EFFECTS OF PREY DENSITY AND TEMPERATURE ON SURVIVAL, GROWTH, AND BEHAVIOUR OF NEWLY HATCHED STRIPED WOLFFISH (Anarhichas lupus)

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EFFECTS OF PREY DENSITY AND TEMPERATURE ON SURVIVAL, GROWTH, AND BEHAVIOUR OF NEWLY HATCHED STRIPED WOLFFISH (*Anarhichas lupus*).

BY

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Dedicated to the life and spirit of Thane F.F. Wiseman

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ABSTRACT

Two of the main factors contributing to the growth and survival of larval fish are food and temperature. Adequate food must be provided when the larvae switch from endogenous to exogenous feeding. Temperature can affect growth rate, survival, and metabolism. Feeding and temperature studies were carried out on newly hatched striped wolffish (*Anarhichas lupus*). In a preliminary study larvae were fed three densities of *Artemia*, 100/l, 300/l, and 900/l. Survival was not significantly different among treatments (mean 19.5±5.78%) but growth rates were affected by prey density. A non-feeding study showed that larvae can survive on their yolk reserves for 2 to 4 weeks with the first mortality occurring at 15 days post-hatch. Larvae raised on a combination of *Artemia* and dry feed showed improved survival over the initial prey density study. The level of *Artemia* influenced growth, survival and weaning time. Final percent survival for larvae fed 900 *Artemia*/l plus dry diet was 94.3% as compared to 52.6% for larvae fed 100 *Artemia*/l plus a dry diet. Growth was also significantly faster for larvae fed at 900/l. Larvae offered a larger density of *Artemia* initially consumed more *Artemia* and weaned themselves onto dry feed two weeks earlier than larvae fed the smaller density of *Artemia*. Larvae were also raised at 3 different temperature regimes, high (8.0-13.5°C), low (4.0-7.8°C), and ambient (3.0-13.5°C). High temperature had the greatest effect on survival in the first 6 weeks.

Survival levelled off after this time. Final survival for high, low, and ambient was 50.5%, 16.0%, and 6.8% respectively. Overall larvae grown at higher temperatures were larger than those at the lower temperature range. Specific growth rate however dropped at higher temperatures later in the study.

The results of this experiment suggest that wolffish larvae should be fed a combination of *Artemia* and dry pellets immediately from hatch. *Artemia* should be continued until about 6 weeks or a length of 30 mm. At this time the larvae should be observed consuming primarily dry food. Temperature should be maintained at 4-8 °C for the first 6 weeks. Evidence suggests that the temperature should not exceed 8°C after 6 weeks however further investigation is needed.

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CHAPTER 1.0 GENERAL INTRODUCTION

HISTORY

Interest in the striped wolffish (*Anarhichas lupus*, Linnaeus 1758) as a new species for aquaculture has increased in recent years. Most of the research on this wolffish has been carried out in Norway and the former Soviet Union. With the decline in the Newfoundland fishery, aquaculture has been identified as a promising economic alternative, and wolffish is one of the species presently being investigated in this province. Research into wolffish culture in Newfoundland began in 1993 at the Wesleyville Marine Finfish Hatchery in Wesleyville, Bonavista Bay. The hatchery was established to work on culture techniques for lumpfish (*Cyclopterus lumpus*) but research soon expanded to include work on other marine species including wolffish and ocean pout (*Macrozoarces americanus*).

Striped wolffish are an attractive candidate for farming in Newfoundland for several reasons. They are native to our waters and therefore likely to have a lower optimum growing temperature than many other cultured species. They also tolerate a wide range in water temperature and oxygen content (Tilseth, 1990), are easy to start-feed (Ringø et al., 1987), are easily weaned to artificial food (Moksness et al., 1989; Tilseth, 1990), produce large, well-developed larvae, have shown good growth rates (Moksness, 1994), tolerate high stocking densities (Tullock et al.,1996) and produce a tasty white flesh.

Wolffish are members of the family Anarhichadidae. These fish inhabit moderately deep water in the North Atlantic and North Pacific Oceans. The three species that live off Canada's Atlantic coast are the striped, spotted (*Anarhichas minor*), and northern

(*Anarhichas denticulatus)* wolffish. There is also some evidence of interspecific forms between *A. lupus* and *A. minor* (Luhmann, 1954). Spotted wolffish inhabit deeper, cooler water than the striped wolffish. The spotted wolffish is of limited economic importance and is usually caught as a by-catch. The northern wolffish is found in Arctic seas on both sides of the North Atlantic. Its flesh is usually a jellied texture and therefore not eaten. Striped wolffish, also known as common wolffish, Atlantic wolffish, ocean wolffish, catfish (Scott and Scott, 1988) ocean catfish, and gray wolffish, is the most common of the three species off Newfoundland (Albikovskaya, 1982).

ECONOMIC IMPORTANCE

The solitary nature of striped wolffish makes it difficult to catch in large numbers and they are typically taken as a by-catch in other groundfish fisheries (Albikovskaya, 1982). Total reported landings for 1993 were only 20,464 metric tonnes with more than half captured in Icelandic waters (FAO, 1995).

Wolffish have a tasty white flesh which can also be smoked, pickled or dried. The liver, bile, and roe can also be utilized and the skin can be tanned into a fine leather (Butt, 1993). Antifreeze proteins present in the blood can be extracted and utilized in the medical and food industries (Fletcher, pers. comm.).

HABITAT

Striped wolffish range from the northwest coast of France, to the Bering and White seas, around Greenland and Iceland, and along the east coast of North America as far south as Cape Cod (Barsukov, 1959). To survive the sub-zero winter temperatures of the North Atlantic wolffish produce high levels of antifreeze proteins. These proteins allow survival at temperatures as low as -1.7°C (King et al., 1989). Off eastern Newfoundland they have been reported at depths of 101-350 m and temperatures of -0.4 to 4.0°C (Albikovskaya, 1982). Trawl studies in the north Atlantic found striped wolffish most abundant at depths less than 100 metres. They were caught in waters with temperatures from -1.3-10.2°C with the greatest catches between 1-4°C (Beese and Kandler, 1969). They are slow swimmers, moving in side to side undulations like an eel (Bigelow and Schroeder, 1953) and prefer rocky bottoms (Pavlov and Novikov, 1993). In Newfoundland waters they inhabit deeper offshore waters but move inshore to depths of 5-15 m in the spring prior to mating (Keats et al., 1985). In Icelandic waters wolffish migration is opposite to that found off Newfoundland with movement into deeper spawning grounds in autumn (Jónsson, 1982). This difference in migration may be due to location of suitable ground for spawning since wolffish tend to spawn in rocky crevaces or burrows. Although typically a solitary species, there is evidence that wolffish may congregate in suitable spawning areas. For example, Powles (1967) reported a high occurrance of *A. lupus* eggs caught in fish nets in an area south of Lahave Bank off southern Nova Scotia, suggesting that the area suppported a spawning aggregation of wolffish. Little is known about the habitat of juvenile wolffish. Both adults and larvae

have been observed in shallow waters, however, extensive searches for newly settled juveniles were unsuccessful. It is likely that juveniles inhabit deeper offshore water and only move inshore when sexually mature (Keats et al., 1986).

MORPHOLOGY

Adult wolffish have an elongate, laterally compressed body with a large rounded head. They are equipped with a variety of large, well-developed teeth designed for feeding on bottom invertebrates. Teeth are shed and replaced yearly during spawning (Templeman, 1986).

Wolffish have a long, single dorsal fin extending to the base of the caudal fin. The anal fin is half the length of the dorsal. The pectorals are large, pelvics are absent, and the caudal fin is small. Wolffish have a thick tough skin, with a thick layer of mucus and few scales (Barsukov, 1959). Its color may vary from slaty to dull olive green to purplish brown. The sides are transversed with 10 or more dark strips (Scott and Scott, 1988). Jónsson (1982) reported an average size of 13.6 cm at one year up to 98.5 cm at 20 years of age with males growing faster than females. The maximum length was considered to be 120 cm.

FEEDING

The diet of adult striped wolffish consists mainly of bottom invertebrates. Analysis of stomach contents of wolffish taken from the Northwest Atlantic showed that 85% of the diet by volume consisted of bottom invertebrates including whelks, brittle stars, scallops,

crabs, and sea urchins. Fish comprised the remaining 15% of the diet with redfish being the main component (12%; Templeman, 1985). Feeding is reduced during spawning and males may not feed at all while guarding eggs (Keats et al., 1985). Analysis of stomach fullness indicated that striped wolffish feed more intensely from summer to autumn than autumn to winter (Albikovskaya, 1983). Food items are typically crushed and eaten. Food is taken from the bottom using canines, broken and crushed with the conical teeth and molars and the fine food parts are scraped from the fragments using the pharyngeal teeth (Barsukov, 1959). The calcareous exteriors of the food are almost completely dissolved by the high concentration of HCl in the stomach. The digestive system is protected from the sharp shell fragments by a well developed epithelial integument and high concentration of mucus secreting cells (Verigina, 1974).

REPRODUCTION

Adult striped wolffish move into Newfoundland waters in early spring. The fish pair off over the summer and spawning takes place usually in September - October (Keats et al., 1985, 1986). In the White Sea spawning occurs from July-September. Adults in this area are sexually mature at 5-7 years at a length of 35 cm (Pavlov and Novikov, 1993).

Evidence suggests that, unlike most fish species, the eggs of wolffish are fertilized internally (Johannessen, et al., 1993; Pavlov, 1994). Reproductive studies by Johannessen et al. (1993) have shown that the testes are small (0.11% of body weight) producing a maximum of 1.5 ml of milt at stripping. Sperm swim actively in undiluted seminal fluid and can

therefore be inseminated into the oviduct without being activated by water. Males also develop a papilla on the urogenital pore which likely functions as a copulatory organ.

Studies of spawning behaviour in the laboratory (Johannessen, et al., 1993) showed that courtship behaviour begins about 4-5 months prior to spawning. Females were observed to move "restlessly" around the chosen male, leaning and rubbing against him. Males were passive except for a repeated "side-bending" behaviour which lasted from 10 minutes to over an hour. Males and females were observed performing a copulatory-like behaviour in which there was close contact between their sexual openings. This occurred 8-15 hours prior to spawning, with the pairs sometimes holding this position for 1-2 minutes.

Thirty to fifty hours prior to spawning the females were observed to perform a series of behaviours. The female went through about 12-24 hours of "side-lying" with little movement, then 3-6 hours of "labour" with intense bending, twisting and shivering with short periods of rest. Copulation occured after this period of labour followed by 8-15 hours of "resting". The actual spawning lasted about 3-7 minutes. Eggs were deposited in a string of mucus. The female then wrapped its body around the eggs and began turning them. Within 6-10 hours the eggs were firmly attached to each other. Females were never observed to protect the eggs (Johannessen, et al., 1993). Males have been observed protecting masses of eggs in burrows and rock crevices in waters off the coast of Newfoundland (Keats et al., 1985; Watkins, pers. comm.). The male of a captive breeding pair in Norway also provided care by aerating the eggs and turning them more or less continuously with its tail. The male enclosed the egg mass in a layer of skin mucus that likely helped to protect against parasites

and pathogens (Ringø and Lorentsen, 1987). Paternal care is the most common form of parental care among fish (Smith and Wooten, 1995). For example male lumpfish (*Cyclopterus lumpus*) provide care by molding the newly fertilized eggs into the crevice of his nest, fanning and puffing on the eggs for aeration, and guarding the eggs by removing invertebrate predators and chasing fish from the nest area (Goulet et al., 1986). However, among fish with internal fertilzation, maternal care is more common (Smith and Wooten, 1995). For example female ocean pout (*Macrozoarces americanus*), a species with similar reproductive and morphological features to wolffish, provide care to their egg mass for up to 3 months. The female pout, like the male wolffish, wraps itself around the eggs and fans them, and likely provides an antiparasitic agent in the skin mucus to keep the eggs free of leeches (Yao and Crim, 1995).

EGGS AND LARVAE

The young of many marine fish species are small and undeveloped with females producing large numbers of pelagic eggs measuring about 1 mm in diameter. At hatch the young are usually 3-5 mm long and live off the yolk sac for a period until the eyes and jaw develop and exogenous feeding begins (Blaxter, 1981).

Wolffish differ greatly from most marine fish. Approximately 2100 eggs/kg (relative fecundity; Pavlov, 1994) are produced, each measuring about 6.0 mm in diameter. The eggs undergo a long incubation period of 7-9 months, and hatch in the spring. Eggs held at

ambient temperatures (as low as -1.5°C) at the Ocean Sciences Centre in Newfoundland hatched from the end of February into April.

Larvae hatch at 20 mm or more with very little yolk. They have large, well-pigmented eyes, darkly pigmented skin with a silvery gut region, and well-developed fins. At hatch they possess about 50 teeth (Barsukov, 1959). Pavlov and Moksness (1994) compared wolffish ontogeny with that of salmonids. They found that the period from egg activation to hatching is twice as long in wolffish as in Atlantic salmon (*Salmo salar*). At hatch, wolffish are more developed with only a remnant of yolk sac remaining. Following Balon (1985) they proposed that wolffish have direct ontogeny, developing directly into a juvenile without a larval period. Fish ontogeny and naming of life stages can be quite complicated and beyond the scope of this study. The fish used in these studies were newly hatched and for simplicity will be referred to as larvae.

Feeding begins within the first few days post-hatch (personal observation). Information on larval feeding in the wild is sparse. However gut analysis of samples from ichthyoplankton surveys off northern Norway revealed that stomach contents consisted mainly of crustaceans (1-3 mm) and fish larvae (6-10 mm) (Falk-Petersen et al., 1990). Gut analysis of 7 larvae and fry by Pavlov and Novikov (1993) showed that larvae feed on crustaceans, fish eggs, and fish larvae. Larvae are predominantly pelagic but also spend a considerable amount of time resting on the bottom.

The studies reported in this thesis had three objectives:

1) To determine the prey density which produced the best growth and survival in larval wolffish.

2) To determine, using behavioural observation, a strategy to wean larvae from live food to a dry diet.

3) To determine the temperature which produced the best growth and survival of larval wolffish.

CHAPTER 2.0 FIRST-FEEDING

2.1 INTRODUCTION

Most marine larval fish hatch with a yolk sac which provides the larva with nutrients during the period from hatch to exogenous feeding. After this, many larvae go through a period of mixed feeding when yolk reserves are reduced and they switch from endogenous to exogenous feeding. This transition is generally considered a "critical period," with an increase in mortality depending on the availability of food (Blaxter, 1981; Kamler, 1992; and May, 1974). During this period there is a point-of-no-return (PNR) which is described as when 50% of the starved larvae are alive but not strong enough to feed (Blaxter and Hempel, 1963).

When culturing fish, optimum conditions must be provided to maximize survival through this early critical period. Information on feeding protocols for newly hatched striped wolffish is limited. Most available information is based on wild-caught or juvenile wolffish and does not consider feeding at hatch or feeding behaviour. In studies where live food was provided, the amount of food was not examined (Moksness 1990; Moksness et al., 1989; and Ringø et al. 1987).

When feeding live food to larval fish it is important to determine the optimum prey density. Increasing prey levels can result in increased rates of survival, growth, and food consumption up to a certain level (Werner and Blaxter, 1981). If levels are too low, larvae may not obtain adequate nutrition. If levels are too high, feeding behaviour may be negatively

affected. For example shorthorn sculpin (*Myoxocephalus scorpius*) larvae were found to have better survival when fed low levels of *Artemia*. It is thought that the larvae become distracted when prey levels are too high and feeding rates decrease (Brown, pers. comm.). High prey levels may also result in incomplete digestion. For example, herring (*Clupea harengus*) larvae feeding at a prey density of 3000/l evacuated their gut more quickly than those fed at lower prey densities. The *Artemia* were not well digested and passed through the gut virtually intact (Werner and Blaxter, 1981). Also since live food production is expensive and labour intensive, optimum levels should be determined to prevent overfeeding and keep operating costs down.

Ringø et al. (1987) found that newly hatched wolffish fed natural zooplankton (*Acartia longiremis* or *Metridia longa*) survived past 120 days while those fed a cod roe diet survived to 50 days post-hatch. In a study by Moksness et al. (1989) survival was better among larvae fed *Artemia* and dry pellets as compared to those fed only pellets. However the groups studied were held under different light and temperature conditions and fed at different frequencies. The group given *Artemia* received this from 24 to 71 days post-hatch and prey density was not reported. Since wolffish begin exogenous feeding within the first few days post-hatch it makes little sense to give the larvae dry pellets first then live prey if the aim is to wean them onto dry feed. In another study by Moksness (1990), wild caught wolffish were fed moist and dry pellets as well as *Artemia* nauplii and natural zooplankton. Total mortality ranged from 49.6 to 69.3%. The larvae were estimated to be about 33 days old at the time of the experiment and therefore possibly past the period of mixed feeding.

In a previous study by the author (Wiseman, 1993), prey levels for newly hatched wolffish were maintained at 80/l and 240/l. Survival at both densities was low. Studies at the Wesleyville Hatchery provided *Artemia* at 300/l and total mortality occurred by Week 5 (Blanchard, 1994). My first experiment was designed to determine the best prey density for larval wolffish.

Determining the age when the yolk is completely absorbed would help identify the time frame when larvae must switch from endogenous to exogenous feeding. A major mortality at this time would indicate that the switch to exogenous feeding was not successful and the fish died of starvation. Pavlov (1986) reported that for White Sea wolffish, held at 7.8°C, the switch to exogenous feeding continues for 10-15 days, ending with complete absorption of the yolk. Ringø et al. (1987) reported that wolffish held at 1-3°C absorbed their yolk in 10-14 days. I conducted a second study to determine how long larval wolffish can survive on their yolk reserves and if yolk absorption rate in wolffish from Newfoundland waters is similar to that found in wolffish from other areas.

2.2 MATERIALS & METHODS

Approximately 30 striped wolffish egg masses were collected from Bauline, Conception Bay in October 1993 by Wesleyville Finfish Hatchery SCUBA divers. They were distributed to Ocean Sciences Centre in Logy Bay, the Marine Institute in St. John's, and the hatchery in Wesleyville. One of the Logy Bay masses was discarded due to fungal contamination. A second mass was incubated in ambient seawater throughout the winter in

a plastic basket with screened sides placed on a wet bench. The basket was siphoned when necessary to remove dead eggs and sediment. The first major hatch was on February 27, 1994 at a temperature of -1.0°C.

For the prey density study, one hundred newly hatched wolffish were placed in each of six 30-litre glass aquaria. A perforated piece of PVC pipe with a capped end was attached to the bottom of the tank. Water entered this pipe through flexible tubing attached to the other end of the pipe. The sides of each tank were covered in black plastic to prevent disturbances. The tanks were placed in wet benches and provided with ambient seawater. Temperature was maintained at approximately 4-6°C by adjusting water flows. Each tank had an airstone. Light was provided by overhead fluorescent tubes as well as daylight from nearby windows (natural photoperiod, 60-100 Lux at midday).

The wolffish were fed three different concentrations of enriched (DHA Super Selco, Inve) *Artemia franciscana* (Instar II), 100/litre, 300/litre, and 900/litre twice a day (10:00 a.m. and 4:00 p.m.). There were two aquaria per treatment. *Artemia* cysts (Sweetwater Express) were decapsulated and hatched daily. On the second day following decapsulation *Artemia* were enriched with DHA Super Selco (Artemia Systems, Sorgeloos et al., 1986) to increase HUFA levels. *Artemia* were washed with filtered seawater to remove enrichment residues and collected in a beaker. Counts of the *Artemia* culture were taken on three 0.1 ml samples and averaged to determine how much volume to add to the tanks. The *Artemia* were cooled in the wet bench for about 10 minutes prior to adding to the tanks. Five hundred ml

of cooled algae (*Isochrysis galbana*) was also added to each tank prior to the morning feeding.

