in the lower density treatments. Such grasping and wrestling amongst seahorses has most commonly been observed in young juvenile seahorses immediately after birth when they are in nearest proximity to each other (Whitley & Allan, 1958; Bellomy, 1969; Giwojna, 1990), and prevention (e.g. supply of holdfasts) is recommended to reduce the chances of resulting mortalities.

The well developed prehensile tail of *H. abdominalis* with its lack of oxidative musculature and substantial population of tonic fibres (Thomson, 1993), allows it to grip with considerable strength to substratum, and conspecifics, for long periods without apparent fatigue. In this study, when juveniles were grasped by another, they were usually grasped around the snout or neck, and sometimes round the body or tail. Seahorses that were grasped were observed to often shake themselves quite vigorously to free themselves. Not only does this expend energy and prevent them from feeding effectively, but if grasped around the snout then ventilation can be stopped or at least temporarily impeded. The vigorous shaking by a grasped juvenile usually resulted in the grasping conspecific letting go within 1–5 seconds. However, if the grasping juvenile was intent on feeding whilst grasping, or if a seemingly weakened juvenile was being grasped, then the period of grasping could extend for 15–30 seconds.

Although speculative, it may be possible to commercially use stocking densities >1 l^{-1} in juvenile *H. abdominalis* 70 mm+ in length without reducing overall growth and survival, as was encountered in this investigation, by increasing the density of anchorage substratum for the seahorses within stocking tanks so that contact between juveniles is reduced. However, an optimal balance between stocking density, cost of increasing holdfast material density, and impact of increased holdfasts on seahorse mobility and feeding efficiency must be found.

Optimal stocking densities for *H. abdominalis* will need to be determined for seahorses of different size, as increasing seahorse size will commensurately require a reduction in stocking density. In addition, optimal balances within the commercial aquaculture environment will need to be determined between the influence of stocking density on seahorse growth and survival in relation to the cost/benefit of the stocking density used. This cost/benefit will vary according to whether the primary purpose of production is for the lower volume but higher return live aquarium trade, or the higher volume but lower return medicinal trade, as well as the nature and efficiency of each different commercial operation.

CHAPTER 4

JUVENILE FEEDING ON LIVE, ARTIFICIAL AND FROZEN FOODS

4.1 Introduction

One of the main bottlenecks in trying to establish biologically and economically viable commercial seahorse aquaculture is that of providing sufficient quantities of nutritionally-complete food. In their natural environment seahorses are visual predators that target live prey such as amphipods, copepods, mysid shrimp, and caridean shrimp (Reid, 1954; Lovett, 1969; Tipton and Bell, 1988; Texeira & Musick, 2001). In the captive environment aquarists, researchers, and commercial culturists have traditionally relied heavily on cultured live foods such as brine shrimp (*Artemia*), copepods, mysid shrimp and amphipods to feed to seahorses, in addition to collecting live wild foods (Correa *et al.*, 1989; Forteath, 1995; Lockyear *et al.*, 1997; Wilson & Vincent, 1998; Hilomen-Garcia, 1999; Payne & Rippingale, 2000; Indiviglio, 2002; Naik *et al.*, 2002; Abbott, 2003).

Although potentially beneficial in terms of increasing the nutritional variety on offer, the harvesting of wild live foods for seahorses is dependent upon an unpredictable resource and may impact upon natural ecosystems, particularly if carried out on a large scale in defined and restricted habitats sensitive to exogenous impacts. In addition, there is the potential to introduce pathogens or other undesirable organisms (e.g. parasitic copepods and hydroids) into the culture environment from wild-caught food (Gardner, 2003).

Culturing large quantities of live food for fish in a commercial environment and ensuring a constant supply can prove difficult and costly (Hamlin & Kling, 2001; Blair *et al.*, 2003; Callan *et al.*, 2003; Khemis *et al.*, 2003; Payne, 2003), and may limit variation in nutrient intake by fish due to inherent characteristics of some live prey (e.g. Lazo *et al.*, 2000). Consequently, any practice that can decrease dependence on live feeds could significantly reduce culture/financial bottlenecks in the rearing of finfish (Hamlin & Kling, 2001). Therefore, considerable effort has been concentrated on developing and testing formulated or artificial feeds for fish (e.g. Applebaum, 1985; Daniels & Hodson, 1999; Buchet *et al.*, 2000; Yufera *et al.*, 2000; Silva, 2001; Blair *et*

al., 2003; Khemis *et al.*, 2003; Ljunggren *et al.*, 2003; Hamre *et al.*, 2005; Wang *et al.*, 2005; Curnow *et al.*, 2006). However, the weaning of fish from live diets to alternative diets such as formulated feeds or frozen (inert) feeds can be a significant culture bottleneck itself, and is dependent upon many factors such as the ontogeny of the fish's digestive system, properties of the feed, fish behaviour, and culture environment (Hart & Purser, 1996; Cañavate & Fernández-Díaz, 1999; Kubitza & Lovshin, 1999; Kestemont & Mélard, 2000; Ljunggren *et al.*, 2003; Herbert & Graham, 2004; Wang *et al.*, 2005; Vega-Orellana *et al.*, 2006). Nutritionally appropriate formulations with appropriate chemosensory attributes must be determined and developed relative to fish ontogeny, species and culture environment in order to maximize fish growth and survival (Yacoob *et al.*, 2001; Hamre *et al.*, 2005).

Seahorses have not generally been reared on artificial foods in commercial aquaculture due to difficulties in getting them to accept non-live foods. There are anecdotal reports of aquarists successfully feeding seahorses on artificial foods, such as fish flake and goldfish granules. There are also general reports of commercial seahorse culturists using artificial foods to some degree, such as shrimp- and fishmeal-based diets (Chen, 1990; Forteath, 2000). However, due to the sensitive nature of commercial operations in relation to divulging successful culture techniques that might benefit potential competitors, enquiries into exactly what artificial foods are used and how they are presented to the seahorses are often not fruitful. There is as yet no commercially available artificial food specifically designed for seahorses.

Frozen foods (inert foods), such as frozen mysids and copepods, can be utilized quite successfully to supplement, or even totally replace feeding live foods to seahorses (Garrick-Maidment, 1997; Forteath, 2000; Indiviglio, 2002; Payne, 2003; Wilson *et al.* 2006). For example, Payne (2003) compared the growth and survival of newborn *H. barbouri* on diets of enriched frozen calanoid copepods (*Gladioferens imparipes*) and enriched live *Artemia* and found no difference in survival rates of juveniles between these diets, although growth was slower on the copepod diet. The main advantages of using frozen foods such as these are that they can be batch-cultured and then frozen in large quantities at times that are convenient to hatchery staff, and may reduce overall production costs (Payne, 2003), in addition to providing the aquaculturists with certainty of food supply. However, the weaning of seahorses onto frozen foods may take time, and can depend on the appearance of the frozen food after the

freezing/thawing process. For example, if frozen mysid shrimp are badly fractured and mangled, the seahorses may refuse to eat them (Mai, 2004a).

As shown in the previous chapters (Chapter 2 & 3) the seahorse *H. abdominalis* can be successfully cultured through to sexual maturity and up to 7 years of age, and with relatively high juvenile survival rates, using a primarily live *Artemia* diet. However, the material/labour costs in establishing and maintaining a healthy *Artemia* culture system could impinge upon the economic feasibility of a commercial venture rearing *H. abdominalis* in New Zealand. In addition, enriched *Artemia* may not actually be the best diet for *H. abdominalis* due to possible nutritional inadequacies that *Artemia* may possess in relation to specific dietary requirements this species may have, as has been discussed for other finfish (Watanabe *et al.*, 1978; Watanabe *et al.*, 1982; Barclay & Zeller, 1996; Furuita *et al.*, 1999). At this stage it is not known what the optimal nutritional profiles for *H. abdominalis* and other seahorses are, although feeding a variety of prey items has been reported as beneficial. For example, *H. kuda* adults fed a combination of highly unsaturated fatty acid-enriched (HUFA) *Artemia,* mysids and *Tilapia* fry bred more often and had bigger brood sizes than those only fed a single diet of the aforementioned prey (Hilomen-Garcia, 1999).

Mixed feeding (or co-feeding) of live and non-live diets is a commonly used strategy to help wean larval fish onto non-live or manufactured foods, and can enhance larval fish growth and survival beyond that achieved by feeding either type of food alone and decrease the duration of weaning (Drouin *et al.*, 1986; Ehrlich *et al.*, 1989; Person Le Ruyet *et al.*, 1993; Abi-Ayad & Kestemont, 1994; Kolkovski *et al.*, 1997a; Kolkovski *et al.*, 1997b; Rosenlund *et al.*, 1997; Cañavate & Fernández-Díaz, 1999; Daniels & Hodson, 1999; Jenkins & Smith, 1999; Lazo *et al.*, 2000; Hamlin & Kling, 2001; Kaiser *et al.*, 2003; Khemis *et al.*, 2003; Ljunggren *et al.*, 2003; Herbert & Graham, 2004; Curnow *et al.*, 2006; Vega-Orellana *et al.*, 2006). For example, Kolkovski *et al.* (1997b) found that the presence of *Artemia* increased dry microdiet assimilation by 30–50% in seabass (*Dicentrarchus labrax*) larvae. Mixed feeding is considered to improve weaning success by: 1) increasing ingestion of non-live diets by stimulating overall feeding response through visual or olfactory stimuli, 2) using the live organisms to supplement and promote appropriate enzymatic activity in the fish to aid in digestion and absorption of formulated diets, and 3) improving assimilation and absorption of formulated diet ingredients by contributing factors such as polar lipids and other fractions (Tandler &

Kolkovski, 1991; Walford *et al.*, 1991; Koven *et al.*, 1993; Cahu & Zambonino Infante, 1994; Kolkovski *et al.*, 1997b; Lazo *et al.*, 2000; Curnow *et al.*, 2006).

This chapter aims to determine whether *H. abdominalis* juveniles can be weaned onto frozen and artificial foods, either alone or mixed with live *Artemia*, in order to obtain an indication as to whether the use of non-live foods may be feasible in commercial aquaculture. The research contained in this chapter is based on data from Woods (2003c). Specifically, hypotheses to be tested were:

1) H_0: Juvenile seahorses cannot be weaned onto an artificial food; H_1: juvenile seahorses can be weaned onto an artificial food.

2) H_0: Juvenile seahorses cannot be weaned onto a frozen food; H_1: juvenile seahorses can be weaned onto a frozen food.

3) H_0: mixed feeding does not affect weaning; H_1: mixed feeding improves weaning success.

4) H_0: age (size) of juveniles does not affect weaning; H_1: age (size) of juveniles does affect weaning.

4.2 Materials and methods

In all experiments, carried out between September 2000 and February 2001, the same tank setup was used. Tanks used were the same 9-l transparent plastic flat-bottomed circular fish bowls as used in Chapter 3, surrounded by a dark blue background to enhance food detection. Water inflow to the tanks was approximately 0.25 l min^{-1}. A 12 h light:12 h dark photoperiod was provided by automatically controlled cool white fluorescent lights (2 x 58 W cool white fluorescent tubes) above the tanks.

A total of five experiments were conducted: 1) newborn juveniles fed artificial food, 2) one month-old juveniles fed artificial food, 3) two month-old juveniles fed artificial food, 4) one month-old juveniles fed frozen food, and, 5) two month-old juveniles fed frozen food. Experiments were conducted sequentially commensurate with the age of the juvenile seahorses, with one and two month-old juveniles tested on the two types of inert foods at the same time within each age class. During each experiment, observations were conducted on feeding in juveniles to determine whether the foods being offered were actually being consumed, and at what rate relative to the other diets. Once a week one individual from each replicate tank was selected at random and

observed for a period of one minute immediately after food was introduced into its tank (focal sampling). The number of feeding strikes that individual seahorse performed in the one minute period was then recorded.

Each experiment was run for 30 days, during which the tanks were inspected daily for mortality, and any excess food and faeces siphoned to waste. The tanks were cleaned thoroughly (detergent/chlorine) each week. After 30 days the surviving seahorses were counted, their standard lengths (SL) measured by placing juveniles on a steel rule covered by seawater in a shallow tray, and wet weights measured on a Mettler microgram balance following quick blotting on a paper towel. Length (Standard length = SL) and wet weight measurements of seahorses are presented as mean ± 1 SE.

Juveniles were sourced from F2 captive seahorses (12–16 cm SL). In all experiments there were no significant differences in either juvenile SL or wet weight between treatments or replicates at the start of each experiment (ANOVA, $P>0.05$). Juveniles were sourced from two broods for the newborn experiment and from three different broods for the remaining four experiments. Prior to experimentation, juveniles were mixed together and then randomly allocated to treatments and replicates within treatments. Juveniles were only used for one experiment, i.e. non-repetitive use of juveniles.

Depending on the age of juvenile seahorses tested, varying sizes of live *Artemia* enriched with Super Selco® (24 h at 26°C) were used as the control diet and for mixed feeding. *Artemia* were offered to seahorses at a rate of 1000 *Artemia* nauplii l^{-1} three times daily (0900, 1200, and 1500-h). Super Selco® (INVE Aquaculture NV) is a self-emulsified fish oil concentrate that has been widely used to enrich *Artemia* prior to feeding them to fish (see Chapter 5). The enrichment concentration used for Super Selco® was as recommended by the manufacturer: 0.6g l^{-1} 24 h^{-1} 100 000 *Artemia*$^{-1}$. *Artemia* nauplii density was not maintained in-between daily rations. Artificial and frozen food were offered at a feeding rate of 33.3% of body weight three times daily (0900, 1200, and 1500-h) giving a total daily ration of 100% of body weight. On weekends, however, all three feed rations were combined and given once in the morning (seahorses could cope with this single feed; juveniles would steadily keep predating upon the *Artemia* which could remain alive in the tanks for 24 h). All uneaten food was siphoned to waste each morning before each daily feed was administered.

4.2.1 Experiments on feeding artificial food

The artificial food tested was "Golden Pearls" from Brine Shrimp Direct, Utah, USA (http://ww.brineshrimpdirect.com; accessed 31/05/05) (proximate analysis: 60% protein, 8% lipids, 15% ash, 8% moisture, Vitamin C 2000 ppm, Vitamin E 400 ppm, astaxanthin 500 ppm) (Fig. 4.1). Two size grades of Golden Pearls were used: GP1 (200–300 μm) and GP2 (300–500 μm). This artificial food was chosen because of its similar colouration to newly hatched *Artemia* nauplii, similar size to nauplii and three-dimensional physical appearance.
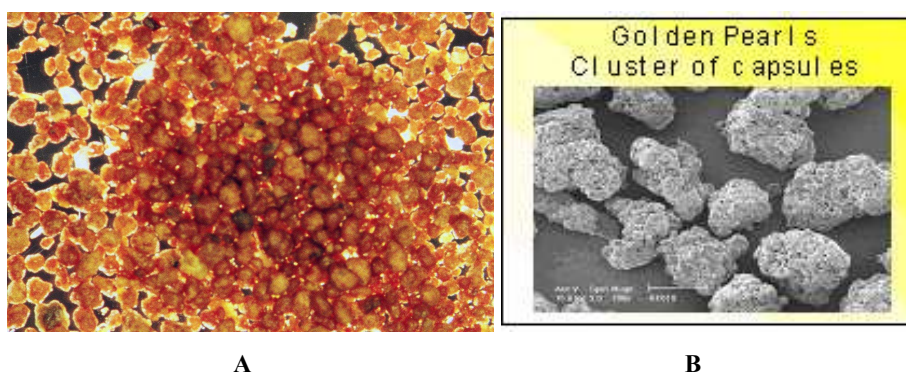
Figure 4.1 Golden Pearls GP2 from Brine Shrimp Direct showing colouration (A), and, (B) agglomerated nature of Golden Pearls in Scanning Electron Microscopy detail (source: (A) Chris Woods, (B) http://www.artemia-international.com; accessed 08/09/05).

Five mixed feeding treatments with three replicate tanks for each treatment were used as follows:

1) fed *Artemia* nauplii only (A)

2) fed Golden Pearls only (GP)

3) fed both *Artemia* nauplii and Golden Pearls initially with Golden Pearls only after 5 days (5A + GP)

4) fed both *Artemia* nauplii and Golden Pearls initially with Golden Pearls only after 10 days (10A + GP)

5) fed both *Artemia* nauplii and Golden Pearls initially with Golden Pearls only after 20 days (20A + GP).

4.2.1.1 Newborn juveniles fed Golden Pearls

Within 12 h of release from the parent male, 150 newborn juveniles (SL = 15.6 ± 0.02 mm, wet weight = 0.007 ± 0.001 g) from two broods were randomly and equally allocated to five mixed feeding treatments with ten seahorses per tank. GP1 were used.

The mean ± 1 SE length of *Artemia* used was 0.58 ± 0.02 mm. The top of the tanks were covered with black plastic to prevent air bubble ingestion by the juveniles when preying on *Artemia* nauplii, as this can reduce survival and growth in newborn *H. abdominalis* (see Chapter 3). Light intensity at the sides of the tanks was 4.8 µmol m^{-2} s^{-1}.

Water parameters (mean ± 1 SE) during the course of the experiments were as follows: temperature 13.0 ± 0.12°C (range 10.5–14.1°C), dissolved oxygen 8.2 ± 0.06 mg l^{-1} (7.8–8.5 mg l^{-1}), salinity 33.6 ± 0.05 ppt (31.26–34.7 ppt), and pH 8.18 ± 0.01 (8.06–8.22).

4.2.1.2 One month-old juveniles fed Golden Pearls

One month-old juveniles (SL = 33.7 ± 0.1mm, wet weight = 0.053 ± 0.001g) previously reared on *Artemia* nauplii were tested using GP1. One hundred and fifty juveniles were randomly and equally allocated to five mixed feeding treatments. The mean length of *Artemia* used was 0.91 ± 0.03 mm. The tops of the tanks in this experiment and all subsequent experiments were not covered by black plastic, as the risk of air-bubble ingestion is less at this age. Light intensity at the water surface was 5.1 µmol m^{-2} s^{-1}.

Water parameters (mean ± 1 SE) during the course of the experiments were as follows: temperature 15.2 ± 0.13°C (range 14–16.5°C), dissolved oxygen 7.9 ± 0.1 mg l^{-1} (7.6–8.3 mg l^{-1}), salinity 33.84 ± 0.04 ppt (32.9–34.4 ppt), and pH 8.16 ± 0.02 (8.09–8.24).

4.2.1.3 Two month-old juveniles fed Golden Pearls

Two month-old juveniles (SL = 55.8 ± 0.1mm, wet weight = 0.24 ± 0.001g) previously reared on *Artemia* nauplii were tested using GP2. Seventy-five juveniles were randomly and equally allocated to one of five mixed feeding treatments. The mean length of *Artemia* used was 1.32 ± 0.05 mm.

Water parameters (mean ± 1 SE) during the course of the experiments were as follows: temperature 17.6 ± 0.1°C (range 16.5–18.2°C), dissolved oxygen 7.82 ± 0.06 mg l^{-1} (7.5–8.2 mg l^{-1}), salinity 33.76 ± 0.04 ppt (33.1–34.87 ppt), and pH 8.17 ± 0.01 (8.11–8.22).

4.2.2 Experiments on feeding frozen food

The frozen food tested was Cyclop-eeze® from Argent Laboratories, Washington USA (http://www.argent-labs.com; accessed 31/05/05) (Fig. 4.2). Cyclop-eeze® are flash-frozen copepods collected from arctic salina in northern Canada (proximate analysis: protein 60%, carbohydrate 3%, ash 3%, lipid 35% - fatty acids 18:3n-3 10.45%, 20:5n-3 11.74%, 22:6n-3 11.09%, astaxanthin 2867 ppm, canthaxanthin 15 ppm). The mean length of copepods was 1.05 ± 0.03 mm. This food was not tested with newborn *H. abdominalis* as the copepods were too large to be ingested by them.

A   B

Figure 4.2 Cyclop-eeze® copepods from Argent Laboratories: (A) Frozen vacuum-packed 1 kg block (source: (A) http://www.argent-labs.com; accessed 05/08/05), and, (B) thawed Cyclop-eeze® copepods (source: Chris Woods).

Five mixed feeding treatments with three replicate tanks for each treatment were used as follows:
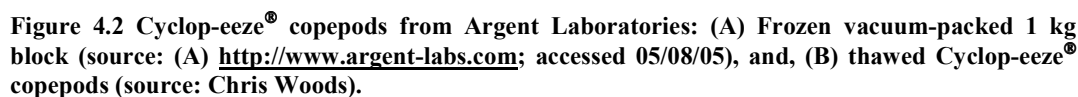
1) fed *Artemia* nauplii only (A)

2) fed Cyclop-eeze® only (C)

3) fed both *Artemia* nauplii and Cyclop-eeze® initially with Cyclop-eeze® only after 5 days (5A + C)

4) fed both *Artemia* nauplii and Cyclop-eeze® initially with Cyclop-eeze® only after 10 days (10A + C)

5) fed both *Artemia* nauplii and Cyclop-eeze® initially with Cyclop-eeze® only after 20 days (20A + C).

4.2.2.1 One month-old juveniles fed Cyclop-eeze®

One month-old juveniles (SL = 33.7 ± 0.1 mm, wet weight = 0.05 ± 0.001 g) previously reared on *Artemia* nauplii were tested using Cyclop-eeze®. One hundred and fifty

juveniles were randomly and equally allocated to five mixed feeding treatments. The mean length of *Artemia* used was 0.92 ± 0.02 mm.

Water parameters (mean ± 1 SE) during the course of the experiments were as follows: temperature 15.2 ± 0.13°C (range 14–16.5°C), dissolved oxygen 7.89 ± 0.09 mg l^{-1} (7.6–8.3 mg l^{-1}), salinity 33.84 ± 0.04 ppt (32.9–34.4 ppt), and pH 8.16 ± 0.02 (8.09–8.24).

4.2.2.2 Two month-old juveniles fed Cyclop-eeze®

Two month-old juveniles (SL = 55.4 ± 0.1 mm, wet weight = 0.239 ± 0.001 g) previously reared on *Artemia* nauplii were tested using Cyclop-eeze®. Seventy-five juveniles were randomly allocated to five mixed feeding treatments. The mean length of *Artemia* used was 1.31 ± 0.04 mm.

Water parameters (mean ± 1 SE) during the course of the experiments were as follows: temperature 17.6 ± 0.1°C (range 16.5–18.2°C), dissolved oxygen 7.81 ± 0.06 mg l^{-1} (7.5–8.2 mg l^{-1}), salinity 33.76 ± 0.04 ppt (33.1–34.87 ppt), and pH 8.17 ± 0.01 (8.11–8.22).

4.2.3 Statistical analyses

Data were analysed with NCSS 2004 (Hintze, J., Number Cruncher Statistical Systems, Kaysville, Utah, USA). Data were tested for normality (Shapiro-Wilk's W test) and homogeneity of variances (Levene's test). Data were transformed appropriately before analysis to ensure data normality and homogeneity of variances. For length and weight comparisons, nested ANOVA's were performed, with post-hoc Tukey HSD (*P* at 0.05) tests to detect where significant differences occurred. For comparison of survival, Kruskal-Wallis tests were performed, with Bonferroni all pairs MCP (*P* at 0.05) tests to detect where significant differences occurred. Students *t*-tests were used to compare observed feeding strike rates of juveniles on live *Artemia*, Golden Pearls or Cyclop-eeze® between each mixed feeding treatment and feeding strike rates on non-live food between the mixed feeding and non-live food only treatment.

4.3 Results

4.3.1 Experiments on feeding artificial food

4.3.1.1 Newborn juveniles fed Golden Pearls

After one month of rearing, juveniles fed *Artemia* only had significantly higher survival than those fed GP1 only and in the mixed feeding treatments (Kruskal-Wallis $H_{4,15}$ = 15.29, $P<0.01$) (Bonferroni all pairs MCP, $P<0.05$) with 50% of juveniles surviving (Fig. 4.3). GP1 only and 5A + GP1 had no surviving seahorses, while the 10A + GP1 and 20A + GP1 mixed feeding treatments had mean survival of 3.3 ± 3.3% and 6.7% ± 6.7%, respectively.

Because of the low survival rates, a statistical comparison of growth between the treatments was deemed to be invalid. SL and wet weight of juveniles after one month in the *Artemia*-only treatment were 32.2 ± 0.3 mm and 0.06 ± 0.001 g respectively. The length and weight of the one juvenile surviving in the 10A + GP1 treatment were 30 mm and 0.04 g respectively, and mean ± 1 SE SL and wet weight of the two surviving juveniles in the 20A + GP1 treatment were 31 ± 0.3 mm and 0.05 ± 0.001 g, respectively.
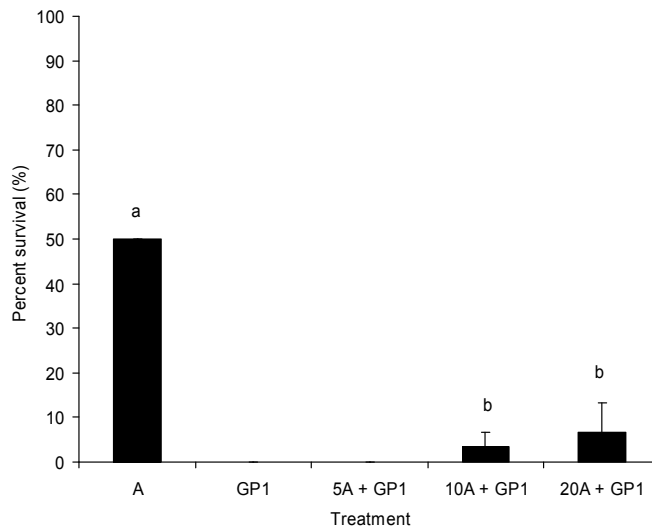
Figure 4.3 Survival (mean ± 1 SE) of newborn *Hippocampus abdominalis* fed either *Artemia* or four different mixed feeding treatments with Golden Pearls size grade 1 for 30 days from 0 to one month-old. Key: A = *Artemia*-only, GP1 = Golden Pearls-only, 5A +GP1 = mixed feeding for 5 days, 10A + GP1 = mixed feeding for 10 days, and 20A + GP1 = mixed feeding for 20 days. Different letters denote significant differences between treatments (*P<0.05*). No letters denotes non-significance.

Observation of feeding behaviour revealed the incidence of feeding strikes to be significantly higher for juveniles feeding on *Artemia* than GP1 in the mixed feeding

treatments (Students t-test, $t_{1,106} = 14.75$, $P<0.001$) (Table 4.1). The mixed feeding of *Artemia* with GP1 appeared to slightly increase the incidence of feeding strikes on the GP1 but this was not significant (Students t-test, $t_{1,106} = 1.19$, $P>0.05$).

Table 4.1 Feeding strike rate (mean ± 1 SE no. strikes min^{-1}) of juvenile *Hippocampus abdominalis* of different ages offered non-live foods and/or *Artemia* nauplii.

Seahorse age (months)	Treatment	Food item	Food item
		Artemia	Golden pearls
0	*Artemia*-only	2.41 ± 0.19	-
	Golden Pearls-only	-	0.16 ± 0.04
	Artemia + Golden Pearls	2.76 ± 0.15	0.33 ± 0.11
		Artemia	Cyclop-eeze®
1	*Artemia*-only	2.61 ± 0.13	-
	Cyclop-eeze®-only	-	0.47 ± 0.06
	Artemia + Cyclop-eeze®	2.28 ± 0.12	0.97 ± 0.14
		Artemia	Golden Pearls
1	*Artemia*-only	2.58 ± 0.1	-
	Golden Pearls-only	-	0.56 ± 0.06
	Artemia + Golden Pearls	2.2 ± 0.09	0.89 ± 0.12
		Artemia	Cyclop-eeze®
2	*Artemia*-only	2.5 ± 0.12	-
	Cyclop-eeze®-only	-	0.53 ± 0.06
	Artemia + Cyclop-eeze®	2.31 ± 0.07	0.92 ± 0.1
		Artemia	Golden Pearls
2	*Artemia*-only	2.53 ± 0.12	-
	Golden Pearls-only	-	0.49 ± 0.06
	Artemia + Golden Pearls	2.26 ± 0.07	0.94 ± 0.1

4.3.1.2 One month-old juveniles fed Golden Pearls

After one month of rearing, there was a significant difference in juvenile survival between dietary treatments (Kruskal-Wallis, $H_{4,15} = 23.05$, $P<0.01$) (Fig. 4.4) with greater survival in the *Artemia*-only treatment than the GP1-only, 5A + GP1 and 10A + GP1 treatments, but not the 20A + GP1 treatment (Bonferroni all pairs MCP, $P<0.05$). Juveniles fed GP1-only had lower survival than the 5A + GP1, 10A + GP1 and 20A + GP1 treatments, and juveniles fed 5A + GP1 had lower survival than the 20A + GP1 treatment (Bonferroni all pairs MCP, $P<0.05$).

There were significant differences among the treatments in both juvenile SL (ANOVA, $F_{4,109} = 11.39$, $P<0.001$) and wet weight ($F_{4,109} = 29.26$, $P<0.001$) (Fig. 4.4). The juveniles fed *Artemia*-only were larger and heavier, with generally increasing length and weight with longer mixed feeding periods amongst the other treatments.

Observation of feeding behaviour revealed the incidence of feeding strikes to be significantly higher for juveniles feeding on *Artemia* than GP1 in the mixed feeding treatments (Students *t*-test, $t_{1,106} = 11.44$, $P<0.001$) (Table 4.1). The mixed feeding of *Artemia* with GP1 significantly increased the incidence of feeding strikes on the GP1 (Students *t*-test, $t_{1,142} = 2.2$, $P<0.05$).
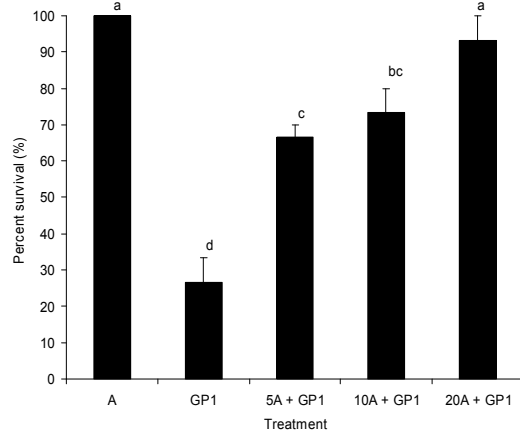
A
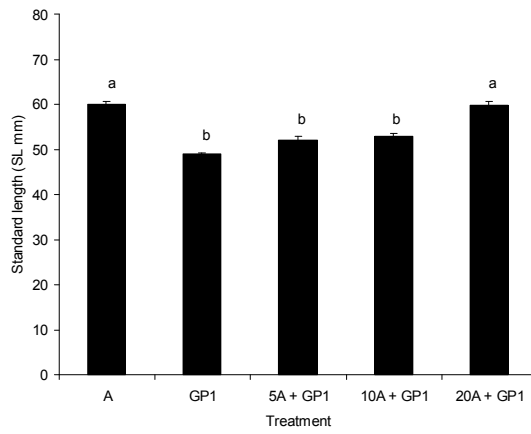
B
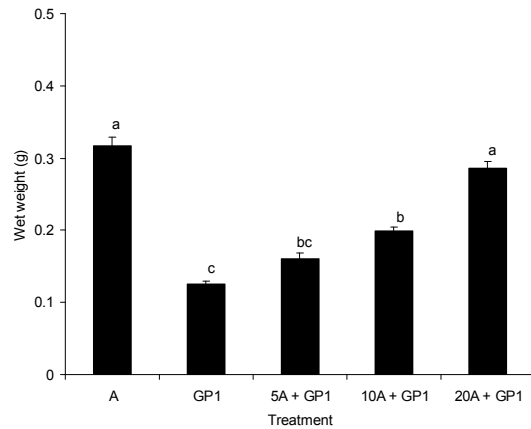
C

Figure 4.4 Survival (A), length (B), and wet weight (C) (mean ± 1 SE) of juvenile *Hippocampus abdominalis* fed either *Artemia* or four different mixed feeding treatments with Golden Pearls size grade 1 for 30 days from one month-old. Key: A = *Artemia*-only, GP1 = Golden Pearls-only, 5A +GP1 = mixed feeding for 5 days, 10A + GP1 = mixed feeding for 10 days, and 20A + GP1 = mixed feeding for 20 days. Different letters denote significant differences between treatments (*P*<0.05). No letters denotes non-significance.

4.3.1.3 Two month-old juveniles fed Golden Pearls

After one month of rearing, there was no significant difference in juvenile survival between dietary treatments (Kruskal-Wallis, $P>0.05$) with mean survival between 67–93% across the treatments (Fig. 4.5).

There were significant differences among the mixed feeding treatments in both juvenile SL (ANOVA, $F_{4,58} = 23.31$, $P<0.001$) and wet weight ($F_{4,58} =315.17$, $P<0.001$) (Fig. 4.5). The juveniles fed *Artemia*-only were larger and heavier, with generally increasing length and weight with longer mixed feeding periods.

Observation of feeding behaviour revealed the incidence of feeding strikes to be significantly higher for juveniles feeding on *Artemia* than GP2 in the mixed feeding treatments (Students *t*-test, $t_{1,106} = 12.03$, $P<0.001$) (Table 4.1). The co-feeding of *Artemia* with GP2 significantly increased the incidence of feeding strikes on the GP2 (Students *t*-test, $t_{1,142} = 3.51$, $P<0.01$).
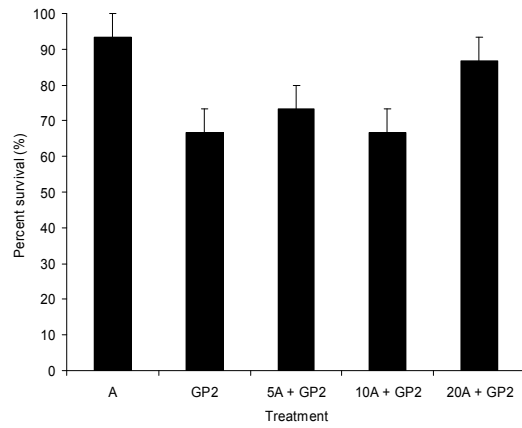
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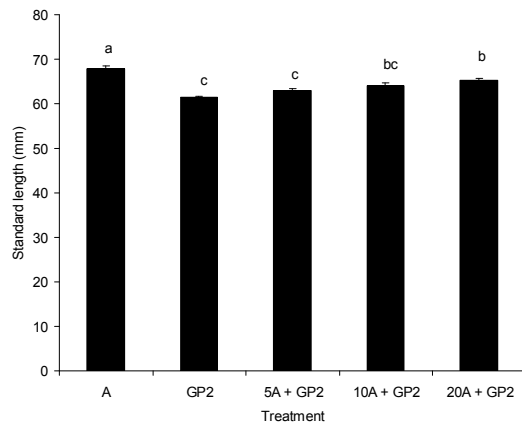
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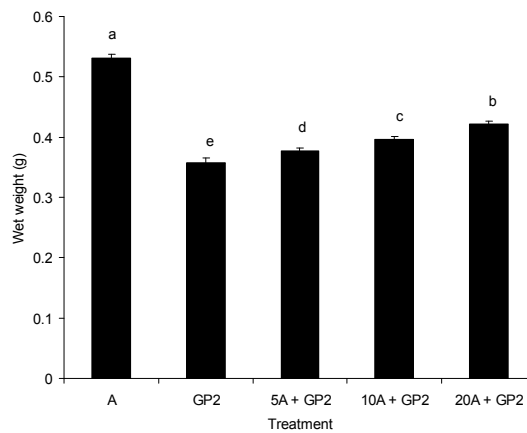
C

Figure 4.5 Survival (A), length (B), and wet weight (C) (mean ± 1 SE) of juvenile *Hippocampus abdominalis* fed either *Artemia* or four different mixed feeding treatments with Golden Pearls size grade 2 for 30 days from two months-old. Key: A = *Artemia*-only, GP2 = Golden Pearls-only, 5A +GP2 = mixed feeding for 5 days, 10A + GP2 = mixed feeding for 10 days, and 20A + GP2 = mixed feeding for 20 days. Different letters denote significant differences between treatments (*P*<0.05). No letters denotes non-significance.

