EFFECTS OF PHOTOPERIOD AND LIGHT INTENSITY ON THE SURVIVAL, GROWTH AND FEEDING BEHAVIOUR OF LARVAL STRIPED WOLFFISH (Anarhichas lupus)

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BY

Kelly J. Moret ©

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Abstract

This thesis investigated the impact of two environmental factors on the performance of larval striped wolffish (*Anarhichas lupus*). Specifically, I describe the impact of photoperiod and light intensity on the growth, survival, and feeding behaviour of larval wolffish.

In the photoperiod experiment, larval wolffish were subjected to photoperiods consisting of 12 hours light/12 hours dark, 18 hours light/6 hours light, or 24 hours continuous light. Results showed that a photoperiod of 18L/6D yielded the best survival and growth after 50 days. Providing 24 hours light, a common technique in larviculture, did not offer any advantage in terms of survival or growth compared to the 18L treatment. The higher performance results seen for the 18L treatment is attributed to the similarity in photoperiod of the natural environment for the species.

The investigation into the effects of light intensity on the survival and growth of larval wolffish compared intensities of 10, 40, 160, 320, 750, and 1200 lux. For all values tested, survival and growth increased with increasing light intensity. A light intensity in the range of 750 lux-1200 lux produced survival rates of approximately 92.0% by day 50.

The effects of light intensity (320 lux, 750 lux, 1200 lux) on the feeding and activity of larvae were also investigated. The frequency of feeding increased with increasing light intensity. The impact of light intensity was most significant during days 30-40, a period corresponding to the switch from endogenous to exogenous feeding in larval wolffish. During this period, the larval in the highest light intensity treatment

(1200 lux) had significantly greater frequencies of feeding compared to the lowest light intensity treatment (320 lux). By the end of the study (day 50), there was no difference observed between treatments in terms of successful or unsuccessful foraging.

For the production of larval wolffish a photoperiod of at least 18L in conjunction with 1200 lux is recommended for maximum growth and survival up to at least day 50 post-hatch.

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I dedicate this thesis to my daughter Catherine. Although she is the reason this thesis was delayed, more importantly, she is my source of inspiration, and the reason I want to strive to be better.....hence the completion of this thesis!!

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Chapter 1: General Introduction-Culturing New Species

Market value, cost of production, and the quality of farmed fish have been identified as decisive factors in assessing a new species' potential for aquaculture development (Tilseth, 1990; Tilseth *et al.*, 1992). In Atlantic Canada biological suitability must be considered an equally important factor as any candidate species investigated for production must be able to tolerate sub-zero (<0°C) temperatures for significant portions of the year (Brown *et al.*, 1992; 1995a). Factors dealing with politics, economics, infrastructure, pollution and disease are also crucial to new species' development and production success (Hempel, 1993). Consequently, the development of a new species will only be lucrative if the animal's biology is suited to the habitat where it will be grown and if cooperative government/research, and industry alliances exists.

In general, there is usually an incentive or motivating factor driving the development or expansion of the aquaculture industry. In 1990, world aquaculture production reached approximately 15 million metric tonnes (mt), by 1998 this expanded to approximately 30.8 million metric tonnes (FAO statistics, 2001). By the year 2025, it is estimated that 62.4 million metric tonnes (mt) of aquaculture products are expected to be produced. Since the world capture fisheries will remain stable at about 100 million metric tonnes (mt), increased market demand will have to be filled through aquaculture production (Hempel, 1993).

Traditionally, the incentive for the aquaculture industry has not been to supply or meet the world demand for seafood products. Instead, the northern and temperate regions

have focussed their attention on high value finfish products. Although the Northern European aquaculture industry has been dominated by salmonids, several factors have prompted research into culturing alternate cold water marine species. Factors such as the fluctuations in the Atlantic salmon (*Salmo salar*) market caused by excessive world supply and decreasing value in the international market (Strand *et al.*, 1995) and decreased landings in the traditional commercial fisheries have served as a strong impetus for expanding cold water production into other species. Similarly, the imposed moratorium on the east coast Canadian groundfish fishery, which resulted in a drastic shortage of once readily available fish, also prompted extensive investigations into cold water aquaculture.

In an effort to deal with the situations mentioned above, the Northern European and Atlantic Canadian aquaculture industries identified several finfish species, namely halibut (*Hippoglossus hippoglossus*), haddock (*Melanogrammus aeglefinus*), cod (*Gadus morhua*), and two species of Atlantic wolffish, the striped wolffish (*Anarhichas lupus*) and the spotted wolfish (*Anarhichas minor*) as potential candidates for cold water development (Tilseth, 1990; Brown *et al.*, 1995a; Stefanussen *et al.*, 1993). Since all these species occur naturally in the cold waters around Atlantic Canada and Northern Europe, their suitability to the existing environment/habitat was not considered an impediment to production.

Research into all aspects of aquaculture production stages (broodstock, egg, larval, juvenile/grow-out) is considered crucial for the success of the industry. Although

literature may be available for each production stage, caution should be exercised when drawing direct comparisons between wild and cultured fish as factors dealing with density, stress, feeding and environmental conditions are often inconsistent (Blaxter, 1975a) and therefore not directly applicable. When comparing wild to reared fish, Blaxter (1975a) noted that variability in behaviour (aggression, feeding), morphology (abnormal fins and heads, pigmentation), physiology (egg size, quality and fecundity) and biochemistry (fat, water, and ash content in the body) does occur despite the fact that growth rates and condition factors of cultured fish exceed those of the wild population. Where inconsistencies occur, it is evident that problem specific research is essential for resolving production impediments.

Norway's success in culturing alternate cold water species is due in part to the strong cooperative effort among scientists, government and industry. Canadian industry has taken the leading role in the selection of new species for culture and has fostered relationships with scientists from provincial and federal governments as well as universities in order to develop research activities. Scientists from both countries agree when considering a new species for production, research and development must occur at each stage of production in order for producers to incorporate science into the rearing protocols.

In summary, striped wolffish is considered an ideal species for aquaculture production in the cold waters of Newfoundland due to its suitability to a cold water environment. However, little research has been conducted on determining the specific

environmental requirements for the various development stages of this species. In particular, having environmental protocols which are easily transferred into production protocols remains an obstacle. The role of light (photoperiod and light intensity) in the culture of this species is one key research area which remains largely unaddressed. Based upon this lack of information, the focus of my thesis is to determine the photoperiod and light intensity requirements of the larval stage and to determine the effect of light intensity on the feeding behaviour of larval wolffish. In the following chapter a technical review, including accomplishments and obstacles to culturing this species will be presented for each stage of production.