An initial sample of twenty wolffish, less than one day post-hatch, was killed with MS-222 and immediately rinsed in distilled freshwater. Standard length (tip of the mouth to the end of the notochord) was measured to the nearest 0.5 mm using a dissecting microscope. Each sample was then placed on preweighed aluminum foil and dried at 90°C for 48 hours before weighing. Five fish per tank were sampled for standard length at weeks 2 and 5. Day one of the experiment was March 1 and it continued until April 7 (Day 38). Mortalities were removed daily and counted. Results were combined in one week intervals.

In the non-feeding study, seventy newly hatched wolffish were stocked in each of two white plastic tanks. To help contend with nitrogen supersaturation in the water, degassers were placed in each tank. They consisted simply of an Erlenmeyer flask placed on a brick in the tank and equipped with an airstone with high airflow. The inflow line was placed in the degasser allowing the water to become well aerated before it entered the tank. Water exited through a small screen at one end of the tank. No food or algae was added to the tanks for the entire study period. Temperature was maintained at 4-6°C. Mortalities were removed and counted daily. Results were combined in one week intervals.

Results were analyzed using SAS/STAT (SAS Institute, 1988). A general linear model was used to determine if prey density or age influenced the survival or growth of the larvae and to test for tank effects. If a significant age*treatment interaction was found, the results were then analyzed using a least squares means test with a Bonferroni corrected P-

value to check for significance at each age. A Bonferroni corrected P-value is found by dividing the original P-value (0.05) by the product of the number of ages times the number of treatments (Sokal and Rohlf, 1995). When testing multiple comparisons a reduced P-value allows a more robust test of significance. Prior to statistical analysis, survival data was ranked transformed since other transformations (i.e. log transformation, square root transformation, etc) did not achieve normality. This rank transformation approach replaces the data with their ranks allowing the usual parametric test to be applied to the ranks (Conover and Iman, 1981). Growth data was log transformed to meet the assumptions of the test.

2.3 RESULTS

2.3.1 SURVIVAL

For the prey density study, survival was determined for each tank by subtracting the number of mortalities from the total (initial total minus sampled fish). An average was then calculated for each treatment.

At the end of the study (Week 6) percent survival was 20.6 (±0.55) for 100 *Artemia*/l, 16.9 (±5.25) for 300 *Artemia*/l, and 21.1 (±11.55) for 900 *Artemia*/l (Fig.1). There was no significant difference in survival between treatments (ANOVA, F=1.71, df=2,18, P=0.2088). The mean survival for the experiment was 19.5 (±5.78)%.

In the non-feeding study mortalities began at 15 days post-hatch (Week 3) and all fish were dead by 32 days post-hatch (Week 5) (Fig.2).

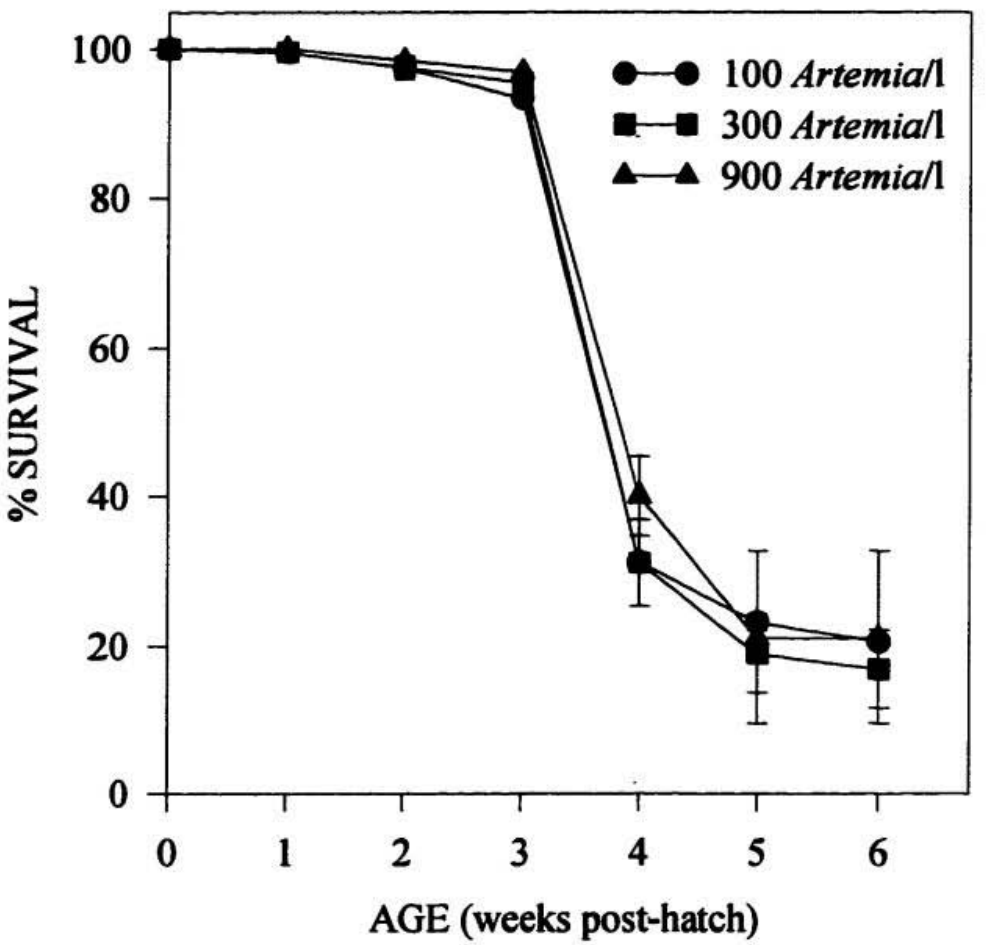

Figure 1:Weekly percent survival (±se) of striped wolffish fed different densities of *Artemia*.

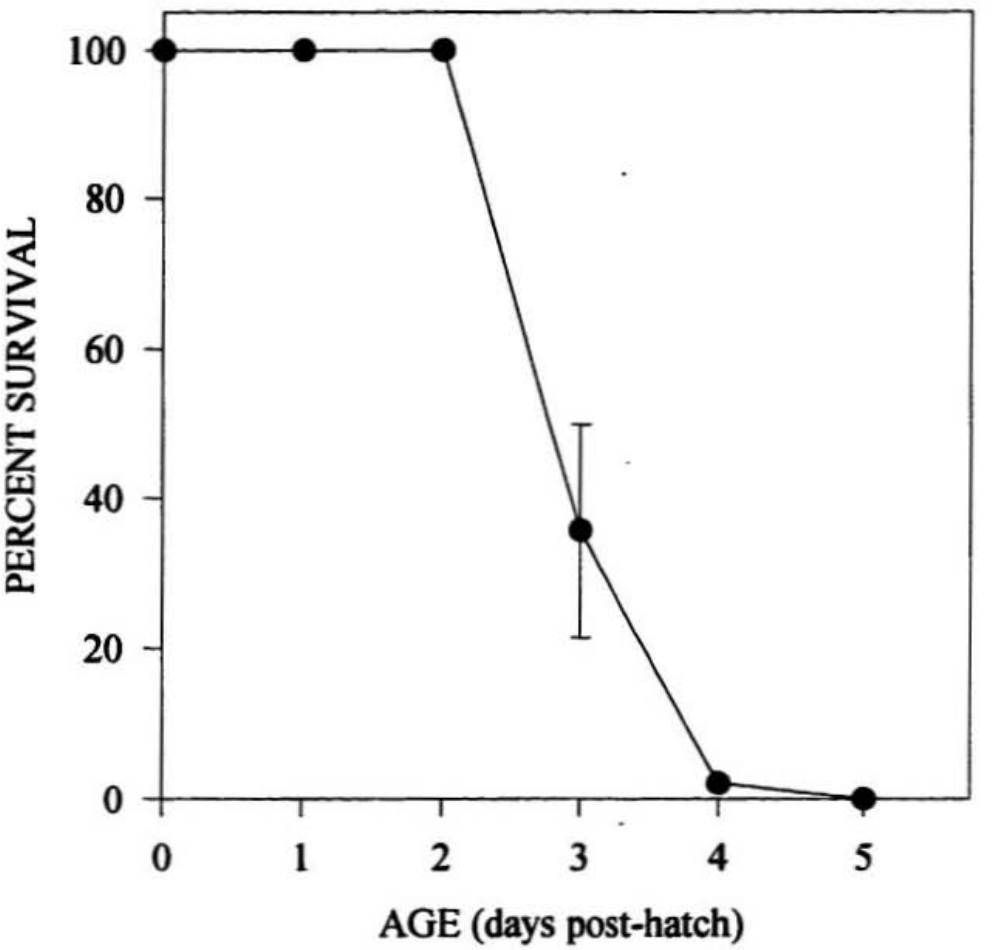

Figure 2: Weekly percent survival (±se) of non-fed striped wolffish.

2.3.2 GROWTH

No significant differences were found between replicate tanks ($P>0.05$). At hatch the larvae were 20.7±0.09 mm (n=20) long. There was a significant difference in standard length between treatment 1 (100/l) and 3 (900/l) at Week 2 and Week 5, and between treatment 2 (300/l) and 3 (900/l) at Week 5 ($P<0.0083$, Bonferroni correction). By Week 5 the larvae measured 22.0±0.27 mm (n=10) for treatment 1, 22.8±0.13 mm (n=10) for treatment 2 and 24.4±0.36 mm (n=10) for treatment 3 (Fig.3)

2.4 DISCUSSION

Survival in the study was low compared to other studies. However, the period of high mortality between weeks 3 and 5 (21-35 days) was similar to that of many wolffish studies. Moksness et al. (1990) found that larvae fed dry pellets alone and dry pellets with *Artemia* showed high mortality between 20 and 40 days. Blanchard (1994) compared a variety of diets consisting of *Artemia*, dry diets, and combinations of live and inert foods. All groups died at the same rate with a peak in mortalities between day 27 and 36.

Prey density did affect growth rate as early as two weeks post-hatch. Prey density has been found to affect growth and survival of many other species. Houde (1978) investigated optimum prey levels in larval bay anchovy (*Anchoa mitchilli)*, lined sole (*Achirus lineatus*), and sea bream (*Archosargus rhomboidalis)*. Over a 16 day period they were fed wild plankton at levels of 50, 100, 1000, and 5000/l for bay anchovy, 50, 100, and 1000/l for lined

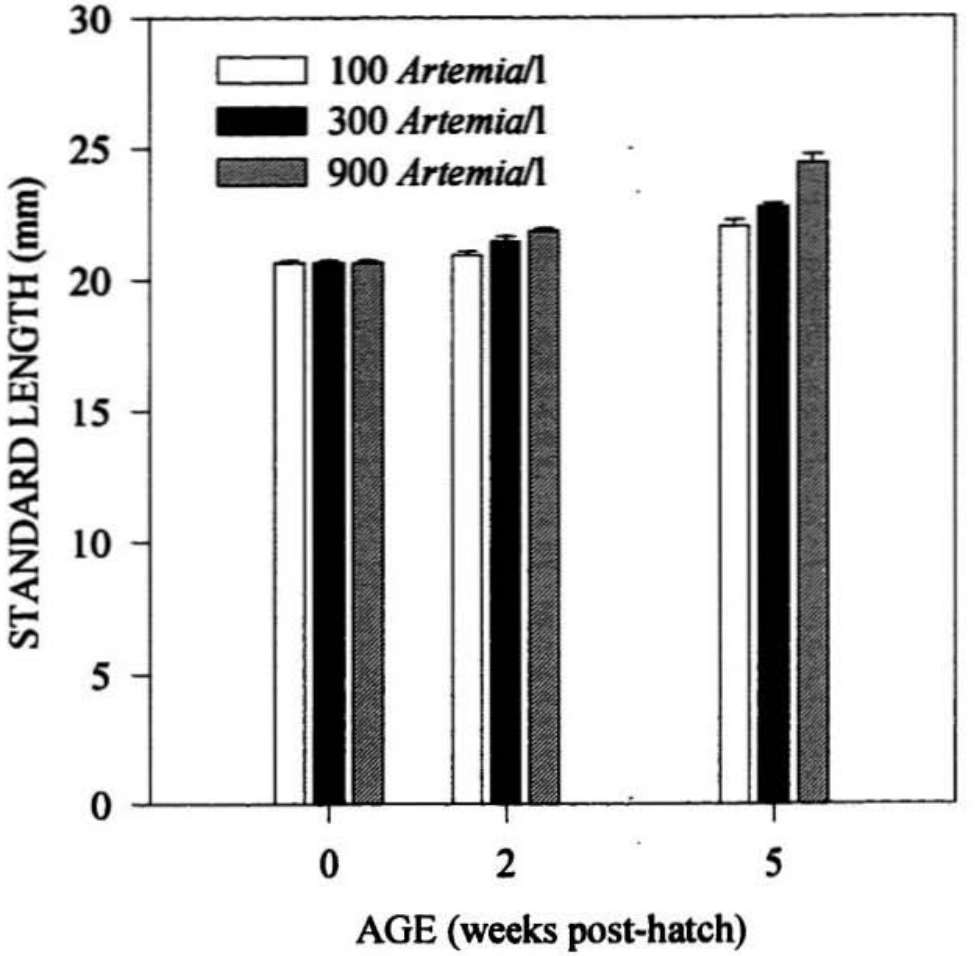

Figure 3: Mean standard length (mm +se) of striped wolffish fed three different densities of *Artemia*.

sole and 10, 25, 50, 100, and 500/l for sea bream. Survival and growth increased with increasing prey concentrations for all three species. Survival rate of sea bream increased from 3.9% at a prey level of 10/l to 12.7% at 50/l and up to 72.4% at a prey level of 500/l. Mean standard length increased from 4.35 mm at 10/l to 7.76 at 500/l. The prey levels at which 10% survival to metamorphosis was predicted were 107/l for bay anchovy, 130/l for lined sole, and 34/l for sea bream.

The question arises as to why survival rate is similar under the three prey densities but growth is faster for larvae fed at 900 *Artemia*/l? All three groups had the same success in switching from an endogenous food source to an exogenous one. Growth was better for larvae fed at 900 *Artemia*/l than those fed 100 *Artemia*/l as early as two weeks post-hatch. In a study by Duray and Bagarinao (1984), milkfish larvae (*Chanos chanos*) were abruptly weaned from rotifers to six different artificial diets. The control was weaned onto *Artemia*. Those weaned onto *Artemia* grew the fastest but survival was low at 42%. Those fish fed an artificial plankton diet were the only group to show a significantly higher survival (63%). They suggested that growth was rapid for those fed *Artemia* because of appropriate predator-prey behavioural characteristics of the nauplii. Milkfish are particulate visual feeders on plankton, so food should be permanently available in the water column.

Artemia may be nutritionally inadequate for some species resulting in starvation when the larvae switch from endogenous to exogenous feeding. In a study by Klumpp and Westernhagen (1986), plaice (*Pleuronectes platessa)* and blenny (*Blennius pavo*) larvae were successfully raised on *Artemia* from first feeding to metamorphosis. In the same study

however, herring (*Clupea harengus*) larvae grew, but showed a sudden high mortality at 38 days after first feeding. It was suggested that a diet of *Artemia* may cause species specific problems and have long-term toxicity or nutritional deficiency for herring. This may also be the case for wolffish in this study as a diet composed solely of *Artemia* may not contain all the nutrients found in the yolk. Therefore when the yolk is exhausted the larvae die.

Newly hatched wolffish larvae can survive on their yolk reserves for 2-4 weeks. The first mortality in the non-feeding study occurred at 15 days post-hatch. This would indicate that for larvae held at 4-6°C, the critical period for successfully switching from yolk utilization to ingesting and digesting exogenous food must occur prior to 15 days post-hatch. A major mortality starting at 15 days or shortly after would indicate that larvae were unsuccessful in switching from endogenous to exogenous nutrition. Strand et al. (1995) reported that the main cause of mortality of wolffish larvae fed formulated dry feeds was failure to initiate feeding. Survival curves were similar in all experimental groups with the greatest mortality between 30 to 40 days. Mortality of the fed groups corresponded closely to the short time span in which the unfed group died of starvation.

In conclusion wolffish show low survival when first fed a diet of only *Artemia*. High mortality in weeks 3 to 5 indicates an unsuccessful switch to exogenous feeding. This is supported by the complete mortality of starved larvae in 2 to 4 weeks. This may be the result of the larvae not receiving enough food or that the diet was inadequate.

CHAPTER 3.0 WEANING

3.1 INTRODUCTION

Typically marine larvae are first fed on live food and later weaned onto prepared pelleted feed. Small marine larvae may be first fed on small natural zooplankton or rotifers (*Brachionus plicatilis*) and gradually weaned to larger zooplankton or *Artemia* and then pelleted feed. The large size and advanced development of wolffish at hatch allow first feeding on *Artemia*. However, in past experiments wolffish larvae fed only *Artemia* show very poor survival. Even when prey levels were 900/l, as in the preliminary experiment described earlier, survival remained low. The use of live food in culturing fish is expensive and labour intensive so weaning to prepared feeds as early as possible is desirable.

Weaning larval fish onto dry food can be difficult. The pellet must be the proper size, texture and odour. It must have all the necessary nutrients with the proper levels of proteins, lipids, minerals, and vitamins, as well as the right compliment of essential amino acids and essential fatty acids. These nutrients must then be easily digested by the rudimentary system of the young larval fish.

This second study will investigate the effect of prey density on behaviour, growth, and survival when *Artemia* are given in combination with a dry diet right from hatch. Detailed behavioural observations are seldom used as a tool in aquaculture studies. Observations of feeding behaviour can help explain growth and survival results. Behaviour studies are particularly useful when the larvae are fed two food types. Important information such as

food preference, feeding success, feeding rates, and weaning times can be gathered for use in developing feeding protocols.

3.2 MATERIALS & METHODS

One hundred newly hatched larvae were placed in individual 30 litre glass aquaria. Flows were adjusted to maintain temperature between 4-7°C. The side and back walls of each aquarium were wrapped in black plastic to provide a dark background and to separate each tank. The front wall was left open to allow for easy observation. All tanks were located in a wet bench which was completely surrounded by a black curtain. Light was provided by overhead fluorescent tubes as well as natural light from nearby windows (natural photoperiod; 60-80 Lux at midday). Five hundred ml of cooled algae (*I. galbana*) was added to each tank daily. Tanks were siphoned daily to remove feces and excess food.

Mortalities were removed daily and counted. Percent survival was determined by subtracting the number of dead fish from the total and dividing by the total (the number taken for dry weights and number missing at the end of the study was subtracted from the initial total of 100 and used as the total for mortalities).

Twenty larvae were sampled at the start of the experiment (age 4 days) and used as initial samples for all tanks. Standard length was measured and dry weights obtained following drying at 90°C for 48 hours on pre-weighed aluminum foil. Five larvae were sampled from each tank every two weeks thereafter.

The experiment consisted of two feeding treatments (duplicate tanks for each treatment). Treatment 1 larvae were fed enriched *Artemia* at a density of 100/l and Treatment 2 at 900/l. These prey densities were the highest and lowest used in the previous prey density experiment. *Artemia* were prepared and counted in the same manner as described earlier. A marine larval dry diet (Lansy N4 500-800 μm) was added to the tanks following the addition of *Artemia*. At each feeding about 0.3 grams (0.6 grams/day) of dry diet was carefully dropped onto the surface of the water so that it floated. This was observed to be in excess of what the larvae would consume since there would be excess food on the bottom of the tank. For most of the experiment all larvae were fed twice a day (10:00 a.m. and 4:00 p.m.). In Week 8 the larvae were observed to be taking most of the food, so an extra feeding of dry diet was added at 12:00 noon.

Feeding observations began 2 Weeks post-hatch and were performed twice a week until the end of the experiment. Following the morning addition of food (*Artemia* and dry pellets) the observer sat quietly in front of the tank. Since larval wolffish are not exclusively pelagic and may sit or rest (ie. in contact with the bottom but with some forward movement to capture food) for periods of time, observations were carried out on fish in both locations. Six fish (three swimming fish and three resting) were arbitrarily chosen and observed for two minutes each. Similar observation times have been used in other larval fish behaviour studies (Brown, 1986; Brown and Colgan, 1984). The time each fish spent swimming or resting, in each two minute observation, was recorded.