4.3.2 Experiments on feeding frozen food

4.3.2.1 One month-old juveniles fed Cyclop-eeze®

After one month of rearing, there was no significant difference in juvenile survival between dietary treatments (Kruskal-Wallis, $P>0.05$) with mean survival between 90–97% across the treatments (Fig. 4.6).

There were significant differences among the treatments in both juvenile SL (ANOVA, $F_{4,141} = 18.24$, $P<0.001$) and wet weight ($F_{4,141} = 10.63$, $P<0.01$) (Fig. 4.6). The juveniles fed *Artemia*-only were larger and heavier, with generally increasing length and weight with longer mixed feeding periods.

Observation of feeding behaviour revealed the incidence of feeding strikes to be significantly higher for juveniles feeding on *Artemia* than Cyclop-eeze® in the mixed feeding treatments (Students t-test, $t_{1,106} = 11.81$, $P<0.001$) (Table 4.1). The mixed feeding of *Artemia* with Cyclop-eeze® significantly increased the incidence of feeding strikes on Cyclop-eeze® (Students t-test, $t_{1,142} = 4.47$, $P<0.001$).
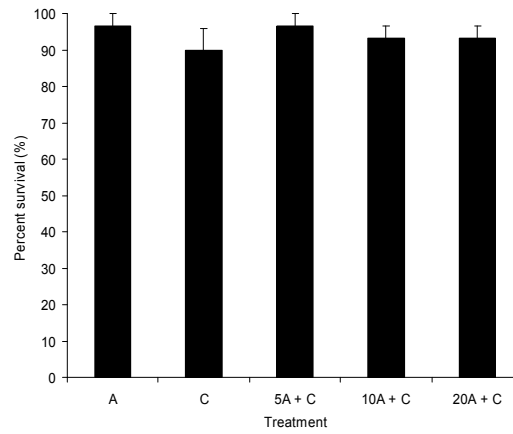
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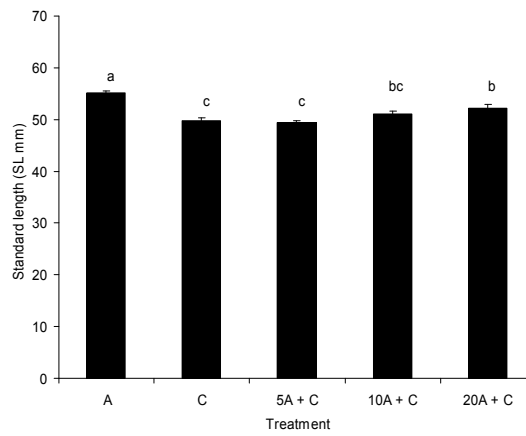
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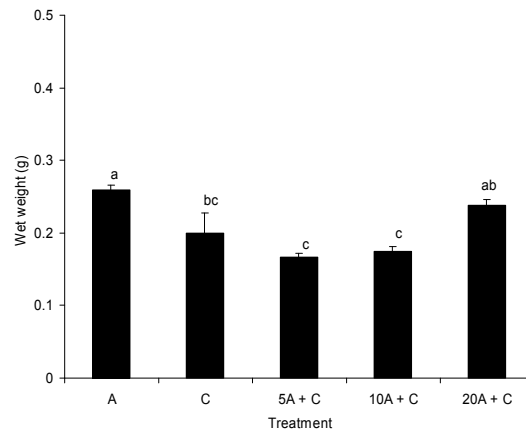
C

Figure 4.6 Survival (A), length (B) and wet weight (C) (mean ± 1 SE) of juvenile *Hippocampus abdominalis* fed either *Artemia* or four different mixed feeding treatments with Cyclop-eeze® for 30 days from one month-old. Key: A = *Artemia*-only, C = Cyclop-eeze®-only, 5A +C = mixed feeding for 5 days, 10A + C = mixed feeding for 10 days, and 20A + C = mixed feeding for 20 days. Different letters denote significant differences between treatments (*P*<0.05). No letters denotes non-significance.

4.3.2.2 Two month-old juveniles fed Cyclop-eeze®

After two months of rearing, there was a significant difference in juvenile survival between dietary treatments (Kruskal-Wallis, $H_{4,15} = 9.09$, $P<0.01$) with greater survival in the *Artemia*-only treatment, compared with juveniles fed Cyclop-eeze®-only, and juveniles in the Cyclop-eeze®-only, 5A + C and 10A + C treatments, but not the 20A + C treatment (Bonferroni all pairs MCP, $P<0.05$) (Fig. 4.7). The 20A + C treatment had greater survival than the Cyclop-eeze®-only and 5A + C treatments.

There were significant differences among the treatments in both juvenile SL (ANOVA, $F_{4,59} = 24.09$, $P<0.001$) and wet weight ($F_{4,59} = 54.13$, $P<0.001$) (Fig. 4.7). The control juveniles fed *Artemia*-only were larger and heavier, with generally increasing length and weight with longer mixed feeding periods.

Observation of feeding behaviour revealed the incidence of feeding strikes to be higher for juveniles feeding on *Artemia* than Cyclop-eeze® in the mixed feeding treatments (Students *t*-test, $t_{1,106} = 10.6$, $P<0.001$) (Table 4.1). The mixed feeding of *Artemia* with Cyclop-eeze® appeared to slightly increase the incidence of feeding strikes on Cyclop-eeze® (Students *t*-test, $t_{1,142} = 3.58$, $P<0.01$).
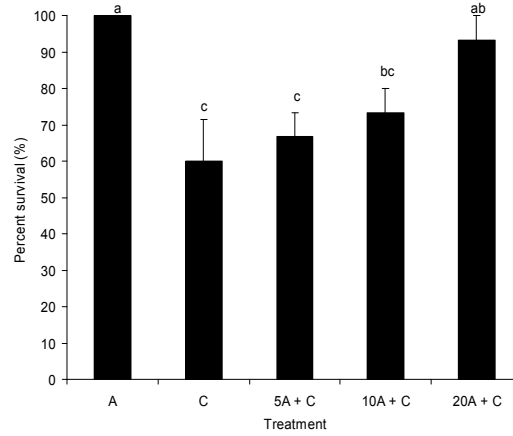
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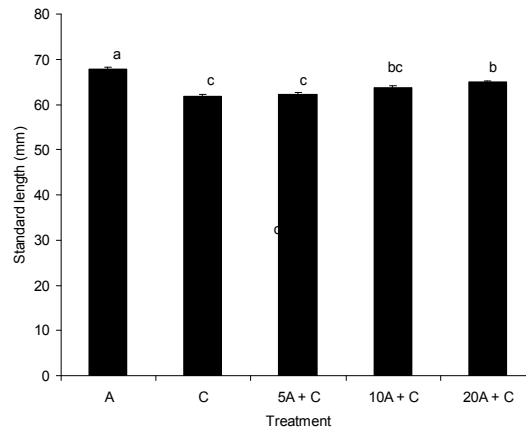
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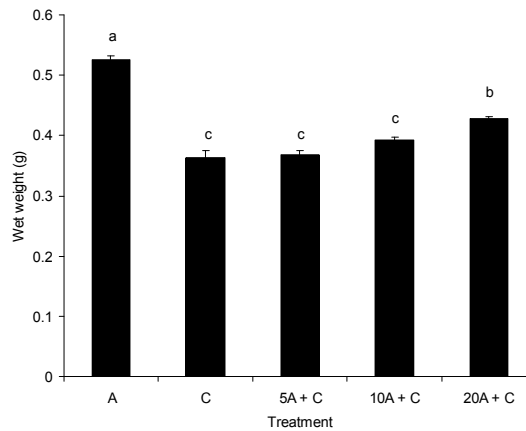
C

Figure 4.7 Survival (A), length (B) and wet weight (C) (mean ± 1 SE) of juvenile *Hippocampus abdominalis* fed either *Artemia* or four different mixed feeding treatments with Cyclop-eeze® for 30 days from two months-old. Key: A = *Artemia*-only, C = Cyclop-eeze®-only, 5A +C = mixed feeding for 5 days, 10A + C = mixed feeding for 10 days, and 20A + C = mixed feeding for 20 days. Different letters denote significant differences between treatments (*P*<0.05). No letters denotes non-significance.

4.4 Discussion

Juvenile *H. abdominalis* one to two months-old were able to be weaned onto both frozen and artificial foods on an experimental scale, but newborn juveniles were not able to be weaned onto the artificial food. Thus, the alternative hypothesis (H$_1$: age (size) of juveniles does affect weaning) was accepted. The ability to utilise non-live foods in the rearing of *H. abdominalis* has important implications for the commercial culture of this species, as the use of non-live foods can dramatically reduce material and labour costs. Non-live foods also provide a more predictable and reliable food source than the culturing or collection of live foods. The alternative hypotheses (H$_1$: juvenile seahorses can be weaned onto an artificial, and H$_1$: juvenile seahorses can be weaned onto a frozen food) were reservedly accepted for juveniles one to two months-old. However, depending on age of the juvenile seahorses and the type of non-live food offered, these weaned *H. abdominalis* juveniles sometimes had lower survival and growth compared to juveniles fed a standard live diet of enriched *Artemia* and these differences must be carefully considered before non-live foods are incorporated as culture practice.

Wilson *et al.* (2006) also examined rearing larger juvenile *H. abdominalis* (1.2 g wet weight) on frozen mysids (*Tenagomysis* sp.), a crumble (<1mm crumble) diet made from mysids, and a commercial crumble diet (NRD, 500–800 μm, INVE Lansy) over a 30-day period compared to juveniles fed live enriched (Super Selco®) *Artemia*. Similar to this study, they found that although survival in these older juveniles was no different for any of the diets, juveniles failed to wean onto the mysid crumble diet and lost weight, whilst juveniles did wean onto the commercial crumble but did not gain any weight. Similar to this study, they also observed that although *H. abdominalis* did wean on to the commercial crumble, the seahorses exhibited a lower feeding strike rate, and juveniles fed live *Artemia* and frozen mysids exhibited the highest growth rates.

A large variety of other marine fish have shown varying degrees of acceptability of non-live foods, particularly where co-feeding is employed. Kolkovski *et al.* (1997a) found that *Artemia* visual and chemical stimuli acted synergistically with each other to increase artificial diet ingestion in sea bream (*Sparus auratus*) larvae. The presence of live *Artemia* elicited a feeding response in *S. auratus* larvae on an artificial diet, which increased artificial diet ingestion by 60%. Larvae could subsequently be weaned entirely onto the artificial diet much more effectively than larvae without exposure to the live

Artemia. Blair *et al.* (2003) found that larval haddock (*Melanogrammus aeglefinus*) readily consumed artificial diets after initial co-feeding with live prey, but exhibited lower growth and survival than larvae fed on live *Artemia* or rotifer diets alone. In contrast, Ljunggren *et al.* (2003) investigated the use of artificial foods for juvenile pikeperch (*Stizostedion lucioperca*) and perch (*Perca fluviatilis*) and found that both species easily accepted formulated feeds, even without co-feeding, without any difference in growth or survival rates compared to those that were co-fed. Further, they found that agglomerated formulated feed gave better weaning results in juveniles of both pikeperch and perch than did non-agglomerated feeds.

In some fish species, reduced growth and survival have been observed when they are presented with artificial and inert diets. A variety of causes have been suggested for this, such as: lack of proper buoyancy (Ruyet, 1989), format instability in water (Ruyet, 1989), timing of weaning in relation to fish ontogeny and weaning duration (Hart & Purser, 1996; Cañavate & Fernández-Díaz, 1999; Jenkins & Smith, 1999; Hamlin & Kling, 2001), diet digestibility in relation to fish digestive enzyme ontogeny (Ruyet, 1989; Jones *et al.*, 1993; Rao, 2003; Vega-Orellana *et al.*, 2006), lack of acceptable visual and chemically stimulatory attributes (Ruyet, 1989; Fredette *et al.*, 2000), and nutritional adequacy of the diet (Ruyet, 1989; Hamre *et al.*, 2005; Wang *et al.*, 2005; Singh & Kahn, 2007). Inadequacies in the nutritional profile of the frozen and artificial diets (e.g. inadequate levels of certain lipids, proteins, carbohydrates, vitamins or trace elements) in relation to the dietary requirements of *H. abdominalis* could be a causative factor in the typically lower survival and growth of juveniles fed these diets (the optimal nutritional profile for *H. abdominalis* has yet to be determined, although a discussion of possible fatty acid and proximate requirements for seahorses is provided in Chapter 5). Ideally, nutritional analyses would have been carried out on both the Golden Pearls and the Cyclop-eeze® (e.g. fatty acids, amino acids) used in this study. Unfortunately, appropriate funding was not available for these at the time of the study. Therefore, growth and survival trends for newborn and juvenile *H. abdominalis* fed these foods can not be related to the nutritional quality of the foods themselves. Additional factors that may affect the efficacy of artificial and inert diets are lack of food palatability, and difficulties with gut-processing and assimilation of these foods by the juvenile seahorses. However, inadequacy in terms of diet presentation, i.e. how "attractive" the frozen and artificial diets are to the juvenile seahorses, is more likely to be the most

significant causative factor (in the absence of nutritional comparisons) in the reduced juvenile survival and growth as observed.

Although juveniles were physically capable of ingesting the size range of non-live diets offered, and did indeed ingest them as confirmed by observations of feeding, there remained a good proportion of these foods which were not consumed. This is in contrast to the juveniles offered *Artemia* nauplii which there was virtually no wastage; *Artemia* nauplii were relentlessly followed and consumed. Observations of feeding showed that when juveniles were presented with the frozen and artificial diets they carefully scrutinised the food before striking. On introduction of food into their tanks juveniles would become alert and both eyes would be directed at the food. The juveniles would then move their bodies to keep the food at the optimal striking distance from their snouts (~ 4–5 mm distance) while they scrutinised it. Juveniles would often inspect and then reject many individual Cyclop-eeze® copepods and Golden Pearls before actually striking and ingesting one. In contrast, juveniles offered *Artemia* would usually attack every nauplii that they visually "locked-on".

Selective planktivory, as exhibited by seahorse juveniles, involves the sensory detection of individual prey/food items from amongst other suspended non-food particles (essentially environmental "noise"), and subsequent discrimination as to whether that prey/food is appropriate to attack, followed then by the actual feeding strike (Cox & Pankhurst, 2000; Strickler *et al.*, 2005). For fish feeding in the water column there may only be a short time interval between encountering a particle in a motionally fluid environment, perceiving it as a desired one (through visual, tactile or chemosensory cues), and attacking it (Strickler *et al.*, 2005).

The head and eye orientation towards individual Cyclop-eeze® copepods and Golden Pearls, but not striking and then turning away, would seem to indicate that these foods did not trigger a feeding strike response for some reason/s. This may be as a result of physical damage to the copepods during freezing and thawing which rendered them "unattractive", or the food being unrecognisable as prey, as in the case of the Golden pearls (unless the water currents moved them in a certain manner which might make them seem alive). Examination of the Cyclop-eeze® copepods under a stereo-microscope revealed that approximately 18% were completely intact upon thawing. Another 38% were reasonably intact (e.g. an antenna missing, or part of the abdomen

missing), while the remaining 44% were in a highly fragmented state (see Fig. 4.2). This freezing/thawing damage may account for the rejection of many Cyclop-eeze® copepods.

The water currents within the tanks kept the copepods in suspension and moving for at least 4 h following introduction into the tanks, so there was ample time for them to be consumed. The water currents did not make the copepods move in a normal copepod manner (cyclopoids are characterized by erratic jumping motions, Confer & Blades, 1975), which may also have affected their "attractiveness" to the juveniles, although this assumes that the juveniles, which had never before been exposed to copepods, possessed a particular search-image for copepods as prey. Jenkins (1987) and Svenning & Borgstrom (2005) suggested that some fish larvae display innate prey behaviours or preferences, whereas others suggest learning plays an important role in prey selection (Werner *et al.*, 1981; Cox & Pankhurst, 2000); such that fish select for and are more effective at capturing familiar food (Checkley, 1982; Wahl *et al.*, 1993; Cox & Pankhurst, 2000). Strickler *et al.* (2005) investigated feeding responses in planktivorous fish (e.g. bluegills, *Lepomis macrochirus*) in relation to both real and virtual copepod and other planktonic prey and hypothesized that particular movement patterns by the prey was the primary factor for triggering feeding strikes. According to Rao (2003), one of the main reasons that artificial diets are not readily accepted by the larvae of some fish, even when they are suitable in terms of size, colour, flavour and nutritional adequacy, is that they show no active or escape behaviour, which is necessary for fish larvae to act.

Likewise, the tank water currents also kept the Golden Pearls in suspension and moving for at least 2 h following introduction into the tanks, after which time there was a gradual settling of particles around the outer edge of the tank bottoms. This settling effect could be a factor in the lower survival and growth of juvenile *H. abdominalis* exposed to these treatments, although juvenile seahorses are capable of ingesting a great many prey in 2 h (e.g. 160–200 *Artemia* nauplii). Lower feeding strike rates would again seem to indicate that a feeding strike response was not triggered by the majority of the Golden Pearls.

The use of artificial diets as early as possible in the rearing process with fish may improve weaning (Watanabe & Kiron, 1994; Cañavate & Fernández-Díaz, 1999),

although species-specific differences in size at weaning must be considered in relation to behavioural, physical and sensory ontogenetic changes (Hart & Purser, 1996; Silva, 2001; Herbert & Graham, 2004). For example, Barlow & Rodgers (1993) found weaning in barramundi (*Lates calcarifer*) most efficient at 17 mm total length, when they are at the transition stage between taking planktonic food and benthic/demersal food items. The reasons why newborn *H. abdominalis* were not able to be weaned onto the artificial food are open to speculation.

The water currents necessary to keep the non-live foods in suspension may have decreased feeding effectiveness in juveniles by moving the food particles too quickly, particularly for the newborn juveniles; water turbulence has been shown to affect feeding behaviour in some fish (Clarke *et al.*, 2005). Newborn *H. abdominalis* are not strong swimmers and the water currents, although gentle near the surface where the juveniles tended to swim and attach, could have caused them to expend excessive energy while hunting the moving food items. However, feeding strike rates in newborn juveniles were similar to those of older juveniles when feeding on *Artemia*, which suggests that they could still competently feed in the water currents and lighting arrangement. The fact that newborn to two month-old juveniles could effectively ingest all the *Artemia* presented to them in the water current pattern utilised in the experiments (the *Artemia* were also constantly swimming as well) would suggest that this is not an over-ruling factor in their case. In contrast, the feeding strike rate of newborn *H. abdominalis* on Golden Pearls was approximately half that of one to two month-old juveniles. Although physically capable of ingesting the smaller grade of Golden Pearls (200–300 μm) (based on physical width of live *Artemia* which they can feed on, and observed expansive capabilities of their snout, Woods, pers. obs.) juveniles may have experienced sufficient doubt as to whether the Golden Pearls were a item of food as discussed earlier. Aside from the use of water currents as used in this investigation to keep the artificial and inert diets moving, the use of mechanical devices (to vibrate the feed particles) and of refractory chemicals in the diets (to create the illusion of movement) have been attempted to increase the visual appeal of artificial or inert diets, but with variable results (Rao, 2003).

Although in many teleost fish vision is the primary sense used in prey detection and capture (Guthrie, 1968; Blaxter, 1969, 1980; Hunter, 1981), there is increasing evidence that many fish, even those with well-developed visual acuity, can use other non-visual

(olfactory or mechano-sensory) senses to detect and "taste" (gustatory senses) their food (Hara, 1971; Caprio, 1978; Harada, 1982; Knutsen, 1992; Batty & Hoyt, 1995; Kolkovski *et al.*, 1997b; Kotrschal *et al.*, 1998; Cox & Pankhurst, 2000; Kohbara *et al.*, 2000; Yacoob *et al.*, 2001; Kohbara *et al.*, 2002; Rao, 2003). For example, the larvae of sole (*Solea solea*) can feed at night using chemo- and mechanoreception (Batty & Hoyt, 1995). Olfactory receptors tend to have broad response spectra to amino acids, sex hormones, pheromones and bile acids, tend to be more sensitive to amino acids than gustatory receptors and show similar response magnitudes among different species (Caprio, 1978, 1984; Ishida & Kobayashi, 1992; Kohbara *et al.*, 2000). Gustatory receptors show considerable species differences (Marui et al., 1983; Caprio, 1984; Hara, 1994; Yacoob *et al.*, 2001), suggesting a role in discriminating food items during uptake and ingestion (Bardach & Villers, 1974). To date, the importance of non-visual senses in feeding has not been investigated for seahorses. *Hippocampus abdominalis* does possess a lateral line composed of small pores with short papillae (Kuiter, 2001) so presumably can detect vibratory stimuli. It also has paired olfactory pits with close-together anterior and posterior nares immediately in front the eyes (Woods, pers. obs.) so presumably have some olfactory capacity. Research into this aspect of the biology of seahorses would be greatly beneficial for the development of effective artificial foods.

The effect of chemical stimulants on increasing artificial diet acceptability in various fish species feeding has been successfully exploited for many years by aquaculturists. According to Lovell (1998), carnivorous fish species show the greatest stimulatory response to alkaline and neutral substances, such as glycine, praline, taurine, valine and betaine, while herbivorous species respond more to acidic compounds such as aspartic and glutamic acids. Such attractants and feeding stimulants can not only assist in food detection and elicitation of a feeding response, but also increase the palatability of the food, thus preventing food rejection following initial ingestion. For example, preliminary trials with artificial diet formulations suitable for adult *H. abdominalis* have revealed that certain dietary ingredients result in the seahorses spitting the artificial diets back out after finding the artificial formats sufficiently visually acceptable to feed on (Woods, unpubl. data), suggesting a negative gustatory response. Fredette *et al.* (2000) found that addition of a commercial feeding stimulant "FinnStim", which contains a betaine-amino acid mixture, to artificial diets increased feed consumption, growth and feed efficiency of juvenile winter flounder (*Pleuronectes americanus*).

In this investigation, juvenile seahorses fed *Artemia* and non-live foods generally experienced increased growth and lower mortality the longer the mixed feeding period before cessation of mixed feeding. The positive relationship between duration of mixed feeding and growth and survival in *H. abdominalis* may simply be a reflection of longer access to a live food which was consumed in greater quantities than the non-live food (as indicated by observations on feeding strike rates), and which may have been more nutritious than the non-live foods offered. Hart & Purser (1996) found a similar pattern with mixed-fed greenback flounder (*Rhombosolea tapirina*), as did Daniels & Hodson (1999) with southern flounder (*Paralichthys lethostigma*).

This study only followed newborn and juvenile seahorse growth and survival for a short period. Whilst the effects of diet on growth and survival were being compared across treatments in time, they were not being compared across equal time duration post feed change where weaning occurred. It would have been interesting to follow the longer term progression of the juveniles on the artificial and frozen diets as there may be nutritional effects (either positive or negative) of artificial diets that do not become apparent for some time. For example, inadequate energy or essential amino acids in the diet may result in protein degradation at a greater rate than protein synthesis, leading to the cessation of growth and weight loss, or the loss of appetite over time with subsequent growth reduction (Singh & Khan, 2007). Ostaszewska *et al.* (2005) compared the effects of feeding pike-perch (*Sander lucioperca* L.) larvae with either live *Artemia* or one of four artificial diets. Whilst they found differences in growth and survival between fish fed the live *Artemia* and some artificial diets, they also found distinct histological differences. Some artificial diets caused liver fatty degeneration, poorly developed intestinal folds, smaller hepatocytes, and retarded development of gastric glands, all of which could influence the subsequent health of pike-perch. Brandsen *et al.* (2005) altered dietary 22:6n-3 fatty acid levels in formulated feeds fed to striped trumpeter (*Latris lineata*) larvae from 5 to 18 days post-hatch. Whilst they found no differences in growth or survival between dietary treatments, they did find that low 22:6n-3 treatment larvae exhibited behavioural differences and problems in lipid assimilation and transport.

The fact that one to two month-old *H. abdominalis* could successfully feed on the non-live diets without mixed feeding (although usually with lower growth or survival) indicates that while mixed feeding is advantageous, weaning can occur without it.

However, by co-feeding frozen and artificial foods with live *Artemia*, the feeding strike rate on the two former foods by juvenile *H. abdominalis* did increase, and this is one of the primary mechanisms for utilizing mixed feeding in weaning. The alternative hypothesis (H_1: mixed feeding improves weaning success) was reservedly accepted. Feeding strike rate on Golden Pearls was increased by 86% on average, whilst on Cyclop-eeze® it was increased by 90% on average. Consequently, this may contribute to increasing growth and survival the longer the live food is present along with the non-live foods. Kolkovski *et al.* (1997b) found a similar trend in the feeding rate on an artificial diet by sea bream (*Sparus auratus*), which was increased by 50-60% in the presence of the visual stimuli of live *Artemia* prey, and by 120% in the presence of both visual and chemical stimuli of live *Artemia* prey. Kolkovski *et al.* (1997b) found that the feeding increase caused by chemical stimuli could be attributed to four metabolites found in the water in which *Artemia* had been reared: betaine and the free amino acids, arginine, alanine, and glycine.

Some researchers have found that a gradual reduction of the live food component during mixed feeding, rather than abrupt cessation as used in this investigation, is an effective method in increasing larval fish growth and survival (Rosenlund *et al.*, 1997; Daniels & Hodson, 1999; Herbert & Graham, 2004; Vega-Orellana *et al.*, 2006). For example, Rosenlund *et al.* (1997) found that halibut (*Hippoglossus hippoglossus*) larvae fed decreasing amounts of *Artemia* along with a dry diet, experienced higher specific growth rates than larvae fed solely with *Artemia* (2.6% vs. 1.7% day^{-1}). Daniels & Hodson (1999) found that in the flounder *P. lethostigma*, optimum growth and survival when weaning post metamorphosis larvae off *Artemia* and onto dry and semi-moist artificial foods occurred at 20 d with abrupt cessation, but that this weaning period could be shortened when gradual weaning was employed. Previous studies have shown that learning to successfully feed on a novel prey/food variously occurred one to five days after exposure to that novel item (Meyer, 1986; Wahl *et al.*, 1993; Cox & Pankhurst, 2000), indicating that a temporal overlap is required during the transition to a weaning diet (Cox & Pankhurst, 2000). It would therefore be worthwhile in the future to compare the success of mixed feeding of *Artemia* and non-live foods in *H. abdominalis* using both abrupt cessation and gradual reduction.

The lower strike rates on the artificial and inert foods observed in this investigation have important culture ramifications. Uneaten food increases the potential for deterioration in

water quality, increase in fish stress, and susceptibility to disease and infection from pathogens, which may then contribute to higher mortality and poorer growth, and increased effort to clean tanks (Leu *et al.*, 1991; Muir & Sutton, 1994; Kaiser *et al.*, 2003). In addition, from a commercial culturist's viewpoint any uneaten food represents an economic loss as the uneaten food has been paid for but produces no revenue through conversion to stock biomass. Therefore, reducing or removing this economic loss by means which increase ingestion rates must be found, and/or that economic loss must be factored into operational costs and offset by increased return elsewhere (e.g. increased seahorse sale price).

In conclusion, this investigation has demonstrated that there is the potential to utilise frozen and artificial diets as a cheaper and more convenient dietary alternative to *Artemia* in the culture of *H. abdominalis*. However, more research is required into improving diet presentation, attractiveness and acceptability, scaling-up of suitable rearing tanks for keeping the food in suspension on a commercial scale, and aligning the nutritional profile of the diet to that required by *H. abdominalis* before a commercial diet can be developed and applied. Whilst the successful development of nutritionally appropriate and acceptable artificial and inert diets for seahorses can have potentially significant economic benefits in their commercial aquaculture, it can also have potential post-farm disadvantages which should be factored into its application. For example, hatchery-reared fish that have been weaned onto non-live foods can exhibit reduced feeding abilities and success when presented again with live prey (Sundström & Johnsson, 2001; Ellis *et al.*, 2002; Wintzer & Motta, 2005). Where seahorse aquaculture is primarily directed at the enhancement of depleted wild stocks it would be more appropriate to either not use artificial or inert foods, or to employ a reconditioning period of the seahorses back onto live foods similar to those will encounter in the wild before they are released.

CHAPTER 5

EFFECT OF LIVE FOOD ENRICHMENT AND USE OF FROZEN MYSIDS ON SEAHORSE GROWTH AND SURVIVAL

5.1 Introduction

5.1.1 Effect of *Artemia* enrichment

As seahorses are predators who primarily use vision to locate their prey, their culture has largely been reliant upon culturing live prey as food, as this elicits a strong feeding response. In chapter 4 it was shown that there is potential for the weaning of *H. abdominalis* juveniles from live foods to frozen foods and artificial diets, which has strong potential to improve the economic viability of commercial seahorse culture. However, the development of attractive and nutritionally appropriate artificial diet formulations that can offer improved growth and survival rates compared to live foods still remains to be achieved. Until this occurs it is prudent to examine the potential for improving the quality of live feeds in order to increase growth and survival rates of seahorses, and reduce the culture costs associated with live food production.

Artemia (Fig. 5.1) possess characteristic attributes that are technically and commercially conducive to them being used as a live food in commercial aquaculture:

1) They are readily available in nitrogen-packaged diapause cyst form, which enables the cysts to be stored (within a certain timeframe) until they are required to be hatched-out, whereupon only 24 hrs is required to produce hatched nauplii of a required biomass.

2) Their size range (0.4–0.5 mm in length at hatch and up to 12 mm in length as adults) allows them to be fed to a range of fish species and life stages.

3) They can act as bioencapsulators, or delivery vehicles, for a range of beneficial nutrients and other agents.

4) They can be fed a wide range of diets (of the appropriate size), from live microalgae to microcapsules, bacteria and yeasts, and byproducts from the food industry.

5) They exhibit both oviparity (diapause cysts) and ovoviviparity (free-swimming nauplii) depending upon environmental conditions; this allows plasticity in the type of offspring production.

6) Provided culture conditions are appropriate, they can be intensively cultured (inoculation densities of 5000-18 000 nauplii l^{-1}) with fast growth (nauplii to adult in around eight days), high fecundity (up to 300 nauplii or cysts every four days from adults), and high survival rates, in a range of scales from small beakers (e.g. 1-l) to large outdoor ponds (e.g. >200 ha).

7) They are tolerant of large ranges in environmental parameters such as oxygen level, salinity and temperature.

8) Moulting to the next instar may be avoided, and energy metabolism (and, therefore, reduction in nutritional value) reduced through chilling to 4–6°C.

9) There is considerable literature dealing with their culture.

| A | B |
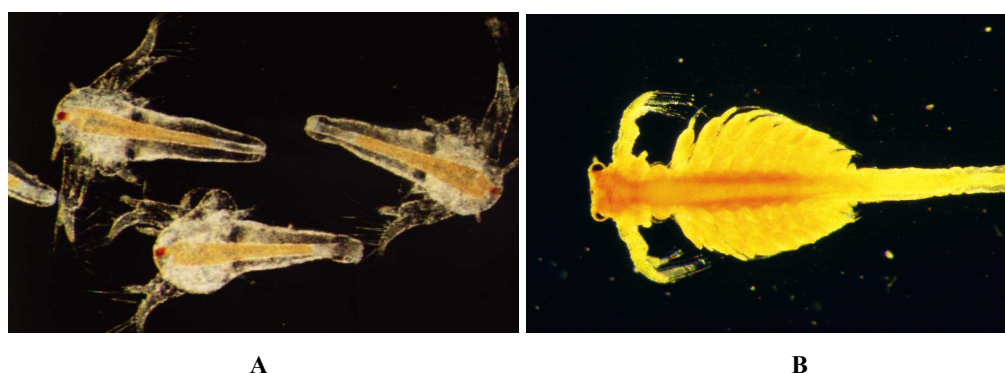
Figure 5.1 *Artemia* sp. instar II nauplii (A), and, late stage nauplii (B) (source: Chris Woods).

Artemia have been recognized as potentially nutritionally inadequate in relation to the dietary requirements of the animal they are being fed to, though their nutritional value can be changed through their diet (Watanabe *et al.*, 1978; Watanabe *et al.*, 1982; Barclay & Zeller, 1996; Van Stappen, 1996; Furuita *et al.*, 1999; Han *et al.*, 2000, 2001). From their second moult (instar II) *Artemia* are non-selective continuous particle feeders; within appropriate culture media *Artemia* non-selectively ingest particles within a size range of 1–50 μm, offering potential to use them as bioencapsulators (Van Stappen, 1996; Sorgeloos *et al.*, 2001; Dhont & Van Stappen, 2003; Lim *et al.*, 2003). This bioencapsulation is commonly referred to as "enrichment". Because *Artemia* provide fish culturists with a convenient "carrier" vehicle for desirable dietary components such as essential fatty acids (EFAs), considerable effort has been put into improving the nutritional value of *Artemia* through enriching them with various media and tailoring their nutritional content to the particular requirements of different fish species (Watanabe *et al.*, 1978; Watanabe *et al.*, 1982; Barclay & Zeller, 1996; Furuita

et al., 1999; Han *et al.*, 2000; Gapasin & Duray, 2001; Sorgeloos *et al.*, 2001; Harel *et al.*, 2002; Shapawi & Purser, 2003; Monroig *et al.*, 2006). *Artemia* can also act as bioencapsulators for a variety of beneficial nutrients, steroid hormones, vitamins, antimicrobial agents, vaccines and probiotics (Mohney *et al.*, 1990; Webster & Lovell, 1990; Campbell *et al.*, 1993; Gatesoupe, 1994; Dixon *et al.*, 1995; Van Stappen, 1996; Sorgeloos *et al.*, 2001; Smith *et al.*, 2004a, b).

As seahorse culture is still a rather nascent activity in many countries (particularly western countries) and is still relatively small in scale compared to other more established finfish industries, there is a relative paucity of published research on seahorse nutrition, and consequently on methods for improving cultured seahorse growth and survival rates through live food production and enrichment. However, the importance of highly unsaturated fatty acids (HUFA) for enriching live foods such as *Artemia* sp. in *Hippocampus* sp. nutrition has been demonstrated (Filleul, 1996; Chang and Southgate, 2001; Thompson, 2002; Shapawi & Purser, 2003). For example, Filleul (1996) found that juvenile *H. abdominalis* growth was increased when the juveniles were fed *Artemia* enriched with higher levels of arachidonic acid (AA), eicosapentaenoic acid (EPA), and somewhat smaller amounts of docosahexaenoic acid (DHA).

