Effect of feeding regimen, temperature and stocking density on growth and survival of juvenile clownfish (*Amphiprion percula*)

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By

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This thesis is dedicated to my family for the continuous support and encouragement they have always given. Thanks.

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Abstract

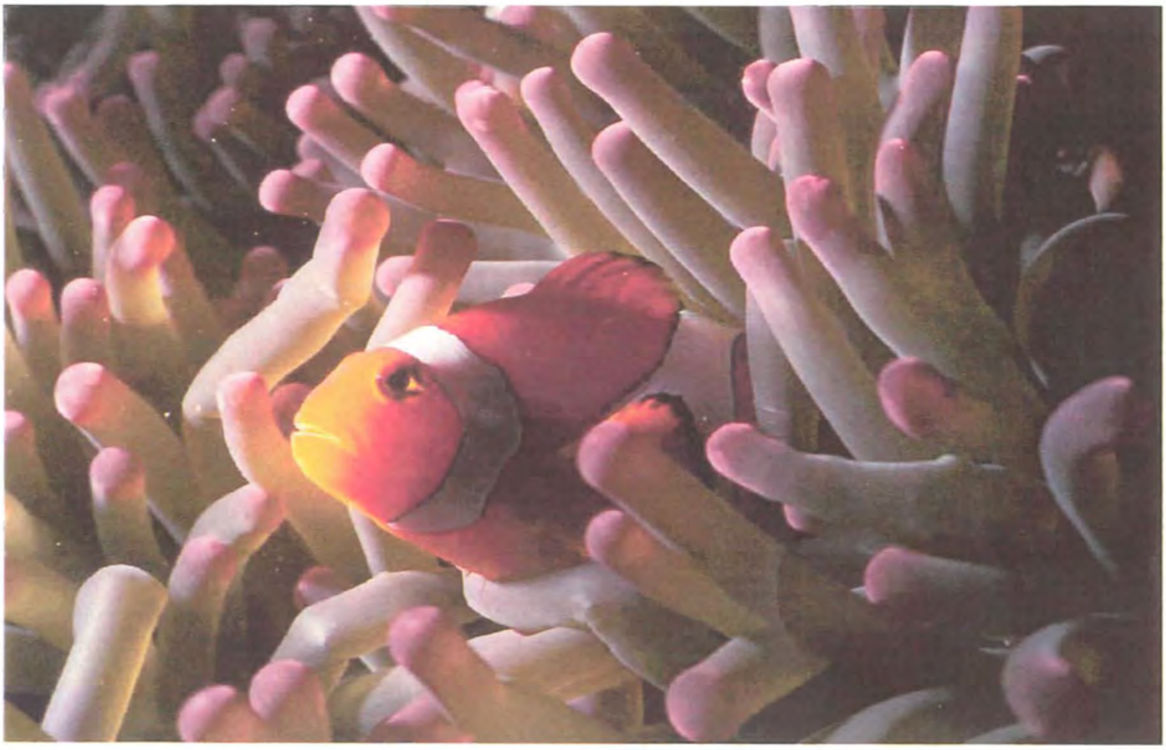
In aquaculture, a thorough knowledge of the specific environmental requirements of a species is needed in order to maximize growth rate and survival. There is a paucity of data regarding the fundamental environmental requirements for the ongrowing phase of clownfish juveniles. This prompted the design of three experiments to determine the best feeding regimen, temperature and stocking density that maximize growth and survival of *Amphiprion percula*.

Ration size and feeding frequency are important factors for optimizing fish growth during the juvenile grow-out phase. A factorial growth trial was conducted to determine the effect of feeding frequency and ration size on the growth of juvenile clownfish (*Amphiprion percula*).Three feeding frequencies (1, 2 and 3 times daily) and six rations (2, 4, 6, 8, 10 and 12 % body weight per day (BW.day^{-1})) were used to test the growth response over a twelve week period. Non-linear regression analysis on the effect of ration, independent of feeding frequency, on growth resulted in a significant ($n = 36$; $r^2 = 68.7$) parabolic model: $\ln y = -0.0302x^2 + 0.5159x + -4.4377$. Maximum growth corresponded to a ration of 8.5% BW.day^{-1}. Survival as a function of ration was significantly lower at 2% BW.day^{-1}. Data were further examined with Analysis of CoVariance (ANCOVA) to determine the effect of ration on growth at each feeding frequency. The combination revealed a maximum growth rate when the fish were fed a ration of 10% BW.day^{-1} divided into two equal meals. The required ration per meal to maintain maximum growth was also found to decrease as feeding frequency increased.

The determination of the best temperature for growth is of great importance due to the direct relationship between fish metabolism and temperature. The thermal preferendum of *A. percula* has already been estimated at 26 ± 0.7°C but it is not known whether this closely approximates the temperature for maximum growth. Sixteen tanks were set to different temperatures ranging between 21.5 and 30.2 °C. Ten juvenile *A. percula* were placed in each tank and growth was measured fortnightly over the course of the 10 week experiment. Non-linear regression analysis resulted in significant models for fish length ($y = -0.0005x^2 + 0.00267x - 0.0338$; $r^2 = 56.7$, $n = 11$) and weight ($y = -0.00016x^2 + 0.0084x - 0.1073$; $r^2 = 61.6$, $n = 11$). These models predict that maximum growth would be at 27.7 and 27.1 °C for length and weight, respectively. Temperature, over the range tested, had no apparent effect on survival. There was no significant difference between the temperatures for maximum growth and the preferred temperature.

The effect of stocking densities ranging from 0.2 $fish.L^{-1}$ to 4.0 $fish.L^{-1}$ on growth were used in the third experiment. The fish were fed to satiation twice daily and growth was measured fortnightly throughout the 8 week experiment. No effects on growth, survival or coefficient of variation were found within the range of stocking densities tested.

Adult *Amphiprion percula* nestling in host anemone



Chapter 1

General Introduction

The global value of the trade in ornamental marine fish and invertebrates was estimated in 1985 to be US$ 24 - 40 million annually (Wood, 1985). More recently, imports into the United States and the European Union were estimated at US$ 8.9 million (Chapman *et al.*, 1997) and US$ 11 million (Bassleer, 1994), respectively for 1992. Andrews (1990) and Hoff (1996) report that marine species are becoming increasingly popular in the aquarium fish trade. Although marine ornamental species are imported in relatively low volumes, the fish generally have a higher value than freshwater species. For example, only 4 % of the total number of ornamental fish imported into the USA in one month were marine, although they accounted for 20% of the total trade value for that month (Chapman *et al.*, 1997). Furthermore, importation trends of ornamental fish indicate that the marine component is becoming increasingly popular relative to the freshwater sector. This is highlighted by Wood (1992) who found that the proportion of marine fish imported into the U.K. as a percentage of the total ornamental fish imports rose from 11.4% to 17% between 1977 and 1989.

It has been estimated that approximately 90% of freshwater aquarium fish within the ornamental trade are captive bred (Andrews, 1990). However, virtually all marine fish in the trade are caught from the wild (Andrews, 1990). Wild capture involves the use of hand nets and chemicals such as sodium cyanide (NaCN), rotenone and quinaldine (Wood, 1985). Sodium cyanide acts on the enzyme systems responsible for respiratory metabolism and has been shown to cause internal damage to the liver, intestine and reproductive organs of fish (Rubec, 1988). It also kills invertebrates and corals (Rubec, 1988) and large scale use for fish capture often contributes significantly to the degradation of coral reefs (Wood, 1985; Andrews, 1990; Tongson and McAllister, 1997). The application of sodium cyanide into an area will result in all the fish in that area being affected. It has been estimated by Rubec (1988) that of all the fish exposed to the toxin at a capture site, only 10% are captured, 50 % die entombed in the coral and the remainder are consumed by predators. Furthermore, of the fish retained, it is estimated that more than 80% die *en route* to the marine hobbyist due to delayed mortality (Rubec, 1988). Vine & Hecht (1998) estimate that less than 2% of the fish imported from Indonesia and Sri Lanka into South Africa survive before reaching the consumer. Such statistics coupled with the depletion of many species and an increase in demand (Wood, 1992) have sparked concern about the impact of the marine aquarium trade on coral reef systems (Rubec, 1988; Medley *et al.*, 1993). Successful aquaculture may reduce current and future pressure on coral reef ecosystems. However, culture of a wide range of marine ornamental species would be required if aquaculture was to offer any meaningful challenge to the wide variety of wild caught species offered in the trade.

Amphiprion percula belongs to the subfamily Amphiprionae (Family Pomacentridae). This subfamily comprises 28 species, 27 belonging to the genus *Amphiprion* and one species of the genus *Premnas*. All species have a symbiotic relationship with sea anemones and *A. percula* has been found to be associated with *Heteractis magnifica* (Quoy & Gaimard, 1833), *Stichodactyla gigantea* (Forsskal, 1775) and *Heteractis crispa* (Ehrenberg, 1834) (Fautin & Allen, 1992). The dependence on sea anemones of members of the Amphiprionae separates them from the rest of the

Pomacentridae family. However, the domino (*Dascyllus trimaculatus*) is a pomacentrid that is often found in association with anemones but is not necessarily dependent on them for survival (Mariscal, 1970).

Amphiprion percula is normally bright orange with three white bars running vertically across the body (see frontispiece). Adults are protandrous hermaphrodites and form permanent pair bonds (Allen, 1972). The female is the larger and more dominant of the pair and smaller subordinate males may also inhabit the host anemone. If the dominant male dies or is removed, the next largest subordinate male will become the dominant (beta) male (Fautin & Allen, 1992; Hattori, 1994). If the dominant female is removed, the dominant male will change sex , while the next largest male will develop into the new alpha male (Fricke, 1979).

Spawning occurs throughout the year, except in the subtropics where reproduction tends to be limited to spring and summer months (Allen, 1972). Natural spawning of *A. perideraion, A. chrysopterus* and *A. clarkii* has been found to be correlated to lunar cycles (Allen, 1972; Ochi, 1985). Under captive conditions, spawning occurs approximately every two weeks (Allen, 1972; pers. obs.). Spawning under natural conditions occurs 2-3 hours after sunrise and may last from 30 minutes to 2 hours (Allen, 1972; Ross, 1978) with between 100 and 1000 eggs being laid per clutch. Clutch size is between 200 and 400 eggs (Allen, 1980). The dominant male attends to the eggs and often fans them with the pectoral fins to ensure adequate water flow over them (pers.obs.). Hatching occurs after sunset, usually between six and eight days after spawning (Allen, 1972; Hoff, 1996). The larval period lasts between 8-12 days (Fautin & Allen, 1992) and larval dispersal is determined by the ocean currents. The short larval period is thought to have limited the distribution of this species to northern Queensland, the islands of New Guinea, New Britain, New Ireland, Vanuatu and the Solomon Islands (Fautin & Allen, 1992).

Reducing the growout period of fish from juvenile to saleable size is relevant to any fish producer and implementation of faster and more efficient production protocols may determine the financial viability of a marine fish grower. Although clownfish are not generally farmed on a large-scale, reduction in the wild stocks through capture for the aquarium trade may, in time, lead to commercial propagation of species of the *Amphiprion* genus. In addition, *Amphiprion* species may serve as candidate species to develop the technology and improve production efficiency. The findings may therefore become applicable to other species. Fundamental to the intensive production of a cultured species, is knowledge of the specific environmental requirements needed to obtain satisfactory survival and enhanced growth (Deacon, 1997).

Sequential determination of the optimal environmental factors to maximize growth is difficult because of potential interactions between the factors tested. For example, this study attempts to determine the optimal feeding regimen, temperature and stocking density of *A. percula*. If an experiment to determine the optimal temperature was conducted first, the results may not be conclusive because the feeding regimen or stocking density may not have been optimum because the optimal level of these factors has not been determined . Essentially, it is difficult to decide which factor needs to be determined first. Deacon (1997), designed a theoretical protocol for the sequential determination of environmental factors based on Fry's (1947, 1971) concepts of limiting factors.

He concluded that controlling factors (temperature) should be examined first, followed by limiting factors (ie. food requirements). Deacon (1997) did not investigate stocking density and it was thus decided, in the present study, to determine the optimal stocking density last and to use the current stocking densities used in the Rhodes University hatchery for the experimental determination of the optimal temperature and feeding regimen.

Research on the temperature preference of *Amphiprion percula* juveniles led to the suggestion of a thermal preferendum of 26 ± 0.7 °C (Lall, 1996). According to a hypothesis by Jobling (1981) and results by Deacon (1997), the preferred temperature and the temperature for optimal growth are similar. It was thus decided to first determine the effects of the limiting factor, food, before the controlling factor, temperature. This is based on the assumption that the thermal preferendum and the optimal temperature for growth of this species are not significantly different.

Food intake is a major factor regulating the growth of fish (Brett, 1979). Growth rate increases with ration from a minimum negative value (zero ration), through the point of zero growth (maintenance ration), up to the maximum where ration is highest (optimal ration) (Brett, 1979). Generally, maximum growth occurs near the limit of maximum food intake (Brett *et al.*, 1969; Andrews & Stickney, 1972; Brett, 1979) and feeding beyond this level would not increase growth, but would lead to food wastage and a possible deterioration of water quality (Tucker *et al.*, 1979; Cole & Boyd, 1986). An important aspect in determining the best feeding regimen for a species is the feeding frequency. An optimal feeding frequency should allow efficient digestion of food to ensure a minimum deprivation time (Deacon, 1997). It has been suggested that appetite increases with gastric evacuation which, in turn, is related to digestion rate and ultimately the metabolic rate (Brett, 1971; Grove *et al.*, 1978). Due to the relationship between temperature and metabolic rate in poikilothermic animals (Fry, 1971), the determination of optimal feeding regimens requires that metabolism is not limited by sub optimal temperatures. Use of the preferred temperature (Lall, 1996) of *A. percula* would allow experiments on the feeding regimen (Chapter 3) to be performed without jeopardizing growth performance due to sub optimal, temperature-related metabolism.

To date, no research has been conducted to determine the effect of feeding rate, ration, or feeding frequency on growth of *A. percula* juveniles, although Gordon (1999) investigated feeding regimens during the weaning stage from live food to dry food.. Detailed knowledge of the feeding regimen over the juvenile ongrowing stage would be beneficial for commercially producing this species in large scale systems. Efficient utilization of food has important economic implications (Cacho *et al.*, 1990) for any aquaculture venture and feeding at the correct level would maintain good growth, survival and size variation whilst limiting food wastage and water deterioration.

It has not been tested whether Lall's (1996) indication of thermal preference of *A. percula* correlates to the temperature for optimal growth. High correlations between the preferred temperature and the temperature at which maximum growth occurs have been recorded for a number of fish species (Jobling, 1981). Knowledge of the optimal temperature for growth is important since it coincides with the level where the rate of metabolic processes is optimal (Brett, 1979). This ensures that all anabolic processes are maximized (Fry, 1971). Furthermore, predictions of how growth is affected at temperatures above and below the optimum have not been made. For example, the trade-offs between lower growth at sub-optimal temperatures versus the cost of heating or cooling the water to achieve the temperature for optimal growth may be an important consideration for selecting a farm site. This prompted the design of an experiment to determine the effect of temperature on growth of *A. percula* juveniles that would allow for the development of a growth/temperature model for this species (Chapter 4).

For a species to be a suitable aquaculture candidate, it must grow at an acceptable rate under conditions of high stocking density (Bjornsson, 1994). Under natural conditions, *A. percula* juveniles recruit individually into sea anemones (Allen, 1972) and do not form schools. However, under captive conditions juvenile clownfish often form tight aggregations of individuals when reared at high densities (pers. obs.). High stocking densities may reduce growth rate due to competition for resources between individuals (Fox & Flowers, 1990) and aggression-related stress (Heath, 1991). Whether *A. percula* exhibit dominant or aggressive behavior when reared at high densities is not known. The objective of the experiment in Chapter 5 is to test whether juveniles of this species are able to be grown at high stocking densities. Furthermore, the experiment is designed to estimate the stocking density at which growth rate and survival is maximized.

To achieve maximum growth and survival of a species in aquaculture, examination of the optimal environmental conditions required by the species is needed. Detailed knowledge concerning the requirements to grow *Amphiprion percula* juveniles is lacking. This study focuses on the effects that feeding regimen, temperature and stocking density have on growth and survival of *A. percula* juveniles.

Chapter 2

General Materials and Methods

Origin of juvenile fish

Amphiprion percula broodstock were kept in 350L plastic containers connected to a 4000L recirculating system at the Rhodes University marine aquarium fish hatchery. Fish were fed twice daily on varying diets including commercial marine flake, frozen *Artemia*, grated shrimp (*Penaeus indicus*), sand mussels (*Donax serra*) and a gelatinised diet. Illumination was provided by natural lighting augmented with metal halide lamps. Eggs were usually spawned in the afternoon and were left in the care of the adults until just prior to hatching, 7 days after spawning.

Eggs were removed from the broodstock tanks on the day of anticipated hatching and were placed into dark-coloured, round 60 L PVC larval rearing tanks. Initially, the tanks contained sterilised seawater. Green water containing predominantly *Nannochloropsis sp.* was added to make up an algal density of approximately 10000 cells.ml^{-1}. The temperature was maintained at 27-28°C with a submersible aquarium heater and the salinity was kept at 35ppt. Green water was added to the tanks during the first five days after hatching (DAH) to maintain the desired concentration of algal cells. From 5 DAH water from the broodstock recirculating system was circulated through the larval tanks at a flowrate of 0.22L.min^{-1}.

The larvae were fed rotifers (*Brachionus sp.*) for the first 7 DAH. Instar I *Artemia fransiscana* (Jamaican strain) were added from 5 DAH until post-metamorphosis. *Artemia* were hydrated, decysted and hatched according to protocols outlined in Sorgeloos *et al.* (1986).

The post-larvae were weaned onto finely crushed commercial AKWA marine fish flake food (see Table 2.1) from 20 DAH. This food was used as the standard food for all experiments and was formulated for tropical marine species.

Table 2.1 Proximate composition of AKWA flake food (g/100g).

Crude protein	46
Crude fat	5
Fibre	5
Max. moisture	8

System design and management.

System A (refer to Figure 2.1)

A closed recirculating system with a total volume of 1300L was used to evaluate the effect of the feeding regimen (Chapter 3) and fish density (Chapter 4) on growth and survival of *A. percula* (Figure 2.1).The system consisted of thirty-six tanks (25x25x20 mm) filled to a volume of 10 L. Water was circulated through the system by a 0.3 kW Nocchi pump (A) at a delivery of 4800 L/hr at a 2 m head. Water flowed into the tanks via 5mm aquarium tubing and aeration was provided by an airstone in the corner of each tank fed from a side-channel blower .A sieve constructed from a section of a 110 mm pipe covered on both sides with 1.5 mm plastic mesh was fitted to the outflow of each tank. This mesh allowed fecal wastes to be removed but prevented the escape of the fish.

