FOOD AND FEEDING REQUIREMENTS OF JUVENILE STRIPED WOLFFISH (Anarhichas lupus)

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by

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Abstract

The striped wolffish (*Anarhichas lupus*) is a candidate species for commercial aquaculture in Newfoundland. It possesses a number of characteristics which facilitate culture, such as large eggs, well developed larvae which readily accept formulated feeds and tolerance to low temperatures. Little research has been conducted to determine the dietary requirements of the juvenile wolffish or the optimum stocking density and feeding frequency.

The effects of three feeding frequencies (two meals/day, one meal/day and one meal/two days) on various growth parameters were investigated. Mean meal size was significantly and inversely affected by the feeding frequency. In addition, total feed consumption over time was directly affected by the meal frequency. The specific growth rate (SGR) was not adversely affected by the decrease in meal frequency or feed intake. Feed and labour costs, therefore, may both be reduced by lowering the frequency, without compromising the SGR.

The stocking density also affected the feed consumption. The smallest mean meal size (3.990 mg/g fish) was consumed by fish stocked at 80 g/L. The largest meals were consumed by fish stocked at 50 g/L (4.955 mg/g) while the meal size of fish stocked at 20 g/L was in between these values. The feed conversion ratio (FCR) decreased significantly when the stocking density was greater than 50 g/L and the protein efficiency ratio (PER) was significantly higher when the stocking density was greater than 50 g/L.

The dietary energy balance, expressed as the protein energy:total energy ratio (PE:TE) had a significant negative influence on the intake of feed, lipid and energy. As the PE:TE increased, the feed, lipid and energy intakes all decrease significantly. The PE:TE had no significant impact on the FCR or PER at either constant 9 °C or ambient temperatures (13.0 °C to 2.0 °C). The production cost (based on feed costs per kilogram of fish produced) was not significantly affected by the PE:TE or by decreasing temperatures.

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1.0 Introduction

The striped wolffish is one of a number of cold-adapted species being investigated for its aquaculture potential in the coastal areas of the North Atlantic, including Newfoundland. Wolffish belong to the family Anarhichadidae and are native to the North Atlantic and Pacific Oceans (Hubbs and Barnhart, 1944; Wilimovsky, 1964). The three species that inhabit Newfoundland waters are the striped or Atlantic wolffish, *Anarhichas lupus* L., the spotted wolffish, *A. minor* Olafsen, and the northern wolffish, *A. denticulatus* Kroyer (Albikovskaya, 1983). All three are common bycatches in otter trawls and gillnets, but the northern wolffish, also known as the jelly cat, is discarded due to poor flesh quality (Templeman, 1966; 1984). The spotted wolfish is the least common of the three. It rarely inhabits water less than 50 m deep or warmer than 5 °C (Albikovskaya, 1982). The frequency of capture of northern wolffish in trawls increases as depth increases, between 151 and 600 m, with some reports of capture in water up to 750 m deep. Northern wolffish are most frequently captured in water colder than 5 °C (Albikovskaya, 1982). *Anarhichas lupus*, also known as the common wolffish, is most commonly found in water up to 350 m deep and colder than 4 °C (Albikovskaya, 1982).

1.1 Biological Adaptations

Wolffish display a range of adaptations to a cold, inshore habitat which are of particular interest to aquaculturists. These include tolerance to cold temperatures by employment of an anti-freeze protein, large eggs, advanced development of larvae at hatching and a relatively minor metamorphosis.

1.1.2 Temperature Tolerance

Tolerance to low water temperatures is of concern to aquaculturists, especially those in northern locales. Ambient seawater temperatures around Newfoundland regularly fall

below 0 °C during the winter. Those farming a species which cannot tolerate such temperatures are obliged to heat the water, adding considerable expense to their operation. An option is to identify a species which not only survives low temperatures, but maintains a reasonable growth rate. One such species is the wolffish.

1.1.2.1 Eggs and Larvae

Temperature tolerance has been investigated by a number of workers. Pavlov and Moksness (1994b) compared striped wolffish egg development at six temperatures ranging from 5 °C to 15 °C. They showed a decreased proportion of normally cleaved eggs at temperatures of 11 °C and higher. A subsequent study examining four temperatures ranging from 9.9 °C to 15.7 °C led to the conclusion that 12.8 °C is likely the upper temperature limit for the incubation of wolffish eggs (Pavlov and Moksness, 1994b). Incubation of fertilized eggs at 1.0, 3.0 and 4.8 °C demonstrated that the lower temperature limit for proper development is likely 3.0 °C (Pavlov and Moksness, 1994b). The effects of temperature on hatching success were studied by maintaining two groups of eggs at a constant temperature of 1 °C or 3 °C and another at 6.5 °C. Approximately two weeks before hatching the eggs held at 6.5 °C were changed to 7 °C or 10 °C water. Those eggs held at 7 °C and 10 °C could not hatch without mechanical pressure on the eggs, so 7 °C was determined to be the upper limit for successful hatching (Pavlov and Moksness, 1994b). The hatchability of eggs incubated at 1 °C and 3 °C was 98.0% and 91.0%, respectively. This is not unlike Atlantic salmon which have a lower incubation limit between 0 °C and 1 °C (Wallace and Heggberget, 1988).

1.1.2.2 Juveniles and Adults

Few temperature tolerance studies have been conducted with juveniles and adults. Moksness (1994) collected wolffish fry in the Barents Sea and maintained them in tanks at ambient temperature for a minimum of 54 months. Based on temperature records and

data from monthly weighings, he concluded that the optimum temperature for wolffish culture is between 7 °C and 9 °C. In the wild, wolffish live at temperatures between -1.9 °C and 9 °C, but generally inhabit water cooler than 4 °C (Albikovskaya, 1982). This temperature tolerance range differs from Atlantic salmon which tolerate temperatures between 5.5 °C and 24 °C but grow most effectively between 10 °C and 17 °C (Piper *et al* 1986). The optimal temperature range for wolffish falls within normal ambient temperatures for the Northwest Atlantic Ocean.

1.1.3 Fecundity

An understanding of the physiology of reproduction in wolffish is important for establishing and maintaining productive broodstock. Wolffish fertilization is internal, so the motility of undiluted sperm can last for several days (at 0 to 4 °C) while the known duration of viability is approximately 10 hours (Pavlov, 1994; Moksness and Pavlov, 1996). Salmon sperm are viable for a minute or less (Pavlov and Moksness, 1994b). Wolffish produce a small amount of sperm, which is less dense than that of Atlantic salmon (Kazakov and Obrazsov, 1990; Pavlov and Radzikhovskaya, 1991). Two main reproductive strategies are evident in nature. The r-strategy, evident in pelagophilous fish such as cod, involves producing large numbers of young and providing little or no parental care. The k-strategy, common to Atlantic salmon (*Salmo salar*), lumpfish (*Cyclopterus lumpus*) and wolffish, involves the production of smaller number of young, with a greater degree of parental investment (Soin *et al.*, 1986). This investment, in the case of fish, may come as a large egg with ample yolk reserves, or care in the form of guarding of the nest and free-swimming young. This is in marked contrast to the strategy of producing hundreds of thousands to millions of pelagic eggs and releasing them for distribution by ocean currents. Turbot (*Scophthalmus maximus*), for example, produce 1 million eggs per kilogram annually while wolffish and Atlantic salmon produce roughly 2000 eggs per kilogram annually (Tilseth, 1990; Pavlov and Moksness, 1994b). Male wolffish guard the egg masses and ensure sufficient water circulation through the mass

(Keats *et al.*, 1985; Ringø and Lorentsen, 1987). Pavlov and Moksness (1995) determined the incubation period (fertilization to 50% hatch) in days (y) to be approximated by the equation:

$$y = 425.28 - 77.875x + 6.584\,x^2 - 0.20325\,x^3, \qquad (1)$$

where x is the temperature (°C). Wolffish larvae hatch at an advanced stage of development, ready for exogenous feeding and closely resemble small adults. Establishment of husbandry conditions for successful reproduction of captive broodstock will eliminate the need for yearly collection of wolffish egg masses.

1.1.4 Egg Size and Larval Development

For aquaculture purposes, eggs should be large and produce well developed larvae (Soin *et al.*, 1986; Tilseth *et al.*, 1992). The size of wolffish eggs facilitates management and handling associated with incubation and wolffish larvae hatch at a more advanced stage of development than do most marine fish (Table 1.1). Halibut larvae (*Hippoglossus hippoglossus*), for example, are still embryos when they hatch, having no functional eyes, jaws or gut (Pittman *et al.*, 1990). Wolffish begin exogenous feeding on *Artemia* within hours of hatching, although start-feeding within six days of hatch is normal (Pavlov and Novikov, 1986). Wolffish readily wean on to artificial feeds (Moksness *et al.*, 1989). In fact, Strand *et al.* (1995) start-fed larvae using a floating, formulated feed in a shallow raceway and had 82% survival to 60 days post-hatch. Yellowtail flounder larvae (*Limanda ferruginea*) begin exogenous feeding at 4-5 days post-hatch and require copepod nauplii <100 μm in size (Smigielski, 1979). They gradually wean onto larger organisms (adult copepods, rotifers and *Artemia* sp.) as they grow and metamorphose (Smigielski, 1979). The metamorphosis between the larval and juvenile stage is very minor in wolffish. Known as direct ontogeny, this characteristic is desirable in potential

Table 1.1: Comparison of egg diameter (mm) and body lengths (mm) at hatch for several cold-water marine fish species.

Species	Egg Diameter (mm)	Length at Hatch (mm)	Source
Atlantic Cod		4.4 ± 0.1[1]	Gotceitas and Brown, 1993
(Gadus morhua)	1.2 - 1.6	3.5 - 4.0	Tilseth, 1990
	1.1 - 1.4	3.6	Rosenlund *et al.*, 1993
Atlantic Salmon[2]	5.0 - 6.0	15.0 - 20.0	Tilseth, 1990
(Salmo salar)	4.6 - 6.6		Thorpe *et al.*, 1984
Halibut		6 - 7	Pittman *et al.*, 1990
(Hippoglossus hippoglossus)	3.0 - 3.5	6.5 - 7.0	Tilseth, 1990
Lumpfish	2.6 ± 0.3	5.8 ± 0.3	Brown *et al.*, 1992
(Cyclopterus lumpus)			
Ocean Pout			
(Macrozoarces americanus)	8.5 ± 0.2	39.2 ± 0.7	Brown *et al.*, 1992
Turbot	0.9 - 1.2	2.7 - 3.0	Tilseth, 1990
(Scophthalmus maximus)			
Wolffish	4.7 - 5.1	21	Pavlov and Moksness, 1994a,b
(Anarhichas lupus)	4.7 - 6.0	20	Pavlov and Novikov, 1986
	5.5 - 6.0	20	Tilseth, 1990
Yellowtail Flounder		2.75	Smigielski, 1979
(Limanda ferruginea)		2.66	Laurence and Howell, 1981
	0.79 - 1.01		Colton and Marak, 1969
	0.68 - 0.76	2.1 - 7.0	Tilseth, 1990

[1] 5 to 7 days post-hatch

[2] Atlantic salmon are anadromous, spending their adult lives in seawater, except for spawning migrations into freshwater streams and rivers.

aquaculture species since metamorphosis may cause many mortalities (Balon, 1985; Soin *et al.*, 1986; Tilseth, 1990).

Reports of cannibalism in wolffish exist (Moksness, 1990), but are not commonplace (Pavlov and Novikov, 1986; Ringø *et al.*, 1987; Moksness *et al.*, 1989). Cannibalism appears to be linked to low stocking densities and to a large size gradient among fish in a tank (Moksness and Pavlov, 1996).

1.1.5 Growth

Juvenile wolffish have been raised in Norway at an ambient temperature ranging from 6.6 ° to 11.7 °C. They were hand-fed moist or dry pellets, with a protein energy: total energy (PE:TE) between 0.50 and 0.63 (Stefanussen *et al.*, 1993). Those fish fed dry pellets had a significantly higher growth rate than those fed moist pellets (0.26 g/day and 0.15 g/day, respectively: Stefanussen *et al.*, 1993). Wolffish larvae have exhibited maximum specific growth rates (SGR) up to 3.34% BW/day during the first 108 days post-hatch and up to 3.6% BW/day from 100 to 150 days post-hatch at ambient temperatures (Moksness *et al.*, 1989). These growth rates were achieved using three dry diets and different feeding regimes and weaning schedules. At optimum rearing conditions, (below 8 °C) Moksness *et al.* (1989) claimed that striped wolffish could reach 2.5 kg within 2 years of hatching.

1.2 Culture Methods

The striped wolffish, with its lean, fine-textured flesh, tolerance to low water temperatures and its hardy, well-developed larvae, is a prime candidate for culture in Newfoundland (Soin *et al.*, 1986; Seafood Leader, 1991). The challenge lies in determining the husbandry practices which will maximize the health, growth and reproductive capabilities of the fish and, ultimately, the profitability of the aquaculture

venture. The egg rearing protocol and the care of larvae are well established and researchers in Newfoundland have achieved 93.5% survival of larvae through metamorphosis using high light intensities, altered photoperiods and artificial feeds (Wiseman and Brown, 1996). The next step is determining the protocol for rearing juveniles.

1.2.1 Stocking Density

Wolffish live solitary lives, normally inhabiting rocky crevices, except when they pair up during spawning season (Moksness and Pavlov, 1996). This raises questions about the potential to culture this species at economically viable stocking densities. Some species do not adapt well to high stocking densities. Very high stocking densities can lead to aggression, increased risk of disease and poor growth rates (Keenleyside and Yamamoto, 1962; Fenderson and Carpenter, 1971; Baker and Ayles, 1990). However, Arctic charr reared at high stocking densities have higher mean weights and lengths than those reared at lower stocking densities under the same conditions (Brown *et al.*, 1992). This may result from the reduction of antagonistic interactions between fish and the decreased time spent swimming, thus decreasing the expenditure of energy.

1.2.2 Diet

In its natural habitat, the striped wolffish consumes a variety of prey similar to those consumed by spotted wolffish (*A. minor*) (Albikovskaya, 1983). These include *Lithodes* spp. and *Hyas* spp. (Decapoda), sea stars (Asteroidea), brittle stars (Ophiura), *Strongylocentrotus* spp. and sand dollars (Echinoidea), clams (Bivalvia) and whelks (Gastropoda) (Albikovskaya, 1983; Templeman 1986). Indigestible body armour protects many of these prey items. Wolffish teeth cannot grind down this skeletal matter, so they crush it to facilitate swallowing. Orlova *et al.* (1989) provided wolffish with meals of natural prey items, some with the shells removed. From their observations of gut passage

times they concluded that, generally, the more indigestible matter a meal contained, the faster it passed through the stomach and intestine. However, when a food bolus passed quickly through the intestine the digestible components often passed undigested.

Feeding natural food is not an option in the context of intensive culture, therefore, it is necessary to develop and evaluate formulated feeds. While there have been a number of studies of larval wolffish nutrition, there have been few investigations of the dietary requirements of juvenile and adult wolffish (Ringø *et al.*, 1987; Moksness *et al.*, 1989; Orlova *et al.*, 1989 and Moksness, 1990). These studies generally used natural prey, such as *Artemia salina*, scallops, mussels, squid, shrimp and echinoderms or commercial salmon feed. Some researchers have formulated their own feeds (Moksness, 1990), but the precise dietary requirements of wolffish are not yet known. Studies of other marine carnivorous fish have shown requirements for minimum dietary levels of 50-60% protein, 10-20% lipid, and maximum levels of carbohydrates, ash and fiber 10-20%, 4%, and 10-25%, respectively on a dry matter basis (Tucker, 1992; Wilson, 1994).

1.2.3 Protein Requirements

Carnivorous fish species have high requirements for dietary protein (Tucker, 1992). Atlantic salmon (*Salmo salar*), for example, require 45% of the dry diet as protein (Lall and Bishop, 1977). Plaice (*Pleuronectes platessa*) fed six diets with a range of protein from 20 to 70% (dry basis) demonstrated optimum weight gain when fed a diet containing 50% protein (Cowey *et al.*, 1972). Animal proteins offer the most nutritionally complete spectrum of amino acids so they are the most commonly used protein. However, they are very expensive and drive up the cost of feeds, so measures to decrease feed costs are needed. First, since excess protein is metabolized for energy or deaminated and excreted, it is wise to determine the protein requirements of the species in question to avoid wastage due to inefficient use. Adron *et al.*, (1976) demonstrated with turbot (*Scophthalmus maximus* L.) that at comparable energy levels, a diet with a lower

protein content is superior to a diet with a higher protein content. Therefore, it appears that the crucial factor is the ratio of protein energy to the total energy (PE:TE). Carnivores digest carbohydrates inefficiently. The required enzymes for carbohydrate metabolism are present, but in very low quantities, compared to herbivorous fish. This is evident in cod which, at low dietary carbohydrate levels have a relatively high carbohydrate digestibility. As the dietary level of carbohydrates increases, the digestibility drops dramatically (Hemre *et al.*, 1989). Therefore, with wolffish, the concern is to find an optimum balance between protein and lipid while keeping carbohydrate values to a minimum.

1.2.4 PE:TE Requirements

Jobling *et al.*, (1991) found that adult cod gain weight rapidly when fed diets with PE:TE = 0.40 to 0.45, without deleterious effects on the hepatosomatic index. Younger cod (50 to 300 grams) show the best results when fed diets with PE:TE $\geq$ 0.56 (Lie *et al.*, 1988). Young plaice (*Pleuronectes platessa*) fed a series of formulated feeds with a PE:TE range from 0.14 to 0.92 showed optimum growth when fed a diet with PE:TE = 0.70 (Cowey *et al.*, 1972). In a similar study using turbot (*Scophthalmus maximus* L.) and a PE:TE range of 0.50 to 0.85 (isoproteic diets with decreasing total energy contents), the authors concluded that the protein efficiency ratio (PER) reached an optimum value when the PE:TE was less than 0.50 (Adron *et al.*, 1976). An investigation of the effects of two water temperatures on the protein requirements of juvenile sea bass *(Dicentrarchus labrax*) was performed. Four dry diets with PE:TE = 0.162, 0.214, 0.263 and 0.313 were formulated and fed as a fixed ration to fish held in a recirculated system. At both temperatures, 15 and 20 °C, the highest weight gain was observed when PE:TE = 0.263. However, based on nutrient and energy utilization efficiencies, a PE:TE = 0.214 was recommended for culturing juvenile sea bass at both 15 °C and 20 °C (Hidalgo and Alliot, 1988).

1.2.4.1 Hepatosomatic Index

The hepatosomatic index (HSI) provides information about the appropriateness of the energy content of the diet which complements growth and performance data. Atlantic salmon, for example, are able to store excess lipids in various locations in the body, such as flesh and liver. They are able to quickly mobilize these lipid reserves when needed. In non-oily fish such as wolffish and cod, excess energy is deposited as fat in the liver. This may impair liver function, waste dietary resources and reduce fillet yields. An experiment with juvenile cod (*Gadus morhua*) was completed using six formulated diets with PE:TE from 0.11 to 0.61. A positive linear relationship between HSI and dietary lipid content was demonstrated (Lie *et al.*, 1988). In feeding trials with turbot (*Scopthalmus maximus* L.) using seven diets with PE:TE between 0.50 and 0.85, the lipid content of the fish increased as the PE:TE decreased. However, at no time was the carcass lipid content of experimental fish higher than that of wild fish (Adron *et al.*, 1976). This result is in sharp contrast to the results of an experiment with juvenile plaice (*Pleuronectes platessa*). Using a dietary PE:TE range between 0.14 and 0.92 over a 12 week period, fish fed each diet showed a significantly higher carcass lipid content than wild plaice (Cowey *et al.*, 1972). These workers concluded that while the carcass lipid levels were related to the diet lipid levels, the total dietary energy content likely exceeded requirements.

1.2.5 Feeding Frequency

The frequency of feeding depends, primarily, on the rate of gastric evacuation. However, a multitude of factors influence the rate of gastric evacuation, including energy content, particle size, digestibility of the food, structure of the alimentary canal, water temperature and the phase of digestion (Tyler, 1970; Grove *et al*, 1978; Persson, 1982; Bromley, 1987; Bromley, 1988; Dos Santos and Jobling, 1988 and Jensen and Berg, 1993). Determination of an appropriate feeding frequency is critical in order to minimize feed wastage and maximize growth. Feeding too frequently results in wasted feed, even if

feeding by hand. Conversely, if meals are too infrequent, the rate of growth could be compromised. Orlova *et al.* (1989), studying striped wolffish, documented food passage times through the gut ranging between 4 and 10 days for natural foods such as scallops with and without shells, mussels, fish and shrimp. Highly digestible food items like formulated pellets could, theoretically, take 10 days to pass through the gut. Halibut, (*Hippoglossus hippoglossus*), voluntarily eat large meals two to three times per week while the lemon sole (*Microstomus kitt* Walbaum) consume small meals every one to one and a half days (Davenport *et al.*, 1990). The authors attribute this to the fact that halibut posses large stomachs and short intestines, as opposed to the small stomach and looping intestine of the lemon sole. Wolffish have small stomachs and intestines which measure an average of 68% of the body length (Verigina, 1974). This defines the need to establish the rate of gastric evacuation and return of appetite.

There are several ways to evaluate the rate of gastric emptying in fish. One common method is to feed fish a meal containing contrast media such as barium sulphate. The progress of the meal (often pre-weighed) through the gastrointestinal tract is recorded by periodic x-rays (Edwards, 1971; Jobling *et al.*, 1977; Grove *et al.*, 1978; Flowerdew and Grove, 1979). This technique is accurate and does not require killing large numbers of fish. Another common method is to serially kill a specified number of fish at pre-determined intervals following a meal and remove, dry and weigh the stomach contents (Jobling, 1980; MacDonald *et al.*, 1982; Persson, 1982; Bromley, 1987; Jensen and Berg, 1993). A third method is the analysis of stomach contents pumped out of an anaesthetized fish at a given time after a pre-weighed meal (Bromley, 1988; Dos Santos and Jobling, 1988).

1.3 Rationale

Since the moratorium on the groundfishery was established in July 1992, fresh, white-fleshed fish are in high demand and are sold at a premium price. The price of fresh cod

(*Gadus morhua*) in Newfoundland markets now exceeds that of salmon, which was once considered a delicacy. This has encouraged aquaculturists and researchers to identify a suitable species for aquaculture that will provide a source of fresh, white-fleshed fish. In addition to high quality flesh, this species must possess characteristics that facilitate culture. Many marine species have a precarious larval development and exhibit mass mortality during metamorphosis. This is currently an area of extensive research. An alternative to resolving these issues is to identify a species with large eggs and well-developed larvae at hatch. The search for such a species has resulted in special attention being given to the wolffishes. The primary marine fish species cultured in Newfoundland currently is the Atlantic salmon. Except for a series of farms using heated effluent from a hydroelectric plant, low temperatures have resulted in slower growth rates than in other, more southerly, locations. Atlantic salmon also have a well-understood, relatively simple larval stage. Newfoundland has an obvious need and capacity for a water-based industry. It appears as though aquaculture is the ideal industry to fill that niche. With ambient temperatures which are considered low by the standards of producers in temperate climates, the culture of a hardy, cold-water species, such as wolffish must be developed. With the extraordinary success of larval wolffish culture to date, there is a need for development of a protocols for the culture of juveniles and adults.