Chapter Two: Technical Review of Wolffish Culture

2.1 Overview

The concept of developing wolffish as an aquaculture species originated in Norway and Russia during the 1980's as fluctuations in the salmon aquaculture industry created greater initiatives to culture alternative species. To date, the vast majority of research and development for this species occurs primarily in Norway but research interest has also spread to Scotland and Atlantic Canada.

Wolffish are members of the family Anarhichadidae, with at least three species living off Canada's Atlantic coast (Scott and Scott, 1988). The species include the striped (*Anarhichas lupus*), spotted (*A. minor*) and the northern (*A. denticulatus*) wolffish. Although both the spotted and striped species are considered aquaculture candidates, the spotted wolffish has become the primary focus of aquaculture research. The solitary and reclusive habit of the fish in combination with low commercial landings (usually reported as a by-catch) create unpredictable market availability, a situation conducive to aquaculture development. Furthermore, the fillets of wolffish are of excellent quality, comprised of firm, white flesh. Fillet products can be smoked, pickled, or dried and even the liver, bile and roe can be utilized. The skin can be tanned into a fine leather (Butt, 1993; Moksness and Pavlov, 1996), and antifreeze proteins in the blood can be extracted and utilized in the biotechnology industry, medical, and food industries (Wiseman, 1997; Brown, 1998). Consequently, the profitability associated with total utilization of the animal highlights the culturing potential for this species.

The natural ecology of wolffish, including habitat preferences (Baruskov, 1959; Beese and Kandler, 1969; Albikovskaya, 1982a; King *et al.*, 1989; Pavlov and Novikov, 1993), distribution and migrations (Powles, 1967; Jonsson, 1982; Templeman, 1984a; Keats *et al.*, 1986a; Ortova *et al.*, 1990), morphology (Barsukov, 1959; Jonsson, 1982; Templeman, 1984b; Scott and Scott, 1988) and feeding (Albikovskaya, 1982b, 1983; Templeman, 1985; Keats *et al.*, 1986b) have been previously studied. For the purposes of this chapter, a review of the biology and ecology of the animal will not be presented. Instead, an overview of each stage of aquaculture production (reproduction, egg development, larval, and juvenile and on-growing) will be presented. A summation of the scientific and industrial breakthroughs which have permitted the culturing of this species, as well as some of the obstacles which still remain for full scale production will also be discussed.

2.2 Broodstock and Reproduction

Establishing a wolffish broodstock which could produce eggs and sperm, and subsequently larvae, on a consistent and reliable basis has plagued aquaculturists since first efforts were made to culture wolffish. Unlike many other fish species, wolffish (in particular female wolffish) do not spawn readily in captivity. In fact, the only documented cases of natural spawning in captivity were reported for fish maintained in a rearing facility for many years (Ringø *et al.*, 1987; Ringø and Lorentsen, 1987). To date, artificial reproduction by insemination is the primary technique used to obtain and fertilize gametes.

The first published report for successfully fertilizing wolffish eggs under artificial conditions and successfully raising the larva, was by Pavlov and Novikov (1986). In addition to successful fertilization, this experiment provided some initial information on sperm physiology. In 1991, a second successful artificial spawning and fertilization from wild-caught broodstock was reported (Pavlov and Radzkihovskaya, 1991). During this time, researchers also started to observe the reproductive behaviour of wolffish.

Kvalsund (1990) was one of the first to suggest that wolffish was an internal fertilizer based upon the following observations: 1) males produced a very small volume of milt (approximately 1.2 ml); 2) the time during which eggs are extruded (15-20 minutes) is inadequate for fertilization of eggs with such a small quantity of sperm; 3) the urogenital papilla which develops in males may function as a copulatory organ; 4) there is periodic close contact of the female and male prior to egg ovulation. Observations on the spawning behaviour of wolffish by Johannesson *et al.* (1993) also supported the hypothesis of internal fertilization.

Additional research conducted by Pavlov (1994a; 1994b) and by Pavlov and Moksness (1994a) further confirmed that wolffish were indeed internal fertilizers. In water, the ability of eggs to be fertilized decreased, but without water, and in ovarian fluid, the capacity for fertilization increased to approximately six hours. A contact period of at least two hours was determined necessary to ensure high fertilization success (90-95%). It has also been determined that a temperature of 0°C allowed sperm to remain fertile for up to ten hours. After this time, sperm viability decreased.

Subsequent research conducted by Pavlov and Moksness (1994b; 1995;1996a)

resulted in rapid improvements and refinements in artificial insemination, and yielded a tremendous amount of information on egg and sperm quality. These advancements were quickly followed by a repeat maturation and spawning of captive broodstock (Pavlov and Moksness, 1996b) and an understanding of the biological, physiological, and environmental factors which affect the quality of gametes and the success of artificial reproductive technology (Pavlov and Moksness, 1994a; 1996a; 1996b; Pavlov *et al.*, 1997).

As a result of this research two methods of artificial fertilization (internal and external) were developed (Pavlov, 1994a; 1996a; 1996b; Pavlov *et al.*, 1997). Internal fertilization is accomplished by introducing milt into the oviduct of the female. Approximately 10-25 ml of sperm solution is injected with a syringe into the genital opening into the middle of the ovary. After insemination, females are kept in holding tanks for four to six hours, after which they are stripped of their eggs. A time frame of four to six hours is used because after six to eight hours, the female releases her eggs into the water and the eggs adhere together.

External fertilization consists of stripping ripe females and males and mixing the gametes (eggs and ovarian fluid with milt). The gametes are placed in a cylinder with the sperm dilutant (Ringer's solution) and the cylinder is inverted up to 20 times during the first hour (fewer inversions thereafter). Gametes are maintained at a temperature of ~4.7°C for four to six hours (a sufficient enough time to ensure approximately 100% fertilization). Fertilized eggs are then removed and distributed over the bottom of special trays to prevent sticking. No differences in the quality of eggs were determined for either

the internal or external fertilization techniques. To ensure good quality of gametes and successful high fertilization rates: 1) the concentration of sperm should exceed $1.0x10^6$ per ml; 2) one female should be fertilized with the sperm of several males; 3) use of the cylindrical chamber for insemination enables the concentration of sperm to be maximized; 4) Ringer's solution is recommended as a dilutant instead of seawater because it enables sperm to live longer in the ovarian fluid and prevents egg swelling; 4) eggs and sperm should be in contact for at least 2 hours to ensure high fertilization success; 6) activation and fertilization of eggs by spermatozoa and the initial development of eggs should occur in the ovarian fluid, however, eggs should be released into seawater before the beginning of cleavage to ensure subsequent normal development; 7) to prevent fertilized eggs from sticking together in clumps, eggs should be placed in static seawater (five to six hours) and then separating individually on a special tray before flushing the remaining ovarian fluid from the egg surface (Pavlov, 1994b; Pavlov and Moksness, 1994a; Pavlov and Moksness, 1996a; 1996b; Pavlov *et al.*, 1997).