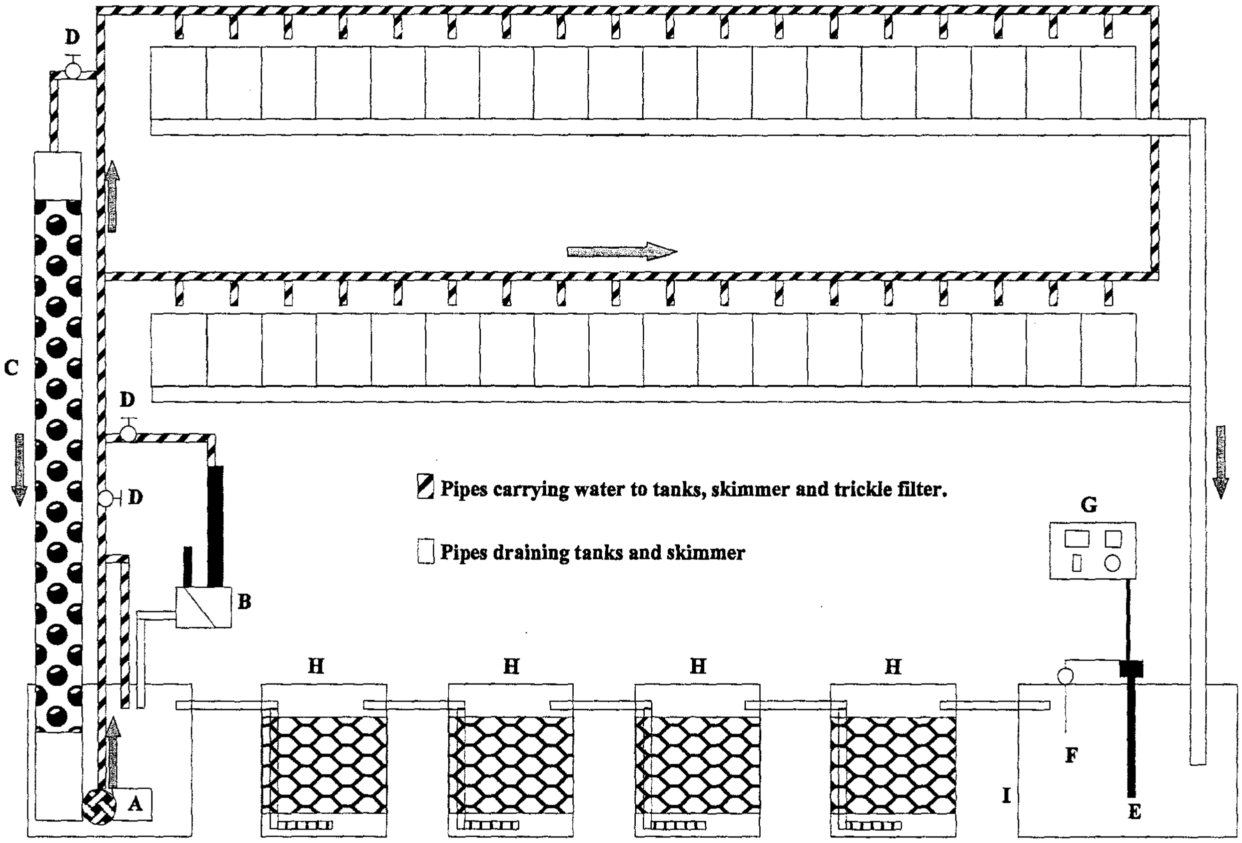


Figure 2.1 Experimental system A design (arrows indicate direction of water flow). Pump (A); foam fractionator (B); trickle filter (C); valves (D);heater element (E); thermostat probe (F); thermostat control (G); settling tank (I); flooded biofilters (H).

Filtration for this system consisted of two main components. First, a 2.5 m trickle filter (C) constructed from 200mm PVC piping in the pump sump. Water was diverted from the tank supply line into the top of the trickle tower. Plastic hair curlers were used as the medium in the trickle tower. Water from the pump was also circulated through a foam fractionator (B) back into the sump. The second filtration component was made up of four rectangular containers (70x39x38) (H) and a 200L (110x47x40) settling tank (I). Water from the aquaria entered the settling tank and flowed into the first of the four submerged filter containers. The first container was filled with polyester wool that traps particulate matter. The remaining three containers were filled with shredded plastic. There was also a layer of crushed oyster shells in the bottom of the second container to buffer the pH.

The water was heated by a 1kW heating element (E) in a vitrasol sleeve which was regulated by an external thermostat (G). Both the element and the thermostat probe (F) were suspended in the settling tank. The ambient temperature of the laboratory in which the system was housed was controlled by an airconditioner. The tanks were illuminated by flourescent tubes (Osram: Biolux) suspended above the tanks. Light intensity was measured at the water surface of each tank with a SL-100 laboratory quantum scalar irradiance meter and was maintained at 1.37 ± 0.29 (3×10^{15}) quanta.sec^{-1}.cm^{-2} .A photoperiod of 12L:12D was maintained by automatic electronic timer switches.

System B (refer to figure 2.2)

The experiment to determine the effect of temperature on fish growth and survival (Chapter 4) was conducted in a 750L recirculating system (figure 2.2). Water was circulated by a 0.3 kW Nocchi

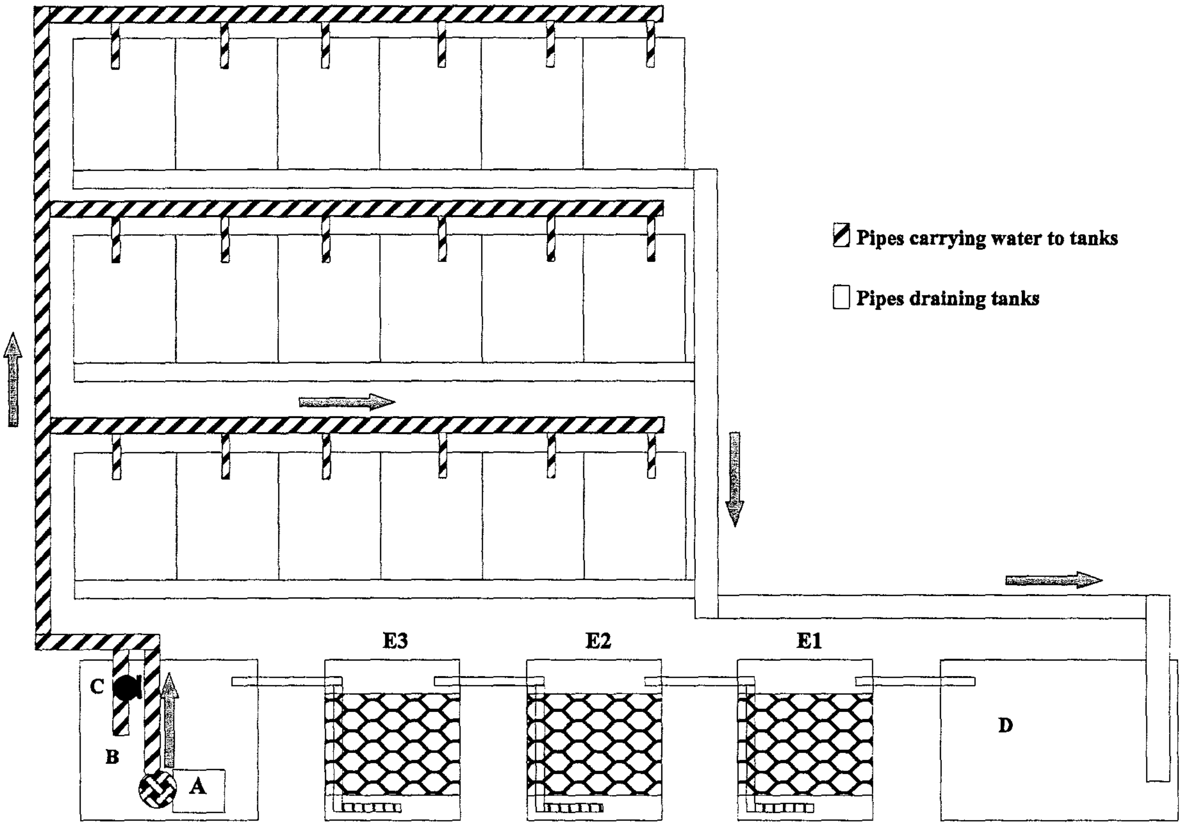


Figure 2.2 Experimental system B design (arrows indicate direction of water flow). Pump (A); flow regulator pipe (B); valve (C); settling tank (D); flooded biofilter containers (E1-E3).

pump (A) supplying eighteen 12L glass aquaria. Each tank was supplied with water via a 5mm inflow pipe and aerated with an airstone. Outflow sieves were the same as described for system A and the outflowing water from all tanks drained into a 100L settlement tank (D), from which it was passed through a series of three separate 100L filter compartments (E1-3) filled with polyester wool, shredded plastic, and oyster shells mixed with shell grit, respectively. The tanks were heated individually as described in the materials and methods section of Chapter 4. Photoperiod was set to 12L : 12D with an electronic timer switch. The tanks were illuminated by two 4-foot flourescent (Biolux) tubes attached to the ceiling of the room. The light intensity was measured in each tank with a QSL-100 laboratory quantum scalar irradiance meter and measured 0.49 ± 0.10 (1×10^{15}) quanta.sec^{-1}.cm^{-2} at the water surface.

Black plastic cardboard was placed between tanks to ensure that fish in adjacent aquaria were visually isolated from each other.

Water quality for both systems was measured weekly and recorded values are presented in their respective chapters. Salinity was measured with a Atago S/MiII handheld refractometer, while total ammonia (NH_4^+/NH_3), nitrite (NO_2^-) and nitrate (NO_3^-) were measured with Interpet aquarium test kits. Water pH was measured weekly with a Horiba pH meter F-8 L and dissolved oxygen was measured with a Oxyguard^R Handy MK III.

Measurement of growth

All fish were weighed and measured at three-week intervals for the feeding regimen experiment (Chapter 3). It was decided thereafter that this procedure was not as stressful for the fish as originally thought, so the fish were weighed and measured fortnightly in subsequent experiments (Chapters 4 & 5). Fish from each tank were caught with a hand net and anaesthetized in a solution of 2-phenoxyethanol at a concentration of 0.2ml.L^{-1} seawater for approximately five minutes. Deacon *et al.* (1997) have shown that regular use of 2-phenoxyethanol does not affect fish growth. All fish in each tank were weighed simultaneously. This was done by netting the anaesthetized fish with a hand net, drying off excess water by patting on dry paper towelling for 30 seconds and then placing the group of fish into a plastic petri dish on a zeroed electronic balance. At the end of each experiment, individual fish weights and corresponding lengths were recorded. These were used to calculate the length-weight models in Chapter 3 and 4.

All fish in each tank were photographed in the petri dish alongside a graduated ruler. This method was used to reduce the amount of time that the fish were out of the water as well as minimizing handling of individual fish. The total length of each fish was measured from the photographs with Mitutoyo Digital callipers and error due to magnification was corrected by taking the mean of three measurements of known lengths off the ruler in each photograph.

Chapter 3

The effect of ration and feeding frequency on the growth and survival of juvenile *Amphiprion percula*

Introduction

In aquaculture, feeding regime is a major factor which can be regulated to manipulate fish growth. Growth is either reproductive or somatic (Jobling, 1994) and juvenile fish direct most energy into somatic growth. Growth performance of captive fish can be manipulated by feeding regime, stocking density and other managerial alternatives (Chua & Teng, 1978).

The relationship between ration and metabolism is the fundamental basis of fish growth (Brett, 1979) and inadequate feeding may be a limiting factor on metabolism (Fry, 1971; Brett, 1979). Thus, the determination of the amount of food or ration required to enhance growth becomes important. Juvenile fish require a high food consumption for growth due to their high metabolic rate (Brett & Groves, 1979, Wurtsbaugh & Cech, 1983). Growth rates of fish are related to metabolic rate (Elliot, 1975; Grayton & Beamish, 1977) and growth is dependent on feeding ration if other potential growth limiting factors are mitigated.

Generally, the relationship between ration size and growth rate is positive, starting from a minimum negative value at zero ration to a maximum at the point of maximum food intake (Elliot, 1975; Huisman, 1976; Wurtsbaugh & Davis, 1977a,b; Chua & Teng, 1982). A plateau is reached beyond the maximum ration, where further intake of food does not lead to an increase in growth rate because the fish are fed in excess of their consumption potential (Talbot, 1994). Although different models have been used to describe the relationship between ration and growth (Huisman, 1976; Cui *et al.*, 1994,1996; Paul *et al.*, 1994; Tuene & Norvedt, 1995), the curvilinear model has been most commonly used to determine the maintenance, optimal and maximum ration requirements of fish (Brett, 1979; Chua & Teng, 1982). Economically, fish should grow at a maximal rate (Cacho *et al.*, 1990) and need to be fed at the correct level to sustain maximum growth rates. Furthermore, the physiological requirements suggest that a sub-optimal ration may result in slow growth and increased size variation (Tyler & Dunn, 1976), while ration in excess of the maximum may decrease digestive efficiency and have a detrimental effect on water quality (Tucker *et al.*, 1979; Cole & Boyd, 1986).

Feeding frequency is also important and is dependent on the evolved feeding strategy of the species (Chua & Teng, 1978). Clownfish are opportunistic (Allen, 1972) and are adapted to many small meals daily. Although *ad libitum* access to food is recommended to eliminate any biases arising from the feeding regime in growth trials (Jobling, 1983), it is not always practical or possible.

The optimal feeding frequency is essentially the lowest frequency that ensures maximum food intake (Grayton & Beamish, 1977) and maximum growth rate (Andrews & Page, 1975). Although there is a positive relationship between feeding frequency and growth (Chua & Teng, 1978) at levels higher than the maximum daily intake, growth increase may become negligible (Tsevis *et al.*, 1992) and may lead to excess lipid accumulation in the liver (Chua & Teng, 1978; Kayano *et al.*, 1993). However, increased feeding frequencies have been found to improve water quality (Yager & Summerfelt, 1994) by reducing variation of fish oxygen consumption (Yager & Summerfelt, 1993) and variations of water quality (Phillips *et al.*, 1998). In addition, high feeding frequencies (4-6 times per day) significantly reduced size variation of *Epinephelus akaara* (Kayano *et al.*, 1993).

Feeding frequency and ration size are important components of any feeding regime prescribed for maximum fish growth. To date, most studies conducted have attempted to determine optimum levels of these two factors separately. Ishiwata (1969a,b in Chua & Teng, 1978) have reported that the required daily food ration is dependent on feeding frequency. The two factors may have a simultaneous effect on the growth rates of fish, and may not be mutually exclusive.

This experiment aims to investigate the effect of ration size and feeding frequency and their combinations on the growth and survival of juvenile *Amphiprion percula.*

Materials and Methods

Juvenile *Amphiprion percula* were reared by protocols described in Chapter 2. Three hundred and sixty 55 day old fish were randomly collected from two combined batches and transferred into thirty-six 10 L glass tanks connected to a 1300 L recirculating system (System A, Chapter 2). Temperature was maintained at 26.2 ± 0.3 °C, which is within the preferred temperature for this species (Lall, 1996), and flow rate remained constant at 0.42 ± 0.08 $L.min^{-1}$. Salinity was maintained at 35 ± 1.2ppt by addition of fresh water. Total ammonia (NH_4^+/NH_3), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations ranged between 0 - 0.1 $mg.L^{-1}$, <0.1 $mg.L^{-1}$ and <25 $mg.L^{-1}$, respectively. Water pH values ranged between 8.15 - 8.3 and dissolved oxygen was always recorded between 90 - 100 % saturation.

Fish were fed crushed AKWA® flake food (Table 2.1, Chapter 2). Fish fed once a day were fed at midday; those fed twice per day were fed at 09h00 and 17h00; and those fed three times per day were fed at 09h00, 12h00 and 17h00.

Fish were weighed and measured at three-week intervals according to the procedure outlined in Chapter 2.

Computation of rations.

The food ration that resulted in best growth performance of *A. percula* was evaluated by offering a standard food (Table 2.1 Chapter 2) at varying percentages of fish body weight per day (BW.day^{-1}). The rations used were 2, 4, 6, 8, 10 and 12 %BW.day^{-1} and were calculated using the average fish weights recorded every three weeks. Since an exact daily ration, proportional to the body weight of a growing fish, cannot be prescribed (Brett and Shelbourne, 1975) because the ration would proportionately decrease as the fish grew during the 3 weeks between data collection. Thus, the average fish weights were predicted for each ration based on the growth performance from the previous three week growth period.

Every three weeks growth rates (G) (% BW.day^{-1}) from the previous periods were calculated as follows:

$$G = \left[\left(\frac{W_e}{W_o} \right)^{\frac{1}{d-1}} - 1 \right] \times 100 \qquad (1)$$

Where:
W_o = initial weight (g)
W_e = final weight (g)
d = days

This growth rate was considered representative for the following growth period and the projected final weight was predicted using the following equation, derived from (1).

$$W_e = W_o \times \left[\left(\frac{g}{100} \right) + 1 \right]^{d-1} \qquad (2)$$

Where:
g = growth rate (%BW.day^{-1})

W_e = projected final weight (g)

W_o = initial weight (g)

d = days

Food was weighed out and fed according to the estimated average fish weight. By calculating the amount of food required in this manner, it was accepted that the fish would be overfed to some unknown degree for the first half of the growth period and then potentially underfed toward the end of the growth period (Figure 3.1).

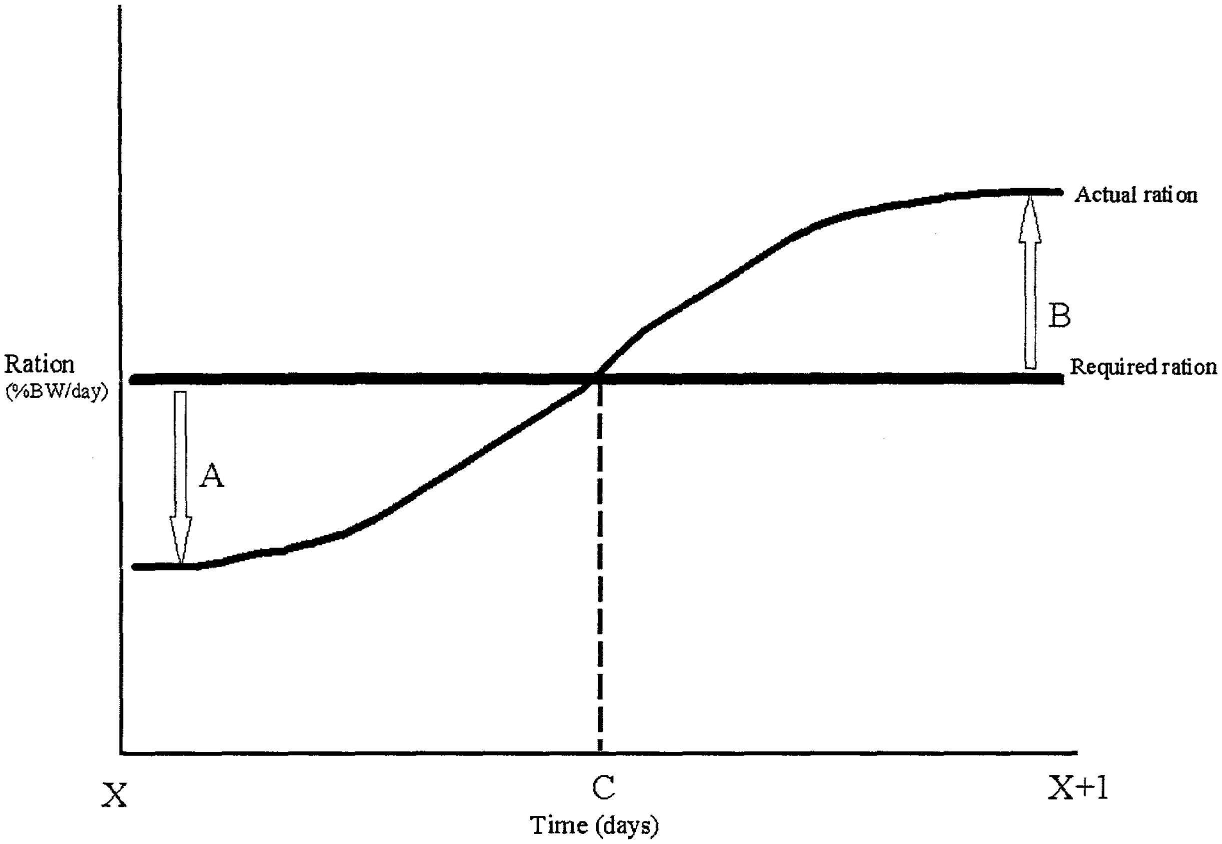


Figure 3.1 Hypothetical curve showing the predicted ration size between data collection where:

A: indicates period when fish were overfed.