The feeding behaviour performed by the larvae was described by six Modal Action Patterns (MAPs). Barlow (1968) defined a MAP as a spatiotemporal pattern of coordinated movement, which clusters about some mode, making the behaviour recognizable. The feeding MAPs recorded were Orient, Fixate, Lunge, Bite, Miss, and Reject (Table 1). The occurrence of each behaviour during the two minute observation period was recorded. A distinction between bites at *Artemia* and bites at dry diet was made. In addition, the MAPs of Orient, Fixate, Lunge, and Bite were combined into a category termed Forage.

Following the observations in each tank the number of fish swimming and resting was determined. Using a hand-held counter the number resting or number swimming (whichever was smallest) was counted. Although not the most accurate method this permitted a fast means of counting the fish before they moved. The number of fish in the other category was determined by adding the mortalities to the number counted and subtracting from the total. Of the fish resting on the bottom the number lying on their side as opposed to those in an upright position was also recorded. This has been described as a behaviour in the larval stage of wolffish (Moksness, et al., 1989).

The experiment was set up for 10 weeks but ended after nine due to technical problems and loss of water flow in one of the tanks. Survival data was collected up to week 9 however growth and behaviour data were only available up to week 8.

Table 1: Operational description of Modal Action Patterns (MAPs) of striped wolffish larvae.

MAP	DESCRIPTION
ORIENT	Response of larva to food item, which involves a movement of the trunk to bring the head of larva facing and in alignment with the prey.
FIXATE	Pause between orientation and food capture. The head of larva faces the prey. Larva focuses on food item. Does not always precede lunge and bite.
LUNGE	A prey-capture response in which the trunk of larva assumed an s-shaped position and precedes a fast forward movement towards food item of more than 1/4 body length.
BITE	Usually preceded by lunge but not always. Involves opening and closing of mouth quickly. No noticeable forward movement (less than 1/4 body length).
MISS	Larva is unsuccessful in capturing food item.
REJECT	Larva ejects or spits out captured food item.

FORAGE = ORIENT + FIXATE + LUNGE + BITE

The data were transformed when necessary to meet the assumptions of the statistical tests. If data were still not normally distributed, they were then rank transformed. Effects of feeding levels and age on survival, growth and behaviour were analyzed using a general linear model. In most analyses a significant age*treatment interaction was found so a least squares means test with a Bonferroni corrected P-value was used to test significance at each age. If no significant age*treatment interaction was found then a Duncan's test was used to test for significance at each age. A linear regression was used to determine if the frequency of foraging increased or decreased over time.

3.3 RESULTS

3.3.2 SURVIVAL

Larvae fed a high density of *Artemia* showed a significantly higher survival from Week 3 onwards ($P<0.0028$) (Fig.4, Table 2). By the end of the study 94.3% (±0.9) of larvae fed 900 *Artemia*/l had survived. For those fed 100 *Artemia*/l, survival was significantly lower ($P< 0.0028$, Bonferroni correction) at 52.6% (±2.6). Mortalities remained low throughout the experiment in the high density *Artemia* tanks but increased continuously after Week 3 for the low density *Artemia* tanks.

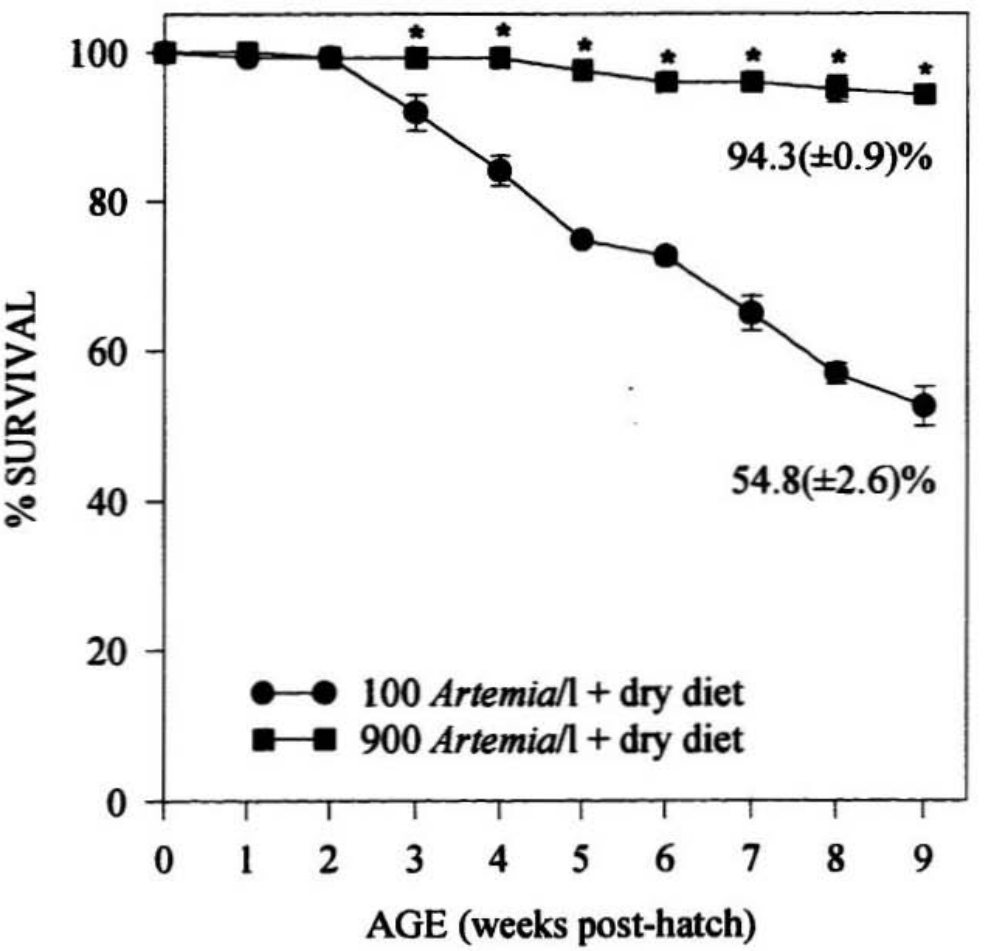

Figure 4: Weekly percent survival (±se) of striped wolffish larvae fed different densities of *Artemia* plus dry diet.

Table 2: Results of ANOVA and probabilities from least squares means analysis, for effects of age (in weeks) and prey density on percent survival of striped wolffish larvae. * indicates a significant difference (P <0.0028, Bonferroni correction).

	ANOVA			Least Squares Means by week								
Source	F	df	P	1	2	3	4	5	6	7	8	9
Treat	965.82	1,18	0.0001	0.7025	0.9796	0.0015*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Age	101.69	8,18	0.0001									
Treat*Age	63.77	8,18	0.0001									

3.3.2 GROWTH

At the start of the study (4 days post-hatch) larvae were 22.2±0.09 mm (n=20) in length (Table 3). From week 4 onwards standard length was significantly higher ($P<0.0063$, Bonferroni correction) in those larvae fed a higher density of *Artemia* (Fig.5, Table 4). By the last sampling day, larvae fed 900 *Artemia*/l measured 36.7±0.56 (n=10) mm whereas those fed 100 *Artemia*/l measured only 30.4±0.72 mm (n=10: $P< 0.0063$, Bonferroni correction), a difference of 6.3 mm.

Initial dry weight at the start of the study was 11.3±0.11 mg (n=20: Table 3). Larvae sampled from the 900 *Artemia*/l treatment had a significantly higher dry weight at week 6 and week 8 ($P< 0.0063$, Bonferroni correction, Table 4). By the last sampling day, larvae fed a high density of *Artemia* (900/l) had a dry weight of 76.9±5.10 mg (n=10), whereas those fed 100/l weighed 41.1±4.01 mg (n=10: Fig.6), a difference of 35.8 mg.

Specific growth rates (Table 5) were calculated from standard lengths (Fig.7) and dry weights (Fig.8) using the formula:

$$SGR = (\log_e Y_2 - \log_e Y_1)/(t_2 - t_1) * 100$$

where t = time and Y = fish size

SGR ranged from 2.68 %/day to 4.25%/day (based on dry weight) among larvae fed 900 *Artemia*/l. For larvae fed 100 *Artemia*/l SGR fluctuated throughout the study with a low

Table 3: Mean standard length (mm) and dry weight (mg) (±se) of striped wolffish larvae fed two different prey densities, 100 Artemia/l and 900 Artemia/l, plus a dry diet.

Age (weeks post-hatch)	100 Artemia/l		900 Artemia/l	
	standard length	dry weight	standard length	dry weight
0	22.2 ±0.09	11.3 ±0.11	22.2 ±0.09	11.3 ±0.11
2	23.1 ±0.31	12.1 ±0.91	24.6 ±0.33	16.9 ±1.01
4	25.6 ±0.36	20.2 ±1.20	28.3 ±0.30	26.9 ±0.90
6	26.7 ±0.67	20.0 ±2.29	32.4 ±0.69	42.4 ±3.62
8	30.4 ±0.72	41.1 ±4.01	36.7 ±0.56	76.9 ±5.10

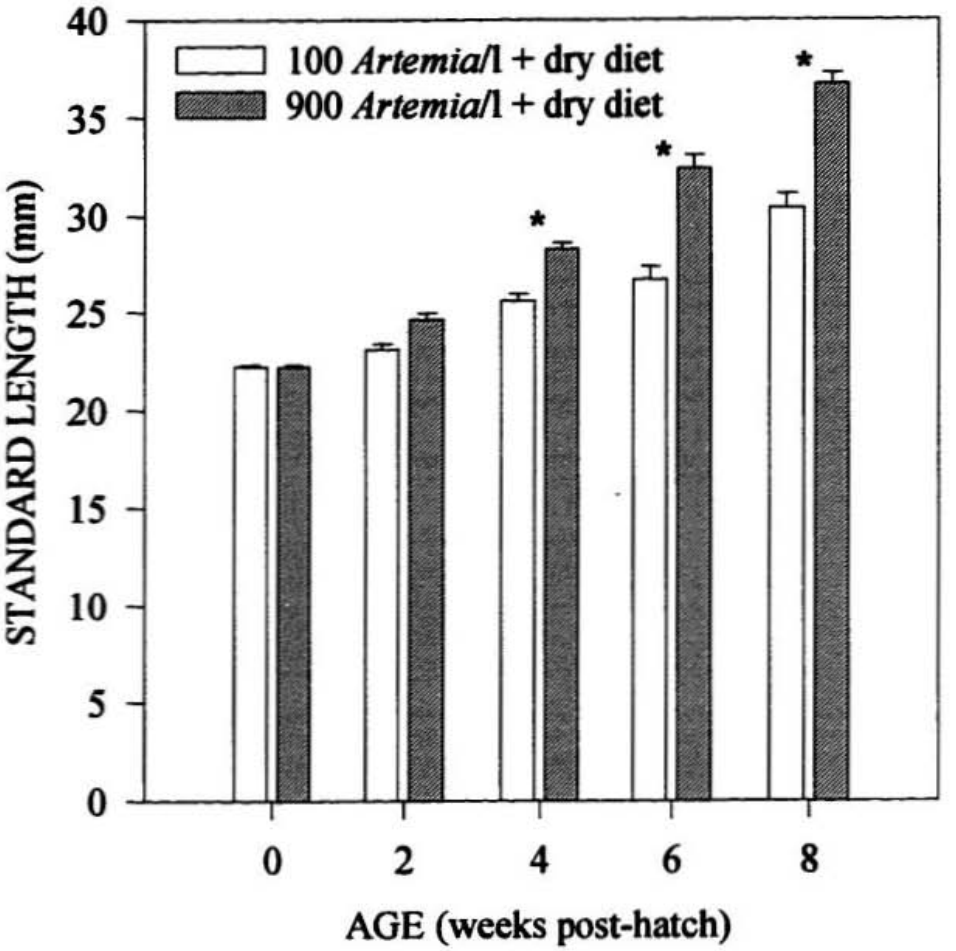

Figure 5: Mean standard length (mm ±se) of striped wolffish fed different densities of *Artemia* plus dry diet. * indicates P <0.0063, Bonferroni correction. (n=10 except n=20 at age=0).

Table 4: Results of ANOVA and probabilites from least squares means analysis for effects of age (in weeks) and prey density on standard length (mm) and dry weight (mg) of striped wolffish larvae fed different densities of Artemia (100/l and 900/l). * indicates a significant difference (P <0.0063, Bonferroni correction).

		ANOVA			Least Squares Means weekly			
Growth Parameter	Source	F	df	P	2	4	6	8
Standard Length	Treat	94.49	1,70	0.0001	0.0113	0.0001*	0.0001*	0.0001*
	Age	116.81	3,70	0.0001				
	Treat*Age	3.5	3,70	0.0199				
Dry Weight	Treat	80.94	1,70	0.0001	0.0520	0.0314	0.0001*	0.0001*
	Age	97.31	3,70	0.0001				
	Treat*Age	7.94	3,70	0.0001				

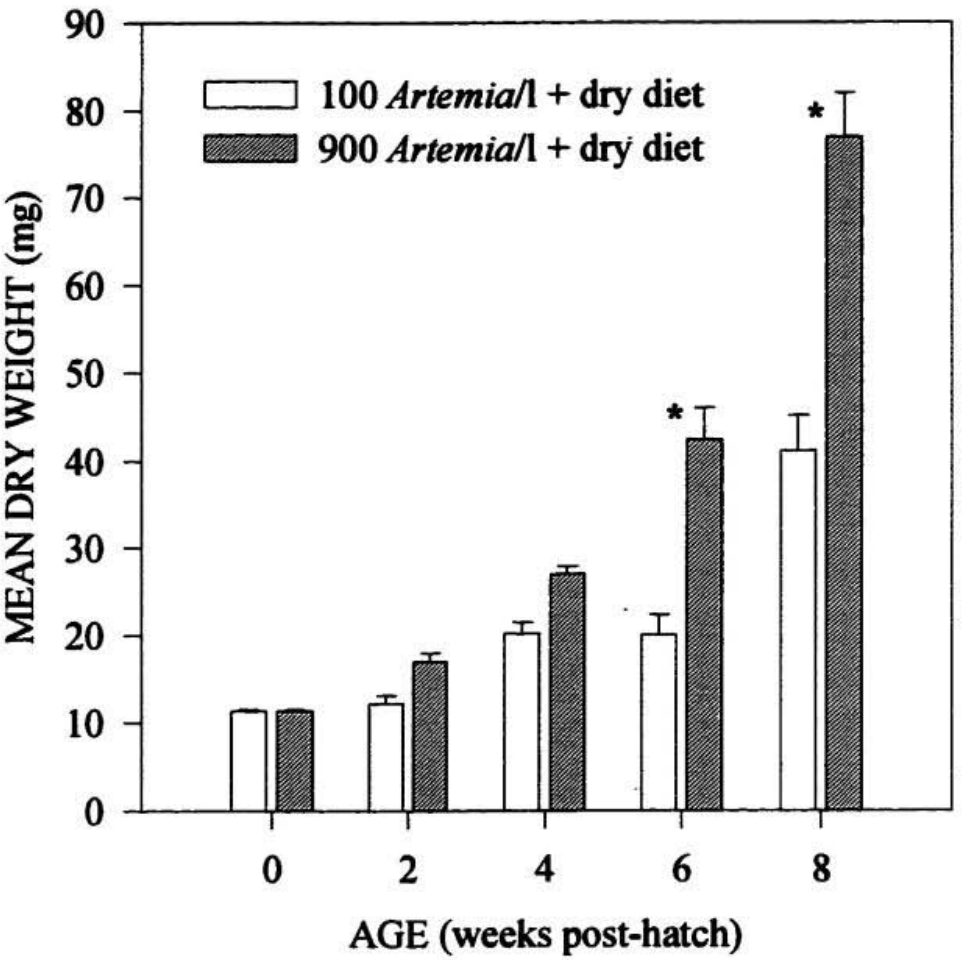

Figure 6: Mean dry weight (mg ±se) of striped wolffish larvae fed different densities of *Artemia* plus dry diet. * indicates P <0.0063, Bonferroni correction (n=10 per week except n=20 at age =0).

Table 5: Specific growth rates (%/day) of striped wolffish larvae fed two different prey densities, 100 Artemia/l and 900 Artemia/l, plus a dry diet, calculated from standard length and dry weight.

Age (weeks post-hatch)	100 Artemia/l		900 Artemia/l	
	standard length	dry weight	standard length	dry weight
0 - 2	0,26	0,46	0,68	2,68
2 - 4	0,73	3,66	1,00	3,32
4 - 6	0,30	-0,07	0,97	3,25
6 - 8	0,93	5,14	0,89	4,25

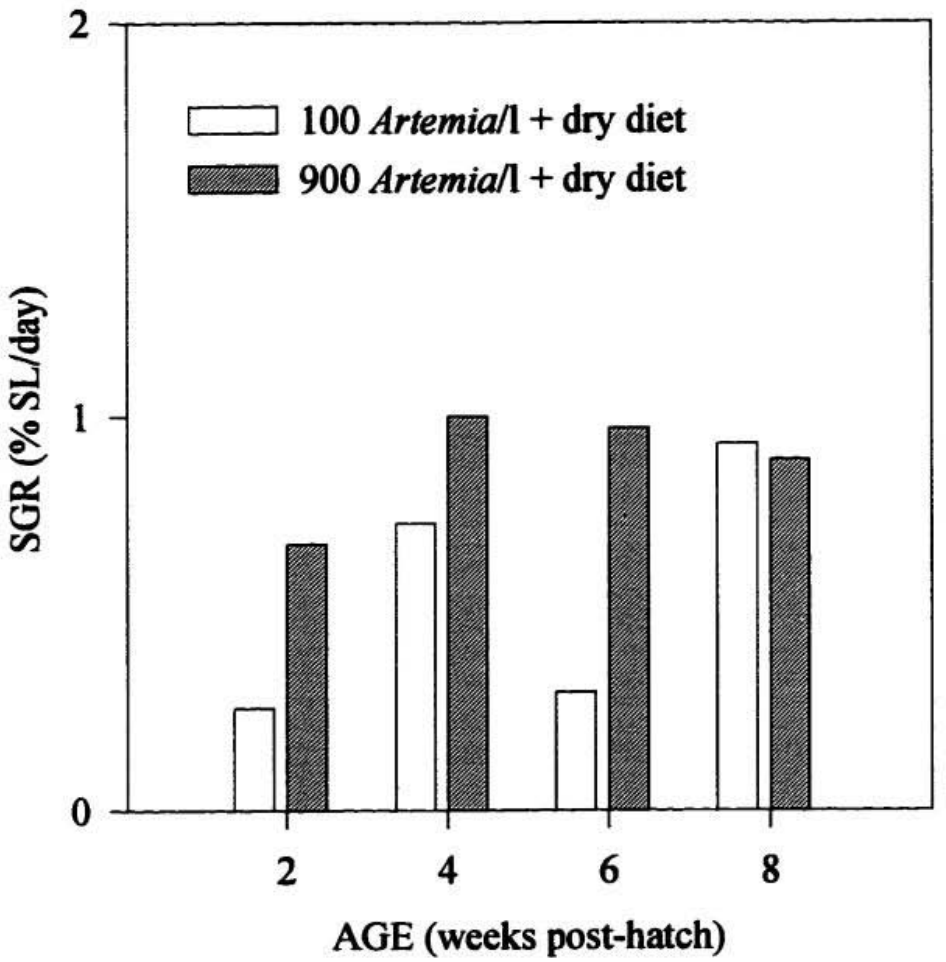

Figure 7: Specific growth rate (%/day) calculated from standard length of striped wolffish fed different densities of *Artemia* plus dry diet.