The purpose of my study was to examine the food and feeding requirements of juvenile striped wolffish under culture conditions with the aim of maximizing growth. The objectives were threefold:

1. **Establish a suitable stocking density for juvenile striped wolffish.**

2. **Determine an appropriate feeding schedule for juvenile striped wolffish.**

3. **Identify an effective range of dietary energy balances for striped wolffish diets.**

2.0 Materials and Methods

2.1 Aquarium Facilities

Experiments were conducted in two facilities. The first was the laboratory at the Fisheries and Marine Institute of Memorial University of Newfoundland in St. John's, which has a seawater recirculation system with temperature regulation. The other facility used was the Wesleyville Marine Fish Hatchery in Badger's Quay, Newfoundland, which is a flow-through system operating at ambient sea water temperature.

2.1.1 Recirculation Facility

A recirculated seawater facility was used for the research completed in St. John's. This laboratory was selected because of the temperature control system which maintains the water temperature within 0.3 °C of the set temperature. The lab was divided into two sides, each of which contained six - 750 L green fiberglass tanks (Figure 2.1). These tanks were set up on two levels, with three tubs on each level. Tanks had individual water supplies and individual drains. The drains joined into a common pipe on each level and the levels then combined to return to the sump through a series of two paired biological filters. Two of the four filters were replaced every two weeks so that the bacteria population on the filter was maintained. The total capacity of the system was 2.7 m^3. Water was transported by truck from the Ocean Sciences Centre, situated on the shores of Logy Bay. Weekly replacement of water in the system was approximately 20%. Replacement was gradual, with several gallons being replaced daily. Once a month a flushing occurred when one third of the water in the system was replaced at once. The tank floors, below the suspended experimental containers, were siphoned regularly to optimize water quality. The salinity was constant at 32 ppt. The dissolved oxygen concentration was never lower than 92% in the tanks and at times the concentration reached 105% saturation, therefore aeration was unnecessary. The photoperiod was

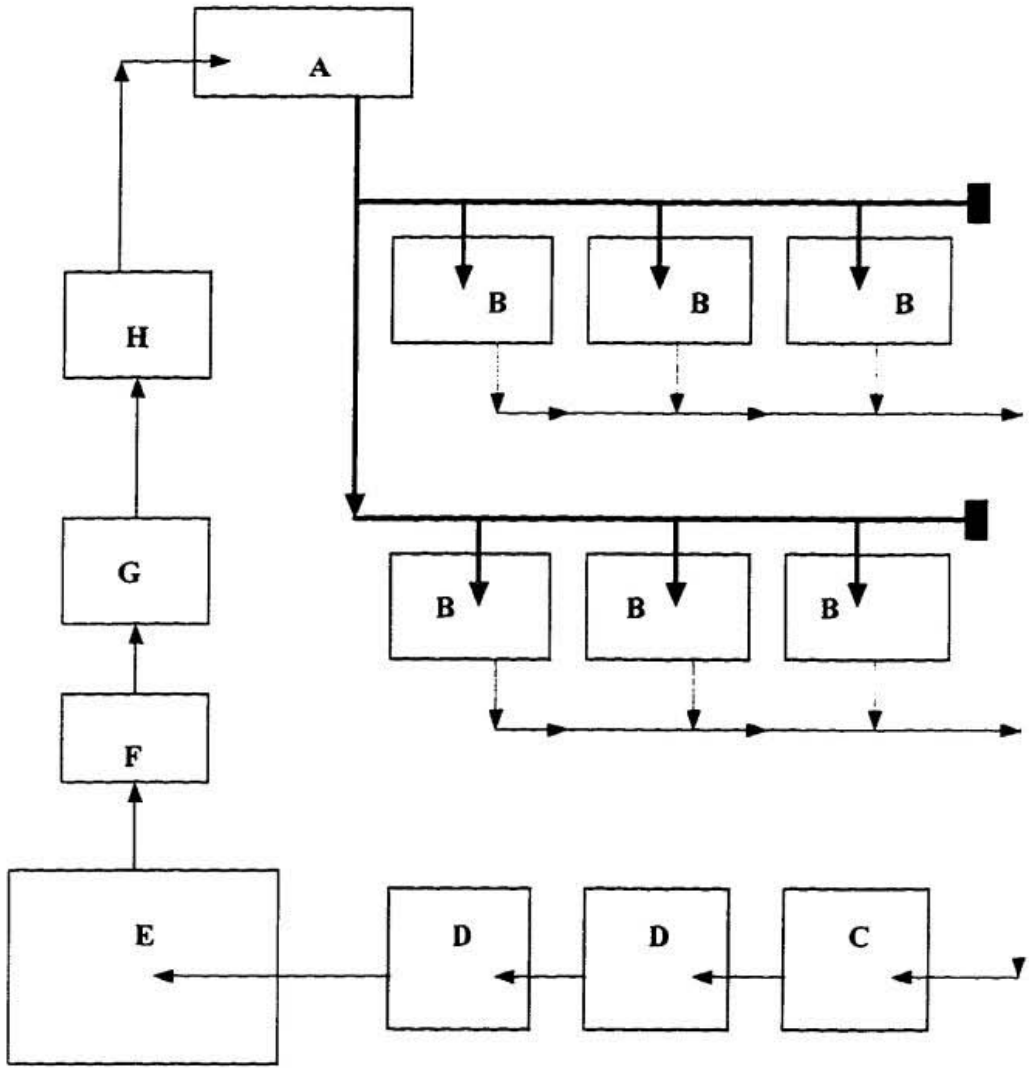


Figure 2.1: Schematic diagram of the recirculation facility. A. header tank; B. rearing tank; C. filter (fiberglass bedding); D. filter (bio-rings); E. sump tank; F. pump; G. chiller unit; H. micro filters.

maintained at 18L:6D. The light intensity over the course of the experiment was constant with a mean of 116.5 lux (S.E. = 14.5).

Experiments in the recirculation system were conducted either in tanks or baskets which were modified to accommodate fish. The tanks used were white 4.5 L plastic containers with an outflow hole cut 4 cm from the bottom. Fiberglass mesh was glued over the outflow so fish and feed would not escape. Six of these containers were strapped together in a circle using electrical ties and a seventh, bottomless tank was attached in the centre to act as ballast, keeping the tanks level at all times (Figure 2.2). The inflow pipe was fitted with six spigots which, when connected to pieces of plastic tubing, delivered water to each tank. The water temperature was maintained at 9.0 ± 0.3 °C throughout the feeding trials by use of a thermostatically regulated chiller unit (Johnson Controls, Milwaukee, Wisconsin).

The baskets were pale blue rectangular plastic containers measuring 23 cm by 35 cm by 15 cm. The sides and bottoms of these pans were cut out leaving a frame on which was glued fiberglass mesh. The mesh (standard mosquito screen) was small enough to prevent feed from passing through the tank floor. This is critical since wolffish are bottom feeders and the total amount of feed consumed was recorded at each meal. The baskets were suspended from wooden dowels such that they were submerged to within 2.5 cm of the tops. A mesh fence was erected around each basket to prevent fish from jumping out of the baskets. String was threaded through the bottom of the fence and tied around the top of the baskets. Fences were held erect using plastic straws placed in holes in the corners of the baskets and by tying the top edges of adjacent fences together. Two baskets were suspended in each tub, such that the volume of water contained in each basket was approximately 7 L. The inflow pipe was fitted with spigots and two inflow hoses were directed into each basket, delivering a total of 2 L/min.

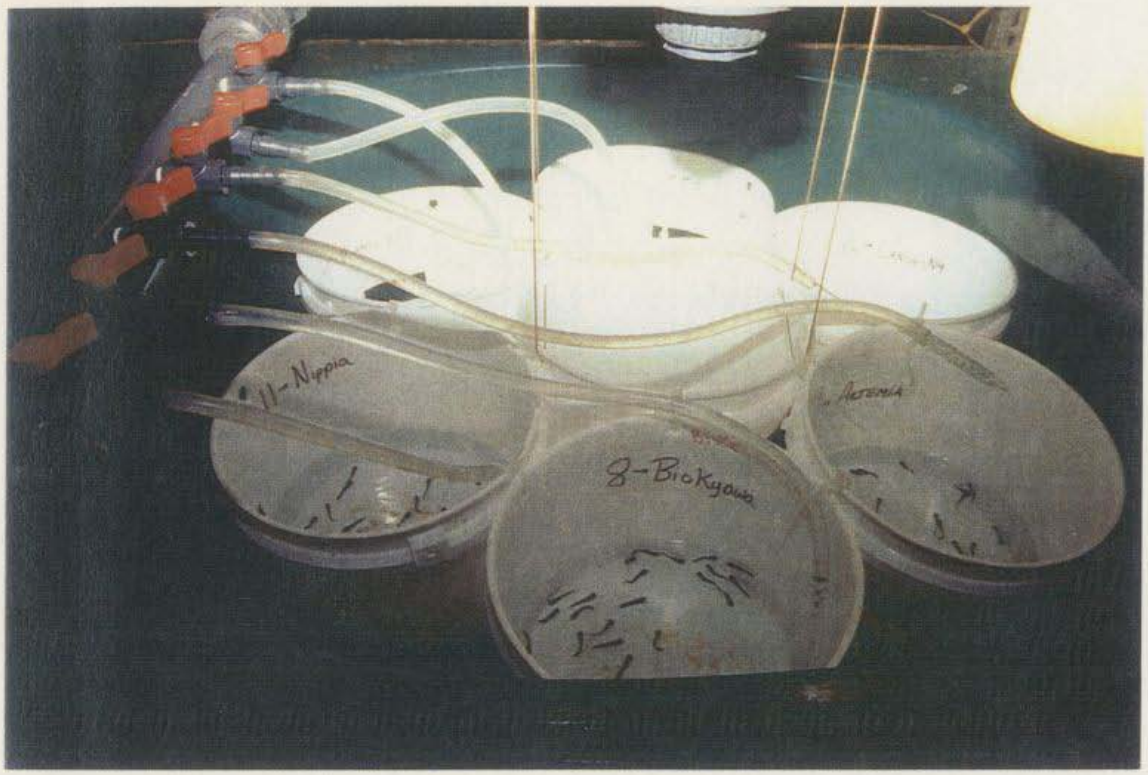


Figure 2.2: Layout of plastic tanks used in the stocking density/feeding schedule trial.

2.1.2 Flow-Through Facility

The flow-through facility at Wesleyville, Newfoundland is an experimental marine fish hatchery. It contains a larval rearing room which is also suitable for juvenile culture. The system is made up of 27 green fiberglass raceways of dimensions 106 cm x 25 cm x 15 cm deep. The length of the raceways and water depth were adjustable based on the requirements for each experiment. The raceways were built into three racks, each with three levels and three raceways per level. Water was pumped directly from 8 m depth outside the hatchery, through a sand filter to the larval rearing room where it was distributed to each raceway. The raceways drained into a common pipe and water was discarded. A plastic slatted screen separated the fish from the outflow, so neither food nor fish entered the drain. There was no water temperature control in the larval rearing room, and the ambient water temperature was recorded daily. Dissolved oxygen levels were consistantly above 90 % saturation. The light intensity was approximately 100 lux and the photoperiod was 18L:6D.

2.2 Fish

Two separate year-classes of fish were used in these experiments. The older (referred to as 1+ for the purpose of this study), came from two egg masses collected by divers off Bauline in Conception Bay, Newfoundland in the autumn of 1993. The egg masses were taken to the recirculation system where they were gently pulled apart and incubated in 4 °C seawater which was biologically filtered and exposed to ultraviolet light. The incubation unit (Heath tray) was covered in black plastic to minimize the exposure of the eggs to light. Eggs were not treated with an antibacterial agent, as recommended by Pavlov and Moksness (1993). One of the two egg masses was destroyed by bacterial infection. Dead eggs were picked daily, and when the fish began to hatch they were placed in the white tanks described previously. These, the older of the two year classes, had a mean hatch date of March 2, 1994. Initially, larvae were fed *Artemia* enriched with

High DHA Super Selco (Artemia Systems, INVE Aquaculture NV, Baasrode, Belgium) which is a commercial blend of polyunsaturated fatty acids. The larvae were soon weaned onto a series of feeds designed for marine fish larvae and produced by Lansy (INVE Aquaculture NV, Baasrode, Belgium). Feeding trials continued until the fish were 60 days old. Following the larval feeding trials the fish were maintained on Lansy diets. A trial using moist feeds was then started. Having been fed on dry feeds, the fish would not accept the moist feed. Despite the fact that various binders and binding techniques were used, the feed consistently crumbled when chewed by the wolffish and, therefore, was not completely ingested. For this reason, all subsequent experiments were conducted using dry, extruded feeds. When not being used in an experiment, fish were maintained on the Hi Pro Salmon Grower Diet (Corey Feed Mills, Fredericton, NB) (Table 2.1). This was supplemented with fresh, frozen feed, such as chopped herring or squid.

When the experiments began with the 1+ fish, the group of fish used in the stocking density trial were approximately 530 days post-hatch. Those involved in the dietary energy trial were approximately 577 days post-hatch. The morphometric summaries of the year classes are shown in Appendix A.

The younger fish (referred to as 0+) were collected in November 1994 and incubated in the same manner as the older fish. However, these eggs were treated with glutaraldehyde in order to prevent bacterial infection. Treatments were applied when the eggs appeared unhealthy. The mean hatch date was February 2, 1995. They were initially fed *Artemia* but were weaned on to one of three commercial marine larvae diets (Lansy; Biokyowa, Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan; or Moore-Clark, St. Andrews, New Brunswick) until 70 days post-hatch. Around that time the fish stopped swimming and eating. They were moved from the white buckets into the green tanks and were fed frozen, chopped herring and squid. Their activity level soon increased and three weeks later they were weaned on to the dry feed once again. Fish hatched at the Wesleyville Hatchery and the Ocean Sciences Centre experienced similar changes in behaviour when

Table 2.1: Ingredients and proximate analysis of experimental diets.

Ingredients[2]		Diet[1] 1	2	3	4	5	6	Salmon Feed
Fish Oil	(%)	22.3	19.2	16.0	13.0	9.9	7.4	
Cellulose	(%)	16.7	13.7	9.9	6.8	3.4	0.0	
Animal Protein Products	(%)	37.0	42.0	47.0	52.0	57.0	62.0	
Plant Protein Products	(%)	14.0	16.0	17.0	19.0	20.0	22.0	
Processed Grain By-products	(%)	9.0	8.1	9.1	8.2	8.7	7.6	
Vitamins and Minerals	(%)	1.0	1.0	1.0	1.0	1.0	1.0	
Proximate Analysis								
Moisture	(%)	6.04	6.07	6.64	6.04	5.53	7.27	6.66
Protein	(%)	36.04	40.62	45.36	44.54	53.48	58.43	52.45
Lipid	(%)	29.99	27.7	26.78	23.67	21.22	16.96	18.07
Carbohydrate[3]	(%)	21.37	18.62	13.05	17.36	10.84	4.40	11.84
Ash	(%)	6.56	6.99	8.17	8.39	8.93	9.94	10.98
Total Energy[4]	(kcal/g)	5.74	5.67	5.62	5.45	5.46	5.35	6.06
PE:TE		0.35	0.40	0.45	0.46	0.55	0.61	0.49

[1] Diets 1-6 were formulated and prepared by Ziegler (Gardners PA). The formulations were based on a commercial salmon feed, therefore, detailed ingredient information is unavailable. The salmon feed is a proprietary commercial diet: Hi Pro Grower Salmonid Diet (Corey Feed Mills, Fredericton, New Brunswick).

[2] All values are given on a dry diet basis.

[3] Carbohydrate content estimated by subtraction.

[4] Total (gross) energy values calculated using standard caloric values (Cho *et al.*, 1982). Protein: 5.6 kcal/g; lipid: 9.5 kcal/g; carbohydrate: 4.1 kcal/g. One kilocalorie is equal to 4.184 kilojoules (Dorland, 1985).

they reached 70 days post-hatch, and high mortalities were recorded. Modification of the feed or tanks when feed consumption patterns change may boost feeding and activity rates and avoid mortalities.

When the dietary energy trial began, the 0+ fish were 209 days post-hatch. These juveniles were fed more intensively than those fish hatched the previous year, and the mean weights and lengths reflect this (Appendix A). The mean weights differed by less than 2 g and there was a full year between hatch dates.

2.3 Experimental Design

2.3.1 Feeding Schedule/Stocking Density Trial

Each of three stocking densities, 20 g/L, 50 g/L and 80 g/L, was randomly assigned to 9 round containers, for a total of 27 containers. Three containers of each group of nine were assigned a feeding schedule; two meals/day, one meal/day and one meal/two days. One hundred and fifty three fish were randomly assigned to the 27 containers to produce stocking densities as indicated. Fish were hand fed according to the schedule assigned to their respective tanks and they were weighed and measured every four weeks for a total of 12 weeks.

2.3.2 Dietary Energy Trial

Two hundred and forty - 0+ fish were evenly distributed among 12 baskets in the recirculated system so that the stocking density was 20 g/L. The six diets were each randomly assigned to two baskets. The feeding trial lasted 12 weeks. Fish lengths and weights were measured at the start of the experiment and then at four week intervals. This trial was repeated in raceways at the Wesleyville Hatchery at ambient water temperatures. A third group of fish, 577 days post-hatch, were assigned to 12 raceways in

Wesleyville at the same stocking density. The six diets were randomly assigned to raceways and morphological measurements were taken every four weeks.

The 240 fish used in the trial at the recirculation facility were subsequently killed for stomach analysis during the gastric evacuation trial. At that time, the livers were weighed in order to determine the hepatosomatic index (liver weight as a percentage of whole body weight).

2.3.3 Gastric Evacuation Trial

The serial slaughter method was chosen for the gastric evacuation trial because of the low cost and reported accuracy of the results. Following a period of starvation, fish were fed to apparent satiation and five fish from each treatment were slaughtered initially and at 8 hour intervals. Fish were frozen immediately following slaughter. This method permitted removal and weighing of the livers to provide data on the changes in hepatosomatic indices due to variations in the dietary energy balance.

2.3.3.1 Temperature Study

Wolffish at the Wesleyville Hatchery lost their appetite when the ambient water temperature reached 1 °C (Watkins, pers. comm.). Pavlov (1995) reported that the optimum temperature range for maximizing growth rates in juvenile wolffish is 10 °C to 14 °C. However, Moksness (1994) reported that the optimum rearing temperature for juveniles is below 10 °C. Based on this information, four temperatures were selected: 2 °C, 5 °C, 9 °C and 12 °C. These values encompass the most likely range of water temperatures encountered under Newfoundland's coastal culture conditions.

2.3.3.2 Dietary Energy Balance Study

Six diets with a range of PE:TE values between 0.35 and 0.61 (Table 2.1) were used to determine whether the dietary energy balance influenced the rate of gastric evacuation. The gross energy contents of the diets were approximately equivalent, so this was ruled out as a possible source of variation in gastric emptying rate. Each diet was fed to 40 previously starved fish which were analysed using the serial slaughter technique using methodology described in Section 2.6.2.

2.4 Feeds

2.4.1 Density/Feeding Schedule Trial

During this trial, the fish were fed 3.0 mm Hi Pro Salmon Grower pellets produced by Corey Feed Mills (Fredericton, New Brunswick). The proximate composition is shown in Table 2.1. This is the same diet they had been fed for the previous three months. It was selected because of its palatability, growth performance and availability.

2.4.2 Dietary Energy Trial

For three months prior to this trial the fish were all maintained on Hi Pro Salmon Grower pellets produced by Corey Feed Mills. Preliminary observations of feeding responses and growth using pellets from three feed manufacturers showed that the Zeigler salmon starter formulation appeared to be the most palatable and produced the best growth rates (Zeigler, Gardners, PA). Six diets were developed specifically for this trial with the assistance of Zeigler's Technical Services department. Zeigler was requested to modify their salmon starter formulation to produce six diets with a range of protein energy: total energy (PE:TE) from 0.40 to 0.65. They modified the base diet by increasing the lipid and cellulose content and decreasing the protein content such that the gross energy remained relatively constant. The compositions of the diets are shown in Table 2.1. Zeigler expressed some concern over the pellet quality of the low-protein diets, but all

diets had high quality pellets, aside from the high protein diets which produced a considerable amount of fines. The fines were sieved off and the feed quality was otherwise excellent. The calculation of energy values was done according to standard values as reported by Cho *et al.* (1982).

2.5 Feeding Protocol

There is some question as to whether observers can determine when fish are satiated. True satiation is the point when the stomachs of all fish under observation are full. Since this is impossible to determine without dissecting the fish, researchers must rely on observable behaviours to determine the point of apparent satiation (Cowey *et al.*, 1972). Wolffish, for example, when they were hungry would swim vertically with their heads protruding above the water surface when someone approached the tank, until food was offered or the researcher moved away from the tank. When food was offered, fish gradually sank or swam to the bottom, following feed pellets. As food continued to be delivered, few fish remained at the surface and the majority were on the bottom chewing pellets. In early stages of a meal, fish which were in the process of chewing and swallowing a pellet oriented themselves toward a new pellet as it sank and even approached that pellet before the first pellet had been swallowed. They frequently spat out the initial pellet to take the new one if pellets were presented quickly. As the fish neared apparent satiation, approaching decreased first, then orienting decreased. At that point, fish no longer swam in the water column. Those fish which had not yet reached apparent satiation generally rested on the bottom with the anterior portion of their bodies propped up on their pectoral fins. Those which were satiated tended to rest completely on their ventral surface or lie on their sides. These postures were not concrete indications of their level of satiation, but provided a clue as to how much more feed should be added. Based on preliminary observations, wolffish did not ingest pellets that had been in the water more than one minute. Therefore, when fish no longer oriented, approached or ingested feed within a minute of presentation, they were considered satiated.

Throughout these experiments fish were fed to apparent satiation rather than being given a fixed percentage of body weight. Preliminary observations indicated that some days very little feed was consumed, and other days an extraordinary amount was consumed. If a fixed percentage body weight was delivered each meal, some days feed would be wasted and other days the fish would be left hungry because they required a larger meal than the prescribed amount. In both cases, the feed conversion ratio data would be distorted and the growth rates adversely affected. A fixed ration is not responsive to fluctuations in appetite and growth is not maximized. It was for this reason, too, that hand-feeding was selected as opposed to automatic feeders. Hand feeding, though extremely time-consuming, allows the researcher to monitor the feed consumption and identify changes which could indicate poor health, changes in water quality or feed requirements.

Experimental containers were siphoned prior to the morning meal, regardless of the feeding schedule assigned to a given tank. Feeding times for fish fed twice per day were 9 a.m. and 3 p.m.. Fish fed once per day were fed at 3 p.m., as preliminary investigation showed that feeding activity was higher when the single daily meal was provided in the afternoon rather than in the morning. For those fed twice per day, the morning meal was the largest 71.6% of the time (n = 729). Feed dishes were weighed initially and following each meal. Fish were not fed the day prior to each weighing to ensure that the gut was free of significant amounts of feed and to disturb the feeding regime as little as possible. Feeding resumed the day following weighing, as scheduled.