B: indicates period where fish were underfed.

C: Approximate time during the growth period when fish were fed at the projected ration

X: Initial inventory

X+1: Final inventory

Data analysis

Log-transformed percentage survival rates were compared between treatments for ration and feeding frequency using Kruskal-Wallis one way analysis of variance on ranks. Differences between treatments were identified with a Student-Newman-Keuls test.

Length frequency histograms were used to describe and compare the condition factors of the fish at each of the six ration levels. The condition factor is a good indicator of the general "well-being or fitness" of an animal (Bolger & Connolly, 1989) and can be used as a comparative tool to assess the effects of varying environmental factors between treatments. Condition factor (CF) was calculated as follows:

$$CF = \frac{\textbf{Weight (g)}}{\textbf{Length (mm)}^3} .10^5$$

Coefficient of variation (CV) of condition factors were calculated for each ration with:

$$CV = \frac{\textbf{Standard Deviation}}{\textbf{Mean}} \textbf{ x100}$$

Non - linear regression was used to establish the relationship between ration (%BW.day^{-1}) and growth rate (mm.day^{-1}). Growth (length and weight) differences between the ration treatments (2 - 12 %BW.day^{-1}) at each feeding frequency (FF) were evaluated by pair-wise comparison of slopes using Analysis of CoVariance (ANCOVA). The error level to reject H_0 (growth rates do not differ between treatments) was set at $P < 0.003$ by dividing the 5% level of confidence by the number of paired comparisons. Growth models for weight and length and the length-weight relationship for *A. percula* were calculated using the Least Squares Method of all the fish in the experiment, except for those fed 2 %BW.day^{-1}.

Results

Survival

Significant differences ($P \leq 0.0129$) in survival at the end of the experiment were found between fish fed at 2 %BW.day^{-1} and those fed at higher rations (Table 3.1). No significant differences ($P = 0.3297$) in fish survival were found between any of the three feeding frequency treatments.

Table 3.1 : Mean percentage log-transformed survival of juvenile *A. percula* fed six rations after 86 days.

Ration (% BW.day $^{-1}$)	**ln survival (%) (± SD)***
2	3.78 ± 0.73^a
4	4.39 ± 0.16^b
6	4.47 ± 0.13^b
8	4.52 ± 0.04^b
10	4.42 ± 0.33^b
12	4.55 ± 0.08^b

* Same letters indicate homogenous groups ($P > 0.05$).

Frequency distribution

Condition factors at the end of the experiment were represented as frequency histograms (Figure 3.2). Juvenile *A. percula* fed at a 2 %BW.day^{-1} ration had a lower mean condition factor (1.52) and a greater condition factor variation (CV=10.3%) than fish fed at all other rations. A daily ration of 6 %BW resulted in the most even distribution and lowest variation (6.5%). The highest average condition factor (2.0) was recorded for fish fed 8 %BW.day^{-1} with a variation of 8.63%.

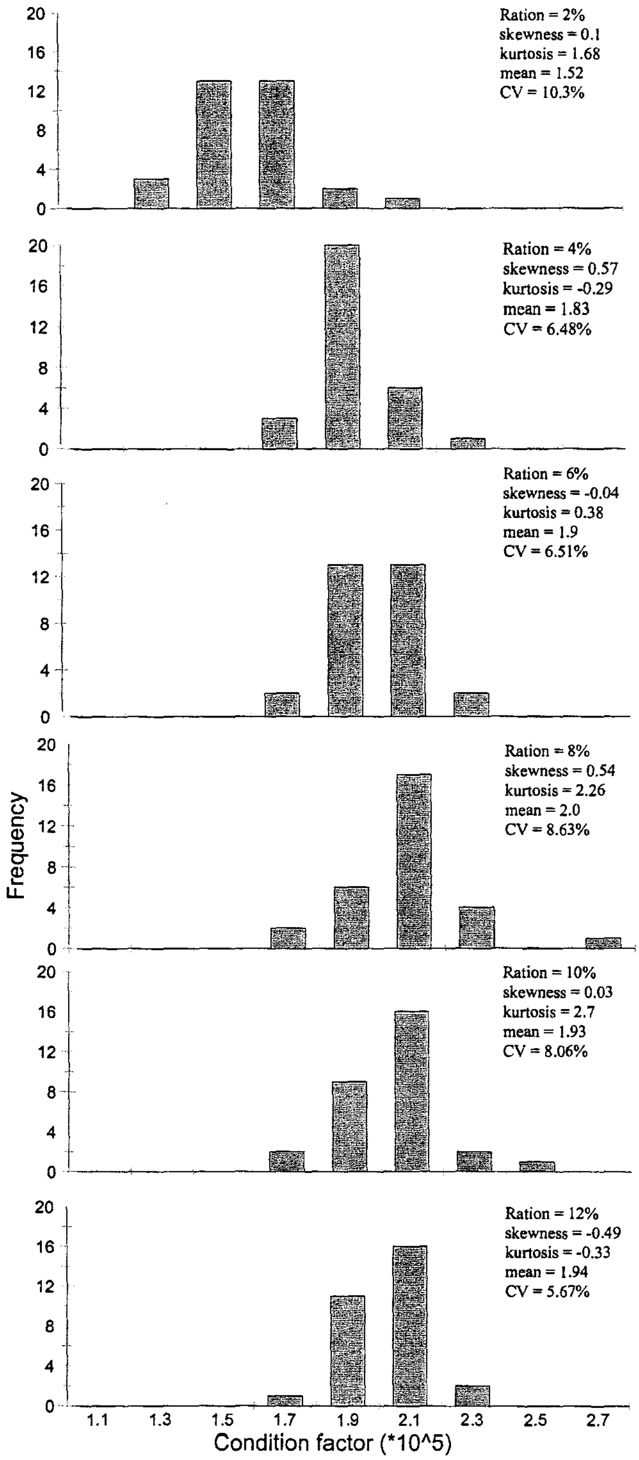


Figure 3.2 Frequency histograms of condition factor (weight/length^3.10^5) for fish fed rations of 2, 4, 6, 8, 10 & 12 %BW.day^{-1} for 86 days.

Growth - ration relationship

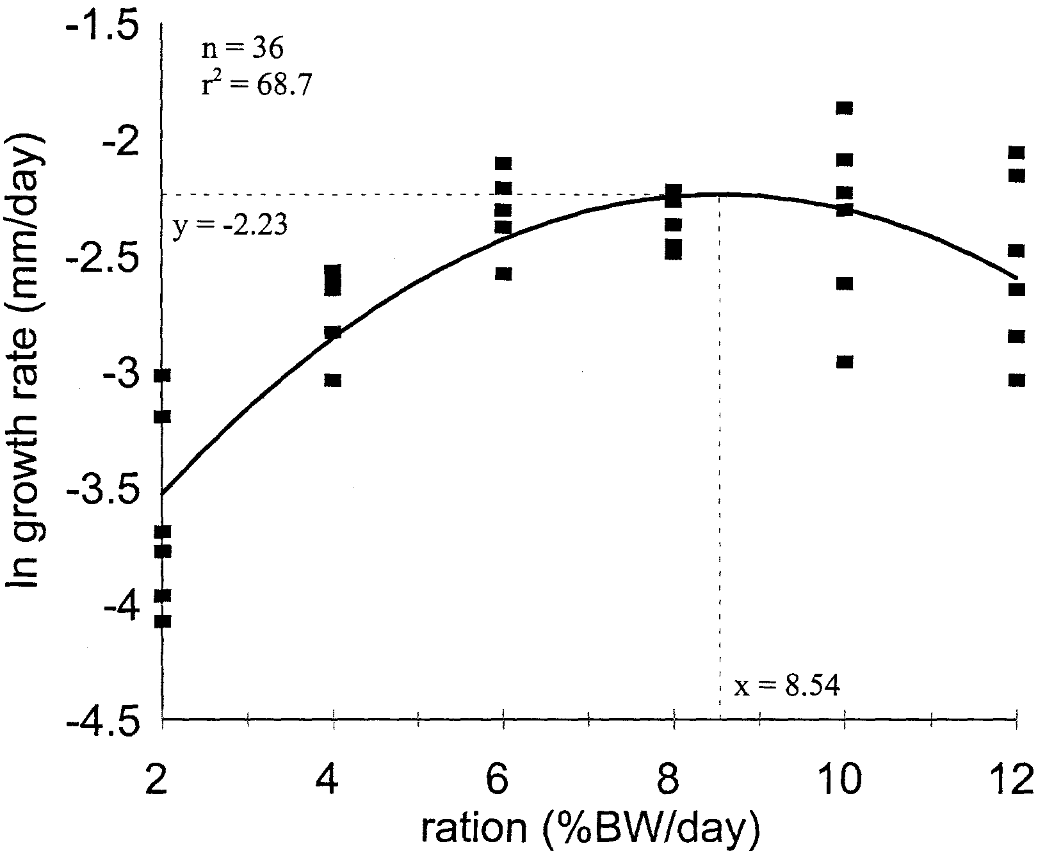


Figure 3.3 The relationship between growth and ration for juvenile *A. percula* after 86 days. x represents the ration that resulted in the highest growth rate (y).

The relationship between growth rate and ration is parabolic (Figure 3.3) and is best described by the polynomial model:

$$\ln y = -0.0302x^2 + 0.5159x + -4.4377 \qquad r^2 = 68.7\% \qquad P < 0.05$$

The maximum ration was calculated from the derivative function of: $x_{max} = \frac{-b}{2a}$

The corresponding point of highest growth rate was calculated with: $y_{max} = c - \frac{b^2}{4a}$

Where a, b and c are co-efficients from $y = ax^2 + bx + c$

The maximum growth rate was estimated at ln(-2.23) mm.day^{-1} at a ration of 8.54 %BW.day^{-1} (Figure 3.3). No significant ($P < 0.05$) relationships could be found that described the effect of feeding frequency on growth. Thus, feeding frequencies of one, two and three times daily did not result in any differences in the growth of juvenile *A. percula.*

Comparison of growth rates by ration at three feeding frequencies

Length

Comparisons of growth (length) responses of *A. percula* juveniles fed rations of 2 - 12 %BW.day^{-1} at feeding frequencies (FF) of 1, 2 and 3 times per day are presented in Tables 3.2, 3.4 and 3.6, respectively.

In fish fed once a day, a ration of 6 %BW.day^{-1} resulted in a significantly ($P < 0.003$) faster growth rate than in fish fed all other rations with the exception of fish fed a daily ration of 8 %BW.day. There were no differences amongst the growth rates of fish fed at the other rations. Growth models for the fish fed at each ration are shown in Table 3.3. Positive growth (length) was recorded at all six rations (Figure 3.4) when fed once per day, and a ration of 2 %BW.day^{-1} resulted in lowest overall growth rate (b = 0.0027 mm.day^{-1}, Table 3.3).

At a feeding frequency of 2 times per day, fish fed at 2 %BW.day^{-1} increased in length at a significantly ($P < 0.003$) slower rate than those fed at rations ranging from 4 - 12 %BW.day^{-1} (Table 3.4, Figure 3.5). In addition, fish fed 10 %BW.day^{-1} were found to grow faster than those fed at 4 and 6 %BW.day^{-1} . The regression models for fish fed at each ration are presented in Table 3.5 and the mean length of fish (± SE) at each ration level are shown in Figure 3.5.

Fish fed 2 %BW.day^{-1} exhibited significantly ($P < 0.003$) lower growth (length) rates than those fed at the higher rations when fed three times daily (Table3.6; b=0.0013 mm.day^{-1}, Table3.7). Mean length was also lowest for fish fed 2 %BW.day^{-1} than at higher rations (Figure 3.6). The highest growth was observed in fish fed 10 %BW.day^{-1} (b=0.0055 mm.day^{-1}, Table 3.7).

Weight

Fish fed 6 %BW.day^{-1} gained weight at a significantly ($P < 0.003$) higher rate than those fed 2 %BW.day^{-1} when fed once daily (Table 3.8). In addition, fish fed at 4 %BW.day^{-1} (b=0.0087 g.day^{-1}), 10 %BW.day^{-1} (b=0.0094 g.day^{-1}) and 12 %BW.day $^{-1}$ (b=0.0101 g.day^{-1}) had low growth rates that were not significantly different from those fed 2 %BW.day^{-1} (b=0.0064 g.day^{-1}). Fish fed at 8 %BW.day^{-1} (b=0.0137 g.day^{-1}) had comparable weight gain to fish fed at 6 %BW.day^{-1} (b=0.0181 g.day^{-1}) (Table 3.9) .

In fish fed twice daily, a ration of 2 %BW.day^{-1} gained weight at a significantly ($P < 0.003$) lower rate than at higher rations of 4 - 12 %BW.day^{-1} (Table 3.10). Best growth was recorded for fish fed 10 %BW.day^{-1} (b=0.0168 mm.day^{-1}, Table 3.11).

When fed 3 times daily, juvenile *A. percula* fed 2 %BW.day^{-1} grew significantly ($P < 0.003$) slower than those fed rations of 4 - 12 %BW.day^{-1} (Table 3.12). Comparably higher rates of weight gain were observed when fish were fed at 6 (b=0.0164 g.day^{-1}) and 10 (b=0.0165 g.day^{-1}) %BW.day^{-1} (Table 3.13).

Table 3.2 Pair-wise comparison of slopes (ANCOVA) of length gain for *A. percula* fed once per day at rations of 2, 4, 6, 8, 10 &12 %BW.day^{-1} over 86 days.

Ration pair (%BW.day^{-1})	F	d$f_{a,b}$	P-value
2 - 4	0.0	1 , 179	0.9996
2 - 6	12.7	1 , 190	0.0005*
2 - 8	3.1	1 , 186	0.0789
2 - 10	0.6	1 , 194	0.4391
2 - 12	0.3	1 , 186	0.6038
4 - 6	17.4	1 , 217	0.0000*
4 - 8	4.3	1 , 213	0.0396
4 - 10	0.8	1 , 221	0.3685
4 - 12	0.4	1 , 213	0.5419
6 - 8	3.8	1 , 224	0.0530
6 - 10	13.1	1 , 232	0.0004*
6 - 12	11.1	1 , 224	0.0019*
8 - 10	1.9	1 , 228	0.1680
8 - 12	1.9	1 , 220	0.1695
10 - 12	0.0	1 , 228	0.5880

* denotes significant difference ($P < 0.003$) between two slopes.

Table 3.3 Growth models describing length of *A. percula* fed once per day at rations of 2, 4, 6, 8, 10 & 12 %BW.day^{-1} over 86 days. a and b represent the y-intercept and slope respectively.

Ration (% BW.day^{-1})	Regression equation (y = a + bx)*
2	$\ln(y) = 2.7315 + 0.0027x^{a}$
4	$\ln(y) = 2.8271 + 0.0027x^{a}$
6	$\ln(y) = 2.6754 + 0.0055x^{b}$
8	$\ln(y) = 2.7726 + 0.0041x^{ab}$
10	$\ln(y) = 2.7871 + 0.0032x^{a}$
12	$\ln(y) = 2.8101 + 0.0031x^{a}$

* same letters indicate homogenous groups ($P < 0.003$)

Table 3.4 Pair-wise comparison of slopes (ANCOVA) of length gain for *A. percula* fed twice a day at rations of 2, 4, 6, 8, 10 &12 %BW.day^{-1} over 86 days.

Ration pair	F	$df_{a,b}$	P-value
2 - 4	14.5	1 , 194	0.0002*
2 - 6	13.9	1 , 200	0.0002*
2 - 8	27.3	1 , 211	0.0000*
2 - 10	54.4	1 , 191	0.0000*
2 - 12	34.1	1 , 211	0.0000*
4 - 6	0.0	1 , 200	0.9614
4 - 8	1.5	1 , 211	0.2154
4 - 10	10.4	1 , 191	0.0015*
4 - 12	3.6	1 , 211	0.0581
6 - 8	1.4	1 , 217	0.2458
6 - 10	9.4	1 , 197	0.0024*
6 - 12	3.3	1 , 217	0.0708
8 - 10	4.2	1 , 208	0.0420
8 - 12	0.5	1 , 288	0.4753
10 - 12	1.7	1 , 208	0.1948

* denotes significant difference ($P < 0.003$) between slopes

Table 3.5 Growth models describing length of *A. percula* fed twice a day at rations of 2, 4, 6, 8, 10 & 12 %BW.day^{-1} over 86 days. a and b represent the y-intercept and slope respectively.

Ration (% BW.day^{-1})	Regression equation (y = a + bx)*
2	$\ln(y) = 2.8214 + 0.0011x^{a}$
4	$\ln(y) = 2.7759 + 0.0037x^{b}$
6	$\ln(y) = 2.7757 + 0.0038x^{b}$
8	$\ln(y) = 2.8436 + 0.0046x^{bc}$
10	$\ln(y) = 2.7387 + 0.0060x^{c}$
12	$\ln(y) = 2.7771 + 0.0051x^{bc}$

* same letters indicate homogenous groups ($P < 0.003$)

Table 3.6 Pair-wise comparison of slopes (ANCOVA) of length gain for *A. percula* fed three times a day at rations of 2, 4, 6, 8, 10 &12 %BW.day^{-1} over 86 days.