Because seahorse nutrition is still a largely unresearched topic, commercial seahorse culturists often use existing, well-known commercial products that have been shown to increase growth and survival in other marine finfish to enrich live foods before feeding to seahorses. However, such commercial products may, or may not, be suitable for optimising seahorse growth and survival, and their relative cost/benefit ratio may significantly impact on the commercial viability of seahorse aquaculture.

Using live *Artemia* as prey, the first aim of this chapter is to determine whether the growth and survival of cultured juvenile *H. abdominalis* is influenced by the type of media used to enrich the *Artemia*. Hypotheses to be tested were:

1) H_0: Juvenile seahorse growth and survival are not different when fed on *Artemia* enriched with different media; H_1: Juvenile seahorse growth and survival are different when fed on *Artemia* enriched with different media.

2) H_0: there is no difference in the relative cost/growth benefit ratio between each enrichment media used; H_1: there is a difference in the relative cost/growth benefit ratio between each enrichment media used.

The research contained in this chapter is based on data from Woods (2003d).

5.1.2 Use of frozen mysids

Mysid shrimp (Mysidacea) inhabit marine, estuarine, brackish and freshwater ecosystems, where they are an important intermediary trophic link (Mauchline, 1980; Allen, 1982; Fulton III, 1984; Patterson, 1996; Roast *et al.*, 2000; Richoux *et al.*, 2004). Mysids have been shown to be able to accumulate substantial amounts of lipids despite their flexible feeding habits, providing a lipid-rich food source (Bradshaw *et al.*, 1990; Richoux *et al.*, 2004), and other beneficial dietary components (such as carotenoids) to their predators (Ibrahim *et al.*, 1984). Mysids are eaten by many predatory fish, and are the dominant food item for some fish species (Griffiths, 1976; Allen, 1982; Grossnickle, 1982; Hostens & Mees, 1999; Garrison & Link, 2000; Szedlmayer & Lee, 2004; Takahashi *et al.*, 2004). Mysid shrimp have also been shown to be important dietary components of some seahorse and seadragon species in their natural environment (Reid 1954; Tipton & Bell 1988; Sanchez-Camar *et al.*, 2005; Vincent *et al.*, 2005), including *H. abdominalis* (Lovett 1969; Flynn & Ritz, 1999; Woods, see Chapter 6).

Mysids have been extensively used in the aquaculture of aquatic organisms such as cephalopods (e.g. cuttlefish and squid) (Akiyama *et al.*, 1997; Domingues *et al.*, 2000; Domingues *et al.*, 2001; Koutea & Boucaud-Camou, 2001) and shrimp (Ogle & Price, 1976). Mysid shrimp, often in frozen form, are also commonly used by aquarium hobbyists, researchers and some commercial aquaculturists, to feed seahorses as an alternative to live prey such as *Artemia*. Some seahorse species can be weaned relatively easily onto frozen mysids and these mysids appear to promote good seahorse growth and survival (Garrick-Maidment, 1997; Fenner, 1998; Hilomen-Garcia, 1999; Forteath, 2000; Indiviglio, 2002; Mai, 2004b; Ingram, 2005). In addition, feeding frozen mysids may also result in lower ammonia production compared with live foods such as *Artemia* (Wilson *et al.*, 2006) (provided they are eaten quickly), which is an important consideration in recirculation and high-loading biomass seawater systems.

In an ideal culture or hobby environment, live foods are often preferred for seahorses in order to promote behavioural alertness (Garrick-Maidment, 1997), and because of water quality concerns if frozen food is left uneaten (Abbott, 2003). However, many aquarists who purchase seahorses choose to provide them with non-live food. Therefore, survival of seahorses sold into the aquarium trade might be enhanced if they are accustomed to non-live foods before they are sold (Payne, 2003). Frozen mysid shrimp (Fig. 5.2) are a popular convenience food used by many aquarium hobbyists, and are often recommended for feeding to captive seahorses (e.g. http://www.aquarticles.com, http://mysid.reed-mariculture.com, http://www.peteducation.com, http://www.seahorse-australia.com.au, http://www.oceanrider.com, http://www.seahorse.org, http://www.seahorseusa.com; accessed 12/09/05). However, despite their widespread use for feeding seahorses, there are few published reports that have qualitatively assessed the actual growth and survival benefits of feeding mysid shrimp (either live or frozen) to seahorses.

Figure 5.2 Commercially available frozen mysids (*Mysis relicta*, Piscine Energetics) (source: Chris Woods).

In commercial aquaculture it is critical to determine how to maximise a cultured organism's growth, survival and health in relation to minimising the amount (and therefore cost) of food required to obtain the former. The effective minimisation of feeding rate, whilst still promoting high growth, survival and fish health (Alcorn *et al.*, 2003), may not only have the effect of reducing feeding costs (De Silva & Anderson, 1995), but also biological loading in recirculation systems and in outflow water from flow-through systems (an important consideration in helping to mitigate any environmental impact of a culture operation) (Timmons *et al.*, 2002).

Many studies have been conducted into the effects of manipulating feeding regimes in a number of cultured fish species in order to improve culture efficiency (e.g. Wang *et al.*, 1998; Saether & Jobling, 1999; Rabe & Brown, 2000; Mihelakakis *et al.*, 2001; Mischke et al., 2001; Webster *et al.*, 2001; Dwyer *et al.*, 2002; Petursdottir, 2002; Cho *et al.*, 2003; Fiogbé & Kestemont, 2003; Jodun, 2004; Rowland *et al.*, 2005). These have highlighted species-specific differences in the effects of feed ration on growth and survival, as well as the interactive effects that variables such as feeding frequency, water temperature, and diet composition have upon the impact of feed ration (e.g. Imsland *et al.*, 2001).

The second aim of work described in this chapter is to: 1) determine the efficacy of using frozen mysids to rear cultured *H. abdominalis* in comparison to a standard diet of enriched live *Artemia*, and, if the use of frozen mysids is efficacious, 2) to investigate the effect of differing daily feed rations on the growth and survival of cultured *H. abdominalis*. Hypotheses to be tested were:

1) H_0: Seahorse growth and survival when fed frozen mysids is inferior to that when fed enriched live *Artemia*; H_1: Seahorse growth and survival when fed frozen mysids is as good as (or even better) to that when fed enriched live *Artemia*.

2) H_0: there are no differences in growth, survival or the relative cost/growth benefit ratio between differing feed rations using frozen mysids; H_1: there are differences in growth, survival or the relative cost/growth benefit ratio between differing feed rations using frozen mysids.

This research is based on Woods & Valentino (2003) and Woods (2005a).

5.2 Materials and methods

5.2.1 Effect of *Artemia* enrichment

Three commercial enrichment products commonly used in finfish aquaculture were tested for their effects on growth and survival in *H. abdominalis*: the self-emulsified fish oil concentrates Super Selco® and DHA Protein Selco® (INVE Aquaculture NV), and Algamac-3050® (Aquafauna Bio-Marine Inc.) which is composed of spray-dried cells of the heterotrophic protist *Schizochytrium* sp.. The growth rate and survival of *H. abdominalis* fed on these commercial enrichment products was compared with that of

H. abdominalis fed *Artemia* reared on a relatively low-cost on-growing diet (a mixture of a proprietary rice bran-based product (EPABSF) and the microalga *Spirulina platensis*). As enrichment media can vary considerably in their purchase cost, the relative cost/growth benefit ratio for each enrichment media is of particular interest to commercial culturists. Therefore at the conclusion of the experiment this was calculated and compared for the different enrichment media. The effect of each enrichment media on the nutritional composition of the *Artemia* was examined through lipid and proximate analyses.

5.2.1.1 Fatty acid analysis of *Artemia*

Artemia salina (GSL cysts from MacKay Marine Brine Shrimp Co. Inc., USA) which had been on-grown on a diet of 90% Eyre Peninsula Aquaculture (Australia) brine shrimp food (EPABSF; proprietary rice bran-based product) and 10% *S. platensis* (Shengli Oilfield Group, China) to a suitable size for the juveniles were enriched for 24 h in aerated 60-l containers at a constant 28°C using the three commercial enrichment preparations. Enrichment concentrations used were as recommended by the manufacturers: 0.6 g l^{-1} 24 h^{-1} 100 000 *Artemia*$^{-1}$ for Super Selco® and DHA Protein Selco®, 0.2 g l^{-1} 12 h^{-1} 100 000 *Artemia*$^{-1}$ for Algamac-3050®. *Artemia salina* reared for 24 h at a constant 28°C on the EPABSF/*S. platensis* on-growing food (0.6 g l^{-1} 24 h^{-1} 100 000 *Artemia*$^{-1}$) were used as the control. Following enrichment, *Artemia* were harvested and rinsed with freshwater.

Lipid analyses were performed by a commercial analytical laboratory (Industrial Research Ltd, Gracefield, Lower Hutt, New Zealand). Triplicate samples of enriched *Artemia* were stored over dry ice. The samples were then freeze-dried for 2 days. Total dry weights were recorded before samples were taken for lipid extraction.

Samples of freeze-dried material (83–129 mg) were weighed into 8 ml test tubes. Methanol (1 ml) and dichloromethane (2 ml) were added, and the test tubes shaken on a vortex mixer and then placed in an ultrasonic bath for 5 min. Each tube was briefly centrifuged and the solvent transferred by Pasteur pipette to a second tube. This procedure was repeated once more.

The solvent (~6 ml) was washed with 1.5 ml of KCl solution (0.88% in distilled water). The two phases did not separate cleanly, so the tube was centrifuged to break the

emulsion. The upper layer (containing non-lipid material) was removed by Pasteur pipette and discarded. The lower layer was washed with 1.5 ml of methanol-water (1:1). The solvent was removed under a stream of argon at 35°C. Final traces of solvent were removed on the freeze drier. The weight of the lipid extract was then recorded.

Fatty acids in the oil (free and bound) were converted to methyl esters with H_2SO_4 in methanol. Petroleum ether (0.5 ml containing the internal standard) and 1% H_2SO_4 in methanol (1 ml) were added to the lipid extract, and the test tube flushed with argon, sealed, and then placed in a water bath at 50°C overnight. Salt solution (NaCl 5%, 2 ml) was then added and the fatty acid methyl esters (FAME) extracted into 2 x 2 ml petroleum ether. The upper phase was removed and washed with sodium bicarbonate solution (2%, 2 ml). GC analysis was performed on a HP-5890 gas chromatograph using an Alltech EC-Wax column (0.25 mm x 30 m) (FID detector). The temperature program was 165°C for 3 min, 4°C min^{-1} to 195°C for 10 min, and 4°C min^{-1} to 225°C for 12 min. Peaks were identified by comparison to cod liver oil used as an internal standard. Nonadecanoic acid (19:0) was used as an internal standard.

5.2.1.2 Proximate analysis of *Artemia*

Analyses (protein, fat, ash and moisture) were performed by a commercial analytical laboratory (AgriQuality Ltd, Auckland, New Zealand) on frozen 100 g samples of enriched *Artemia*. Analysis was conducted on duplicate subsamples from the 100 g mass. Method protocol was not provided, but method references (Association of Official Analytical Chemists International) provided were as follows: ash (JAOAC 45, 548 (1962)), fat (AOAC 922.06, 948.15), protein (AOAC Official Methods of Analysis 15th Ed 968.06), and moisture (AOAC 930.15).

5.2.1.3 Seahorse growth and survival

In September 2001, 144 third generation captive-bred (F3) six month-old juvenile *H. abdominalis* (mean ± 1 SE standard length = 70.5 ± 1.2 mm, mean wet weight = 0.59 ± 0.03 g, mean Condition Factor (CF) = 0.17 ± 0.01) were randomly allocated to one of the four enrichment treatments, with four replicate tanks per treatment (nine seahorses per replicate tank). There were no significant differences in juvenile length, weight or Condition Factor (CF) between treatments or replicates at the start of the experiment (ANOVA, *P*>0.05).

Tanks used were 9-l transparent plastic flat-bottomed circular fish bowls (as used in Chapters 3 & 4) supplied with seawater heated to a constant 18°C and filtered to 20 μm. Water flow through the tanks was approximately 0.25 l min^{-1}. Individual strands of separated black shade-cloth (1 mm width) attached to the base of the seawater inflow line provided holdfasts for the seahorses.

A photoperiod of 12 h light:12 h dark was provided by automatically controlled cool white fluorescent (2 x 58W) tubes above the tanks at an approximate intensity of 6.5 μmol m^{-2} s^{-1} at the water surface. Tanks were inspected daily and excess waste/faeces siphoned out. Tanks were completely drained and cleaned every two weeks (chlorine/detergent).

Juvenile seahorses were fed twice daily (typically at 0900 and 1400-h) to excess with the enriched *Artemia* (mean ± 1 SE length = 2.11 ± 0.02 mm). *Artemia* were not temperature-acclimated to 18°C from their culture temperature of 28°C during feeding, but no dropping-out of suspension of the *Artemia* was observed. Feeding to excess in this study was defined as feeding to such a level that there were always *Artemia* present in the tanks between 0900 and 1700-h, and that that level was sufficient to cause a marked reduction in attack response one hour after each feed (from >4 strikes min^{-1} to <1 strike min^{-1}). Unlike some fish, *H. abdominalis* need not cease attacking prey even when the digestive tract is full; seahorses may continue to attack, spitting out attacked prey and then attacking new prey with the same result (Woods, pers. obs.). Therefore, feeding to excess was used rather than feeding to satiation.

The experiment was concluded after 90 days. Final juvenile length and wet weight were recorded. Mean Specific Growth Rate for each tank was calculated (SGR % increase in body weight day^{-1}) = $((\ln W_f - \ln W_i)/t)$ x 100, where W_f = final wet weight, W_i = initial wet weight, and t = number of days. Condition Factor (CF) was also calculated for individual juveniles as follows: (CF) = 10^{-6} (whole seahorse wet weight (g)/standard length (mm)$^{3.5}$).

Mean ± 1 SE water parameters during the experiment were as follows: dissolved oxygen 8.07 ± 0.05 mg l^{-1} (range 7.6–8.4 mg l^{-1}), salinity 34.12 ± 0.02 ppt (32.3–34.72 ppt), and pH 8.16 ± 0.01 (8.12–8.2).

5.2.1.4 Statistical analyses

Statistical analyses were conducted using NCSS 2000 (Hintze, J., Number Cruncher Statistical Systems, Kaysville, Utah, USA) and SYSTAT 10.0 (SPSS Inc., Chicago, Illinois, USA). Data were tested for normality (Shapiro-Wilk's W test) and homogeneity of variances (Levene's test). Data were transformed where appropriate before analysis to ensure data normality and homogeneity of variances. Differences in percent lipid composition (arcsine square-root transformed data) of the enriched *Artemia* for the major fatty acids oleic acid, 18:1n-9, linoleic acid, LA, 18:2n-6, linolenic acid, ALA, 18:3n-3, arachidonic acid, AA, 20:4n-6, eicosapentaenoic acid, EPA, 20:5n-3 and docosahexaenoic acid, DHA, 22:6n-3, as well as total percent PUFA and n-3 HUFA composition, were tested using Kruskal-Wallis ANOVA with post-hoc Tukey HSD (P at 0.05). Differences in percent proximate composition (arcsine square-root transformed data) of the enriched *Artemia* were tested using Kruskal-Wallis ANOVA with post-hoc Tukey HSD (P at 0.05). Differences in seahorse SL and wet weight were tested for using nested ANOVA with post-hoc Tukey HSD (P at 0.05). For the CF data, an Analysis of Covariance (ANCOVA) was performed on log-transformed data with log-length as a covariate to treatment to test for any differences in data slope between stocking density treatments. This revealed no significant interaction effects (ANCOVA, P>0.05) and confirmed the assumption of homogeneity of slopes, allowing ANOVA to be performed for comparison of CF at a standardized log-transformed mean with post-hoc Scheffe's test (P at 0.05). Differences in mean SGR for tanks were tested using Kruskal-Wallis ANOVA with post-hoc Tukey HSD (P at 0.05).

5.2.1.5 Cost/benefit analysis of enrichment

To determine the relative value of each of the enrichments used and their resulting growth benefit for seahorses, a cost/benefit (CB) ratio was calculated for each enrichment medium at the conclusion of the experiment. The kg^{-1} cost (excl. shipping, taxes and customs clearance costs) of the enrichments used in this investigation at the time of their purchase (August 2001) from commercial retailers was as follows: $NZ270 kg^{-1} ($US113.4 kg^{-1}) for both Super Selco$^{®}$ and DHA Protein Selco$^{®}$, NZ$103.6 kg^{-1} ($US43.5 kg^{-1}) for Algamac-3050$^{®}$, and $NZ11 kg^{-1} (US$4.62 kg^{-1}) for the EPABSF/*S. platensis* mixture. Using these costs (in relation to the enriching concentrations/enriching volumes used as outlined earlier) and the growth rates obtained in this investigation, a cost/benefit (CB) value can be calculated as such: CB (NZ$) = cost of actual enrichment used (NZ$) seahorse^{-1} day^{-1}/mean unit increment (i.e. mm or

mg) increase day^{-1}. The labour cost in preparing the enrichments and enriching the *Artemia* for each enrichment treatment was approximately the same and was not factored into cost/benefit.

5.2.2 Use of frozen mysids

5.2.2.1 Fatty acid analysis of mysids

Fatty acid analyses were performed by a commercial analytical laboratory (Industrial Research Ltd, Gracefield, Wellington) as in section 5.2.1.1 on samples of enriched *A. salina* and frozen mysids. Triplicate samples of *A. salina* enriched for 24 h at 28°C on Algamac-3050$^{®}$, and triplicate samples of the frozen mysids were stored over dry ice to ensure they were completely frozen. The samples were then freeze-dried for 2 days. Total dry weights were recorded, then samples taken for lipid extraction.

5.2.2.2 Proximate analysis of mysids

Analyses (protein, fat, ash, and moisture) were also performed by a commercial analytical laboratory (AgriQuality Ltd, Auckland) as in section 5.2.1.2 on frozen 100 g samples of the *A. salina* enriched for 24 h at 28°C on Algamac-3050$^{®}$, and frozen mysids. Analysis was conducted on duplicate subsamples from the 100 g mass.

5.2.2.3 Seahorse growth and survival fed live *Artemia* vs. frozen mysids

Ninety captive-bred ten month-old *H. abdominalis* (mean ± 1 SE SL = 113.6 ± 0.6 mm, wet weight = 2.92 ± 0.07 g, Condition Factor (CF) = 0.19 ± 0.003) were randomly allocated to one of three treatments with a daily wet weight food ration of 25% of their wet body weight: 1) fed live *A. salina* only, 2) fed frozen mysids only, or, 3) fed live equal weights of live *A. salina* and frozen mysids.

In the first feeding treatment, *A. salina* (GSL cysts from MacKay Marine Brine Shrimp Co., Inc., USA) which had been reared on a diet of 90% EPABSF/10% *S. platensis* to a suitable size (mean ± 1 SE length = 4.8 ± 0.5 mm), were enriched for 24 h at a constant 28°C using Algamac-3050$^{®}$. Following enrichment, *A. salina* were harvested and rinsed with freshwater prior to being used as food. In the second treatment, the mysid *Amblyops kempi* (Holt & Tattersall, 1905) was used as food. This was the Hikari (Kyorin Co. Ltd, Himeji City, Hyogo Prefecture, Japan) brand of mysid shrimp, imported frozen and distributed by Biosuppliers, Auckland, New Zealand. The daily

ration of frozen mysids required was thawed in seawater before being fed to the seahorses. In the third feeding treatment, the *A. salina* + mysid treatment, the 25% body weight ration was split 50:50 between *A. salina* and mysids.

Tanks used were as described in section 5.2.1.3. Seawater was heated to a constant 18°C and filtered to 20 μm. Water flow through the tanks was approximately 0.25 l min^{-1}. There were six replicate tanks randomly assigned per treatment, with five seahorses per tank. There were no significant differences in seahorse length, weight, or condition factor (CF) between treatments or replicates at the start of the experiment (ANOVA, $P>0.05$). Sex ratio of the seahorses used for the experiment was 50:50, with three tanks in each treatment with 2 males-3 females, and the other three tanks with 3 males-2 females. The maintenance of sexually mature *H. abdominalis* in such a culture situation has been proven not to result in any sex-related growth differences (Woods, 2003b). Seahorses were fed once at approximately 1000-h each day. Uneaten food and faeces were siphoned from each tank approximately 6 h later.

Monthly censuses were conducted to measure growth (SL and wet weight). SL was measured to the nearest mm by placing seahorses on a plastic ruler immersed in a shallow tray of seawater. Wet weight was measured on a Mettler P440 balance following blot-drying of seahorses. Wet weights were conducted 16 h after the last access of seahorses to food. Following each census, the daily wet weight ration of each diet was adjusted according to average seahorse wet weight increase for each treatment to maintain the 25% ration (i.e. monthly ration adjustment).

A photoperiod of 12 h L:12 h D was provided by timer-controlled 2 x 58W cool white fluorescent tubes above the tanks at a mean intensity of 9.0 μmol photons m^{-2} s^{-1} at the water surface. Tanks were inspected daily and uneaten food and faeces siphoned away. Tanks were completely drained and cleaned every week with chlorine and detergent.

If mortalities occurred these were removed. In order to prevent potential increased growth rates in seahorses with increased access to food through the loss of competing tank-mates, dead seahorses were replaced with identifiable (i.e. unusually slender bodies or long snouts) seahorses of the same size in order that original stocking densities were maintained. Replacement seahorses were not included in final analyses.

Each week, uneaten mysids and *A. salina* from a single feed ration were siphoned from three tanks in each treatment at 1500-h. The wet weight of these mysids and *Artemia* was recorded and they were then placed on pre-weighed filter papers and dried at 60°C for 24 h. Dry weight measurements were used to calculate the proportion of food being offered that was consumed in relation to the calculated dry weight of seahorses (based on dry weight = 24% of wet weight of *H. abdominalis* if dried at 60°C for 48 hours, Woods 2002). Three months with weekly uneaten ration sampling gave 12 consumption measurements per tank. These were then averaged within tanks to derive one average consumption measure and this was then compared against measured final mean weight increase for each tank; this result was then averaged for each treatment. The feed conversion ratio (FCR) is $CC/(W_f-W_i)$, where CC = mean cumulative dry weight of food consumed, W_f = mean final seahorse calculated dry weight per replicate tank, and W_i = mean initial seahorse calculated dry weight per replicate tank. The FCR results were presented as ratios (dry weight (g) of food required to increase dry seahorse weight by 1 g, e.g. 1:1).

The experiment was concluded after 90 days. Final seahorse SL and wet weight were recorded. Mean specific growth rate (SGR% increase in body weight day^{-1}) for each tank's seahorses was calculated after 90 days.

Mean water parameters during the experiment were: temperature 18.3 ± 0.01°C (range 17.8–18.5°C), dissolved oxygen 7.6 ± 0.1 mg l^{-1} (7.09–8.1 mg l^{-1}), salinity 34 ± 0.1 ppt (33.6–34.33 ppt), and pH 8.1 ± 0.01 (8.05–8.2).

5.2.2.4 Effect of mysid ration

Seventy-two captive-bred eleven month-old *H. abdominalis* (n = 36 males, 36 females, mean ± 1 SE SL = 124.8 ± 1.05 mm, wet weight = 4.16 ± 0.1 g, CF = 0.19 ± 0.003) were randomly allocated to one of four daily feeding rations: 1) 5%, 2) 10%, 3) 15%, or, 4) 20% of seahorse wet body weight fed as frozen mysids (*A. kempi*). The daily ration of frozen mysids required was thawed in seawater prior to being fed to the seahorses.

There were three replicate tanks randomly assigned per treatment, with six seahorses per tank, and three male and three female seahorses per tank. There were no significant differences in seahorse SL or wet weight between treatments, and within or between sexes or replicates at the start of the experiment (ANOVA, $P>0.05$).

Seahorses were rendered individually identifiable by injecting Visible Implant fluorescent Elastomer (VIE) marks into different segments of the anterior-most left lateral tail segments (Fig. 5.3). VIE has been proven to have extremely high retention and visibility in this body location of *H. abdominalis* without significant impact upon seahorse growth or survival (Woods & Martin-Smith, 2004).

Seahorses were fed twice, at 0900 and 1400-h, each day with each treatment's feed ration divided equally amongst these two feedings (e.g. 5% feed ration = 2.5% at each feeding), except for the weekends when only one feed was conducted. Uneaten food and faeces were siphoned away from each tank each morning before feeding.
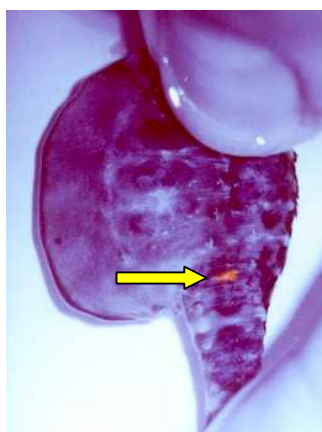
Figure 5.3 VIE-tagged *Hippocampus abdominalis* seen under blue LED illumination, with the VIE mark indicated by the yellow arrow (source: Chris Woods)

Monthly censuses were conducted to measure growth (SL and wet weight) as in the previous experiment. Wet weights were conducted 24 h after the last access of seahorses to food. Following each census, the daily wet weight ration of each ration treatment was adjusted according to average seahorse wet weight increase for each treatment to maintain the treatment rations (i.e. monthly ration adjustment).

Seahorses were maintained in 23-l translucent white low-density polyethylene tanks (40 cm high x 30 cm wide). To aid in visual detection of the pale mysids by the seahorses, the tanks were tinted blue with the external application of Plasti-kote® T-35 Astro Blue spray paint on the side and bottom of the tanks. The tanks were aerated and supplied with flow-through ambient seawater filtered to 20 μm at a flow rate of 0.25 l min^{-1}.

Holdfasts for the seahorses to attach to were provided through synthetic aquarium plants (Red Ludwigia and Corkscew Val).

A photoperiod of 12 h L:12 h D was provided by timer-controlled 6 x 55W cool white fluorescent tubes above the tanks at an of 11.4 µmol photons m^{-2} s^{-1} at the water surface. Tanks were completely drained and cleaned every two weeks with chlorine and detergent.

The treatment of any mortalities and weekly calculation of FCR for each treatment were conducted as in the *Artemia* vs. mysid experiment (section 5.2.2.3) except that any replacement seahorses were also VIE-tagged seahorses. These replacement seahorses were not included in any final analyses.

The experiment was concluded after 90 days. Final seahorse SL and wet weight were recorded. SGR and CF were then calculated for each individual seahorse.

Mean water parameters during the experiment were: temperature 15.9 ± 0.05°C (range 14.1–17.6°C), dissolved oxygen 9.5 ± 0.5 mg l^{-1} (9.2–9.9 mg l^{-1}), ammonia 0.07 ± 0.01 mg l^{-1} (0.0–0.1 mg l^{-1}), salinity 35.18 ± 0.05 ppt (34.8–35.4 ppt), and pH 8.14 ± 0.01 (8.03–8.18).

5.2.2.5 Statistical analyses

Statistical analyses were conducted as in 5.2.1.4, with the addition of testing for differences in FCR between replicate tanks (5.2.2.3) and individual seahorses (5.2.2.4) using Kruskal-Wallis ANOVA with post-hoc Tukey HSD ($P = 0.05$).

5.2.2.6 Cost/benefit analysis of mysid ration

To determine the relative value of each of the four feed rations used and their resulting growth benefit for seahorses, a cost/benefit (CB) value was calculated for each ration at the conclusion of the experiment. The cost (per kg excl. shipping) of the frozen mysids procured at the time of their purchase (August 2001) from the commercial supplier was NZ$40 kg^{-1} (US$16.8 kg^{-1}). Using this cost and the growth rates obtained in this investigation, a cost/benefit (CB) value can be calculated for each ration as: CB (NZ$) = cost (NZ$) of frozen mysids offered per seahorse per day/mean unit increment (i.e. mm

or mg) increase per day. The CB was calculated based on both the total amount of mysids offered to the seahorses, and the amount of mysids actually consumed.

5.3 Results

5.3.1 Effect of *Artemia* enrichment

5.3.1.1 Fatty acid analysis of *Artemia*

Both similarities and marked differences in the lipid profiles of the enriched *A. salina* were observed (Table 5.1). For example, Algamac-3050® and Super Selco® enriched *A. salina* had the highest percentages of DHA, 22:6n-3 (Kruskal-Wallis ANOVA, $H_{3,12}$ = 9.97, $P<0.05$), while Super Selco® enriched *A. salina* had the highest percentage of EPA, 20:5n-3 (Kruskal-Wallis ANOVA, $H_{3,12}$ = 9.35, $P<0.05$). EPABSF/*S. platensis* enriched *A. salina* had the highest percentages of C18 FAs, particularly the EFA linoleic acid, 18:2n-6 (Kruskal-Wallis ANOVA, all $P<0.05$). There were no significant differences in percentage composition of AA, 20:4n-6 (Kruskal-Wallis ANOVA, $P>0.05$). Percentages of polyunsaturated fatty acids (PUFA) in enriched *A. salina* were highest in Super Selco® and Algamac-3050® enriched *A. salina* (Kruskal-Wallis ANOVA, $H_{3,12}$ = 9.26, $P<0.05$). Percentages of n-3 HUFA were highest in Super Selco® and Algamac-3050 enriched *A. salina* (Kruskal-Wallis ANOVA, $H_{3,12}$ = 9.7, $P<0.05$).

Table 5.1 Fatty acid composition (%) of *Artemia salina* enriched for 24 h at 28°C. Data are the mean ± 1 SE of triplicate samples. Statistical comparison of some major fatty acids (%) as outlined in Materials and Methods: row values with different superscripts are significantly different (*P*<0.05).

Fatty acid	Super Selco[®]	DHA Protein Selco[®]	Algamac-3050[®]	EPABSF/*S. platensis*
14:0	0.89 ± 0.34	0.98 ± 0.37	2.5 ± 0.38	0.99 ± 0.38
16:0	10.86 ± 1.33	15.03 ± 1.4	16.43 ± 1.16	13.2 ± 1.28
16:1*n*-9	0.73 ± 0.07	0.79 ± 0.1	0.53 ± 0.08	1.29 ± 0.14
16:1*n*-7	2.59 ± 0.38	5.07 ± 0.8	2.32 ± 0.4	2.89 ± 0.49
18:0	6.52 ± 0.42	7.52 ± 0.04	6.14 ± 0.12	6.76 ± 0.25
18:1*n*-9	21.31± 2.22[ab]	25.5 ± 2.44[ab]	16.26 ± 2.74[b]	29.36 ± 2.41[a]
18:1*n*-7	5.97 ± 0.61	6.03 ± 0.13	5.08 ± 0.33	5.59 ± 0.09
18:2*n*-6	11.76 ± 2.13 [ab]	14.43 ± 2.72 [ab]	9.82 ± 2.68 [b]	23.11 ± 2.88 [a]
18:3*n*-3	0.93 ± 0.06 [ab]	1.33 ± 0.09[a]	0.65 ± 0.13[b]	1.1 ± 0.14[a]
20:4*n*-6	3.25 ± 0.75[ns]	2.75 ± 0.62 [ns]	5.56 ± 0.91 [ns]	2.47 ± 0.74 [ns]
20:5*n*-3	17.81 ± 1.37[a]	10.01 ± 0.9[b]	10.56 ± 1.41[bc]	5.4 ± 1.58[c]
22:5*n*-3	1.37 ± 0.19	0.66 ± 0.08	0.61 ± 0.1	0.07 ± 0.07
22:6*n*-3	8.21 ± 1.06[a]	3.84 ± 1.17[b]	12.82 ± 1.24[a]	1.68 ± 1.03[b]
DHA:EPA	0.46	0.38	1.21	0.31
Oleic acid:DHA	2.6	6.64	1.27	17.48
EPA/AA	5.48	3.64	1.9	2.19
% known	95.29 ± 0.35	95.55 ± 0.24	90.74 ± 0.46	94.87 ± 0.43
% PUFA	44.23 ± 1.99[a]	33.62 ± 0.53[b]	41.04 ± 0.5[a]	34.02 ± 0.55[b]
% *n*-3 HUFA	29.23 ± 2.71[a]	16.44 ± 2.09[b]	25.66 ± 2.18[ab]	8.42 ± 2.67[c]
% MUFA	32.56 ± 2.31	38.12 ± 2.05	24.36 ± 2.43	39.63 ± 2.08
% SFA	18.5 ± 2.07	23.81 ± 1.76	25.34 ± 1.63	21.22 ± 1.66
% lipid DW	13.5	10.8	13.1	8.9
% lipid WW	0.87	0.65	0.68	0.45

5.3.1.2 Proximate analysis of *Artemia*

Artemia salina from all four enrichment treatments had similar (non-significantly different, Kruskal-Wallis ANOVA, *P*>0.05) wet and dry percentage composition of protein, fat, carbohydrate, ash and moisture (Table 5.2).

Table 5.2 Proximate analysis (%) of *Artemia salina* enriched for 24 h at 28°C. Figures in brackets () are calculated dry weight percentages. Statistical comparison as outlined in Materials and Methods: row values with different superscripts are significantly different (*P*<0.05).

Component	Super Selco®	DHA Protein Selco®	Algamac-3050®	EPABSF/*S. platensis*
Protein (%)	3.8 (57.6)	4.1 (56.9)	3.9 (58.2)	4.1 (53.2)
Fat (%)	0.76 (11.5)	0.8 (11.1)	0.77 (11.5)	0.73 (9.5)
Carbohydrate (%)	0.34 (5.2)	0.7 (9.7)	0.33 (4.9)	1.27 (16.5)
Ash (%)	1.7 (25.8)	1.6 (22.2)	1.7 (25.4)	1.6 (20.8)
Moisture (%)	93.4	92.8	93.3	92.3

5.3.1.3 Seahorse growth and survival

After three months there was a significant difference in juvenile SL between the enrichment treatments (ANOVA, $F_{3,144}$ = 6.39, *P*<0.01) (Table 5.3), with juveniles in the DHA Protein Selco® and Algamac-3050® treatments longer than those juveniles in the Super Selco® treatment, but not longer than juveniles in the EPABSF/*S. platensis* treatment (Tukey HSD, *P*<0.05). Based on mean standard length increases for each treatment, this equates to calculated daily length increases of 0.33 mm for Super Selco®, 0.41 mm for DHA Protein Selco®, 0.40 mm for Algamac-3050®, and 0.36 mm for the EPABSF/*S. platensis* enrichment.

There was also a significant difference in juvenile wet weight between the treatments (ANOVA, $F_{3,144}$ = 12.76, *P*<0.001) (Table 5.3), with juveniles in the DHA Protein Selco®, Algamac-3050®, and EPABSF/*S. platensis* treatments all significantly heavier than juveniles from the Super Selco® treatment (Tukey HSD, *P*<0.01). Based on mean standard wet weight increases for each treatment, this equates to calculated daily weight increases of 1.83 mg for Super Selco®, 2.63 mg for DHA Protein Selco®, 2.65 mg for Algamac-3050®, and 2.45 mg for the EPABSF/*S. platensis* enrichment.