2.6 Sampling

2.6.1 Weighing

Fish were weighed and measured one day prior to the start of the experiment and every 28 days thereafter over an 84 day period for a total of four measurements. Fish were

individually anaesthetised in a bath of 25 mg/L MS-222 (Sigma Chemical Company, St. Louis, MO, USA). Each fish was removed from the bath, and gently blotted with a paper towel, then placed on a scale with a ruler covered with a transparent plastic sheet. Mass (in grams) and standard length from the edge of the upper jaw when the mouth is closed, to the tip of the notochord (in centimeters) were taken and the fish were briefly examined for signs of aggression (scars or torn fins) or disease before being placed in an aerated recovery bath.

Wolffish were remarkably tolerant of anaesthesia and handling. Fish have been observed feeding within two hours of being anaesthetized. More than five thousand fish were anaesthetized over the course of this research and only a single fish died following the procedure.

2.6.2 Gastric Evacuation Trials

Fish were starved for 5 days prior to the start of the experiment. At 4 p.m. on the day the experiment started, fish were fed to apparent satiation with the diet they had been consuming for the past three months and the tank was immediately siphoned. Five randomly selected fish were removed from each tank at eight-hour intervals beginning fifteen minutes following the meal and every eight hours thereafter. The fifteen minute delay in taking the first sample was to ensure that pellets had been completely swallowed and had moved into the stomach. Fish were killed with an overdose of MS-222, and were immediately placed in plastic bags, sealed, then frozen in a deep freezer with their heads slightly elevated. This position reduced any risk of ingested feed leaking out of the fish stomachs, since there is no valve between the esophagus and the stomach (Verigina, 1974). There is a pyloric valve between the stomach and the intestine, so there was little concern of losing stomach contents into the intestine. Fresh livers were generally friable so freezing made the samples more manageable. Freezing also prevented leakage from stomachs, since the contents were often watery.

For analysis, fish were set out on the laboratory bench for 10 minutes until the layer of skin and muscle covering the stomach thawed. The fish were weighed and the standard length was recorded. A transverse ventral incision was made between the opercula, followed by a medial, longitudinal incision to the anal pore. The body wall was pulled away, revealing the body cavity. The liver, which covers the esophagus and most of the stomach, was removed and weighed. The stomach was removed by severing the esophagus next to the stomach and the intestine immediately posterior to the pyloric valve. Since the stomach was still frozen at this point, there was no concern about contents leaking out. A small incision to the stomach wall allowed the contents to be simply squeezed out of the stomach onto a pre-weighed filter paper in a Buchner funnel. The stomach wall was rinsed with distilled water and the frozen stomach contents were thawed and distributed around the filter paper using distilled water. These papers were suction-filtered and oven dried at 60 °C for three days prior to re-weighing (Dos Santos and Jobling, 1988). The dry weight of the stomach contents was calculated by subtraction.

2.6.3 Diet Analysis

Proximate analyses of dietary moisture, protein, lipid and ash were completed in triplicate for every diet and mean values are reported in Table 2.1. The moisture content was determined by drying pre-weighed samples in an oven set at 105 °C until the dry weight was constant. Samples were cooled in a dessicator to minimize the adherence of water to the sample or sample dish. The protein content of dried samples was determined using the Kjeldahl method (Tecator Digestion System 20, 1015 digester, Sweden; Tecator Kjeltec System 1028 Distilling Unit, Sweden). Total nitrogen was converted to crude protein by multiplying by 6.25 on the assumption that the protein in the feed is approximately 16% nitrogen. The crude lipid content of each diet was determined using a hexane-based Soxhlet lipid extraction apparatus (Tecator Soxtec System HT 1043 Extraction Unit, Sweden). The ash content was measured by placing a pre-weighed crucible and dried diet

sample in a muffle furnace (Thermolyne, Sybron Corporation, Dubuque, Iowa, USA) set at 450 °C overnight, cooling in a dessicator and reweighing the crucible and sample. The carbohydrate content of the diets was estimated by subtracting the sum of the other nutrients from 100. The gross energy of all diets was calculated by multiplying the percent protein in the diet by 5.6 kcal/gram, the percentage of lipid by 9.5 kcal/gram and the percentage of carbohydrate by 4.1 kcal/gram. The sum of these values equals the gross dietary energy per 100 grams.

2.7 Calculations

2.7.1 Condition Index

The condition index (CI) relates the fish weight to its length. High CI values indicate a high weight per unit length, which is generally a favourable characteristic. The CI of each fish was calculated using the following formula:

$$CI = (W/L^3) * 100, \quad (2)$$

where W is wet weight (g) and L is standard length (cm) (Goddard, 1996).

2.7.2 Specific Growth Rate

The specific growth rate (SGR) describes the daily rate of growth as a percent of body weight. It was calculated according to the following formula:

$$SGR = ((\ln Wt_2 - \ln Wt_1) / (t_2 - t_1)) * 100, \quad (3)$$

where Wt_1 and Wt_2 are the wet weights (g) of the individuals at day t_1 and t_2 (Goddard, 1996).

2.7.3 Feed Conversion Ratio

The feed conversion ratio (FCR) describes how efficiently the feed is converted to body weight. Ideally, the FCR for a dry diet is 1.0 or less. The FCR for this and subsequent trials was calculated as follows:

$$FCR = \text{feed ingested (g)} / (TW_2 - TW_1 \text{ (g)}), \quad (4)$$

where TW_1 and TW_2 are the sum of the weights of fish in a tank (g) at the beginning and end of the feeding trial (Goddard, 1996).

2.7.4 Protein Efficiency Ratio

The protein efficiency ratio (PER) gives an indication of the weight gain per gram of protein ingested. The PER for each tank was calculated as follows (Papoutsoglou *et al.*, 1987):

$$PER = 100 * (TW_2 - TW_1 \text{ (g)}) / (\text{feed ingested (g)} * \% \text{ protein}), \quad (5)$$

where TW_1 and TW_2 are the sum of the weights of fish in a tank (g) at the beginning and end of the feeding trial.

2.7.5 Hepatosomatic Index

The liver weight relative to the total fish body weight is indicated by the hepatosomatic index (HSI). This index may be an indirect determinant of the degree of lipid deposit in the liver. The HSI was calculated using the following formula:

$$HSI = (L_W/W) * 100, \quad (6)$$

where L_W is the liver weight (g) and W is the weight of the whole fish (g) (Stefanussen *et al.*, 1993).

2.7.6 Cost of Production

The cost of production was calculated based only on feed costs and growth rates in order to evaluate the effectiveness of the diets. No factors such as pumping or heating costs were taken into account. The production cost was calculated using the formula:

$$\$/kg \text{ produced} = \text{cost of food } (\$/kg) * FCR; \quad (7)$$

where the cost of food ($/kg) was provided by the manufacturer, and FCR was calculated by Equation 4.

2.8 Statistical Analysis

2.8.1 Feeding Trials

Treatment means of the condition indices, specific growth rates, and hepatosomatic indices were based on measurements of all the individual fish within each tank. Feed conversion ratios and protein efficiency ratios were calculated on a per tank basis. Analysis of variance (ANOVA) was done using SPSS software (SPSS, 1994; SPSS Inc., Chicago, Illinois) with $\alpha = 0.05$. Insignificant factors were pooled and reanalysed. Tukey's B multiple range test was used to determine the nature of significant treatment differences. All percentage data were arcsine transformed prior to analysis (Sokal and Rohlf, 1969). All means are reported with ± standard error.

2.8.2 Gastric Evacuation Trials

Stomach contents at each sampling time were expressed in grams and as a percentage of body weight. Using regression analysis, the rate of evacuation was calculated based on the percent body weight of food remaining in the stomach and the weight of meal remaining in the stomach. Regression coefficients were calculated using linear and logarithmic models. All percentage data were arcsine transformed prior to analysis.

3.0 Results

3.1 Stocking Density/ Feeding Schedule Trial

3.1.1 Observations

Aggression was noted among fish held at 20 g/L, especially those fed once every two days. This aggression was generally initiated by the largest fish in the tank, who would swim towards another with its mouth open. The aggressor generally did not bite the other fish, but simply hit them with an open mouth between the pectoral fins and the anal pore. No wounds or torn fins were observed at any time. The aggressive behaviour was more prevalent during feeding but was not limited to this time. When aggression was evident, only the aggressor consumed feed.

The feeding schedule was a factor in the rate of feed consumption. Fish fed twice per day generally waited for the pellet to reach the bottom of the tank and made no response to the pellet for up to 10 seconds. Those fish fed once daily and once every second day were fed in the afternoon based on observations that they were more active in the afternoon and consumed meals slightly faster. This was important, because when they were slightly lethargic in the morning, the feed sits on the tank bottom longer and likely becomes less palatable. As a rule, when fish fed every second day were offered feed, they fed more actively than fish fed more frequently. Fish fed once every second day swam to the water surface whenever the technician approached, regardless of whether food was offered or not. This behaviour was less evident in fish fed once daily and was never noted in fish fed twice daily.

3.1.2 Morphometrics

The initial mean lengths and weights of fish are shown in Table 3.1. Tank effects were not significant for both length and weight ($p > 0.05$, three way ANOVA). The mean lengths and weights of fish stocked at 20 and 50 g/L were not statistically different and both were significantly lower than the mean length and weight of fish stocked at 80 g/L (Tukey's B). These similarities and differences remained constant as the fish grew throughout the experiment. There was no significant difference detected in the initial or final mean condition index among the three stocking densities ($p > 0.05$, one way ANOVA; Table 3.2). In one treatment (20 g/L stocking density, one meal/2 days) there was a significant decrease in the CI over the course of the 12 week trial ($p = 0.037$, one way ANOVA).

The three feeding schedules did not produce significant differences in weights between treatments (Table 3.1). The initial mean condition indices of the wolffish did not differ significantly between feeding schedule treatments ($p > 0.05$, one way ANOVA). Following the twelve week trial, the mean condition index of only one treatment changed significantly (stocking density = 20 g/L, one meal per two days) (Table 3.2). This treatment produced a decrease in the condition index from 1.011 ± 0.034 to 0.909 ± 0.030 ($p < 0.05$, one way ANOVA).

3.1.3 Specific Growth Rate

SGR values equal to or less than 0 were not included in the analysis since they represented fish that were not feeding or were not healthy. Tank effects were not significant ($p > 0.05$, three-way ANOVA, Appendix B), therefore, data was pooled. Neither stocking density nor feeding schedule produced significantly different SGR values ($p > 0.05$, two way ANOVA) (Figure 3.1). The mean SGR for the fish used in this experiment was 0.399 ± 0.013 %BW per day ($n = 391$).

Table 3.1: Initial and final lengths (cm) and weights (g) of wolffish A: stocking density trial (mean ± standard error); B: feeding schedule trial (mean ± standard error).

A.

Stocking Density	Initial Length (cm)	Final Length (cm)	Initial Weight (g)	Final Weight (g)	n
20 g/L	8.95 ± 0.15	9.55 ± 0.19	7.18 ± 0.30	8.10 ± 0.46	27
50 g/L	9.28 ± 0.09	9.94 ± 0.12	7.96 ± 0.21	9.70 ± 0.37	63
80 g/L	11.01 ± 0.10	11.97 ± 0.13	12.75 ± 0.29	15.83 ± 0.46	63

B.

Feeding Schedule	Initial Length (cm)	Final Length (cm)	Initial Weight (g)	Final Weight (g)	n
2 meals/day	9.94 ± 0.17	10.77 ± 0.20	9.76 ± 0.45	12.45 ± 0.20	51
1 meal/day	9.95 ± 0.16	10.62 ± 0.20	9.76 ± 0.42	11.84 ± 0.63	51
1 meal/2 days	9.92 ± 0.16	10.74 ± 0.21	9.86 ± 0.46	11.54 ± 0.65	51

Table 3.2: Comparison of initial and final condition indices by feeding schedule and stocking density treatment (mean ± standard error).

Feeding Schedule	Stocking Density (g/L)	Number of Fish	Initial Condition Index	Final Condition Index	Significance[1]
Two meals / day	20	9	1.003 ± 0.011	0.962 ± 0.038	n.s.d.
	50	27	0.979 ± 0.012	0.951 ± 0.027	n.s.d.
	80	27	0.949 ± 0.023	0.948 ± 0.030	n.s.d.
One meal / day	20	9	0.962 ± 0.019	0.941 ± 0.027	n.s.d.
	50	27	0.993 ± 0.019	0.927 ± 0.022	n.s.d.
	80	27	0.945 ± 0.027	0.942 ± 0.024	n.s.d.
One meal / 2 days	20	9	1.011 ± 0.034	0.909 ± 0.030	p = 0.037
	50	27	0.987 ± 0.019	0.930 ± 0.026	n.s.d.
	80	27	0.960 ± 0.021	0.975 ± 0.022	n.s.d.

[1] n.s.d. = no significant difference, $p > 0.05$, one way ANOVA.

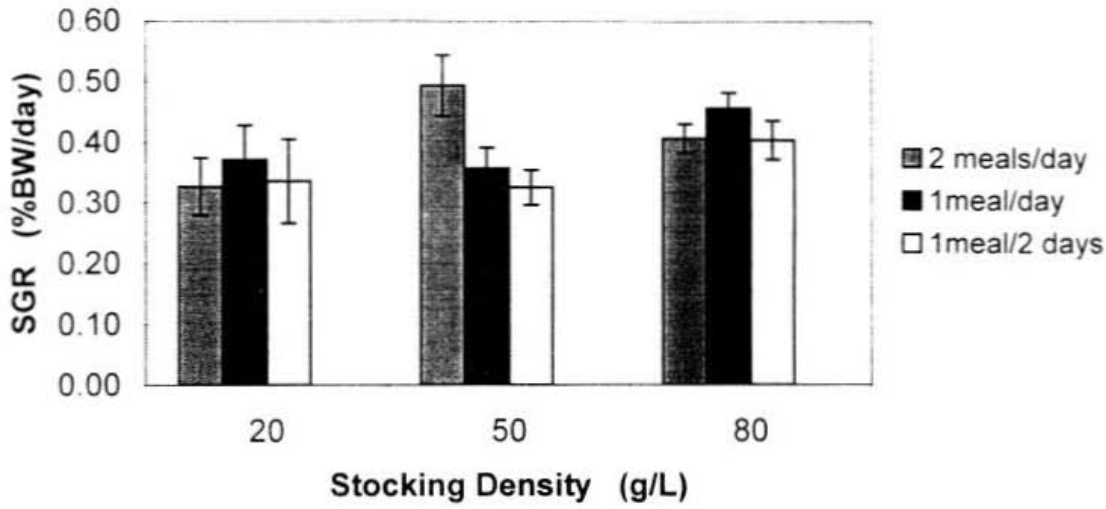


Figure 3.1: Mean specific growth rates (SGR) of 0+ wolffish held at three stocking densities and fed according to three feeding schedules at 9 °C. Vertical bars represent standard error.

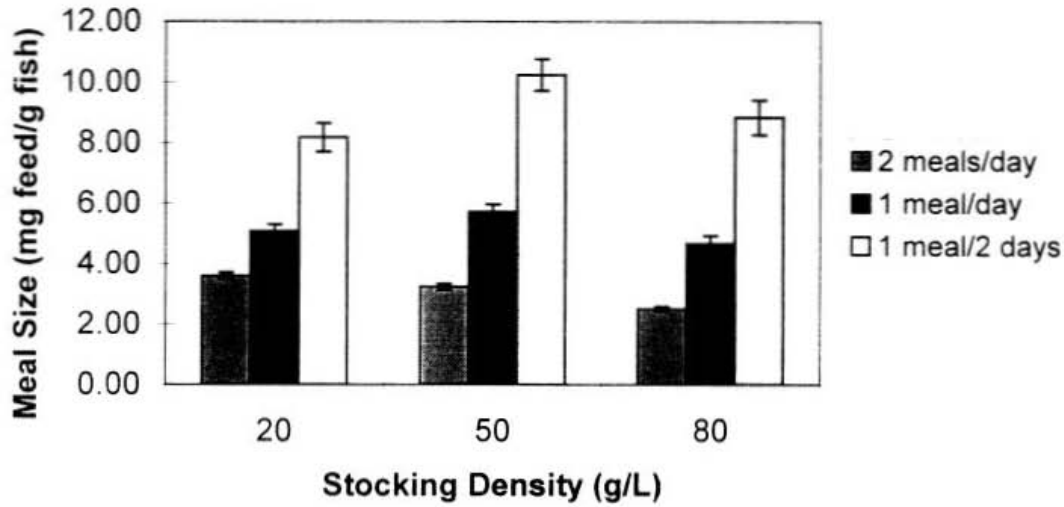


Figure 3.2: Mean meal size (mg feed/g fish) of 0+ wolffish stocked at three densities and fed according to three feeding schedules at 9 °C. Vertical bars represent standard error.

3.1.4 Feed Consumption

The following factors; tanks, stocking density and feeding schedule, were examined in relation to feed consumption by juvenile wolffish. All factors had a highly significant influence on feed consumption by the wolffish ($p < 0.01$, 4 way ANOVA) (Appendix C). Tank effects were significant ($p = 0.009$, one way ANOVA). The mean feed intake per meal by wolffish increased significantly ($p < 0.0001$, one way ANOVA) as the experiment progressed (Figure 3.2). Stocking density significantly affected feed intake ($p < 0.0001$, one way ANOVA). In tanks fed twice daily, the mean meal size eaten by the wolffish was lowest (2.493 ± 0.085 mg/g) in those stocked at 80 g/L and highest (3.567 ± 0.117 mg/g) in those stocked at 20 g/L. However, under the other two feeding regimes, mean meal size was highest in tanks stocked at 50 g/L. Feed consumption was inversely and significantly correlated with the feeding frequency ($p < 0.0001$, one way ANOVA). Those stocked at 80 g/L and fed twice per day had a mean feed intake per meal of 2.493 ± 0.085 mg/g, while those fed once every 2 days (also at 80 g/L) consumed more than three times as much food per meal on average (8.836 ± 0.576 mg/g). Similarly, those stocked at 50 g/L showed a threefold difference feed consumption per meal between fish fed twice daily and those fed every two days. At 20 g/L stocking density, the fish fed once every second day consumed, on average, only twice as much feed per meal as the fish fed twice daily.

3.1.5 Feed Conversion Ratio

Upon calculation of various growth parameters, some tanks were found to have biologically impossible values or values indicative of unhealthy fish within a tank. Such tanks were excluded from subsequent analysis. Tanks with negative FCR values and values greater than 10.0 were excluded. Negative values are biologically impossible and values greater than 10.0 were assumed to represent tanks containing unhealthy fish. Tank effects were not evident ($p > 0.05$, three-way ANOVA, Appendix D), therefore data was pooled. Stocking density significantly affected the FCR (Figure 3.3). Fish stocked at 20

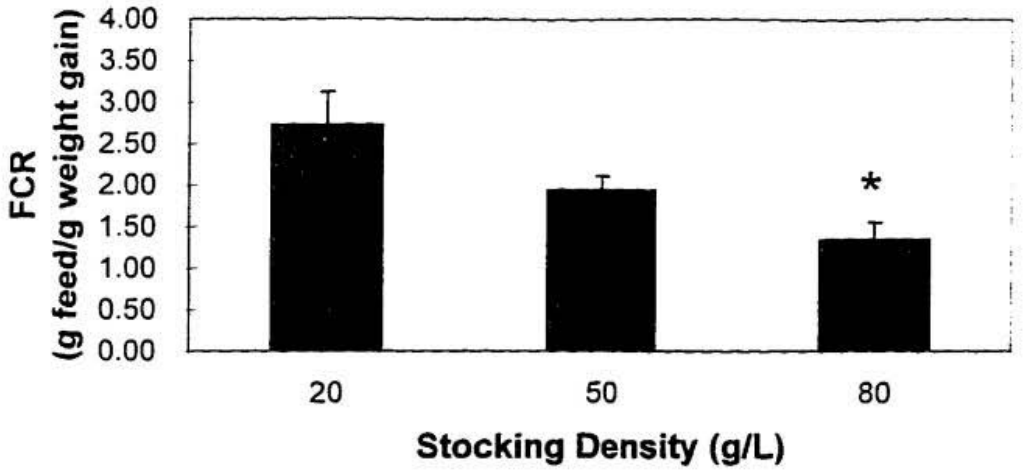


Figure 3.3: Mean feed conversion ratios (FCR) of tanks of 0+ wolffish held at three stocking densities. n (20g/L) = 21; n (50 g/L and 80 g/L) = 26; Vertical bars represent standard error. (* denotes statistically different values).

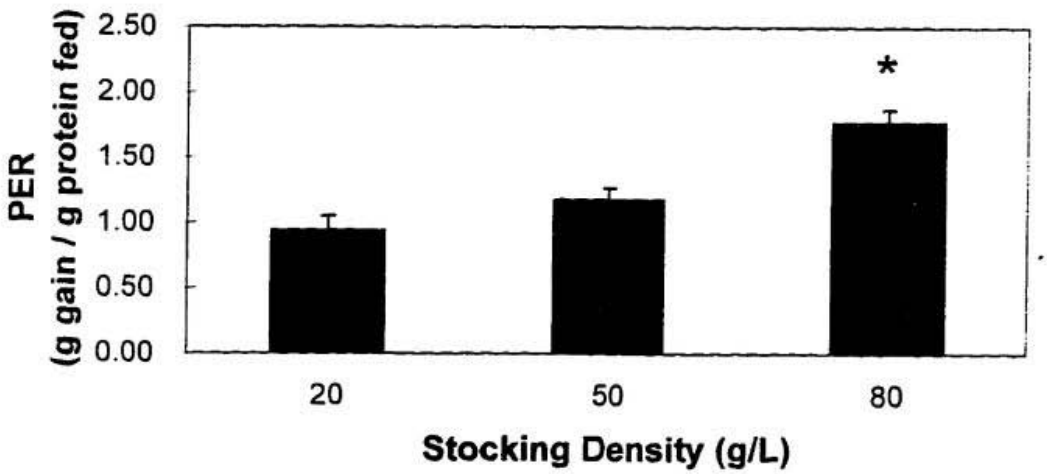


Figure 3.4: Mean protein efficiency ratios (PER) of tanks of 0+ wolffish held at three stocking densities. n (20g/L) = 21; n (50 g/L and 80 g/L) = 26; Vertical bars represent standard error. (* denotes statistically different values).

g/L and 50 g/L had similar feed conversion ratios and both were significantly higher than the FCR of fish stocked at 80 g/L ($p < 0.05$, Tukey's B post hoc test). The FCR was not significantly affected by the feeding schedules ($p > 0.05$, three way ANOVA) (Appendix D).