Ration pair	***F***	**d*f*$_{a,b}$**	**P-value**
2 - 4	9.2	1 , 210	0.0028*
2 - 6	32.8	1 , 210	0.0000*
2 - 8	15.0	1 , 206	0.0001*
2 - 10	35.7	1 , 210	0.0000*
2 - 12	18.6	1 , 214	0.0000*
4 - 6	5.6	1 , 228	0.0184
4 - 8	1.2	1 , 224	0.2751
4 - 10	7.2	1 , 228	0.0078
4 - 12	0.7	1 , 232	0.4022
6 - 8	1.1	1 , 224	0.2959
6 - 10	0.1	1 , 228	0.7089
6 - 12	3.1	1 , 232	0.0808
8 - 10	1.8	1 , 224	0.1764
8 - 12	0.2	1 , 228	0.6732
10 - 12	4.5	1 , 232	0.0358

* denotes significant difference ($P < 0.003$) between slopes.

Table 3.7 Growth models describing length of *A. percula* fed three times a day at rations of 2, 4, 6, 8, 10 & 12 %BW.day^{-1} over 86 days. a and b represent the y-intercept and slope respectively.

Ration (% BW.day^{-1})	**Regression equation (y = a + bx)***
2	$\ln(y) = 2.8214 + 0.0013x^{a}$
4	$\ln(y) = 2.7929 + 0.0036x^{b}$
6	$\ln(y) = 2.7399 + 0.0053x^{b}$
8	$\ln(y) = 2.7598 + 0.0045x^{b}$
10	$\ln(y) = 2.6849 + 0.0055x^{b}$
12	$\ln(y) = 2.7255 + 0.0042x^{b}$

* same letters indicate homogenous groups ($P < 0.003$)

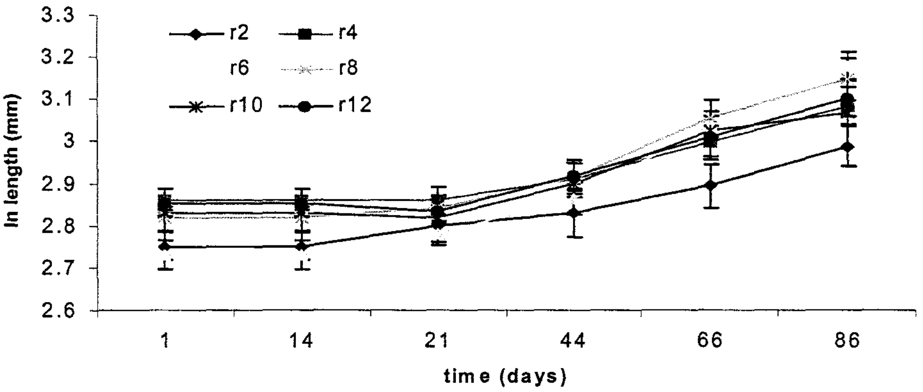


Figure 3.4 Mean length (± SE) of *A. percula* juveniles fed rations of 2, 4, 6, 8, 10 & 12 %BW.day^{-1} once daily. Daily ration (%BW.day^{-1}) is denoted by r

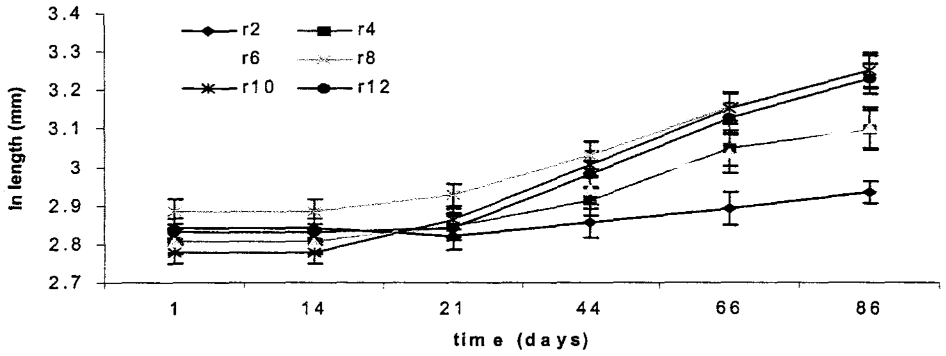


Figure 3.5 Mean length (± SE) of *A. percula* juveniles fed rations of 2, 4, 6, 8, 10 & 12 %BW.day^{-1} twice daily. Daily ration (%BW.day^{-1}) is denoted r.

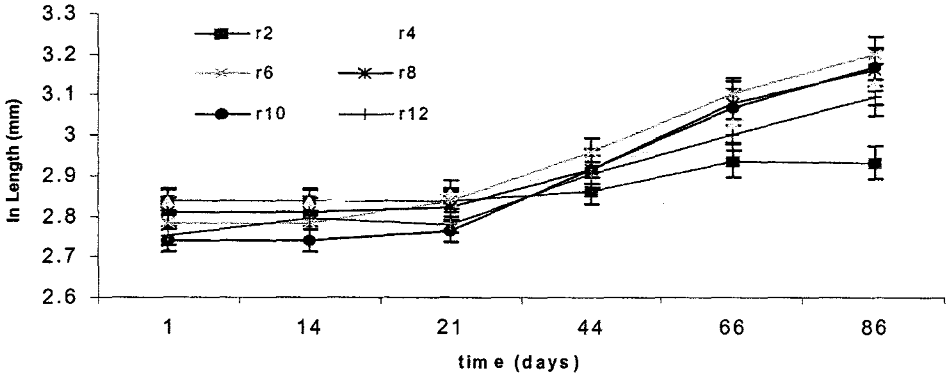


Figure 3.6 Mean length (± SE) of *A. percula* juveniles fed rations of 2, 4, 6, 8, 10 & 12 %BW.day^{-1} three times daily. Daily ration (BW.day^{-1}) is denoted by r.

Table 3.8 Pair-wise comparison of slopes (ANCOVA) of weight gain for *A. percula* fed once per day at rations of 2, 4, 6, 8, 10 &12 %BW.day^{-1} over 86 days.

Ration pair	***F***	**d$f_{a,b}$**	**P-value**
2 - 4	1.2	1 , 16	0.2878
2 - 6	14.3	1 , 16	0.0016*
2 - 8	6.6	1 , 16	0.0207
2 - 10	1.8	1 , 16	0.1966
2 - 12	2.1	1 , 16	0.1636
4 - 6	11.1	1 , 16	0.0042
4 - 8	3.9	1 , 16	0.0661
4 - 10	0.2	1 , 16	0.688
4 - 12	0.4	1 , 16	0.5158
6 - 8	1.7	1 , 16	0.2172
6 - 10	8.8	1 , 16	0.0091
6 - 12	6.1	1 , 16	0.0249
8 - 10	2.6	1 , 16	0.1262
8 - 12	1.4	1 , 16	0.2503
10 - 12	0.1	1 , 16	0.7503

* denotes significant difference ($P < 0.003$) between two slopes.

Table 3.9 Growth models describing weight of *A. percula* fed once per day at rations of 2, 4, 6, 8, 10 & 12 %BW.day^{-1} over 86 days. a and b represent the y-intercept and slope respectively

Ration (% BW.day^{-1})	**Regression equation (y = a + bx)***
2	$\ln(y) = -2.6065 + 0.0064x^{a}$
4	$\ln(y) = -2.4667 + 0.0087x^{ab}$
6	$\ln(y) = -2.9149 + 0.0181x^{b}$
8	$\ln(y) = -2.5351 + 0.0137x^{ab}$
10	$\ln(y) = -2.4996 + 0.0094x^{ab}$
12	$\ln(y) = -2.3917 + 0.0101x^{ab}$

* same letters indicate homogenous groups ($P < 0.003$)

Table 3.10 Pair-wise comparison of slopes (ANCOVA) of weight gain for *A. percula* fed twice a day at rations of 2, 4, 6, 8, 10 &12 %BW.day^{-1} over 86 days.

Ration pair	***F***	**d$f_{a,b}$**	**P-value**
2 - 4	25.2	1 , 16	0.0001*
2 - 6	35.2	1 , 16	0.0000*
2 - 8	60.5	1 , 16	0.0000*
2 - 10	66.1	1 , 16	0.0000*
2 - 12	39.9	1 , 16	0.0000*
4 - 6	0.5	1 , 16	0.4863
4 - 8	3.6	1 , 16	0.0750
4 - 10	7.6	1 , 16	0.0139
4 - 12	3.4	1 , 16	0.0821
6 - 8	1.7	1 , 16	0.2607
6 - 10	4.4	1 , 16	0.0525
6 - 12	1.6	1 , 16	0.2275
8 - 10	1.2	1 , 16	0.2905
8 - 12	0.1	1 , 16	0.7429
10 - 12	0.3	1 , 16	0.5758

* denotes significant difference ($P < 0.003$) between two slopes

Table 3.11 Growth models describing weight of *A. percula* fed twice a day at rations of 2, 4, 6, 8, 10 & 12 %BW.day^{-1} over 86 days. a and b represent the y-intercept and slope respectively

Ration (% BW.day^{-1})	**Regression equation (y = a + bx)***
2	$\ln(y) = -2.4376 + 0.0018x^{a}$
4	$\ln(y) = -2.5272 + 0.0111x^{b}$
6	$\ln(y) = -2.5306 + 0.0125x^{b}$
8	$\ln(y) = -2.3208 + 0.0147x^{b}$
10	$\ln(y) = -2.4556 + 0.0168x^{b}$
12	$\ln(y) = -2.4993 + 0.0155x^{b}$

*same letters indicate homogenous groups ($P < 0.003$)

Table 3.12 Pair-wise comparison of slopes (ANCOVA) of weight gain for *A. percula* fed three times a day at rations of 2, 4, 6, 8, 10 &12 %BW.day^{-1} over 86 days.

Ration pair	***F***	**d$f_{a,b}$**	**P-value**
2 - 4	15.4	1 , 16	0.0012*
2 - 6	76.8	1 , 16	0.0000*
2 - 8	25.1	1 , 16	0.0001*
2 - 10	39.9	1 , 16	0.0000*
2 - 12	17.4	1 , 16	0.0007*
4 - 6	7.8	1 , 16	0.0131
4 - 8	1.8	1 , 16	0.1966
4 - 10	5.4	1 , 16	0.0334
4 - 12	1.5	1 , 16	0.2339
6 - 8	1.0	1 , 16	0.3350
6 - 10	0.0	1 , 16	0.9699
6 - 12	0.6	1 , 16	0.4568
8 - 10	0.8	1 , 16	0.3926
8 - 12	0.0	1 , 16	0.9477
10 - 12	0.5	1 , 16	0.4909

* denotes significant difference ($P < 0.003$) between two slopes.

Table 3.13 Growth models describing weight of *A. percula* fed three times a day at rations of 2, 4, 6, 8, 10 & 12 %BW.day^{-1} over 86 days. a and b represent the y-intercept and slope respectively

Ration (% BW.day^{-1})	**Regression equation (y = a + bx)***
2	$\ln(y) = -2.3728 + 0.0016x^{a}$
4	$\ln(y) = -2.4242 + 0.0098x^{b}$
6	$\ln(y) = -2.6090 + 0.0164x^{b}$
8	$\ln(y) = -2.4508 + 0.0138x^{b}$
10	$\ln(y) = -2.7416 + 0.0165x^{b}$
12	$\ln(y) = -2.7328 + 0.0140x^{b}$

* same letters indicate homogenous groups ($P < 0.003$)

Length weight relationship

No observable differences were identified between condition factors of the fish at each ration (Table 3.14). Thus the rations were pooled, resulting in the correlation presented in Figure 3.7. Fish fed at 2 %BW.day^{-1} were excluded due to poor growth performance. A log transformation of both axes resulted in a highly significant (correlation coefficient = 0.986) linear relationship between length and weight for juvenile *A. percula*. The best regression model for these data is $\ln(y) = -11.1091 + 3.0804 \ln(x)$ where y and x are fish weight (g) and length respectively.

Table 3.14 Length weight relationship for juvenile *A. percula* fed at 4, 6, 8, 10 &12% BW.day^{-1} after 86 days.

Ration (%BW.day^{-1})	**$F_{1,28}$**	**Regression equation**	**R^2 (%)**
4	751.1	$\ln(y) = -10.579 + 2.888 \ln(x)$	96.41
6	2962.1	$\ln(y) = -11.664 + 3.268 \ln(x)$	99.06
8	831.2	$\ln(y) = -10.957 + 3.044 \ln(x)$	96.74
10	1027.2	$\ln(y) = -10.653 + 2.932 \ln(x)$	97.35
12	1444.0	$\ln(y) = -11.224 + 3.125 \ln(x)$	98.11

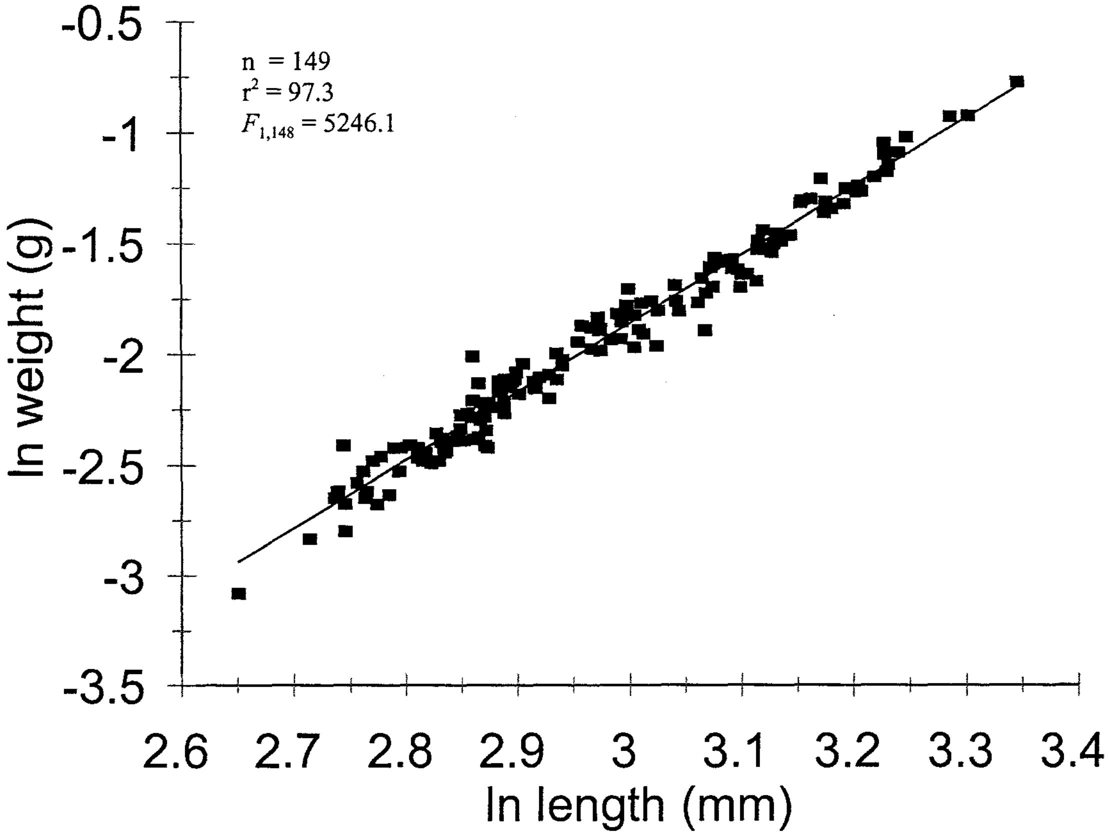


Figure 3.7 The relationship between length and weight for juvenile *Amphiprion percula* fed rations between 4 and 12 %BW.day^{-1} after 86 days of growth.

Discussion

The maximum daily ration, independent of feeding frequency, that resulted in the highest growth rate in juvenile *Amphiprion percula* was estimated at 8.54 %BW.day^{-1}. The growth/ration relationship for *A. percula* was parabolic, which is similar to the relationship established for carp (*Cyprinus carpio*) (Huisman, 1976). In contrast, curvilinear relationships for sockeye salmon (*Oncorhynchus nerka*) (Brett, 1979), rainbow trout (*O. mykiss*) (Huisman, 1976; Wurtsbaugh & Davis, 1977a,b), sea bass

(*Dicentrarchus labrax*) (Hidalgo *et al.*, 1987) and estuarine grouper (*Epinephelus salmoides*) (Chua & Teng, 1982) were found to be the best representation of the growth/ration relationship. Furthermore, Cui *et al.* (1994, 1996), Tuene and Nortvedt (1995) and Paul *et al.* (1994) found that the growth/ration relationship was linear for grass carp (*Ctenopharyngodon idella*), white sturgeon (*Acipenser transmontanus*), and Atlantic (*Hippoglossus hippoglossus*) and Pacific halibut (*H. stenolepis*), respectively. However, although *ad libitum* feeding was used (Cui *et al.*, 1994, 1996), it appears that the fish were not fed at or above the maximum ration.

The parabolic relationship for *A. percula* juveniles indicated that growth was depressed above a maximum ration. It may be suggested that this is a result of the "glutton effect" where food is utilized less efficiently at high meal sizes (Brett & Groves, 1979) or that a portion of the food is not ingested (Cui, *et al.* 1996) above a given ration level. Excess unconsumed food was not recorded due to the small particulate size. Thus it cannot be quantified if fish fed at rations in excess of the maximum did in fact ingest all the food offered.

Growth rates below the maximum ration decreased indicating that ration size was a limiting factor for the growth of juvenile *A. percula.* Food conversion ratio (FCR) could not be determined as the uneaten food particles were too small to be collected efficiently. FCR would be expected to increase once the maximum ration has been exceeded as growth efficiency, the efficiency with which food is converted into growth, decreases for fish fed above maximum rations (Talbot, 1994).

Condition factor of juvenile *A. percula* followed a similar pattern to the growth/ration relationship with the highest condition recorded for fish fed at 8 %BW.day^{-1} and lowest at 2 %BW.day^{-1}. Similar positive relationships between increasing condition factor and increasing ration were found for flounder (*Pseudopleuronectes americanus*) and cod (*Gadus morhua*) by Tyler & Dunn (1976) and Edwards *et al.* (1972), respectively. Reduced condition factor at the lower ration level (2 %BW.day^{-1}) indicates that food was limiting (Jobling, 1996) and resulted in a length increase without a corresponding increase in weight.

High survival of fish is expected for fish maintained in adequate to optimal culture conditions. Survival of *A. percula* juveniles was significantly less when fed at 2 %BW.day^{-1} compared with those fed at the higher rations tested. The fish fed at 2 %.BW.day^{-1} had a lower condition factor and were less active than those at higher ration levels. Mortality of these fish was most likely a result of starvation. Thus 2 %BW.day^{-1} may be near to or above the maintenance ration for this species since the lowest survival and condition factor were measured at this level.

The above discussion refers to the effect of ration, independent of the feeding frequency, on the growth of *Amphiprion percula* juveniles. In contrast, however, when feeding frequency was considered as a factor independent of ration level, no significant effect on growth or survival of *A. percula* were found. Due to the factorial design, some fish in each of the feeding frequency treatments were fed at potentially limiting rations (2 and 4 %BW.day^{-1}) for growth. This may have been a major contributing factor to the large variances in growth at each feeding frequency treatment which consequently led to no differences being found.

Other authors have determined the optimal feeding frequency for the best growth of various species (Table 3.15). Furthermore, increased feeding frequency has been found to reduce size variation

(Kayano *et al*., 1993), because smaller fish have more opportunity to be in the feeding area and are able to receive an adequate amount of food per day (Thomassen & Fjaera, 1996).