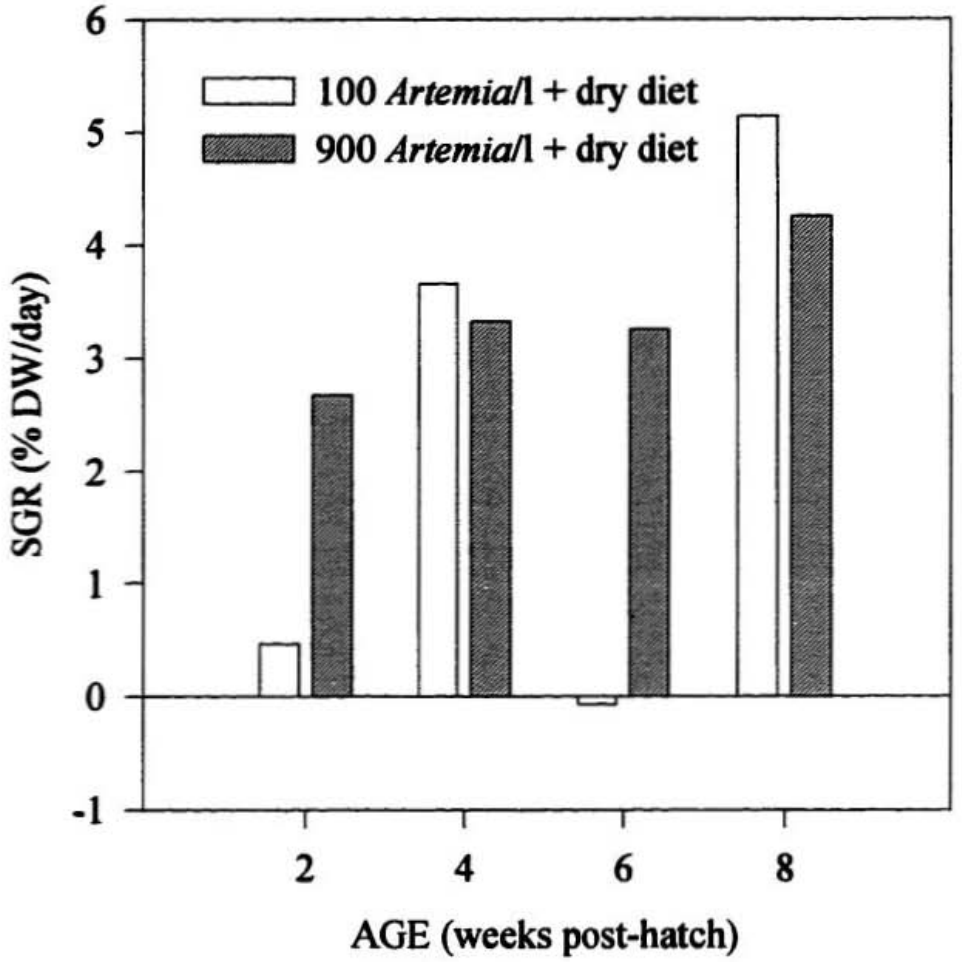

Figure 8: Specific growth rate (%/day) calculated from dry weight of striped wolffish larvae fed different densities of *Artemia* plus dry diet.

of -0.07 %/day (based on dry weight) calculated from weeks 4 to 6 to a high of 5.14%/day in weeks 6 to 8.

3.3.3 BEHAVIOUR

Larvae fed 900 *Artemia*/l showed a significantly higher frequency of each MAP (Orient, Fixate, Lunge, Bite) at 2 weeks post-hatch ($P < 0.0031$, Bonferroni correction). In weeks 3 to 9 there were no significant differences ($P > 0.0031$, Bonferroni correction) (Fig.9a, b, c, d; Table 6). Similar results were obtained for foraging (Fig.10, Table 6). Foraging among those larvae fed 900 *Artemia*/l decreased significantly over the experiment (Linear Regression, slope= -0.325429, $P = 0.0001$). Larvae fed 100 *Artemia/l* maintained their level of foraging behaviour over the experiment (slope= -0.053911, $P=0.3168$).

Among larvae fed 100 *Artemia*/l a significant difference ($F=4.83$, df=3,736, $P=0.0025$) was found between frequency of MAPs over the experiment (Fig. 11a). The larvae performed more Orient and Fixate behaviours than Lunge and Bite behaviours ($P < 0.05$, Duncan multiple range test). Larvae in the 900 *Artemia*/l treatment showed no significant difference ($F=2.21$, df=3,736, $p=0.0858$) among frequencies of MAPs (Fig.11b).

Larvae fed 900 *Artemia/l* had a significantly higher frequency of bites toward *Artemia* in weeks 2 and 3 ($P < 0.0031$, Bonferroni correction) than those from the 100 *Artemia*/l treatment (Fig.12a, Table 6). There was no significant difference during the rest of the experiment ($P > 0.0031$, Bonferroni correction). There was no difference in frequency of bites

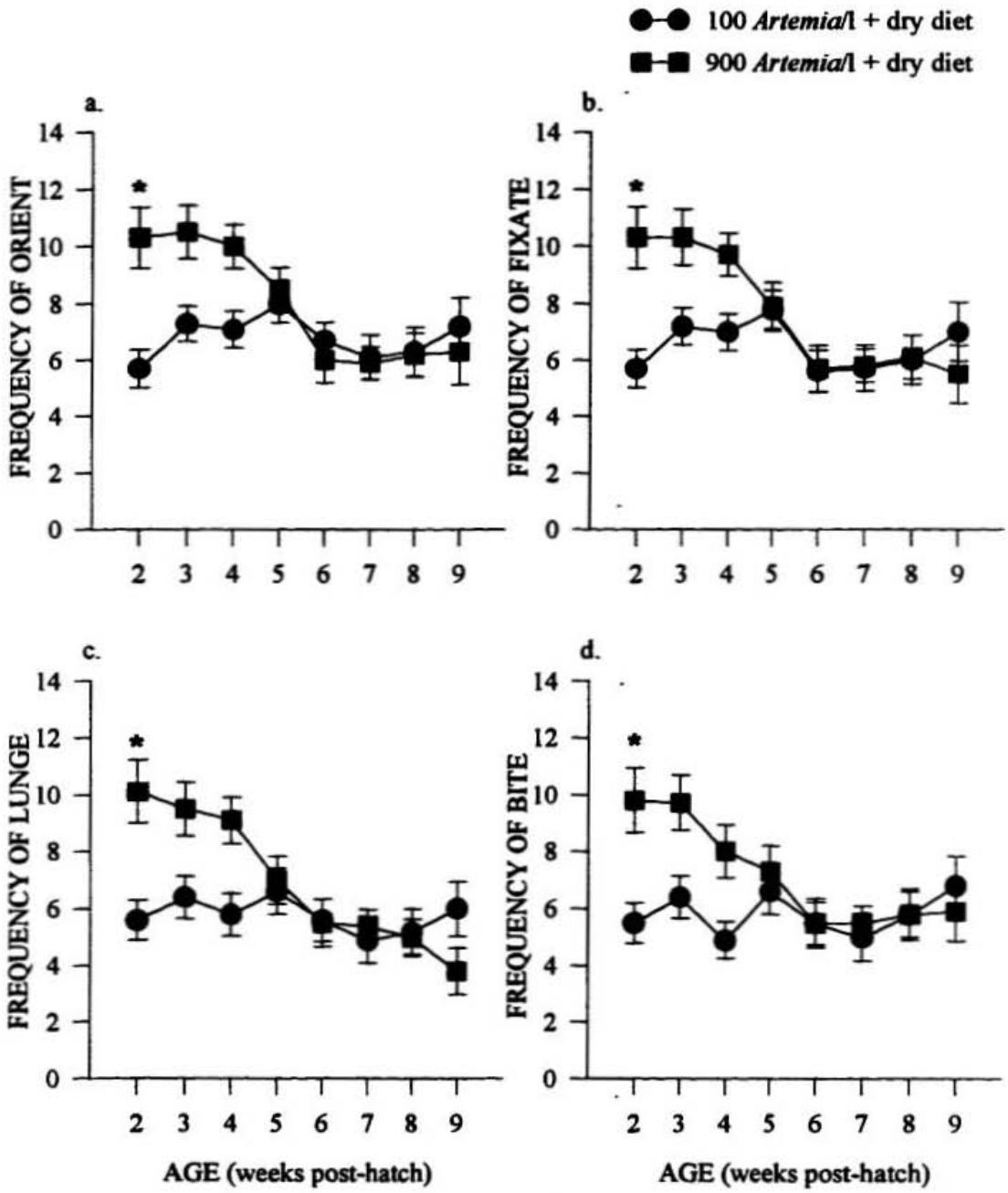

Figure 9: Mean weekly frequency (±se) of a) Orient, b) Fixate, c) Lunge, and d) Bite in a 2 minute observation period for striped wolffish fed different densities of *Artemia* plus dry diet. * indicates P <0,0031, Bonferroni correction (n=24 per week).

Table 6: Results of ANOVA and probabilities from least squares means analysis, for effect of age (in weeks) and prey density on frequency of Model Action Patterns (MAPs) among striped wolffish larvae fed different densities of Artemia (100/l and 900/l). * indicates a significant difference (P<0.0031, Bonferroni correction).

		ANOVA			Least Squares Means by week							
MAP	Source	F	df	P	2	3	4	5	6	7	8	9
Orient	Treat	5.31	1,366	0.0218	0.0009*	0.0197	0.0133	0.6624	0.4825	0.8062	0.9733	0.2680
	Age	4.26	7,366	0.0002								
	Treat*Age	2.79	7,366	0.0078								
Fixate	Treat	5.12	1,366	0.0242	0.0012*	0.0327	0.0209	0.9579	0.9907	0.9869	0.8402	0.1233
	Age	5.03	7,366	0.0001								
	Treat*Age	2.55	7,366	0.0141								
Lunge	Treat	5.73	1,366	0.0172	0.0021*	0.0234	0.0050	0.6702	0.8168	0.5802	0.9647	0.0268
	Age	5.19	7,366	0.0001								
	Treat*Age	3.22	7,366	0.0025								
Bite	Treat	11.04	1,366	0.0010	0.0005*	0.0078	0.0105	0.5834	1.0000	0.6808	0.6808	0.4509
	Age	2.67	7,366	0.0104								
	Treat*Age	2.35	7,366	0.0235								
Forage	Treat	6.59	1,366	0.0107	0.0021*	0.0195	0.0094	0.7230	0.7838	0.8466	0.8085	0.1892
	Age	3.89	7,366	0.0004								
	Treat*Age	2.48	7,366	0.0168								
Bite@ Dry diet	Treat	2.63	1,366	0.1054	0.2237	0.0429	0.4753	0.8868	0.0183	0.0051	0.0485	0.8532
	Age	15.56	7,366	0.0001								
	Treat*Age	3.00	7,366	0.0044								
Bite@ Artemia	Treat	4.18	1,366	0.0417	0.0001*	0.0002*	0.0297	0.8184	0.0516	0.1689	0.3201	0.6739
	Age	21.84	7,366	0.0001								
	Treat*Age	5.81	7,366	0.0001								

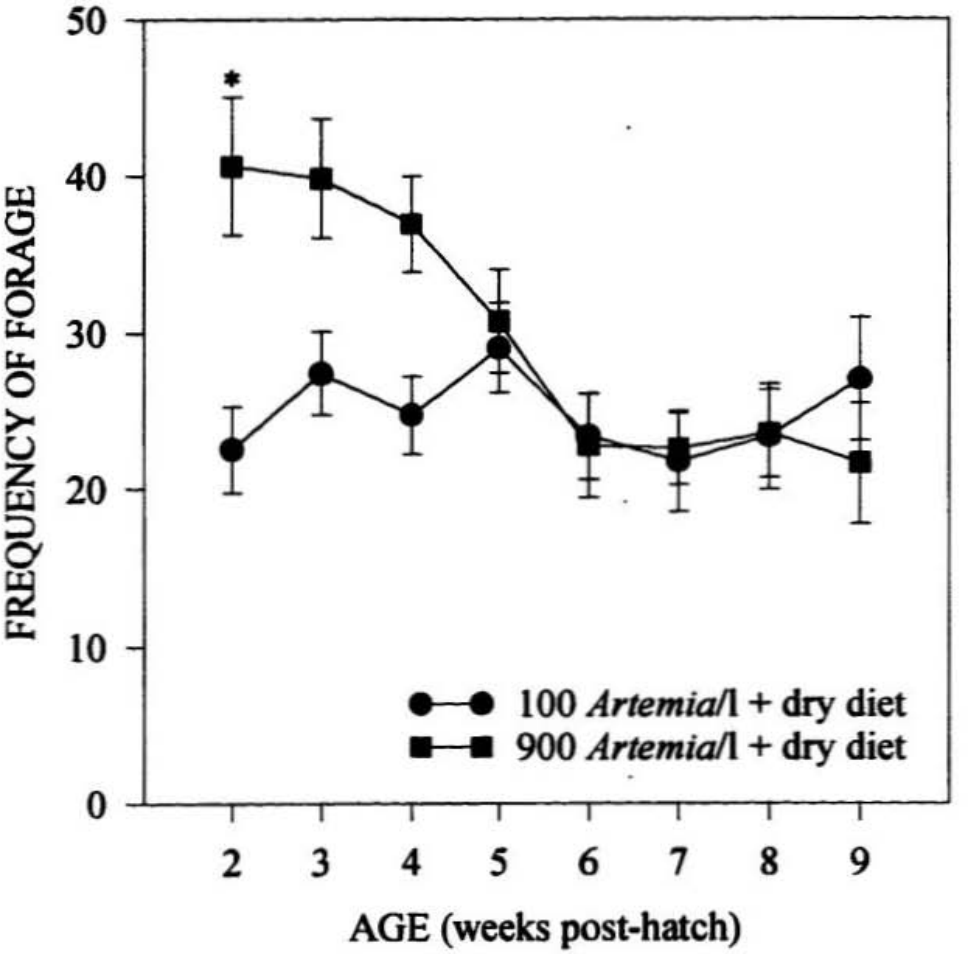

Figure 10: Mean weekly frequency (±se) of Forage in a 2 minute observation period for striped wolffish fed different densities of *Artemia* plus dry diet. * indicates P <0.0031, Bonferroni correction (n=24 per week).

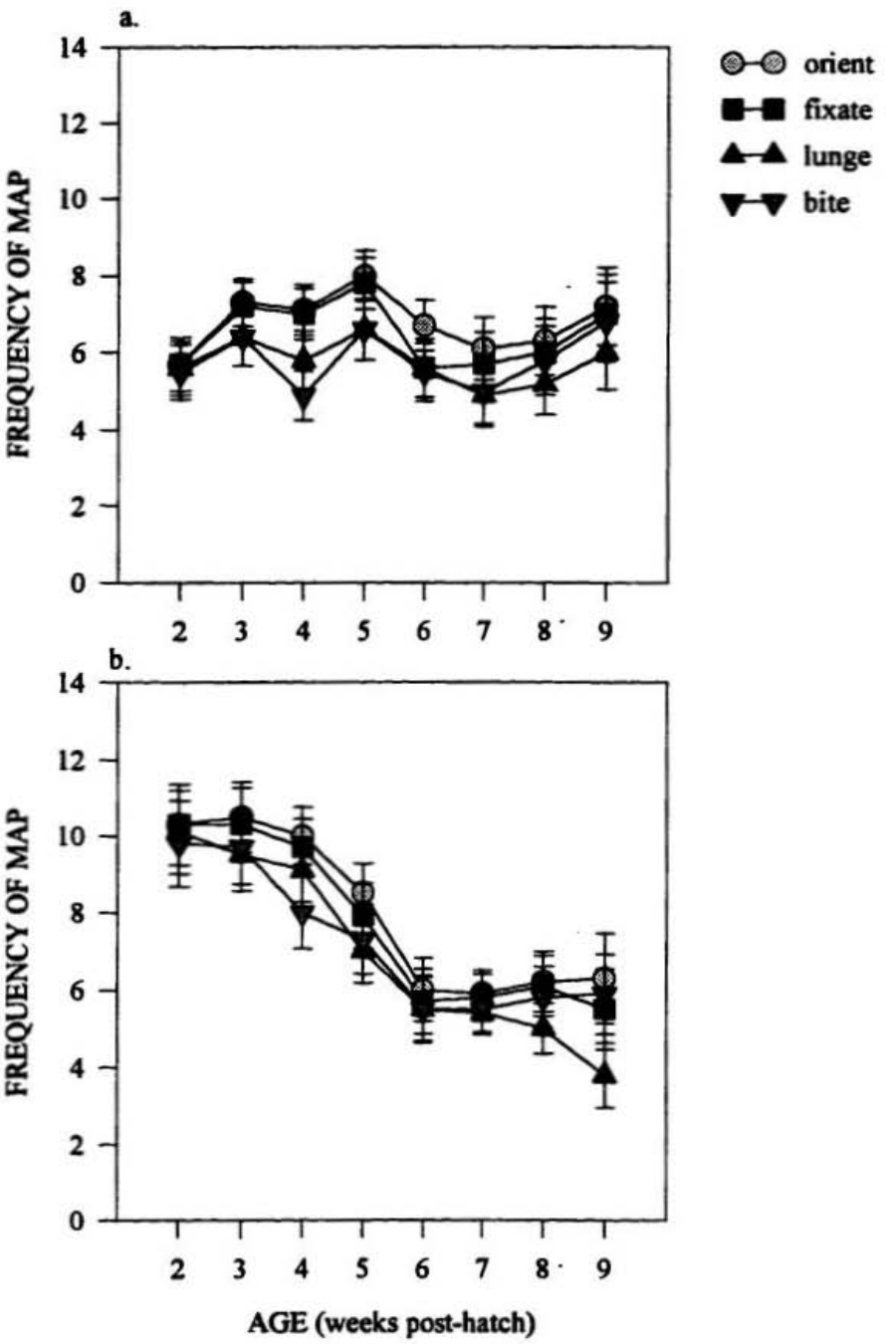

Figure 11: Mean weekly frequency (±se) of each MAP (Orient, Fixate, Lunge, Bite) in a 2 minute obsevation period for striped wolffish larvae in a) Treatment 1, 100 *Artemia*/l plus dry diet and b) Treatment 2, 900 *Artemia*/l plus dry diet (n= 24 per week).