The importance of environmental influences on the successful spawning and fertilization of wolffish was presented by Pavlov and Moksness (1994a, 1996a) and Pavlov *et al.* (1997). At temperatures below 10°C egg fertility is increased and is accompanied by fewer resorbed eggs. Normal egg ripening in females requires that broodstock be kept at a temperature below 10°C for at least four months before ovulation. For spermatozoa, which are activated in seminal plasma, sperm are able to remain motile for up to ten days, provided they are kept at temperatures close to 0°C. Motility, and viability decrease significantly as the temperature increases.

More recent research on temperature effects on broodstock have indicated that the temperatures experienced by the broodstock during breeding season affects both the final maturation, the timing of ovulation, and egg quality (Tveiten and Johnsen, 1999; Tveiten *et al.*,1999; 2001).

Photoperiod had no effect on spawning males (Pavlov *et al.*, 1997). Photoperiod affected female maturation time and resulted in a protracted period of egg maturation, thereby confirming an endogenous rhythm in the control of reproduction (Pavlov and Moksness, 1994a). There is also evidence that the intensity of light may affect spawning and egg quality. In a technical report by Moksness and Pavlov (1996), they stated that strong light intensities caused premature release of eggs of lower quality. Unfortunately, no experiments were conducted on the effects of light intensity on spawning and gamete quality.

Other variables such as rearing female broodstock in the presence of males, and diet quality and composition fed to the broodstock are believed to be important factors in obtaining high quality viable eggs year-round (Pavlov and Moksness, 1994a).

2.3 Egg Incubation

For wolffish, the period of egg incubation from fertilization to hatch, is approximately 1000 degree days. Egg masses which have been incubated under ambient water temperatures experienced normal egg hatching and subsequent larval development, despite negative ambient water temperatures. Pavlov and Moksness (1995) attempted to determine the incubation temperatures which could shorten embryonic development

while ensuring normal ontogeny. The results indicated that successful incubation of eggs was possible at temperatures between 5-11°C but the highest incubation occurred at lower temperatures (between 5-7°C). A temperature of 9°C seems to be the upper limit for normal development. Beyond this temperature, many fin rays (particularly pre-caudal parts of dorsal and anal fins) were absent.

The results suggest a temperature regime for minimizing wolffish egg incubation time could be: incubate at 7°C from egg fertilization to the morula stage (2 days); incubate at 11°C from this stage to 50% vascularization of the yolk sac (30 days); incubate at 7°C from the latter stage to formation of rays and caudal and pectoral fins (57 days); 11°C from the latter stage to hatching (104 days).

Pavlov and Moksness (1993) stated that bacteria living on the surface of wolffish eggs may cause low gas exchange, gradual destruction of egg membranes, premature hatching of embryos, and high mortality. They recommended treating eggs with a gluteraldehyde bath. Current practice (Falk-Petersen *et al.*, 1999) is to use a gluteraldehyde treatment of 150 parts per million twice a month to deal with the growth of microorganisms on eggs. The authors state that premature hatching has been a problem in individual egg bathes although they do not fully understand the reasons for the premature hatching.

Breaking the egg masses into smaller portions, and incubating these pieces in Heath Tray ™ units (standard egg incubation equipment used for salmonids) was a good method for egg incubation in Newfoundland. The units consisted of several trays, and had a high water flow which cascaded down through the trays. High water flow, along

with an air hose for each tray, and regular cleaning of the systems resulted in high proportions of normal larvae, with little egg mortality, and little bacterial infection (Halfyard, 1995, pers. comm.; Watkins, 1995 pers. comm.; Wiseman, 1997).

2.4 Larval Stage

At hatching, wolffish larvae are approximately 20 mm long, they have large pigmented eyes, pigmented skin, developed fins, approximately 50 teeth, and little to no yolk sac (Barsukov, 1959; Moksness and Pavlov, 1996). This contrasts with the majority of marine finfish larvae, which generally hatch at 2-5 mm in length, with little or no pigmentation, poorly developed sensory systems (e.g. poor eyes and vision), and have a huge yolk sac. Most of these fish larvae live off their huge yolk sac until a functional mouth and digestive system develops and the larvae are able to switch from endogenous to exogenous feeding. Generally these larvae, undergo an energetically demanding metamorphosis, and survival beyond this stage is very low (Blaxter, 1981). Larval wolffish however, are capable of eating within days after hatching and do not go through a distinctive and stressful metamorphosis.

In general, marine larvae are usually start-fed on small, wild or cultured plankton (e.g., rotifers), and are successively weaned onto larger, cultured zooplankton (e.g., *Artemia*) before weaning onto commercial pellets. The advanced "juvenile-like" stage of larval wolffish, as well as a review of the diets from wild-caught larvae (Falk-Petersen *et al.*, 1990) suggested larvae are capable of eating large prey items soon after hatching, thereby eliminating the need for smaller live-food items.

Preliminary research on first-feeding in larval wolffish indicated that high survival was possible if larvae were fed a diet of wild zooplankton and that other diets such as *Artemia,* fish products, or commercial pellets compromised survival during this period (Ringø *et al.*, 1987). Ringø *et al.* (1987) found newly hatched wolffish fed diets of wild zooplankton had a survival rate of 97%, at day 120 post-hatch, compared to larvae fed on a prepared cod roe diet which had 0% survival, at day 50 post-hatch. Diets of dry pellets (varying moisture levels) alone, or in combination with *Artemia*, failed to achieve the survival rates obtained by Ringø *et al.* (1987). In the initial study (Moksness *et al.*, 1989) higher survival rates were obtained when larvae were fed *Artemia* in combination with the dry pellets versus dry pellets alone, implying *Artemia* provided some additional benefit to first-feeding larvae than dry pellets alone could not provide.

Additional studies conducted by Blanchard (1994) and Wiseman (1997) using only *Artemia* as the diet for first-feeding wolffish larvae, revealed that regardless of prey density, survival rates were much lower for wolffish fed *Artemia* than for wolffish fed wild zooplankton (Ringø *et al.*, 1987). Wiseman (1997) conducted experiments where larvae were fed varying densities of enriched *Artemia* (100 per/L, 900 prey/L) in combination with a commercial diet feed to excess. At the end of the study (9 weeks post-hatch), larvae fed prey at high densities (900/L and dry food) had a final survival of 94.3%. Results from the behavioural analysis revealed that larvae fed significantly more on *Artemia* during the critical period (up to 5 weeks post hatch) (Wiseman, 1997). However, by weeks 6-7 the larvae fed equally on *Artemia* and dry feed and by week 7, the larva preferred dry food, having weaned themselves off *Artemia* (Brown *et al.*, 1997).

The wolffish's ability to "self-wean" in the presence of suitable diets, can be considered a benefit with respect to potential commercial production.