Table 3.15 Optimal feeding frequencies for growth of eight fish species.

Species	**FF$_{range}$**	**FF$_{opt}$**	**Author**
Oncorhynchus mykiss	1 - 6	2	Grayton & Beamish, 1977
Epinephelus tauvina	1/5 - 3*	1/2*	Chua & Teng, 1978
Micropogonias furnieri	1/3 - 2*	1	Aristizabal Abud, 1990
Oreochromis mossambicus	4 - 10	8	Thorpe & Hecht, 1992
Dicentrarchus labrax	0 - 4	3	Tsevis *et al*., 1992
Epinephelus akaara	1 - 8	4-6	Kayano *et al*., 1993
Clarias fuscus	1 - 3	3	Buurma & Diana, 1994
Oncorhynchus mykiss	10 , 20	10	Vega *et al*., 1994
Lates calcarifer	1 - 3	3	Aranyakananda *et al*., 1995

Note: FF - refers to feeding frequency (no. of meals per day)
* Fractions refer to no. of feedings/no. of days

Other factors associated with higher feeding frequencies include a reduced variability of oxygen consumption (Yager & Summerfelt, 1993) by stabilizing metabolic rates (Yager & Summerfeld, 1994; Guinea & Fernandez, 1997) and water quality, especially total ammonia excretion which has been shown to be less variable (Phillips *et al*., 1998). These factors however, were not determined in the present study, but may need to be addressed in future feeding studies of *A. percula.*

A daily ration of 8.5 %BW.day^{-1} resulted in maximum growth irrespective of feeding frequency. If, however, the effect of feeding frequency is included into the analysis, new interactions are revealed.

At a feeding frequency of once per day, juvenile clownfish exhibited maximum growth when fed a ration of 6 %BW.day^{-1}. Furthermore, maximum rates of growth were attained when fish were fed rations of 10 and 6-10 %BW.day^{-1} at feeding frequencies of 2 and 3 times per day respectively. As the feeding frequency decreased, the ration requirement for maximum growth decreased as well. There was a decrease of food required per meal as the time interval between meals was lessened (Table 3.16) because food can only be processed at a specific rate governed by the metabolism of the fish. Larger rations required (6 %BW.day^{-1}) per meal at a single feeding per day may indicate that those fish responded to long intervals between feedings by becoming hyperphagic. This occurs when fish are fed infrequently and overeat in an attempt to sustain themselves over long meal intervals (Jobling, 1994). This was also noted by Grayton & Beamish (1977) when trout were fed infrequently and led to a prolonged period of egestion of feces after a meal.

Table 3.16 Per meal percentage food required for maximum growth at three feeding frequencies in juvenile *A. percula*.

No. of meals per day	**Growth ration$_{max}$ [a]**	**% fed per meal[b]**
1	6	6
2	10	5
3	6 - 10	2 - 3.3

[a] The ration (%BW.day^{-1}) required for maximum growth
[b] The amount (%BW) required for maximum growth per meal

Due to the small size of *Amphiprion percula* juveniles accurate determination of weight can be difficult. Thus a length-weight relationship for this species was calculated to enable potential growers to calculate ration requirements as a percentage of body length. A high correlation between length and weight was recorded ($r^2 = 97.2\%$) and the slope value from the regression was calculated at 3.08.

Ration size did not influence the relationship between weight and length in fish fed above 2 %BW.day^{-1}. In these fish, significantly lower survival and growth was observed at all feeding frequencies. Thus, data from this ration were not included in the length-weight regression analysis. In conclusion, the highest rate of growth was found when fish were fed twice per day at a ration of 10 %BW.day^{-1}, and together with the establishment of a weight-length model, these findings could be applied to large scale production of this species.

Chapter 4

The effect of temperature on the growth of juvenile *Amphiprion percula*

Introduction

Temperature is an important factor that regulates the metabolic rate (Fry, 1971; Beitinger & Fitzpatrick, 1979; Brett, 1979), physiological state (Iwata *et al.*, 1995) and growth (Cech *et al.*, 1984; Buckel *et al.*, 1995; Brander, 1995; Imsland *et al.*, 1996; Ellis *et al.*, 1997) of fish. Thus, knowledge of the thermal regime required for growth and survival is fundamental for successful culture of any fish species (Berg, 1990; Iwata *et al.*, 1995).

Generally, growth tends to increase with an increase in temperature within species dependent limits (Jobling, 1983). Initially, food is processed faster at an increasing temperature due to the higher metabolic rate (Peck, 1989). However, as the temperature approaches the upper limit of the preferred thermal range the fish will become stressed, resulting in slower growth and eventually in death.

Oxygen consumption increases with temperature (Hettler, 1976; Konstantinov, 1990; Iwata *et al.*, 1994), but the oxygen carrying capacity of the water decreases as less oxygen can be dissolved. Furthermore, rising temperature affects the efficiency with which hemoglobin binds to oxygen (Jobling, 1994). Thus, at high temperatures and less dissolved oxygen in the water, fish tend to respire more to receive sufficient oxygen. This uses up metabolic energy that could otherwise be used for growth and in extreme cases may cause metabolism to become catabolic (Iwata *et al.*, 1994).

Temperature also moderates the catalytic properties of enzymes (Jobling, 1994). In fish, changes in temperature result in changes of the relative proportions of two or more enzyme isoforms so that enzyme activity is optimized depending on the ambient temperature (Jobling, 1994). This mechanism probably evolved to reduce the chances of failure of enzyme activity at temperatures beyond the limits of the natural temperature range.

It is thus evident that temperature exerts considerable influence on fish physiology and growth. Earlier temperature/growth related research has focused primarily on commercial foodfish species (see review by Brett, 1979) because of the advantages gained by farmers from knowing the thermal range of the species they are growing. It is important that the temperature for maximum growth is determined for each species and not hypothesized by making inferences to species with similar natural distributions or species with a wide natural distribution and very variable temperature range. In addition, we cannot assume that the average temperature found in a species natural range is the optimal temperature at which it should be grown in captivity.

The relationship between temperature and growth rate is typically described by a parabola (Brett, 1979). This has been demonstrated for *Clarias gariepinus* (Britz & Hecht, 1987) and *Cichlasoma urophthalmus* (Martinez-Palacios *et al.*, 1996) juveniles. These results indicate that growth rate increases with temperature up to a specific maximum, whereafter it drops as increasing temperatures become physiologically limiting. The apex temperature defines the level at which maximum growth occurs (Brett, 1979, Deacon, 1997).

The paucity of empirical data on the effect of temperature on the growth rate of *Amphiprion percula* prompted the design of this study which aims at modeling growth as a function of temperature. Ultimately, the study aims at determining the optimal temperature for growth of this species.

Materials and methods

System design and management

Sixteen 12L glass aquaria connected to a 750L recirculating system were used to test the effect of temperature on growth of juvenile *A. percula*. System design (Figure 2.2) and system management are described in Chapter 2. Nitrogenous waste products, salinity, oxygen saturation and pH measurements were recorded weekly in the system. Ammonia (NH_4^+/NH_3) and nitrite (NO_2^-) levels were below 0.1mg.L^{-1} and nitrate was maintained below 20 mg.L^{-1}over the duration of the experiment. The pH ranged between 8.1 and 8.28. Lighting was controlled with a timer switch set to 12L:12D. Average salinity was 34.5 ± 1.8 ppt ($\bar{x}$ ± standard deviation) and water lost to evaporation was replaced with dechlorinated tap water. Oxygen saturation was found to be above 90 % for all measurements taken.

Experimental fish

Sixty-four-day-old juvenile *Amphiprion percula* (n=160) that had been reared at the Rhodes University marine hatchery according to protocols described in Chapter 2, were used in the study. They were selected randomly from a single batch. The fish had been weaned onto the experimental diet (Table 2.1) one month prior to the start of the experiment. Rearing temperature of the culture water was 26° C before the study and the fish were acclimated to the system to avoid potential temperature-related stress. Ten fish were assigned to each tank and before release into their respective tanks, they were placed in a plastic bag containing 4 liters of their culture water. The bags remained in their experimental tanks for 12 hours to ensure that the water temperature matched that of the tank water. Air was bubbled into each bag with an airstone during this period to ensure oxygen saturation levels did not become limiting.

During acclimation, the metabolic rate will initially change abruptly (acute response) to adjust to the new temperature (Jobling, 1994). This may be followed by the metabolic rate gradually stabilizing at a new level once the fish is acclimated (Jobling, 1994), a process called partial compensation which may take from a few days to a few weeks (Jobling, 1994). Therefore, the fish in the present study were allowed to adjust to the new temperatures for 7 days, during which they were monitored for any potential signs of stress.

The fish were fed twice daily at a ration of approximately 8 %BW.day^{-1} and rations were adjusted according to fortnightly biomass estimations based on measurement of individual lengths and total weight of fish per tank. The method used to weigh and measure the fish is described in Chapter 2.

Temperature profiles

Each tank was heated with a submersible thermostatically controlled aquarium heater set to the temperature chosen for the respective tank. All tanks were connected to a recirculating system to ensure equal water quality in all tanks. The system was housed in a temperature-controlled room with ambient temperature set to 13° C. The flow rate into each tank averaged 0.12 ± 0.03 L.min^{-1} which was adequate for the heater in the tank to maintain the desired temperature. Airstones were placed in the corner of each of the tanks to maintain high oxygen levels and in an attempt to inhibit any possible temperature stratification. An alcohol thermometer was glued to the inside front glass of each tank and opaque plastic sheeting was placed between tanks to prevent visual contact between fish in adjacent tanks.

Lall (1996) demonstrated that the preferred temperature of *Amphiprion percula* juveniles is 26 ± 0.7°C. This temperature was used in the present study as the median experimental temperature and heaters in the other tanks were set to provide for temperatures between 20 and 32 °C (Table 4.2). The temperature of each tank was recorded twice daily with alcohol thermometers that had been calibrated against a Bailey BAT-12 thermocouple (0.1° C accuracy). Differences between the thermometers and the thermocouple were plotted over a range of temperatures from 5° - 60° C to ascertain if differences between the thermometer and the thermocouple were linear. The temperature reading for each thermometer was corrected according to the measured difference.

At the end of the experiment, a final check of the alcohol thermometers was made with a highly accurate biochemical mercury thermometer. For tank 10 (Table 4.1) a 5.5°C difference was recorded. Further comparisons led to exclusion of tanks 2, 6 and 10 from the data set (Table 4.1) for which a difference of more than 10% was recorded between the alcohol and the mercury thermometers. In addition, data from tanks 3 and 16 (Table 4.1) were excluded from further analysis because their heaters were malfunctioning. All temperatures recorded from the other tanks were adjusted according to their differences to the biochemical mercury thermometer (Table 4.1).

Table 4.1 Average temperatures of each tank measured with alcohol and mercury thermometers.

Tank no.	**Alcohol thermometer[a] (°C)**	**Mercury thermometer[b] (°C)**
1	22.5	23.5
4	20.5	21.5
5	22.5	24.5
7	27	29.5
8	30.5	30.2
9	26	26.5
11	26	28
12	24.5	25.8
13	26.5	28.5
14	24	25.5
15	28.5	29.2

[a] average temperature measured daily over 75 days (calibrated with a thermocouple)
[b] adjusted temperature at the end of the experiment
[c] total mortality due to heater malfunction

Disease

On day 47 the heater in tank 3 overheated and burst. This killed the fish in that tank and tripped the electricity in the entire system. Due to the low ambient temperature (± 13° C) of the room in which the system was housed, there was a drop of temperature in all experimental tanks. Although the problem was rectified within 6 hours, the water temperature in most of the tanks had dropped by up to 8° C. Many fish showed signs of *Oodinium* infection three days after the incident. Copper citrate was added to the water at a concentration of 0.2 $mg.L^{-1}$ Cu for 10 days (Stoskopf, 1993). Thereafter, the water in the system was replaced and the fish appeared to have recovered, although some mortalities were occasionally recorded. Final survival was thus highly variable (Table 4.2).

Statistical analysis

Survival of *A. percula* juveniles grown at the different temperatures was not used as a comparative index for determining the optimal temperature due to disease related mortalities.

Growth rates ($mm.day^{-1}$) of the fish in each tank were determined using linear regression of individual lengths from each measurement period against time. The slope of the regression model is used to estimate the growth rate in $mm.day^{-1}$. The resulting estimate of growth was plotted against temperature and the relationship was modeled using non-linear regression. Similar analysis was performed using the total weight of all fish per tank.

The relationship between weight and length was determined by log-transforming both variables and fitting a least square regression on the transformed data. Furthermore, condition factor (CF) of the fish in each treatment was calculated with:

$$CF = \frac{\textbf{Weight(g)}}{\textbf{Length (mm)}^3} \; .10^5$$

Coefficient of variation (CV) per tank was calculated with:

$$CV = \frac{\textbf{Standard Deviation}}{\textbf{Mean}} \; \mathbf{x100}$$

Results

Good growth was found for fish maintained between 27 and 27.7° C. For example, growth in length and weight was 0.0032 $mm.day^{-1}$ (Figure 4.1) and 0.0067$g.day^{-1}$ (Figure 4.2), respectively. Lowest growth was found at the lowest temperature (21.5° C). A decrease in growth was also found at temperatures exceeding the range for maximum growth.

Analysis of length and weight data resulted in a significant correlation ($r^2 = 94.11$) (Figure 4.3) with the prediction: $\ln(y) = 3.089 \ln(x) - 11.29$.

No relationship could be found that reliably predicts the condition of *A. percula* as a function of temperature ($F_{1,83} = 2.74$, $P = 0.101$) (Figure 4.4). Furthermore, coefficient of variation of weight and length for *A. percula* were not correlated to temperature ($F_{1,9} = 0.46$, $P < 0.51$; $F_{1,9} = 0.17$, $P < 0.69$) , respectively.

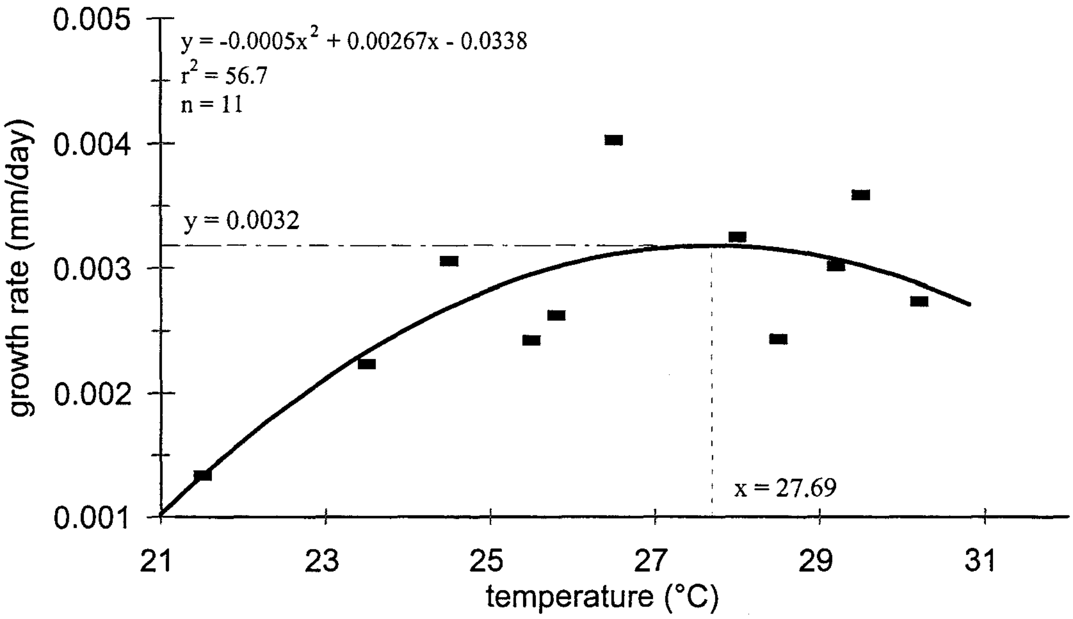


Figure 4.1 The relationship between growth in length and temperature for juvenile *A. percula*. x and y represent the temperature for which highest growth rate was estimated.

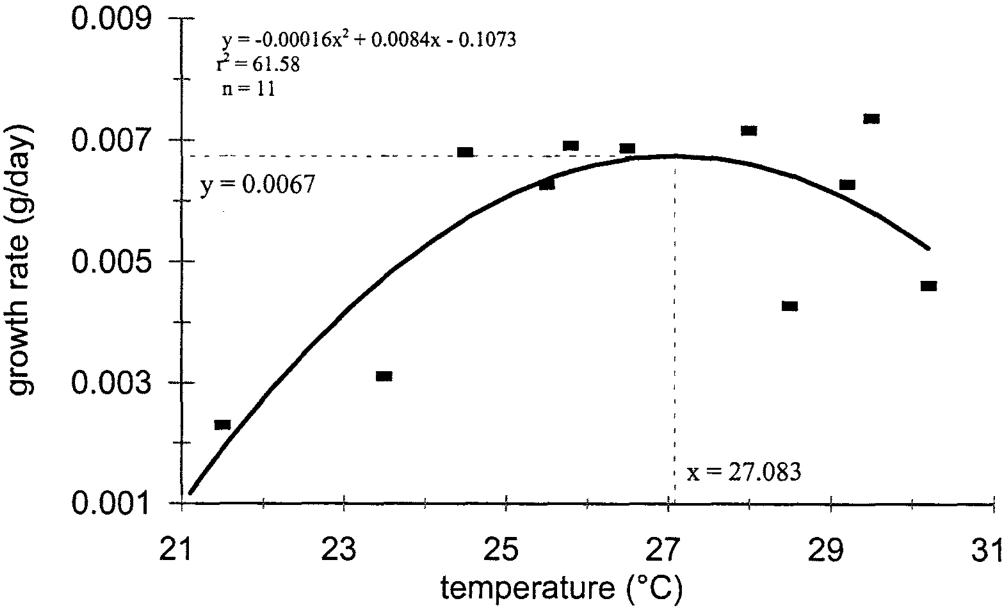


Figure 4.2 The relationship between growth (weight) and temperature for juvenile *A. percula*. x and y represent the temperature at which the highest growth rate occurred.