3.1.6 Protein Efficiency Ratio

Negative protein efficiency ratios (PER) and those equal to or greater than 4.0 were not included in this analysis. Negative values are biologically impossible and values equal to or greater than 4.0 corresponded to tanks discarded due to unacceptable FCR values. Tank data was pooled due to the absence of significant tank effects ($p > 0.05$, three way ANOVA, Appendix E). The PER was significantly affected by the stocking density (Figure 3.4). Fish stocked at 20 g/L and 50 g/L were not statistically different (0.936 ± 0.115, $n = 23$ and 1.175 ± 0.090, $n = 25$, respectively). The PER of fish stocked at 80 g/L was significantly higher than that of fish held at lower stocking densities (1.770 ± 0.102, $n = 26$) ($p = 0.006$, one way ANOVA, Tukey's B Multiple Range Test). No significant effects due to the feeding schedule were evident ($p > 0.05$, three way ANOVA) (Appendix E).

3.2 Dietary Energy Balance Trial

3.2.1 Temperatures

Temperatures in the recirculation system were maintained at 9.0 ± 0.1 °C, with a range from 8.7 °C to 9.3 °C. The ambient water temperature in Swain's Island Tickle dropped from 13.0 °C to 2.0 °C during the twelve week trial, which started on September 10, 1995 (Figure 3.5).

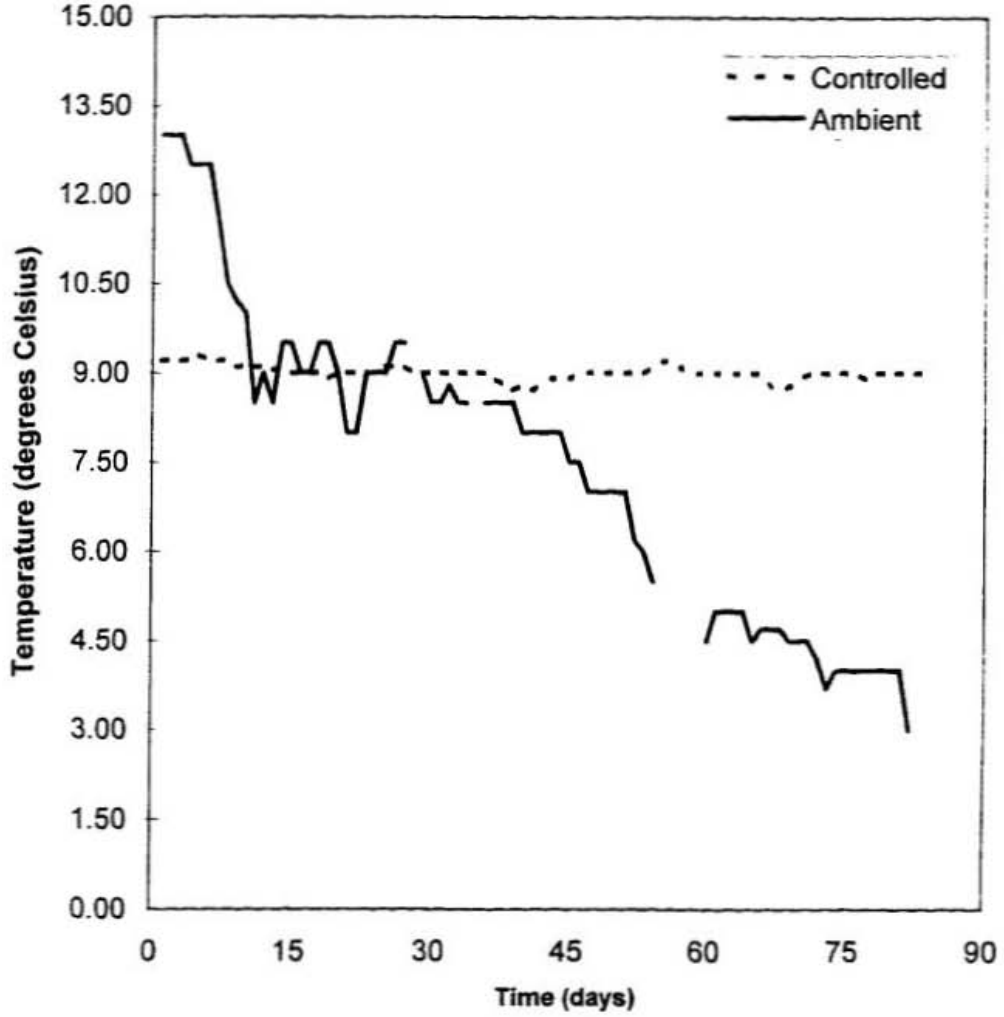


Figure 3.5: Temperature profile of recirculated, thermostatically controlled system (St. John's) and the ambient seawater at Wesleyville, Newfoundland. Starting date: September 10, 1995.

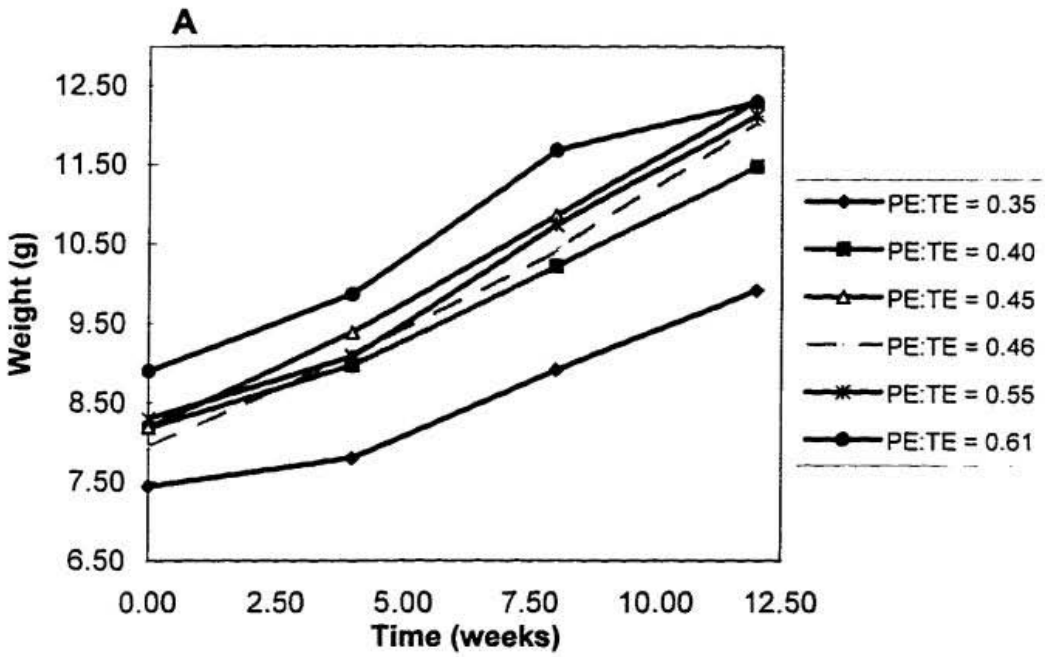
3.2.2 Morphometrics

Growth trials conducted in the recirculating system using 0+ fish showed relatively consistent growth (Figure 3.6; Table 3.3). The mean lengths throughout the trial were significantly affected by diet, the stage of the trial (number of weeks) and the tank ($p < 0.01$, $p = 0.009$ and $p < 0.01$, respectively, three way ANOVA; Appendix F). These tank effects were not evident in the weight data ($p = 0.998$, three way ANOVA; Appendix G). The mean weights per tank in all diet treatments increased significantly during the 12 week trial (Figure 3.6). In the trial conducted at ambient temperature using 0+ fish, the rate of growth decreased as the trial progressed and temperature dropped (Figure 3.6B; Table 3.4).

Significant tank effects influenced the mean lengths and weights of 1+ fish at ambient temperature. In order to establish equivalent initial stocking densities with a relatively small number of fish, different numbers of larger and smaller fish were used in each treatment. This renders the mean fish length and weight data meaningless. Therefore, total weights (on a per-tank basis) are shown in Figure 3.7. Total lengths and weights (on a single tank basis) are shown in Table 3.5. In addition, because the diets were randomly assigned and distributed anonymously to the tanks, one diet (PE:TE = 0.45) was mistakenly fed to three tanks rather than two for the duration of the experiment. Consequently, one diet (PE:TE = 0.55) was offered to only one tank. Total weights per tank are shown to avoid further confusion.

The condition indices (CI) of fish fed diets of PE:TE = 0.35, 0.40 and 0.46 at a constant temperature did not change significantly over the course of the 12 week growth trial (Table 3.6A). Those fish fed diets with PE:TE = 0.45, 0.55 and 0.61 at a constant temperature exhibited a significant increase in condition index over the course of the experiment.

Figure 3.6: Weight change (g) of wolffish fed six diets with a range of PE:TE values under two temperature regimes. (A: 0+ wolffish held at 9 °C, n = 40; B: 0+ wolffish held at ambient seawater temperature, n = 40]


A
12.50
11.50
10.50
9.50
8.50
7.50
6.50
Weight (g)
0.00
2.50
5.00
7.50
10.00
12.50
Time (weeks)
PE:TE = 0.35
PE:TE = 0.40
PE:TE = 0.45
PE:TE = 0.46
PE:TE = 0.55
PE:TE = 0.61

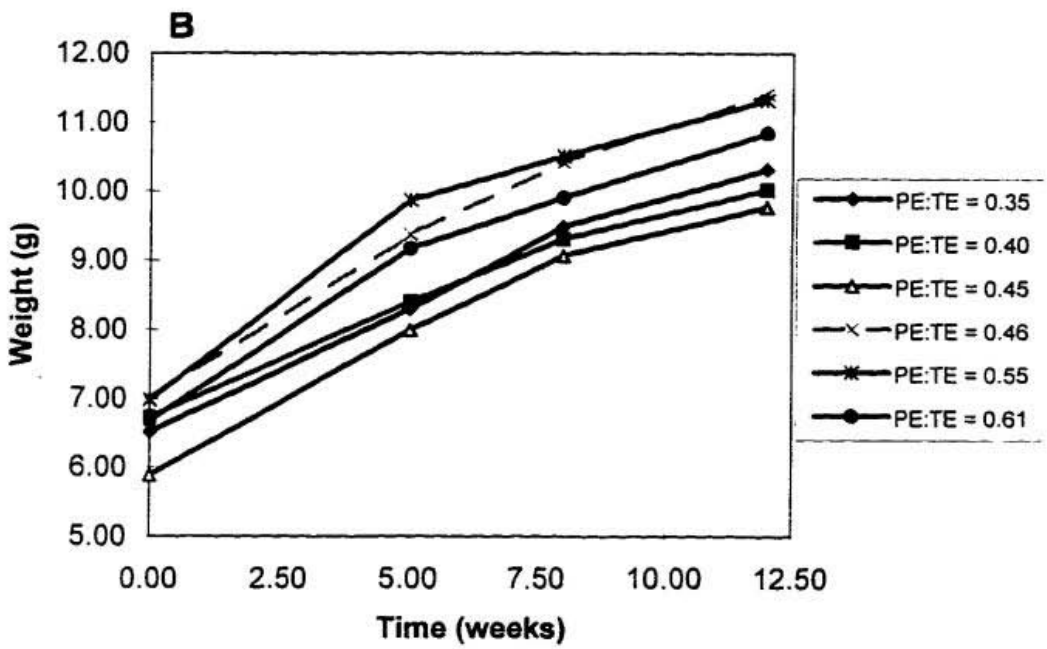

B
12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
Weight (g)
0.00
2.50
5.00
7.50
10.00
12.50
Time (weeks)
PE:TE = 0.35
PE:TE = 0.40
PE:TE = 0.45
PE:TE = 0.46
PE:TE = 0.55
PE:TE = 0.61

Table 3.3: Morphometrics of 0+ wolffish, held at 9 °C, fed six diets with a range of PE:TE values, throughout the 12 week trial (mean ± standard error). Table A: Length (cm); Table B: Weight (g).

A	DIETS (PE:TE)					
Time (weeks)	**0.35**	**0.40**	**0.45**	**0.46**	**0.55**	**0.61**
0	9.073 ± 0.093	9.490 ± 0.125	9.600 ± 0.087	9.430 ± 0.097	9.615 ± 0.112	9.798 ± 0.119
4	9.438 ± 0.095	9.755 ± 0.085	9.910 ± 0.077	9.788 ± 0.096	9.935 ± 0.130	10.218 ± 0.120
8	9.480 ± 0.099	10.125 ± 0.124	10.173 ± 0.096	10.263 ± 0.115	10.065 ± 0.119	10.290 ± 0.120
12	9.877 ± 0.109	10.483 ± 0.113	10.475 ± 0.111	10.615 ± 0.107	10.454 ± 0.142	10.653 ± 0.110

B	DIETS (PE:TE)					
Time (weeks)	**0.35**	**0.40**	**0.45**	**0.46**	**0.55**	**0.61**
0	7.440 ± 0.217	8.175 ± 0.218	8.193 ± 0.213	7.938 ± 0.231	8.303 ± 0.289	8.900 ± 0.281
4	7.808 ± 0.235	8.973 ± 0.180	9.385 ± 0.248	9.113 ± 0.257	9.303 ± 0.307	9.873 ± 0.293
8	8.905 ± 0.280	10.220 ± 0.370	10.858 ± 0.310	10.422 ± 0.332	10.738 ± 0.448	11.683 ± 0.415
12	9.919 ± 0.384	11.540 ± 0.503	12.320 ± 0.414	12.049 ± 0.394	12.129 ± 0.505	12.329 ± 0.376

Table 3.4: Morphometrics of 0+ wolffish held at ambient temperature, fed six diets with a range of PE:TE values, throughout the 12 week trial (mean ± standard error). Table A: Length (cm); Table B: Weight (g).

A	DIETS (PE:TE)					
Time (weeks)	**0.35[2,3]**	**0.40**	**0.45[2]**	**0.46[2]**	**0.55**	**0.61**
0	8.335 ± 0.157	8.245 ± 0.233	7.900 ± 0.154	8.435 ± 0.147	8.765 ± 0.134	8.065 ± 0.197
5[1]	9.175 ± 0.159	8.800 ± 0.223	8.631 ± 0.167	8.985 ± 0.142	9.600 ± 0.151	8.868 ± 0.265
8	9.565 ± 0.180	9.300 ± 0.203	9.032 ± 0.169	9.405 ± 0.464	10.010 ± 0.162	9.316 ± 0.246
12	9.940 ± 0.176	9.570 ± 0.207	9.442 ± 0.168	10.255 ± 0.200	10.250 ± 0.175	9.674 ± 0.244

B	DIETS (PE:TE)					
Time (weeks)	**0.35[2,3]**	**0.40**	**0.45[2]**	**0.46[2]**	**0.55**	**0.61**
0	6.460 ± 0.391	6.385 ± 0.498	5.715 ± 0.355	6.900 ± 0.373	7.045 ± 0.341	6.270 ± 0.436
5[1]	8.480 ± 0.517	7.325 ± 0.491	7.037 ± 0.425	8.590 ± 0.142	9.410 ± 0.457	8.026 ± 0.603
8	9.815 ± 0.647	8.790 ± 0.536	8.011 ± 0.453	10.210 ± 0.665	10.410 ± 0.603	8.658 ± 0.633
12	10.715 ± 0.642	9.615 ± 0.585	9.442 ± 0.528	11.055 ± 0.682	11.285 ± 0.703	9.674 ± 0.729

[1] Transportation was unavailable during week 4, so measurements were made at week 5.
[2] Between weeks 5 and 8 one mortality was noted in this treatment.
[3] Between weeks 8 and 12 two mortalities were found in this treatment.

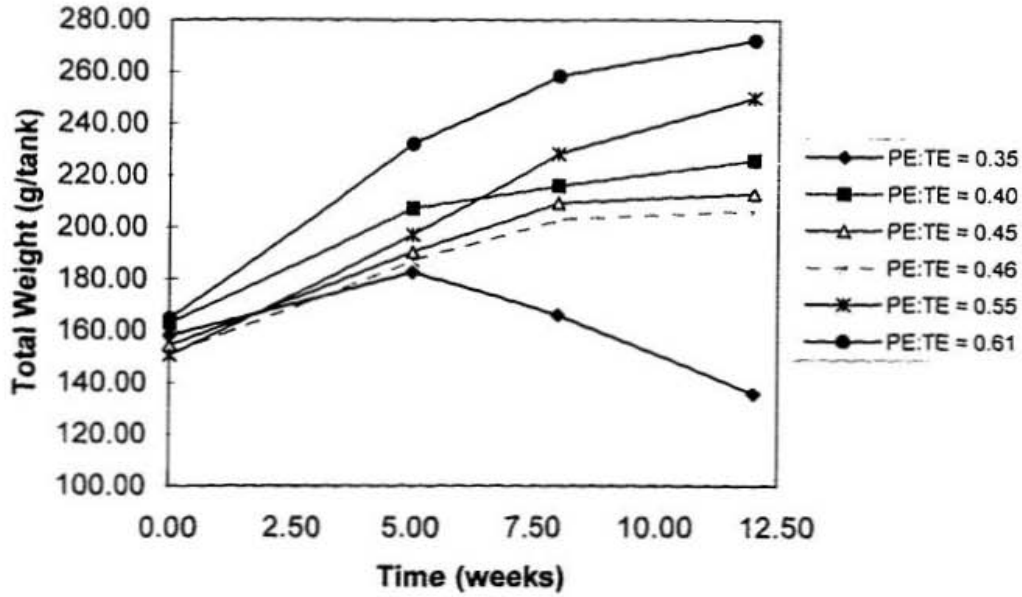


Figure 3.7: Weight change of 1+ wolffish fed six diets with a range of PE:TE values at ambient seawater temperatures. Total weight per tank (g). For PE:TE = 0.35 and 0.46, n = 13; for PE:TE = 0.40 and 0.65, n = 18; for PE:TE = 45, n = 22 and for PE:TE = 0.60, n = 9. (PE:TE = 0.35, 0.45 and 0.46 each had one mortality between week 5 and week 8. PE:TE = 0.35 had two mortalities between week 8 and week 12).

Table 3.5: Total tank weights (g) of 1+ wolffish held at ambient temperature, fed six diets with a range of PE:TE values, throughout the 12 week trial.

	DIETS (PE:TE)					
Time (weeks)	**0.35**	**0.40**	**0.45**	**0.46**	**0.55**	**0.61**
0	158.1	163.1	154.3	151.0	150.6	164.7
5[1]	182.6	207.1	190.4	187.0	197.0	232.0
8	166.1	215.8	209.3	203.0	228.2	258.1
12	135.7	225.8	213.0	206.4	250.0	272.3

[1] Transportation was unavailable during week 4, so measurements were made at week 5.

Table 3.6: Initial and final condition indices (CI) of wolffish fed six diets with a range of PE:TE values for twelve weeks. (Mean ± standard error, n = 40 at beginning of trial). Table A: 0+ fish held at 9 °C; Table B: 0+ fish held at ambient temperature; Table C: 1+ fish held at ambient temperature.)

Table 3.6A

PE:TE	Initial CI	Final CI	Significance[1]
0.35	0.989 ± 0.011	1.013 ± 0.005	n.s.d.
0.40	0.993 ± 0.062	0.976 ± 0.013	n.s.d.
0.45	0.924 ± 0.017	1.065 ± 0.022	$p < 0.000$
0.46	0.946 ± 0.020	0.992 ± 0.016	n.s.d.
0.55	0.926 ± 0.018	1.046 ± 0.017	$p < 0.000$
0.61	0.944 ± 0.018	1.014 ± 0.020	$p = 0.012$

[1] n.s.d = no significant difference, $p > 0.05$, one way ANOVA

Table 3.6B

PE:TE	Initial CI	Final CI	Significance[1]
0.35	1.119 ± 0.018	1.054 ± 0.018	$p = 0.009$
0.40	1.084 ± 0.022	1.046 ± 0.019	n.s.d.
0.45	1.138 ± 0.018	1.024 ± 0.016	$p < 0.000$
0.46	1.113 ± 0.019	1.071 ± 0.019	n.s.d.
0.55	1.078 ± 0.017	1.010 ± 0.015	$p = 0.003$
0.61	1.147 ± 0.021	1.055 ± 0.031	$p = 0.016$

[1] n.s.d = no significant difference, $p > 0.05$, one way ANOVA

Table 3.6C

PE:TE	Initial CI	Final CI	Significance[1]
0.35	1.019 ± 0.040	0.999 ± 0.037	n.s.d.
0.40	1.008 ± 0.028	1.043 ± 0.033	n.s.d.
0.45	1.055 ± 0.049	1.056 ± 0.035	n.s.d.
0.46	0.945 ± 0.019	0.995 ± 0.047	n.s.d.
0.55	0.982 ± 0.036	1.022 ± 0.040	n.s.d.
0.61	0.989 ± 0.026	1.000 ± 0.041	n.s.d.

[1] n.s.d = no significant difference, $p > 0.05$, one way ANOVA

Under ambient water temperatures, all diets except for PE:TE 0.40 and 0.45 produced a significant decrease in the mean CI of 0+ fish (Table 3.6B). Diets with PE:TE 0.40 and 0.45 produced no significant change in the condition index.

The CI of 1+ fish held at ambient temperature was not significantly affected by tank replicates ($p > 0.05$, one way ANOVA). No significant differences were apparent among diet treatments ($p > 0.05$, one way ANOVA) for either initial or final CI data (Table 3.6C).

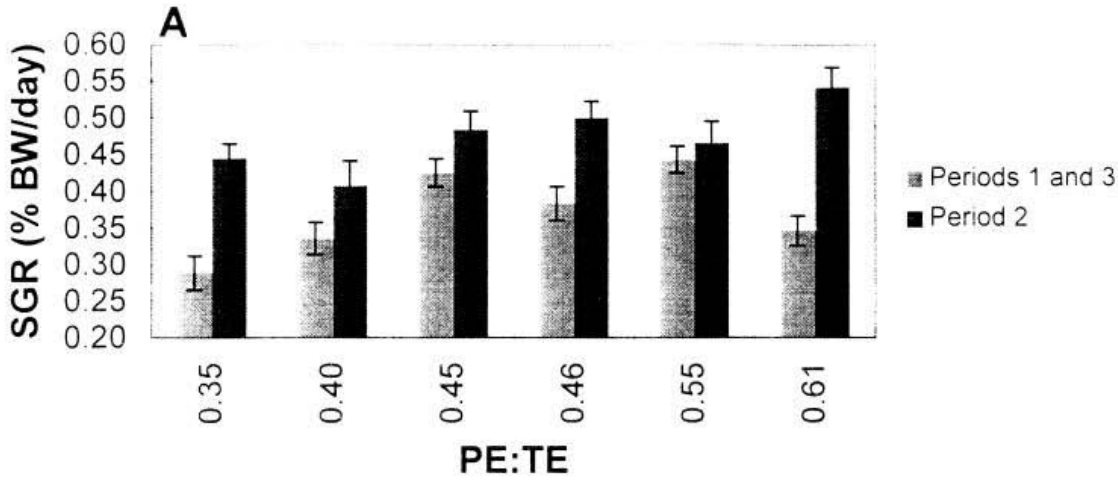
3.2.3 Specific Growth Rate

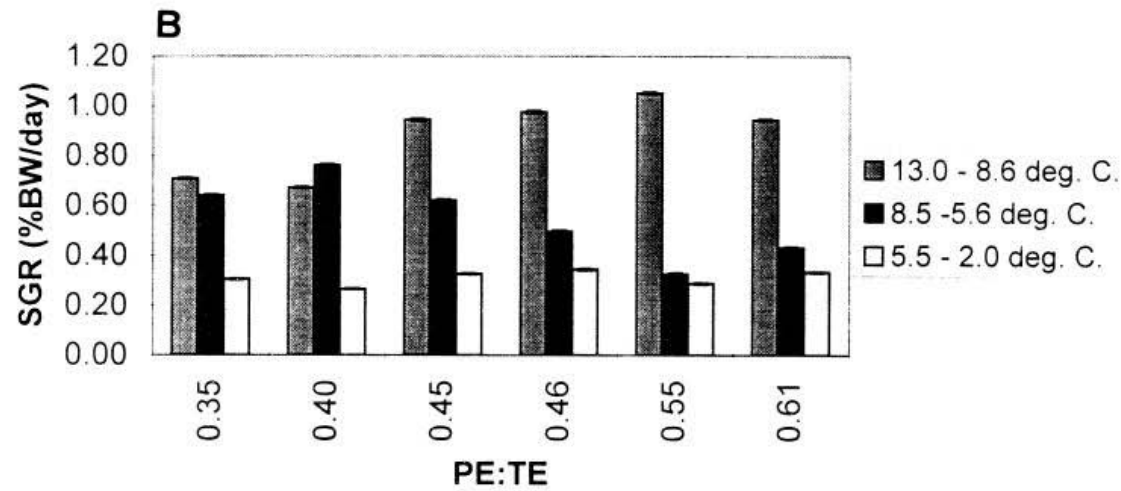
In the trial conducted at a constant temperature, replicates were not statistically different and were pooled (Figure 3.8A). Time was found to be a significant factor ($p < 0.05$, three way ANOVA) and further investigation showed that the SGR during the second period (weeks 4 to 8) was significantly higher than in the other two periods. Periods one and three, which were statistically similar, were pooled (Figure 3.8A).