More recent studies conducted by Hendry and Halfyard (1998) compared the growth and survival rates of larvae fed three different diets. Results indicate the survival of the larvae was greatest with enriched *Artemia*/dry diet (>90%), followed by unenriched *Artemia*/dry diet (>80%), and finally the dry pellet only (76%). These results support the theory that the presence of live food during the first feeding stage promotes the instinctive predatory response while the *Artemia* (particularly, the enriched form) offers some nutritional contribution to the larvae (Brown *et al.*, 1995b; Hendry and Halfyard, 1998).

In Norway, a study conducted by Strand *et al.* (1995) demonstrated it was possible to start-feed larvae and obtain high survival by using only a commercial diet. In this report, larvae were fed two commercial diets (diet A: floating pellet, diet B: sinking pellet) for 60 days post-hatching. At the end of the study, both growth and survival were higher among larvae fed diet A (final survival 82%). The authors postulated that a floating diet stimulated a higher start-feeding incidence due to larvae more readily attacking the floating diet. Start-feeding larval wolffish solely on commercial pellets is the preferred and most commonly used technique in Norway.

One constant noted among all the feeding studies was the time frame during which significant mortalities occurred (Moksness *et al.* 1989 (days 20-49); Blanchard, 1994 (days 27-36); Strand *et al.*, 1995 (days 22-40); Wiseman, 1997 (days 21-35). Day 20-40 post-hatch therefore represent a critical period for larval wolffish growth and survival. This time period corresponds to the total absorption of the yolk sac and a switch

from endogenous to exogenous feeding. High mortalities observed at this time were attributed to a failure of larvae to initiate feeding and an unsuccessful switch to the exogenous food provided (Strand *et al.*, 1995; Wiseman, 1997).

In addition to ensuring optimal dietary requirements, factors such as optimal environmental and rearing conditions also affect the growth and survival of a species. For example, temperature is known to be an important variable in rearing larval fish as it can affect incubation time, size at hatch, yolk utilization efficiency, growth, feeding rates, time to metamorphosis, behaviour, swimming speed, digestion rate, gut evacuation, and metabolic demand (Blaxter, 1988). For most species, growth rate tends to increase with increasing temperatures until the optimal growth rate is reached and beyond this "optimal temperature" growth rate decreases (Jobling, 1983).

Various studies have indicated that wolffish are capable of tolerating a wide range of water temperatures (1.0-13.7°C) during rearing (Stefanussen *et al.*, 1993; Ringø *et al.*, 1987; and Moksness, 1994). A study by Moksness (1994) on the growth rates of striped and spotted wolffish recommended a temperature of between 7-9°C. It was recommended that rearing temperatures not exceed 10°C, especially for the spotted wolffish, which appear to have a lower optimal temperature of the two species.

Temperature studies conducted by Wiseman (1997) on larval wolffish showed that for the first 6 weeks post hatch, temperatures should be between 4-8°C. Temperature affected survival of larval wolffish up to 6 weeks post-hatch but not after this period. These results indicated that fish reared in lower than optimal rearing temperatures may have been unsuccessful in their transition from endogenous to exogenous feeding and the

inadequate rearing temperatures prevented the larvae from feeding at a level that met their metabolic demands.

2.5 Juvenile/On-Growing

For production purposes, one of the first studies on the feeding behaviour of juvenile wolffish was conducted by Ortova *et al.* (1989). This study revealed that the frequency of food ingestion depends on water temperatures where juveniles held at high temperatures (6-10°C) feed daily. However, at low temperatures (0-2°C) the intervals between ingestion of food are increased to 2-3 days.

Diet composition and the processing technique used to formulate the commercial diets are crucial to maximizing juvenile production. According to Stefanussen *et al.* (1993) in order to achieve a high growth rate in wolffish, the feed composition should have a high protein concentration (>50%) and a low carbohydrate content (<20%). High fat content in the feed results in higher fat content in the fillet and an enlarged liver, whereas the water content in the feed did not affect the growth rate. Moksness *et al.* (1995) compared moist (squid diets), regular fish meal diets, and low temperature (LT) processed dry pellets and found there were no differences in growth, feed conversion, protein efficiency rate, or productive protein values between the moist and LT diets. However, the LT had better results in all parameters when compared to the regular diet. With respect to rations, Ortova *et al.* (1989) found that for adult wolffish daily feeding rations were maximal at 9-10°C and that 0-1°C was close to the critical temperature for feeding.

higher densities by using re-sorting of fish to prevent starvation and mortalities of the smallest fish. Moksness and Pavlov (1996) reported that yearlings and adults have been successfully maintained without mortalities at stocking densities of 100 kg/m^2. Fam (1997) determined 50g/L to be sufficient, while increased densities lowered feed conversion ratios and lower densities affected the protein efficiency ratio.

Determining the optimal environmental requirements is essential for any stage of production. For wolffish juveniles, little is known about their light requirements. Pavlov (1995) determined that newly-hatched larvae react positively to light but during the course of ontogenesis they become increasingly demersal and the role of light and vision in the search for food decreases. It was also noted that in winter and autumn, juveniles periodically discontinue feeding and that growth rates decreased implying a seasonal rhythm possibly linked to the decreasing photoperiod at this time. Moksness and Pavlov (1996) noted that the positive reaction of juveniles to light disappears in fish greater than 1 g in weight and longer than 50-60 mm. They also suggested that continuous light was important during the pelagic phase of wolffish and that the strong reactions of larvae and juveniles to light could be used as a management tool for their behaviour.

Feeding frequency studies for juvenile wolffish determined that feeding rates varied from every day for smaller wolffish to every other day for larger fish (Steinarrson and Moksness, 1996). Studies conducted by Ortova *et al.* (1989) and Fam (1997) confirm that feeding of large juveniles need only occur every second day.

2.6 Conclusion

In summary, much of the rearing technology for this species has been determined. The role of light (photoperiod and light intensity) consistently remains an area which needs to be addressed for each stage of production. In the following chapters the role of light on larval production is examined. The objectives of my study are: 1) to determine the role of photoperiod on growth and survival of larval wolffish; 2) to determine the role of light intensity on the growth, survival, and feeding behaviour of larval wolffish; and 3) to recommend light protocols which can be incorporated into hatchery culture technology.

Chapter 3: The Effect of Photoperiod on Larval Wolffish Growth and Survival

3.1 Introduction

Wolffish have been identified as a strong candidate for cold water aquaculture due in part to an inconsistent market supply and its potential biological suitability to cold-water rearing. Relative ease in larval and juvenile production, good quality flesh (fillet), and small landed quantities from the commercial fisheries have highlighted the wolffish's appeal as an aquaculture candidate. Research on the culture of striped (*Anarhichas lupus*) and spotted wolffish (*Anarhichas minor*) is presently underway in Newfoundland, Quebec, Norway, and Scotland.