In terms of CF, there was a significant difference between the treatments (ANOVA, $F_{3,144}$ = 4.4, *P*<0.05) (Table 5.3), with juveniles in the DHA Protein Selco® and EPABSF/*S. platensis* treatments having higher CF's than juveniles in the Super Selco® treatment, but not the Algamac-3050® treatment (Scheffe, *P*<0.05).

Comparison of mean daily SGR revealed a significant difference between the treatments (Kruskal-Wallis ANOVA, $H_{3,16}$ = 8.14, *P*<0.05) (Table 5.3), with juveniles in the Super

Selco® treatment having a lower daily SGR than the other three treatments (Tukey HSD, $P<0.05$).

No mortalities were recorded in any of the enrichment treatments.

Table 5.3 Final standard length (SL mm), wet weight (g), Condition Factor (CF) and mean SGR (% increase in body weight day^{-1}) of juvenile *Hippocampus abdominalis*. Values are mean ± 1 SE. Column values with different superscripts are significantly different ($P<0.05$).

Enrichment	SL (mm)	Weight (g)	CF	SGR
Super Selco®	99.2 ± 1.5^c	2.20 ± 0.1^b	0.224 ± 0.006^b	0.40 ± 0.02^b
DHA Protein Selco®	105.4 ± 1.5^{ab}	2.96 ± 0.1^a	0.244 ± 0.006^a	0.47 ± 0.01^a
Algamac-3050®	107.4 ± 1.2^{ab}	2.98 ± 0.12^a	0.231 ± 0.007^{ab}	0.45 ± 0.02^a
EPABSF/*S. platensis*	103.3 ± 1.6^{bc}	2.80 ± 0.14^a	0.246 ± 0.008^a	0.42 ± 0.01^a

5.3.1.4 Cost/benefit analysis of enrichment

Marked differences in cost/benefit ratio for each treatment at the conclusion of the experiment were observed (Table 5.4). The EPABSF/*S. platensis* enrichment was the most cost effective enrichment (lowest CB) for seahorse length and seahorse weight increase per day, followed in order by Algamac-3050®, DHA Protein Selco®, and then Super Selco®.

Table 5.4 Cost/benefit (CB) value for each enrichment used: CB (NZ$) = cost of actual enrichment used (NZ$) seahorse^{-1} day^{-1}/mean unit increment (i.e. mm or mg) increase day^{-1}.

Variable	Super Selco®	DHA Protein Selco®	Algamac-3050	EPABSF/*S. platensis*
CB mm day^{-1}	$0.217	$0.176	$0.069	$0.012
CB mg day^{-1}	$0.038	$0.033	$0.009	$0.0009

5.3.2 Use of frozen mysids

5.3.2.1 Fatty acid analysis of mysids

Marked differences in fatty acid profiles between the *A. salina* and mysids were observed (Table 5.5). Enriched *Artemia* had the highest percentages of oleic acid, 18:1n-9, and linoleic acid, 18:2n-6 (Kruskal-Wallis ANOVA, $H_{1,5}$ = 4.35, $P<0.05$ for both), but a lower percentage of linolenic acid, 18:3n-3 (Kruskal-Wallis ANOVA, $H_{1,5}$ = 4.5, $P<0.05$). Mysids had a higher percent composition of both EPA, 20:5n-3 (Kruskal-Wallis ANOVA, $H_{1,5}$ = 3.97, $P<0.05$), and DHA, 22:6n-3 (Kruskal-Wallis

ANOVA, $H_{1,5}$ = 3.97, $P<0.05$), but a lower composition of AA, 20:4n-6 (Kruskal-Wallis ANOVA, $H_{1,5}$ = 4.35, $P<0.05$). Percent n-3 HUFA and total PUFA in mysids were significantly greater than that of *Artemia* (Kruskal-Wallis ANOVA, $H_{1,5}$ = 3.97, $P<0.05$ and $H_{1,5}$ = 4.1, $P<0.05$, respectively). Both DHA:EPA and oleic acid:DHA ratios were higher in enriched *Artemia*, although their EPA/AA ratios were lower than that of the mysids, which were particularly high.

Table 5.5 Fatty acid composition (%) of *Artemia salina* enriched for 24 h at 28°C on Algamac-3050®, and frozen mysids (*Amblyops kempi*). Data are the mean ± 1 SE of 3 samples. Statistical comparison of some major fatty acids (%) as outlined in section 6.2.3: row values with different superscripts are significantly different ($P<0.05$).

Fatty acid	*Artemia*	Mysids
14:0	2.5 ± 0.38	2.28 ± 0.09
16:0	16.43 ± 1.16	23.97 ± 0.2
16:1n-9	0.53 ± 0.08	0
16:1n-7	2.32 ± 0.4	4.15 ± 0.06
18:0	6.14 ± 0.12	2.51 ± 0.02
18:1n-9	16.26 ± 2.74[a]	7.12 ± 0.09[b]
18:1n-7	5.08 ± 0.33	3.59 ± 0.06
18:2n-6	9.82 ± 2.68 [a]	1.18 ± 0.03[b]
18:3n-3	0.65 ± 0.13[a]	1.41 ± 0.04[b]
20:4n-6	5.56 ± 0.91[a]	0.84 ± 0.01[b]
20:5n-3	10.56 ± 1.41[a]	22.6 ± 0.19[b]
22:5n-3	0.61 ± 0.1	0.68 ± 0.01
22:6n-3	12.82 ± 1.24[a]	22.76 ± 0.14[b]
DHA:EPA	1.21	1.01
Oleic acid:DHA	1.27	0.31
EPA/AA	1.9	26.9
% known	90.74 ± 0.46	96.17 ± 0.05
% n-3 HUFA	25.66 ± 2.18[a]	49.07 ± 0.3[b]
% PUFA	41.04 ± 0.5[a]	51.29 ± 0.29[b]
% MUFA	24.36 ± 2.43	15.73 ± 0.11
% SFA	25.34 ± 1.63	29.15 ± 0.42
% lipid DW	8.89	9.13
% lipid WW	0.53	0.75

5.3.2.2 Proximate analysis of mysids

Overall, enriched *A. salina* and frozen mysids had similar wet and dry percentage composition of protein, fat, carbohydrate, ash and moisture (Table 5.6). Although, the

frozen mysids appeared to have a higher percentage of carbohydrate and ash with lower moisture content this was non-significant at the very low level of replication (Kruskal-Wallis ANOVA, $P>0.05$).

Table 5.6 Proximate composition (%) of *Artemia salina* enriched for 24 h at 28°C on Algamac-3050®, and frozen mysids (*Amblyops kempi*). Data are the mean of duplicate subsamples. Figures in brackets () are dry matter percentages.

Component	*A. salina*	Mysids
Protein (%)	3.9 (58.2)	6.5 (58.7)
Fat (%)	0.77 (11.5)	0.86 (7.8)
Carbohydrate (%)	0.33 (4.9)	0.64 (5.8)
Ash (%)	1.7 (25.4)	3.1 (27.9)
Moisture (%)	93.3	88.9

5.3.2.3 Seahorse growth and survival fed live *A. salina* vs. frozen mysids

After three months, there were no significant differences in seahorse SL, wet weight, or CF between the treatments (ANOVA, $P>0.05$) (Table 5.7) (Figs 5.4 & 5.5). As a secondary test, Kruskal-Wallis ANOVAs ($P = 0.05$) were performed on the mean differences in initial and final SL, wet weights, and CFs per replicate tank for each treatment to determine if there were any proportional differences; there were no significant differences.
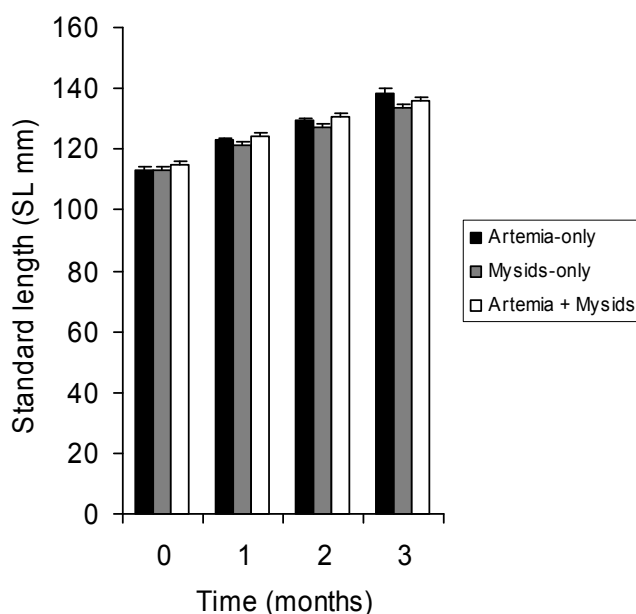
Figure 5.4 Increase in mean ± 1 SE standard length (mm) of *Hippocampus abdominalis* fed *Artemia salina*-only, frozen mysids-only, or *A. salina* + frozen mysids, at 25% body weight daily ration for 90 days.
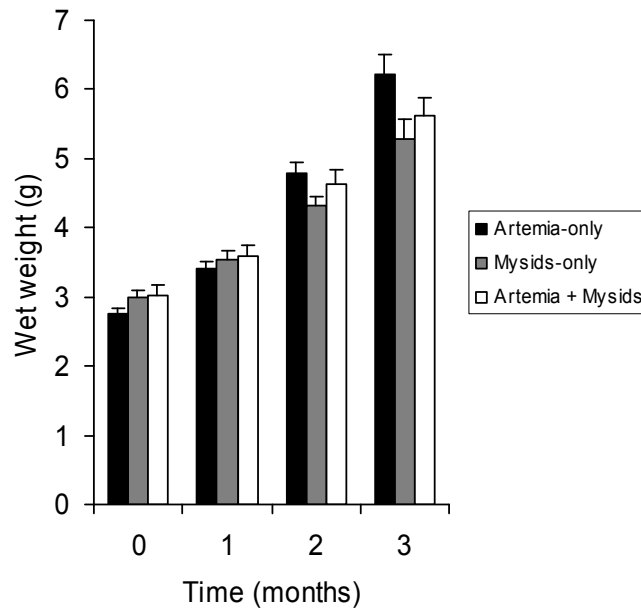
Figure 5.5 Increase in mean ± 1 SE wet weight (g) of *Hippocampus abdominalis* fed *Artemia salina*-only, frozen mysids-only, or *A. salina* + frozen mysids, at 25% body weight daily ration for 90 days.

Comparison of mean daily SGR revealed significant differences between the treatments (Kruskal–Wallis ANOVA, $H_{2,17} = 11.2$, $P<0.01$), with seahorses in the *A. salina*-only treatment having a higher SGR than in the mysid only treatment and the *A. salina* + mysid treatment (Tukey HSD, $P<0.05$). It is possible that non-significant differences in initial and final wet weights may have become compounded in calculating SGR to derive a significant result, in comparison to the non-significant differences in length and wet weight by themselves.

Collection of uneaten food over the course of the experiment revealed that all *A. salina* presented to the seahorses were consumed within the 6 h feeding period, both in the *A. salina*-only treatment and the *A. salina* + mysid treatment. Fine mesh screens (250 μm) on the tanks ensured retention of the size of *Artemia* used. In the mysid only treatment, mean percent consumption was 54.5 ± 3.1%, while in the *A. salina* + mysid treatment mean percent consumption of mysids was 48 ± 4.2%.

Calculation of mean FCR revealed significant differences between the treatments (Kruskal-Wallis ANOVA, $H_{2,17} = 7.15$, $P<0.05$), with seahorses in the *A. salina*-only treatment having a lower FCR (more efficient) than in the mysid-only treatment, but not the *A. salina* + mysid treatment (Tukey HSD, $P<0.05$) (Table 5.7).

In terms of survival, there were no differences in percent survival (Kruskal-Wallis ANOVA, $P>0.05$), with only one mortality in the *A. salina* + mysid treatment. One replicate tank (five seahorses) in the mysid-only treatment was lost in the third month of the experiment when the water inflow line was accidentally knocked out overnight. As this was not related to the experimental treatments this loss was not included in the survival analysis.

Table 5.7 Final measurements of Condition Factor (CF), percent survival, mean specific growth rate (SGR), and mean food conversion ratio (FCR) of *Hippocampus abdominalis* fed *Artemia salina* only, frozen mysids only, or *A. salina* + frozen mysids, at 25% body weight daily ration for 90 days. Rows with different superscripts are significantly different ($P<0.05$).

	Artemia-only	Mysids-only	*Artemia* + mysids
CF	0.2 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
% survival	100	100	96.7 ± 3.7
SGR	0.97 ± 0.03^a	0.69 ± 0.05^b	0.74 ± 0.03^b
FCR	$6.14{:}1^a$	$8.4{:}1^b$	$7.3{:}1^{ab}$

5.3.2.4 Effect of mysid ration

After three months, there were no significant differences between feed ration treatments SL (ANOVA, $P>0.05$), with seahorses increasing from a mean $\pm$ 1 SE SL of 124.8 ± 1.05 mm to 147.3 ± 1 mm (Fig. 5.6). As a secondary test, a one-way ANOVA ($P = 0.05$) was performed on the differences in initial and final SL for each treatment to determine if there were any proportional differences; there were no significant differences. There was a significant difference in weight between feed rations (ANOVA, $F_{3,72} = 8.32$, $P<0.01$), with seahorses in the 10–20% feed ration treatments significantly heavier than those in the 5% treatment after three months (Tukey HSD, $P<0.05$) (Fig. 5.7). The weight difference between treatments also resulted in significant differences in CF (ANOVA, $F_{3,72} = 5.7$, $P<0.01$) (Table 5.8), with the seahorses fed the 10–20% rations having significantly higher CFs than seahorses in the 5% ration after three months, but not the 10% ration (Scheffe, $P<0.05$). There were no intersexual differences in final seahorse length, weight or CF (ANOVA, $P>0.05$).
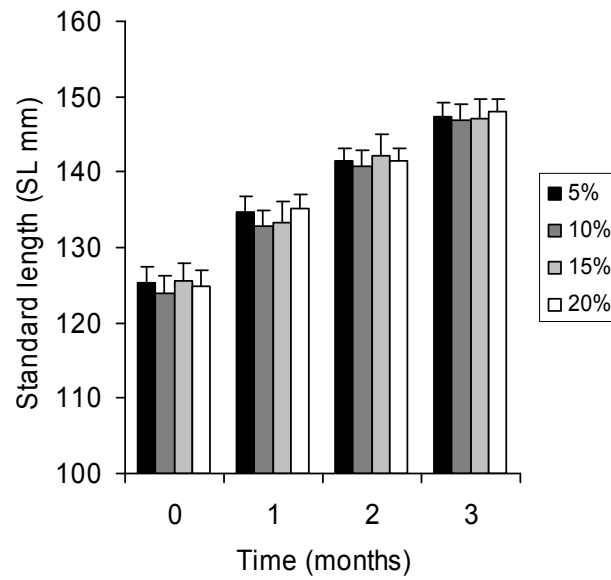
Figure 5.6 Increase in mean ± 1 SE standard length (mm) of *Hippocampus abdominalis* fed rations of 5, 10, 15 and 20% wet body weight of frozen mysids once-daily.
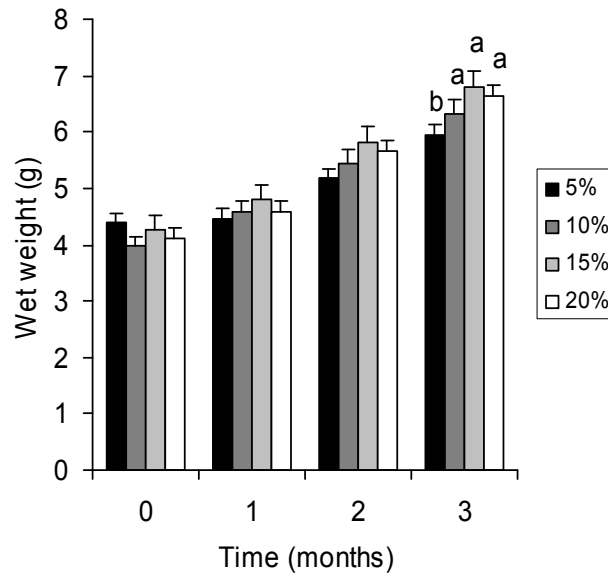
Figure 5.7 Increase in mean ± 1 SE wet weight (g) of *Hippocampus abdominalis* fed rations of 5, 10, 15 and 20% wet body weight of frozen mysids once-daily. For final measurements (Month 3), different letters denote significant differences between treatments (*P*<0.05).

Table 5.8 Final Condition Factor (CF), SGR (% increase in body weight day^{-1}), and dry weight feed conversion ratio (FCR) for cultured *Hippocampus abdominalis* fed four feed rations (5, 10, 15, and 20% wet body weight once-daily), expressed as mean ± 1 SE. FCRs are given both for the total amount of mysids offered and the actual amount of mysids consumed. Feed rations are expressed as % wet body weight (bw). Column values with different superscripts are significantly different (*P*<0.05).

Ration	CF	SGR (%)	FCR offered	FCR actual
5% bw	0.15 ± 0.004[b]	0.35 ± 0.01[b]	7.45 ± 0.9:1[a]	7.4 ± 0.87:1
10% bw	0.17 ± 0.007[ab]	0.55 ± 0.02[a]	9.31 ± 0.83:1[a]	6.51 ± 0.58:1
15% bw	0.18 ± 0.007[a]	0.52 ± 0.06[a]	13.1 ± 1.42:1[b]	7.59 ± 0.82:1
20% bw	0.17 ± 0.004[a]	0.55 ± 0.03[a]	17.9 ± 1.45:1[c]	7.88 ± 0.64:1

Based on the total amount of mysids offered to each seahorse in the four feed rations, there was a significant difference in FCR (ANOVA, $F_{3,72}$ = 28.18, *P*<0.001), with the 5% and 10% rations having more efficient (lower) FCRs than the 15–20% rations (Table 5.8). However, there was an increasing wastage of mysids associated with increasing feed ration (the more mysids that were supplied the greater the proportion of these that went uneaten). For the 5, 10, 15, and 20% feed rations the average % daily consumption of the mysid ration was 99.5, 70, 58.1, and 44.1%, respectively. Based on the actual amount of mysids consumed, there were no significant differences in FCRs between the treatments (ANOVA, *P*>0.05) (Table 5.8). There was a significant difference in SGR between the four feed rations (ANOVA, $F_{3,72}$ = 3.88, *P*<0.05), with the 5% feed ration having a lower SGR than the 10–20% feed rations (Tukey HSD, P<0.05) (Table 5.8).

5.3.2.5 Cost/benefit analysis of mysid ration

Calculation of the cost/benefit value (Table 5.9) between the different feed ration treatments showed that for both seahorse SL and wet weight, increasing feed ration did not result in a commensurate increase in length or weight that outweighed the actual cost of offering more mysids per seahorse per day based on the total amount of mysids offered. With actual mysid consumption taken into account in relation to the mean increase in SL and weight recorded for each treatment (Table 5.9), the cost/benefit value still appears to economically favour the 5% ration for length increase, and both the 5 and 10% feed rations over the 15 and 20% rations for weight increase. Survival was 100% across all treatments.

Table 5.9 Mean ± 1 SE increase in seahorse length and weight on which cost/benefit (CB) value for four feed rations tested for cultured *Hippocampus abdominalis* (5, 10, 15, and 20% wet body weight, bw, daily) is calculated: CB (NZ$) = cost (NZ$) of frozen mysids used per seahorse per day/mean unit increment (i.e. mm or mg) increase per day. Values are calculated both for the total amount of mysids offered, and the actual amount of mysids consumed in each treatment.

	5% bw	10% bw	15% bw	20% bw
Increase in length (mm)	22.4 ± 0.93	22.8 ± 0.93	21.6 ± 0.87	23.2 ± 0.87
CB mm day^{-1} offered	$0.037	$0.074	$0.116	$0.154
CB mm day^{-1} actual	$0.037	$0.051	$0.068	$0.068
Increase in weight (g)	1.62 ± 0.1	2.5 ± 0.12	2.53 ± 0.21	2.58 ± 0.1
CB mg day^{-1} offered	$0.052	$0.067	$0.099	$0.139
CB mg day^{-1} actual	$0.052	$0.047	$0.058	$0.061

5.4 Discussion

5.4.1 Effect of *Artemia* enrichment

5.4.1.1 Fatty acid and proximate composition of enriched *A. salina*

Lipids are important sources of metabolic energy, are essential components of cell membranes, serve as carriers for fat-soluble vitamins, and are the source of EFAs. Fatty acids are necessary for maintaining cell membrane integrity, lipid transport, pigmentation and are the building blocks for many hormones (Sargent *et al.*, 1997; Copeman & Parrish, 2002, Brandsen *et al.*, 2005; Villalta *et al.*, 2005a). The EFAs for fish are considered to comprise polyunsaturated fatty acids (PUFA) with carbon chain lengths of 18 and highly unsaturated fatty acids (HUFA) with carbon length chains of 20 and 22, of both *n*-3 and *n*-6 series. These FAs cannot be synthesized do novo by fish, though some fish can convert C-18 PUFA into longer chain fatty acids of the same series (Sargent *et al.*, 1998). There appear to be major differences between freshwater and marine fishes, where freshwater fish require either linoleic acid (LA) or linolenic (ALA) acid, or both, and marine fish require either DHA or EPA, or both (Sargent *et al.*, 1997). According to Sargent *et al.* (1997), marine fish naturally contain high levels of DHA and EPA, therefore they have high nutritional requirements for these fatty acids, as well as for other fatty acids considered to be essential for fish growth (e.g. AA and ALA) (Sargent *et al.*, 1997; Corraze, 2001; Brandsen *et al.*, 2005). For example, Watanabe *et al.* (1989) found that juvenile striped jack (*Pseudocaranx dentex*) fed EFA-deficient diets suffered higher mortality and slower growth than those fed diets containing EPA and DHA. Consequently, research on enriching *Artemia* has concentrated on increasing their content of the FAs DHA, EPA and AA (Han *et al.*,

2001; Ritar *et al.*, 2004). However, the relative importance of particular FAs can vary between fish species. For example, Villalta *et al.* (2005b) found that Senegal sole (*Solea senegalensis*) larvae required negligible amounts of DHA for normal development and related this to the predominance of EPA rather than DHA in the benthic fauna this species normally consumes. In contrast, other flatfish such as yellowtail flounder (*Limanda ferruginea*) with longer larval stages than *S. senegalensis* need high exogenous supplies of DHA (Copeman *et al.*, 2002).

In the three enriching and one on-growing media treatments, the Algamac-3050® and Super Selco® enriched *A. salina* had the highest percentages of DHA, while Super Selco® enriched *A. salina* had the highest percentage of EPA. There were no significant differences in percentage composition of AA. Percentages of polyunsaturated fatty acids (PUFA) were highest in Super Selco® and Algamac-3050® enriched *A. salina*, as were percentages of *n*-3 HUFA. On a simplistic "more is better" basis, overall the Super Selco® and Algamac-3050 enriched *A. salina* with their higher content of EFAs might be expected to promote better growth and survival in *H. abdominalis* (although at some threshold level the effect of increasing the amount of certain EFAs may become negative).

However, a major problem in determining the effects of various HUFA in fish is that the requirement for any given HUFA is determined not only by its absolute amount in the diet, but also by the absolute amounts of other HUFA in the diet due to such factors as competitive inhibitions (Rainuzzo *et al.*, 1997; Sargent *et al.*, 1999; Corraze, 2001). For example, EPA competes with AA for the same metabolic enzymes. The DHA:EPA ratio has been particularly focused on in the past decade (Izquierdo, 1996; Rainuzzo *et al.*, 1997; Rodríguez *et al.,* 1997; Sargent *et al.*, 1997; Gapasin & Duray, 2001; Copeman *et al.*, 2002). Ibeas *et al.* (1997) found that best growth performance in juvenile sea bream (*S. aurata*) occurred with an EPA/DHA ratio of 2:1, while Copeman *et al.* (2002) found growth and survival in larval yellowtail flounder (*L. ferruginea*) was higher when the fish were fed rotifers with a DHA:EPA ratio of 8.2:1 compared to rotifers with a ratio of 1.9:1. The lowest DHA:EPA (at different *n*-3 percentage composition) ratio (0.31:1) in this investigation occurred in EPABSF/*S. platensis* on-grown *Artemia*, while the highest (1.21:1) occurred in the Algamac-3050® enriched *Artemia*. In her studies with eggs and newborn of *H. abdominalis*, Thompson (2002) found the best performing enrichment diets contained DHA:EPA ratios <1:1, and postulated that seahorses may have a lower

DHA:EPA target ratio than most of the other marine fish larvae studied to date. This appears to be supported by indicative sampling of some of the natural prey of *H. abdominalis* (see Chapter 6).

Other EFA ratios focused on in marine finfish nutrition include oleic acid (18:1(*n*-9)):DHA (Takeuchi *et al.*, 1996; Furuita *et al.*, 1999), and EPA:AA (Sargent *et al.*, 1997). Oleic acid:DHA ratios in this investigation varied between 1.27:1 and 17.48:1, while EPA:AA ratios varied between 1.9:1 and 5.5:1. Thompson (2002) found newborn *H. abdominalis* had a EPA:AA ratio of 0.72:1 while eggs had a ratio of 6.81:1 and postulated that ideal diets for *H. abdominalis* should contain elevated levels of AA. In this study, the Algamac-3050® enriched *A. salina* possessed the most "traditionally" favourable EPA:AA ratio of 1.9:1. However, concentrating on single EFA ratios may be too simplistic, as there may be complex interactions and interspecific differences between multiple EFA indices (Sargent *et al.*, 1997; Furuita *et al.*, 1999).

In addition to lipids, growth, body composition, feed utilization and survival in fish is closely associated with the protein and energy content of their feed. For most omnivorous or carnivorous marine fish studied to date, approximate nutrient requirements on a dry weight basis are: protein 40–60%, carbohydrate 10-20%, lipid 10–20%, fibre 4%, and ash 10–25% (e.g. de Silva & Anderson, 1995; Morais *et al.*, 2001; Watanabe *et al.*, 2001; Skalli *et al.*, 2004; Usman *et al.*, 2005). For example, for seabass (*Lates calcarifer*) fingerlings percentage dietary inclusions of 40–45%, 15–18%, and <20% for protein, lipids and carbohydrate respectively are recommended (Boonyaratpalin, 1997). However, within these general requirements there are often interspecific and ontogenetic differences and requirements outside these ranges, as well as interactive effects between different levels of each dietary component (e.g. protein-sparing effects by carbohydrate or lipid level: Skalli *et al.*, 2004; Kim & Lee, 2005). An example of ontogenetic proximate dietary differences is that of Atlantic halibut (*Hippoglossus hippoglossus*), where Hatlen *et al.* (2005) found that small (60 g) halibut experienced greater growth with greater food conversion on a diet with 56% protein compared with a diet containing 41% protein, but that in larger fish (800 g) this effect did not occur.

The dry proximate percent composition of the enriched *A. salina* in this investigation appears to fall within general marine fish nutritional requirements, except for the

carbohydrate levels in the Super Selco[®], DHA Protein Selco[®] and Algamac-3050[®] enriched *A. salina*, which were lower than 10%. All the enriched *A. salina* in this investigation had similar (non-significantly different) percentage compositions of protein, ash and moisture, although EPABSF/*S. platensis* enriched *A. salina* appeared slightly non-significantly lower in percentage fats and higher in carbohydrates. This is interesting given differences in some of these components within the enrichments themselves. For example DHA Protein Selco[®] contains approximately 27% crude protein, whereas Super Selco[®] contains no measurable protein (http://www.inve.com; accessed 28/04/06). This indicates that although enrichment for 24 h with the four different products was sufficient to alter the EFA levels, the basic proximate composition of *A. salina* remained largely unaltered by this enrichment, and, therefore unlikely to have influenced growth and survival in the different enriching treatments.

Most of the research conducted on the effects of different protein and energy levels in marine fish has been conducted on species cultured for human consumption, where the emphasis is on increasing flesh/muscle quality and quantity (e.g. Chou *et al.*, 2001; Watanabe *et al.*, 2001; Hatlen *et al.*, 2005; Ozorio *et al.*, 2006; Wang *et al.*, 2006). However, given the morphology of seahorses, with their extensive bony body structure, lack of large muscle fillets (apart from inside their muscular prehensile tail, Thomson, 1993), and relatively sedentary lifestyle, the relative importance of protein, carbohydrates and fats in seahorse diets may be quite different from most food fish. Nonetheless, the ideal absolute levels and relative proportions of protein, carbohydrates and fats should still be determined for seahorses for the optimization of growth and survival. Determining proximate dietary requirements for seahorses is also important with regards to the economical efficiency of commercial culture particularly if inert diets are to be developed, as the greatest ingredient cost is often incurred by meeting the protein and energy specification of the diet (de Silva & Anderson, 1995; Kim & Lee, 2005; Usman *et al.*, 2005).

For the commercial aquaculturist, the focus of which enrichment medium is best suited to its particular cultured species often lies not in the resulting biochemical composition of its prey and that prey's theoretical biochemical value in relation to other cultured species, but which enrichment promotes best growth and survival relative to its cost.

5.4.1.2 Seahorse growth and survival

In this investigation, the use of three commercial *Artemia* enrichment products (Super Selco®, DHA Protein Selco®, and Algamac-3050®) and a cheap *Artemia* on-growing diet used as an enrichment were shown to promote growth (with no mortality) in cultured juvenile *H. abdominalis* fed solely on live *A. salina* enriched with these media. Super Selco® enriched *Artemia* produced the lowest growth therefore the alternative hypothesis (H$_1$: Juvenile seahorse growth and survival are different when fed on *Artemia* enriched with different media) is accepted.

In commercial aquaculture, in addition to any differences in growth and survival obtained in marine fish after enriching *Artemia* with an enrichment product such as one of those used in this investigation, the actual cost/benefit relationship of that enrichment product requires examination. In this investigation, the cheapest enrichment (EPABSF/*S. platensis*) was the most cost-effective for *H. abdominalis*, at the feeding rate used in this investigation, both for length and weight increase. The alternative hypothesis (H$_1$: there is a difference in the relative cost/growth benefit ratio between each enrichment media used) is accepted. Not only is this enrichment the most cost-effective, the growth rates obtained by the seahorses on the DHA Protein Selco® and Algamac-3050®, although slightly better than that of seahorses on EPABSF/*S. platensis*, were not statistically significantly better.

These results would seem to question the need for costly enrichment of *A. salina* following on-growing on the EPABSF/*S. platensis* mixture for juvenile *H. abdominalis*. This has important ramifications for the economical efficiency of the commercial culture of *H. abdominalis*. The sourcing of appropriate cheap plant-processing by-products from within New Zealand's existing pastoral industries could dramatically improve the economical efficiency of seahorse culture, compared with using more expensive imported conventional enrichments. For example, Tyler (1996) found that finely ground wheat pollard was a suitable food for *A. franciscana*, although *Artemia* growth was better when the wheat pollard was supplemented with the microalga *Dunaliella salina*. Job *et al.* (2002) also questioned the need for expensive imported proprietary *Artemia* enrichments, and tested the effect of using cheap (US$1.40 kg^{-1}) *Artemia* enrichments derived from locally sourced fish and shrimp (*Acetes* sp.) on juvenile growth and survival of the tropical seahorse *H. kuda*. They found growth of juvenile *H. kuda* was reasonably rapid (0.9–1.53 mm day^{-1}) and without difference

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when fed *Artemia* enriched with either fish or shrimp enrichments, but that juvenile survival was higher on the shrimp-enriched *Artemia*.

Thompson (2002) and Shapawi & Purser (2003) also examined the effect of varying *Artemia* enrichment on *H. abdominalis*. Thompson (2002) tested the effects of *A. salina* enrichment on juvenile seahorses up to 42 days of age. She found that *A. salina* enriched with fish oils (cod (*Gadus morhua*), squid (*Todarodes pacificus*) and hoki (*Macruronus novaezelandiae*)) produced better growth and survival than unenriched *A. salina*, and that seahorse survival and growth were greater for juvenile seahorses fed *A. salina* enriched with Super Selco® compared with *A. salina* enriched with hoki oil. The main EFA differences between in the *A. salina* enriched on these two diets was a greater amount of AA, EPA, DHA and total *n*-3 HUFA in the Super Selco® treatment. She also found that the different microalgae used to enrich *A. salina* also affect juvenile seahorse growth, with *A. salina* enriched with *Tetraselmis chui* and *Isochrysis galbana* exhibiting better growth and survival than juveniles fed *A. salina* enriched with *Dunaliella tertiolecta*.

Using four week-old juveniles with a feed rate of 5% body weight seahorse^{-1} day^{-1} on a dry weight feed to wet weight seahorse basis, Shapawi & Purser (2003) compared the commercial enrichments A1-Selco®, A1-DHA Selco®, Algamac-2000® and Algamac-3010®, with cultured live microalgae *Isochrysis galbana* (Tahitian strain) and *Pavlova lutheri* in a 1:1 mixture. They found enriched *A. franciscana* supported good growth and survival. They noted few differences in terms of growth and survival in seahorses fed *A. franciscana* enriched with the four commercial enrichments, although overall A1-Selco® and Algamac-3010® appeared marginally better. The commercial enrichments produced better growth than *A. franciscana* enriched with the mixed microalgae and unenriched *A. franciscana*.

Thompson (2002) found that juvenile *H. abdominalis* exhibited better growth and survival when fed *A. salina* that had been enriched for 24 h on Super Selco® compared with *A. salina* that had only been enriched for 4 h with this same feed, and that the fatty acid profile of the latter closely resembled that of unenriched *A. salina*. The benefit of longer enrichment periods is currently a contentious area of aquaculture research; there appears to be as a wide range in recommended enrichment durations as a function of a number of variables such as *Artemia* sp. strain, enrichment medium, target species,

bacterial load, enrichment vessel shape and aeration level, and temperature. For example, Narcisco *et al.* (1999) examined HUFA content and DHA:EPA improvements of *A. franciscana* with various commercial oils, with enrichment durations of between 9 to 48 h. They found that the *n*-3 HUFA content and DHA:EPA ratio increased after up to 33 h of enrichment. In contrast, McEvoy *et al.* (1995) observed catabolism of HUFAs at a duration of 19 h enrichment and recommended stopping enrichment at 18 h. Ritar *et al.* (2004) compared *Artemia* enriched on the microalga *Chaetoceros muelleri*, Algamac-3050® and a squid oil emulsion, for durations up to 36 h. They found that bacterial loading increased significantly for each enrichment treatment by 24 to 26 h, but that bacterial loading was lowest in the *C. muelleri* enrichment and highest in the Algamac-3050® enrichment where it caused major *Artemia* sp. mortalities. They also found that the EPA content in *Artemia* from all three enrichments increased with enrichment duration, but that DHA content of the *C. muelleri*-enriched *Artemia* did not. The enrichment period of 24 h as used here for all enrichments was chosen because it is the commonly used enrichment duration for *Artemia* (Sorgeloos *et al.*, 2001). However, it may be worthwhile investigating the optimal enrichment duration for each of the enrichment media used in this investigation.