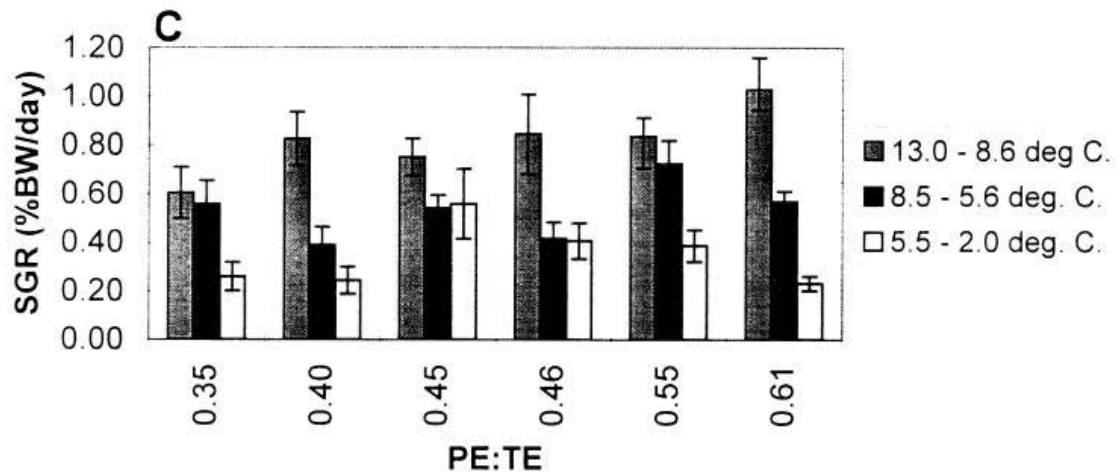
In the trial held at ambient temperature with 0+ fish, there were no significant tank replicate effects ($p > 0.05$, three way ANOVA), so replicates were pooled (Appendix H). Diet effects were not significant ($p > 0.05$, two way ANOVA), but the time effect was significant. This corresponds with the decrease in temperature (Figure 3.8B). The SGR in each period decreased significantly.

Examination of the SGR of 1+ fish at ambient temperature revealed no significant replicate effects ($p > 0.05$, three way ANOVA), therefore replicates were pooled. The two way ANOVA confirmed that diet effects were not significant ($p > 0.05$) but that the time (i.e., temperature) was a significant factor ($p < 0.001$; Figure 3.8C).

Figure 3.8: Specific growth rates (SGR) of 0+ and 1+ wolffish fed six diets with a range of PE:TE values under two temperature regimes. (A: 0+ wolffish held at 9 °C, n = 40; B: 0+ wolffish held at ambient seawater temperature, n = 40; C: 1+ fish at ambient seawater temperature. For PE:TE = 0.35 and 0.46, n = 13; for PE:TE = 0.40 and 0.65, n = 18; for PE:TE = 45 n = 22 and for PE:TE = 0.60, n = 9.)


A
SGR (% BW/day)
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
Periods 1 and 3
Period 2


B
SGR (%BW/day)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
13.0 - 8.6 deg. C.
8.5 -5.6 deg. C.
5.5 - 2.0 deg. C.


C
SGR (%BW/day)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
13.0 - 8.6 deg C.
8.5 - 5.6 deg. C.
5.5 - 2.0 deg. C.

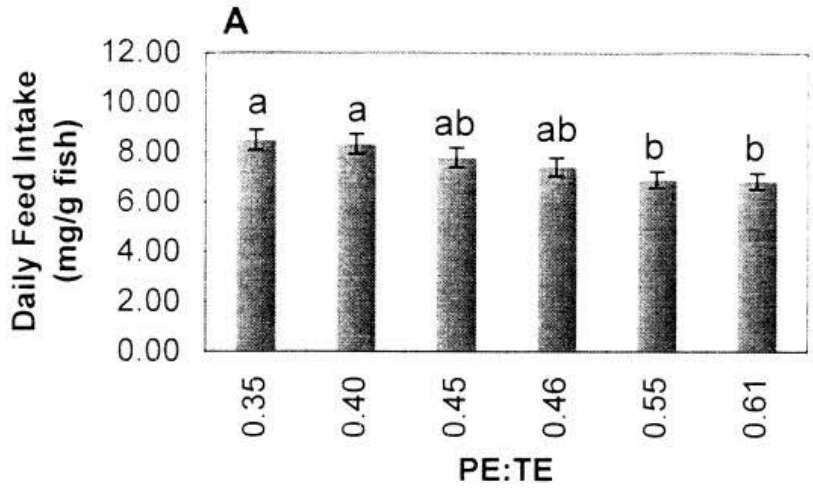
3.2.4 Feed Consumption

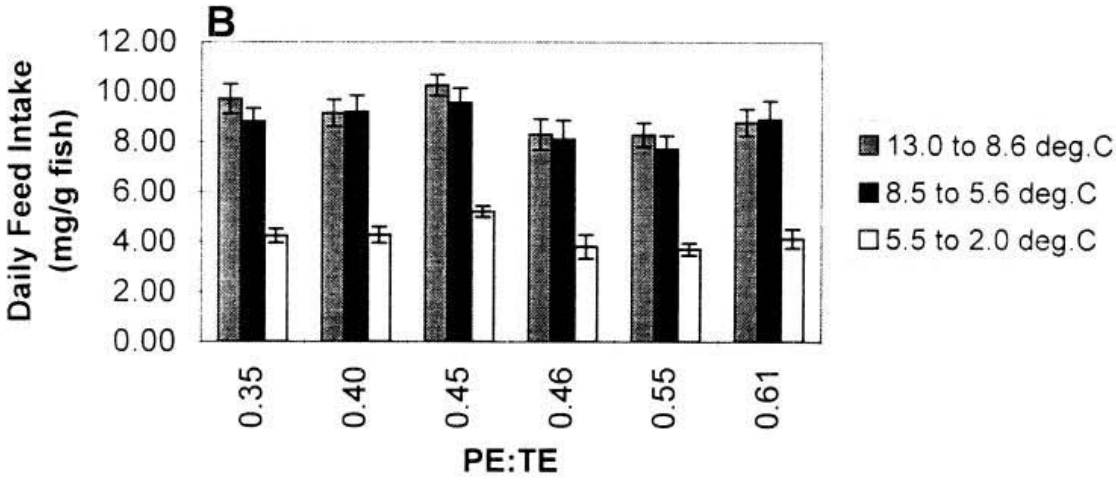
At constant temperature, neither replicate nor time effects were significant for feed consumption by 0+ fish. Mean daily consumption of feed decreased as the dietary PE:TE increased (Figure 3.9A). Post hoc analysis indicated that consumption of diets with PE:TE = 0.55 and 0.61 was significantly lower than diets with PE:TE = 0.35 and 0.40.

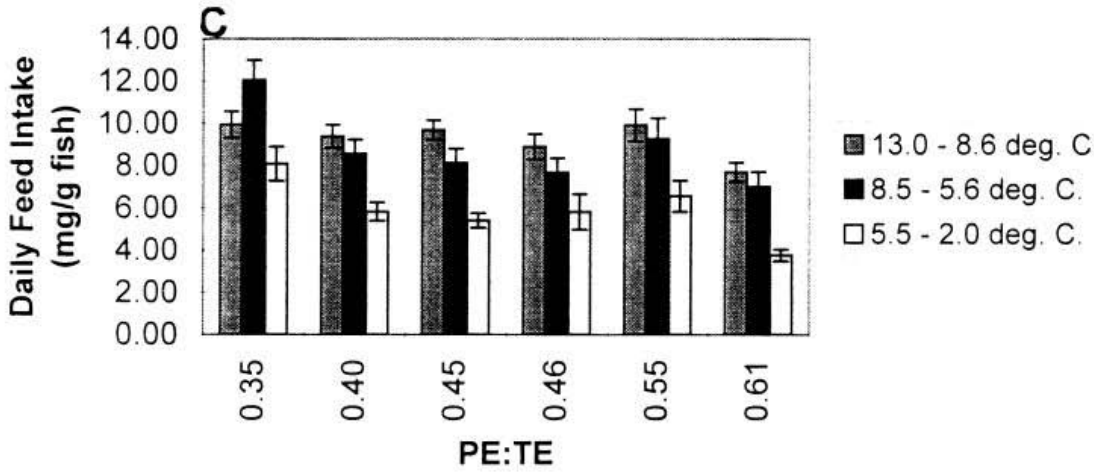
Significant replicate effects were found in the experiment using 0+ fish at ambient temperature ($p < 0.01$, three way ANOVA; Appendix I). The mean intake values for replicates A and B were 7.825 ± 0.999 mg/g and 6.917 ± 0.740 mg/g, respectively. Consumption rates during periods one and two were not significantly different (9.075 ± 0.982 mg/g and 8.711 ± 1.064, respectively; $p > 0.05$, one way ANOVA). Both were significantly higher than the consumption during period three (4.231 ± 0.341; $p < 0.001$, one way ANOVA; Figure 3.9B). Of all diets in period three, the consumption of diet PE:TE = 0.45 was significantly higher than the consumption of diets with PE:TE = 0.46 and 0.55 (Tukey's B).

No significant effects were found for the feed consumption by 1+ fish ($p > 0.05$, three way ANOVA) so replicates were pooled. Both diet and time were significant factors ($p < 0.001$ and $p < 0.01$, respectively, two way ANOVA; Appendix J), and the interaction of the two factors was not significant. No periods within any diet treatment had statistically similar rates of consumption ($p > 0.05$, one way ANOVA). The 1+ fish showed a more consistent decrease in feed consumption as the PE:TE value increased, than did the 0+ fish. The mean daily feed intake by 1+ fish significantly decreased as the ambient temperature decreased. During the first period, when the ambient temperature fell from 13.0 °C to 8.6 °C, the feed intake was not significantly different than the intake during the second period (ambient temperatures 8.5 °C to 5.6 °C). Therefore, feed intake values for the first two periods were pooled (Figure 3.9C). For all diets, the feed intake during the

Figure 3.9: Mean daily feed intake (mg feed/g fish) by 0+ and 1+ wolffish fed six diets with a range of PE:TE values under two temperature regimes. (A: 0+ wolffish held at 9 °C, n = 168; B: 0+ wolffish held at ambient seawater temperature; C: 1+ fish at ambient temperature. In Figures B and C, period 1: n = 70, period 2: n = 42, period 3: n = 56.) Vertical bars represent standard error. Similar letters denote statistically similar values.


A
Daily Feed Intake (mg/g fish)
12.00
10.00
8.00
6.00
4.00
2.00
0.00
a
a
ab
ab
b
b
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE


B
Daily Feed Intake (mg/g fish)
12.00
10.00
8.00
6.00
4.00
2.00
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
13.0 to 8.6 deg.C
8.5 to 5.6 deg.C
5.5 to 2.0 deg.C


C
Daily Feed Intake (mg/g fish)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
13.0 - 8.6 deg. C.
8.5 - 5.6 deg. C.
5.5 - 2.0 deg. C.

third period, when the temperature ranged from 5.5 °C to 2.0 °C, was significantly lower than during the previous two periods.

3.2.5 Daily Lipid Intake

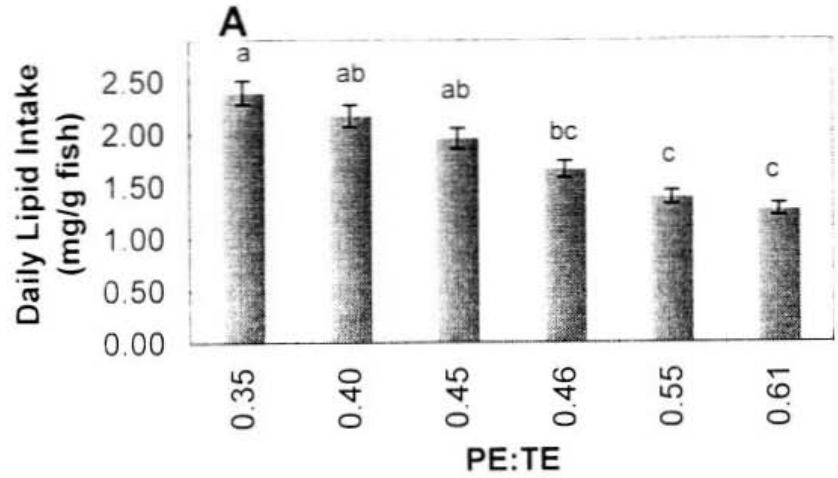
The daily lipid intake by six-month-old fish held at 9 °C was not significantly different in the replicates or over time ($p > 0.05$, three way ANOVA). The lipid intake, however, decreased significantly as the PE:TE increased ($p < 0.0001$, one way ANOVA; Figure 3.10A). Fish fed diet PE:TE = 0.35 consumed a significantly greater amount of lipid daily ($2.397 \pm 1.625 * 10^{-4}$ mg/g), than fish fed diets with PE:TE ≥ 0.45 (Tukey's B).

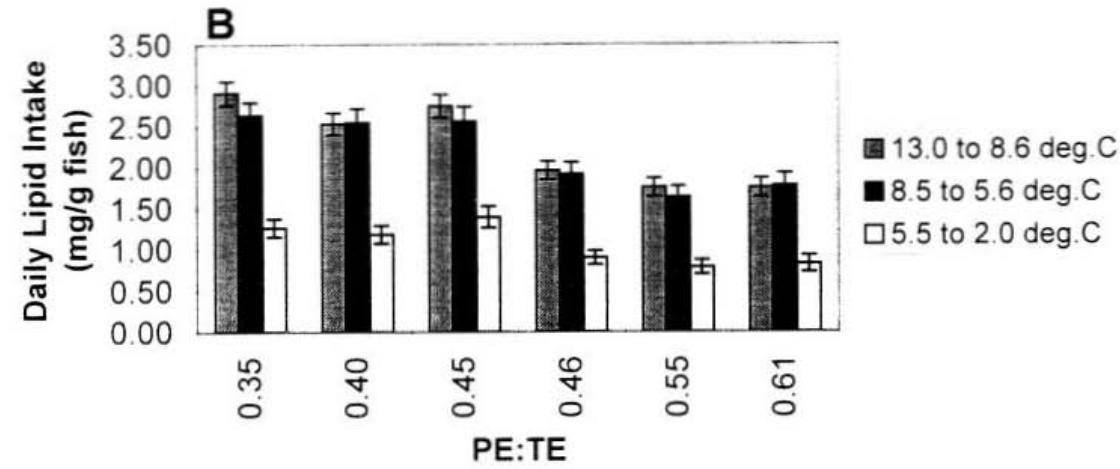
Similarly, the lipid intake by fish fed diet PE:TE = 0.40 ($2.171 \pm 1.356 * 10^{-4}$ mg/g) was significantly greater than that of fish fed diets PE:TE ≥ 0.55. Finally, lipid intake by fish fed diet PE:TE = 0.55 ($1.385 \pm 5.277 * 10^{-5}$ mg/g) was significantly greater than that of fish fed diet PE:TE = 0.61 ($1.270 \pm 4.417 * 10^{-5}$ mg/g).

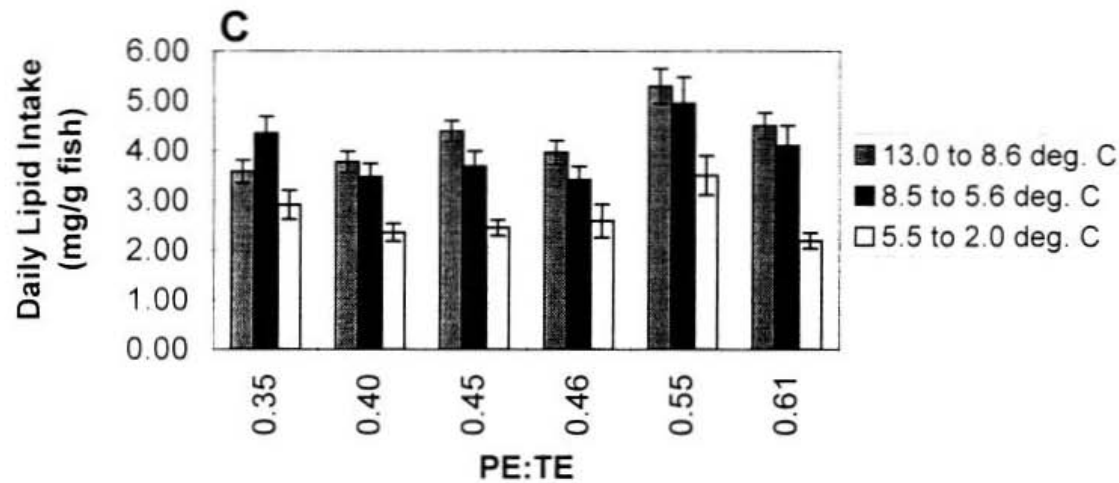
Likewise, the daily lipid intake by 0+ fish at ambient temperature decreased significantly as the PE:TE increased ($p < 0.01$, two way ANOVA; Figure 3.10B). The decreasing temperature also caused a significant reduction in the lipid intake. The first two periods had statistically similar lipid intake values for each diet ($p > 0.05$, one way ANOVA). Lipid intake during period three was significantly lower than the first two, approximately half the intake during the first two periods. Fish fed diet PE:TE = 0.45 consumed 2.679 ± 0.172 mg lipid/g each day in periods one and two and 1.397 ± 0.120 mg lipid/g in period three.

Tank effects did not significantly affect the lipid intake of 1+ fish at ambient temperature ($p > 0.05$, three way ANOVA). Diet and time were both significant factors ($p < 0.001$ and $p < 0.01$, respectively, two way ANOVA). With the exception of fish fed diet PE:TE

Figure 3.10: Mean daily lipid intake (mg lipid/g fish) by 0+ and 1+ wolffish fed six diets with a range of PE:TE values under two temperature regimes. (A: 0+ wolffish held at 9 °C, n = 168; B: 0+ wolffish held at ambient seawater temperature; C: 1+ fish at ambient temperature. In Figures B and C, period 1: n = 70, period 2: n = 42, period 3: n = 56.) Vertical bars represent standard error.


A
Daily Lipid Intake (mg/g fish)
2.50
2.00
1.50
1.00
0.50
0.00
a
ab
ab
bc
c
c
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE


B
Daily Lipid Intake (mg/g fish)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
13.0 to 8.6 deg.C
8.5 to 5.6 deg.C
5.5 to 2.0 deg.C


C
Daily Lipid Intake (mg/g fish)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
13.0 to 8.6 deg. C
8.5 to 5.6 deg. C
5.5 to 2.0 deg. C

= 0.35, which had a higher lipid intake in period two than in period one, the lipid intake decreased significantly as time passed and the temperature decreased (Figure 3.10). The highest mean daily lipid intake was seen in fish fed diet PE:TE = 0.55, which consumed 5.294 ± 0.350 mg lipid/g fish in period one and 3.505 ± 0.394 mg lipid/g fish in period three.

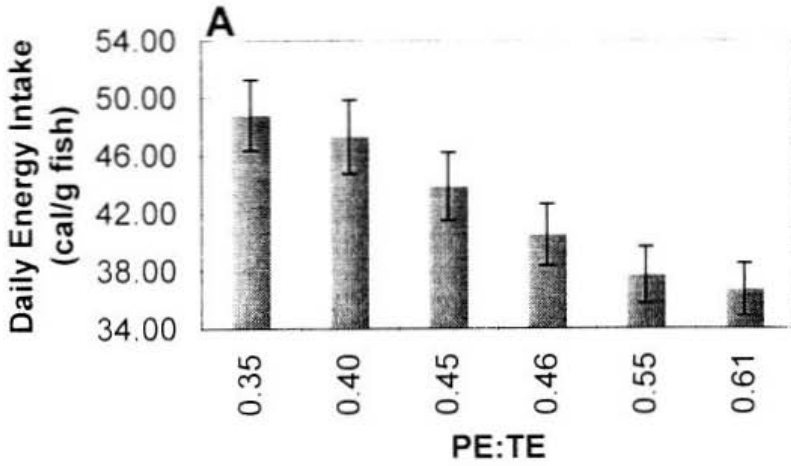
3.2.6 Daily Energy Intake

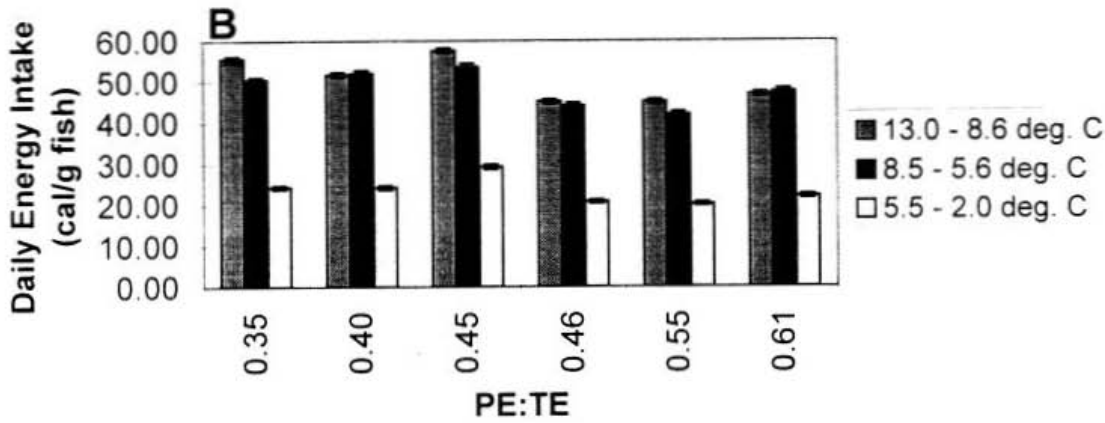
In fish held at constant temperature there were no significant differences in daily energy intake among replicates nor over time ($p > 0.05$ for each factor, three way ANOVA). As the PE:TE increased the daily energy intake decreased significantly ($p < 0.0001$, one way ANOVA; Figure 3.11A). Diet PE:TE = 0.35 was consumed at a significantly higher rate than diets PE:TE ≥ 0.46 (Tukey's B). Also, diet PE:TE = 0.40 was consumed at a higher rate than diets PE:TE ≥ 0.55 (Tukey's B).