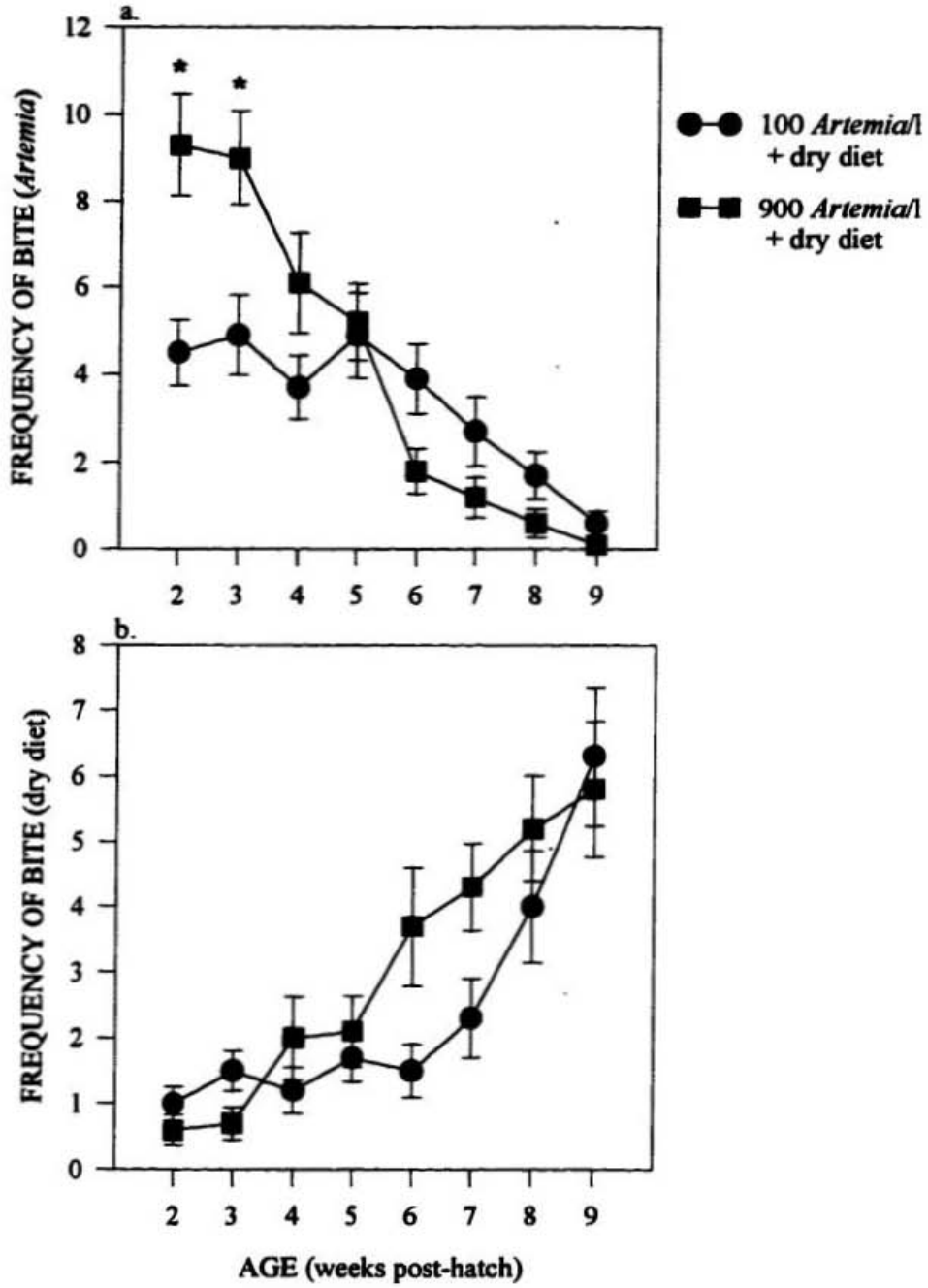

Figure 12: Mean weekly frequency (±se) of Bites at a) *Artemia* and b) dry diet in a 2 minute observation period for striped wolffish larvae fed different densities of *Artemia* plus dry diet (n= 24 per week).

at dry diet between treatments over the experiment (P> 0.0031, Bonferroni correction) (Fig.12b, Table 6).

Considering only those larvae which were swimming during the treatment, there were significantly more bites made at *Artemia* in weeks 2 and 3 (P< 0.0031, Bonferroni correction) among larvae fed *Artemia* at 900/l than among those fed the lower level (Fig.13a, Table 7). There was no difference during the rest of the study (P> 0.0031, Bonferroni Correction). There was also no significant difference in frequency of bites toward dry pellets between treatments (Fig.13b) (P> 0.0031, Bonferroni correction; Table 7).

Among resting fish there was no significant difference between treatments in frequency of bites at *Artemia* (Fig. 14a) (F=1.44, d,f=1,174, P=0.2312) or dry diet (Fig. 14b) (F=1.82, d,f=1,174, P=0.1786).

Among swimming fish fed 100 *Artemia*/l (Fig. 15a) there was no significant difference in bites at *Artemia* or dry diet for the first 7 weeks (P>0.0031, Bonferroni correction; Table 8). By weeks 8 and 9, significantly more (P< 0.0031, Bonferroni correction) bites where made toward dry diet. In the high food tanks (Fig.15b) swimming larvae made more attempts towards *Artemia* in Weeks 2 and 3 (P<0.0031, Bonferroni correction; Table 8) and more bites towards dry diet from Week 6 (P<0.0031, Bonferroni correction) onward. There was no significant difference in bites in Weeks 4 and 5 (P>0.0031, Bonferroni correction).

Among resting fish fed the low density of *Artemia*, significantly more bites were made towards *Artemia* during the first 6 weeks (Fig. 16a) (P<0.0031, Bonferroni correction; Table 8). In weeks 7-9 there was no significant difference in bites at the food items (P> 0.0031,

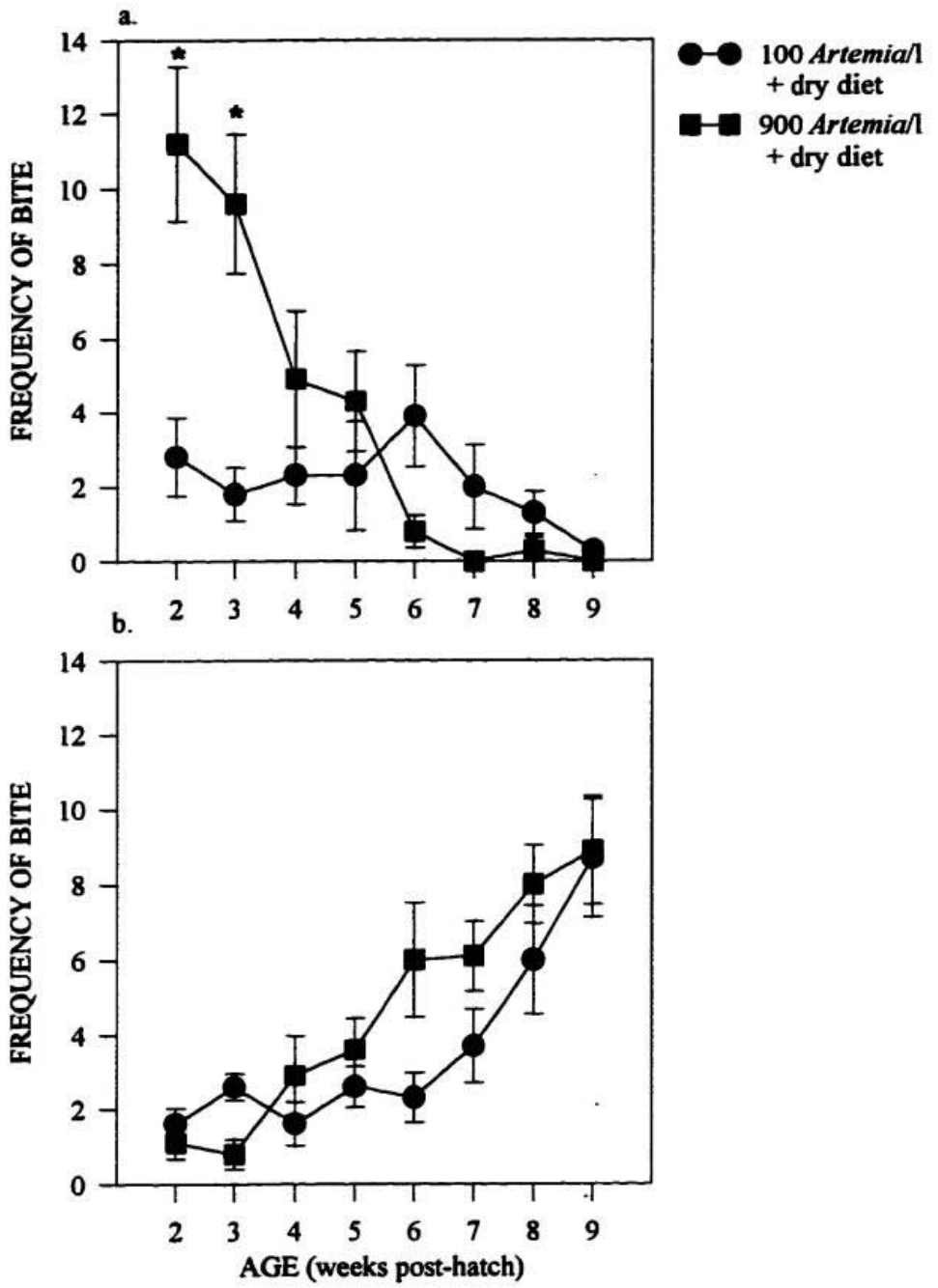

Figure 13: Mean weekly frequency (±se) of Bites at a) *Artemia* and b) dry diet in a 2 minute observation period for striped wolffish larvae fed different densities of *Artemia* plus dry diet. Observations on swimming larvae only. * indicates P<0.0031, Bonferroni correction (n= 12 per week).

Table 7: Results of ANOVA and probabilites from least squares means analysis, for effects of age (in weeks) and prey density on frequency of Bite (MAP) among striped wolffish larvae (swimming only) fed different densities of Artemia (100/l and 900/l). Each food item (Artemia and dry diet) analysed separately. * indicates a significant difference (P <0.0031, Bonferroni correction).

		ANOVA			Least Squares Means by week							
Food Item	Source	F	df	P	2	3	4	5	6	7	8	9
Artemia	Treat	3.71	1,174	0.1064	0.0001*	0.0001*	0.2260	0.1799	0.0335	0.0977	0.3064	0.6599
	Age	9.65	7,174	0.0001								
	Treat*Age	5.93	7,174	0.0035								
Dry Diet	Treat	2.63	1,174	0.0558	0.4360	0.0042	0.2535	0.4551	0.0058	0.0449	0.1511	0.9056
	Age	14.08	7,174	0.0001								
	Treat*Age	3.18	7,174	0.0001								

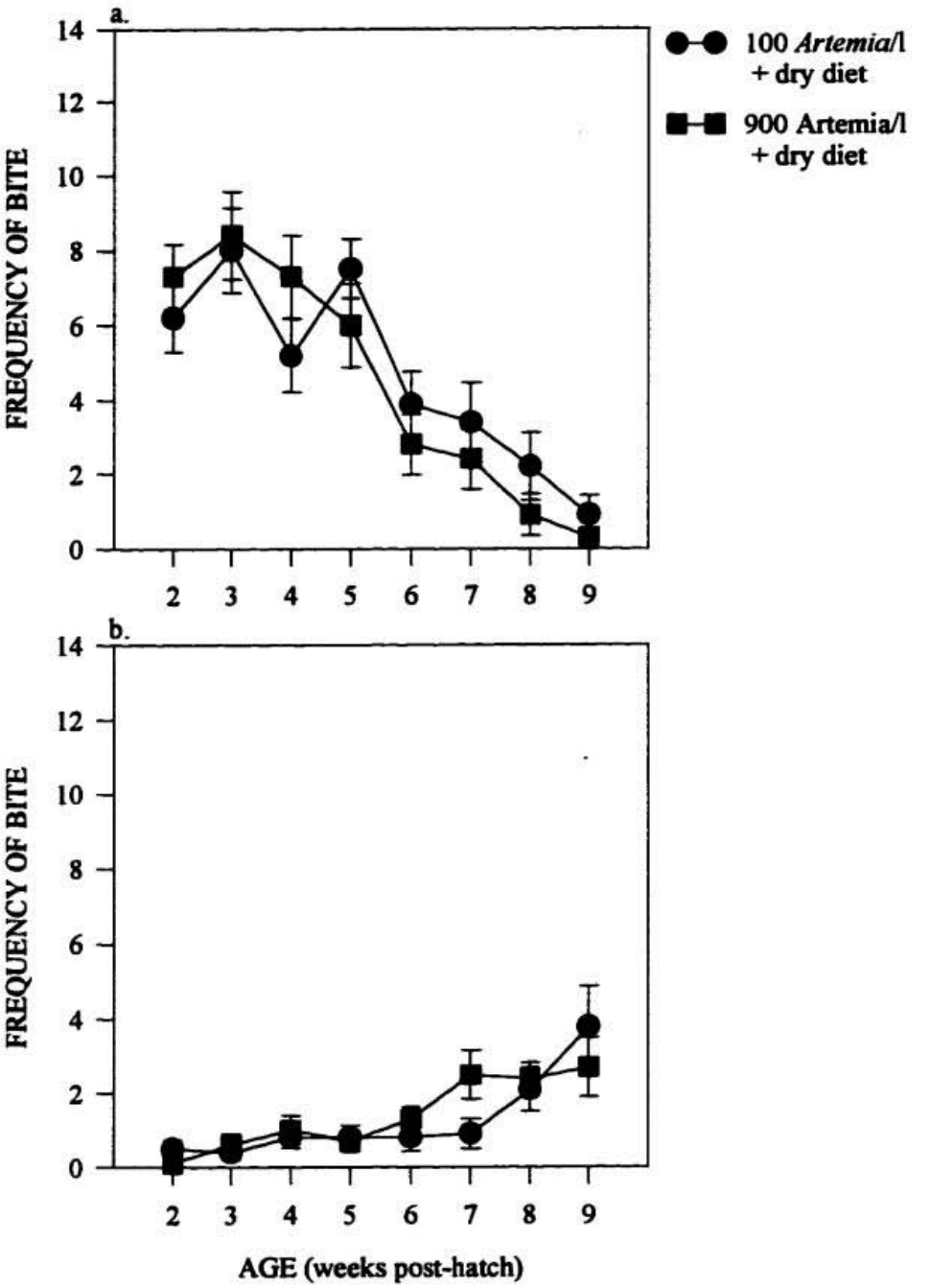

Figure 14: Mean weekly frequency (±se) of Bites at a) *Artemia* and b) dry diet in a 2 minute observation period for striped wolffish larvae fed different densities of *Artemia* plus dry diet. Observations on resting larvae only (n=12 per week).

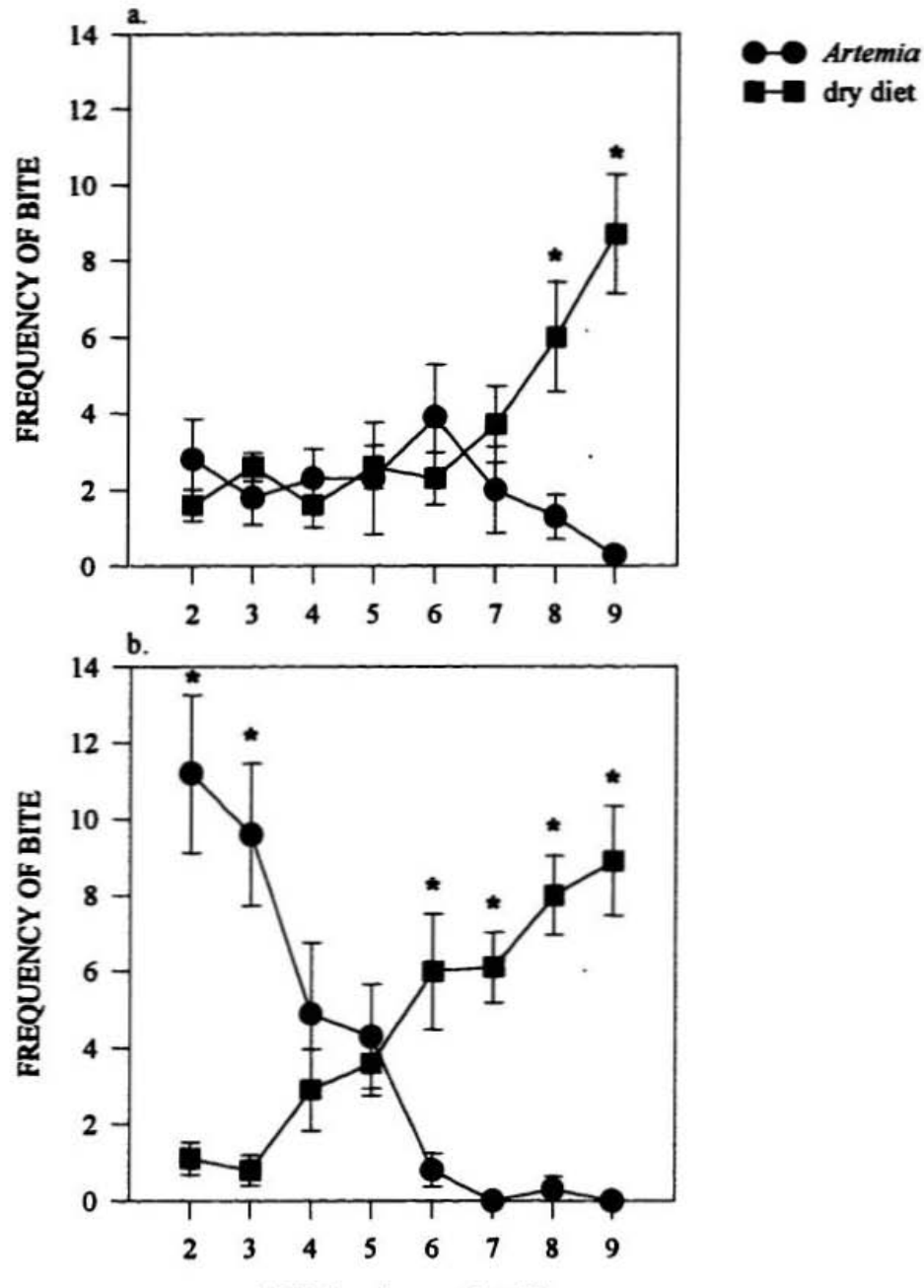

Figure 15: Mean weekly frequency (±se) of Bites at *Artemia* and dry pellets in a 2 minute observation period for striped wolffish larvae fed at a level of a) 100 *Artemia*/l plus dry diet and b) 900 *Artemia*/l plus dry diet. Observations on swimming larvae only. * indicates P <0.0031, Bonferroni correction (n=12 per week).

Table 8: Results of ANOVA and probabilites from least squares means analysis, for effects of age (in weeks) and food item (Artemia or dry diet) on frequency of Bite (MAP) among striped wolffish larvae fed different levels of Artemia (100/l and 900/l). Each location (swimming or resting) and treatment analysed separately. * indicates a significant difference (P <0.0031, Bonferroni correction).