This investigation dealt only with the effects of varying *A. salina* enrichment on growth and survival of *H. abdominalis*. There was no quantification or qualification of any internal or physiological variables which may have important long-term effects. For example, Brandsen *et al.* (2005) found that in larval striped trumpeter (*Latris lineata*) that although feeding rotifers enriched with varying levels of DHA produced differences in larval growth or survival, larvae fed rotifers enriched with lower levels of DHA exhibited poorer swimming ability and gut physiology indicative of affected lipid assimilation and transport ability. Therefore, a definitive conclusion as to the direct benefits of each of the enriching products used in this investigation cannot be made.

It is likely that the relative importance of different enrichments will vary with regards to the ontogeny and reproductive stage of the animal in question (Izquierdo, 1996). For example, in broodstock nutrition lipids are critically important sources of metabolic energy for gonad formation and for the formation of cell and tissue membranes (Watanabe *et al.*, 1984; Sargent, 1995). Harel *et al.* (1994) recommended a minimum of 0.42% HUFA in the diets of seabream (*Sparus aurata*) broodstock for good quality eggs, while Li *et al.* (2005) found that deficient or excess dietary *n*-3 HUFA in *Plectorhynchus cinctus* caused reduced spawning performance, with best spawning

performance (i.e. number of buoyant eggs and larvae surviving at three days post hatch) at *n*-3 HUFA content of 2.4 and 3.7%. Thompson's (2002) finding of relatively high amounts of AA in the eggs of *H. abdominalis* (8.2% of fatty acid content and a EPA:AA ratio of 6.81:1) would appear to indicate that this is an important FA in prenatal development of this species. Therefore, the EPABSF/*S. platensis* enriched *A. salina* which was slightly lower in total percent composition of lipids, and quite poor in some EFAs such as AA, might not be as effective in adult broodstock conditioning as it was in overall growth promotion.

Marine fish larvae also appear to have a higher requirement for *n*-3 HUFA levels than juveniles, possibly because larvae need *n*-3 HUFA primarily for metabolism and membrane construction in accordance with their higher growth rate and/or for the higher levels of DHA for neural development (Izquierdo, 1996). The *n*-3 HUFA requirements reported for larvae of various species of marine fish range from 0.3 to 39 g kg^{-1} on a dry weight basis (Izquierdo, 1996). As seahorses do not have a larval phase and their development is effectively complete (with appropriate organ development such as an open and well differentiated digestive tract (Filleul, 1996)) upon release from the male seahorse's brood pouch, their requirement for *n*-3 HUFA may be reduced. Thompson (2002) found that newborns had lower EPA and DHA content compared to the eggs, but with an elevated AA content in newborns, possibly indicating a requirement for this EFA at first feeding.

In addition to the effect of prey nutritional value on animal growth, prey densities can also affect growth rate by affecting assimilation efficiencies. For example, Werner and Blaxter (1980) noticed that in larval herring (*Clupea harengus*), which have a straight tube gut similar to that of seahorses and pipefish, increasing prey density was associated with decreasing state of prey digestion. Ryer and Boehlert (1983) found that in the pipefish *Syngnathus fuscus* gut evacuation rate was positively related with gut content. *Artemia* can be harder for fish to digest compared to other live prey such as copepods and, when prey densities are high, may actually pass through the digestive tract intact (Luizi *et al.*, 1999; Payne & Rippingale, 2000), and sometimes even still alive (Woods, pers. obs.). Although not qualified, as *H. abdominalis* was fed to excess in this investigation, it is likely that optimal prey digestion and absorption did not occur. This may mean that the full nutritional benefit of the various enrichments used was not realised.

This investigation did not seek to define which particular EFAs, ratio or absolute amounts are essential for optimal growth and survival in *H. abdominalis* (the primary objective was to compare the efficacy of some commonly used *Artemia* enrichments). That requires more refined study, with multivariate dose-response manipulations and biochemical analysis of target animal tissues (Sargent *et al.*, 1997). However, it is interesting to note that the EPABSF/*S. platensis* enriched *A. salina* had the lowest levels of many EFAs, as well as the lowest DHA:EPA ratio and greatest oleic acid:DHA ratio, yet *A. salina* enriched with EPABSF/*S. platensis* still promoted good seahorse growth with no mortalities. This suggests that, as far as EFA requirement for somatic growth is concerned, late-stage *H. abdominalis* juveniles do not require large amounts of EFA, or the classical ideal EFA indices as demonstrated for the larval stages of some marine fishes (Izquierdo, 1996; Coutteau, *et al.*, 1997; Sargent *et al.*, 1999). However, the results obtained in this study are only applicable to the age and size of the seahorses tested; nutritional requirements for seahorses are likely to vary with factors such as ontogeny and reproductive state, as has been demonstrated for other fish species (e.g. Izquierdo *et al.*, 2001; Plante *et al.*, 2007).

Both Thompson (2002) and Shapawi & Purser (2003) have questioned the need for high DHA:EPA ratios in their respective enrichment studies on *H. abdominalis*, although Chang & Southgate (2001) and Thompson (2002) maintain that newborn *Hippocampus* spp. do require a certain amount of *n*-3 HUFA and other EFAs to maximize growth and survival. Thompson (2002) speculated that juvenile seahorses may have lower dietary EFA requirements compared with other cultured marine teleosts due to their extensive prenatal development. Chang & Southgate (2001) recommended a dietary inclusion of >9.3 mg DHA g^{-1} dry weight, whilst Thompson (2002) recommended an inclusion of EPA and DHA at around 0.15% diet dry weight, AA at 0.2% diet dry weight, and *n*-3 HUFA content of 0.3 to 0.6% of diet dry weight. It is interesting to note that the Super Selco® treatment exhibited the lowest growth, but that the *A. salina* enriched with Super Selco® exhibited the highest *n*-3 HUFA levels (including the highest EPA level). As an excess of *n*-3 HUFA has been shown to cause negative effects in some fish (Corraze, 2001; but see Copeman *et al.*, 2002), it would be worthwhile investigating whether high *n*-3 HUFA levels do have a growth-retarding effect in *H. abdominalis*.

In conclusion, all four *Artemia* enrichments used in this investigation promote good growth and excellent survival in juvenile *H. abdominalis*, and therefore can be used in

the culture of this seahorse species. DHA Protein Selco® and Algamac 3050® were the best in terms of increasing seahorse growth, with the EPABSF/*S. platensis* enrichment performing virtually as well. Super Selco® produced the lowest growth rates of all the enrichments. However, on a cost per unit increment increase, the EPABSF/*S. platensis* enrichment, was the most cost-effective for seahorse length and weight increase. Given interspecific differences in nutritional requirements among different fish species and the lack of clear correlation between level of traditionally highly emphasized EFAs and growth in *H. abdominalis*, there is still considerable research required to determine what proximate dietary components and EFAs are indeed essential and their absolute and relative levels for maximizing growth, survival and health of captive *H. abdominalis*.

Optimizing the nutritional value of live foods used in seahorse culture, in the most cost-effective manner possible, should significantly increase seahorse farm production capacity, quality of seahorses produced and the potential economic viability of commercial farms. However, seahorse culturists may seek to partially supplement, or even totally replace, live foods with artificial or inert diets to further increase these. Frozen mysid shrimp, which are relatively widely available and have been widely used historically to rear seahorses, can provide commercial culturists with an alternative to live food, but their efficacy and relative cost-effectiveness remains largely untested to date.

5.4.2 Use of frozen mysids

5.4.2.1 Live *Artemia* vs. frozen mysids

In this study, feeding live enriched *Artemia*-only, frozen mysids-only, or a combination of the two did not result in significant differences in seahorse length or weight increase between these treatments, although SGR was higher in the *Artemia*-only treatment. In addition, there were no differences in survival, with almost 100% survival being recorded across the three treatments. This demonstrates that frozen mysids can be used as food in rearing *H. abdominalis* as an alternative to live *Artemia*. The alternative hypothesis (H_1: Seahorse growth and survival when fed frozen mysids is as good as (or even better) to that when fed enriched live *Artemia*) is accepted.

However, there is a larger issue to consider other than demonstrating the usefulness of frozen mysids in promoting good growth rates and high survival in seahorses – that of

the ability to wean seahorses onto non-live foods. This ability is of vital importance to economically feasible and responsible seahorse culture for two main reasons:

1) First, the use of non-live foods (either partially or wholly as a substitute for live foods) could significantly reduce culture costs by mitigating the plant space, materials and labour costs involved in producing live foods, although this is highly dependent upon a number of factors such as price paid for the frozen foods, cost of labour, cost of *Artemia* diets and efficiency of bulk-live-food producing units. The storage and use of frozen foods also provides the culturist with a more reliable and known quantity and quality of food for seahorse production. Even if seahorses can be weaned entirely to a non-live diet, it is still recommended to add some live food component to the diet to maintain a healthy interest in feeding by seahorses, as seahorses fed exclusively on frozen food have been observed to exhibit a decreased interest in feeding (Giwojna, 1990).

2) Second, the ability to wean cultured seahorses onto non-live foods prior to sale in the aquarium trade should significantly improve their chances of survival (Payne, 2003). This is because many aquarium owners who purchase seahorses do not have the time, inclination, or resources to culture sufficient quantities of their own live food. They may also not be able to access or afford to buy live foods from commercial retailers in the quantities that maintaining healthy seahorses requires. Therefore, seahorses that already accept non-live foods should have higher chances of surviving post-sale. As seahorses have sometimes been classed as "difficult" fish to maintain successfully in aquaria (e.g. Mai, 2004b), any factor which improves their survival and makes them easier to maintain in aquaria may help increase sales.

Because of their size, commercially available frozen mysids such as those used in this investigation are not suitable for feeding whole to early juvenile seahorses. The mysids used in this study were approximately 10 mm in length, and are readily accepted by *H. abdominalis* as small as 80–90 mm SL, but were too large for seahorses smaller than this to ingest (Woods, pers. obs.). The feeding of live prey, such as enriched *Artemia* nauplii, to pelagic newborn *H. abdominalis* is still a desirable practice in the culture of this species, as newborn *H. abdominalis* find it very difficult to feed on non-live diets which require moving water currents to keep the food in suspension (see Chapter 4). As they increase in size and become stronger swimmers and more experienced at food

capture, juvenile *H. abdominalis* can be successfully fed on non-live diets (i.e. at one and two-months of age, Chapter 4) that are smaller than the frozen mysids used in this investigation. As the juveniles of *H. abdominalis* and other species (Mai, 2004b) grow in size they tend to utilize all the water space within their tanks and appear quite amenable to benthic feeding, where movement of the food from currents appears to be less critical (although still useful).

As discussed in Chapter section 5.4.1.1, fish naturally contain high levels of the highly unsaturated fatty acids (HUFA) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Because of this, they consequently have high nutritional requirements for these fatty acids, as well as for other fatty acids such as arachidonic acid (AA) and linolenic acid, which are termed essential fatty acids (EFA) (Sargent *et al.*, 1997). In this study, the highest EPA and DHA percentages were found in the frozen mysids, which also contained the highest percentage of *n*-3 HUFAs. However, these higher percentages of EFAs in the mysid shrimp did not result in increased growth rates. This lack of correlation between higher levels of EFAs and increased seahorse growth is similar to growth results when *H. abdominalis* was fed *Artemia* fed different enrichments (section 5.4.1.3), and would appear to support the contention of some other researchers (i.e. Thompson, 2002; Shapawi & Purser, 2003) that seahorses such as *H. abdominalis* may not require large amounts of EFA, or the classical ideal EFA indices as demonstrated for the larval stages of some marine fishes.

According to Richoux *et al.* (2004), seasonal changes in the availability of essential fatty acids provide a dietary influence on seasonal lipid patterns in *Mysis mixta*, whereby rapid storage of lipids occurs during periods of high abundance of specific fatty acids. If this also occurs in other mysid species which are collected and sold in frozen form as fish food, then harvest of these may need to be targeted at certain times in the year when the occurrence of desirable fatty acids in the mysids is highest. However, caution should be exercised as to where mysids are harvested from as they may act as vectors of certain toxins contained within the food they consume (Engström-Öst *et al.*, 2002).

Although there were no differences in growth rate in seahorses fed *Artemia*-only or a combination of *Artemia* and frozen mysids, there may be physiological benefits from mixed feeding. For example, in broodstock nutrition lipids are critically important

sources of metabolic energy, in gonad formation, and in the formation of cell and tissue membranes (Watanabe *et al.*, 1984; Sargent, 1995). Hilomen-Garcia (1999) reported that established mating pairs of captive seahorses (*H. kuda*) fed a combination diet of HUFA-enriched *Artemia* (at 15% body weight), and mysids (at 6% body weight) or *Tilapia* fry (at 5% body weight) showed more parturition events and greater brood size than those fed on an HUFA-enriched *Artemia*-only diet (at 30% of body weight). Thus, mixed feeding of the enriched *Artemia* and frozen mysids (or other suitable foods), such as used in this study, with their different HUFA profiles could well be a beneficial practice.

In other commercially cultured finfish species that have been extensively researched, FCRs on non-live diets have become very efficient through a combination of environmental, husbandry and dietary manipulations. For example, Webster *et al.* (2001) obtained a FCR of 1.7 in hybrid sunshine bass fed once a day to satiation on a commercial pellet diet (EXT 400). Although not grossly inefficient, the FCRs recorded here for *H. abdominalis* (from 6.14:1 to 8.72:1 on a dry weight basis) should be targeted for improvement, as feed efficiency can strongly impact upon the commercial viability of a culture operation. As seahorses are likely to be inefficient feeders because of their associated feeding morphology (they lack masticatory structures and a true stomach, Lourie *et al.*, 1999), the impact of feeding rate and regime, and associated culture environment and husbandry variables on cultured seahorse FCR should be investigated further.

Optimal feeding rates (i.e. % body weight per day to feed) and regimes (i.e. how many times to feed each day) have not yet been determined for most cultured seahorse species. Based on observed activity patterns in juvenile cultured *H. abdominalis*, Ouyang (2005) suggested that once-daily feed rations of 3 to 5% wet body weight in enriched live *Artemia* might be suitable rations, but that 1% could be an insufficient ration. In order to maximise economic benefit, it is imperative to determine the point at which the least amount of food fed to seahorses results in the most efficient conversion to growth (i.e. maximise FCR). Feeding past this point is economically wasteful. It appears from observations on the actual consumption of the mysid ration that the feed rate of 25% wet body weight, as used in this investigation, represents overfeeding, with inefficient food conversion; lower feeding rates may improve FCR ratios. The fact that only approximately 50% of the mysids offered in this experiment were actually

consumed indicates that a more appropriate wet body weight ration might be closer ≤12.5% on a once-daily basis.

5.4.2.2 Effect of mysid ration

For commercial seahorse farms, the possibility that increasing food ration may increase seahorse growth and decrease time to reach market size must be examined. However, if there is no production benefit (i.e. increased growth rates which allow a shorter culture duration, and therefore lower associated labour and plant running costs per seahorse, until market sale) to be gained from feeding seahorses increased feed rations that are equal to or greater than the proportionate increase in feeding cost, then the use of such increased feeding rations must be questioned. For cultured *H. abdominalis*, in terms of increase in seahorse length, there was no growth advantage to feeding seahorses more than 5% of their wet body weight per day in a single feed. However, there was a wet weight gain and CF advantage associated with increasing feed ration >10%. For each commercial operation the costs and benefits to particular feed rations must be carefully calculated and considered.

For the medicinal market, small dried seahorses do not sell for high prices (where they are sold cheap in bulk or incorporated into encapsulated proprietary medicines); the highest prices are usually paid for larger seahorses which meet particular market requisites (Vincent, 1996). Large *H. abdominalis* (i.e. 20 cm+ in SL) that meet the required market requisites are sold on a per piece/size basis or per weight basis in the medicinal market. If sold on a per weight basis, then increased weight gain can be of economic benefit when supplying this market. Therefore, the use of a higher daily feed ration >10% body weight may be of advantage. However, if seahorses are sold on an individual basis (i.e. as part of a high-value packaged presentation) then this weight increase advantage may well be negated.

For the aquarium market, *H. abdominalis* is usually sold when it is >10 cm SL (CITES Appendix II-listing currently offers a universal 10 cm minimum height (see Chapter 7) size restriction for all wild-caught seahorses, but legitimate aquaculture operations may trade seahorses at any size). Unlike food-fish culture operations that are geared to producing as large and heavy a fish as possible in the shortest possible time — as their product is sold on a per weight basis — in the aquarium seahorse trade the overall size and weight of seahorses are of secondary concern. The primary physical attributes that

command higher prices in this trade are attributes such as colouration and body patterning, body shape, body condition (i.e. condition factor), and body ornamentation (e.g. dermal cirri, coronet shape) (G. Leveridge, Seahorse New Zealand, pers. comm.). This reduces any advantage to the use of higher feeding rations to increase weight gain, although there may some benefit due to the increase in CF associated with higher feed ration provided the effects on the primary physical attributes between feed rations are equal. Although seahorses on the 5% ration had a lower CF, they appeared to be healthy-looking with bright colouration and filled-out abdomens characteristic of this species and were not emaciated with shrunken abdomens which are often a sign of underfeeding and poor health.

Based on the total amount of mysids offered to seahorses in the respective feed rations, FCRs became less efficient as feed ration increased as did the cost/benefit ration. The alternative hypothesis (H_1: there is a difference in the relative cost/growth benefit ratio between each enrichment media used.) is accepted. On an economic basis this would favour not implementing higher feed rations given the limited growth benefits obtained with these higher feed rations, although any reductions in associated labour and plant running costs produced from the higher feed rations must be considered. However, the significantly lower FCRs in the higher feed rations are an artefact of the reducing proportion of mysids that were consumed in the higher feeding rations. When mysid consumption was taken into account there were no significant differences in FCR between the rations, although FCR did appear to be becoming less efficient at the 20% feed ration. This trend in increasing FCR with higher feed ration is supported by the results in section 5.4.2.3, where the ration of 25% wet body weight fed once a day to *H. abdominalis* resulted in a FCR of 8.72:1, with approximately 50% of the mysids consumed.

In addition to the economic wastage of food remaining uneaten as was observed in the 10-20% feed rations, the impact of uneaten mysids on water quality must be considered; uneaten food may seriously impact on water quality (Timmons *et al.*, 2002; Foss *et al.*, 2006). In this investigation, although there were an increased proportion of mysids that were not eaten with increasing feed ration, this did not affect the water quality (as determined by inter-tank ammonia and oxygen level testing) and did not affect seahorse growth. This is most likely due to the regular removal of faeces and uneaten mysids, as well as the seawater system being a flow-through system. In a commercial operation,

particularly a large-scale one, periods between regular waste removal and tank cleaning may be extended due to commercial pressure to reduce labour costs; this may lead to increased tank fouling which can lead to reduced water quality, which in turn can affect seahorse health, growth and survival. Therefore caution must be exercised in implementing a feed ration where food wastage occurs.

As this study demonstrated, the cost/benefit ratio of implementing feed rations >5% body weight on a once-daily basis does not appear to be advantageous for the culture of *H. abdominalis*. Significantly greater proportionate increases in seahorse growth with respect to the increased cost of providing a feed ration >5% of were not achieved. Even with the actual consumption of the total daily feed ration taken into account, the cost/benefit value still appears to economically favour the 5% ration for length increase, and both the 5 and 10% feed rations over the 15 and 20% rations for weight increase. The next logical steps in optimising the feeding regime for *H. abdominalis* in commercial culture are to test the interactive effects of culture environment and husbandry, feeding frequency and diet composition with feed ration in relation to growth and survival in seahorses, with real associated commercial labour and plant costs factored-in to the analysis.

CHAPTER 6

NATURAL DIET AND MALE REPRODUCTIVE OUTPUT OF WILD
SEAHORSES

6.1 Introduction

The exploitation of seahorses for the traditional medicinal, curiosity and ornamental aquarium fish markets (see Marichamy *et al.*, 1993; Vincent, 1996; Lourie *et al.*, 1999) has focused attention on the large gaps in our knowledge of seahorse biology and ecology in their natural environments. Recently, a summary of what is known about seahorses' life-history traits and behavioural characteristics was synthesised and compared with other marine teleosts (Foster & Vincent, 2004).

6.1.1 Natural diet

The qualitatitive and quantitative examination of the natural diet for any fish species is an important step in helping to understand complex trophic interactions and temporal/spatial/ontogenetic/sexual variations in species biology, as well as an important tool in species conservation and fisheries management (Nelson, 1979; Ryer & Boehlert, 1983; Tipton & Bell, 1988; Olaso *et al.*, 1999; Arnal & Côte, 2000; Castillo-Rivera *et al.*, 2000; Rikardsen *et al.*, 2000; Villarroel & Acuña, 2000; Xie *et al.*, 2000; Delvaria & Agostinho, 2001; Denny & Schiel, 2001; Gasket *et al.*, 2001). If that fish species is also of interest to commercial aquaculturists, such as is the case with seahorses, then an understanding of its natural diet is also of benefit to the aquaculturist as an indicator to what type and size range of prey organisms/foods might be utilized in culturing that species. Natural prey may also be used as a reference from which the lipid and fatty acid composition of cultured live feed organisms can be modeled, or compared against (Rainuzzo *et al.*, 1997; Sargent *et al.*, 1999).

Seahorses are ambush predators which predominantly rely on stealth and camouflage to approach their prey (James & Heck, 1994; Flynn & Ritz, 1999; Lourie *et al.*, 1999). They feed upon a wide range of epifaunal and planktonic prey, particularly crustaceans such as copepods, amphipods, isopods, and caridean, euphausid and mysid shrimps (Reid 1954; Lovett 1969; Tipton & Bell 1988; Do *et al.*, 1998; Texeira & Musick 2001; d'Entremont, 2002; Kendrick & Hyndes, 2005; Felício *et al.*, 2006). Seahorses may

change their diet ontogenetically with smaller individuals consuming smaller prey (Kanou & Kohno, 2001; Texeira & Musick, 2001; Foster & Vincent, 2004). For *H. abdominalis* there is little known about its natural diet.

The research in the previous chapters has demonstrated that *H. abdominalis* may be reared to maturity primarily on a diet of *Artemia* sp., a prey organism they would not normally encounter in their natural habitat but which is used extensively in its commercial culture and associated research (Hilomen-Garcia, 1999; Forteath, 2000; Chang & Southgate, 2001; Job *et al.*, 2002; Shapawi & Purser, 2003; Wong & Benzie, 2003). However, to the commercial culturist, knowledge of *H. abdominalis*' natural diet is highly desirable, particularly if the preferred or commonly predated organisms identified could be cultured *en masse* as a more "natural" and possibly more nutritious alternative to *Artemia*.

6.1.2 Reproductive output

As with natural diet, knowledge of the natural reproductive output of *H. abdominalis* is also of interest to commercial seahorse culturists. This knowledge can provide natural benchmarks against which commercial aquaculturists can measure their operational success. For example, if a commercial operation is producing fewer juveniles per male seahorse than wild males of comparable size, and those juveniles produced are also of smaller size at birth, then this may point to aspects of the culture operation (e.g. nutrition, water quality, tank design, and possibly genetic stock) which could be improved.

Poortenaar *et al.* (2004) investigated ovarian morphology, reproductive condition and sex steroid levels in female *H. abdominalis* from Wellington Harbour. They found that reproductively active females are present throughout the year, with relatively large eggs compared to other teleosts. The reproductive output of male *H. abdominalis* has not previously been documented.

Knowledge of male seahorse reproductive output is important because seahorses, as members of the Syngnathidae family of fishes, exhibit the characteristic reproductive trait of this family: advanced paternal care of the developing embryos (Vincent 1990; Vincent *et al.*, 1992; Lourie *et al.*, 1999; Wilson *et al.*, 2001; Foster & Vincent, 2004).

The male seahorse receives the eggs from the female into his enclosed brood pouch where they remain post-fertilisation until the fully developed and independent juveniles are released. Therefore, male seahorses are an important factor in determining reproductive output. Aside from possible commercial gain from obtaining knowledge of natural male seahorse reproductive output/capacity, it is also of importance regarding the regulation and sustainability of trade in wild-caught seahorses (see Chapter 7).

Knowledge of both the natural diet and the reproductive output of *H. abdominalis* is crucial to both the development of the commercial aquaculture of this species, and to the management and protection of this species in relation to the CITES Appendix II-listing of all seahorse species. The aim of this chapter is to:

1) Determine the natural diet of adult *H. abdominalis* (both sexes) from Wellington Harbour.
2) Determine the reproductive output of adult male *H. abdominalis* from Wellington Harbour.

For natural diet, information was sought on what prey items were consumed and whether there were any differences in these between sexes and different sized seahorses. For reproductive output, information was sought on the relationship between juvenile characters such as number and size, and parent male characters such as size and pouch volume to either support or question the validity of the recommended 10 cm mHT size restriction for the exploitation of wild seahorses. This research is based on Woods (2002, 2005b).

6.2 Materials and methods

6.2.1 Natural diet

Seahorses (*n* = 59) used for diet analysis were captured as part of a separate study investigating the reproductive endocrinology of wild female seahorses in Wellington Harbour from August 2000 to July 2001 (Poortenaar *et al.*, 2004), between 0900 and 1300-h. These seahorses were captured by hand on snorkel/SCUBA from amongst macroalgal stands *(Macrocystis pyrifera, Carpophyllum flexuosum, C. maschalocarpum, Sargassum sinclairii)* (0.5–6 m depth) in Wellington Harbour (174°50' S, 41°17.50' E). All seahorses encountered, apart from brooding males, were

collected; brooding male seahorses were not collected for dissection and dietary analysis in an effort to minimize research impact (see 6.2.2 below). Following transfer from the field back to the laboratory seahorses were anaesthetised with AQUIS® and their wet weights and standard lengths (SL) recorded.

Following blood sampling for the endocrinology work, the seahorses were killed by excess anaesthetisation and immersion in 10% formalin (max. of 2 h following capture; blood sampling was not feasible in the field because of the delicacy with which caudal punctures must be made). The seahorses' abdomens were then opened with a ventral incision along the keel line and the digestive tract removed and fixed in 10% formalin. The time delay in fixing from time of original collection meant that the processes of digestion and food assimilation were still occurring during this delay. This made prey identification more difficult and the estimated values for both digestive tract fullness and actual dry weight of digestive tract contents should therefore be regarded as conservative. Faecal excretion in the time between collection and fixing was not measured.

Fullness of the intact digestive tract was visually estimated as a percentage based on the relative turgidity along the gut length without increment classes (e.g. completely flaccid and flat along entire length = 0% full; very turgid along entire length = 100% full). A lengthwise incision along the gut was then made and the contents flushed out onto a gridded (1 x 1 mm grid) counting tray. These contents were viewed under a light stereo-microscope and identified to the lowest taxonomic level possible. Contents were assessed as an estimation of the percent volume each type of food item contributed to the total volume of gut contents (percentage of equivalent number of grids with each content type out of the total number of grids covered by gut contents), and their frequency of occurrence. For example, if 0.5 of one 1 x 1 mm grid was covered by food item A, and a total of 10 grids were covered by all food items, then food type A = 5% of digestive tract contents. The gut contents were then placed on pre-weighed filter papers and dried to constant weight (24 h at 60°C).

6.2.1.1 Fatty acid comparison of natural prey to food used in seahorse aquaculture
To gain an indication as the nutritional value of food items used in the aquaculture of *H. abdominalis* in relation to the natural prey of wild *H. abdominalis*, a sample of two of the more common prey items of *H. abdominalis* (the mysid *Tenagomysis similis* and the

caridean shrimp *Hippolyte bifidirostris* — see 6.3.1) were collected by hand-netting from the same sampling area from which the seahorses were collected for diet analysis. Fatty acid analyses were performed on triplicate samples of *T. similis* and *H. bifidirostris* by a commercial analytical laboratory (Industrial Research Ltd, Gracefield, Wellington) as in Chapter 5. The fatty acid profiles of *T. similis* and *H. bifidirostris* were then compared against those of *Artemia* enriched with Algamac-3050® and commercially supplied frozen mysids, *Amblyops kempi* (see Chapter 5).

6.2.2 Reproductive output of male seahorses

Brooding male seahorses ($n = 46$) were hand-collected by snorkel/SCUBA between 0900 and 1300-h from the same habitat area in Wellington Harbour as in 6.2.1 from January 2000 to February 2003 (Table 6.1). All pregnant male seahorses encountered were collected. Whether or not a male seahorse was brooding was determined *in situ* using visual characteristics such as colour and turgidity of the pouch (see Woods 2000a). Despite exhibiting promiscuous courtship behaviour *in situ*, *H. abdominalis* has been shown to mate monogamously in Tasmania (Wilson & Martin-Smith, *in press*), Australia. It is not known whether New Zealand populations of *H. abdominalis* mate monogamously. Therefore, it is not known whether the removal of pregnant males for this study may have significantly affected local reproductive partnerships.

The location of collection for each male was recorded on a dive slate using identifiable shore landmarks for visual triangulation. This was deemed feasible as an approximate (± 5 m) method of collection reference as the macroalgal stands searched were restricted to a narrow (3–10 m wide) band following the shoreline, and the collector was very familiar with the area.

Brooding males were removed and transported in sealed snap-lock plastic bags containing seawater back to the laboratory. Standard length (SL cm) of each male was recorded against a steel ruler (± 0.5 mm). Maximum time between collection and transport to the laboratory was 2 h. No mortalities occurred and, after initially reclusive behaviour, seahorses in the laboratory were observed to exhibit apparently normal behaviour and feeding.

Table 6.1 Collection events and number (events/no. collected) of brooding *Hippocampus abdominalis* males collected in each month over the entire sampling period (January 2000 to February 2003).

Month	2000	2001	2002	2003	Total
January	2/4	2/5	1/0	1/3	6/12
February	3/7	1/3	1/1	1/2	6/13
March	1/2	1/5	1/0	-	3/7
April	0/0	1/3	1/1	-	2/4
May	1/0	3/1	0/0	-	4/1
June	0/0	1/2	0/0	-	1/2
July	0/0	1/0	0/0	-	1/0
August	1/1	1/0	1/0	-	3/1
September	1/1	1/0	0/0	-	2/1
October	2/1	0/0	1/1	-	3/2
November	3/1	0/0	1/1	-	4/2
December	1/0	0/0	1/1	-	2/1

Brooding seahorses were maintained in land-based tanks to examine their brood characteristics. This was preferred to collection and maintenance in sea-based cages in their natural environment because: (1) the slim girth of juvenile *H. abdominalis* when first released requires mesh no larger than 1.5 mm at the largest aperture (Woods, unpubl. data) — this size mesh fouls rapidly thus reducing water quality inside the cages; (2) land-based maintenance allows greater monitoring and control of environmental variables; and (3) the daily monitoring for birthing events was possible in a land-based situation, but not in a sea-based situation owing to funding constraints, availability of assistance, and weather conditions.

Brooding males were maintained at the NIWA Mahanga Bay hatchery in Wellington in aerated 75-l round white polyethylene plastic tanks (one male per tank) supplied with flow-through ambient seawater filtered to 20 µm at a flow rate of 1 l min^{-1} and exposed to a 14 h L:10 h D photoperiod. These tanks had a central upstand overflow and attachment substratum of 20 mm polyethylene mesh and macroalgae from natural seahorse habitat. Seahorses were fed brine shrimp (*Artemia* sp.) 3–11 mm in length enriched with Algamac-2000 and Super Selco®, at an approximate rate of 100 brine shrimp seahorse^{-1} day^{-1} in one feeding. Supplemental feeds of amphipods (*Orchestia chilensis*, 2–8 mm in length) at the rate of 10–30 seahorse^{-1} day^{-1} in one feeding were also given. This feeding regime equates to 15–25% of wet body weight seahorse^{-1} day^{-1},

which has been shown to be *in excess* of requirements (Woods, 2005a). Water parameters (mean ± 1 SE) were as follows: water temperature 14.7 ± 0.1°C (range 10.4–18.4°C), dissolved oxygen 7.3 ± 0.4 mg l^{-1} (range 7.5–8.4 mg l^{-1}), salinity 34.0 ± 0.0 ppt (range 31.3–34.9 ppt), and pH 8.2 ± 0.0 (range pH 8.1–8.2).

Tanks were checked daily for release of juvenile seahorses and any release of premature juveniles or eggs. Tank setup ensured that any release of premature juveniles or eggs would be readily observed (i.e. tank bottoms and water surface free from visual obstruction). Upon release of their progeny (during darkness between 1800 and 0700-h), adult males were removed from the tank and their pouches flushed out with seawater to ensure there were no remaining unborn juveniles. It was typical for male *H. abdominalis* to release all their juveniles in one night, rather than over consecutive nights. The wet weight (WW) to 0.1 g of each male was recorded on a Mettler P440 balance following removal from the water and gentle shaking-off of excess water.

The juveniles were removed from the tank as soon as possible (within 2 h of light) A random sample of five juvenile from each brood were euthanised in AQUIS$^®$ and their SL measured against a steel ruler (± 0.5 mm). Dry weights (DW) (60°C for 24 h) were recorded with the use of a Mettler microgram balance (± 1 μg) for each juvenile sampled, in preference to WW because of rapid desiccation of the slim juveniles.

An approximate brood pouch volume (ml) was calculated for each male after the release of juveniles as follows: brood pouch volume = brood pouch length x width x depth x 1.3 (Woods, unpubl. data from a study on actual brood pouch volumes in *H. abdominalis*). Brood pouch length was measured in a straight line from the pouch opening to the bottom point where the pouch joins the tail, pouch width as the widest lateral distance, and pouch depth as the dorso-ventral distance at the widest point (see Vincent, 1990). As the skin of the brood pouch may have become stretched during brooding, there exists the potential for these pouch measurements to be different from those in non-brooding males. Following these measurements, the parent male seahorse and the remaining balance of his progeny were returned to the original collection location (± 5 m) the same day as juvenile release (with no juvenile mortality) in an attempt to minimise research impact on the resident population.

As an example of how knowledge of wild male seahorse reproductive output may act as a useful comparative benchmark for commercial seahorse aquaculturists, the reproductive output data generated from the above wild seahorses were compared against those of cultured male *H. abdominalis* (F1–F3, whose original parents were originally collected from the same location as the wild seahorses) reared between 1998 and 2002 at NIWA's Mahanga Bay facility ($n = 42$). Mean ± 1 SE SL and wet weight for these cultured male seahorses were 16.1 ± 0.5 cm SL (range 11.7–25.6 cm) and 17.6 ± 1.8 g (range 2.6–53.9 g) respectively. Comparisons in the number of juveniles produced per brood, and the mean SL (cm) and DW (mg) of juveniles per brood upon birth were made between the wild and cultured *H. abdominalis*.

6.2.3 Statistical analyses

All data were analysed using Statistica 6.1 (Statsoft, Tulsa, Oklahoma, United States). Data were tested for normality (Shapiro-Wilk's W test) and homogeneity of variances (Levene's test). Data were transformed appropriately before analysis to ensure normality and homogeneity of variances.

Possible sexual differences in diet composition were tested using two-tailed Students *t*-test on arcsine-square root transformed data. For the purposes of this sex comparison, given the very unequal number of females to males, an equal number of females within the size range of the male seahorses were randomly selected. Any males collected that were brooding were excluded from the comparison to remove any possibility of diet differences that might occur. Possible ontogenetic variations in diet were examined using regression analysis.