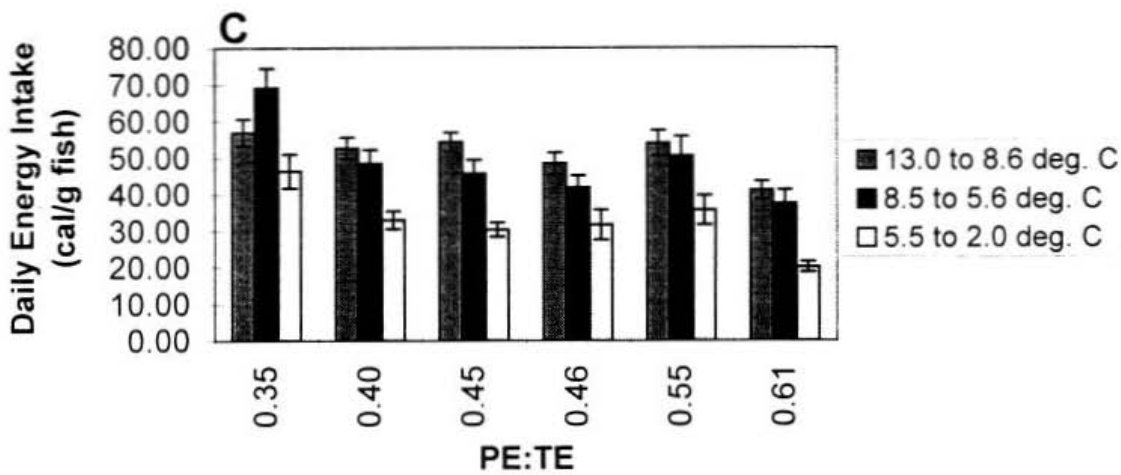
The energy intake of 0+ fish at ambient temperature was significantly influenced by diet ($p < 0.01$, two way ANOVA) and time ($p < 0.001$, two way ANOVA), but no tank effects were apparent ($p > 0.05$) (Figure 3.11B). The decreasing temperature had an effect such that the energy intakes for periods one and two, covering a temperature range 13.0 °C to 5.5 °C, were statistically similar (pooled value = 49.654 ± 0.948 cal/g), but the mean value for period three was significantly lower (23.503 ± 0.768 cal/g). No interactions between diet and period were found ($p > 0.05$, two way ANOVA).

Replicates were not a significant factor in the energy intake of 1+ fish at ambient temperature ($p > 0.05$, three way ANOVA). Diet and time were significant factors in the daily energy intake ($p < 0.05$ for both factors, two way ANOVA). In period one the fish fed diet PE:TE = 0.35 consumed 56.986 ± 3.621 calories/g each day, significantly more than those fed diet PE:TE = 0.61 which consumed 41.138 ± 2.409 calories/g each day

Figure 3.11: Mean daily energy intake (calories/g fish) by 0+ and 1+ wolffish fed six diets with a range of PE:TE values under two temperature regimes. (A: 0+ wolffish held at 9 °C, n = 168; B: 0+ wolffish held at ambient seawater temperature; C: 1+ fish at ambient temperature. In Figures B and C, period 1: n = 70, period 2: n = 42, period 3: n = 56.) Vertical bars represent standard error.


A
Daily Energy Intake (cal/g fish)
54.00
50.00
46.00
42.00
38.00
34.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE


B
Daily Energy Intake (cal/g fish)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
13.0 - 8.6 deg. C
8.5 - 5.6 deg. C
5.5 - 2.0 deg. C


C
Daily Energy Intake (cal/g fish)
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
13.0 to 8.6 deg. C
8.5 to 5.6 deg. C
5.5 to 2.0 deg. C

(Tukey's B). Similarly, during period 3, the fish fed diet PE:TE = 0.35 consumed 46.371 ± 4.611 calories/g daily compared to the 20.107 ± 1.427 calories/g consumed daily by the fish fed diet PE:TE = 0.61 (Figure 3.11C). Post hoc analysis (Tukey's B) revealed that the energy intake when PE:TE > 0.54 was significantly lower than when PE:TE < 0.41 (Figure 3.11). No interaction between diet and time was evident ($p > 0.05$, two way ANOVA).

3.2.7 Hepatosomatic Index

Replicate effects were not significant ($p > 0.05$, two way ANOVA). Mean hepatosomatic indices of 0+ fish held at 9 °C were plotted against the dietary PE:TE treatment (Figure 3.12). The hepatosomatic index of fish fed PE:TE = 0.35 (4.645 ± 0.127 %BW), was significantly higher than the next closest hepatosomatic index value (PE:TE = 0.55 ; 4.246 ± 0.096 %BW; Tukey's B). Fish fed diets with PE:TE = 0.45 and 0.55 had statistically similar hepatosomatic index values as did fish fed diets with PE:TE = 0.40 and 0.46, with both pairs of values being significantly different from each other. Despite the significant results, no trend was evident ($p > 0.05$, regression ANOVA). Although the liver does not appear to be affected by changes in the dietary lipid balance, observations during dissections revealed white fatty deposits in the mesentery associated with the intestines. No analysis was done regarding the composition of these deposits and limited notes were made on the dietary PE:TE value of the fish in which these deposits were found.

When the hepatosomatic index values were plotted against the daily lipid intake, no trend was evident ($p > 0.05$, regression ANOVA; Figure 3.13). Likewise, a plot of the hepatosomatic indices by daily energy intake showed no correlation ($p > 0.05$, regression ANOVA; Figure 3.14).

Figure 3.12: Hepatosomatic indices (%BW) of 0+ wolffish, held at 9 °C, in relation to the dietary PE:TE value. Vertical bars represent standard error.

Figure 3.13: Hepatosomatic indices (%BW) of 0+ wolffish, held at 9 °C, in relation to the mean daily lipid consumption (mg lipid/g fish). Vertical bars represent standard error.

Figure 3.14: Hepatosomatic indices (%BW) of 0+ wolffish, held at 9 °C, in relation to the mean daily energy consumption (calories/g fish). Vertical bars represent standard error.

Figure 3.12

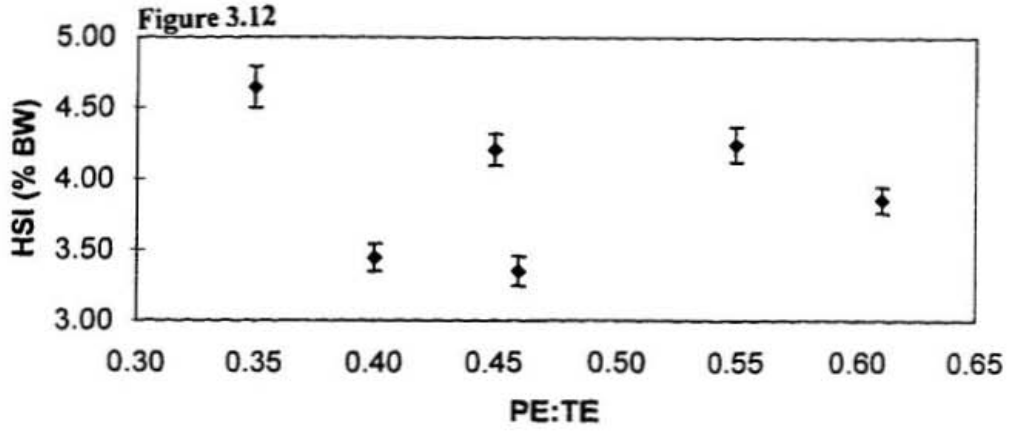


Figure 3.13

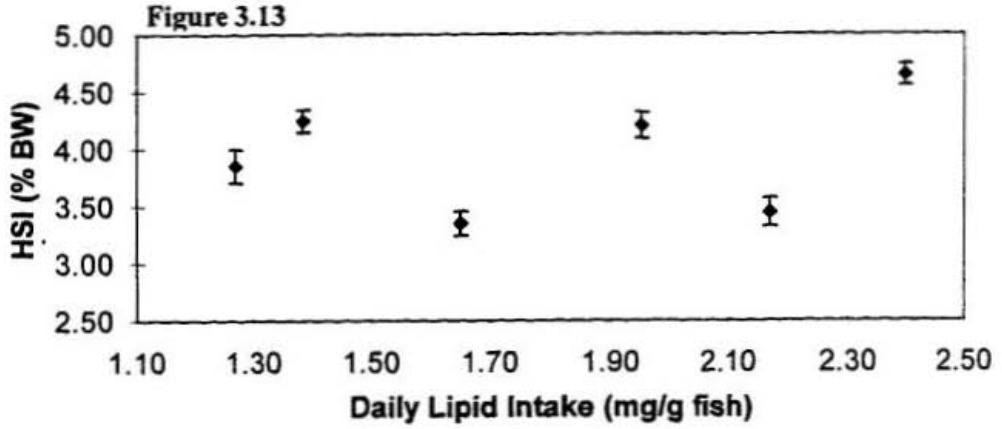
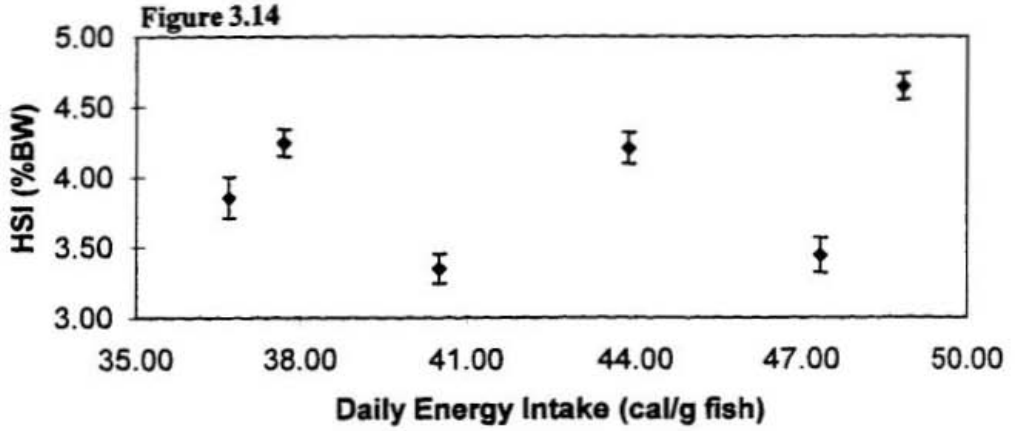


Figure 3.14



3.2.8 Feed Conversion Ratio

Replicates for each diet were not statistically different, so results for each diet were pooled ($p > 0.05$, three way ANOVA). No diet effects were significant ($p > 0.05$, two way ANOVA). Time was a significant factor ($p = 0.017$, two way ANOVA), with a significantly lower FCR in period two (1.196 ± 0.049) than in periods one and three, which were not statistically different according to post hoc analysis (2.471 ± 0.409, and 2.674 ± 0.528, respectively; Figure 3.15A). In period two, no diet was converted more efficiently than another ($p > 0.05$).

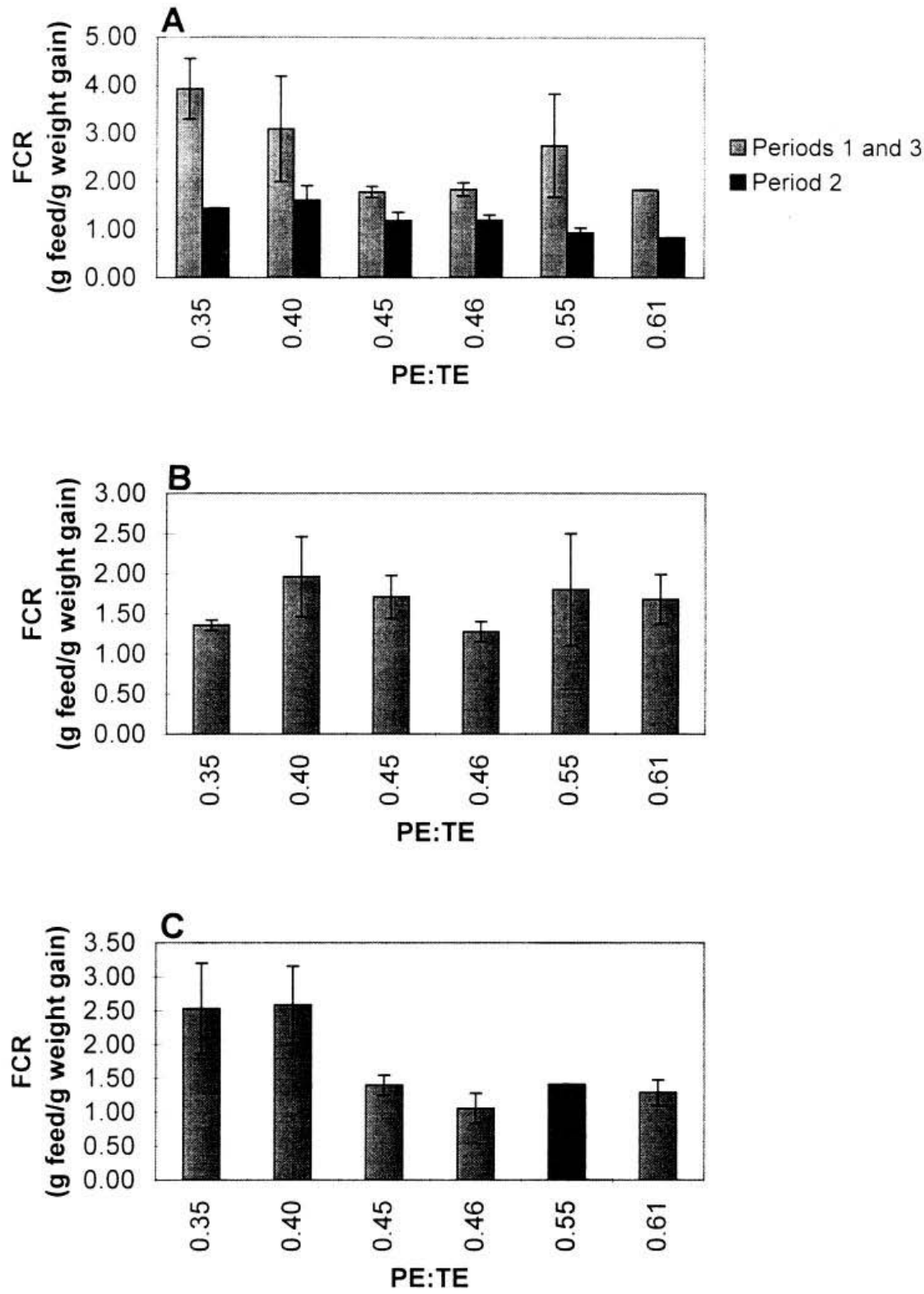
At ambient temperatures, the 0+ fish showed no significant difference in the feed conversion ratio between replicates, diets and time periods ($p > 0.05$, three way ANOVA) (Figure 3.15B). The overall mean FCR for all diets and periods was 1.635 ± 1.152 ($n = 35$).

A similar situation was evident in the 1+ fish maintained at ambient temperatures (Figure 3.15C). No significant differences were found in the feed conversion ratios among replicates within diets nor among diets ($p > 0.05$, three way ANOVA). The mean FCR was 1.712 ± 0.186.

3.2.9 Protein Efficiency Ratio

Replicates of each diet treatment in the constant temperature trial with the 0+ fish were not statistically different, therefore PER values were pooled ($p > 0.05$, three way ANOVA). Again, the results during period two (2.115 ± 0.163) were higher than the results in periods one and three, which were subsequently pooled (pooled PER = 1.133 ± 0.088). No significant differences were detected in the PER among the diet treatments [p

Figure 3.15: Feed conversion ratios (FCR) of 0+ and 1+ wolffish fed six diets with a range of PE:TE values under two temperature regimes. (A: 0+ wolffish held at 9 °C, n = 2 (periods 1 and 3), n = 2 (period 2); B: 0+ wolffish held at ambient seawater temperature, n = 4; C: 1+ fish at ambient seawater temperature, n = 3 for PE:TE = 0.45, n = 1 for PE:TE = 0.55 and n = 2 for remaining diets. Vertical bars represent standard error.


A
FCR
(g feed/g weight gain)
5.00
4.00
3.00
2.00
1.00
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
Periods 1 and 3
Period 2
B
FCR
(g feed/g weight gain)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
C
FCR
(g feed/g weight gain)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE

> 0.05, one way ANOVA (periods one and three pooled) and p > 0.05, one way ANOVA (period two); Figure 3.16A].

At ambient temperatures the PER of 0+ fish held in replicate tanks within diet treatments were not statistically different and no differences were attributable to decreasing temperatures (p > 0.05 for each factor, three way ANOVA; Figure 3.16B). The mean PER for the trial was 1.774 ± 0.128 (n = 35).

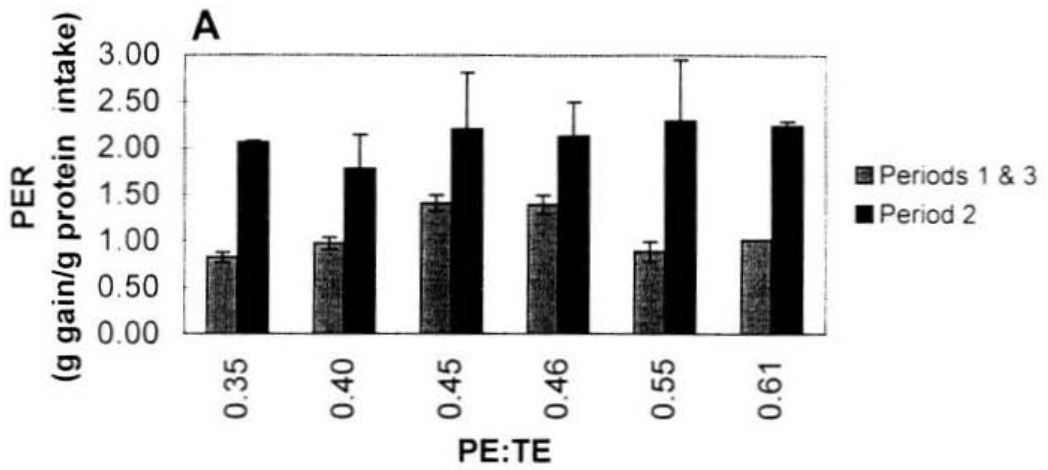
At ambient temperature, 1+ fish showed no significant differences in PER between replicates, nor with decreasing temperatures (p > 0.05, three way ANOVA; Figure 3.16C). There were no significant differences in PER among diet treatments (p > 0.05, one way ANOVA). The mean PER for the trial was 1.665 ± 0.142 (n = 27).

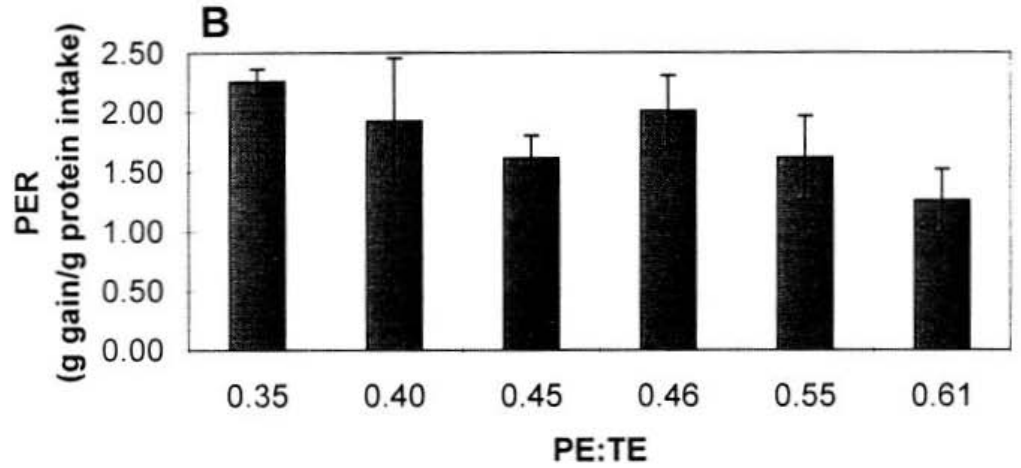
3.2.10 Production Cost

The production cost of 0+ fish at 9 °C was not significantly affected by replicate effects (p > 0.05, three way ANOVA), so the tank data was pooled. Diets were not a significant factor (p > 0.05, two way ANOVA), but the period significantly influenced the production cost (p = 0.017, two way ANOVA; Figure 3.17A). Periods one and three were statistically similar (pooled value = 3.402 ± 0.315 $/kg), and were significantly higher than period two (1.591 ± 0.167 $/kg; Tukey's B).

The cost of producing 0+ fish at ambient temperature was not significantly affected by tank effects, time or diets (p > 0.05 for all factors, three way ANOVA). The mean cost of production was 1.976 ± 0.121 $/kg (Figure 3.17B).

Figure 3.16: Protein efficiency ratios (PER) of 0+ and 1+ wolffish fed six diets with a range of PE:TE values under two temperature regimes. (A: 0+ wolffish held at 9 °C, n = 4 (periods 1 and 3) and n = 2 (period 2); B: 0+ wolffish held at ambient seawater temperature; n = 6; C: 1+ fish at ambient seawater temperature. n = 3 for PE:TE = 0.45, n = 1 for PE:TE = 0.55 and n = 2 for remaining diets. Periods refer to 4 week intervals during the 12 week trial. Vertical bars represent standard error.