			ANOVA			Least Squares Means by weeks							
Location	Treat.	Source	F	df	P	2	3	4	5	6	7	8	9
Swim	100/l	Treat	17.42	1,176	0.0001	0.9741	0.1203	0.6236	0.0866	0.0436	0.0754	0.0010*	0.0001*
		Age	0.00	7,176	1.0000								
		Treat*Age	3.51	7,176	0.0015								
Swim	900/l	Treat	17.9	1,176	0.0001	0.0001*	0.0001*	0.5517	0.9052	0.0001*	0.0001*	0.0001*	0.0001*
		Age	0.00	7,176	1.0000								
		Treat*Age	18.39	7,176	0.0001								
Rest	100/l	Treat	73.15	1,176	0.0001	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0117	0.5572	0.0067
		Age	0.00	7,176	1.0000								
		Treat*Age	9.8	7,176	0.0001								
Rest	900/l	Treat	28.06	1,176	0.0001	0.0001*	0.0001*	0.0001*	0.0001*	0.1190	0.6592	0.0001*	0.0001*
		Age	0.00	7,176	1.0000								
		Treat*Age	18.56	7,176	0.0001								

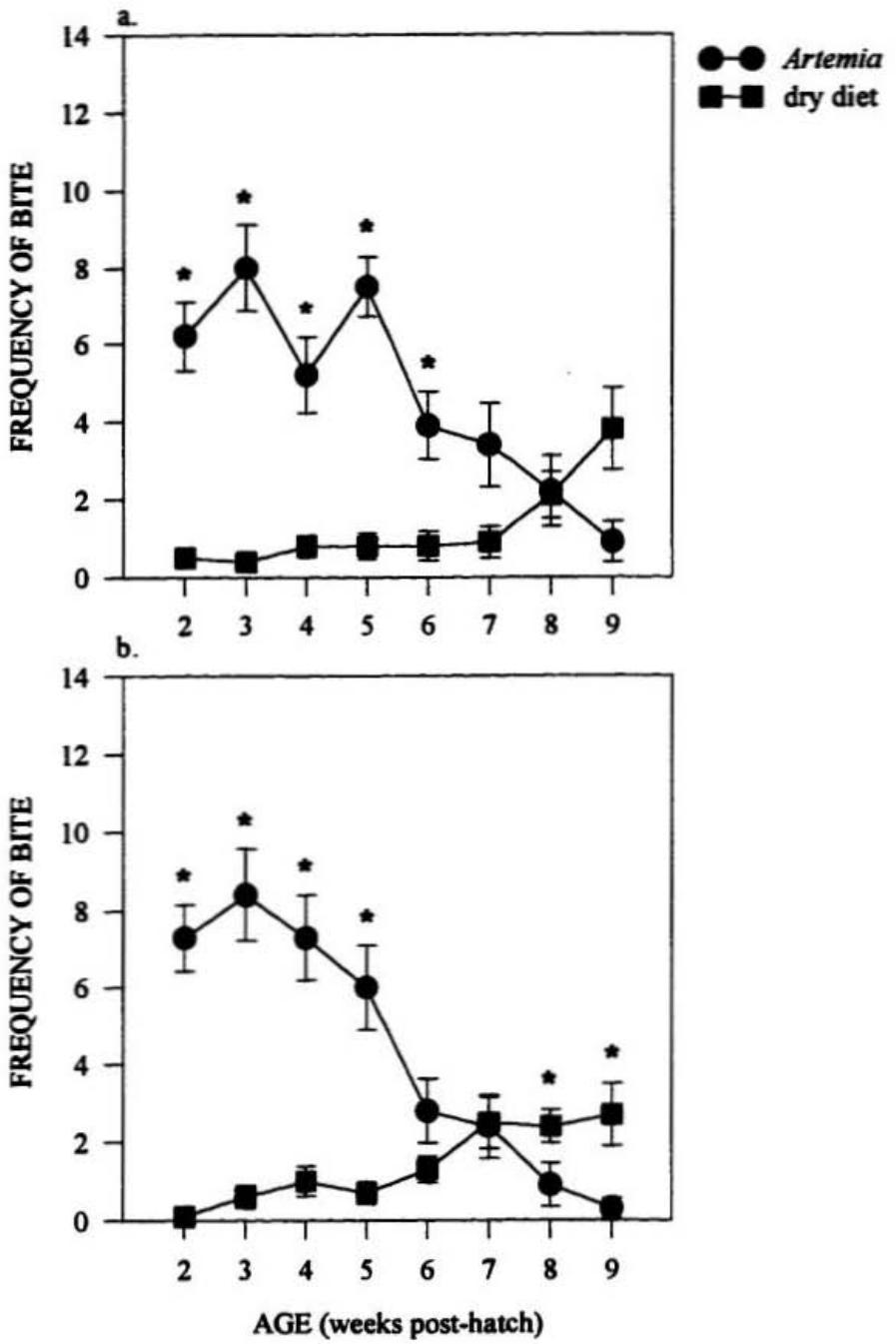

Figure 16: Mean weekly frequency (±se) of Bites at *Artemia* and dry pellets in a 2 minute observation period for striped wolffish larvae fed at a level of a) 100 *Artemia*/l plus dry diet and b) 900 *Artemia*/l plus dry diet. Observations on resting larvae only. * indicates P <0.0031, Bonferroni correction (n=12 per week).

Bonferroni correction). Resting fish in the tanks provided with the higher density made significantly more bites toward *Artemia* in weeks 2 to 5 (Fig. 16b) ($p<0.0031$, Bonferroni correction; Table 8) but made significantly more bites toward dry pellets in the last two weeks of the experiment ($p<0.0031$, Bonferroni correction).

The overall success at ingesting a food item was determined by subtracting the total number of misses and rejects (spit out food item) from the total number of bite attempts (at times larvae missed both *Artemia* and dry diet but only rejected dry diet, never *Artemia*). An average success rate (±se) was calculated. Percent ingestion success of *Artemia* was 99.5% ±0.45 for larvae in the low *Artemia* treatment and 98.3% (±0.58) for larvae in the high *Artemia* treatment. Ingestion success of dry diet was 94.3% ±3.99 among larvae fed the low *Artemia* density and 93.4% ±4.33 among larvae fed the high *Artemia* density.

The percentage of fish swimming (Fig.17) was significantly higher ($P< 0.0031$, Bonferroni correction) in the low density tanks (100/l) than the high density tanks (900/l) up to Week 9 ($P>0.0031$, Bonferroni correction; Table 9). No aggression was observed in any of the tanks until Week 8. Until this time, resting fish appeared to be randomly distributed on the bottom. When aggression was first observed, bottom fish were more evenly distributed and many fish had torn fins. It appeared that attacking fish were attempting to displace fish from their position. Attacked fish would often retaliate. There seemed to be no difference in size of fish in an aggressive encounter.

Of the fish resting on the bottom only fish fed 100 *Artemia*/l were observed lying on their sides (Fig.18). When touched with a pipette these fish would quickly swim away. This

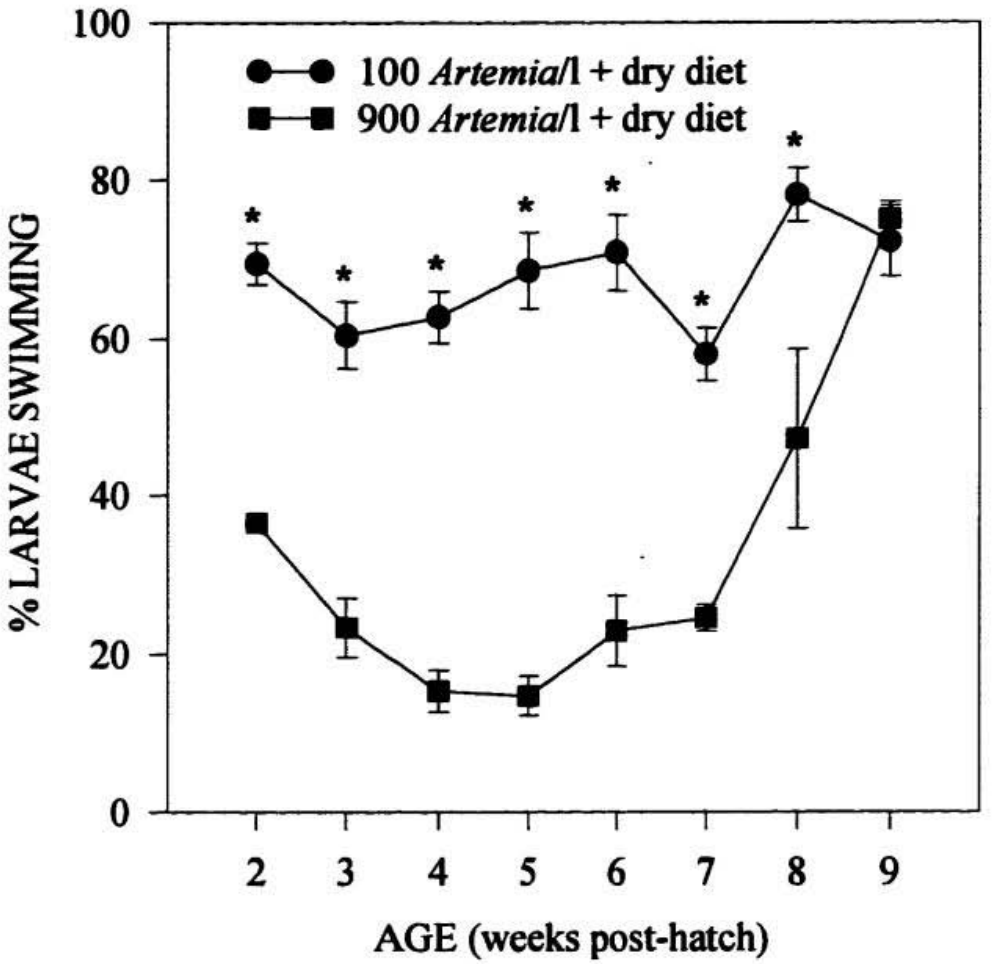

Figure 17: Percent of striped wolffish larvae swimming (weekly mean ±se) in each feeding treatment. * indicates P <0.0031, Bonferroni correction (n=4 per week).

Table 9: Results of ANOVA and probabilities from least squares means analysis, for effects of age (in weeks) and prey density on percent of striped wolffish larvae fed different densities of Artemia (100/l and 900/l) that are swimming. * indicates a significant difference (P <0.0031, Bonferroni correction).

	ANOVA			Least Squares Means by week							
Source	F	df	P	2	3	4	5	6	7	8	9
Treat	289.81	1,48	0.0001	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.7348
Age	16.97	7,48	0.0001								
Treat*Age	9.85	7,48	0.0001								

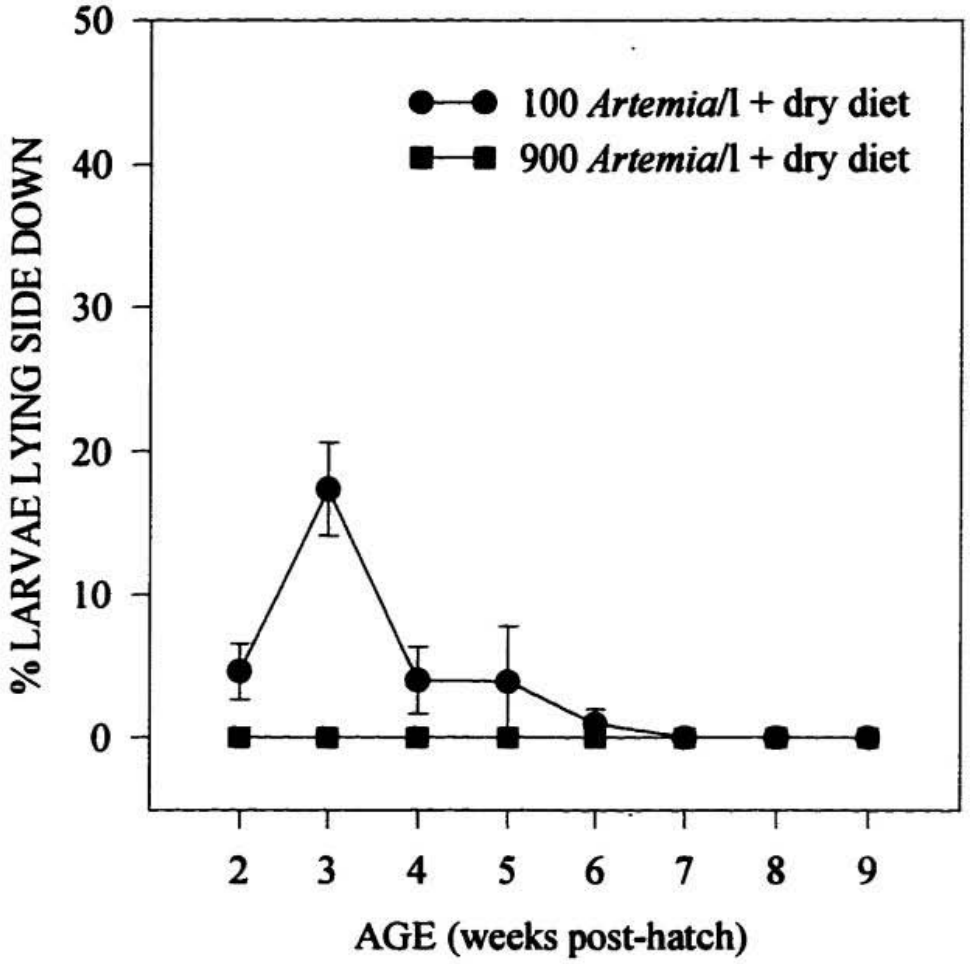

Figure 18: Percent of resting (bottom) striped wolffish larvae lying sideways at the end of each observational period in each feeding treatment (n=4 per week).

side-lying behaviour peaked at 17.4% (±3.24) in Week 3, corresponding with the time mortalities started to occur. No live fish were ever observed lying on their sides in the high *Artemia* tanks.

Overall, significantly more foraging MAPs were performed by swimming larvae at the end of the study in Weeks 8 and 9 ($P<0.0031$, Bonferroni correction) than by resting larvae (Fig.19). Swimming larvae maintained their level of foraging throughout the study (slope= -0.000586, P=0.9915). Resting larvae decreased foraging frequency over the study period (slope= -0.379925 P=0.0001).

3.4 DISCUSSION

From this study two conclusions can be drawn. One is that wolffish larvae will take dry diet as well as *Artemia* at first feeding and perform better on this combination in comparison to larvae fed only *Artemia*. The second is that the level of live prey is important when trying to wean larvae onto dry feed. As shown previously, a diet of only *Artemia* fed through the period when larvae are switching from endogenous to exogenous feeding is inadequate and results in low survival. However wolffish larvae will grow and have high survival when fed a high level of *Artemia* nauplii in combination with a dry diet from hatch.

Larvae of other species have shown improved survival when fed live and dry foods in combination. American shad (*Alosa sapidissima*) larvae fed prepared dry diets (*Artemia* flakes and AP-100) in combination with *Artemia* nauplii showed 66 and 84% survival respectively compared to 47% survival for larvae fed *Artemia* only at a rate of 18 *Artemia*/fish

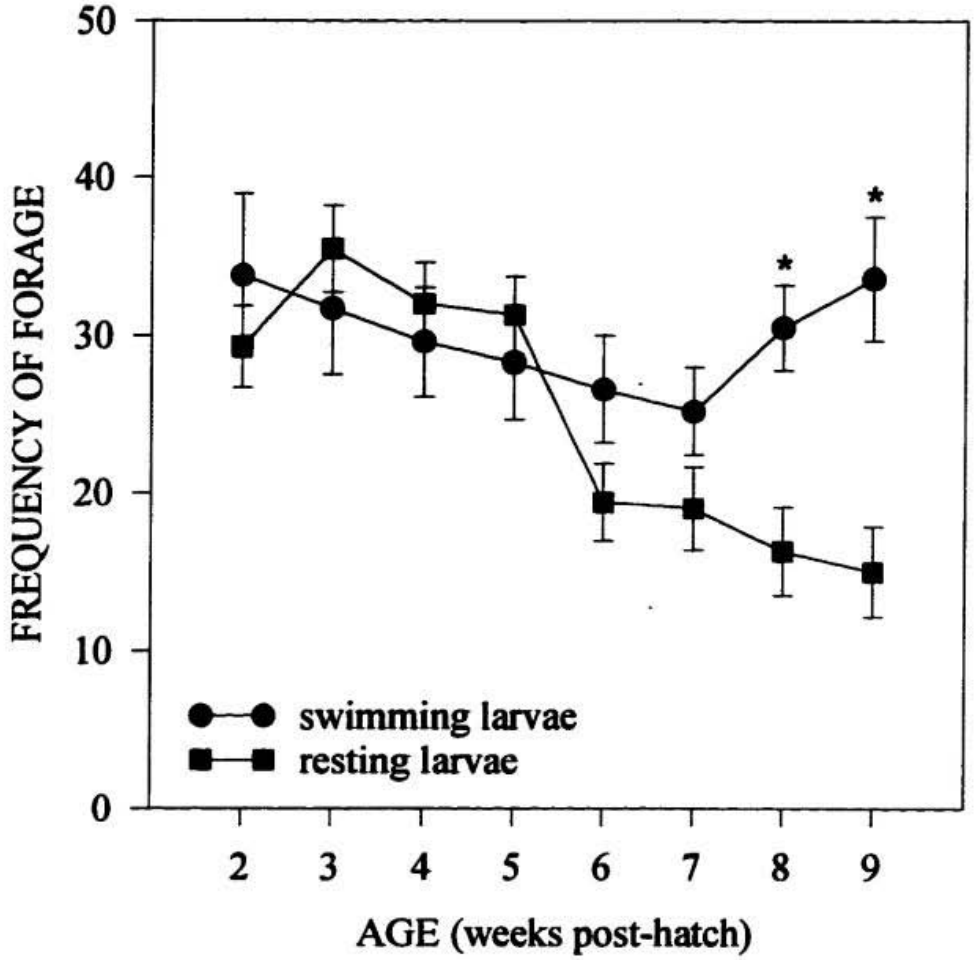

Figure 19: Mean weekly frequency (±se) of Forage in a 2 minute observational period of striped wolffish larvae swimming and resting. * indicates P <0.0031, Bonferroni correction (n=24 per week).

daily (Wiggins et al., 1986). Goldfish (*Carassius auratus*) larvae fed a combination of *Artemia* and dry feed showed better growth than larvae fed dry feed only. This combination also appeared to reduce cannibalism during the first few weeks (Kestemont, 1995). African catfish (*Clarias lazera*) fed trout starter and *Artemia* for 3 weeks showed 87% survival compared to 10% survival on trout starter alone. Even larvae fed for shorter periods on *Artemia* or even on frozen *Artemia* showed higher survival than those on only dry feed (Hogendoorn, 1980).

The survival of wolffish offered a high level of *Artemia* in the present study did not show the usual pattern of high mortalities through the critical period. Several studies (Moksness et al., 1989; Strand et al., 1995) have shown an increase in mortalities between 20 to 40 days post-hatch. In the present study, survival was still 94.3% after 9 Weeks. This indicates a successful switch from endogenous to exogenous feeding.

Behavioural observations showed that wolffish larvae are very successful (>90%) in capturing food items. Larvae fed high prey levels initially take more *Artemia* than dry diet. Overall foraging was initially higher in the high food tanks but the frequency decreased over the study. Those fed less *Artemia* maintained their level of foraging throughout the study. The greatest difference was seen among the swimming fish. All larvae were found to gradually increase their preference for dry food over time and, in effect, weaned themselves. In the high prey density tanks, swimming fish weaned onto the dry feed two weeks earlier (at week 6, 32.4 mm long), than swimming fish in the low prey density tanks (at week 8, 30.4 mm long). Resting fish in high food tanks weaned one week earlier than resting fish in the

low food tanks.. The earlier weaning is likely related to the faster growth of larvae fed more *Artemia*. More rapidly growing larvae are capable of ingesting and digesting the dry feed earlier than the smaller larvae.

Why does a higher level of *Artemia* increase survival and growth of larvae? There are several factors which may help explain this.

It may simply be that *Artemia* on their own are nutritionally inadequate as a first feed but in combination they compliment the dry feed. They may provide a good source of vitamins. For example vitamin C is important in the formation of collagen in connective tissue including cartilage, bone, and dermis. Whitefish (*Coregonis lavaretus*) larvae were found to store significantly more ascorbate (vitamin C) in the body during the first days of feeding when fed *Artemia* than when fed only commercial or lab prepared diets. Microparticulate processing techniques used in larval diet preparation contribute to vitamin C deterioration (Dabrowski, 1990).

The enriched *Artemia* may also provide a good source of highly unsaturated fatty acids or HUFAs. Asian seabass (*Lates calcarifera*) fed live food enriched with the HUFAs, 20:5ω-3 (eicosapentaenoic acid or EPA) and 22:6ω-3 (docosahexaenoic acid or DHA) were found to have a higher resistance to stress and successfully reached metamorphosis. Larvae fed HUFA deficient nauplii died before reaching metamorphosis (Dhert et al., 1990). Gilthead seabream (*Sparus aurata*) fed rotifers enriched with high levels of EPA and DHA showed better growth with a lower moisture content and higher total lipid levels than larvae

fed lower levels of these fatty acids (Koven et al., 1990). Studies on the HUFA requirements for wolffish would be useful in determining the best food for these fish.

The larvae may also utilize free amino acids (FAA) present in the live prey as an energy source. Studies on halibut (*Hippoglossus hippoglossus)* (Fyhn, 1989; Ronnestad et al., 1993) and cod (*Gadus morhua*) (Fyhn and Serigstad, 1987) larvae showed that the levels of FAA in larvae at hatch are greatly depleted soon after hatch without a net protein increase indicating that the FAA are used for something other than growth. Cod eggs were found to contain about 200 nmol of FAA at spawning which decreased by 175 nmol during the egg stage and first 5 days of larval life. The uptake of oxygen by the eggs was calculated to account for 85% of the oxygen required to catabolize the missing FAA (Fyhn and Serigstad, 1987). The digestive tract of many marine fish larvae is morphologically and functionally incomplete when the endogenous nutrients are nearing exhaustion and exogenous feeding begins (Fyhn, 1989). With the low proteolytic capacity at this time larvae may require an external supply of FAA until the intestine is adequately differentiated. Fyhn suggested that since marine invertebrates also contain high intracellular concentrations of FAA, the natural prey of marine larvae such as copepods are a likely source. If *Artemia* also contain high levels of FAA and wolffish larvae utilize them as an energy source, this may help explain the better results among larvae fed high levels of *Artemia*.