Differences in percent lipid composition (arcsine square-root transformed data) of the wild *T. similis* and *H. bifidirostris*, and the enriched *Artemia* and frozen mysids for the major fatty acids oleic acid, 18:1*n*-9, linoleic acid, 18:2*n*-6, linolenic acid, 18:3*n*-3, arachidonic acid (AA), 20:4*n*-6, eicosapentaenoic acid (EPA), 20:5*n*-3, docosahexaenoic acid (DHA), 22:6*n*-3, as well as total percent polyunsaturated fatty acids (PUFA) and *n*-3 highly unsaturated fatty acids (HUFA) composition were tested using Kruskal-Wallis ANOVA with post-hoc Tukey HSD (*P* at 0.05).

For reproductive output, data were tested for normality (Shapiro-Wilk's W test) and homogeneity of variances (Levene's test). Data were transformed appropriately before analysis to ensure normality and homogeneity of variances (all variables except parent male SL required Log_e transformation for normality). Owing to the low numbers of brooding males caught in individual months (Table 6.1), no attempt was made to analyse data on a monthly basis. However, all data for the total collection period were combined and stratified into season (Spring: September-November; Summer: December-February; Autumn: March-May; Winter: June-August) and analysed with single factor Analysis of Variance (ANOVA) to determine whether there were any seasonal differences in brood characteristics (i.e. length and weight of parent males, number of juveniles, length and weight of juveniles, number of juveniles in relation to brood pouch volume). As the conception dates were not known for the captured males it was not deemed valid to test for relationships between numbers of juveniles per brood, mean juvenile SL, mean juvenile DW, in relation to the number of days the brooding adults were kept in captivity.

For the comparison of reproductive output in terms of the number of juveniles produced per brood between wild and cultured *H. abdominalis*, an Analysis of Covariance (ANCOVA) was performed on log-transformed data, with parent male SL as a covariate to treatment, to test for any differences in data slope between wild and cultured treatments. This revealed no significant interaction effects (ANCOVA, $P>0.05$) and confirmed the assumption of homogeneity of slopes, so a single factor ANOVA was performed for comparison of the number of juveniles produced per brood at a standardized log-transformed mean with post-hoc Scheffe's test (*P* at 0.05). Student's *t*-tests were used to compare mean juvenile SL and DW per brood between wild and cultured seahorses.

6.3 Results

6.3.1 Natural diet

A total of 59 adult seahorses (mean ± 1 SE, SL = 19.2 ± 0.4 cm (range 13.2–27.4) and mean wet weight = 15.4 ± 1.0 g (range 3.5–31.0)) were collected. No juvenile seahorses were observed. Of the 59 seahorses collected, 44 were female and 15 male.

The guts of these seahorses contained mostly crustaceans (Table 6.2, Fig. 6.1). Within the crustaceans, amphipods (caprellids and ischyrocerids), decapods (caridean shrimp), and peracarids (mysid shrimp) dominated in both frequency of occurrence and percent volume of gut contents. Within the dominant dietary items, particular species were dominant prey. For example, for amphipods the caprellid *Caprella equilibra* and the ischyrocerid *Ischyrocerus longimanus* were dominant prey. For mysids and carideans, *Tenagomysis similis* and *Hippolyte bifidirostris* respectively, were dominant prey. Although also consumed, prey items such as copepods, tanaids, isopods (e.g. *Cymodocella* sp.), euphausids, polychaetes, and teleosts were only minor dietary items in terms of percent volume and frequency of occurrence.

There were no significant dietary differences between male and female seahorses (student's *t*-test, *P*>0.05).

There was a significant decrease in the estimated proportion of total gut volume of crustaceans consumed with increasing SL (Fig. 6.2). This was caused by larger seahorses consuming a lesser proportionate amount of amphipods (Fig. 6.3). With increasing SL there appeared to be a greater amount of decapods (e.g. *H. bifidirostris*) consumed relative to other prey taxa, but this was not a significant relationship ($r^2 = 0.036$, *P*>0.05).

Table 6.2 Gut contents of *Hippocampus abdominalis* collected from Wellington Harbour (*n* = 59): *F*, % frequency of occurrence; PV, mean ± 1 SE % by volume.

Digestive tract contents	*F* (%)	PV (%)
CRUSTACEA	100	66.05 ± 3.28
Copepoda	6.8	1 ± 0.88
Cyclopoida	3.4	0.1 ± 0.09
Harpacticoida	3.4	0.9 ± 0.88
Amphipoda	49.2	22.64 ± 4.19
Corophioidea	1.7	0.11 ± 0.11
Caprellidae	25.5	11.98 ± 3.19
Gammaridae	13.6	2.48 ± 1.04
Ischyroceridae	18.6	6.97 ± 2.43
Hyalidae	3.4	1.39 ± 1.1
Amphithodea	3.4	0.24 ± 0.17
Talitroidea	3.4	0.17 ± 0.15
Tanaid	6.8	0.17± 0.1
Isopoda	6.8	0.98 ± 0.51
Sphaeromatidae	3.4	0.34 ± 0.27
Flabellifera	1.7	0.4 ± 0.4
Decapoda	52.5	30.84 ± 4.44
Brachyura	5.1	1.27 ± 1.07
Caridea	47.5	28.22 ± 4.38
Peracarida	20.3	8.44 ± 2.8
Mysidacea	20.3	8.43 ± 2.8
Cumacea	5.1	0.19 ± 0.12
Eucarida	1.7	0.59 ± 0.59
Euphausiacea	1.7	0.59 ± 0.59
ANNELIDA	1.7	0.25 ± 0.25
Polychaeta	1.7	0.25 ± 0.25
PISCES	5.1	2.08 ± 1.24
Tripterygidae	5.1	2.08 ± 1.24
INORGANIC (sand)	6.8	3.59 ± 2.03
UNIDENTIFIABLE	94.9	27.9 ± 2.74
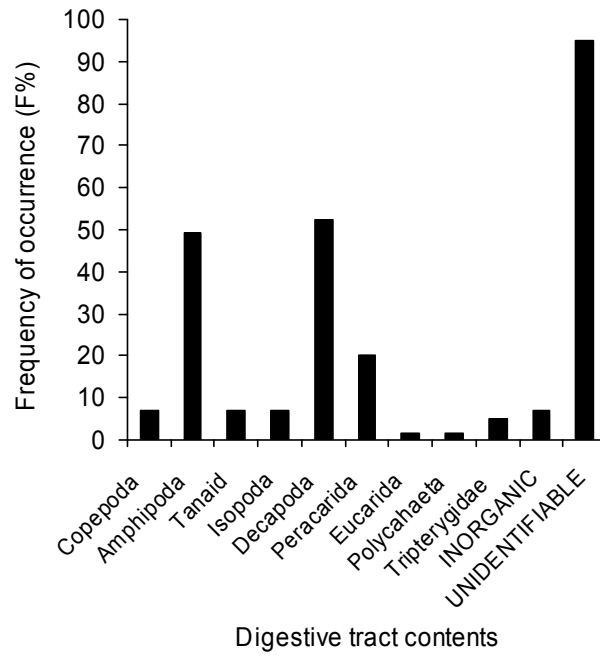
A
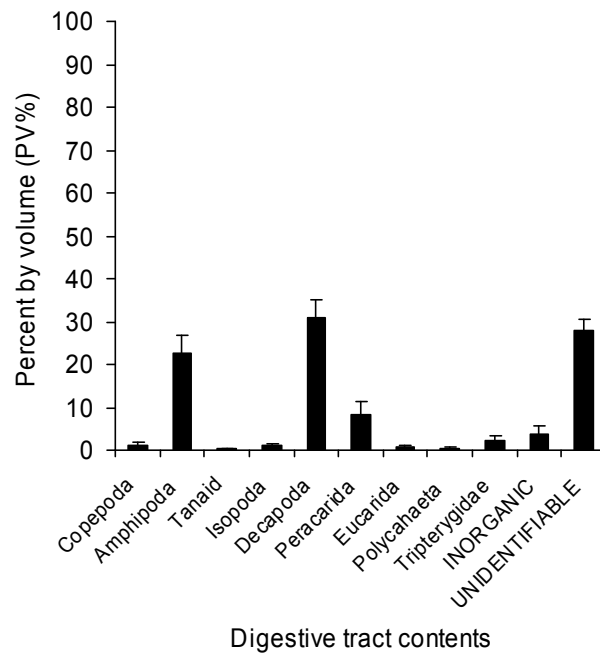
B

Figure 6.1 Gut contents of *Hippocampus abdominalis* collected from Wellington Harbour (*n* = 59) grouped by major taxa: (A) frequency of occurrence (*F* %); (B) percent by volume (PV%), mean ± 1 SE % by volume.
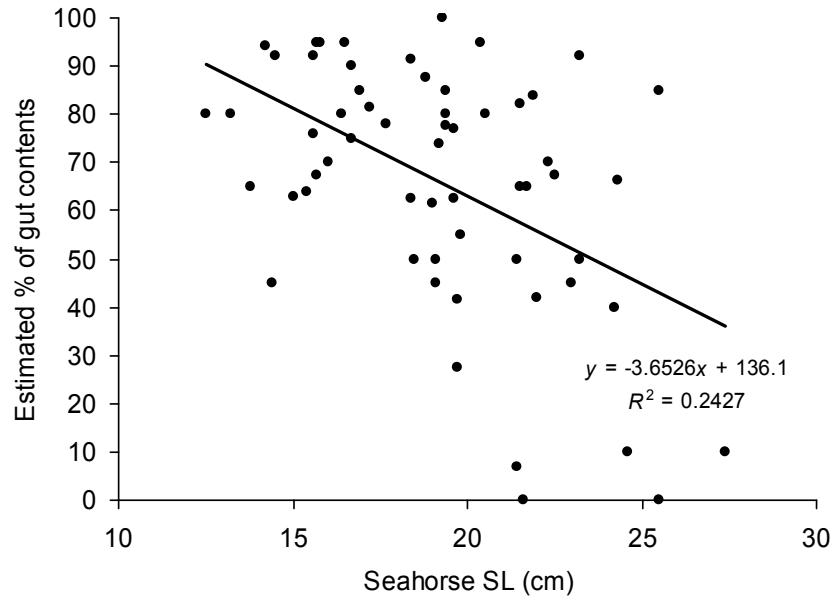
Figure 6.2 Relationship between estimated mean percent gut contents volume of crustaceans in *Hippocampus abdominalis* from Wellington Harbour (*n* = 59), and seahorse length (SL).
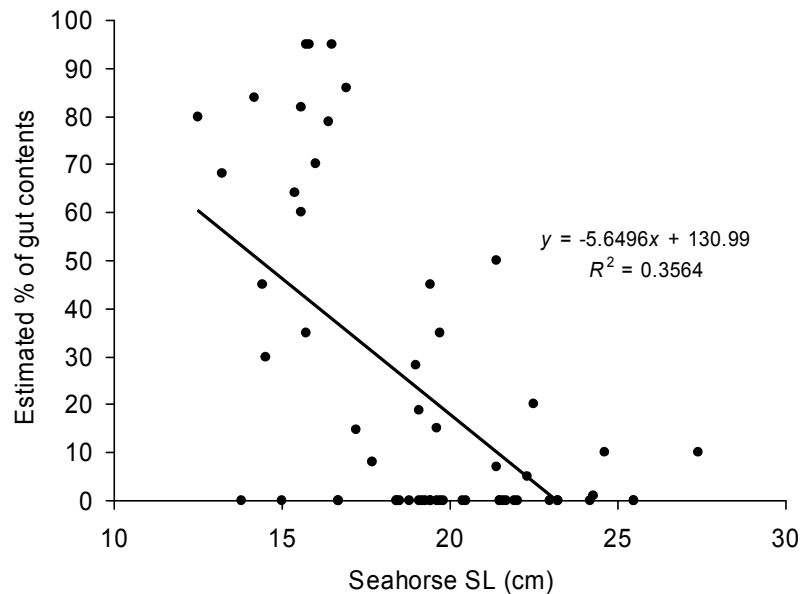
Figure 6.3 Relationship between estimated mean percent gut contents volume of amphipods in *Hippocampus abdominalis* from Wellington Harbour (*n* = 59), and seahorse length (SL).

Examination of diet composition month by month was not possible due to low (e.g. *n* = 2) capture rates in some months summed over 2000 to 2003. However, grouping of seahorses into seasonal groups (spring: September-November (*n* = 17); summer: December-February (*n* = 16); autumn: March-May (*n* = 16); winter: June-August (*n* =

10)) did appear to show some differences in prey consumption although these were non-significant (ANOVA, *P*>0.05). For example, within the major prey taxa the overall consumption of crustaceans appeared to decrease during winter (Fig. 6.4). This decrease appears to be caused by a decrease in consumption of amphipods during this time. Consumption of decapods was lowest in autumn. Peracarid consumption appeared relatively constant during the year.
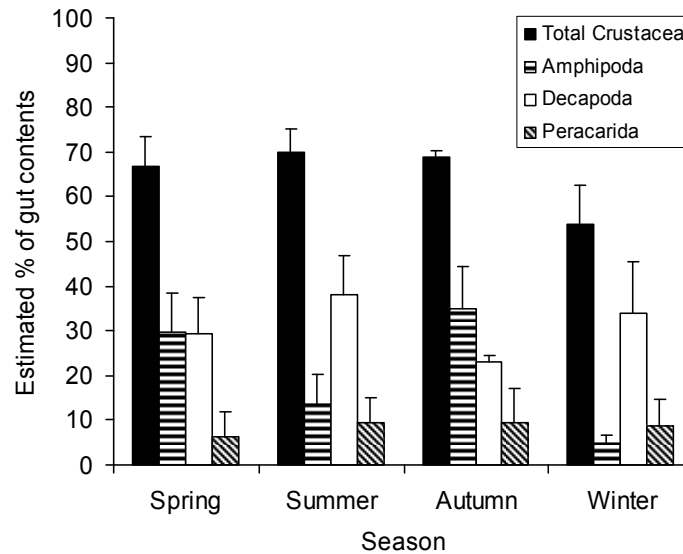
Figure 6.4 Estimated seasonal percent gut contents volume (mean ± 1 SE) of the major prey taxa in *Hippocampus abdominalis* from Wellington Harbour (*n* = 59), during the sampling period of 1 year (spring: September-November; summer: December-February; autumn: March-May; winter: June-August).

Mean percent fullness of the gut was 49.2 ± 2.4% and mean dry weight of gut contents was 0.18 ± 0.1 g. Theoretically, if a dry weight to wet weight conversion factor for adult seahorses is used (dry weight = c. 24% of wet weight for adult *H. abdominalis*, see Woods 2002), then the mean wet weight of digestive tract contents was 4.9% of the calculated mean wet body weight of the seahorses sampled (recorded mean 0.18 g dry weight of gut contents/calculated mean seahorse dry weight of 3.7 g).

In 42% of the seahorses examined, an unidentified pedunculate digenean parasite was found within the digestive tract attached to the gut wall or associated with the digestive tract contents. Although not counted as a dietary item, this parasite made up a mean 6.5 ± 1.5% of the gut content volume. The parasite reached up to 7 mm in relaxed length

and could number up to 15 individuals within a single seahorse. This parasite is still in the process of being identified.

6.3.1.1 Fatty acid comparison of natural prey to food used in seahorse aquaculture

Enriched *Artemia* had the highest percentages of oleic acid, 18:1n-9, and linoleic acid, 18:2n-6 (Kruskal-Wallis ANOVA, $H_{1,3}$ = 9.36, P < 0.05 for both), but a lower percentage of linolenic acid, 18:3n-3 (Kruskal-Wallis ANOVA, $H_{1,3}$ = 9.36, P<0.05) (Table 6.3). Enriched *Artemia* and *H. bifidirostris* had the highest percentages of arachidonic acid (AA), 20:4n-6 (Kruskal-Wallis ANOVA, $H_{1,3}$ = 9.50, P<0.05). Enriched *Artemia* had the lowest percentage composition of eicosapentaenoic acid (EPA), 20:5n-3 (Kruskal-Wallis ANOVA, $H_{1,3}$ = 10.39, P<0.05), while frozen mysids had the highest percentages of docosahexaenoic acid (DHA), 22:6n-3 (Kruskal-Wallis ANOVA, $H_{1,3}$ = 9.46, P<0.05). Frozen mysids also had the highest percentages of n-3 highly unsaturated fatty acids (HUFA) and total polyunsaturated fatty acids (PUFA) (Kruskal-Wallis ANOVA, $H_{1,3}$ = 10.39, P<0.05 and $H_{1,3}$ = 9.50, P<0.05, respectively). Both DHA:EPA and oleic acid:DHA ratios were higher in the enriched *Artemia*, although their EPA/AA ratios were lower than those of the other three foods, particularly both types of mysids.

Table 6.3 Fatty acid composition (%) of wild mysid *Tenagomysis similis*, wild caridean shrimp *Hippolyte bifidirostris*, *Artemia* enriched for 24 h at 28°C on Algamac-3050®, and frozen mysids (*Amblyops kempi*). Data are the mean ± 1 SE of 3 samples. Statistical comparison of some major fatty acids (%) as outlined in section 7.2.1.1: row values with different superscripts are significantly different (*P*<0.05).

Fatty acid	*T. similes*	*H. bifidirostris*	*Artemia*	Frozen mysids
14:0	1.67 ± 0.05	2.69 ± 0.01	2.5 ± 0.38	2.28 ± 0.09
16:0	26.02 ± 0.47	20.45 ± 0.11	16.43 ± 1.16	23.97 ± 0.2
16:1n-9	0.1 ± 0.1	0	0.53 ± 0.08	0
16:1n-7	4.63 ± 0.31	5.97 ± 0.02	2.32 ± 0.4	4.15 ± 0.06
18:0	2.94 ± 0.08	6.09 ± 0.02	6.14 ± 0.12	2.51 ± 0.02
18:1n-9	7.09 ± 0.08[b]	9.15 ± 0.04[b]	16.26 ± 2.74[a]	7.12 ± 0.09[b]
18:1n-7	4.77 ± 0.26	5.14 ± 0.08	5.08 ± 0.33	3.59 ± 0.06
18:2n-6	1.11 ± 0.00[b]	1.78 ± 0.02[b]	9.82 ± 2.68[a]	1.18 ± 0.03[b]
18:3n-3	1.12 ± 0.05[ab]	1.43 ± 0.01[a]	0.65 ± 0.13[b]	1.41 ± 0.04[a]
20:4n-6	1.9 ± 0.05[b]	5.25 ± 0.06[a]	5.56 ± 0.91[a]	0.84 ± 0.01[b]
20:5n-3	19.67 ± 0.11[b]	18.3 ± 0.1[b]	10.56 ± 1.41[c]	22.6 ± 0.19[a]
22:5n-3	0.92 ± 0.03	0.56 ± 0.02	0.61 ± 0.1	0.68 ± 0.01
22:6n-3	17.07 ± 0.90[b]	13.31 ± 0.02[b]	12.82 ± 1.24[c]	22.76 ± 0.14[a]
DHA:EPA	0.87	0.73	1.21	1.01
Oleic acid:DHA	0.42	0.69	1.27	0.31
EPA/AA	10.35	3.49	1.9	26.9
% known	93.67 ± 0.27	92.42 ± 0.03	90.74 ± 0.46	96.17 ± 0.05
% n-3 HUFA	41.08 ± 1.14[b]	34.61 ± 0.18[c]	25.66 ± 2.18[d]	49.07 ± 0.3[a]
% PUFA	44.23 ± 0.96[b]	42.17 ± 0.12[c]	41.04 ± 0.5[c]	51.29 ± 0.29[a]
% MUFA	18.31 ± 0.75	20.4 ± 0.32	24.36 ± 2.43	15.73 ± 0.11
% SFA	31.13 ± 0.58	29.84 ± 0.10	25.34 ± 1.63	29.15 ± 0.42
% lipid DW	11.2	13.0	8.89	9.13
% lipid WW	2.2	2.7	0.53	0.75

6.3.2 Reproductive output of male seahorses

A total of 46 brooding males were collected. The SL (mean ± 1 SE) of brooding males was 18.1 ± 0.6 cm (range 10.5–26.4 cm) and mean WW 17.6 ± 1.6 g (range 3.1–44.4 g). Males as small as 8.3 cm SL were observed in the field, but 10.5 cm SL was the smallest brooding male observed and collected. Brooding males were found in most months when collections were made, apart from July when one collection was attempted between 2000 and 2003 (Table 6.1). There were no significant seasonal differences detected in any brood characteristics (P>0.05). Consequently, all data were pooled for subsequent analysis.

The duration of brooding by pregnant wild males in the laboratory was 18.7 ± 1.6 (mean ± 1 SE) days (range 1–47 days). Mean (± 1 SE) number of juveniles released in captivity per brood was 271.2 ± 27 (range 37–744). Regression analysis revealed a positive relationship between the number of juveniles released per brood and parent male SL, with larger males producing greater numbers of juveniles per brood (r^2 = 0.50, P<0.001) (Fig. 6.5). The number of juveniles per brood was also positively related with parent male WW (r^2 = 0.46, y = 0.7714x + 1.4355, P<0.001), and brood pouch volume (r^2 = 0.50, y = 0.7122x + 3.825, P<0.001), with increasing number of juveniles per brood with increasing male SL and brood pouch volume (cc). Pouch volume (mean ± 1 SE) of parent males was 11.7 ± 1.3 ml (range 1.6–34.4 ml). Pouch volume and male SL were positively related, with larger males having pouches of larger volume (r^2 = 0.67, P<0.001) (Fig. 6.6). Parent male SL and WW were also positively related (r^2 = 0.86, y = 0.1532x - 0.0951, P<0.001) with WW increasing with increasing male SL.
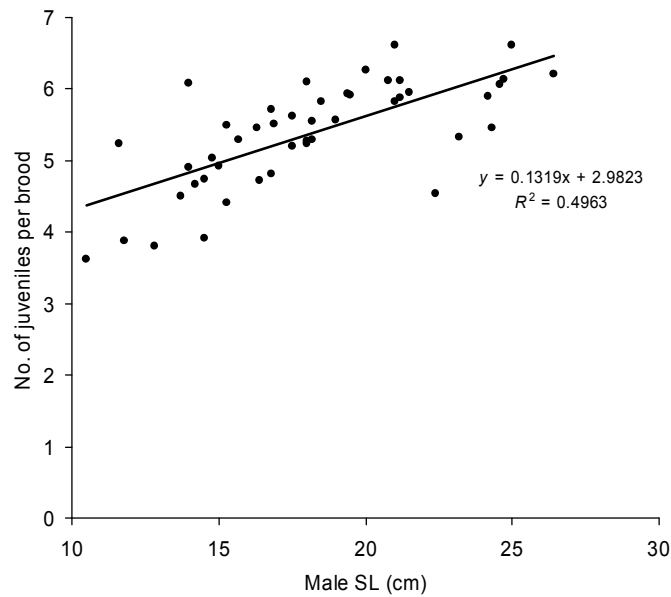
Figure 6.5 Relationship between total number of juveniles per brood ($\log_e$ transformed) and parent male standard length (SL cm) in *Hippocampus abdominalis* from Wellington Harbour, New Zealand (*n* = 46).
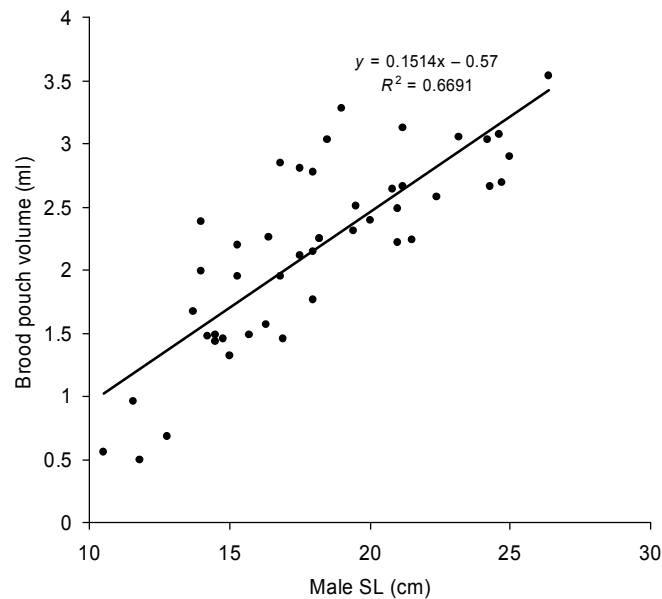
Figure 6.6 Relationship between brood pouch volume ($\log_e$ transformed) (ml) and parent male standard length (SL cm) in *Hippocampus abdominalis* from Wellington Harbour, New Zealand (*n* = 46).

SL (mean ± 1 SE) of juveniles was 16.7 ± 0.2 mm (range 12.8–22.4 mm) and mean DW was 1.2 ± 0.1 mg (range 0.6–2.5 mg). Regression analysis revealed that there was a positive relationship between mean juvenile SL and mean juvenile DW ($r^2 = 0.32$, $y = 1.6872x - 4.5542$, $P<0.001$) with juvenile DW increasing with juvenile SL.

There was no significant relationship between mean juvenile SL and the number of juveniles per brood ($r^2 = 0.00$, $P>0.05$) or mean juvenile DW and number of juveniles per brood ($r^2 = 0.01$, $P>0.05$). There was also no significant relationship between mean juvenile SL and the number of juveniles per ml of pouch volume ($r^2 = 0.01$, $P>0.05$) or mean juvenile DW and the number of juveniles per ml of brood pouch volume ($r^2 = 0.01$, $P>0.05$), i.e. for any given pouch volume, there was no difference in juvenile size or weight in relation to the number of juveniles being brooded. There was no significant relationship between mean juvenile SL and parent male SL ($r^2 = 0.00$, $P>0.05$) or mean juvenile dry weight and parent male SL ($r^2 = 0.08$, $P>0.05$). Flushing of pouches following release of juveniles did not produce any more pouch contents. Overall, the number (mean ± 1 SE) of premature juveniles (0.6 ± 0.2% of total pouch contents) and undeveloped eggs (0.5 ± 0.1%) released alongside fully developed juveniles was low, with 1.1 ± 0.2% (mean ± 1 SE) of each brood as non-viable.

Comparison of the reproductive output of wild and cultured *H. abdominalis* revealed that at a standardized log-transformed mean of 5.1 mm SL (which equates to a non-transformed mean SL = 17.18 cm), wild *H. abdominalis* had a significantly higher reproductive output (ANOVA, $F_{1,85} = 6.3$, $P<0.01$) with an average 190.5 juveniles per brood compared with 138 juveniles per brood for the cultured seahorses (Fig. 6.7). Wild seahorses had a larger mean size than the cultured seahorses (Students *t*-test, $t_{1,86} = 2.6$, $P<0.05$), but this does not affect the testing of regression slopes. Comparison of the mean juvenile SL and dry weight at birth between the offspring of the wild and cultured seahorses (16.7 ± 0.2 and 16.2 ± 0.2 mm SL, and, 1.2 ± 0.1 mg and 1.2 ± 0.0 mg respectively)) revealed no significant differences in either (Students *t*-test, $t_{1,86} = 1.8$ and $t_{1,86} = 0.5$, $P>0.05$ respectively).
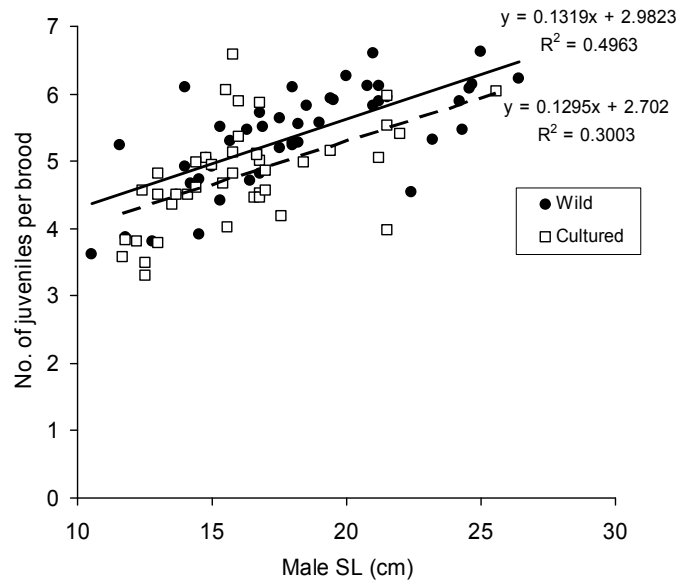
Figure 6.7 Comparison of the total number of juveniles per brood (log_e transformed) and parent male standard length (SL cm) in wild *Hippocampus abdominalis* from Wellington Harbour, New Zealand (*n* = 46) and cultured *H. abdominalis* (*n* = 42).

6.4 Discussion

6.4.1 Natural diet

Hippocampus abdominalis collected from Wellington Harbour preyed predominantly on crustaceans, in particular amphipods such as caprellids and ischyrocerids, caridean shrimp, and mysid shrimp. The predominance of epibiotic and epibenthic crustaceans in the diet of *H. abdominalis* is a reflection of their method of predation and habitat. In Wellington Harbour, *H. abdominalis* was found predominantly amongst stands of the macroalgae *C. flexuosum* and *C. maschalocarpum*. Amongst such macroalgae, seahorses were observed hunting around algal blades for prey such as *H. bifidirostris*, as well as around the surrounding substratum while hitched to the macroalgae, e.g. hunting the epibenthic swarming mysid *T. similis* over sand whilst attached to the macroalgae fringing the sand. Individuals were only occasionally observed out in the open on sandy bottoms, or swimming near macroalgae.

Seahorses ingest their prey via a strong suction action down their tubular snout (Bergert & Wainwright, 1997), and because they lack teeth and any internal masticatory structures, prey items can generally be found intact, which greatly enhances prey identification. As observed during collections, *H. abdominalis* generally ingests its prey whole in one strike. The exception to this is large specimens of *H. bifidirostris*, which

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are often broken into two pieces, with the tail and abdomen separated from the cephalothorax. This was not observed in the smaller shrimp found in the gut contents and would seem to indicate that the seahorses break large shrimp into two pieces before ingestion. This behaviour has been observed in the laboratory for *H. abdominalis* preying on large glass shrimp (*Palaemon affinis* 15–25 mm in length, Woods pers. obs.), as well as on mysid shrimp (Ocken, 1994). Most other seahorse species do not exhibit such prey-breaking behaviour (Foster & Vincent, 2004), although it has also been observed in *H. reidi* (Felício *et al.* 2006). With smaller prey, such as caprellid and ischyrocerid amphipods, these were usually completely intact inside the seahorse's digestive tract, indicating they are either small enough to fit whole directly down the seahorse' snout, or that they are labile enough to be folded compactly upon themselves (e.g. caprellid amphipods) during ingestion.

The presence of sand in the gut contents was relatively uncommon and generally did not constitute a large proportion of gut contents where it did occur, although three individuals which were unusually found on an open sandy area without extensive macroalgae cover did contain reasonably large quantities of sand in their guts (e.g. 80% volume), along with epibenthic organisms. This would appear to indicate that the ingestion of sand occurred during ingestion of prey on the sand bottom. Sand contained within the digestive tract could potentially facilitate digestion of prey through abrading gut contents, but its ingestion does not appear to be deliberately for this purpose.

Other researchers have also found that the diet of seahorses is dominated by crustaceans (Reid, 1954; Lovett, 1969; Tipton & Bell, 1988; Texeira & Musick, 2001; Kendrick & Hyndes, 2005; Felício *et al.*, 2006). However, the importance of specific types of crustaceans in seahorse diets varies with prey availability and abundance, as well as with the size of the seahorse species that consumes them. For example, Tipton & Bell (1988) found that in the small seahorse *H. zosterae* (usually <3.5 cm SL) harpacticoid copepods were the dominant prey item, with amphipods consumed to a much lesser extent and only more so in larger individuals. Kendrick & Hyndes (2005) found the diet of another small seahorse species *H. breviceps* to be dominated by gammarid amphipods, with caprellid amphipods and harpacticoid copepods consumed to a lesser extent. For *H. abdominalis*, which is a much larger seahorse than *H. zosterae* and *H. breviceps*, copepods were a very minor dietary component, with a greater amount of larger prey items such as amphipods, mysids, and caridean shrimp, up to 2 cm in length.

Ontogenetic variations in diet have been observed for many fish species, which is a reflection of a change in feeding capabilities (e.g. change in mouth gape) and/or habitat shifts (Tipton & Bell, 1988; Olaso *et al.*, 1999; Castillo-Rivera *et al.*, 2000; Garcia *et al.*, 2005; Nunn *et al.*, 2007). For example, in the pipefish *Syngnathus scovelli*, increasing pipefish length was related with an increasing frequency of consumption of amphipods, shrimp, ostracods, and crustacean eggs, with smaller pipefish consuming a greater proportion of copepods. In *H. abdominalis*, smaller adult seahorses consumed a larger proportion of total gut volume of amphipods than did larger seahorses. During this investigation, only adult seahorses were collected; early juveniles (1.5–4 cm SL) and late juveniles (4–8 cm SL) were not observed and collected, due to the initial pelagic behaviour of the former (see Chapter 2) and still relatively small size of the latter in a complex macroalgal habitat. However, it is likely that the diet of these juveniles would differ from that of adult seahorses, particularly in the initial pelagic phase where zooplankton assemblages are predated on.

Seasonal changes in food habits of fish may be caused by seasonal changes in the availability and vulnerability of prey, or habitat shifts by the fish themselves (Xie *et al.*, 2000). For example, in the flatfish *Citharichthys spilopterus*, penaeid and peracarid crustaceans were more important during the rainy season, whereas fish, alpheids, palaemonids, and copepods were dominant in the dry season (Castillo-Rivera *et al.*, 2000). In adult *H. abdominalis* from Wellington Harbour, where there appears to be no seasonal habitat shift, consumption of amphipods peaked in spring and autumn, whereas consumption of decapods was lowest in autumn. It is not known what the temporal variations in relative abundance are for these prey taxa in Wellington Harbour. In contrast, consumption of peracarids (mysids) was relatively constant during the year, as these occur in abundance around *H. abdominalis*'s natural habitat during the year in Wellington Harbour (Woods, pers. obs.).

Sexual differences in diet may occur where there are differences in overall behaviour, physical differences, or spatial or seasonal habitat segregation (Molinero & Flos, 1991; Castillo-Rivera *et al.*, 2000). In this investigation, no sexual differences in diet for *H. abdominalis* were observed. It would be interesting to examine whether the diet of brooding male seahorses differs from that of females and non-brooding males. This was not possible in this investigation as the decision was made to non-destructively assess the reproductive output of male seahorses. For example, in *H. zosterae*, male metabolic

rate increases from 10 to 52% over pre-gravid levels (Masonjones, 2001), while Felício *et al.* (2006) found that reproductive male *H. reidi* exhibited higher feeding frequency than non-reproductive males. This may therefore affect feeding behaviour.

For the pipefish *Syngnathus fuscus*, Ryer & Boehlert (1983) determined that natural daily ration calculations yielded estimates of c. 4% of body weight day^{-1} (dry weight food/dry weight of pipefish), which they found similar to other teleosts (e.g. Adams, 1976). In this investigation, calculated daily ration for *H. abdominalis* was found to be 4.9% of seahorse wet weight. This is likely to be an underestimate as discussed in the methods section. Daily ration may change ontogenetically between larval and juvenile stages of different fish species (Peters & Kjielson, 1975). It would therefore be interesting to determine whether daily food ration changes between small juvenile and adult *H. abdominalis*.