A
PER
(g gain/g protein intake)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Periods 1 & 3
Period 2
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE


B
PER
(g gain/g protein intake)
2.50
2.00
1.50
1.00
0.50
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE

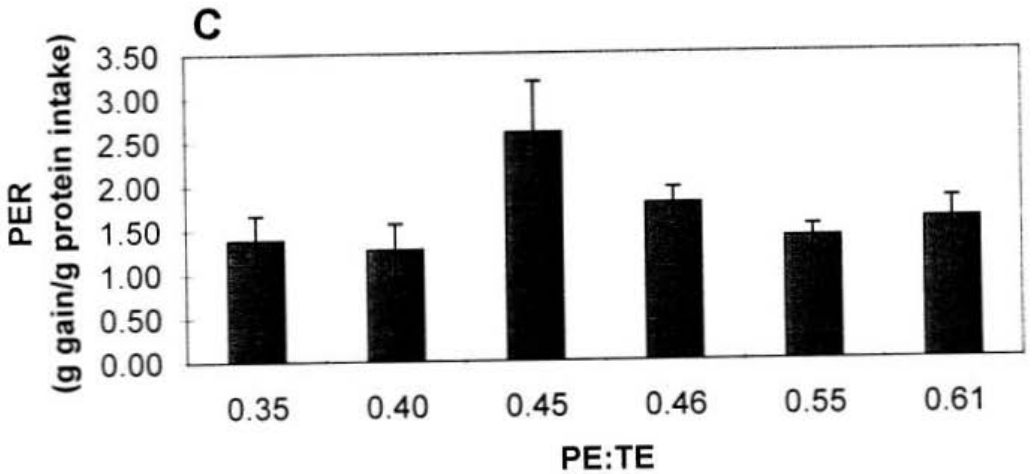
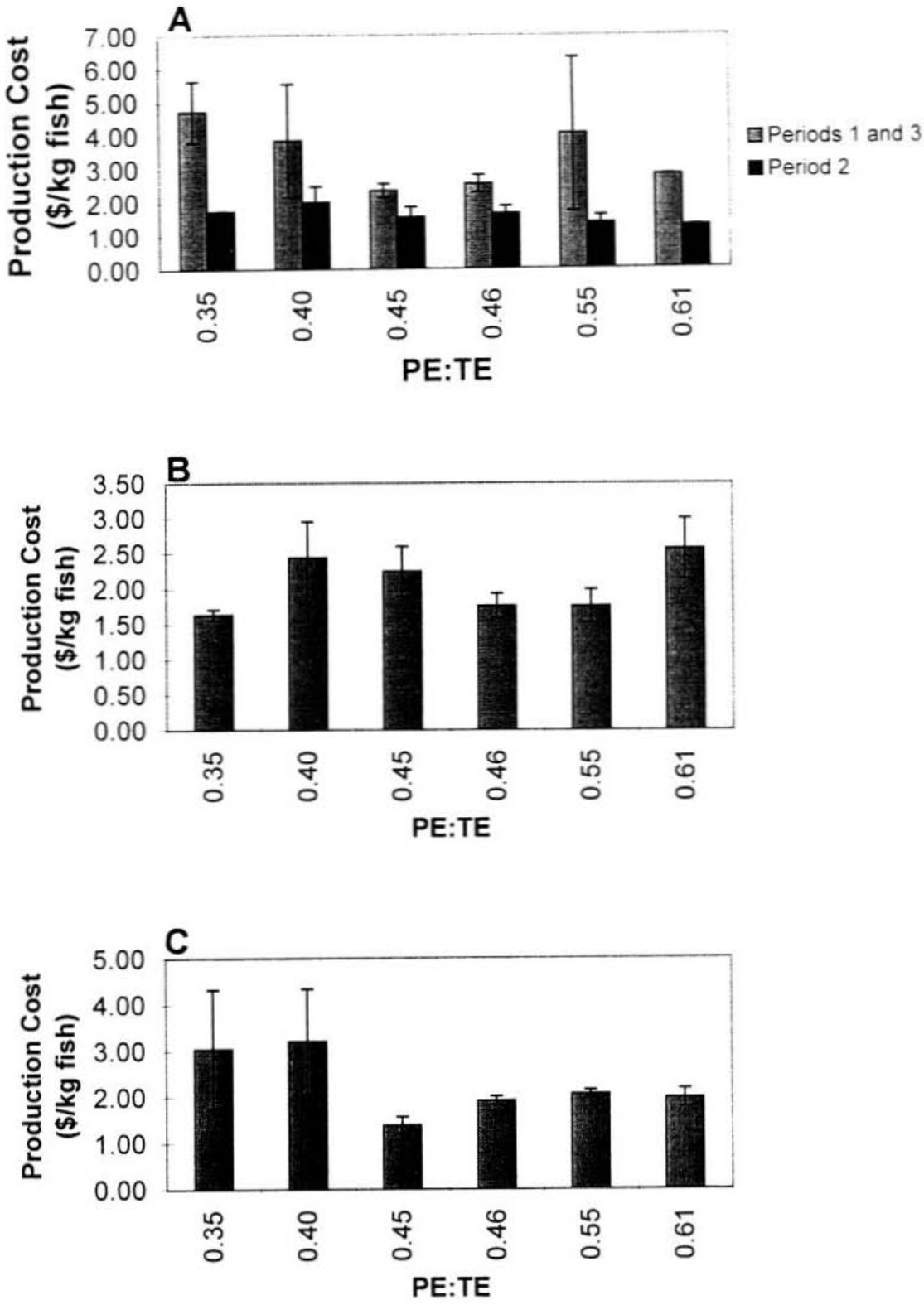

C
PER
(g gain/g protein intake)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE

Figure 3.17: Cost of production ($/kg) of 0+ and 1+ wolffish fed six diets with a range of PE:TE values under two temperature regimes. A: 0+ wolffish held at 9 °C, B: 0+ wolffish held at ambient seawater temperature; C: 1+ fish at ambient seawater temperature. Only feed costs are taken into account. Vertical bars represent standard error.


A
Production Cost ($/kg fish)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
Periods 1 and 3
Period 2
B
Production Cost ($/kg fish)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE
C
Production Cost ($/kg fish)
5.00
4.00
3.00
2.00
1.00
0.00
0.35
0.40
0.45
0.46
0.55
0.61
PE:TE

At ambient temperature, the production cost of 1+ wolffish was independent of tank effects, time and diet effects ($p > 0.05$ for all factors, three way ANOVA). The mean value for the treatment was 2.270 ± 0.219 $/kg (Figure 3.17C).

3.3. Gastric Evacuation Trial

3.3.1 PE:TE Trial

Initial feed intake by 0+ wolffish, expressed as both grams and %BW, was not linearly related to fish size ($p > 0.05$, regression ANOVA), so no corrections were made prior to analysis. Linear and logarithmic equations were calculated to fit the gastric evacuation profile and in all cases, the R value and regression ANOVA indicated that the linear equation was the more accurate, with one exception (Table 3.7). The evacuation rate of fish fed diet PE:TE = 0.45, expressed as a percent body weight was better described by a logarithmic equation (Equation 8) than a linear equation (Equation 9).

$$y = -2.8757x^{-0.3020}; \quad R^2 = 0.3467, \quad p < 0.001 \tag{8}$$

$$y = -0.0003x + 0.0121; \quad R^2 = 0.2752, \quad p < 0.001 \tag{9}$$

The linear gastric evacuation rates in grams per hour are shown in Figure 3.18 and are shown as a percent body weight in Figure 3.19. The initial intake values have been set to an arbitrary value in order to demonstrate the relative rates of evacuation.

3.3.2 Temperature Trial

Initial feed intake by 0+ wolffish, expressed as both grams and percent body weight, was not linearly related to fish size ($p > 0.05$, regression ANOVA), so no corrections were made prior to analysis. Linear and logarithmic equations were calculated to fit the gastric evacuation profile and in all cases, the R value and regression ANOVA indicated that the

Table 3.7: Equations, R^2 values and p values for gastric evacuation of juvenile wolffish fed six diets with a range of PE:TE values at 9 °C. A: Stomach contents in grams; B: Stomach contents as %BW. For each equation, n = 40.

A

PE:TE	Equation	R^2	p
0.35	y = -0.00196x + 0.09375	0.45823	< 0.0001
0.40	y = -0.00239x + 0.13937	0.22832	0.0018
0.45	y = -0.00315x + 0.14688	0.32618	0.0001
0.46	y = -0.00142x + 0.09977	0.11314	0.0389
0.55	y = -0.00220x + 0.10888	0.21937	0.0023
0.61	y = -0.00209x + 0.09852	0.30758	0.0002

B.

PE:TE	Equation	R^2	p
0.35	y = -0.00017x + 0.00905	0.39071	< 0.0001
0.40	y = -0.00022x + 0.01226	0.23850	0.0014
0.45	[1]y = -0.00025x + 0.01205	0.27518	0.0005
0.46	y = 0.00011x + 0.00747	0.14152	0.0167
0.55	y = - 0.00019x + 0.00862	0.31004	0.0002
0.61	y = -0.00018x + 0.00831	0.32725	0.0001

[1] Logarithmic equation ($y = -2.87573x^{-0.3020}$) had R^2 of 0.34668 and $p < 0.0001$.

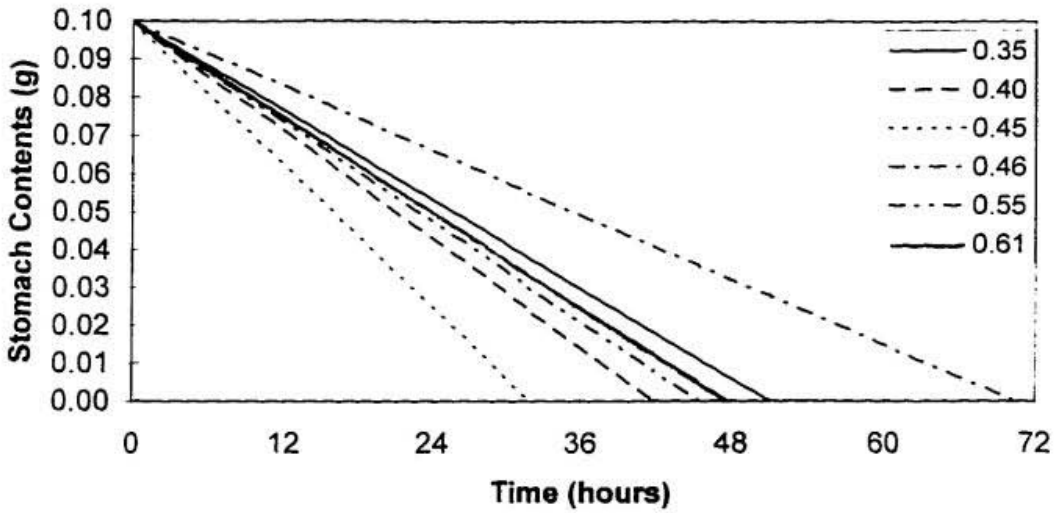


Figure 3.18: Comparison of gastric evacuation rates (g/hr) of 0+ wolffish at 9 °C in relation to dietary PE:TE value. Initial meal size fixed at 0.10 g.

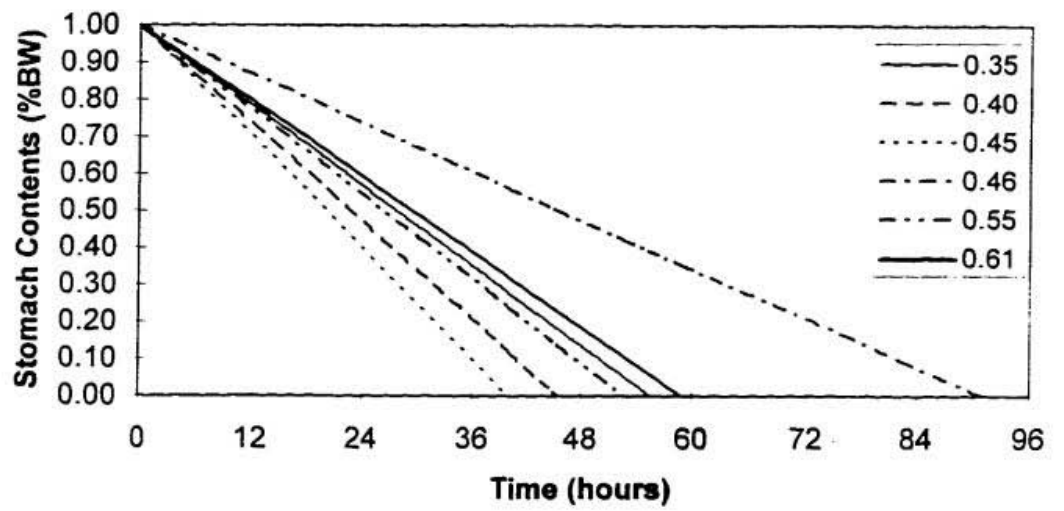


Figure 3.19: Comparison of gastric evacuation rates (%BW/hr) of 0+ wolffish at 9 °C in relation to dietary PE:TE value. Initial meal size fixed at 1.00 %BW.

linear equation had a higher correlation in every case (Table 3.8). The linear gastric evacuation rates are shown in Figures 3.20 and 3.21. The initial intake values have been set to an arbitrary value in order to demonstrate the relative rates of evacuation.

Table 3.8: Equations, R^2 values and p values for gastric evacuation of juvenile wolffish fed a commercial salmon feed at three temperatures. A: Stomach contents in grams; B: Stomach contents as %BW. For each equation, n = 40.

A

Temp. (°C)	Equation	R^2	p
9.0	y = -0.00451x + 0.25440	0.55793	< 0.0001
5.0	y = -0.00331x + 0.24952	0.31519	< 0.0001
2.0	y = -0.00255x + 0.15448	0.35139	< 0.0001

B.

Temp. (°C)	Equation	R^2	p
9.0	y = -0.00044x + 0.02398	0.66592	< 0.0001
5.0	y = -0.00027x + 0.02162	0.26842	0.0002
2.0	y = -0.00025x + 0.01517	0.32833	< 0.0001

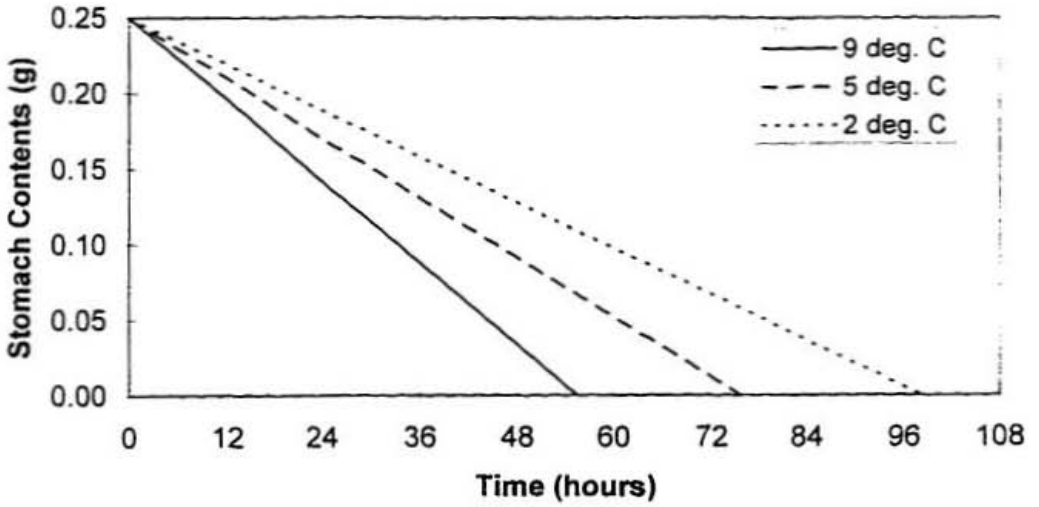


Figure 3.20: Comparison of gastric evacuation rates (g/hr) of 0+ wolffish at three temperatures. Initial meal size fixed at 0.25 g.

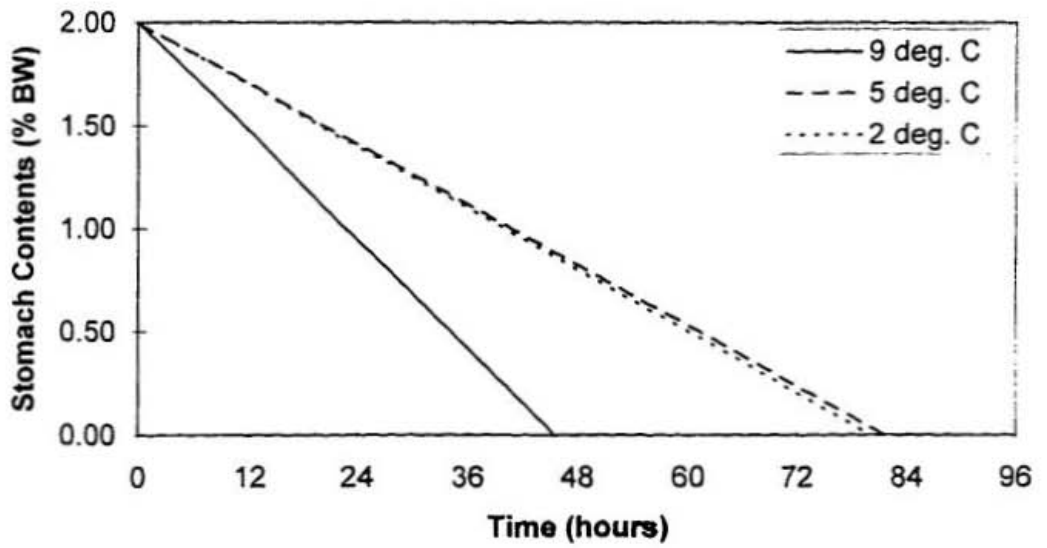


Figure 3.21: Comparison of gastric evacuation rates (%BW/hr) of 0+ wolffish at three temperatures. Initial meal size fixed at 2.00 %BW.

4.0 Discussion

4.1 Stocking Density

The aggression observed among juvenile wolffish fish held at low densities was curious. Though not gregarious in the wild, they are not outwardly aggressive. However, little information from the wild is available on larval and juvenile wolffish and it may be that aggression is important for newly settled fish. Perhaps, like Arctic charr, the peak growth rate occurs at very high densities. This is made evident by the decrease in aggressive interactions among Arctic charr stocked at the two higher densities, as noted by Brown *et al.* (1992). No observations were reported as to the size of the aggressor so as to determine if the aggression was carried out by the biggest fish. Davenport *et al.* (1990) reported a feeding hierarchy in halibut.

Stocking density had a significant influence on the FCR and PER in wolffish. The higher the density, the more efficiently feed and protein were used. Again, the decrease in aggressive interaction may be the primary factor responsible. If so, it is noteworthy that a behavioural factor has a significant physiological response.

Orlova *et al.* (1989) reported a reduced feeding activity and, in some cases, cessation of feeding in wolffish held alone. When stocked with more than one fish in a tank the feeding activity level of the fish increased dramatically. This is in contrast to the current results. While the rate of feeding, itself, was low, fish held at the lowest stocking density did not necessarily consume the least feed per meal. When fed twice per day, the 20 g/L treatment had a significantly higher mean meal size than the two higher stocking densities. When fed less frequently, the low density fish consumed statistically similar mean meal sizes to the 80 g/L fish. Persson (1982) reported an increase in the rate of food consumption when the roach (*Rutilus rutilus*) is kept in schools. Similarly, in the

current study with wolffish, the rate of feeding was higher in the high density tanks, but the total consumption, though much slower in the low density tanks, was greater. Further investigations of growth, health and yield of wolffish at stocking densities equal to and greater than 80 kg/m^3 are recommended.

4.2 Feeding Schedule

The current gastric evacuation data shows that at 9 °C, approximately 48 hours were required to digest a large meal of formulated feed. Orlova *et al.* (1989) using wolffish fed a large meal of scallops at 1.0 °C to 2 °C, discovered that wolffish refused to eat for three days. In that study the first indication of feces was after 3.5 days and normal feeding did not occur until after 4.5 days. Therefore, it seems unreasonable to expect appetite to return within hours of a meal. This corroborates the current finding that decreasing the meal frequency to once every two days did not have a negative impact on the SGR of juvenile wolffish. Lied *et al.* (1985) concluded that the feed conversion efficiency of cod suffered when the fish were fed twice every day. They extended the time between meals to four days and though the growth rate slowed, the feed conversion was very efficient. Lie *et al.* (1988) found a similar FCR in cod whether they were fed each day or every second day. When they fed cod every third day, the FCR did not increase. Furthermore, weight gain was reduced, therefore there was no advantage to feeding cod every third day. Similarly, with wolffish, there was no advantage (nor disadvantage) to a reduced feeding frequency, over the range tested, as far as the SGR and FCR were concerned. To a commercial farmer, however, this translates to reduced feed and labour costs. However, reducing the feeding frequency to once every three days or more may have a negative impact on the SGR and FCR of juvenile wolffish.

Davenport *et al.* (1990) found that halibut retained large meals in their stomachs for approximately four days. Meals offered at 3 day intervals were not consumed by all fish.

This is similar to observations in the current study, where fish fed twice per day did not ravenously approach each meal, whereas a high proportion of fish fed once every two days fed actively.

Tuene and Nortvedt (1995) studied the feed intake of Atlantic halibut fed once per day at 8-9 °C and found that the maintenance ration was 0.126 %BW/day. A comparison may be made directly with wolffish in the current experiment which were fed a similar diet (turbot: 5.71 kcal/g, PE:TE = 0.58; wolffish: 6.06 kcal/g, PE:TE = 0.49) once a day and held at the same temperature. Wolffish consumed nearly four times the daily ration of the halibut. The SGR values were superior in the wolffish (halibut: 0.287 %BW/day; wolffish: 0.399 %BW/day). Feed conversion ratios for halibut [calculated from gross feed conversion efficiency (GFCE) values] ranged between 0.88 and 1.09, while the FCR of wolffish ranged from 1.34 to 2.72 (higher stocking densities lead to lower FCR). In this study, the halibut converted their feed more efficiently.

4.3 PE:TE

The optimum growth rates in my study were found when the PE:TE was between 0.45 and 0.55. The corresponding carbohydrate values were between 10 and 17% dry weight. The PE:TE = 0.61 diet had only 4.4% carbohydrate by weight, so evidently, carbohydrate levels in the wolffish diet may reach 17% without impairing growth. Hemre *et al.* (1989) increased the carbohydrate energy content of cod diets from 0 to 30% and growth was not affected nor did the carbohydrate appear to have a protein sparing effect. However, this may be due to the decreased digestibility of carbohydrate as the concentration in the diet increased.

Lie *et al.* (1988) investigated the effects of a variety of dietary PE:TE on the performance of cod. They conclude that weight gain in cod is highest when PE:TE = 0.42. The feed conversion ratio was significantly lower when PE:TE = 0.42, than when PE:TE = 0.27 or

0.56. The protein efficiency ratio was significantly higher at PE:TE = 0.27 and 0.42 than at PE:TE = 0.56. Like cod, wolffish are a non-oily fish and the results in the current study were very similar to those reported by Lie *et al.*, (1988).

Aksnes *et al.* (1996) fed Atlantic halibut four diets with PE:TE values between 0.49 and 0.76 (protein incrementally substituted for lipid) and found no significant effect on growth or feed conversion (feed conversion = FCR^{-1}). However, using three diets ranging from PE:TE = 0.39 to 0.49, (lipid constant at 25% dry weight) both growth and feed efficiency increased significantly. Based on these, and other results, they concluded that the PE:TE has a significant linear effect on the feed efficiency, but not on the SGR. Also, they found no link between the PE:TE and feed intake. The current study affirms their conclusion regarding SGR, but the PE:TE variations did not significantly influence either the FCR or the feed intake of juvenile wolffish in the current study.