Artemia may also be involved with enzyme activity. In at least some marine species the pancreas is well developed at hatch. Digestive enzymes, including pepsin, trypsin, chymotrypsin, and amylase are usually present (Govoni et al., 1986). The activity of the

digestive enzymes appears to be low at first feeding with an increase before metamorphosis. There are two possible reasons for this increase in activity. The larvae may utilize enzymes naturally present in the ingested food (Dabrowski and Glogowski, 1977). Alternatively, the ingested food may stimulate the production of enzymes from the liver, pancreas, and mucosal epithelium (Govoni et al., 1986). Walford and Lam (1993) attributed digestion of rotifers in sea bass (*Lates calcalrifer*) to a combination of both of these possibilities. Larvae which are fed a low density of live food may not be receiving adequate amounts of enzyme or be feeding on enough *Artemia* to induce enzyme production. If this is the case, these larvae will not be capable of properly digesting the dry food once they are weaned. Behavioural results support this possibility since there was no difference between treatments in frequency of bites at dry pellets. The larvae in both treatments were consuming the same amount of dry food but those which had taken more *Artemia* were doing better.

The larvae may require the stimulation of prey items moving in the water column to induce feeding. Most marine larval fish feed on live feed, thus their visual (and other sensory systems) would be adapted to detect moving prey. At hatch, wolffish have large pigmented eyes and teeth that may function in grasping zooplankton or even small larvae of other fish.

The observations showed larvae weaned themselves onto the dry pellets instead of continuing to choose the *Artemia*. However there were no observations taken after all of the pellets were either eaten or had sunk to the bottom and were of little interest to the larvae. Analysis of gut contents of fish that died when the water shut off in Week 9 showed many with guts full of *Artemia*. This indicates that the larvae are still capturing the *Artemia* when

all the dry food is gone. It is possible that initially the movement of the swimming *Artemia* in the water column stimulates the larvae to feed. The relatively motionless floating dry pellets may not be viewed as prey. The larvae would have to learn by trial and error that the dry pellets are a food source. As the larvae grow and learn to eat the pellets, they may then cue in on the pellet particles more easily than *Artemia* when they are first added to the tanks.

The occurrence of larvae lying on their sides has been described in the literature as normal behaviour (Moksness et al., 1989). This study provides evidence that this behaviour is an indicator of morbidity since it peaked just prior to the peak in mortality. Fish fed the high concentration of *Artemia* were never observed in this position and these same fish showed few mortalities (94.3% survival). Fish that do not successfully switch to exogenous feeding would weaken as they exhaust their yolk reserves and may be unable to maintain an upright position when resting on the bottom.

For most of the study a higher percentage of larvae fed at the lower *Artemia* level were swimming as compared to those fed the higher level. This is likely a response to increase the chance of capturing food. The result in this case is that the larvae use more energy in search of food, resulting in slower growth and survival. Similar results were found for plaice (*Pleuronectes platessa*) larvae (Wyatt, 1972). Plaice make spasmodic, wriggling movements followed by periods of inactivity lasting several minutes. Fed larvae were found to decrease the duration of resting periods over time and maintain the duration of swimming periods. In comparison, starved larvae showed no change in the duration of resting periods and an increase in the duration of swimming periods. Munk and Kiørboe (1985) found that

swimming activity was 100% higher among larval herring (*Clupea harengus*) at low food densities than at the highest concentration. They also found that the water volume searched by larvae increased when prey density decreased.

By the end of the study more of the fish in the higher density tanks were swimming. This coincided with the occurrence of aggressive behaviour. The fish may have been attempting to settle into a more benthic phase and set up territories. With the small bottom area of the aquaria there did not seem to be enough room for all the fish to set up territories so many of the fish were forced off the bottom and had to stay in the water column. Earlier in the study the larvae could be seen lying very close to, and many times, over other larvae. Towards the end of the study, when the aggression began, the fish were evenly distributed on the bottom. Similar behaviour was described by Moksness (1989) in what he called the "first bottom stage." Wolffish measuring 50-100 mm spent most of their time on the bottom but swam regularly, especially when hungry. The larvae characteristically distributed themselves over the bottom with great distances between them and were aggressive to other fish coming too close. Pavlov et al. (1987) described wolffish measuring 25-30 mm switching to a pre-benthic lifestyle and occupying definite territories. It is also possible that as the larvae in the present study were weaned on dry food their demand for food began to exceed the amount added to the tanks and they began to attack the other larvae as a food source. No cannibalism was observed in this experiment but it did occur among fish from the same batch held in other tanks and in other studies (Moksness, 1990, Moksness et al., 1989).

CHAPTER 4.0 TEMPERATURE

4.1 INTRODUCTION

Water temperature can affect many aspects of the early life of fish. These effects include incubation time, size at hatch, yolk utilization efficiency, growth, feeding rates, time to metamorphosis, behaviour, swimming speed, digestion, gut evacuation, and metabolic demand (Blaxter, 1988).

Growth rate tends to increase with increasing temperature until the optimum is reached and then it decreases (Jobling, 1983). Polo et al. (1991) raised gilthead seabream (*Sparus aurata*) larvae at nine different temperatures ranging from 12-30°C. The optimum temperature range was found to be 16-22°C. Outside of this range, mortality increased as did developmental abnormalities, including wrinkled finfold, skeletal deformities, spinal curvature, and large pericardial cavity. Growth rate and yolk absorption rate increased with increasing temperature. However, conversion efficiency index (growth rate/yolksac concumption rate) was highest at 16°C. In a study by Hart and Purser (1995), greenback flounder (*Rhombosolea tapirina*) larvae were reared at 9, 12, 15, 16.5, and 18°C. Larvae raised at 18°C were smaller than those at 9, 12, and 15°C. Yolk absorption was found to be most efficient at 15°C. These larvae grew fastest and were the largest at the time of final yolk absorption.

Increased growth rate can reduce the time to metamorphosis for some species. Laurence (1978) found that cod (*Gadus morhua*) hatched and reared at 7°C reached metamorphosis at 52 days while those at 10°C metamorphosed at 44 days. Similarly winter

flounder (*Pleuronectes americanus* formerly *Pseudopleuronectes americanus*) larvae metamorphosed at 80 days at 5°C and at 49 days at 8°C (Laurence, 1975)

Water temperature may also have an indirect effect on fish larvae. Temperatures which are either too high or too low may stress the fish making them susceptible to disease. Another factor to consider is that oxygen becomes less soluble as water temperature rises. It is important to determine an optimum temperature for rearing larvae to help keep operating costs down while maximizing production. The objective of this study was to determine a suitable rearing temperature for wolffish larvae.

Studies have indicated that wolffish are capable of tolerating a range of water temperatures from 1.0-13.7°C (Moksness, 1994; Ringø et al, 1987; and Stefanussen et al, 1993). Most studies, however, involve juveniles and there is little information available on the effect of temperature on larval wolffish, especially through the mixed feeding stage. Also, most of the literature deals with fish from European waters which may require different growing conditions than fish from Newfoundland waters.

In this study I examined the effect of temperature on the growth and survival of larval wolffish through the "critical period," when larvae switch from endogenous to exogenous feeding.

4.2 MATERIALS & METHODS

Eggs were collected as described earlier, held at the Wesleyville Marine Finfish Hatchery, and incubated throughout the winter at ambient temperature. Intact egg masses

were held in perforated trays and incubated in small troughs with a constant supply of ambient seawater. Hatching began in late April, 1994.

The experiment was carried out in nine, 1 metre diameter by 0.5 metre high, circular dark green tanks enclosed in a room. Three temperature ranges were investigated in triplicate: 8.0-13.5°C (high), 4.0-7.8°C (low) and 3.0-13.5°C (ambient). The high and low temperature tanks were supplied by a recirculation system controlled by a Neslab unit. Water level was held at approximately 5 cm in each tank for a total volume of 40 litres. The fish were exposed to a 12hL:12hD cycle.

Five hundred and twenty-three newly hatched wolffish were transferred to each of the tanks. Due to the high number of larvae required, the tanks were stocked over a two week period as the larvae were hatching. The larvae were apportioned evenly to tanks as they became available. Day 0 of the study was determined when 50% of the fish were moved to the experimental tanks.

Larvae were initially fed enriched *Artemia* at a density of 900/l, as well as Lansy marine larval diet (W3, 300-500 μm) starting at a level of approximately 2% body weight per day. Feedings were hourly, between 9:00 a.m. and 5:00 p.m. Addition of *Artemia* was discontinued at approximately 4 weeks, when the larvae were observed to feed primarily on the dry feed (data from the weaning experiment had not been analyzed by this point). Larvae were weaned onto a larger pellet (N4, 500-800 μm) at 6 weeks. All tanks were siphoned clean of feces and excess food daily.

Samples of newly hatched larvae were taken from the four egg masses which supplied the experiment. Ten larvae from each of these masses (40 in total) were considered the hatch sample. Subsequent samples (10 per tank) were arbitrarily taken in three week intervals from the start of the experiment. Including initial sampling, the tanks were sampled five times. Lethal samples were taken using MS-222 to kill the fish. The fish were immediately rinsed in distilled freshwater and measured for standard length (to the nearest 0.5 mm) using a dissecting microscope. Larvae were then wrapped in pre-weighed foil, put in a cooler and immediately returned to the OSC for drying (48 hours at 90°C) and weighing.

In week 10 a sample was removed for a separate lipid analysis study (Halfyard, unpublished). A total of 46 fish from the high and low temperature tanks was sampled.

Mortalities were removed and counted daily. At the end of the experiment the surviving fish were counted after the last samples were taken. Mortality rate was determined by subtracting the fish sampled plus fish missing from the original total.

The data were square root transformed when necessary to meet the assumptions of the statistical tests. If data were still not normally distributed, they were then rank transformed. Effects of temperature and age on survival and growth were analyzed using a general linear model. In all analyses, a significant age*treatment interaction was found so a least squares means test with a Bonferroni corrected P-value was used to test significance at each age.

4.3 RESULTS

4.3.1 TEMPERATURE

In the high temperature tanks the temperature remained constant, approximately 8.0°C, up to week 6 (Fig. 20). Due to abnormally high ambient water temperatures in 1994, problems were encountered with the Neslab unit. Loss of temperature control in the high temperature tanks started in Week 6. The temperature in the high temperature tanks after Week 6 followed the ambient temperature and increased to a mean of 13.5°C in week 12. In the low temperature tanks the mean temperature in the first five weeks remained between 4.0-6.0°C and then increased to less than 7.8°C (Fig. 20). Mean ambient temperature increased from 3.0-13.5°C over the experiment.

4.3.2 SURVIVAL

Percent survival levelled off in all three treatments after week 6 (Fig.21). By the end of the study, survival in the high temperature tanks was significantly higher ($P<0.0014$, Bonferroni correction) than both low temperature and ambient tanks at 50.5±1.65% (Table 10a,10b). Survival in the low temperature tanks (16.0±2.33%) was significantly higher ($P<0.0014$, Bonferroni correction; Table 10a,10b) than in the ambient tanks (6.8±0.82%).

4.3.3 GROWTH

No significant tank effect was found for growth ($P> 0.05$). The initial size of larvae at the start of the experiment was 21.3±0.13mm (Fig. 22, Table 11). The high temperature

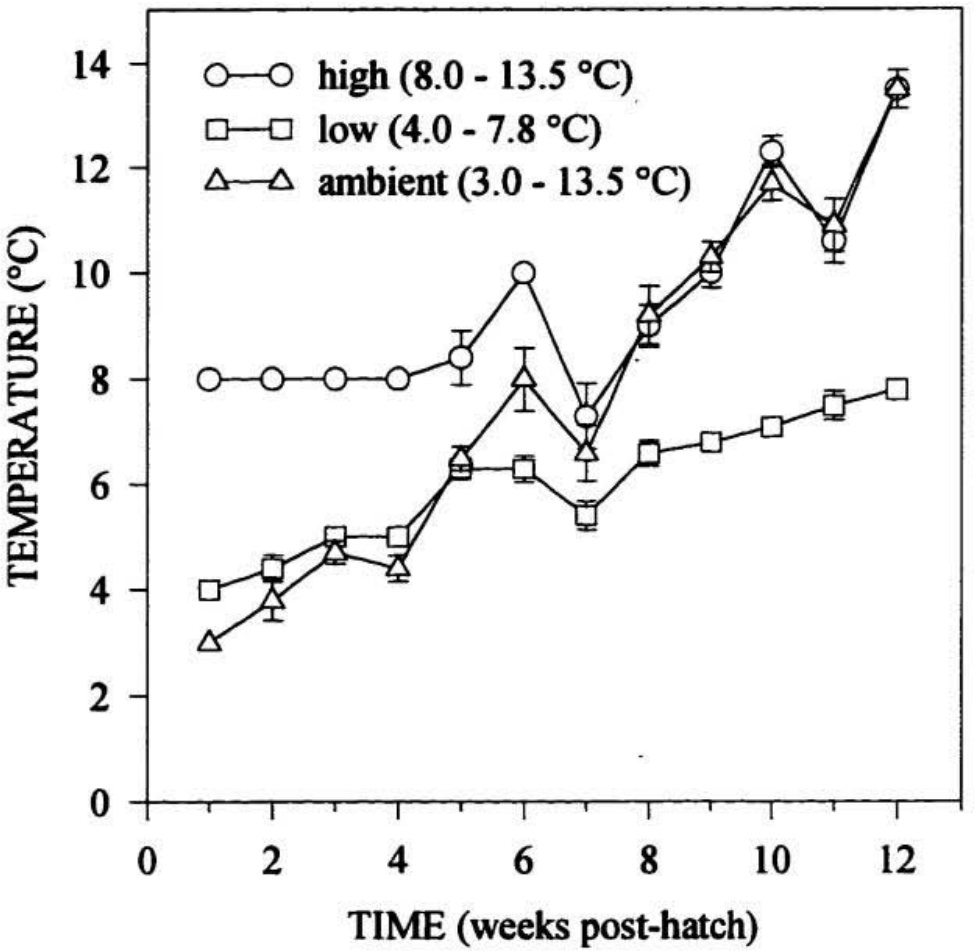

Figure 20: Mean weekly temperature (°C) for striped wolffish larvae held at 3 temperature ranges over 12 weeks. Note: temperature control was lost in high tanks from Week 6 onwards.

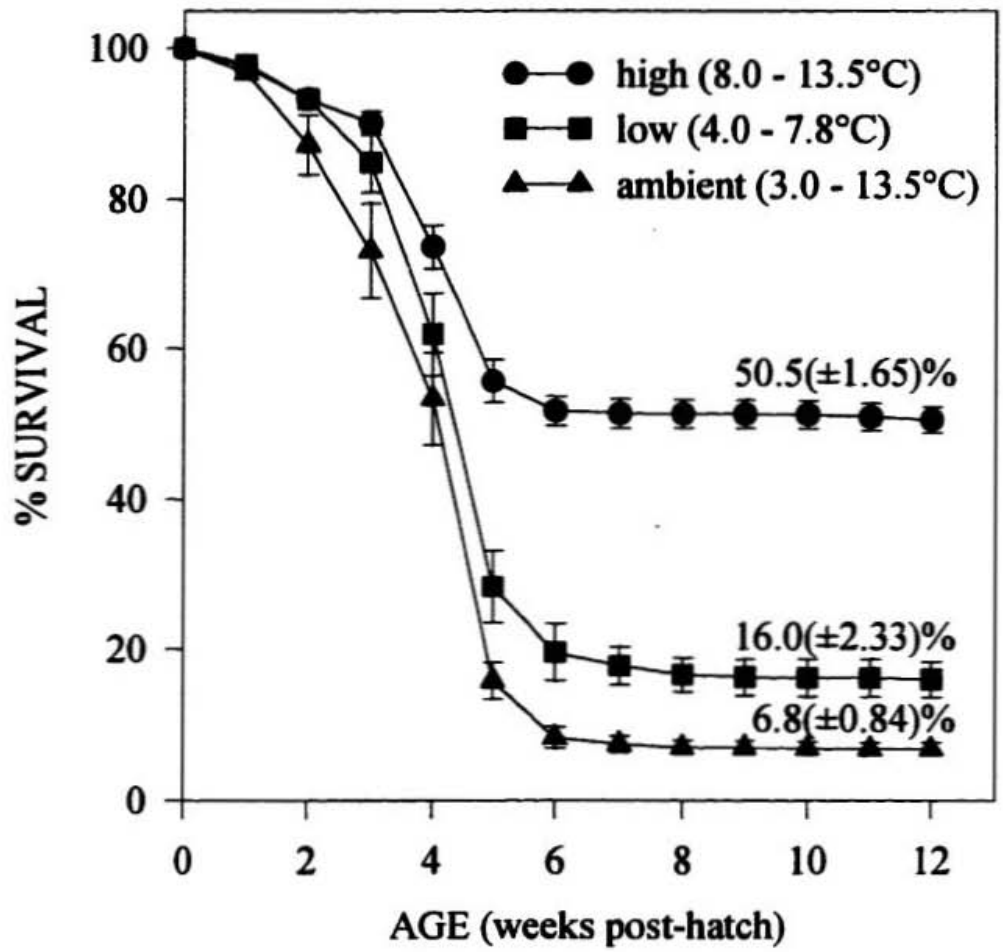

Figure 21: Weekly percent survival (±se) of striped wolffish larvae held at 3 different temperature ranges over 12 weeks.

Table 10a: Results of ANOVA for effects of age (in weeks) and temperature on percent survival of wolffish larvae held at 3 temperarure ranges.

ANOVA Source	F	df	P
Treat	554.89	2,72	0.0001
Age	266.66	11,72	0.0001
Treat*Age	17.26	22,72	0.0001

Table 10b: Probabilities from least squares means analysis, for effects of age (in weeks) and temperature on percent survival of wolffish larvae held at 3 temperarure ranges. * indicates a significant difference (P <0.0014), Bonferroni correction).

Least Squares Means Comparison	Week 1	2	3	4	5	6	7	8	9	10	11	12
High/Low	0.9636	0.9910	0.3956	0.0327	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
High/Ambient	0.9105	0.3354	0.0049	0.0002*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Low/Ambient	0.8744	0.3410	0.0439	0.0845	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*

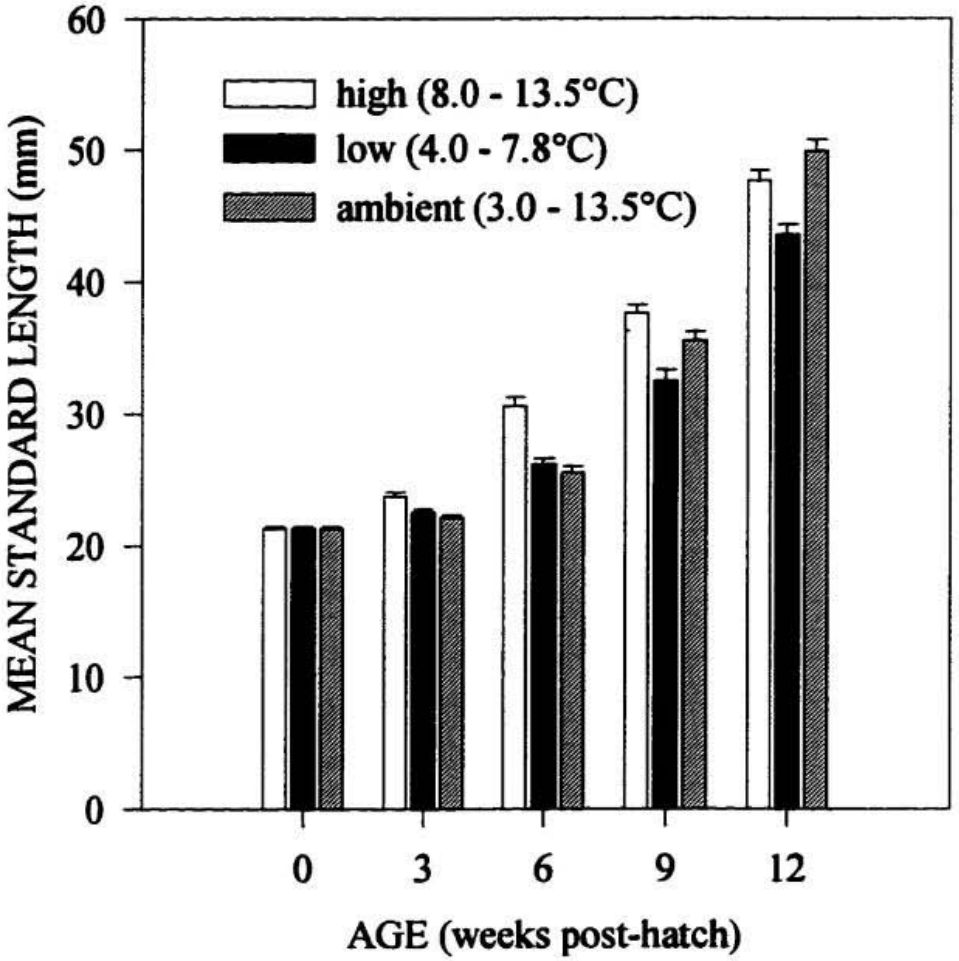

Figure 22: Mean standard length (mm +se) of striped wolffish larvae held at 3 different temperature ranges over 12 weeks (n=30 except n=40 at age=0).