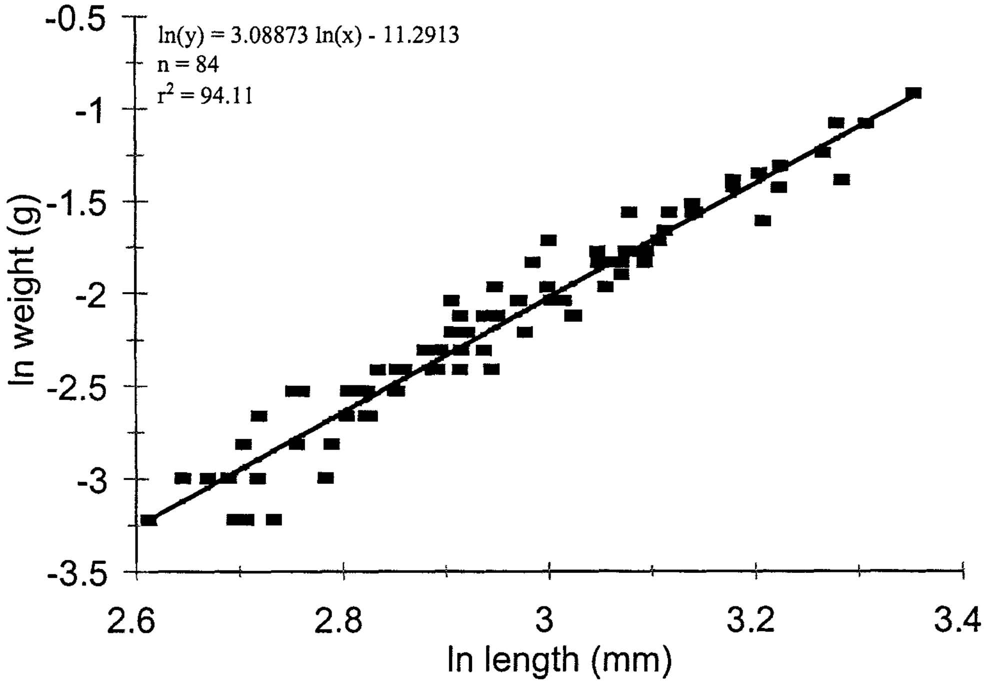


Figure 4.3 The relationship between length and weight of *A. percula* juveniles grown at temperatures between 21.5° C and 30.2° C for 75 days.

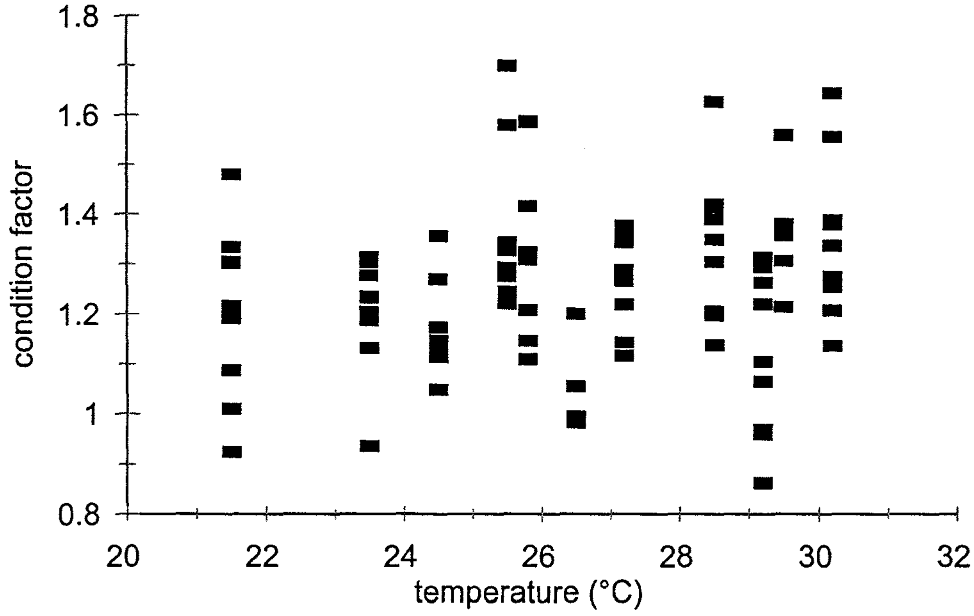


Figure 4.4 The effect of temperature on the condition factor ((weight/length^3) x 10^5) of *A. percula* juveniles at the end of the 75 day study.

Table 4.2: Coefficient of variation (standard deviation x 100/mean) of lengths and weights of *A. percula* juveniles at different temperatures at the end of the 75 day experiment.

		Coefficient of variation	
Temperature (°C)	**n**	**length (mm)**	**weight (g)**
21.5	10	10.66	39.20
23.5	9	19.02	57.57
24.5	8	21.37	74.54
25.5	10	15.88	46.17
25.8	9	9.16	30.46
26.5	4	15.21	38.45
28	9	11.69	37.60
28.5	9	18.34	57.00
29.2	9	16.83	63.50
29.5	5	5.38	16.26
30.2	10	15.93	40.35

Discussion

The drop in temperature on day 47 could have affected the results since the effect of the temperature change was not the same for all treatments (ie treatments with higher temperatures may have experienced a greater drop in temperature). It is not clear if this has influenced the variability shown in the growth models. In addition, it could not be tested if a drop in temperature from an established high level would have had a different effect on the fish than from a lower level. Had the effect of temperature change at high levels on growth been evident, we would have expected an increased variation of the results in the high temperature treatments which is not indicated in Figure 4.2. The estimated optimum temperature coincides closely with that given by Lall (1996) and the shape of the model is comparable to that shown by other authors (Britz & Hecht, 1987). Yet, the incident could have contributed to an increase of the variance in growth at different temperatures. The results provide an indication of an optimal temperature for growth that may need to be revised in future experiments. We suggest an hypothesis which is here made available for possible rejection by other authors in future experiments.

The parabolic relationship between growth and temperature established in the present study was similar to that found for *Clarias gariepinus* larvae and post-larvae (Britz & Hecht, 1987) and for juvenile *Cichlasoma urophthalmus* (Martinez-Palacios *et al.*, 1996). Temperature appears to affect certain physiological processes at the extremes of the tested temperature range, where decreased growth is observed. In addition, a parabolic growth/temperature relationship indicates that the temperature limits of the species have been tested. The positive linear relationship between temperature and growth described for *Oreochromis aureus* by Sonderberg (1990) suggest that growth rate will increase indefinitely with increasing temperature. This is likely to be an erroneous conclusion if the predictions from the model were extended beyond the range for which it was tested. Thus, the results may be different if he had used a wider temperature range in his experiment.

As temperature controls the rate of chemical reactions (Fry, 1971), fish have generally adapted to a specific thermal range in which they optimize physiological performance (Konecki *et al.*, 1995). This range is defined by the incipient lethal temperature range within which at least 50 % of the population could survive indefinitely (Jobling, 1981). The temperatures tested in this study were probably within the lethal temperature range for this species because no increase in mortality was recorded at the extremes of the range tested (21.5 - 30.2 °C).

Although growth is an important consideration in aquaculture, a further aspect this study was concerned with is the effect of environmental factors on fish condition and size variation. Measuring condition factor allows assessment of the relationship between weight and length of an individual or for a population. This is useful if an environmental variable tested in a study controls the length or weight development of the fish. For example, low temperature may reduce fish weight by lowering the metabolic rate and total food consumption, while the length may be determined by stronger genetic factors (Kane, 1988; Klontz *et al.*, 1992) . This would result in a fish that continues to grow in length but does not increase in weight proportionally. Thus, the ratio of weight to length may differ for a fish grown at a lower temperature compared to the same fish when grown at optimal temperatures. However, the results from this study show that temperature did not influence condition of *A. percula* juveniles. Judging by the wide variation of condition indices recorded, it is possible that some other environmental or genetic factor masked the potential effect of temperature on fish condition factor. Previous studies have found that the increased rate of metabolism at higher temperatures leads to a more efficient utilization of the ration (Borghetti & Canzi, 1993), increased lipid deposition (Yang *et al.*, 1997) and an increase of voluntary food intake (Burel *et al.*, 1996), consumption (Letcher & Bengston, 1993) and *ad libitum* feeding rates (Malloy & Targett, 1991). These results suggest that the ration may have become limiting at higher temperatures in the present study, leading to increased social interactions and competition and resulting in high condition factor variation . However, feeding was always in excess (per. obs.), thus, at any temperature there was enough food and any competition or increased social interactions did not affect average condition factor. It is also possible that temperature fluctuations caused by system failure during the experiment may have had some effect.

Many authors have found that the temperature for maximum growth closely approximates the temperature preference of a species (Brett, 1971; Jobling 1981; Britz & Hecht, 1987; Radenko & Alimov, 1992; Deacon, 1997). The temperature preference or final thermal preferendum is the temperature, when given a choice, where fish of a particular species will ultimately congregate (Reynolds & Casterlin, 1979). The zone of thermal preference for *A. percula* was determined at 26 ± 0.7 °C by Lall (1996) which is in line with the temperature established for maximum growth in the present experiment (27 - 27.7 °C). This is confirmed by using the five percent confidence limits for the final thermal preferendum (26 ± 0.7 °C) and the optimal temperature for growth (27.7 ± 1.4 °C) which are very closely related to each other. This supports Jobling's (1981) hypothesis which suggests that the determination of the final preferendum may be a rapid method for the assessment of the temperature to promote maximum growth of fish.

The results of this study show that growth of juvenile *A. percula* can be maximised if they are kept at approximately 27.7 °C. The suggestions that can be made from this study have potential economic applications for farmers of this species. For example, a 68% length increase could be gained if *A. percula* juveniles are grown at 27.7°C rather than at 21°C. Furthermore, a 21% growth (length) advantage would be realized if the water was heated to 27.7 from standard room temperature (24°C). Similar growth deficits would be found at temperatures exceeding the optimal temperature for growth due to the parabolic nature of the model. These are important considerations for potential producers of this species as motivation for a particular site must be related to specific cost benefits from heating or cooling the ambient water.

Chapter 5

The effect of stocking density on growth, size variation, condition and survival of juvenile *Amphiprion percula.*

Introduction

Knowing the maximum stocking density at which to grow a fish species without compromising growth rate is important for any fish producer. Most density-related research has been conducted on food-fish species to obtain the highest biomass of fish per given volume or area.

Previous studies on the effect of density on fish growth indicate that results are species-specific. Some studies have found negative correlations between growth and increasing stocking density under intensive culture of Arctic charr (Christiansen *et al.*, 1992), rabbitfish (El-Sayed *et al.*, 1995), African catfish (Hengsawat *et al.*, 1997), hybrid tilapia (Siddiqui, 1997) and gilthead sea-bream (Canario *et al.*, 1998). Other studies on Florida red tilapia (Watanabe *et al.*, 1990), rainbow trout (Bagley *et al.*, 1994) and largemouth bass (Tidwell *et al.*, 1998) have found no correlation between growth and stocking density. Other studies have found positive correlations between growth rate and stocking density for Arctic charr (Jorgenson *et al.*, 1993), European sea bass (Papoutsoglou *et al.*, 1998) and hybrid catfish (L. Oellermann, Rhodes University, pers. comm.). These results show that density dependent growth is species specific and that predictions for maximum growth performance with respect to density must be made for each species separately and generalizations must be avoided.

An increase in stocking density can affect the behavioral interactions between fish which may lead to increased size variability (Jobling & Reisnes, 1986). In contrast, size variation of Florida red tilapia (Watanabe *et al.*, 1990) and Arctic charr (Siikavuopio & Jobling, 1995) has been found to decrease at high densities, possibly because aggressive interactions are reduced as the development of a social hierarchy is being prevented (Heath 1991). It is unknown whether the degree and kind of aggressive interactions between individual *A. percula* juveniles is influenced by stocking density under aquarium conditions. The objective of this study was to quantify growth and the development of size variation at different stocking densities in *Amphiprion percula* juveniles. In addition, survival was used as a further indicator for the importance of density in the culture of this species.

Methods and materials

Experimental system

Eighteen 10L glass aquaria connected to a 1100L recirculating system described in Chapter 2 (System A, Figure 2.1) were used for this experiment. System design and management procedures are described in Chapter 2. Weekly measurements of total ammonia (NH_4^+/NH_3), nitrite (NO_2^-), nitrate (NO_3^-), salinity, percentage oxygen saturation and pH were done to check if the system operated within suitable limits for the study animals (table 5.1). Photo-period was maintained at 12L:12D with an electronic timer switch.

Table 5.1. Limits and/or ranges of water quality values sampled weekly from the sump of the experimental system

Parameter	**Limits or range**
total ammonia (NH_4^+/NH_3)	< 0.1 mg.L^{-1}
nitrite (NO_2^-)	< 0.1 mg.L^{-1}
nitrate (NO_3^-)	< 20 mg.L^{-1}
salinity	34.2 - 35.6 ppt
oxygen (% saturation)	90 - 96 %
pH	8.14 - 8.46
temperature	26.9 - 27.8 °C

Fish stocking densities

Forty-day-old juvenile *Amphiprion percula* from a single batch were moved into the experimental tanks at ten stocking densities. These fish were reared according to protocols described in Chapter 2 and had been weaned onto the experimental diet from 20 days after hatch (Table 2.1). Ten densities were tested ranging from 0.2 fish.L^{-1} to 4 fish.L^{-1} (Table 5.2). The lower densities (0.2; 0.5; 0.8 fish.L^{-1}) were replicated to have a larger sample size of pooled data. Due to a shortage of fish, the runs at densities > 1 fish.L^{-1} could not be replicated.

Growth measurement

All fish were photographed every two weeks according to techniques described in Chapter 2, except at week 6 when symptoms of *Oodinium* infection were observed. The fish were treated with copper citrate at 0.2 mg.L^{-1} Cu for ten days (Stoskopf, 1993). The water in the system was subsequently completely replaced and no mortalities were observed during and following the treatment. After 60 days in the experimental tanks, all fish were removed and the final lengths and body depths were recorded. In previous chapters (Chapter 3 & 4) total length was recorded for each fish and an average weight per fish was calculated by weighing all individuals in each tank and dividing the total biomass by the number of fish. Since measuring total weight of the fish from each tank was potentially inaccurate, especially at the low densities, fish depth was recorded for each fish. This measurement was taken from the base of the second dorsal spine across the body to the posterior base of the pelvic fin. Without knowing average fish mass it was not possible to calculate rations for specific tanks and thus the fish were fed in excess of what they were able to eat twice daily. Uneaten food and fecal matter was siphoned out of the tanks once weekly.

Table 5.2: Stocking densities used in the 60-day growth trial of *Amphiprion percula* juveniles, with the number of tanks used and the total number of fish used when pooling replicates.

Density (fish.L^{-1})	**No. of tanks per treatments**	**No. of fish treatment**
0.2	6	12
0.5	3	15
0.8	2	16
1.0	1	10
1.5	1	15
2.0	1	20
2.5	1	25
3.0	1	30
3.5	1	35
4.0	1	40

Statistical analysis

All length, depth and survival data from replicate densities were pooled for all analyses. Percentage survival was calculated by:

$$\text{Percentage survival} = \frac{n_{\text{final}}}{n_{\text{initial}}} \times 100$$

Where: n_{initial} = number of fish at the beginning of the experiment

n_{final} = number of fish at the end of the experiment

Growth rates (mm.day^{-1}) for each treatment were calculated using linear regression. The regression models were established based on log-transformed length and body depth values from each measurement period over the course of the experiment. The slope of the regression model was used to estimate growth rate (mm.day^{-1}).

Coefficient of variation (CV) of final length and body depth was calculated for each density using:

$$CV = \frac{\text{Standard Deviation} \times 100}{\text{Mean}}$$

Fish weight was not determined in this experiment which prohibited the use of the commonly used condition factor equation (Chapter 2). Thus, the ratio of fish depth (mm) to fish (mm) length was used.

Results

Survival percentages ranged from 87 to 100 % (Table 5.3). Lowest survival was found in fish reared at a density of 1.5 fish.L^{-1}.No mortality was recorded in five of the ten densities and no correlation existed between survival and stocking density ($P>0.05$, $r^2=7.28\%$).

Table 5.3: Percentage survival of *A. percula* juveniles at ten experimental densities at the end of the eight week experiment. $n_{initial}$ and n_{final} represent numbers of the fish at the beginning and at the end of the experiment, respectively.

Density (fish.L^{-1})	**$n_{initial}$**	**n_{final}**	**% survival**
0.2	12	12	100
0.5	15	15	100
0.8	16	15	94
1.0	10	10	100
1.5	15	13	87
2.0	20	20	100
2.5	25	24	96
3.0	30	27	90
3.5	35	32	91
4.0	40	40	100

Growth in total length and body depth is presented in Figures 5.1 and 5.2, respectively. Growth in length increased from 6.75 μm.day^{-1} at 0.2 fish.L^{-1} to a maximum of 9.24 μm.day at 1.0 fish.L^{-1}. Thereafter, growth rate decreased to 6.93 μm.day^{-1} at 2.0 fish.L^{-1} before increasing again to 7.84 μm.day^{-1} at 3.0 fish.L^{-1}. Lowest growth rates of 6.58 and 6.33 μm.day^{-1} were recorded at 3.5 and 4.0 fish.L^{-1} respectively. Body depth measurements revealed a similar pattern with the highest growth rates occurring at 1.0 (8.33 μm.day^{-1}) and 3.0 fish.L^{-1} (7.17 μm.day^{-1}). However, the lowest rate of body depth growth was found at 2.0 fish.L^{-1} (4.9 μm.day^{-1}).

Stocking density did not have a significant effect on the size variation of length and depth of juvenile *A. percula* (Figures 5.1 & 5.2). A relatively high variation was measured in fish grown at 1.0 fish.L^{-1} with values of 12.50% for total length and 14.47% for body depth. Furthermore, lowest variation of 7.25% and 7.91% for total length and body depth were recorded at 0.5 and 1.5 fish.L^{-1} respectively.

Density did not affect the depth:length ratio of the fish and no significant models could be determined to accurately represent the data (Figure 5.3).

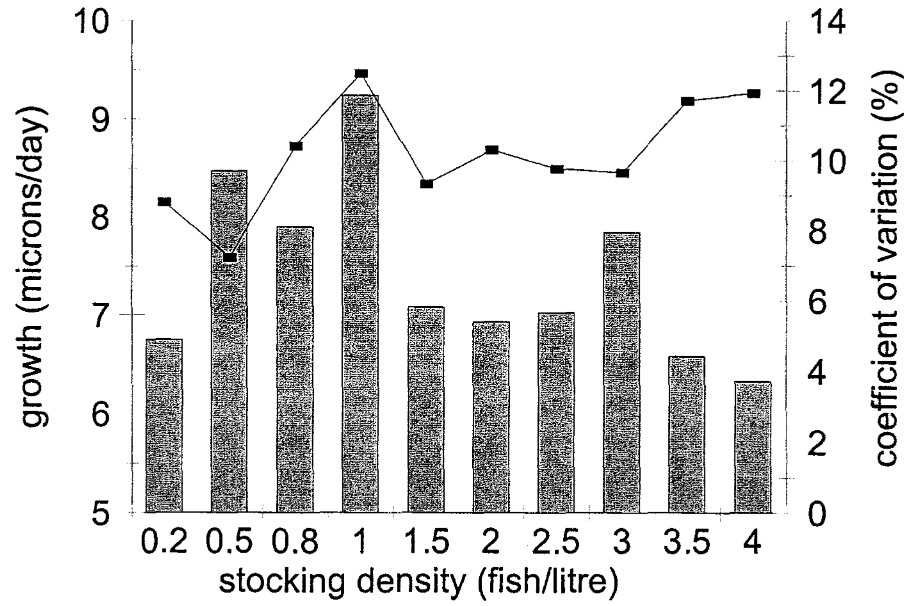


Figure 5.1: Growth rate and coefficient of variation of total length of *Amphiprion percula* juveniles after 8 weeks at ten stocking densities. Bars are y1-axis and line is y2-axis.