Many of the culture techniques dealing with reproduction (Pavlov, 1994a,b; Pavlov and Moksness, 1994a; 1994b; 1996a; 1996b), egg incubation (Pavlov and Moksness, 1993; 1994a; 1995), temperature requirements (Pavlov and Moksness, 1994a,b; 1995; 1996a,b; and Wiseman 1997), diet and feeding protocols (Moksness *et al.*, 1989; Ortova *et al.*, 1989; Stefanussen *et al.*, 1993; Moksness, 1994; Pavlov and Moksness, 1994a; Moksness *et al.*, 1995; Pavlov, 1995; Strand *et al.*, 1995; Wiseman, 1997), stocking densities (Pavlov, 1995), and growth rates (Stefanussen *et al.*, 1993; Moksness, 1994; Moksness *et al.*, 1995; Pavlov, 1995), have been determined for this species.

Despite the tremendous amount of research conducted on wolffish, the role of photoperiod in wolffish culture remains largely unknown. For broodstock, it is known

that under natural conditions spawning is apparently synchronized by the decrease in day length during the summer and autumn months (Moksness and Pavlov, 1996). During laboratory trials Moksness and Pavlov (1996) experimentally altered the photoperiod of their broodstock (from 18L:6D to 6L:18D) and observed a failure of fish to spawn in over 50% of the females subjected to the altered photoperiod. These results indicated that the light cycle plays a role in the determination of wolffish spawning time and suggests the possibility for the management of final maturation by seasonality (day length). Despite the limited information on the effects of photoperiod on wolffish, the effects of photoperiod on other fish species have been extensively studied.

Several authors have shown that photoperiod has a significant effect on the biology and behaviour of fish. Photoperiod and light intensity may affect growth and survival via a number of physiological pathways. For example, Fuchs (1978) stated light stimuli affects sensory receptors in fish (eyes, pineal gland), and induces changes in their physiology. Photoperiod and light may also exert direct effects on the behaviour of an organism, not necessarily linked to any endogenous rhythm (Richus and Winn, 1979). Such behaviours include the activity of fish (Schwassmann, 1971; Britz and Piennar, 1992), reproduction (gonad maturation, gamete production/fecundity, delaying or synchronicity of the spawning seasons; Baggerman, 1980; Ridha and Cruz, 2000; Loir *et al.*, 2001; Rodriguez *et al.*, 2001), physiology (e.g. thyroxine levels; Noeske and Spieler, 1983), feeding behaviour (Schwassmann, 1971; Tandler and Helps, 1985), responses to visual stimuli, diurnal rhythm, and vertical migration, (Blaxter, 1966; 1968a,b; 1973; 1975b; Rahmann *et al.*, 1979).

The response of fish to light is not only species specific but varies with the stage of development. The responses to light levels may be consistent throughout the development stages or it may vary. For example, yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*) develop abnormally in the presence of light (Bolla and Holmefjord, 1988), whereas juvenile-adult stage halibut exposed to continuous light (24 light) had higher growth rates compared to those raised under shorter photoperiods of 8 hours light/16 hours darkness (Simensen *et al.*, 2000). Variation in light requirements within a species can also be seen for yellowtail flounder. Experiments conducted on yellowtail larvae (*Pleuronectes ferrugineus*) demonstrated that higher growth and survival rates were obtained under continuous light (Puvanendran, 1999b; unpublished data) whereas for the juvenile stage (same spawning batch as the larvae described above) a photoperiod of 12 hours light produced comparable growth and survival rates to those raised under continuous light (Purchase and Brown, 1997).

The nature and extent of the effect of increased photoperiod on growth and survival of a species can vary greatly despite similarities in development, habitat, morphology and physiology (Barlow *et al.*, 1995). As an example, Tandler and Helps (1985) demonstrated that for the first 12 days, larval gilthead sea bream (*Sparus aurata*) survive better under 24 hours light versus 12 hours light. However, Dowd and Houde (1980) showed that 13 hours of light resulted in the best growth and survival of larval sea bream (*Archosargus rhomboidalis*) up to day 16 post-hatch. For larvae of yellowtail flounder (*Pleuronectes ferrugineus*) continuous light resulted in improved growth, survival and specific growth rates (Puvanendran, 1999b pers. comm.) however, in

summer flounder (*Paralichthys dentatus*), continuous light offers no benefit to growth or survival (Huber *et al*, 1999). Consequently, selecting an optimal photoperiod for maximum growth and survival, for any new species should be based on experimentation.

No studies have been conducted on larval wolffish concerning the effects of light on the survival or growth of larvae. During larval studies, photoperiods ranging from 16 hours to 24 hours light have been used without explanation (Moksness *et al.*, 1989; Moksness, 1990; Strand *et al.*, 1995; Moksness and Pavlov, 1996; Wiseman, 1997). The objective of this study was to determine the effect of photoperiod on the growth and survival of larval wolffish. The hypothesis that increased photoperiod will result in increased growth and survival of wolffish larvae was tested.

3.2 Materials and Methods

3.2.1 Egg Collection and Larval Selection

During October 1994, wolffish egg masses were collected by SCUBA divers in Bauline, Conception Bay, Newfoundland. Egg masses were transported to the Wesleyville Marine Finfish Hatchery, in Wesleyville, Newfoundland (Fig.1). At the hatchery, egg masses were broken into single layers of eggs and incubated in up-welling vertical tray incubators at a density of approximately 1000 eggs/tray until hatching. Ambient seawater was used during incubation. To prevent bacterial infection, all egg masses were disinfected twice weekly using a gluteraldehyde-seawater bath (Salvesen and Vadstein, 1995). Disinfection ended when the larvae started hatching.