Table 11: Mean standard length (mm) and dry weight (mg) (±se) of striped wolffish larvae held at 3 temperature ranges.

Age (weeks post-hatch)	High (8.0 - 13.5°C) Standard Length	Dry Weight	Low (4.0 - 7.8°C) Standard Length	Dry Weight	Ambient (3.0 - 13.5°C) Standard Length	Dry Weight
0	21.3 ±0.13	12.3 ±0.16	21.3 ±0.13	12.3 ±0.16	21.3 ±0.13	12.3 ±0.16
3	27.3 ±0.36	15.2 ±1.02	22.5 ±0.22	11.5 ±0.76	22.1 ±0.19	9.7 ±0.53
6	30.6 ±0.71	38.5 ±2.95	26.1 ±0.45	23.5 ±1.80	25.5 ±0.46	21.9 ±1.65
9	37.6 ±0.71	87.3 ±5.12	32.5 ±0.86	57.2 ±5.11	35.6 ±0.65	79.9 ±5.43
12	47.7 ±0.75	209.3 ±11.95	43.6 ±0.79	163.7 ±10.95	49.9 ±0.91	261.2 ±15.37

larvae grew faster than the low temperature larvae and were significantly longer from weeks 3 to 9 ($P<0.0042$; Table 12). In weeks 3 and 6 the high temperature larvae were also significantly longer than larvae raised at ambient temperature ($P<0.0042$, Bonferroni correction; Table 12). Ambient larvae were significantly longer ($P<0.0042$, Bonferroni correction) than those at low temperature in weeks 9 and 12. On the last sampling day in week 12 high, low, and ambient temperature fish were 47.7±0.75 mm, 43.6±0.79 mm, and 49.9±0.91 mm in length respectively.

Initial dry weight was 12.3±0.16 mg (Table 11). Dry weight (Fig.23) decreased for both low and ambient fish during the first 3 weeks. Dry weight for high temperature fish was significantly higher than that for low temperature fish from week 6 onwards ($P<0.0042$, Bonferroni correction; Table 12). The relationship between dry weight of high and ambient fish changed throughout the study. In week 6 high temperature fish were significantly heavier but in week 12 the opposite was true. Ambient fish also had a significantly higher dry weight than fish raised at the low temperature in weeks 9 and 12 ($P<0.0042$, Bonferroni correction). By week 12 high, low, and ambient fish weighed 209.3±11.95 mg, 163.7±10.95 mg, and 261.2±15.37 mg respectively.

From Week 0 to 3 SGR based on both standard length (Fig. 24) and dry weight (Fig. 25) was highest in fish from the high temperature tanks. SGR based on dry weight was negative for both low and ambient fish in the first 3 weeks (Table 13).

Table 12: Results of ANOVA and probabilities from least squares means analysis, for effects of age (in weeks) and temperature on standard length (mm) and dry weight (mg) of striped wolffish larvae held at 3 temperature ranges. * indicates a significant difference (P <0.0042), Bonferroni correction).

		ANOVA			Least Squares Means by week				
Growth Parameter	Source	F	df	P	Comparison	3	6	9	12
Standard Length	Treat	35.01	2,342	0.0001	High/Low	0.0019*	0.0001*	0.0001*	0.0044
	Age	824.28	3,342	0.0001					
	Treat*Age	6.58	6,342	0.0001	High/Ambient	0.0001*	0.0001*	0.0582	0.2295
					Low/Ambient	0.3536	0.2188	0.0008*	0.0001*
Dry Weight	Treat	24.03	2,342	0.0001	High/Low	0.2302	0.0019*	0.0001*	0.0001*
	Age	786.07	3,342	0.0001					
	Treat*Age	9.68	6,342	0.0001	High/Ambient	0.0727	0.0005*	0.2791	0.0001*
					Low/Ambient	0.5502	0.6935	0.0004*	0.0001*

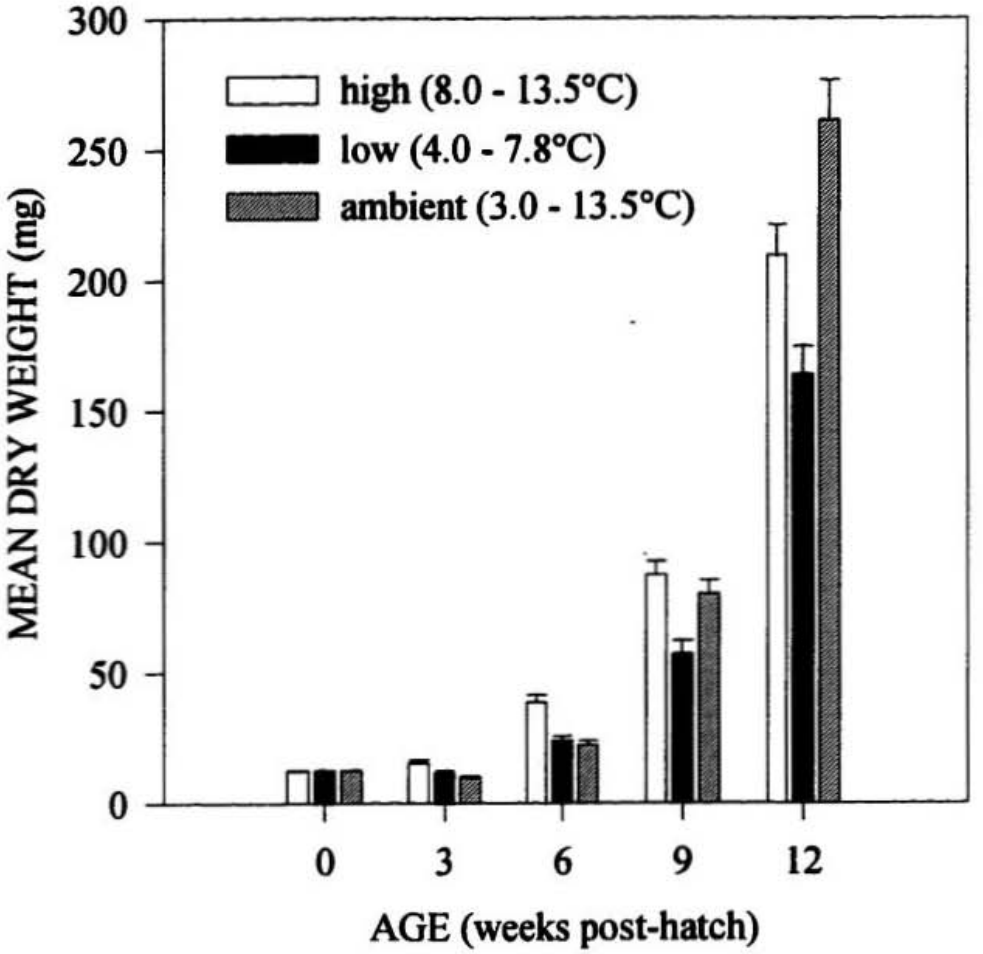

Figure 23: Mean dry weight (mg ±se) of striped wolffish larvae held at 3 different temperature ranges over 12 weeks (n=30 except n= 40 at age=0).

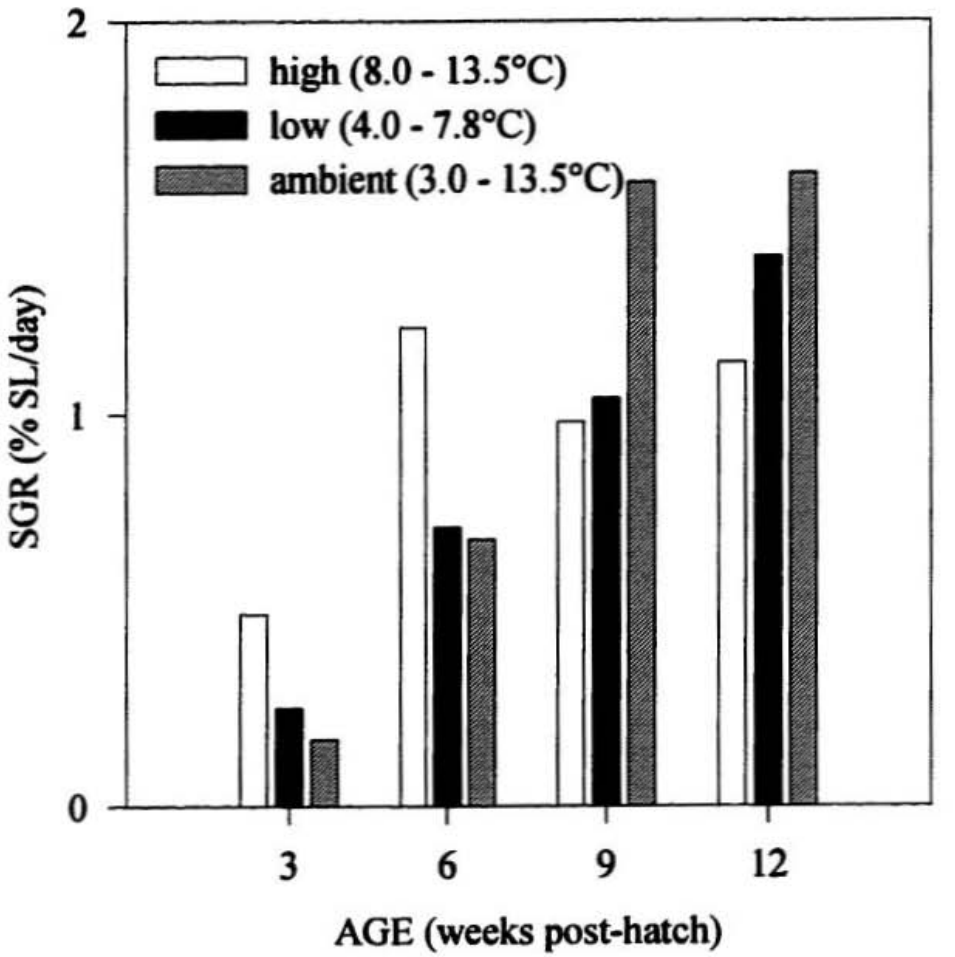

Figure 24: Specific growth rate (%/day) calculated from standard length of striped wolffish larvae held at 3 different temperature ranges over 12 weeks.

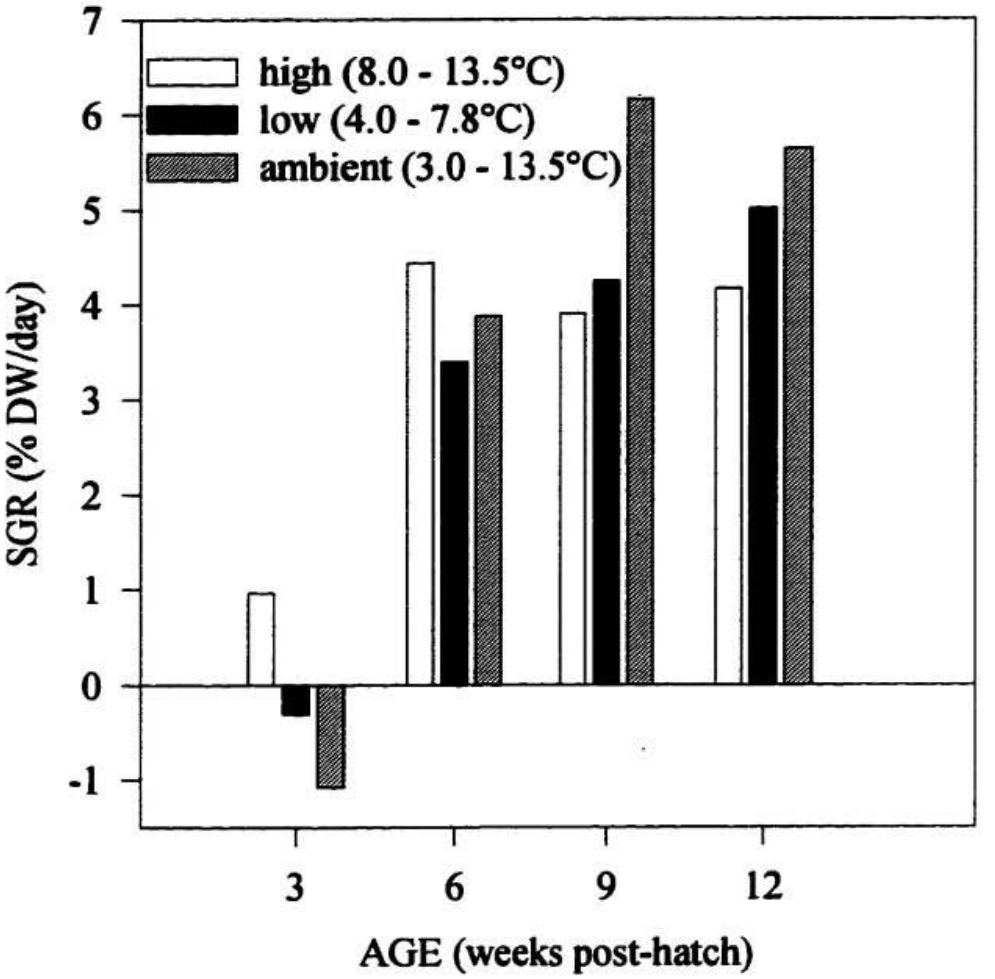

Figure 25: Specific growth rate (%/day) calculated from dry weight of striped wolffish larvae held at 3 different temperature ranges over 12 weeks.

Table 13: Specific growth rates (%/day) of striped wolffish larvae held at 3 temperature ranges, calculated from standard length and dry weight.

Age (weeks post-hatch)	High (8.0 - 13.5°C)		Low (4.0 - 7.8°C)		Ambient (3.0 - 13.5°C)	
	Standard Length	Dry Weight	Standard Length	Dry Weight	Standard Length	Dry Weight
0-3	0.49	0.96	0.25	-0.31	0.17	-1.08
3-6	1.22	4.43	0.71	3.40	0.68	3.88
6 - 9	0.98	3.90	1.04	4.24	1.59	6.16
9 - 12	1.13	4.16	1.40	5.01	1.61	5.64

4.4 DISCUSSION

The results of this experiment demonstrate that temperature has an effect on wolffish survival from hatch through the first 6 weeks. Survival was relatively unaffected by temperature over the last 6 weeks. Even with the increase in temperature in the high and ambient temperature tanks mortality had virtually ceased at all temperatures. In accordance with previous studies, major mortality between 20 and 40 days would indicate unsuccessful transition from endogenous to exogenous feeding. Temperature had a clear effect on the survival rate through this period of mixed feeding. Fish held at low temperatures had the lowest rates of survival, while over half of those held at 8.0-13.5°C survived.

Larvae held at the highest temperature range were longer and heavier than those raised in the lower and ambient temperature tanks at 6 weeks. It is likely that these larvae fed more and utilized these food sources more efficiently during this period of mixed feeding. Paul (1983) found that pollock (*Theragra chalcogramma*) larvae reared at 5°C were more successful at capturing copepod nauplii than those reared at 3°C when fed at low prey densities. Tandler et al. (1989) found the rate of feeding on rotifers and *Artemia* in gilthead seabream (*Sparus aurata*) to be positively correlated with exposure to a high temperature regime. In another study newly hatched goldfish (*Carassius auratus*) larvae were raised at 20, 24, and 28°C and fed a mixed diet of *Artemia* and dry feed. Food utilization, assessed as feed:gain ratio, protein efficiency ratio, and apparent net protein utilization, increased at the higher temperatures studied (Kestemont, 1995)

It is difficult to determine if yolk utilization was affected by temperature in this study. Calculation of yolk sac volume using height and length of the yolk sac would have been difficult if not impossible due to the dark pigmentation of the larvae. Yolk utilization has been found to increase with increasing temperature for many marine species, including summer flounder (*Paralichthys dentatis*) (Johns et al., 1981) and yellowtail flounder (*Pleuronectes ferrugineus* formerly *Limanda ferruginea*) (Howell, 1980). Yolk utilization efficiency tends to be highest in an intermediate range of temperatures within a larva's zone of temperature tolerance (Johns et al., 1981).

Growth was faster in the high temperature tanks throughout most of the experiment. Although the temperatures were similar, the ambient fish were larger than the high temperature fish at the end of the experiment. This is likely due to the low densities in the ambient tanks compared to the high temperature tanks. Aggression and cannibalism was observed during the experiment so lower densities may have resulted in less competition for food and space.

The SGR during the first 3 weeks following hatch was highest for fish held at higher temperatures, indicating that they began feeding soon after hatch. Negative SGRs (dry weight) for both low temperature and ambient fish indicate that the fish were not feeding at a level high enough to meet metabolic needs. SGR was lower for larvae held at the high temperature range after week 6 but continued to increase for larvae at the low temperature range. This may indicate that temperatures above 8.0°C are too high for optimum growth.

SGR was highest for larvae in the ambient tanks but densities were very low in these tanks by this point.

Combining the high survival in the high temperature tanks with the high survival in the weaning study carried out at 4-7°C, the temperature through the first 6 weeks should be within 4-8°C. After 6 weeks the higher temperatures appear to lower growth rates. Evidence suggests that temperatures should not go much higher than 8°C after 6 weeks however further study is needed before any conclusions can be made.

CHAPTER 5.0 CONCLUSIONS

The feeding and temperature studies reported in this thesis have provided useful information on the early rearing of wolffish larvae. Striped wolffish are very well developed at hatch compared to most marine larvae and capable of feeding when only days old. A first-feeding diet of only *Artemia* is inadequate and larvae do much better on a combined diet of *Artemia* and dry feed from hatch. The level of *Artemia* is important, as higher levels result in faster growth, higher survival, and earlier weaning. Temperature has the most effect in the first 6 weeks of larval life. During the first 6 weeks larvae held at about 8°C showed the best growth and survival. During the last 6 weeks temperature had no effect on survival but growth rates decreased at higher temperatures. Taking into consideration the results in the weaning study (4-7°C), rearing temperature should be between 4-8°C for the first 6 weeks. After 6 weeks temperature should probably go no higher than 8°C but further investigation into this area is required.

A feeding strategy for larval wolffish should include:

- start feeding within the first few days of hatch.
- feed enriched *Artemia* at a level of 900/l at least twice a day (depending on flow rates and tank design).
- add a small amount of dry feed (300-500μm) to the tanks at the same time *Artemia* is added.
- observe feeding behaviour and increase level of dry feed accordingly.

- do not decrease *Artemia* levels until about 6 weeks post-hatch or about 30 mm in length when they should be observed ingesting mostly dry feed.

- increase the number of feeding times as the larvae wean onto the dry feed to help prevent aggression and cannibalism.

- keep water temperature at 4-8°C especially in the first 6 weeks.

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