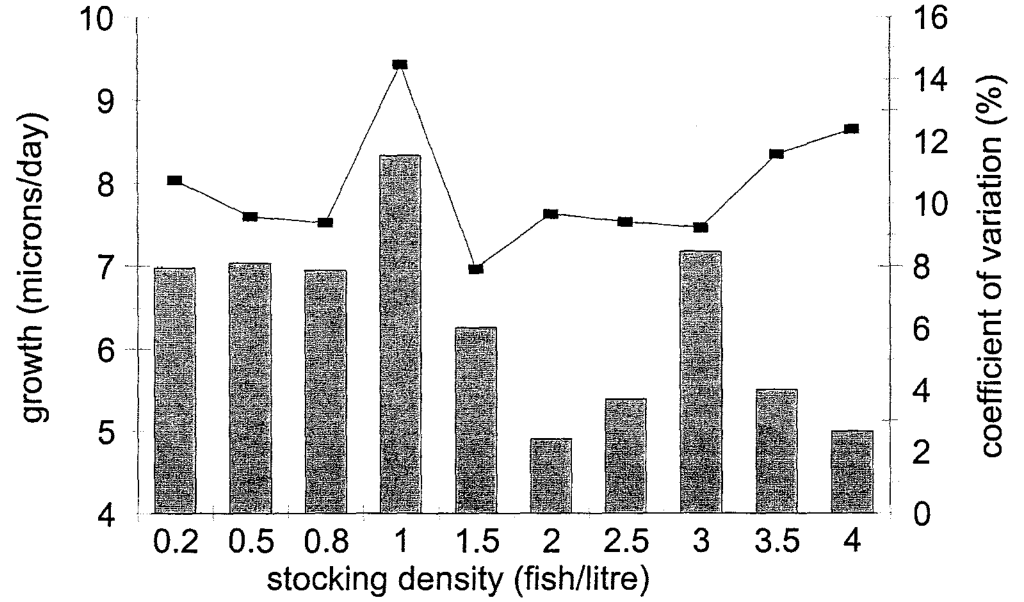


Figure 5.2: Growth rate and coefficient of variation of body depth of *Amphiprion percula* juveniles after 8 weeks at ten stocking densities. Bars are y1-axis and line is y2-axis.

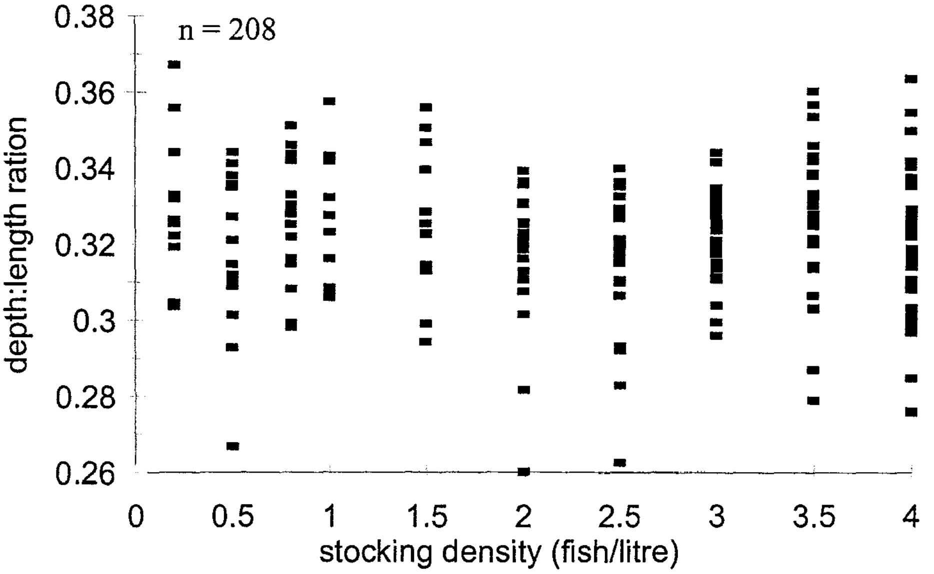


Figure 5.3: Depth/length ratio of individual *A. percula* juveniles at the end of the 8 week experiment.

Discussion

The results from this study indicate that stocking density did not affect the growth of juvenile *A. percula*. Although two peaks of growth were observed within the range of densities tested, one at 1.0 fish.L^{-1} and at 3 fish.L^{-1} (Figures 5.1 & 5.2) there did not seem to be a consistent relationship between density and growth.

Juveniles of this species exhibit pronounced schooling behavior in artificial culture environments (pers. obs.). Khan (1994) has hypothesized that an "optimum degree of grouping" exists in a population of fish and that growth would be reduced at densities above and below this optimum range. Growth reduction beyond the optimal density may be attributed to the formation of dominance hierarchies (Papoutsoglou *et al.*, 1998) or because there may be more competitive interactions between individuals at higher densities (Canario *et al.*, 1988). Examination of the growth rate results from the present study for densities ranging from 0.2 to 4.0 fish.L^{-1} does not allow us to predict an optimal density for growth, which may indicate that the range of densities tested may not have been wide enough.

An important consideration in the determination of an optimal density for the grow-out of *A. percula* juveniles is size variation. The coefficient of variation values for each density tested in this study are variable and do not appear to be density related. It has been suggested that an increase in the coefficient of variation is a symptom of competition (Jobling 1985) because intensified competition accentuates individual differences in members of a population (Craig and Fletcher, 1984). This suggests that there were no differences in competition between the densities tested in this study. However, although food was not limiting, there was always a proportion of dominant fish in each tank (pers. obs.). The extreme of this was observed in the tanks with the lowest fish density (0.2 fish.L^{-1}) where one of the two individuals was always more dominant and would often chase the subordinate into a tank corner. At the highest density (4.0 fish.L^{-1}), the fish tended to form an aggregation with few dominant individuals within the group. This may be further evidence that experimenting with higher densities may be possible, because some sort of social hierarchy existed at all densities tested and this appeared to change as density increased.

Survival did also not appear to be density related (Table 5.3). High mortalities could be expected when density related hierarchies are formed which leads to reduced feeding and possibly growth suppression of low ranking fish (Ejike and Schreck, 1980). Although dominant fish were observed in the tanks (pers. obs.), the interactions were not physically aggressive and it is doubtful that this could have caused elevated mortality. It is also unlikely that any subordinate fish died as a result of starvation.

The depth:length ratio was not correlated with density. This may have been because the relationship may not have been a good indicator of fish condition as the morphometric measurement of body depth may not be a reliable measurement site. Furthermore, any differences between treatments of the depth:length ratio could have been masked by high variations within each treatment.

In summary, based on the available data, an optimal density for growing *A. percula* juveniles has not been established. The results show that any of the densities tested may be used for growing juvenile *A. percula,* although it would make more economic sense if 4.0 fish.L^{-1} was used. This density would provide comparable growth and survival to the lower densities, while producing the most fish per unit volume. In addition, the fish at the higher densities tested (3.0 - 4.0 fish.L^{-1}) appeared to be more active than those at the lower densities. Fish at the low densities (0.2 and 0.5 fish.L^{-1}) generally exhibited shy behavior and tended to hide as a response to any movement outside the tank. Further research into growth and survival at higher densities needs to be conducted in order to establish where the optimal stocking density exists for this species.

Chapter 6

General Discussion and Overview

Feeding regimen

The studies of the feeding requirements of juvenile *Amphiprion percula* have revealed an interaction between feeding frequency and ration size. For example, a ration of 8.54% BW.day^{-1} would result in optimal growth independent of feeding frequency. Thus, this ration size could be fed once a day or divided into portions to be fed throughout the day at a frequency to be determined by another experiment. The factorial design of the experiment in Chapter 3 allowed us to test the combined effect of ration and feeding frequency on growth. Brett (1971) and Tyler and Dunn (1976) found that sockeye salmon (*Oncorhynchus nerka*) and winter flounder (*Pseudopleuronectes americanus*) respectively, consumed higher rations per meal as feeding frequency decreased. Similarly, when growth was optimal, clownfish consumed larger rations per meal as feeding frequency decreased (Table 3.16). Thus, studies to determine the effect of feeding regimens on food consumption of any fish species should address combinations of ration and feeding frequency as each is apparently influenced by the other.

Temperature

Ambient temperature controls the rate of biochemical (metabolic) reactions in fish (Fry, 1971; Brett, 1979). According to Deacon (1997) and Fry (1971), temperature is the most important environmental factor due to its effect on fish metabolism. In the present study, the effect of feeding regimens was tested at a previously established preferred temperature (Lall, 1996), which is known to relate to the temperature for maximum growth (Brett, 1971; Jobling, 1981; Britz & Hecht, 1987; Radenko & Alimov, 1992; Deacon, 1997). The results from the temperature experiment suggested that the optimal temperature for growth and the preferred temperature were very similar, thus confirming the predictions from the results of Chapter 3. Furthermore, the suggested growth/temperature model for *A. percula* can be used for experimental designs in future studies, and is worth testing under commercial farming conditions.

Stocking density

Feeding and temperature were considered as the two most important factors affecting the growth of juvenile *A. percula*. The determination of the optimal stocking density was the next factor to be tested due to the commercial implications for potential growers of this species. The success of intensive culture of any species is based on whether that species can be cultured at a financially viable density. The densities used in Chapter 5 were based on the densities used for the on-growing of this species at the Rhodes University marine aquarium fish hatchery (usually between 5 and 15 fish.L^{-1}). Even though a wide range of densities was used in the experiment, no correlation between density and growth could be established. It is therefore possible that the optimal density for the intensive rearing of this species may exceed 4 fish.L^{-1}. Alternatively, if the optimal density had been within the range tested, it may have been masked by the variations in growth, size and depth:length ratios. Such variability may not have been related to population density, but rather to other factors. For example, personal observations suggested that at higher densities, the fish tended to be more tolerant to stressors outside the tanks. These fish did not attempt to hide in the corners of the tanks at the sign of movement when being fed. This suggests that glass tanks may not have been the ideal vessels in which to conduct this trial because fish at lower densities may have grown better under different conditions.

Experimental design

Experimental determination of optimal environmental conditions to grow a particular species involves the use of a range of the factor being investigated. For example, the study reported on in Chapter 4 investigated the effect of temperature on juvenile clownfish growth and it was estimated that the temperature for optimal growth was approximately 27.7°C. The commonly applied design (ie: Soderberg, 1990; Deacon, 1997) for this kind of experiment is to have a series of pre-selected replicated temperatures to be more statistically robust. The approach in Chapter 4 was different and was similar to work performed by Iwata *et al.* (1994;1995) in that no replication within treatments was used. This experimental design was used for two reasons: first, each tank was heated by its own thermostatically controlled heater. It is difficult to accurately set the thermostat to constantly keep a preselected temperature and even more difficult to set two or more tanks to have the same exact temperature throughout the duration of the study. Second, use of non-replicated temperatures allows more temperatures to be studied within a range, especially if the number of experimental tanks or the availability of fish is limited. This means that an optimal temperature from within a range can be more accurately estimated using regression analysis. In this study, the range of temperatures included the preferred temperature established by Lall (1996). For other fish species where there is no knowledge of the preferred thermal range, the use of non-replicated temperatures would also allow for wider ranges of temperatures to be investigated. This is especially important for preliminary studies.

A similar unreplicated experimental design was used in Chapter 5, where all except the three lowest densities were replicated. The stocking density at which *A. percula* juveniles grow optimally is unknown and thus the widest possible range of stocking densities was used. To limit variation between treatments, only fish from the same batch of eggs were used. The average clutch size for this species is 200-400 eggs (Allen, 1972) and it was not common to have more than 300 juveniles survive from one batch (pers. obs.). Thus, the experimental design was influenced by the number of available fish. If all stocking densities used in Chapter 5 had been duplicated, 380 fish would have been required for the experiment.

Implications

The primary aim of this research was to determine the optimal feeding regimen, temperature and stocking density for growing juveniles of *Amphiprion percula.* It has been established that feeding a dry commercial diet twice a day at approximately 10%BW.day^{-1} would result in highest growth. Superior growth rates to this might be encountered if the fish were fed a diet of live food (eg. *Artemia* sp) as this would be more similar to their natural diet (see: Allen, 1972). However, it is not always viable to feed living food organisms on a large intensive scale. The feed used in Chapter 3 is broadly formulated for marine ornamental species according to the manufacturers and is not specifically formulated for clownfish.

Modeling the effect of temperature on growth predicted that approximately 27.7°C would result in optimal growth of *A. percula* juveniles. This temperature should give best growth results irrespective of which diet is fed because the optimal temperature is thought to correspond to the temperature where all physiological and biochemical reactions are optimal (Deacon, 1997). If production of this species is to take place in a location where the ambient water would need to be heated, the model could be used to predict fish growth rates to determine the economic trade-offs between heating costs and potentially slower growth.

Conclusions

The feeding regimen that would result in optimal growth of *A. percula* juveniles has been established at a ration of 10 %BW.day^{-1} divided into two meals per day. Survival was significantly lower in fish fed a ration of 2 %BW.day^{-1} than for any of the higher rations tested. In addition, a coefficient between length and weight has been established which enables the required daily ration to be calculated as a percentage of fish body length. The temperature for optimal growth of this species has been estimated at approximately 27.7 °C and a model describing the relationship between temperature and growth has been developed. No effect between growth and stocking density was found and further research into this aspect of clownfish culture is required.

Chapter 7

References

Allen, G.R., 1972. *The anemonefishes. Their classification and biology.* T.F.H. Publications, Neptune City. 286pp.

Allen, G.R., 1980. *Anemonefishes of the world.* Aquarium systems, Mentor, Ohio. 101pp.

Andrews, C., 1990. The ornamental fish trade and fish conservation, *Journal of Fish Biology* (Suppl. A), 37: 53-59.

Andrews, J.W. & Page, J.W., 1975. The effect of frequency of feeding on culture of catfish. *Transactions of the American Fisheries Society*, 104: 317-321.

Andrews, J.W. & Stickney, R.R., 1972. Interactions of feeding rates and environmental temperature on growth, food conversion, and body composition of channel catfish. *Transactions of the American Fisheries Society*, 101: 94-99.

Aranyakananda, P., Moree, N. & Chareonwattanakul, Y., 1995. Effects of feeding frequency and stocking density on growth of juvenile sea bass. *Thai Journal of Aquatic Science*, 1(2): 218.

Aristizabal Abud, E.O., 1990. Effect of feeding frequency in juvenile croaker, *Micropogonais furnieri* (Desmarest) (Pisces: Sciaenidae). *Journal of Fish Biology*, 37: 987-988.

Bagley, M.J., Bentley, B. & Gall, G.A.E., 1994. A genetic evaluation of the influence of stocking density on early growth of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 121: 313-326.

Bassleer, G., 1994. The international trade in aquarium/ornamental fish. *INFOFISH International*, 5: 15-17.

Beitinger, T.L. & Fitzpatrick, L.C., 1979. Physiological and ecological correlates of preferred temperature in fish. *Zoology*, 19: 319-329.

Berg, O.K., Finstad, B., Grande, G. & Wathne, E., 1990. Growth of Atlantic salmon (*Salmo salar* L.) In a variable diel temperature regime. *Aquaculture*, 90: 261-266.

Bjornsson, B., 1994. Effects of stocking density on growth rate of halibut (*Hippoglossus hippoglossus* L.) reared in large circular tanks for three years. *Aquaculture*, 123: 259-270.

Bolger, T. & Connolly, P.L., 1989. The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology*, 34: 171-182.

Borghetti, J.R. & Canzi, C., 1993. The effect of water temperature and feeding rate on the growth of pacu *(Piaractus mesopotamicus)* raised in cages. *Aquaculture*, 114: 93-101.

Brander, K.M., 1995. The effect of temperature on growth of Atlantic cod (*Gadus morhua* L.). *ICES Journal of Marine Science*, 52: 1-10.

Brett, J.R., 1971. Satiation time, appetite and maximum food intake of sockeye salmon (*Oncorhynchus nerka*). *Journal of the Fisheries Research Board of Canada*, 28: 409-415.

Brett, J.R., 1979. Environmental factors and growth. In: *Fish Physiology*, Vol. VIII (Hoar, W.S., Randall, D.J. & Brett, J.R., eds.), Academic Press, New York. pp. 599-675.

Brett, J.R. & Groves, D.D., 1979. Physiological energetics. In: *Fish Physiology*, Vol. VIII (Hoar, W.S., Randall, D.J. & Brett, J.R., eds.), Academic Press, New York. pp. 599-675.

Brett, J.R. & Shelbourn, J.E., 1975. Growth rate of young sockeye salmon, *Oncorhynchus nerka*, in relation to fish size and ration level. *Journal of the Fisheries Research Board of Canada*, 32(11): 2103-2110.

Brett, J.R., Shelbourne, J.E. & Shoop, C.T., 1969. Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. *Journal of the Fisheries Research Board of Canada*, 26: 2363-2394.

Britz, P.J. & Hecht, T., 1987. Temperature preferences and optimum temperature for growth of african sharptooth catfish (*Clarias gariepinus*) larvae and post larvae. *Aquaculture*, 63: 205-214.

Buckel, J.A., Steinberg, N.D. & Conover, D.O., 1995. Effects of temperature, salinity, and fish size on growth and consumption of juvenile bluefish. *Journal of Fish Biology*, 47: 696-706.

Burel, C., Ruyet, P-L.,Gaumet, F., Le Roux, A., Severe, A. & Boeuf, G., 1996. Effects of temperature on growth and metabolism in juvenile turbot. *Journal of Fish Biology*, 49: 678-692.

Buurma, B.J. & Diana, J.S., 1994. Effects of feeding frequency and handling on growth and mortality of cultured walking catfish *Clarias fuscus*. *Journal of the World Aquaculture Society*, 25(2): 175-182.

Cacho, O.J., Hatch, U. & Kinnucan, H., 1990. Bioeconomic analysis of fish growth: effects of dietary protein and ration size. *Aquaculture*, 88: 223-238.

Canario, A.V.M., Condeca, J., Power, D.M. & Ingleton, P.M., 1998. The effect of stocking density on growth in the gilthead seabream, *Sparus aurata* (L.). *Aquaculture Research*, 29: 177-181.

Cech, J.J., Mitchel, S.J. & Wragg, T.E., 1984. Comparative growth of juvenile white sturgeon: effects of temperature and hypoxia. *Estuaries*, 7(1): 12-18.

Chapman, F.A., Fitz-Coy, S.A., Thunberg, E.M. & Adams, C.M., 1997. United States of America trade in ornamental fish. *Journal of the World Aquaculture Society*, 28: 1-10.

Chua, T.E. & Teng, S.K., 1978. Effects of feeding frequency on the growth of young estuary grouper, *Epinephalus tauvina* (Forskal), cultured in floating net-cages. *Aquaculture*, 14: 31-47.

Chua, T.E. & Teng, S.K., 1982. Effects of food ration on growth , condition factor, food conversion efficiency, and net yield of estuary grouper, *Epinephelus salmoides* Maxwell, cultured in floating net-cages. *Aquaculture*, 27: 273-283.