At 0-12 hours post-hatch, larvae from three egg masses were randomly selected

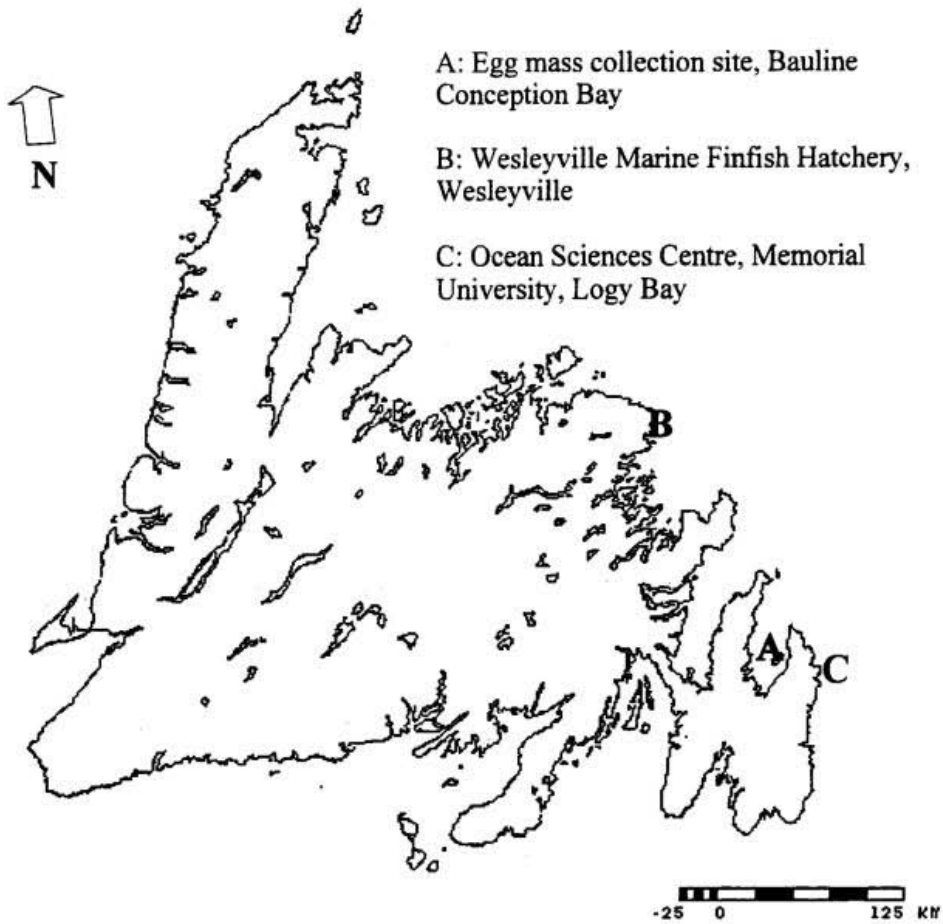

Figure 1: Geographic locations of the egg mass collection site (A) and research facilities (B and C) for experimental protocols.

from the incubation trays and transferred to the Ocean Sciences Centre, Memorial University, Logy Bay, Newfoundland, for experimentation.

Fish were randomly distributed in the experimental tanks at 6-14 hours post-hatch and acclimatized for an additional 24 hours prior to starting the experiment. The initial stocking density for the tanks was 5 fish/L (Pavlov, 1995) with equal proportions of the three egg masses stocked in each tank. Mortalities observed during this time were removed and replaced with new fish. Day 1 represents the day on which the experiments started (28-40 hours post-hatch).

3.2.2 Photoperiod Protocol

Three photoperiods: 12 hours light/12 hours dark (12L; 8:00 a.m.-8:00 p.m.), 18 hours light/6 hours dark (18L; 8:00 a.m.-2:00 a.m.), and 24 hours light (24L) were selected for experimentation. To avoid possible light interference, each photoperiod trial was conducted in a separate light-controlled room.

A light intensity of 750 lux was used for all treatments and was achieved by placing incandescent bulbs approximately 1 metre above the test tanks. A 20 minute twilight period (180 lux) was activated before and after the main lights were turned on/off in order to avoid light-shocking the larvae (Mork and Gulbrandsen, 1994). All lights were controlled electronically with a timer. Light intensities (recorded in units of lux) were measured at the water's surface using a SPER Scientific Light Meter.

Nine (3 replicate tanks per treatment) flow-through, rectangular, glass aquaria (45 cm x 30 cm x 30 cm, 30 L) were used as experimental tanks. The four walls (sides) of

the glass tanks were covered with black plastic to control outside disturbances and limit mirror reflections within the tanks (Pearce, 1991; Barlow *et al.*, 1995; Naas *et. al.*, 1996). The mean ambient sea water temperature was 6.0°C (range: 1.8-12.0°C). Water flows were adjusted daily to maintain a temperature between 6-8°C (Wiseman, 1997).

Each treatment received three daily feedings of *Artemia franciscana* nauplii (1000 prey/L) and a commercial dry pellet fed to excess (Wiseman, 1997), during light hours (10:00 a.m., 2:00 p.m., 6:00 p.m.). *Artemia* decapsulation and enrichment were in accordance with *Artemia* Systems standard manual (Sorgeloos *et al.*, 1986). A fourth (dry food only) supplemental feeding was given to all treatments at 5:30 a.m. which coincided with the dark hours of the 12L and 18L treatments. Dry pellets were observed to sink to the bottom of the tanks shortly after introduction. A previous weaning study conducted on the feeding behaviour of larval wolffish demonstrated that they can feed on dry food on the bottom of the tanks (Wiseman, 1997), thereby ensuring that fish in each treatment had an opportunity to feed on the fourth supplemental ration. The photoperiod experiment was terminated on day 50.

3.2.3 Measurements and Analysis

Survival

All tanks were siphoned daily, prior to first feeding, to remove excess feed and feces. Mortalities observed during cleaning were removed and recorded.

Growth Measurements

Initial (day 1) size measurements were conducted on a sub-sample of larvae from each of the three egg masses. Thirty fish (10 from each egg mass) were measured for standard length and wet weight. In order to avoid introducing potentially stressed or moribund fish into a treatment, none of the fish used in the initial measurements were placed in the experimental tanks. Subsequent growth measurements were performed on sub-samples of fish (10 larvae) randomly chosen from each experimental tank. Measurements were recorded every ten days until the experiment was terminated. Protocols were as follows:

Wet weight

Wet weights were recorded for each of the 10 larva in the sub-sample. Fish were removed from the experimental tanks with a dip-net and excess sea water was removed from each larva by gently towel drying the fish. Fish were placed in a pre-weighed, petri dish which was filled with sea water. A top loading Mettler PC 4400 scientific balance was used to record measurements to the nearest hundredth (0.01) of a gram (g).

Standard length

To determine standard length, individual fish were transferred from the weighing dish to a measuring dish. The measuring dish was a modified petri dish equipped with a flexible, plastic, holding chamber (used to enclose the larvae and prevent it from swimming), and a metric ruler for measuring the lengths of the fish. Based upon

preliminary trails with this apparatus, wolffish larvae ceased swimming when surrounded by the "chamber " and remained stationary until it was removed. The apparatus did not injure any fish during sampling. Standard lengths were recorded in millimetres to the nearest 0.5 millimetres (mm).

Specific Growth Rates

Specific growth rates were determined for all treatments based upon an equation by Buckley *et al.* (1997):

$SGR=\{(\log_e S_2 - \log_e S_1) / T_2 - T_1\} *100$, where

S_1= initial fish measurement (wet weight or standard length);

S_2= final fish measurement (wet weight or standard length);

T_1= initial time; and

T_2= final time.

Specific growth rates were calculated at ten day intervals for the duration of the experiment.