Isonitrogenous diets given to turbot resulted in increased weight gain and protein efficiency ratio (PER) as the energy level increased (Adron *et al.*, 1976). This was not accompanied by an increase in body fat, therefore, the protein sparing effect of lipid was evident. In addition, these same workers, using isocaloric diets with 35% and 50% protein (dry weight basis) discovered a higher PER using the lower protein diet. This was not the case in my study, where no significant differences in PER were evident due to variations in PE:TE, at either 9 °C or ambient temperature.

4.4 Gastric Emptying

According to Jensen and Berg (1993), a large variation in the size of the initial meal consumed by fish following a period of starvation is a common occurrence. In the current study, the initial fed intakes appeared to vary considerably, but were, in fact, statistically similar. In addition, over the weight range selected, fish weight was not a factor in the initial meal size, either as a meal weight or percent body weight.

The feed type did not appear to have a significant effect on the rate of gastric evacuation in the current study. However, Orlova *et al.* (1989), also working with wolffish concluded that the indigestible matter in the diet increased the rate of evacuation through the intestines. This was not directly noted in the current study because it dealt exclusively with the evacuation of meals from the stomach and the feeds used were highly digestible. Orlova *et al.* (1989) also observed protracted evacuation of high-fat meals from the wolffish stomach, in other words the stomach was emptied within one day and the meal remained in the intestines 8 to 10 days. A similar phenomenon appears to exist in higher vertebrates (Hunt and Stubbs, 1975; Burn-Murdoch *et al.*, 1978). According to Jobling (1980), the rate of food movement from the stomach into the duodenum in fish is such that the energy flow is constant. Therefore, the rate of passage is increased when food is of low nutrient density.

Davenport *et al.* (1990) starved halibut for eight days at 10 °C and then fed to satiation. They discovered that the entire meal remained in the stomach for 12 hours and some food remains in the stomach up to four days. However, halibut have a very large stomach and consume their food whole, hence a long period of digestion in the stomach is required. Lemon sole, on the other hand, with small stomachs and relatively long intestines, ingest small, frequent meals which leave the stomach after about two days (Davenport *et al.*, 1990).

In the current study, at 9.0 °C, using diets with a range of PE:TE values, a linear model for gastric emptying provided the best fit in all but one case. The slopes ranged from an evacuation rate of 1.42 mg/hr to 3.15 mg/hr (0.011 %BW/hr to 0.025 %BW/hr). Bromley (1987), using turbot with a mean weight of 0.42 kg, found a mean evacuation rate of 0.47 g/hr or 0.112 %BW/hr. The evacuation rate of the turbot, as related to fish weight, decreased with increasing fish size, while the absolute evacuation rates increased with increasing fish size. Similarly, Bromley (1988) found a linear relationship in the gastric

evacuation rate of whiting (mean weight 268 g) held at 10 °C. In that study the average rate of gastric emptying was 0.31 g/hr. Jobling (1987) determined that evacuation of dry pellets may be accurately described by a square-root equation or a linear equation, as was found in the current study. The fact that the fastest and slowest gastric evacuation rates in the current trial were found using diets PE:TE = 0.45 and 0.46, respectively, confirms the conclusion of Jobling (1980) that the rate of gastric evacuation is independent of dietary energy level and composition.

The current study demonstrated a linear, temperature-dependent rate of gastric evacuation in wolffish. The evacuation rates of wolffish at 5 °C and 2 °C were very similar, but may be confounded by the fact that the fish held at 2 °C ate half the amount of feed consumed by the fish at 5 °C. Tyler (1970) using cod (mean weight 229 g) determined that a logarithmic curve was best except when the temperature was 15 °C or 19 °C, when the linear and logarithmic models were equally acceptable. Persson (1982), using roach (*Rutilus rutilus*) also found that exponential models provided a better fit to gastric evacuation data than linear models. Likewise, Jobling *et al.*, (1977) determined that the gastric evacuation rate in the dab is best described by an exponential model. They noted that larger meals increased the rate of gastric evacuation at a given temperature. This does not explain the results of the current study, which showed that the evacuation rates of wolffish at 5 °C and 2 °C were nearly identical even though the fish at the colder temperature consumed half the amount of the fish at 5 °C. Generally, the smaller the meal size, the slower the rate of gastric evacuation (Tyler, 1970; Flowerdew and Grove, 1979).

4.5 Hepatosomatic Index

In the current investigations on the effects of varying dietary PE:TE on HSI, no significant relationship between the HSI values and the dietary PE:TE, nor the lipid intake and the energy intake were found. Cod, as a non-oily fish, store most of their lipid

reserves in their livers. The lipid content of cod livers ranges between 50 and 60%, while the lipid content in the muscle is less than 1% (Lie *et al.*, 1988). Normal hepatosomatic index values range from 8-12% in cod while those recorded in wolffish in the current study range between 3 and 5%. Lie *et al.* (1988) also found a linear relationship between the total fat consumed and the hepatosomatic index in cod. In wolffish the relationship was neither linear nor logarithmic. However, the narrow range of hepatosomatic index values found in wolffish may reflect the fact that the lipid content of the diets used ranged only from 19.96 to 29.99% of the dry diet. This is a much smaller range of dietary lipid than was used by Lie *et al.* (1988) in the cod study (11 to 61% fat energy). They concluded that a hepatosomatic index of less than 10% could be achieved by using a diet with 25% or less dietary lipid. Their general recommended formulation for cod diets is 60% protein, 25% fat and 15% carbohydrates, which gives a PE:TE = 0.529. This was within the range of dietary PE:TE values used in the current study. However, no recommendation of the dietary formulation can be made based on the wolffish HSI results alone.

The lack of definitive HSI results may be due to the fact that the trial was too short for a significant change in HSI to occur. Berge and Storebakken (1991), conducted a similar trial using halibut and two diets (PE:TE = 0.62 and 0.52) and found no significant difference in the HSI after fourteen weeks. However, Aksnes *et al.* (1996) fed halibut six diets with a range of PE:TE values (0.49 to 0.76) for 523 days and no significant differences were found in the HSI. Instead, they discovered highly significant differences in the lipid content of eviscerated fish and the weight of the entrails. Similarly, wolffish fed diets with low PE:TE values had fat deposits associated with the mesentery around the intestines. In future studies, more attention should be given to the intestines and their role in lipid storage.

4.6 Aquaculture Implications

Wolffish have a life history and biological characteristics that make them ideal for culture purposes, especially in coastal areas of the north Atlantic Ocean. With larval rearing protocols firmly established, and the current advances in juvenile and on-growing procedures, large-scale culture of wolffish is becoming economical. Once requirements for reproduction in captivity have been determined, a sustainable broodstock can be established to supply eggs and eliminate the need for yearly egg collection.

The results of the current study provide a framework for the commercial culture of wolffish. For juvenile and on-growing wolffish, stocking densities of 80 g/L or greater are recommended, as is a meal frequency of 1 meal/two days. These represent the lowest recommended values. Further research is warranted to determine if higher stocking densities and lower meal frequencies are beneficial and practical. Growth parameters do not seem to be affected by the use of a flow through system or re-circulated water supply provided that good water quality is maintained. Recommended rearing temperatures range between 13.0 °C and 5.6 °C. Practically speaking, wolffish farmers could rely on an unheated ambient water supply until the temperature falls below 5.6 °C. Controlled temperature trials below 5.6 °C should be conducted in order to clearly establish the temperature at which the feed intake and growth rate decrease to a level significantly lower than that of fish held at higher temperatures.

Regarding feeds for use with wolffish, emphasis should be placed on dry, extruded feeds with a PE:TE between 0.45 and 0.55, based on the good growth rates achieved using those diets in the current study. There appears to be no advantage in growth or feed conversion to use diets with a higher PE:TE. Though the cost per unit of feed decreases with lower PE:TE feeds, the reduced growth rates and increased feed intake make their use impractical.

5.0 Conclusions

5.1 Stocking Density / Feeding Schedule Trial

1. Low stocking density (20 g/L) combined with a low feeding frequency (one meal/two days) may lead to aggressive, non-injurious, interaction among juvenile wolffish.

2. Juvenile wolffish appeared to feed more actively in the afternoon than in the morning.

3. Feeding rate of juvenile wolffish was inversely related to meal frequency.

4. The SGR of juvenile wolffish was unaffected by stocking density and feeding schedule.

5. The condition index of juvenile wolffish was not significantly affected by feeding schedule or stocking density.

6. Stocking density significantly affected the amount of feed consumed by juvenile wolffish. The lowest meal size was found with fish stocked at 80 g/L ($3.990 \pm 6.029*10^{-4}$ mg/g) and the highest meal size was consumed by fish stocked at 50 g/L ($4.955 \pm 6.235*10^{-4}$ mg/g).

7. Meal frequency significantly influenced the amount of feed ingested. Fish fed once every two days consume a larger meal than those fed twice a day, but over the same time period (two days), fish fed twice a day actually consumed more feed than those fish fed once in two days.

8. Since feed consumption decreased as feeding frequency decreased (Conclusion 7) and the SGR was unaffected by changes in feeding frequency (Conclusion 4), then money may be saved by feeding wolffish less frequently (i.e., labour costs are reduced as are feed costs).

9. The FCR of 0+ wolffish decreased significantly when the stocking density was greater than 50 g/L.

10. The PER was significantly higher when the stocking density was greater than 80 g/L.

5.2 Dietary Energy Balance Trial

1. A decrease in water temperature significantly decreased the SGR of juvenile wolffish, but did not significantly influence the condition index.

2. Dietary PE:TE had no significant impact on the SGR of 0+ or 1+ fish at 9 °C or at ambient temperature.

3. Feed consumption was inversely, and significantly, related to the dietary PE:TE values. Ingestion of diets PE:TE = 0.35 and 0.40 were significantly higher than that of diets PE:TE = 0.55 and 0.61.

4. Intake of lipid and energy decreased significantly as the PE:TE increased.

5. In 0+ wolffish at ambient temperature, daily lipid intake and energy intake in each diet treatment remained constant between 13.0 °C and 5.5 °C. Lipid and energy intakes, when temperature dropped below 5.5 °C, was roughly half that of the higher

temperature periods. The response for 1+ fish was similar, but the intake during the coldest period was not always significantly different than the period before (the warmer of the two).

6. No significant effects on energy intake were produced by an interaction between PE:TE and time (i.e. decreasing temperature). This lack of correlation indicated that energy requirements of juvenile wolffish did not change as the temperature decreased.

7. The HSI of 0+ wolffish held at 9 °C ranged between 4.645 %BW and 3.349 %BW. This was a small range for lean-fleshed marine fish and no significant regression effects on the HSI were evident with respect to dietary PE:TE, lipid intake and energy intake.

8. Dietary PE:TE values had no significant impact on the FCR at either ambient or constant temperatures.

9. The PER of juvenile wolffish was not significantly affected by either dietary PE:TE or decreasing temperatures.

10. The cost of production was not significantly affected by dietary PE:TE based on this set of experiments. A prolonged, large-scale experiment would, however, more accurately determine the production cost.

11. The cost of production did not decrease significantly as the temperature decreased.

5.3 Gastric Evacuation Trials

1. Temperature had a significant, direct effect on the initial feed intake.

2. Gastric evacuation rates of wolffish expressed in grams per hour or percent BW per hour were better represented by linear equations than logarithmic equations.

3. Although gastric evacuation rates varied with dietary PE:TE values, there was no direct correlation between the two factors.

4. The rate of gastric evacuation decreased with decreasing temperature. The rates at which fish at 5 °C and 2 °C emptied their stomachs were very similar, but the fish held at 2 °C, in reality, consumed half of the amount of food consumed by the fish held at 5 °C.

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APPENDICES

Appendix A: Weights and lengths of juvenile wolffish by year class and rearing method.

Year Class	Age (days)	Facility	n	Length (cm)	Weight (g)	Condition Index
0+	209	Recirculated	240	9.4 ± 0.1	8.2 ± 0.1	0.95 ± 0.01
1+	530	Recirculated[1]	153	10.0 ± 0.1	9.9 ± 0.3	0.97 ± 0.01
1+	577	Flow-Through	91	12.3 ± 0.2	20.7 ± 1.1	1.01 ± 0.02

[1] Fish fed Hi-Pro Salmon Grower diet (Corey Feed Mills, Fredericton, NB), once every two days for approximately 400 days. Fish were not fed to apparent satiation.

Appendix B: Three way ANOVA for the SGR of 0+ wolffish fed commercial salmon feed by stocking density[a], feeding schedule[b] and replicate[c] at 9 °C.

Source of Variation	Sum of Squares	df	Mean Squares	F	Sig. of F
Main Effects	0.000	6	0.000	1.385	0.219
Density	0.000	2	0.000	2.028	0.133
Schedule	0.000	2	0.000	1.678	0.188
Replicate	0.000	2	0.000	1.140	0.321
2-Way Interaction	0.000	12	0.000	1.447	0.143
Density - Schedule	0.000	4	0.000	3.319	0.011
Density - Replicate	0.000	4	0.000	0.282	0.889
Sched. - Replicate	0.000	4	0.000	0.879	0.476
3-Way Interaction	0.000	8	0.000	1.392	0.198
Dens.-Sched.-Rep.	0.000	8	0.000	1.392	0.198
Explained	0.000	26	0.000	1.490	0.061
Residual	0.002	364	0.000		
Total	0.002	390	0.000		

[a]Refers to stocking densities 20 g/L, 50 g/L and 80 g/L.
[b]Refers to feeding schedules: two meals/day, one meal/day and one meal/two days.
[c]Refers to three replicates per treatment.

Appendix C: Four way ANOVA for the daily feed intake of 0+ wolffish fed commercial salmon feed by stocking density[a] , feeding schedules[b], period[c] and replicates[d] at 9 °C.

Source of Variation	Sum of Squares	df	Mean Squares	F	Sig. of F
Main Effects	0.012	8	0.002	140.083	0.000
Schedule	0.011	2	0.006	496.549	0.00
Density	0.000	2	0.000	17.522	0.000
Period	0.001	2	0.000	37.963	0.00
Replicate	0.000	2	0.000	9.865	0.000
2-Way Interaction	0.001	24	0.000	4.320	0.000
Schedule - Density	0.000	4	0.000	7.467	0.000
Schedule - Period	0.000	4	0.000	5.028	0.000
Schedule - Replicate	0.000	4	0.000	1.652	0.159
Density - Period	0.000	4	0.000	4.223	0.002
Density - Replicate	0.000	4	0.000	7.063	0.000
Period - Replicate	0.000	4	0.000	0.452	0.771
3-Way Interaction	0.001	32	0.000	3.928	0.000
Sched. - Dens. - Per.	0.001	8	0.000	5.777	0.000
Sched. - Dens. - Rep.	0.001	8	0.000	7.684	0.000
Sched. - Per. - Rep.	0.000	8	0.000	0.830	0.576
Dens. - Per. - Rep.	0.000	8	0.000	1.462	0.166
4-Way Interaction	0.000	16	0.000	1.248	0.222
Sched.-Dens.-Per.-Rep.	0.000	16	0.000	1.248	0.222
Explained	0.015	80	0.000	16.656	0.000
Residual	0.028	2475	0.000		
Total	0.042	2555	0.000		

[a]Refers to stocking densities 20 g/L, 50 g/L and 80 g/L.

[b]Refers to feeding schedules: two meals/day, one meal/day and one meal/two days.

[c]Refers to three- four week periods.

[d]Refers to three replicates per treatment.

Appendix D: Three way ANOVA for the FCR of 0+ wolffish fed commercial salmon feed by stocking density[a], feeding schedules[b] and replicate[c] at 9 °C.

Source of Variation	Sum of Squares	df	Mean Squares	F	Sig. of F
Main Effects	16.621	6	2.770	2.289	0.052
Density	14.993	2	7.496	6.195	0.004
Schedule	0.441	2	0.221	0.182	0.834
Replicate	1.234	2	0.617	0.510	0.604
2-Way Interaction	4.868	12	0.406	0.335	0.978
Density - Schedule	0.585	4	0.146	0.121	0.974
Density - Replicate	3.630	4	0.907	0.750	0.563
Sched. - Replicate	0.816	4	0.204	0.169	0.953
3-Way Interaction	4.387	8	0.548	0.453	0.882
Dens.-Sched.-Rep.	4.387	8	0.548	0.453	0.882
Explained	27.115	26	1.043	0.862	0.652
Residual	54.450	45	1.210		
Total	81.565	71	1.149		

[a]Refers to stocking densities 20 g/L, 50 g/L and 80 g/L.

[b]Refers to feeding schedules: two meals/day, one meal/day and one meal/two days.

[c]Refers to three replicates of each treatment.

Appendix E: Three way ANOVA for the PER of 0+ wolffish fed commercial salmon feed by stocking density[a], feeding schedules[b] and replicates[c] at 9 °C.

Source of Variation	Sum of Squares	df	Mean Squares	F	Sig. of F
Main Effects	5.528	6	0.921	1.601	0.169
Density	0.130	2	0.065	0.113	0.894
Schedule	4.346	2	2.173	3.775	0.030
Replicate	1.034	2	0.517	0.898	0.414
2-Way Interaction	6.364	12	0.530	0.921	0.534
Density - Schedule	3.925	4	0.981	1.705	0.165
Density - Replicate	1.489	4	0.372	0.647	0.632
Sched. - Replicate	1.612	4	0.403	0.700	0.596
3-Way Interaction	2.470	8	0.309	0.536	0.823
Dens.-Sched.-Rep.	2.470	8	0.309	0.536	0.823
Explained	14.369	26	0.553	0.960	0.534
Residual	26.480	46	0.576		
Total	40.850	72	0.567		

[a]Refers to stocking densities 20 g/L, 50 g/L and 80 g/L.

[b]Refers to feeding schedules: two meals/day, one meal/day and one meal/two days.

[c]Refers to three replicates per treatment.

Appendix F: Three way ANOVA for length of 0+ wolffish fed six formulated diets[a] with a range of PE:TE values, over three - 4 week periods and tank replicates[b] at 9 °C.

Source of Variation	Sum of Squares	df	Mean Squares	F	Sig. of F
Main Effects	169.136	9	18.793	37.376	0.00
Diet	53.202	5	10.640	21.162	0.00
Tank	3.484	1	3.484	6.929	0.009
Time	109.839	3	36.613	72.817	0.00
2-Way Interaction	14.260	23	0.620	1.233	0.206
Diet - Replicate	5.320	5	1.064	2.116	0.061
Diet - Time	4.796	15	0.320	0.636	0.847
Tank - Replicate	3.984	3	1.328	2.641	0.048
3-Way Interaction	3.057	15	0.204	0.405	0.978
Dens.-Sched.-Rep.	3.057	15	0.204	0.405	0.978
Explained	196.931	47	4.190	8.333	0.000
Residual	477.670	950	0.503		
Total	674.601	997	0.677		

[a]Refers to isoenergetic diets with PE:TE values: 0.35, 0.40, 0.45, 0.46, 0.55 and 0.61.

[b]Refers to two replicates per treatment.

Appendix G: Three way ANOVA for weight of 0+ wolffish fed six formulated diets[a] with a range of PE:TE values, over three- 4 week periods and replicates[b] at 9 °C.

Source of Variation	Sum of Squares	df	Mean Squares	F	Sig. of F
Main Effects	2216.641	9	246.293	50.677	0.00
Diet	429.954	5	85.991	17.693	0.00
Period	0.000	1	0.000	0.000	0.998
Replicate	1783.232	3	594.411	122.304	0.00
2-Way Interaction	64.086	23	2.786	0.573	0.947
Diet - Period	13.501	5	2.700	0.556	0.734
Diet - Replicate	48.037	15	3.202	0.659	0.826
Period - Replicate	3.027	3	1.009	0.208	0.891
3-Way Interaction	31.341	15	2.089	0.430	0.971
Diet-Per.-Rep.	31.341	15	2.089	0.430	0.971
Explained	2418.019	47	51.447	10.586	0.000
Residual	4617.090	950	4.860		
Total	7035.109	997	7.056		

[a]Refers to isoenergetic diets with PE:TE values: 0.35, 0.40, 0.45, 0.46, 0.55 and 0.61.

[b]Refers to two replicates per treatment.

Appendix H: Two way ANOVA for the arcsine of the SGR of 0+ wolffish fed six formulated diets[a] with a range of PE:TE values over three periods[b] at ambient temperature.

Source of Variation	Sum of Squares	df	Mean Squares	F	Sig. of F
Main Effects	0.004	7	0.001	96.652	0.000
Diet	0.000	5	0.000	2.062	0.068
Period	0.004	2	0.002	333.288	0.000
2-Way Interaction	0.001	10	0.000	15.413	0.00
Diet - Period	0.001	10	0.000	15.413	0.00
Explained	0.005	17	0.000	48.931	0.000
Residual	0.004	658	0.000		
Total	0.008	675	0.000		

[a]Refers to isoenergetic diets with PE:TE values: 0.35, 0.40, 0.45, 0.46, 0.55 and 0.61.

[b]Refers to the following period lengths: Period 1 = 5 weeks, Period 2 = 3 weeks, Period 3 = 4 weeks.

Appendix I: Three way ANOVA for the feed intake by 0+ wolffish fed six formulated diets[a] with a range of PE:TE values over three periods[b] and replicates[c] at ambient temperature.

Source of Variation	Sum of Squares	df	Mean Squares	F	Sig. of F
Main Effects	5298.551	8	662.319	47.109	0.00
Diet	321.984	5	64.397	4.58	0.000
Replicate	4809.940	1	2404.97	171.059	0.00
Period	166.627	2	166.627	11.852	0.001
2-Way Interaction	261.125	17	15.36	1.093	0.356
Diet - Replicate	37.583	5	3.758	0.267	0.988
Diet - Period	171.423	10	34.285	2.439	0.033
Replicate - Period	52.119	2	26.06	1.854	0.157
3-Way Interaction	30.589	10	3.059	0.218	0.995
Diet-Rep.- Per.	30.589	10	3.059	0.218	0.995
Explained	5644.914	35	161.283	11.472	0.000
Residual	13159.532	676	14.059		
Total	18804.446	711	19.366		

[a]Refers to isoenergetic diets with PE:TE values: 0.35, 0.40, 0.45, 0.46, 0.55 and 0.61.

[b]Refers to the following period lengths: Period 1 = 5 weeks, Period 2 = 3 weeks, Period 3 = 4 weeks.

[c]Refers to two replicates per treatment.

Appendix J: Two way ANOVA for the feed intake by 1+ wolffish fed six formulated diets[a] with a range of PE:TE values over three periods[b] at ambient temperature.

Source of Variation	Sum of Squares	df	Mean Squares	F	Sig. of F
Main Effects	2971.562	7	424.509	20.708	0.00
Diet	1182.443	5	236.489	11.536	0.000
Period	1977.102	2	988.551	48.223	0.00
2-Way Interaction	266.325	10	26.633	1.299	0.226
Diet - Period	266.325	10	26.633	1.299	0.226
Explained	3377.891	17	198.699	9.693	0.00
Residual	19556.434	954	20.499		
Total	22934.325	971	23.619		

[a]Refers to isoenergetic diets with PE:TE values: 0.35, 0.40, 0.45, 0.46, 0.55 and 0.61.

[b]Refers to the following period lengths: Period 1 = 5 weeks, Period 2 = 3 weeks, Period 3 = 4 weeks.