Cole, B.A. & Boyd, C.E., 1986. Feeding rate, water quality, and channel catfish production in ponds. *Progressive Fish Culturist*, 48: 25-29.

Craig, J.F., & Fletcher, J.M., 1984. Growth and mortality of zebrafish, *Brachydanio rerio* (Hamilton Buchanan), maintained at two temperatures and on two diets. *Journal of Fish Biology*, 25: 43-55.

Cristiansen, J. S., Svendsen, Y. S. & Jobling, M., 1992. The combined effects of stocking density and sustained exercise on the behaviour, food intake, and growth of juvenile Arctic charr (*Salvelinus alpinus* L.). *Canadian Journal of Zoology*, 70: 115-122.

Cui, Y., Hung, S.S.O. & Zhu, X., 1996. Effect of ration and body size on the energy budget of juvenile white sturgeon. *Journal of Fish Biology*, 49: 863-876.

Cui,Y., Chen, S. & Wang, S., 1994. Effect of ration size on the growth and energy budget of the grass carp, *Ctenopharyngodon idella* Val. *Aquaculture*, 123: 95-107.

Deacon, N., 1997. Determination of the optimum environmental requirements of juvenile marine fish: the development of a protocol. PhD Thesis, Rhodes University, Grahamstown, South Africa.

Deacon, N., White, H. & Hecht, T., 1997. Isolation of the effective concentration of 2-phenoxyethanol for anaesthesia in the spotted grunter, *Pomadasys commersonnii*, and its effect on growth. *Aquarium Sciences and Conservation*, 1: 19-27.

Edwards, R.R., Finlayson, D.M. & Steele, J.H., 1972. An experimental study of the oxygen consumption, growth, and metabolism of the cod (*Gadus morhua*). *Journal of Experimental Marine Biology and Ecology*, 8: 299-309.

Ejike, C. & Schreck, C.B., 1980. Stress and social hierarchy rank in coho salmon. *Transactions of the American Fisheries Society*, 109: 423-426.

Elliot, J.M., 1975. The growth rate of brown trout (*Salmo trutta* L.) fed on reduced rations. *Journal of Animal Ecology*. 44: 823-842.

Ellis, S.C., Watanabe, W.O. & Ellis, E.P., 1997. Temperature effects on feed utilization and growth of postsettlement stage Nassau grouper. *Transactions of the American Fisheries Society*, 126: 309-315.

El-Sayed, A. M., Mostafa, K.A., Al-Mohammadi, J.S., El-Dehaimi, A.A. & Kayid, M., 1995. Effects of stocking density and feeding levels on growth rates and feed utilization of rabbitfish *Siganus canaliculatus*. *Journal of the World Aquaculture Society*, 26(2): 212-216.

Fautin, D.G. & Allen, G.R., 1992. *Field guide to anemonefishes and their host sea anemones.* Western Australian Museum, Perth. 160pp.

Fox, M.G. & Flowers, D.D., 1990. Effect of fish density on growth, survival, and food consumption by juvenile walleyes in rearing ponds. *Transactions of the American Fisheries Society*, 119: 112-121.

Fricke, H.W., 1979. Mating system, resource defence and sex change in the anemonefish *Amphiprion akallopisos. Zeitschrift fur Tierpsychologie.*, 50: 313-326.

Fry, F.E.J., 1947. Effects of the environment on animal activity. *University of Toronto Student Biological Services*, 55: 1-62.

Fry, F.E.J., 1971. Effect of environmental factors on the physiology of fish. In: *Fish Physiology,* Vol VI (Hoar, W.S. & Randall, D.J., eds.), Academic Press, New York. pp 1-98.

Gordon, A.K., 1999. The effect of diet and age-at-weaning on growth and survival of clownfish *Amphiprion percula* (Pisces: Pomacentridae). M.Sc. Thesis, Rhodes University, Grahamstown, South Africa, 85pp.

Grove, D.J., Loizides, L.G. & Nott, J., 1978. Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri. Journal of Fish Biology*, 12: 507-516.

Grayton, B.D. & Beamish, F.W.H., 1977. Effects of feeding frequency on food intake, growth and body composition of rainbow trout (*Salmo gairdneri*). *Aquaculture*, 11: 159-172.

Guinea, J. & Fernandez, F., 1997. Effect of feeding frequency, feeding level and temperature on energy metabolism in *Sparus aurata. Aquaculture*, 148: 125-142.

Hattori, A., 1994. Inter-group movement and mate acquisition tactics of the protandrous anemonefish, *Amphiprion clarkii*, on a coral reef, Okinawa. *Japanese Journal of Ichthyology,* 41(2):159-165.

Heath, R.G.M. (ed.), 1991. Aquaculture '90. Proceeding of a symposium. Aquaculture Association of South Africa, 114-123.

Hettler, W.F., 1976. Influence of temperature and salinity on routine metabolic rate and growth of young Atlantic salmon. *Journal of Fish Biology*, 8: 55-65.

Hengsawat, K., Ward, F.J. & Jaruratjamorn, P., 1997. The effect of stocking density on yield, growth and mortality of African catfish (*Clarias gariepinus* Burchell 1822) cultured in cages. *Aquaculture*, 152: 67-76.

Hidalgo, F., Alliot, E. & Thebault, H., 1987. Influence of water temperature on food intake, food efficiency and gross compostion of juvenile sea bass, *Dicetrarchus labrax*. *Aquaculture*, 64: 199-207.

Hoff, F.H. Jr., 1996. Conditioning, spawning and rearing of fish with emphasis on marine clownfish. Aquaculture Consultants Inc. Dade City, 212pp.

Huisman, E.A., 1976. Food conversion efficiencies at maintenance and production levels for carp, *Cyprinus carpio* L., and rainbow trout, *Salmo Gairdneri* Richardson. *Aquaculture*, 9: 259-273.

Imsland, A.K., Sunde, L.M., Folkvord, A. & Stefansson, S.O., 1996. The interaction of temperature and fish size on growth of juvenile turbot. *Journal of Fish Biology*, 49: 926-940.

Ishiwata, N., 1969a. Ecological studies on the feeding of fishes - VII. Frequency of feeding and satiation amount. *Bulletin of the Japanese Society of Scientific Fisheries*, 35(10): 979-984. (From Chua & Teng, 1978; paper not available to author)

Ishiwata, N., 1969b. Ecological studies on the feeding of fishes - VII. Frequency of feeding and growth. *Bulletin of the Japanese Society of Scientific Fisheries*, 35(10): 985-990. (From Chua & Teng, 1978; paper not available to author)

Iwata, N., Kikuchi, K. & Kurokura, H., 1995. Growth of Japanese flounder *Paralichthys olivaceus* at different temperatures. *The Israeli Journal of Aquaculture*. 47(3-4): 178-184.

Iwata, N., Kotaro, K., Honda, H., Kiyono, M. & Kurokura, H., 1994. Effects of temperature on the growth of Japanese flounder. *Fisheries Science*, 60 (5): 527-531.

Jobling, M., 1981. Temperature tolerance and the final thermal preferendum - rapid methods for assessment of optimal growth temperature. *Journal Fish Biology*, 19: 439-455.

Jobling, M., 1983. A short review and critique of methodology used in fish growth and nutrition studies. *Journal of Fish Biology*, 23: 685-703.

Jobling, M., 1985. Physiological and social constraints on growth of fish with special reference to Arctic charr, *Salvelinus alpinus* L. *Aquaculture*, 44: 83-90.

Jobling, M., 1994. *Fish bioenergetics*. Chapman & Hall, London, pp 309.

Jobling, M., 1996. *Environmental biology of fishes*. Chapman & Hall, London. pp 455.

Jobling, M. & Reinses, T.G., 1986. Physiological and social constraints on growth of Arctic charr, *Salvelinus alpinus* L.: an investigation of factors leading to stunting. *Journal of Fish Biology*, 28: 379-384.

Jorgensen, E.H., Christiansen, J.S. & Jobling, M., 1993. Effects of stocking density on food intake, growth performance and oxygen consumption in Arctic charr (*Salvelinus alpinus*). *Aquaculture*, 110: 191-204.

Kane, T.R., 1988. Length-weight relationship of hatchery reared Atlantic salmon. *Progressive Fish Culturist*, 50: 28-30.

Kayano, Y., Yao, S., Yamamoto, S. & Nakagawa, H., 1993, Effects of feeding frequency on the growth and body constituents of young red-spotted grouper, *Epinephalus akaara. Aquaculture*, 110: 271-278.

Khan, M.S., 1994. Effect of population density on the growth, feed and protein conversion efficiency and biochemical composition of a tropical freshwater catfish, *Mystus nemurus* (Cuvier & Valenciennes). *Aquaculture and Fisheries Management*, 25: 753-760.

Klontz, G.W., Maskill, M.G. & Kaiser, H., 1992. The effects of reduced continuos versus intermittent feeding of steel head trout. *Progressive Fish Culturist*, 53: 229-235.

Konstantinov, A.S., Zdanovich, V.V. and Tikhomirov, D.G., 1990. The effect of temperature fluctuations on metabolic rate and energetics of juvenile fish. *Journal of Ichthyology*, 30(1): 38-47.

Konecki, J.T., Woody, C.A. & Quinn, T.P., 1995. Temperature preference in two populations of juvenile coho salmon, *Oncorhynchus kisutch. Environmental Biology of Fishes*, 44: 417-421.

Lall, A., 1996. Thermoregulatory behavior of *Amphiprion percula*: A contribution towards the identification of optimal conditions for culture. M.SC thesis, Rhodes University, Grahamstown, South Africa, 39 pp.

Letcher, B.H. & Bengtson, D.A., 1993. Effects of food density and temperature on feeding and growth of young inland silversides (*Menidia beryllina*). *Journal of Fish Biology*, 43: 671-686.

Malloy, K.D. & Targett, T.E., 1991., Feeding, growth and survival of juvenile summer flounder *Paralichthys dentatus*: experimental analysis of the effects of temperature and salinity. *Marine Ecology Progress Series*, 72: 213-223.

Mariscal, R.N., 1970. The nature of the symbiosis between Indo-Pacific anemone fishes and sea anemones. *Marine Biology*, 6(1): 58-65.

Martinez-Palacios, C.A., Chavez-Sanchez, M.C. & Ross, L.G., 1996. The effects of water temperature on food intake, growth and body compostition of *Cichlasoma uropthalmus* (Gunther) juveniles. *Aquaculture Research*, 27: 455-461.

Medley, P.A., Gaudian, G. & Wells, S., 1993. Coral reef fisheries stock assessment. *Reviews in Fish Biology and Fisheries*. 3: 242-285.

Ochi, H., 1985. Temporal patterns of breeding and larval settlement in a temperate population of the tropical anemonefish, *Amphiprion clarkii*. *Japanese Journal of Ichthyology*, 32: 248-257.

Papoutsoglou, S.E., Tziha, G., Vrettos, X. & Athanasiou, A., 1998. Effects of stocking density on behavior and growth rate of European sea bass (*Dicentrarchus labrax*) juveniles reared in a closed circulated system. *Aquaculture Engineering,* 18: 135-144.

Paul, A.J., Paul, J.M. & Smith, R.L., 1994. Energy and ration requirements of juvenile pacific halibut *(Hippoglossus stenolepis)* based on energy consumption and growth rates. *Journal of Fish Biology,* 44: 1023-1031.

Peck, L.S., 1989. Feeding, growth and temperature in the ormer, *Haliotis tuberculata* (L.). *Progress in Underwater Science*, 14: 95-107.

Phillips, T.A., Summerfelt, R.C. & Clayton, R.D., 1998. Feeding frequency effects on water quality and growth of walleye fingerlings in intensive culture. *Progressive Fish Culturist*, 60(1): 1-8.

Radenko, V.N. & Alimov, I.A., 1992. Significance of temperature and light for growth and survival of larvae of silver carp, *Hypophthalmichthys molitrix. Journal of Ichthyology, 32(1)*: 16-27.

Reynolds, W.W. & Casterlin, M.E., 1979. Behavioral thermo-regulation and the "final preferendum paradigm". *American Zoology*, 19: 211-224.

Ross, R.M., 1978. Reproductive behaviour of the anemonefish *Amphiprion melanopus* on Guam. *Copeia,* 4: 103-107.

Rubec, P.J., 1988. The need for conservation and management of Philippine coral reefs. *Environmental Biology of Fishes*, 23: 141-154.

Siddiqui, A.Q., Al-Harbi, A.H. & Al Hafedh, Y.S., 1997. Effects of stocking density on patterns of reproduction and growth of hybrid tilapia in concrete tanks in Saudi Arabia. *Asian Fisheries Science,* 10: 41-49.

Siikavuopio, S. & Jobling, M., 1995. The effects of stocking density on survival and growth of wild-caught Arctic charr. *Nordic Journal of Freshwater Research*, 71: 419-423.

Stoskopf, M. 1993. *Fish medicine.* W.B. Saunders Company. 882pp.

Soderberg, R.W., 1990. Temperature effects on the growth of blue tilapia in intensive aquaculture. *Progressive Fish Culturist*, 52: 155-157.

Sorgeloos. P., Lavens, P., Leger, Ph., Tackaert, W. & Versichele, D., 1986. *Manual for the culture and use of brine shrimp* Artemia *in aquaculture.* Artemia Reference Centre, University of Ghent, Belgium, 319pp.

Talbot, C., 1994. How growth relates to ration size. *Fish Farmer*, 17(1): 45-46.

Thomassen, J.M. & Fjaera, S.O., 1996. Studies of feeding frequency for Atlantic salmon (*Salmo salar*). *Aquaculture Engineering*, 15(2): 149-157.

Thorpe, P.S. & Hecht, T., 1992. Feeding frequency, daily ration and feed utilisation of an optimal dry diet by early juvenile *Oreochromis mossambicus. Water S.A.*, 18(1): 53-56.

Tidwell, J.H., Webster, C.D., Coyle, S.D. & Schulmeister, G., 1998. Effect of stocking density on growth and water quality for largemouth bass *Micropterus salmoides* growout in ponds. *Journal of the World Aquaculture Society*, 29(1): 79-83.

Tongson, E.E. & McAllister, D.E., 1997. Net vs. cyanide capture of marine ornamental fishes. *AQUARAMA conference proceedings*, AQUARAMA 5 (4): 1-16.

Tsevis, N., Spiros, K. & Conides, A., 1992. Food conversion budget in sea bass, *Dicentrarchus labrax*, fingerlings under two different feeding frequency patterns. *Aquaculture*, 101: 293-304.

Tucker, L., Boyd, C.E. & McCoy, E.W., 1979. Effects of feeding rate on water quality, production of channel catfish, and economic returns. *Transactions of the American Fisheries Society*, 108: 389-396.

Tuene, S. & Nortvedt, R., 1995. Feed intake, growth and feed conversion efficiency of Atlantic halibut, *Hippoglossus hippoglossus*. *Aquaculture Nutrition*, 1:27-35.

Tyler, A.V. & Dunn, R.S., 1976. Ration, growth, and measures of somatic and organ condition in relation to meal frequency in winter flounder, *Psuedopleuronectes americanus*, with hypotheses regarding population homeostasis. *Journal of the Fisheries Research Board of Canada*, 33(1): 63-75.

Vega, R., Valdebenito, I., Alfaro, D. & Cardenas, S., 1994. Effect of feeding frequency on growth and survival of rainbow trout (*Oncorhynchus mykiss*) farmed at Lautaro fish farming experimental station, Chile. *Aquaculture*, 124: 291.

Vine, N.G. & Hecht, T., 1998. A preliminary survey of the extent of the marine ornamental fish trade in South Africa and the implications for management. Unpublished Report. Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, South Africa. 4pp.

Watanabe, W.O., Clark, J.H., Dunham, J.B., Wicklund, R.I. & Olla, B.L., 1990. Culture of Florida red tilapia in marine cages: the effect of stocking density and dietary protein on growth. *Aquaculture*, 90: 123-134.

Wurtsbaugh, W.A. & Cech, J.J., 1983. Growth and activity of juvenile mosquitofish: temperature and ration effects. *Transactions of the American Fisheries Society*, 112: 653-660.

Wurtsbaugh, W.A. & Davis, G.E., 1977a. Effects of fish size and ration level on the growth and food conversion efficiency of rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology*, 11: 99-104.

Wurtsbaugh, W.A. & Davis, G.E., 1977b. Effects of temperature and ration level on the growth and food conversion efficiency of *Salmo gairdneri*, Richardson. *Journal of Fish Biology,* 11: 87-98.

Wood, E., 1985. Exploitation of coral reef fishes for the aquarium trade. *Marine Conservation Society*, Herefordshire, United Kingdom. 121pp.

Wood, E., 1992. Trade in tropical marine fish and invertebrates for aquaria. Proposed guidelines and labeling scheme. *Marine Conservation Society*, Herefordshire, United Kingdom, 35pp.

Yager, T.K. & Summerfelt, R.C., 1993. Effects of fish size and feeding frequency on metabolism of juvenile walleye. *Aquaculture Engineering*, 12: 19-36.

Yager, T.K. & Summerfelt, R.C., 1994. Effects of feeding frequency on metabolism of juvenile walleye. *Aquaculture Engineering*, 13: 257-282.

Yang, S.D., Huang, D.U., Liu, F.G. & Liao, F.G., 1997. The effect of temperature on growth of silver perch *Bidyanus bidyanus*. *Journal of Taiwan Fisheries Research* (Keelung), 3(1): 19-30

Appendix 1

Early into the write up stage of this thesis, the author was wracked with some terrible pain in the lower abdominal region. Below is what was removed:

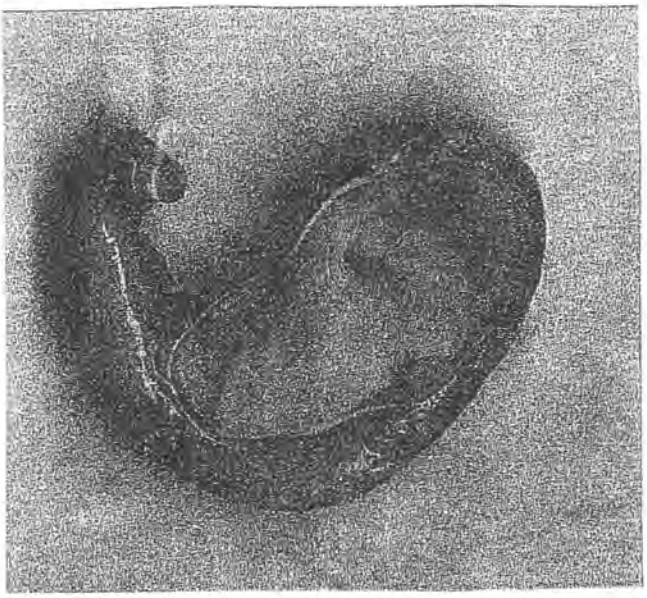


Figure 1. The author's one and only appendix was removed after 26 years and 2 months in the experiment. Whether appendix size was affected by ration or feeding frequency is unknown. Pain was significantly reduced ($P < 0.05$, n=1, $r^2 = 1$) after the operation.