Statistical Analysis

All data were first tested for normality. Data which were not normally distributed were transformed (survival data: arcsin transformed, and growth data: log transformed) to meet the assumptions of the test. A two-way ANOVA (SAS version 6.1, Cary, NC) was used for all comparisons.

Where significant day*treatment interactions occurred, a Tukey's Test for

Multiple Comparisons was performed among treatment means for each sample day. Level of significance was set at $\propto$=0.05.

3.3 Results

Survival

Photoperiod had a significant effect on the survival of larval wolffish (Table 1). Although a significant difference in survival was seen among treatments for day 10 (Table 2) the mortalities recorded during this time were directly attributed to siphoning errors and not treatment effect. Statistically significant differences in survival were seen among treatments between days 30-50 of the experiment. According to the Tukey's results (Tables 2, 3) for days 30-50 inclusive, both the 18L and the 24L treatments had a significantly higher survival rate than the 12L treatment (Fig. 2).

Although no statistically significant difference in survival was recorded between the 18L and the 24L treatments throughout the entire experiment, significant mortalities commenced on day 30 for the 12L treatment and continued until the experiment was terminated on day 50. Final survival rates for each treatment were as follows: 18L (66.89%); 24L (58.67%); and 2L (34.22%).

Wet Weight

Photoperiod had a significant effect on the overall weight of larval wolffish (Table 1, Fig. 3). No significant differences in wet weight were observed among treatments for day 10 (Table 2). Although statistically significant differences were observed on day 20,

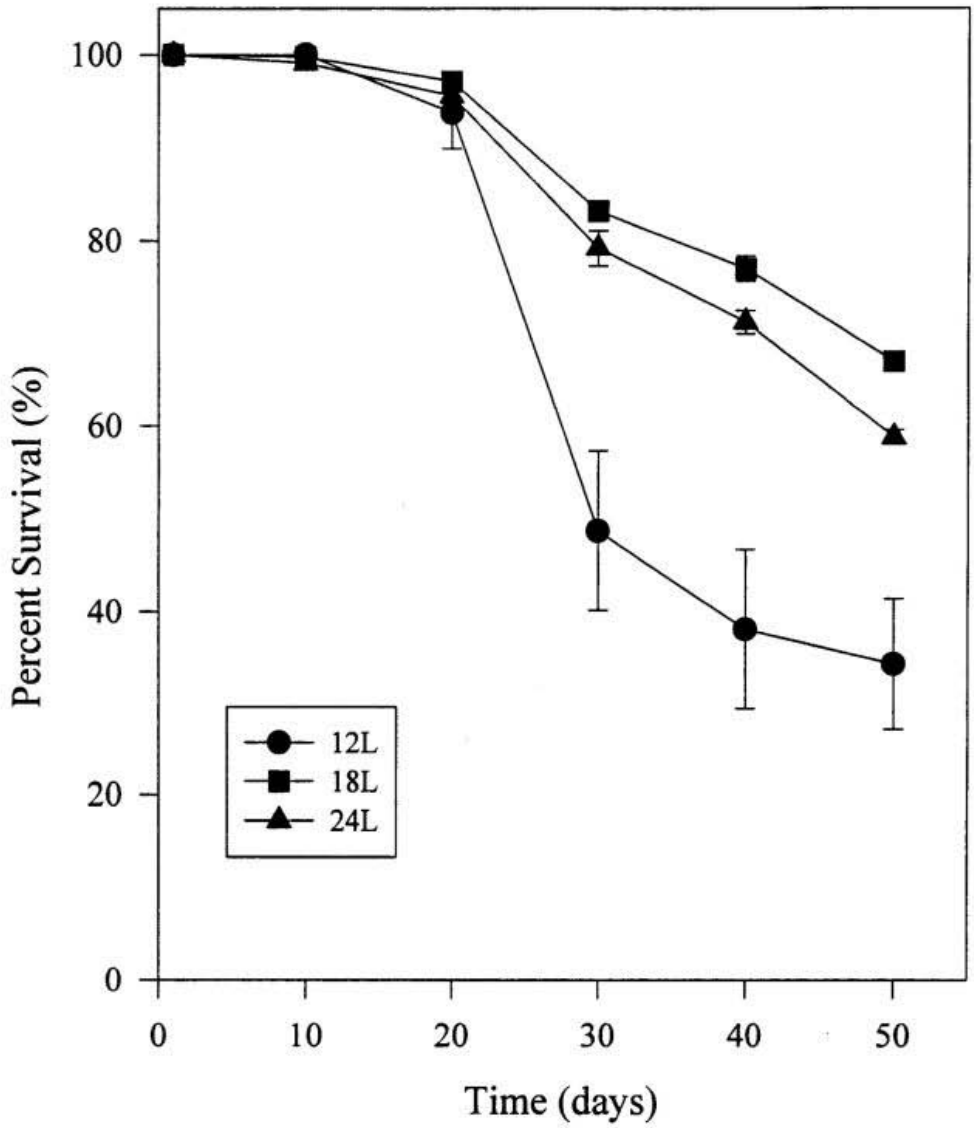

Figure 2: Mean percent survival (%) of larval wolffish over time (days) for photoperiod experiment (vertical bars represent standard error, n=30 for each data point).