Comparison of the fatty acid profiles of two prey items (the mysid shrimp *T. similis* and the caridean shrimp *H. bifidirostris*) of *H. abdominalis* in the wild and two foods used in its culture (*Artemia* enriched on Algamac-3050$^{®}$ and the frozen mysids (*A. kempi*) — see Chapter 5) revealed that overall *n*-3 HUFA levels were lowest in the *Artemia* enriched on Algamac-3050$^{®}$ and percent of PUFAs highest in the frozen mysids. Enriched *Artemia* and *H. bifidirostris* had the highest percentages of AA, 20:4*n*-6, while the frozen mysids had the highest percentages of EPA, 20:5*n*-3, and DHA, 22:6*n*-3.

Marine fish are thought to have high nutritional requirements for the EFAs DHA and EPA, as well as for other fatty acids such as AA, and linolenic acid, 18:3*n*-3 (Watanabe *et al.*, 1989; Sargent *et al.*, 1997; Corraze, 2001). However, the research in Chapter 5 demonstrated that late-stage *H. abdominalis* juveniles do not require large amounts of EFA for somatic growth, or the classical ideal EFA indices as demonstrated for the larval stages of some marine fishes (Izquierdo, 1996; Coutteau, *et al.*, 1997; Sargent *et al.*, 1999). Both Thompson (2002) and Shapawi & Purser (2003) have questioned the need for high DHA:EPA ratios in their respective enrichment studies on *H. abdominalis*, although Chang & Southgate (2001) and Thompson (2002) maintain that newborn *Hippocampus* sp. do require a certain amount of *n*-3 HUFA and other EFAs to maximize growth and survival.

Seasonal variations in the fatty acid levels of organisms such as mysid and caridean shrimp are well documented (Bradshaw *et al.*, 1990; Richoux *et al.*, 2004; Yanar & Çelik, 2005), therefore the value of the fatty acid comparison conducted in this study is useful only as an indicative "snapshot". However, there is considerable scope to alter fatty acid profiles of cultured live foods through a range of dietary and environmental manipulations (Hoff & Snell, 1987; Furuita *et al.*, 1999; Navarro *et al.*, 1999; Sorgeloos *et al.*, 2001; Copeman *et al.*, 2002; Lubzens & Zmora, 2003; Støttrup, 2003; Calado *et al.*, 2005a, b). Given that both *Artemia* enriched on Algamac-3050® and the frozen mysids (*A. kempi*) have been shown to promote good somatic growth with high survival in cultured *H. abdominalis*, the comparative fatty acid profiles of these foods with the two wild foods suggests two things relevant to the commercial culture of *H. abdominalis*: 1) with regards to some fatty acids regarded as necessary or beneficial to marine fish growth, *Artemia* enriched on Algamac-3050® and the frozen mysids (*A. kempi*) can be as good as wild foods, and, 2) it may be beneficial to domestically culture some of the wild prey items of *H. abdominalis* as an alternative to the current importation of *Artemia* cysts and frozen mysids.

6.4.2 Reproductive output of male seahorses

As with knowledge of seahorse natural diet, knowledge of the natural reproductive output of *H. abdominalis* is important for providing natural benchmarks against which commercial aquaculturists can measure their operational success and highlight areas for improvement. *Hippocampus abdominalis* is a very large seahorse species, reaching up to 35 cm SL (Lourie *et al.*, 2004). The size at sexual maturity for wild *H. abdominalis* has not yet been determined, but the size at which 50% of cultured male *H. abdominalis* theoretically reach sexual maturity, signified by the first development of a brood pouch, is 7.3 cm SL. However, in the laboratory, seahorses usually do not start breeding till they reached 9–10 cm SL (Woods, unpubl. data). If these culture data are comparable to that of *H. abdominalis* in the wild, the size range of brooding seahorses found in this study (10.5–26.4 cm SL) appears to be a reasonable representation of the reproductive size range for this species and that first development of a brood pouch at 7.3 cm SL does not necessarily mean the males are reproductive at this size. Thangaraj *et al.* (2006) examined the onset of sexual maturity in cultured *H. kuda*. They found that although males first showed brood pouch development at c. 42 mm length, they did not

begin courtship displays until c. 90 mm length, and first received eggs from females at c. 100 mm length when females were c. 110 mm in length.

Brood size of male *H. abdominalis* in Wellington Harbour was positively related with male size in terms of SL and weight, with larger males producing larger broods. Strawn (1958) found a similar trend in *Hippocampus zosterae* from Florida as did Texeira & Musick (2001) in *Hippocampus erectus* from Chesapeake Bay. This relationship may be explained by brood pouch volume, and hence egg-carrying capacity, increasing as males increase in overall size. However, it is likely that larger brood sizes in larger male seahorses are the result of these males mating with larger females with greater reproductive output, rather than larger male brood pouch volume directly. There is some evidence that larger male seahorses pair preferentially with larger females (this has been observed in captive *H. abdominalis* but not yet for wild *H. abdominalis*, Woods, pers. obs.) and brood size in the preliminary investigation (Chapter 2) was more strongly related with parent female size than parent male size. Vincent & Giles (2003) found this for *H. whitei*, for which female size was the key determinant of the number of young released by the male, not male size or pouch volume.

The average number of juveniles produced per brood by *H. abdominalis* in this study was 271, which falls within the normal range of juveniles produced by other seahorse species of 100–300 juveniles per brood (Foster & Vincent, 2004). The largest brood size recorded in this study (744) is below the maximum recorded for captive *H. abdominalis* (1116 in Foster & Vincent, 2004). According to Foster & Vincent (2004), the size of newborn seahorses is relatively conserved across the genus when compared with the spread in adult sizes of the various *Hippocampus* species. Juvenile size at birth is variable across seahorse species, ranging from 2 to 16 mm in length and appears to be positively related with increasing latitude rather than adult size (Foster & Vincent, 2004). The exceptions to this trend appear to be the very small *H. bargibanti* and the very large *H. abdominalis* which produce smaller and larger than average sized juveniles respectively. Certainly, the average juvenile size at birth in this study (16.7 mm SL) is large when compared with the other seahorse species newborns which vary between 5 and 11.8 mm in length (Foster & Vincent, 2004). Foster & Vincent (2004) compared the reproductive output and parental investment in seahorses against a range of marine teleosts. They found that seahorses have larger diameter eggs than other marine teleosts (controlling for fish size) but appeared to have lower fecundity. This

greater investment in egg size resulted in seahorses having larger young at birth compared with other marine teleosts, even those which also exhibit parental care (Foster & Vincent, 2004). The implication to this is that at birth a size advantage may translate to an increased survival advantage, as mortality rates generally decrease with increasing body size (Ahnesjö, 1992; Houde, 1997).

The eggs of seahorses enter the brood pouch of males with their maternally-provided yolk supply, thus the nutritional status of the female's egg supply will be a major determinant in the resulting quality of juveniles. Along with this maternal influence on egg quality, the male seahorse's brood pouch supplies essential environmental conditions and other elements which affect egg development (Linton & Soloff, 1964; Boisseau, 1967; Melamed *et al.*, 2005). With increasing numbers of embryos brooded, there is the possibility that a resource or supply limitation (e.g. oxygen supply) could occur, with competition among the juveniles for that resource or supply affecting their development (Vincent, 1990; Dzyuba *et al.*, 2006). For example, in captive *H. fuscus*, Vincent (1990) found that the mean length of brooded young was inversely related with brood size, whilst Ahnesjö (1992) observed that in the pipefish *Syngnathus typhle*, the number of newborns brooded by male *S. typhle* was negatively related with the weights of both newborn and 2-week old juveniles, as well as juvenile growth rate.

In this study, mean SL and DW of juvenile *H. abdominalis* did not significantly correlate with parent male size, the number of juveniles being brooded, brood pouch volume of the parent male, or the combination of these latter two variables (number of juveniles per brood/brood pouch volume) within the size range sampled; males of any size produced the same size and weight juveniles. The reason for the lack of a relationship between juvenile size and number of juveniles per brood/brood pouch volume is open to speculation. Vincent (1990) observed that male *H. abdominalis* have proportionately larger brood pouches than other seahorse species, and their pouch is divided by 3–5 septa instead of the normal one. She suggested that the extra septa and large pouches provide larger internal volume for brooding, and that male *H. abdominalis* have a low level of male investment in developing young. It is possible the brood sizes encountered in this study were not large enough in relation to brood pouch volume for resource or space competition to be a significant factor amongst the developing juveniles, and/or egg quality from female seahorses was of a quality such that resource or space competition effects were ameliorated.

The number of non-viable eggs, and premature/under-developed juveniles expelled by brooding *H. abdominalis* was low (combined 1.1 ± 0.2% mean ± 1 SE of pouch contents). As the tank setup ensured that any release of premature juveniles or eggs would be readily observed and tanks were checked daily, it is unlikely that any premature release of juveniles or eggs would have been undetected. Strawn (1958) found that less than 1% of eggs in the pouches of males were infertile during the main part of the breeding season in wild *H. zosterae*. Texeira & Musick (2001) found non-fertilised eggs frequently in wild *H. erectus* (i.e. occurring in 50% of males examined and ranging in percentage of total pouch contents from 1.7 to 33%). In captive *H. fuscus*, Vincent (1990) found that males failed to incubate on average between 9 and 48% of their broods, with lower failure rates in mating pairs that had been together longer.

Possible reasons for brood losses may include such variables as poor quality brood incubation and poor initial egg quality and, in captive seahorses, culture deficiencies such as insufficient feeding (Vincent, 1990). The low brood failure rate of *H. abdominalis* may indicate the production of good quality eggs by females or the provision of a good brood environment by the males. However, this low brood failure rate might not be the full story, as there may be mortality in the pouch during pregnancy which is not traceable at parturition. Hence, the brood failure rate enumerated here should be considered a conservative value.

It is interesting to note that when collections were made, brooding male *H. abdominalis* were found throughout the year (e.g. from January 2000-February 2003). Generally, duration of breeding season is longer in seahorses in tropical than temperate waters with representative breeding durations of 6–12 months for tropical species and 4–8 months for temperate species (Foster & Vincent, 2004). *Hippocampus abdominalis* appears the exception to this pattern. The reasons for this long breeding duration are not yet known. The occurrence of brooding males throughout the year mirrors another study on wild female *H. abdominalis* from Wellington Harbour, which demonstrated the presence of reproductively active females throughout the year, with the gonado-somatic index (GSI) peaking in spring/summer (Poortenaar *et al.*, 2004). In retrospect, it would have been beneficial to have recorded data on the total number and sizes of all seahorses observed during collections, particularly non-brooding males, to determine whether the proportion of males brooding changes temporally. However, even though brooding male

H. abdominalis were found throughout the year, there appeared to be a greater ratio of brooding males caught per collection event during the months January to April (Table 6.1). The ratio of number of brooding males:collection event was approximately 2.1:1 for January to April months, and the reverse (1:2.1) for May to December months. These suggestive peaks in male and female reproductive patterns would appear to support the manipulation of culture photoperiod (e.g. as in Chapter 2) to maximize reproduction.

Comparison of the reproductive output of wild male *H. abdominalis* with that of cultured males revealed that the output of cultured males was approximately 27% lower than that of the wild males. However, there was no difference in the size and weight of the juveniles produced between wild and cultured males, or the number of non-viable eggs, and premature/under-developed juveniles expelled by brooding wild and cultured male *H. abdominalis*. This suggests a limitation in the number of eggs being received by the cultured males, rather than a difference in the quality of brooding by the different males or egg quality and would support the role of females as the primary determinants of reproductive output for seahorses. For *H. whitei*, female size is the key determinant of the number of young released by the male (Vincent & Giles, 2003). Hence, the larger broods in wild male *H. abdominalis* may be the result of these males mating with larger females in the wild with greater reproductive output. However, this cannot be qualified for this investigation, as the size of the wild male's mates is not known in this study.

Alternatively, the cultured female *H. abdominalis* may have been producing fewer eggs to transfer to males. Reasons for reduced fecundity in fish can include environmental variables such as photoperiod and temperature, stress and nutrition (feed rate, nutrient imbalance or restriction) (e.g. Harris, 1984; Deacon & Keast, 1987; Fernández-Palacio *et al.*, 1995; Siddiqui *et al.*, 1997; Ridha *et al.*, 1998; Al Hafedh *et al.*, 1999; Pottinger & Carrick, 2000; Izquierdo *et al.*, 2001; Kruger *et al.*, 2001; Takasuka *et al.*, 2005). For example, Li *et al.* (2005) found that either deficient or excess dietary *n*-3 HUFA had a negative effect on egg and subsequent larval quality in the marine teleost *Plectorhynchus cinctus*. Given that the cultured seahorses were maintained in seawater pumped from the general area in which the wild seahorses were collected and this hatchery seawater generally only experienced a maximum difference of 1°C from the field seawater (J. Illingworth, NIWA, pers. comm.), temperature appears unlikely to be a factor. Stress is also probably not a causative factor as the cultured seahorses were

conditioned to a captive environment with good water quality and a low relatively low stocking density (typically <1 seahorse 50 l^{-1} for broodstock).

Differences in nutrition would be more likely to be a causative factor as the cultured seahorses were fed a restricted diet, with ration and feeding times determined by the researcher. Photoperiod may also be a factor, as the cultured seahorses were typically maintained at 10 h L:14 h D with fluorescent lighting, compared to the much higher intensity natural light with temporal variation experienced by the wild seahorses. Determining reasons for potentially lower reproduction in cultured seahorses is important as the maximization of healthy and sustainable reproductive output can affect the economic efficiency of commercial culture, and requires further investigation.

CHAPTER 7

GENERAL DISCUSSION

7.1 Introduction

For the development of any new aquaculture species there are specific areas of key knowledge that must be acquired to enable the commercial aquaculture of that species to be biologically and economically viable. Specific areas of key knowledge relevant to the development of commercial aquaculture of the seahorse *Hippocampus abdominalis* in New Zealand are:

1) Broodstock e.g. environmental and physical requirements for breeding, reproductive characteristics and fecundity, stocking density, tank design, and water quality.

2) Initial juvenile rearing e.g. diets and their cost/benefit relationships, factors affecting initial survival, stocking density, tank design, and water quality.

3) On-growing to market size e.g. diets and their cost/benefit relationships, maximising growth and survival rates, stocking density, tank design, and water quality.

4) Market e.g. current and predicted demand and supply, economic business models, nature of product required or desired, product production cost and sale price, and relevant business contacts.

The extent to which the research contained in this thesis successfully acquired commercially-relevant aquaculture knowledge on *H. abdominalis* is now discussed. The research scope of this thesis did not include the specific acquisition of market knowledge, however, it is also discussed.

7.2 Broodstock

7.2.1 Captive broodstock

The breeding of large *H. abdominalis* in captivity has proven to be a problematical area for commercial aquaculture in some instances (Vincent, 1996). In the preliminary investigation (Chapter 2), the breeding of large *H. abdominalis* in captivity proved reasonably straightforward on a diet of predominantly enriched live *Artemia*. However, the successful completion of ritualized courtship behaviour and egg transfer from female to male *H. abdominalis* was strongly influenced by vertical tank dimension, with a vertical tank height of 1 m required for egg transfer in the large broodstock.

For many marine temperate species, photoperiod has been shown to be a primary environmental cue for the synchronization of reproductive cycles (e.g. Bye, 1984; Bromage *et al.*, 1990). Temperature is more important in final oocyte maturation and ovulation timing (e.g. Bye, 1990). Knowledge of photoperiod and temperature effects on reproduction allows culturists to manipulate these variables to trigger, maintain or prevent breeding in finfish (Ridha & Cruz, 2000; Migaud *et al.*, 2004; Biswass *et al.*, 2005; Fontaine *et al.*, 2006; Olivotto *et al.*, 2006). For example, Martin-Robichaud & Berlinsky (2004) found that haddock (*Melanogrammus aeglefinus* L.) broodstock could be phase-shifted photothermally to both advance and protract breeding season *ex situ*. They suggested by phase-shifting different groups of fish photothermally, year-round breeding might be achieved with the seasonally-breeding haddock. In the wild, reproductive female and male *H. abdominalis* occur year-round (Poortenaar *et al.*, 2004; Chapter 6; Woods, 2005b), but there appears to be both an increase in female GSI and incidence of male brooding during warmer spring/summer months. This combined with observations from the preliminary investigation which indicated apparent courtship and breeding photoperiod trigger-points, suggest that photoperiod is an important factor in reproduction for *H. abdominalis*. Strawn (1958) found that a photoperiod trigger-point for *in situ* breeding of *H. zosterae* was 11.1 h light, with no breeding at less than this and about two-thirds of males examined breeding at over 12 h light. Strawn (1958) observed that temperature did not appear to be as critical as photoperiod but that it may be important as a wider limit. For *H. abdominalis*, the photoperiod trigger-point for the initiation of breeding appears to be 11 h light:13 h dark, with breeding commencing when the light duration exceeds 11 h day^{-1} (Chapter 2). Commercial culturists of *H. abdominalis* may wish to further investigate photoperiod/thermal manipulation to optimize production of *H. abdominalis* year-round.

A "biological zero point" of 6.52°C was indicated for brooding in *H. abdominalis*, with brood duration decreasing with increasing temperature up to 19°C. Minimum surface seawater temperatures around mainland New Zealand do not typically descend below 7°C (http://www.niwascience.co.nz) but culturists will either want to position culture facilities as far way from the "biological zero point" of 6.52°C as is practicable in relation to other culture considerations, or artificially maintain their seawater at a suitable temperature (e.g. 18°C for constant breeding and short brood duration) to optimise breeding. It would be useful to determine the upper temperature limits for breeding *H. abdominalis* as this may affect the location of seahorse farms, particularly in the far north of New Zealand, and the need for water-cooling equipment. For example, breeding in captive *H. abdominalis* has been observed to cease when the water temperature exceeds 24°C in northern New Zealand (J. Shirley, Pahia Aquarium, pers. comm.).

Competition between multiple males courting the same female was observed, which could prevent transition to the final courtship stage of vertical rising and egg transfer. In large-scale seahorse farms with a species such as *H. abdominalis*, which does not appear to be rigidly monogamous, captive broodstock are often kept in large tanks and left to breed without intervention and resulting newborn juveniles are collected daily (B. Gray, The Seahorse Farm, pers. comm.). This minimizes handling and labour costs, and allows seahorses to pair-up according to attributes such as compatible mate size, but it may result in lower reproductive success due to competitive interactions. For small-scale seahorse farms culturing seahorse species where pair-bonds are important (e.g. *H. kuda*), maintaining pair-bonded mates separately generally results in greater productivity (G. Leveridge, Seahorse New Zealand, pers. comm.) and can have other benefits such as monitoring selected blood-lines. It would be worthwhile investigating whether maintaining broodstock *H. abdominalis* in small or large groups actually affects broodstock productivity.

Broodstock *H. abdominalis* produced a mean brood size of 269 juveniles per brood. This quantity of juveniles is sufficiently large to allow a commercial operation to quickly establish production and closed captive life cycles, whereby wild or purchased seed broodstock are no longer needed, or at least their need minimized as some fresh genetic input may be desirable to maintain sufficient genetic diversity and prevent in-breeding. For example, if males mate and produce juveniles every two months this can

produce approximately 1600 juveniles year^{-1} adult male^{-1}. If only 10% of these juveniles survive to one year of age and a size appropriate for the aquarium trade (>10 cm SL and sexually mature), this still means that each male seahorse produces 160 sexually mature and saleable progeny per year.

Overall, this preliminary study demonstrated that breeding in *H. abdominalis* can be achieved relatively easily once certain basic conditions are provided. This was further proven with successful breeding in successive captive generations derived from the original wild broodstock. Successful large-scale captive breeding of *H. abdominalis* has also proven to be relatively straightforward in a commercial setting (B. Gray, The Seahorse Farm, pers. comm.).

7.2.2. Reproductive output of wild male seahorses

Knowledge of wild seahorse reproductive output can be beneficial to commercial culturists to provide natural benchmarks against which they can measure their operational success. However, there have been few studies on the wild reproductive output of most seahorse species (but see Strawn, 1958; Texeira & Musick, 2001; Vincent & Giles, 2003), and until this study and that of Poortenaar *et al.* (2004) there was no wild reproductive output data for *H. abdominalis*. The reproductive output of wild male *H. abdominalis* was compared against that of cultured male *H. abdominalis* reared at NIWA's Mahanga Bay facility and revealed that the output of cultured males was approximately 27% lower than that of wild males, although there were no differences in the quality (size and weight) of the juveniles produced. This suggests a limitation in the number of eggs being received by cultured males, rather than a difference in the quality of brooding or the eggs. The reasons for this are unknown and require further research, as the maximization of healthy and sustainable reproductive output can affect the economic efficiency of commercial seahorse culture. However, as female seahorses are the primary determinant of male reproductive output, such reproductive research should focus foremost on understanding and maximizing their reproductive output.

Generally, in finfish culture larger fish are used as broodstock as they are often more fecund and may produce better quality offspring (e.g. Wroblewski *et al.*, 1999; Estay *et al.*, 1999, 2004). This positive adult size/reproductive output relationship was also found to be true for wild male *H. abdominalis*. This has also been observed in other

seahorse species such as *H. erectus* (Texeira & Musick, 2001) and *H. kuda* (Dzyuba *et al.*, 2006). The consequence of this for fish culturists generally is the requirement to retain large animals to maximize reproductive output, but this may require large broodstock tanks and special feeds. However, for seahorses this is less of an issue due to their sedentary nature, apparent lack of territorial behaviour and ability to reproduce on a largely standard diet of enriched *Artemia*. Consequently, seahorse culturists may choose to stock more fecund larger broodstock without necessarily increasing tank space to a large degree or using specialist broodstock diets, although the latter may benefit from further investigation.

The year-round breeding of *H. abdominalis* in the wild as demonstrated in Chapter 6 is also a pattern observed by commercial culturists of *H. abdominalis*, albeit usually with a reduction in the frequency of reproduction in captivity during periods of cold temperatures. From a commercial viewpoint, continuous aquaculture reproduction may be desirable to ensure a continuous supply to the markets. However, it may also be desirable to have periods of "rest and recovery" (facilitated by sex separation, or reduced photoperiod and water temperature) where broodstock can recover, build up condition and invest resources into gametogenesis before reproduction is allowed to occur again. Migaud *et al.* (2002) found that longer periods of cooler water in Eurasian perch (*Perca fluviatilis*) produced greater gametogenic investment. If periods of "rest and recovery" are employed, continuous supply could still be maintained if different groups of broodstock are allowed to alternately breed and rest. This may actually prove more beneficial in long-term production if the resulting progeny are subsequently of better quality, with corresponding faster growth rates, survival and healthier appearance. It would be interesting and beneficial to commercial culture to compare the effects of continuous vs. periodic reproduction in cultured *H. abdominalis*.

Knowledge of wild seahorse reproductive output is also of importance regarding the regulation and sustainability of trade in wild-caught seahorses. All seahorse species are currently listed on CITES Appendix II (http://www.cites.org) which requires any trade to be determined as being non-detrimental to exploited populations. To issue a Non-Detriment Finding (NDF) the relevant CITES regulatory body must have a reasonable knowledge of the biology and ecology of the seahorse species concerned. In lieu of a NDF, a minimum seahorse height, such as the 10 cm minimum height (mHT) recommended by CITES (see: http://www.cites.org/eng/notif/2004/033.pdf, accessed 10

March 2005) may be used. The 10 cm mHT size restriction is an across-species compromise that provides one value for all species to allow a reproductive output that will sustain an exploited population (Martin-Smith *et al.*, 2004; Foster & Vincent, 2005).

When the CITES 10 cm mHT size restriction is translated to SL (11.56 cm SL, conversion factor = HT/0.865, Woods unpubl. data) and plotted against brood sizes of male *H. abdominalis* from Wellington Harbour (Fig. 7.1), it appears that this size restriction is not an adequate protective measure for *H. abdominalis* at this location. The data suggest that the 10 cm mHT restriction leaves the most productive males open to exploitation. However, without further data from around New Zealand it is difficult to suggest what an appropriate revised mHT might be for *H. abdominalis*. In relation to the data obtained from Wellington seahorses, mHT would have to be increased to at least 20 cm SL (17.5 cm mHT), to double the reproductive output of protected males currently protected by the 10 cm mHT. A smaller increase in the limit by, for example, 2–5 cm would represent relatively little gain in individual reproductive output, but would be beneficial in leaving a larger number of reproductive individuals in the population.

Altering mHT to a higher limit will not reduce the incidental capture of *H. abdominalis* during relatively non-selective commercial fishing such as trawling or dredging. However, in non-commercial take, an altered mHT could be a useful management tool as fishing in this instance is usually targeted and selective (e.g. hand-collection whilst snorkeling), which would allow seahorses not meeting the mHT to be left undisturbed *in situ*.
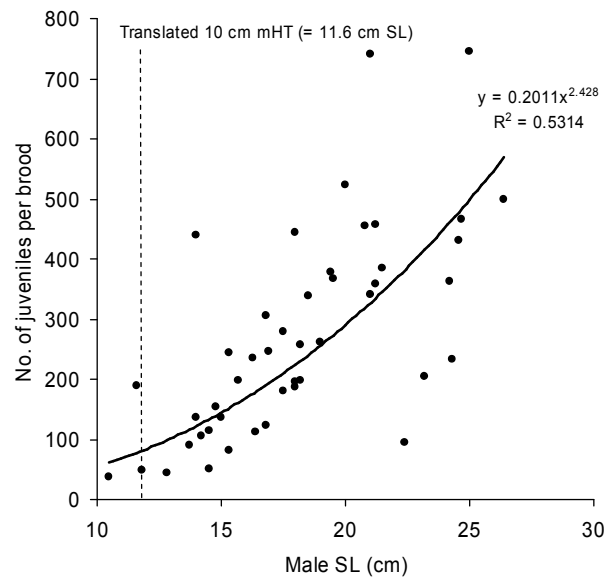
Figure 7.1 Relationship between total number of juveniles per brood and parent male standard length (SL cm) in *Hippocampus abdominalis* from Wellington Harbour, New Zealand (*n* = 46). The CITES 10 cm minimum height restriction (mHT) is translated into SL = 11.6 cm SL.

7.3. Initial juvenile rearing

7.3.1. Initial survival and growth

In the aquaculture of many finfish species, larval production is a major bottleneck reflecting the fact that marine fish usually produce large numbers of very small eggs that generate very small, vulnerable, free-living and rapidly growing offspring which may need to pass through metamorphosis before progressing to the juvenile phase (Sargent *et al.*, 1997, Rønnestad *et al.*, 1999; Gisbert *et al.*, 2004; Nan Chen *et al.*, 2006). However, as seahorses produce well-developed independent juveniles at birth, means of avoiding high mortality rates should be more easily found for seahorses.

The initial rearing of juvenile *H. abdominalis* in the preliminary investigation proved problematic in terms of achieving high survival, with juvenile mortality highest in the first few months following birth. However, observations of these preliminary juveniles indicated problems with prey capture could be largely responsible for the high initial mortalities. As seahorses are visual feeders, the effects of tank background colour and source of illumination were investigated for early juvenile *H. abdominalis*.

Through examining the effects of tank colour on predation, it was determined that tank background had an important effect on both attack rate and capture success on *Artemia*

nauplii for newborn and juvenile *H. abdominalis*. The effect of tank colour has been little studied in seahorses. However, Martinez-Cardenas & Purser (2007) also tested the effect of tank background colour on feeding activity, growth and survival in juvenile *H. abdominalis* but found no differences in feeding activity, growth or survival. The reasons for this difference in results are not known although the different enrichments used in each study resulted in the *Artemia* having different coloured guts. This may have influenced *Artemia* visibility for the seahorses and therefore had an effect on feeding.

It was also found that top-illumination of tanks attracted *Artemia* nauplii to the water surface, which in turn resulted in higher incidences of air bubble ingestion in early juveniles as they attacked the *Artemia* at the water surface. Air bubble ingestion severely compromised juvenile buoyancy control and blocked their gut. By using simple glass aquaria with side illumination and the above-waterline area blacked-off, initial juvenile *H. abdominalis* survival rates were shown to be dramatically improved upon those encountered in the preliminary investigation. These simple findings on how to improve initial juvenile survival were consequently implemented at two commercial farms rearing *H. abdominalis* (South Australian Seahorse Marine Services in Australia and The Seahorse Farm in New Zealand) to improve their culture success.

The elucidation of what variables, either species-specific or generic, significantly affect juvenile seahorse survival has significantly advanced in the last 10 years (e.g. salinity in *H. kuda*; Hilomen-Garcia *et al.*, 2003). It is now not uncommon for commercial farmers to consistently achieve survival rates of >40-50% per brood to market size (G. Leveridge, Seahorse New Zealand; B. Gray, Seahorse New Zealand; M. Payne, Kalbarri Seahorse Sanctuary, pers. comm.) and this has considerably improved the economic viability of commercial seahorse culture.

In commercial aquaculture, it is desirable to determine a suitable density at which fish should be stocked to maximize biomass production and minimize water usage, without compromising the health or quality of the fish. With increasing stocking density, both juvenile *H. abdominalis* growth rates and survival decreased. This appears largely due to the physical impedance of feeding, rather than direct competition for food. At higher stocking densities there was greater occurrence of juveniles grasping and wrestling with each other. This grasping-effect is unique to seahorses compared with other cultured

finfish because of their well developed prehensile tails, and therefore seahorses have unique husbandry requirements for high-density culture. Although research such as this demonstrates a clear growth and survival advantage to using low stocking densities (e.g. 1 or 2 juveniles l^{-1} for juvenile *H. abdominalis* 70 mm in length), within the commercial environment there is a strong economic driver to increase stocking densities. Optimal balances within the commercial aquaculture environment need to be established between the influence of stocking density on seahorse growth and survival in relation to the cost/benefit of the stocking density used.

7.3.2. Initial juvenile diet

Culturing large quantities of nutritious live food for fish in a commercial environment and ensuring a constant supply can prove difficult and costly (Lazo *et al.*, 2000; Hamlin & Kling, 2001; Blair *et al.*, 2003; Callan *et al.*, 2003; Khemis *et al.*, 2003; Payne, 2003). Considerable effort has therefore been concentrated on developing and testing formulated or artificial feeds for finfish species (e.g. Applebaum, 1985; Daniels & Hodson, 1999; Buchet *et al.*, 2000; Yufera *et al.*, 2000; Silva, 2001; Blair *et al.*, 2003; Khemis *et al.*, 2003; Ljunggren *et al.*, 2003; Hamre *et al.*, 2005; Wang *et al.*, 2005).

In Chapter 3 the suitability of frozen and artificial foods for feeding to *H. abdominalis* juveniles, either alone or mixed with live *Artemia* was tested. Using two commercially available inert diets (Golden Pearls and frozen copepods) it was demonstrated that one and two month-old juvenile *H. abdominalis* could ingest and survive on these inert foods. Co-feeding the inert diets with live *Artemia* improved feeding on the inert foods. However, growth and survival rates of one and two month-old juveniles on the inert diets were inferior to those fed only on live enriched *Artemia*.

Given the relative ease with which early instar *Artemia* nauplii can be mass-produced, there may not be an immediate economic benefit to weaning early juvenile *H. abdominalis* onto inert diets. For example, with premium grade *Artemia* cysts around NZ$0.11 g^{-1} (US$0.07 g^{-1}), 1 g of cysts would produce approximately 245 000 instar II nauplii (http://www.brinsehrimpdirect.com; accessed 7 April, 2006), which at a feeding rate of 1000 to 2000 *Artemia* nauplii juvenile seahorse^{-1} d^{-1} (Tung, 2005) would result in a number of juveniles fed to diet cost (NZ$) ratio of 564 to 1126 juveniles:NZ$0.01. In contrast, the Golden Pearls at NZ$0.07 g^{-1} (US$0.05 g^{-1}) (http://www.brinsehrimpdirect.com; accessed 7 April, 2006), if fed at a conservative

wet body weight ration of 1% seahorse^{-1} for one month-old *H. abdominalis* (0.05g wet weight) would result in a number of juveniles fed to diet cost (NZ$) ratio of 262 juveniles:NZ$0.01. This is clearly not as cost effective as using *Artemia* nauplii on a simple cost/benefit basis, although culture costs associated with rearing *Artemia* would need to be added to the calculation for a real assessment of diet cost-effectiveness. Exposing early juveniles to inert diets may have a longer term benefit of predisposing larger seahorses to accept inert diets. This is an important marketing consideration when supplying seahorses to the aquarium trade where aquarium owners may not culture or have access to supplies of live foods, or may prefer to use inert diets.

7.4 On-growing to market size

7.4.1 Effect of *Artemia* enrichment

Artificial diets are used in rearing many commercial finfish species as a means of reducing culture cost and improving feed reliability, as well as offering improved growth rates due to often more efficient Feed Conversion Ratios (FCR) associated with their higher nutrient content and nutrient availability (e.g. Hillestad *et al.*, 1999; Cho *et al.*, 2006; Ozorio *et al.*, 2006). However, seahorses are visual feeders that are strongly attracted to moving prey and have generally proved very difficult to wean onto artificial diets, particularly in the early juvenile stage. Therefore, in the captive environment aquarists, researchers, and commercial culturists have traditionally relied heavily on cultured live foods such as brine shrimp (*Artemia*), copepods, mysid shrimp and amphipods to feed to seahorses, in addition to collecting live wild foods (Correa *et al.*, 1989; Forteath, 1995; Lockyear *et al.*, 1997; Wilson & Vincent, 1998; Hilomen-Garcia, 1999; Payne & Rippingale, 2000; Indiviglio, 2002; Naik *et al.*, 2002; Abbott, 2003).

Until the development of suitable artificial diet formulations that can offer improved growth and survival rates compared to live foods for *H. abdominalis*, it is prudent to examine the potential for improving the quality of currently used live feeds and reduce their associated culture costs. In Chapter 5, the growth rate and survival of *H. abdominalis* fed *Artemia* enriched with three commercial enrichment products (Super Selco®, DHA Protein Selco® and Algamac-3050®) was compared with that of *H. abdominalis* fed *Artemia* reared on a low-cost on-growing diet (a mixture of a proprietary rice bran-based product (EPABSF) and *Spirulina platensis*).

Artemia enriched with all three commercial enrichment products and the EPABSF/*S. platensis* on-growing diet promoted growth (with no mortality) in juvenile *H. abdominalis*. Fatty acid analyses showed that the EPABSF/*S. platensis* enriched *Artemia* had the lowest levels of many EFAs, as well as the lowest (and theoretically poorest) DHA:EPA ratio, yet *Artemia* enriched with the EPABSF/*S. platensis* promoted good seahorse growth with no mortalities. This suggests that late-stage *H. abdominalis* juveniles did not require large amounts of EFA, or the target EFA indices demonstrated for some other commercially farmed fishes. This is supported by Thompson (2002) and Shapawi & Purser (2003) who suggest that *H. abdominalis* may have a lower DHA:EPA target ratio and lower dietary EFA requirements due to its greater degree of parental investment in its young. On a cost/benefit basis the low-cost EPABSF/*S. platensis* on-growing diet was also the most cost-effective for *H. abdominalis* at the feeding rate used in this investigation, with comparable or better growth rates than seahorses fed *Artemia* enriched with the more expensive *Artemia* enrichments. This has important ramifications for the economical efficiency of the commercial culture of *H. abdominalis* in terms of possibly sourcing appropriate cheap plant-processing by-products from within New Zealand's existing pastoral industries for on-growing/enriching suitable live prey.

If commercial seahorse culturists wish to culture *Artemia* as the main food source for their seahorses, then they must employ production systems which maximize *Artemia* biomass whilst minimizing associated production costs. Ideally, they should also reduce their reliance on the importation of *Artemia* cysts, as dramatic seasonal fluctuations in international *Artemia* cyst price and availability have occurred (Sorgeloos *et al.*, 2001; Dhont & Van Stappen, 2003; Lim *et al.*, 2003). In many tropical and sub-tropical countries, *Artemia* biomass can be cultured and harvested in outdoor solar salt ponds and saline lagoons, supplying large quantities of *Artemia* to aquaculture industries such as shrimp farming (Van Stappen, 2003; Camara *et al.*, 2004). Outdoor pond production offers the benefits of large-scale culture and lower operating costs as local climatic conditions drive production. Tyler (1996) investigated intensive production of *A. franciscana* cysts in outdoor ponds in Western Australia with a view to developing large-scale *Artemia* production techniques separate from the normal salt production industry. He established relatively low-cost techniques that enabled the production of 1 kg day^{-1} of cysts from an outdoor 0.08 ha pond.

An example of solar ponds where a natural *Artemia* (*A. franciscana*) biomass occurs in New Zealand is the Dominion Salt Ltd's solar salt works at Lake Grassmere. Haslett & Wear (1985) examined *A. franciscana* biomass production in Lake Grassmere between 1980 and 1982. They found *A. franciscana* biomass was highest during late spring and summer, with the largest estimated biomass production from 238.5 ha of the Lake Grassmere ponds of 12 000 kg dry weight of *Artemia* in January. To give an appreciation of what this type of *Artemia* biomass might mean to a seahorse culturist, 12 000 kg dry weight of *Artemia* (=108 000 kg wet weight based on 90% moisture content in *Artemia*), if frozen and stored, would feed approximately 26 300 large *H. abdominalis* (20 cm SL, 20 g wet weight) at a 5% daily ration for one year. Burkhart Fisheries Ltd currently utilise this resource at Lake Grassmere (http://www.nzartemia.co.nz; accessed 23/08/05) for the harvesting and packaging of *A. franciscana* cysts for domestic and export markets. In New Zealand, aside from the Lake Grassmere resource the commercial potential of solar *Artemia* production has not received any significant attention to date. As a commercial means of supplying international demand as well as potential local demand, this aspect of *Artemia* production may warrant further attention.

Intensive tank culture of *Artemia* offers several advantages over traditional solar pond production: 1) it can be independent of season or climate, 2) it permits frequent harvesting and inoculation, and a controlled production of specific size *Artemia* with a desired nutritional profile, and 3) it permits better control over bacteria and diseases (Naegel, 1999; Van Stappen, 2003). It can also operate on a much more intensive scale within a smaller space footprint. Consequently, worldwide there has been more emphasis on developing intensive *Artemia* production systems for commercial operations (Lavens *et al.*, 1986; Dhert *et al.*, 1992; Lim *et al.*, 2001; Kolkovski *et al.*, 2004). For example, Lim *et al.* (2001) described a small footprint (400 m^2) indoors system for the production of on-grown *Artemia* with a payback period of 1.2 years and mean production rate of 3 kg m^{-3} on a 12-day cycle. Zmora & Shpigel (2006) tested an outdoors intensive recirculation with 600 and 3000-l tanks, which was capable of producing between 31 and 40 kg m^{-3} after 17 to 20 days. Intensive systems are the most likely that many seahorse culturists may employ, and require further research and development if their full potential is to be realised.

7.4.2. Use of frozen mysids

As the production of live foods can be expensive and the production of artificial diets which produce equivalent or superior growth and survival rates compared to live foods for *H. abdominalis* has yet to be achieved, the suitability of frozen diets as an intermediate step between live foods and artificial foods requires examination. The use of frozen copepods (Cyclop-eeze®) for early juvenile *H. abdominalis* did not offer equivalent or superior growth and survival rates compared to live *Artemia* nauplii. However, frozen mysids (*Amblyops kempi*) were shown to be an acceptable alternative to live enriched *Artemia* for older juvenile *H. abdominalis*, providing comparable rates of seahorse growth and survival. The acceptance of frozen mysids by *H. abdominalis* provides culturists with a feed that may help reduce culture costs. It also has potential health benefits for this species as mysids can provide a lipid-rich food source along with other beneficial dietary components such as carotenoids (Ibrahim *et al.*, 1984; Bradshaw *et al.*, 1990; Richoux *et al.*, 2004).

The acceptability of frozen mysids by cultured *H. abdominalis* also has important aquarium sales ramifications. Seahorses which are already weaned to accept frozen foods before sale may be more marketable than those that are not, as aquarists may perceive this as reducing the chances of their newly purchased seahorses declining in health or even dying because they prefer to use (or only use) frozen foods. It also benefits the seahorses themselves, as it increases their chances of survival whilst moving from culture facility to wholesaler/retailer and finally the aquarium owner, where the use of frozen foods is common (Payne, 2003).

Research into optimal feed-specific feeding regimes is essential for increasing potential productivity and economic viability of commercial farms as well as reducing associated biological-loading and outflow-wastes in seawater systems. For cultured *H. abdominalis*, Feed Conversion Ratios (FCR) became less efficient as feed ration increased based on the total amount of mysids offered to seahorses, although when actual mysid consumption was taken into account there were no significant differences in FCR between rations. Examination of the cost/benefit values of implementing once-daily feed rations frozen mysids revealed that >5% wet body weight rations do not appear to be advantageous in terms of deriving greater seahorse growth per unit cost of food.

Mysid shrimp are a natural prey for *H. abdominalis* so there is potential to use endemic mysids as a feed (see section 7.4.3) rather than importing frozen mysids. If seahorses are to be reared on frozen mysids, then it may be advantageous to culture/harvest the mysids at a time when their nutritional profile best matches seahorse dietary requirements, as ontogenetic and seasonal changes in mysid nutritional composition have been observed (Richoux *et al.*, 2004). This could then be exploited to possibly increase seahorse growth and/or reduce the overall feeding rate by providing a richer food source. Alternatively, frozen mysids may be enriched prior to feeding, by soaking them in appropriate enrichments.

However, if multiple large-scale commercial seahorse culture ventures become established in New Zealand utilizing frozen mysids as part of their culture practice, then this will increase the demand for mysids. Currently, frozen mysids are imported from countries such as Japan and the USA where they are harvested from the wild. However, the sustainability of mysid wild-harvest and its flow-on trophic effects have received scant attention to date. The importation of frozen mysids can be costly and is subject to monetary exchange fluctuations and strict biosecurity importation requirements. Therefore, aquaculture production of mysids within seahorse farms may be more desirable if these organisms are to be used as food and economically effective mysid culture techniques can be developed.

7.4.3 Natural seahorse diet

Elucidation of the natural diet of seahorses has two main benefits. Firstly, prey organism type, size, nutritional composition and likely feed ration can be determined, which can then assist culturists in determining feeding regimes and foods used for seahorses. Secondly, it can identify natural prey that could potentially be cultured as food.

The natural diet of *H. abdominalis* in Wellington Harbour consists mainly of epibenthic and epifaunal crustaceans. Other studies on seahorse natural diet have also found that crustaceans are the main food item for seahorses (Reid 1954; Lovett 1969; Tipton & Bell 1988; Do *et al.*, 1998; Texeira & Musick 2001; d'Entremont, 2002; Kendrick & Hyndes, 2005; Felício *et al.*, 2006). For *H. abdominalis*, there were no significant dietary differences between male and female seahorses found. This is an indicator to culturists that different diets for male and female seahorses may not be required; the

provision of different diets would increase associated feeding costs. However, this lack of sexual difference in seahorse diet in the wild may be a reflection of prey availability rather than choice. It would be beneficial to investigate whether cultured seahorses exhibit any sex-related diet preferences when offered a range of different feeds to determine whether culturists may need to provide different diets to the sexes, and whether particular feeds influence reproductive output.

As discussed in Chapter 5, when using frozen mysids as a feed the most appropriate feed ration for cultured *H. abdominalis* appeared to be 5–10% of wet body weight. Examination of wild *H. abdominalis* revealed a calculated daily ration to be 4.9% wet weight, although this is likely to be an underestimate. It would seem reasonable for commercial culturists of *H. abdominalis* to maintain this 5–10% of wet body weight daily feed ration until further research into the optimization of feed regimes is conducted.

As is commonly observed in other finfish and seahorse species (Tipton & Bell, 1988; Olaso *et al.*, 1999; Castillo-Rivera *et al.*, 2000; Kendrick & Hyndes, 2005), there were some ontogenetic differences in the diet of wild *H. abdominalis* (e.g. smaller *H. abdominalis* consumed a greater amount of amphipods). This is logical given that the size range of potential prey increases with seahorse size, as snout ingestion capacity increases. Indeed, it is common practice for commercial culturists to increase the size range of food offered as their seahorses increase in size. However, a maximum size of feed is usually reached at a smaller size in the culture environment compared to the wild due to the maximum size of cultured prey such as *Artemia*, or due to the high cost of culturing very large prey. For example, *Artemia* reach a maximum length of 12 mm, whereas the caridean shrimp predated on by wild adult *H. abdominalis* achieves up to 20 mm in length. As a consequence, cultured seahorses may have to expend more energy hunting and processing more, and smaller, prey (e.g. *Artemia*) compared with their wild counterparts. Consequently, this is where the development of appropriate inert diets and alternative live food species (based on information from the seahorse's diet in the wild) may significantly aid commercial seahorse culture.

Current live food culture for seahorses relies on a limited number of well known species for which there is available culture information. The majority of the research contained in this thesis focuses on the exploitation of two food types commonly used for feeding

seahorses: *Artemia* and frozen mysid shrimp. As an alternative to the current importation of *Artemia* cysts and frozen mysids it may be more beneficial to develop culture techniques for some of the wild prey items of *H. abdominalis* (e.g. caridean shrimp, mysid shrimp and caprellid amphipods).

Culture techniques for marine shrimp, particularly tropical penaeid shrimp for bait and human consumption, have rapidly advanced in the last 20 years (Sorgeloos & Leger, 1992; McVey, 1993; Treece, 1993; Kongkeo, 1997; Bratvold & Browdy, 2001; Samocha *et al.*, 2002; Hari *et al.*, 2004; Hernandez-Llamas *et al.*, 2004; Robinson *et al.*, 2005). More recently, investigations have been conducted into culture techniques for ornamental shrimp species (e.g. Rhyne & Lin, 2004; Calado *et al.*, 2005b). Given the large amount of information available on shrimp culture in general, there is good reason to speculate that the development of culture techniques for shrimp such as *H. bifidirostris*, which are eaten by wild *H. abdominalis*, should be possible. It is interesting that given the large scale that commercial shrimp farms often operate at, that no seahorse culturist appears as yet to have linked their production with this activity. It would seem a logical step to do so if the larval shrimp supply and relative cost of shrimp larvae is sufficient to economically sacrifice them as seahorse feed.

There has been a concentration of effort on developing effective culture techniques for mysid shrimp because of their nutritional value to cultured predators (Kreeger *et al.*, 1991; Akiyama, 1997; Domingues *et al.*, 1998, 1999, 2001; Domingues *et al.*, 2000) and widespread use as a test organism in toxicology studies (Nimmo & Hamaker, 1982; Cripe 1994; Nipper & Williams, 1997; Garnacho *et al.*, 2001). Mysid culture systems capable of comparatively low biomass production are successful and widely employed (Retsema & Neff, 1980; Leger & Sorgeloos, 1982; Ward, 1984). But as yet, economically viable large-scale high biomass units that can replace traditional *Artemia* culture systems are still to be developed. Further efforts need to be directed at producing economically viable mysid culture technology.

Culture techniques for a variety of amphipod species have tended to focus on small-scale culture for use in eco-toxicological studies (Ikeda, 1992; Environment Canada, 1997; Hyne & Everett, 1998; Tsoi & Chu, 2005), though some larger-scale pond production has been examined (Danielssen *et al.*, 1990). Recent interest in amphipod

culture has come from the marine hobby trade and large-scale amphipod culture is in need of investigation for commercial application (Hoff & Snell, 1987).

For natural prey such as caprellid amphipods there is little information available on culture techniques. Caprellids are common members of many littoral habitats, and are particularly abundant in epibiotic fouling communities where they may form an important food source for various fish species (McCain, 1969; Caine, 1991; Thiel *et al.*, 2003; Willis *et al.*, 2004; Buschbaum & Gutow, 2005). Caprellids exhibit a range of different feeding techniques, but they commonly feed on suspended materials or prey on other organisms such as epibionts (Caine, 1974, 1977). Caprellids are well adapted to clinging to various substrata (Takeuchi & Hirano, 1992, 1995) and are capable of reaching high abundances (e.g. 4.9 cm^{-1} on *Zostera marina* blades, Caine, 1987). This would tend to suggest that if the right environmental conditions are provided, then caprellid culture could be possible provided enough food (e.g. microalgae or detrital build-up) and substratum (e.g. fibrous ropes) are present. It may even be possible to incorporate caprellid culture into polyculture recirculation systems as a suspended solids/detrital removal component of the system.

7.4.4 Inert diets for on-growing
The development of appropriate inert diets to feed larger size seahorses (e.g. >80 mm SL) is probably where greatest economic benefit can be gained in commercial seahorse culture, due to the need of larger seahorses for greater quantities of food of larger particle size. Culturing larger live food requires feed materials, plant space and labour. This tips dietary cost-effectiveness back in favour of inert feeds. However, in comparison to cultured finfish fish such as salmon which can be readily weaned *en masse* on to artificial diets which bear no resemblance to natural foods, acceptance of inert diets by seahorses is strongly influenced by the appearance of the diet and resemblance to natural foods (as seen in Chapter 3). Fortuitously, with larger seahorses greater diet format (e.g. appearance and shape) can be tested for acceptance because the sizes of food items are larger and therefore more easily varied in the manufacturing process.

As part of a separate research effort, the development of a suitable inert diet format for large *H. abdominalis* (20 cm SL) was tested using water-stable formulations of variable colouration whose dimensions approximated frozen mysids that the seahorses had been

weaned on to (Woods, unpubl. data). Some diets contained a small amount of poppy seeds. The premise behind including poppy seeds in some diets was to simulate the dark compound eyes of the mysid shrimp. It was found that *H. abdominalis* would ingest artificial diets that closely resembled mysid shrimp (i.e. similar colour and with poppy seeds), particularly when they were presented along with real mysids and when seahorses were kept in groups rather than singly. The conclusion from this small-scale trial was that an inert diet format can be produced for larger seahorses that will be ingested if it approximates a food familiar to seahorses. The increased diet ingestion in a group situation appears to be the result of feeding competition whereby seahorses in a group environment when competing for food, appear to spend less time scrutinising the food. In a group environment (as will be used in commercial culture), seahorses also appeared to be alerted to the presence of food earlier by the behaviour of their tank mates.

Recent advances in production technology have allowed the recreational fishing tackle manufacturing industry to produce visually realistic soft-bodied lures. Some of these lures also incorporate chemical fish attractants, and may be composed in part or entirely of edible materials. It would seem a logical progression to utilize such production technology to produce suitable inert diets for seahorses, as seahorses appear to pay particular attention to the physical appearance of their food.

7.4.5 Growth rate

Improving growth rates is important in commercial aquaculture as this reduces the time taken till market size is achieved and therefore potentially increases production capacity. In the preliminary investigation (Chapter 2), the size of surviving first generation seahorses at one year of age (>10 cm SL) would be large enough to sell into the aquarium trade, but not to the medicinal trade. The growth rate of juvenile *H. abdominalis* in comparison to tropical seahorse species, which currently dominate both aquarium and medicinal trades, was slow. For example, Job *et al.* (2002) achieved a SL of 11 cm in *H. kuda* at around 85 days after birth. However, the on-growing of seahorses from one to seven years of age demonstrated the relative hardiness and suitability of this species to long term captivity. The growth rates obtained also demonstrated that seahorses of a size more targeted to the medicinal trade (>20 cm SL) could be achieved on a relatively simple live food diet in 3–5 years. These growth rates should be considered as conservative as culture variables such as food and temperature

were not manipulated or optimised. It should be possible to reduce the time taken until market size is reached through optimizing culture variables for *H. abdominalis*. For example, by manipulating seawater temperature and diet, large *H. abdominalis* >20 cm SL have been produced in a commercial setting at less than 2 years of age (B. Gray, The Seahorse Farm, pers. comm.).

7.4.6 Commercial on-growing

Even if premium prices for *H. abdominalis* for the medicinal trade can be obtained, economically viable large-scale land-based culture of seahorses for this trade is still difficult in New Zealand. Flat coastal property with good water quality is costly to purchase. Gaining resource consent to develop new aquaculture ventures can be a lengthy and expensive process. Materials and utilities, labour and food production are also relatively expensive compared to many developing countries. An alternative to land-based culture that may enable large-scale culture of large seahorses for the medicinal trade to be more economically viable is that of sea-based on-growing of cultured juvenile seahorses to a large market size in sea-cages.

The advantage in using sea-cages to on-grow juvenile seahorses is that they need not be provided with food, removing the expensive feeding component that is encountered in land-based systems. In sea-cages the seahorses feed on mobile fouling organisms that colonise waterborne structures as part of the normal epifaunal community (see Fig. 7.2). This is in marked contrast to other sea-cage based industries such as salmon farming where exogenous feeding is required and where there may be concerns regarding extra nutrient inputs into the ecosystem. However, resource consents/local council approval, impact assessments and permit variations to existing marine farms must still be obtained.

The natural food supply of mobile colonizing organisms within sea-cages can be further enhanced and supplemented through the use of artificial illumination. For example, in a small-scale trial using artificial illumination within sea-cages containing adult *H. abdominalis*, the presence of 3-LED Electralume® lights attracted increased numbers of prey such as fish larvae, gammarid amphipods, and crustacean larvae into the sea-cages, and resulted in significant increases in adult seahorse length and weight compared with cages with no lighting (C. Woods, unpubl. data).
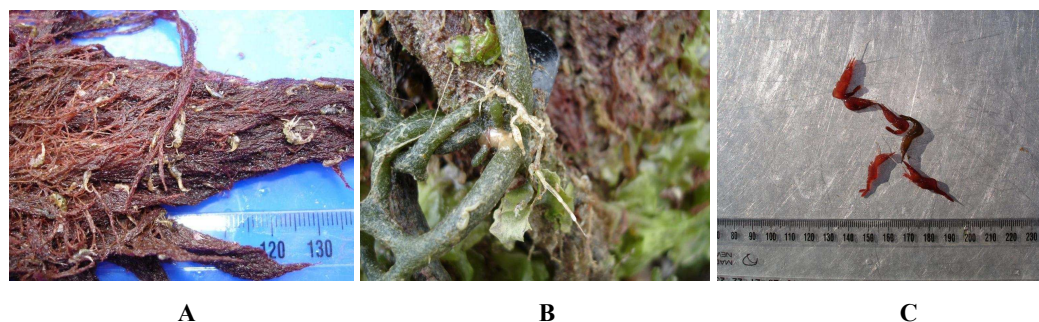
Figure 7.2 Example of mobile epifauna that can act as food for seahorses in sea-cages: A) corophioid amphipods, B) the caprellid *Caprella equilibra*, and C) the shrimp *Hipployte bifidirostris* (source: Chris Woods).

Sea-cage culture involves no water reticulation costs and can utilize existing structures such as mussel long-lines and salmon cages. It can also offer commercial aquaculture companies with a diversified portfolio into a lower volume/higher value product compared with shellfish species such as Greenshell™ mussels. Totally sea-cage-based culture would not be practical in New Zealand due to the small size of juvenile seahorses when first born (the slim girth of juvenile *H. abdominalis* when first released requires mesh no larger than 1.5 mm at the largest aperture (Woods, unpubl. data)) — this size of mesh fouls rapidly, thus reducing water quality inside the cages. Sea-cages are more suited to culturing seahorses at an age where mesh size can be big enough not to foul too quickly, and the seahorses are able to survive the stresses associated with transport to the sea-cage site from the hatchery, e.g. ~8 cm SL (~6–8 months of age).

The use of sea-cages to on-grow seahorses has received little attention to date, although Santos (2000) did examine the on-growing of juvenile *H. kuda* in sea-cages in seagrass and mangrove habitats in the Philippines. Santos (2000) found that juvenile *H. kuda* in small sea-cages in a seagrass habitat achieved an average growth rate of 0.94 mm d^{-1} over a five week period with an average survival of 57.6%. However, juveniles in sea-cages at the mangrove site experienced 100% mortality after two weeks where there was greater turbidity and lower current velocity, with an unqualified greater amount of cage fouling thought by Santos to have restricted water flow into the cages. Shallow sea-cages are being used by a commercial fish farm in Singapore (San Lay Marine Culture) to on-grow cultured tropical seahorses, where seahorses feed on zooplankton and mobile fouling epifauna.

In collaboration with commercial mussel and oyster culture companies, NIWA has conducted two investigations into the small-scale on-growing of *H. abdominalis* in sea-cages on commercial farms. These have demonstrated the technical feasibility of seahorse sea-cage on-growing in New Zealand waters in association with existing marine aquaculture species.

Sea-cage on-growing of seahorses is not without risk. Potential disadvantages to sea-cage rearing of seahorses include: 1) vulnerability of stock to detrimental environmental fluctuations and extreme events (e.g. salinity, saltation and storm events, toxic algal blooms and fluctuations in food supply) and 2) public disturbance and theft. Failure to properly manage the colonisation balance between edible mobile fouling organisms and inedible sessile fouling organisms (which may also possess toxic compounds) in sea-cages will result in a decrease in food availability and a reduction in water flow through the cages. However, proper maintenance of caging mesh and cycling-over of pre-fouled structures with the required abundances and variety of edible organisms can avoid these problems and keep seahorses well-fed.

7.5. Market

7.5.1 Current seahorse aquaculture in New Zealand

Seahorse aquaculture has significantly progressed beyond the stage that it was at in 1997 when the research contained within this thesis was initiated. It is now at the stage where it is both technically and economically feasible; cultured seahorses are being produced for domestic and export sale within a number of countries, such as Australia, England, Ireland, New Zealand, and Sri Lanka. For example, in Australia in 2004 there were at least four commercial seahorse ventures producing captive seahorses for the domestic Australian market (Martin-Smith & Vincent, 2006).

New Zealand currently only has one small-scale commercial seahorse farm in operation — Seahorse New Zealand Ltd. This company has been successful in turning exotic seahorse importation into an aquaculture export business. Run by a single person, this company produces tropical *H. kuda* for export (mainly to the USA) in a small recirculating land-based seawater system. It is one of the leading international suppliers of cultured *H. kuda* to the aquarium trade and has also exported cultured *H. barbouri* and *H. reidi*. All live foods (*Artemia*, mysids and rotifers) are produced on-site in plastic

tunnel-houses on solar-cultured microalgae, with weaning of seahorses to frozen *Artemia* and mysids prior to shipping to increase their marketability. This company is a prime example of how small-scale ventures can sometimes economically operate very effectively, with minimal overhead and operating costs, small infrastructure and self-employment.

Until 2006, there was also one other commercial seahorse farm operating in New Zealand. The Seahorse Farm in Napier was originally developed as a lobster on-growing facility in 1996, but this did not prove economically viable at the time. The Seahorse Farm turned to culturing *H. abdominalis* in the late 1990s and reportedly reached an annual production of around 50 000 seahorses between 2002-2005, for sale to the domestic and export aquarium and medicinal trades (Ingram, 2005), making it one of the largest seahorse farms internationally. Pairs of *H. abdominalis* were sold to the domestic aquarium market at $NZ50 per pair (Ingram, 2005). Even seahorses that died during culture were dried and sold as curios for up to $NZ30 each. Diversification of the farm to include a range of other products such as paddle crabs (*Ovalipes catharus*) for the restaurant trade and native freshwater fish species such as Inanga (*Galaxias maculatus*) for the domestic aquarium trade, and tourist services such as fee-to-see public tours and a retail shop all produced additional revenue for the farm, as well as spreading investment risk away from a single product (de Zylva, 2003). However, in February 2006, this venture ceased trading due to the long-time financial backer withdrawing.

7.5.2 Future directions in seahorse aquaculture in New Zealand

Production of large *H. abdominalis* for the medicinal trade is where more research is required in order to improve growth rates whilst minimizing associated culture costs. The question as to whether *H. abdominalis*, as a seahorse species not traditionally traded in south-east Asian TM on a large scale, can achieve premium medicinal prices and therefore be produced economically in countries such as Australia and New Zealand needs to be considered by culturists considering production. Informed market research needs to be conducted, in which effective medicinal trade contacts are established and nurtured.

In New Zealand and Australia there have already been several examples of over-capitalised companies entering into attempted commercial production of *H. abdominalis*

with anticipated end-prices of >NZ$1000 kg^{-1} dried, without actually having conducted detailed market research and secured agreed pricing prior to production. When investment and production has occurred, and prices subsequently offered have been significantly less than the >NZ$1000 kg^{-1} anticipated, the economic viability of the venture has been seriously affected (e.g. Seahorse Aquaculture Pty Ltd in Tasmania, Australia, Willis & O'Sullivan, 2003). However, high prices for dried *H. abdominalis* in the medicinal trade can be obtained, even in New Zealand. For example, in 2005 *The Seahorse Farm* reported that wholesale door sales for dried *H. abdominalis* of NZ$1000–1500 kg^{-1} (with 200–400 g being the usual quantity sold per purchase) were regularly occurring for domestic medicinal usage (B. Gray, The Seahorse Farm, pers. comm.).

With New Zealand's increasing Asian population, the medicinal trade in seahorses has now become a domestic trade, albeit at a relatively smaller volume than the TM trade in Asian countries such as China and Taiwan. Domestic sales of cultured *H. abdominalis* to TM shops in Auckland have occurred (B. Gray, The Seahorse Farm, pers. comm.). However, with the demise of The Seahorse Farm the only source of cultured *H. abdominalis* in New Zealand has stopped. This has meant that dried seahorses have to be imported; these are usually wild-caught seahorses that do not conform to CITES requirements and are subsequently confiscated at the border when intercepted by customs officials. Given New Zealand's increasing Asian population and their commensurate community integration, it would seem sensible to develop relevant aquaculture contacts and business investment within this community.

Historically, traditional Chinese medicine (TCM) practitioners have relied upon proven (or presumptive) efficacy rather than actual biochemical composition to determine which natural products they use. However, in many Asian countries there has been an increase in the testing of natural products, including syngnathids (e.g. Qu *et al.*, 1991; Yu *et al.*, 1995; Zhang *et al.*, 2003), for their biochemical composition and related therapeutic effects, along with an increasing use of packaged proprietary medicines. As animal biochemical composition may be influenced by diet, the diet-influenced biochemical composition of seahorses may become a valuable marketing tool as already occurs with other foods (e.g. the marketing of foods with higher *n*-3 and *n*-6 fatty acid content for cardiovascular health and brain development). This is where cultured seahorses may have a competitive marketing advantage over wild seahorses and it

would be beneficial to test whether dietary nutritional differences are incorporated into seahorses.

With the renewed interest in many western countries regarding utilizing natural products for their medicinal or nutraceutical properties, a large variety of marine organisms are being tested for beneficial properties (Faulkner, 2000), and this includes seahorses. Within the last few years several commercial companies in New Zealand have tested seahorses for anti-cancer and testosterone-boosting properties. Therefore, the future therapeutic value of seahorses may extend well beyond the TCM market to a wider global market.

A conservative estimate of the annual wholesale value of the world trade in ornamental fish puts it as >$US1 billion, with >1.5 billion fish traded annually at a retail value of $US4.5–6 billion and an annual market growth rate of 8% (Singh, 2005; Yap, 2005). Tropical freshwater species dominate this trade (80–90%), although the interest in marine species is growing. The three largest consumer markets in this trade are the USA, Japan and the European Union (Singh, 2005). Although not currently a large proportion in the aquarium trade by numbers, seahorses are nonetheless rated as a premium aquarium fish (Singh, 2005). Given the size of the international aquarium market and the increasing size and diversity of countries involved, there appears to be considerable scope to increase the market for aquarium seahorses, particularly as many of the historical culture problems involved with seahorse culture are being overcome.

Internationally, live sale retail prices for cultured *H. abdominalis* in the aquarium trade can reach up to US$190 per seahorse (http://www.underwaterexotics.com; accessed 12/07/06), but more often they retail for US$30–60. Unreliability of supply, the fact they are a temperate species, and that their colouration is generally not as consistent and bright as premium tropical species have affected their widespread acceptability and desirability to date. Reliability and consistency of supply to international aquarium markets needs to improve. Better marketing of *H. abdominalis* as to its relative ease of maintenance in the aquarium is also required to improve its market acceptability, although for some aquarists in hotter climates the need to dedicate an aquarium with a chiller unit to house *H. abdominalis* separate from tropical species may still be a hard selling point. Extremely colourful specimens of *H. abdominalis* do exist, often with striking spotting and banding patterns, suggesting that manipulation of dietary,

environmental and genetic variables could result in more consistent and brighter overall colouration for this species, leading to greater market desirability.

Genetic effects on growth are commonly exploited in the aquaculture environment, with selection involving the change of heredity of the cultivated species, and development of breeds/lines/strains adapted to captive environmental conditions and realization of maximal potential of desired characteristics (Hickling, 1962; Kirpichnikov, 1981; Weatherley & Gill, 1987; Vandeputte, 2003). For example, certain colours and patterns are a major pricing influence for ornamental koi carp, leading to investigations into the genetic determinants of colour and pattern in order for these to be manipulated (David, 2004). This is also seen in seahorses, where certain attributes command high prices. For example, some cultured seahorses can retail for up to US$300 each (i.e. http://www.oceanrider.com, OceanRider Fire Reds; accessed 5 February 2005). Draco Marine in Maryland, USA, market *H. erectus* with ornate dermal cirri at an extra US$10 per seahorse, while red *H. reidi* are sold for US$25–35 more per seahorse compared with yellow *H. reidi* (http://www.dracomarine.org; accessed 15/05/06). *Hippocampus abdominalis* exhibits large variation in morphology and colouration, thus offering a wide selection of traits to enhance and refine into a wide range of marketable "types".

Even though New Zealand is geographically isolated from the major live aquarium markets, modern air-travel brings most of these markets within approximately 24 to 48 h from the farm gate. Seahorses appear to be relatively hardy fish during shipment, with standard shipment procedures involving placing seahorses in oxygen-enriched plastic bags contained within polystyrene boxes. Commercial live shipments of cultured *H. abdominalis* from New Zealand have occurred to such countries as Japan and the USA, with high survival reported upon arrival (B. Gray, The Seahorse Farm, pers. comm.). This also appears to be the case for tropical species, where well-packaged cultured seahorses may survive 72 h in-transit with 100% survival upon arrival at the aquarium shop (G. Leveridge, Seahorse New Zealand, pers. comm.).

For the aquarium trade, cultured fish offer an advantage in that they are conditioned to a captive environment, which makes them better able to thrive in the aquarium setting, and during transport and transfer along the chain of custody (Clement, 2001). Progressively, there are increasing recommendations that aquarium owners purchase captive-bred rather than wild-caught seahorses (e.g. http://www.worldofseahorses.com,

; accessed 17 March 2005), along with expected recommendations from commercial culturists themselves (e.g. ; accessed 15/05/06). Such recommendations should see an increasing desirability for cultured seahorses.

According to Yap (2005), a market driver amongst buyers is how healthy the fish meant for export are; Singapore exporters use a benchmark of up to 97% survival rate of the fish being exported. Seahorses are ideally suited to such a benchmark due to their physiology which appears conducive to low stress during handling and transport (Wright, 2005; Wright *et al.*, 2007). This is potentially a strong selling point for cultured seahorses. Accreditation with ISO 9002 Quality Management System is also probably beneficial to ensure high quality product export and acceptability with buyers (Yap, 2005).

The CITES Appendix II-listing of all seahorses reinforces the market advantage to cultured seahorses for the aquarium trade as it is easier to prove that seahorse aquaculture venture is not detrimental to seahorse wild stocks compared to actual wild catches, and cultured seahorses can be legally traded below the CITES mHT size restriction. All international shipments of seahorses are inspected for appropriate CITES documentation. Absent or inappropriate CITES documentation from an unfamiliar authority can result in the seizing of shipments, which can mean seahorse deaths or a decline in health during holding.

As seahorse farms become more efficient and established, the numbers of cultured seahorses available in the aquarium trade will increase. This needs to be backed up by better buyer education on seahorse requirements and feed-supplies for seahorse buyers to maintain healthy seahorses, and therefore a reputable product. The relatively small aquarium market in New Zealand is easy to saturate, so larger export markets such as the USA, Europe and Asia are important target markets for any seahorses culturist in New Zealand to consider. This is especially so for tropical seahorse varieties which have a wider marketability compared to temperate species.

Increasing cultured seahorse production for the aquarium market will potentially result in individual price reductions for species or varietals which become "commonly" available (i.e. supply & demand). This has been observed recently for *H. kuda* where the

recent influx of cultured specimens from Sri Lanka saw an initial price drop of around US$5-10 per seahorse for western culturists of *H. kuda* (G. Leveridge, Seahorse New Zealand, K. Doyle, Seahorse Ireland, pers. comm.). The response from these western culturists has been to reposition their products (which are often more colourful and appear in better condition post transit) as higher-end specimens.

Undoubtedly, new seahorse species (particularly small cryptic species) will be found (or reclassified) as efforts to resolve seahorse taxonomy continue, such as *H. denise* described recently by Lourie & Randall (2003) and *H. colemani* by Kuiter (2003). New species could offer seahorse aquaculturists with new product lines as long as initial collection for culture purposes does not detrimentally affect the wild population. Recently, a small cryptic seahorse, tentatively identified as *H. jugumus* (R. Kuiter, pers. com.), was photographed in the Poor Knights reserve. *Hippocampus jugumus* is known from only a single specimen collected from Lord Howe Island as far back as 1925 (Kuiter, 2003). If this seahorse does represent a hitherto unknown population, then this could represent a presently untraded (and therefore novel) species that could be exploited by aquaculturists in New Zealand.

7.6 Thesis conclusion

The central null hypothesis to the research in this thesis was that: H_0 "*H. abdominalis* cannot be cultured in captivity, with low juvenile survival and growth to maturity". The central alternative hypothesis was that: H_1 "*H. abdominalis* can be cultured in captivity, with high juvenile survival and growth to maturity". It is proposed that the central alternative hypothesis (H_1 "*H. abdominalis* can be cultured in captivity, with high juvenile survival and growth to maturity") generally be accepted although further improvements in juvenile growth rates are required.

The seahorse *H. abdominalis* is now being commercially cultured for sale to the live aquarium and dried medicinal markets. Many of the initial culture difficulties (e.g. poor breeding and high initial juvenile mortality) which dogged the first commercial culture attempts with *H. abdominalis* (Vincent, 1996) have now been resolved. The research in this thesis has significantly contributed to this aquaculture development, as acknowledged recently by Koldewey (2005). However, further culture research is still

required to reduce time till market size is reached and reduce associated culture costs by optimising on-growing techniques.

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