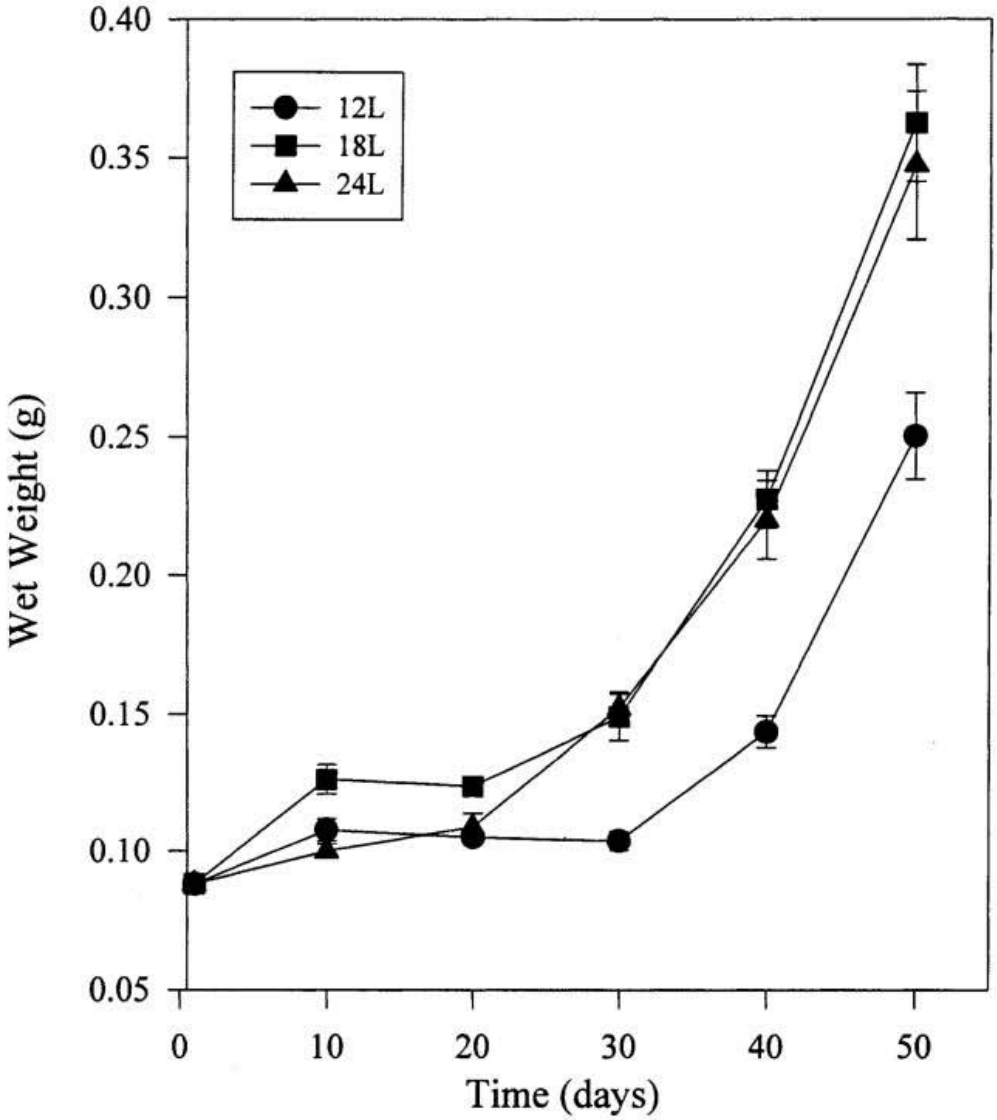

Figure 3: Mean wet weight (g) of larval wolffish over time (days) for photoperiod experiment (vertical bars represent standard error, n=30 for each data point).

Table 1: Results of Two Way Analysis of Variance (ANOVA) on the mean percent survival, mean wet weight, and mean standard length of larval wolffish for the photoperiod experiment (significance level $p<0.05$, * denotes a significant difference).

EXPERIMENT	SOURCE	DF	F-VALUES	p-VALUES
Mean Percent Survival	Treatment	2	32.56	0.0001*
	Day	4	128.48	0.0001*
	Treatment*Day	8	6.03	0.0001*
Mean Wet Weight	Treatment	2	52.72	0.0001*
	Day	4	272.81	0.0001*
	Treatment*Day	6	6.12	0.0001*
Mean Standard Length	Treatment	2	22.85	0.0001*
	Day	4	462.66	0.0001*
	Treatment*Day	8	3.37	0.0072*

Table 2: Results of Tukey's Studentized Range Test on the mean percent survival, mean wet weight, and mean standard length of larval wolffish, for each sample day separately, for the photoperiod experiment (significance level $p<0.05$, * denotes significant difference).

VARIABLE TESTED	DAY	DF	F-VALUES	p-VALUES
Mean Percent Survival	10	2	7.00	0.0270*
	20	2	0.31	0.7450
	30	2	15.25	0.0044*
	40	2	16.58	0.0036*
	50	2	14.96	0.0047*
Mean Wet Weight	10	2	3.90	0.0822
	20	2	6.46	0.0319*
	30	2	20.44	0.0021*
	40	2	150.59	0.0001*
	50	2	12.58	0.0071*
Mean Standard Length	10	2	1.65	0.2690
	20	2	4.07	0.0764
	30	2	12.51	0.0072*
	40	2	2.61	0.1527
	50	2	11.76	0.0084*

Table 3: Tukey's groupings for the mean percent survival, mean wet weight, and mean standard length for the photoperiod experiment (Note: means with the same letter, for each age, are not significantly different, $p<0.05$).

Time (days)	Tukey's groupings (means) for each sample day			
	Treatment	Survival (%)	Wet Weight (g)	Std. Length (mm)
10	12L/12D	100.00^{A}	0.11^{C}	20.07^{E}
	18L/6D	99.78^{AB}	0.13^{C}	20.28^{E}
	24L/0D	99.11^{B}	0.10^{C}	20.53^{E}
20	12L/12D	93.78^{A}	0.11^{CD}	21.05^{E}
	18L/6D	97.11^{A}	0.12^{C}	21.55^{E}
	24L/0D	95.57^{A}	0.10^{D}	20.92^{E}
30	12L/12D	48.67^{B}	0.11^{D}	22.25^{F}
	18L/6D	93.78^{A}	0.15^{C}	24.22^{E}
	24L/0D	84.00^{A}	0.15^{C}	24.00^{E}
40	12L/12D	38.00^{B}	0.14^{D}	25.11^{E}
	18L/6D	76.89^{A}	0.23^{C}	27.88^{E}
	24L/0D	71.11^{A}	0.22^{C}	26.82^{E}
50	12L/12D	34.22^{B}	0.25^{D}	29.01^{F}
	18L/6D	66.89^{A}	0.36^{C}	32.48^{E}
	24L/0D	58.67^{A}	0.35^{C}	31.85^{E}

the Tukey's groupings (Table 3) show the actual difference in the wet weights was minimal (12L (0.11g); 18L (0.12g); 24L (0.10g). Although no significant differences in wet weight occurred between the 18L and 24L treatments during days 20-50, significant differences in mean wet weight occurred between these treatments and the 12L treatment during days 30-50. Consistently, the 12L treatment had a significantly lower wet weight than either the 18L or 24L treatments.

Standard Length

Photoperiod had a significant effect on the standard length of larval wolffish for days 30 and 50 of the experiment (Table 2). For each of these sampling periods, the differences in standard length between the 18L and 24L treatments were not significant but were significantly greater than the standard lengths for 12L treatment (Fig. 4, Table 3). No significant differences were observed among treatments for days 10, 20, or 40.

Specific Growth Rate (SGR; % wet weight/day)

Results from the ANOVA (Table 4) show a significant interaction between day and treatment. However, day effect, not photoperiod treatment has the most significant influence on the wet weight-SGR. Although, negative SGR's were seen for both the 12L and 18L treatments on day 20 (indicating a loss in weight for the fish; Fig.5), day 30 was the only time period in which a significant difference occurred among treatments (Table 5). Larvae sampled from the 12L treatment had a lower growth rate (0.18% wet weight/day) than either the 18L (1.90%) or the 24L (3.39%) treatment. There were no

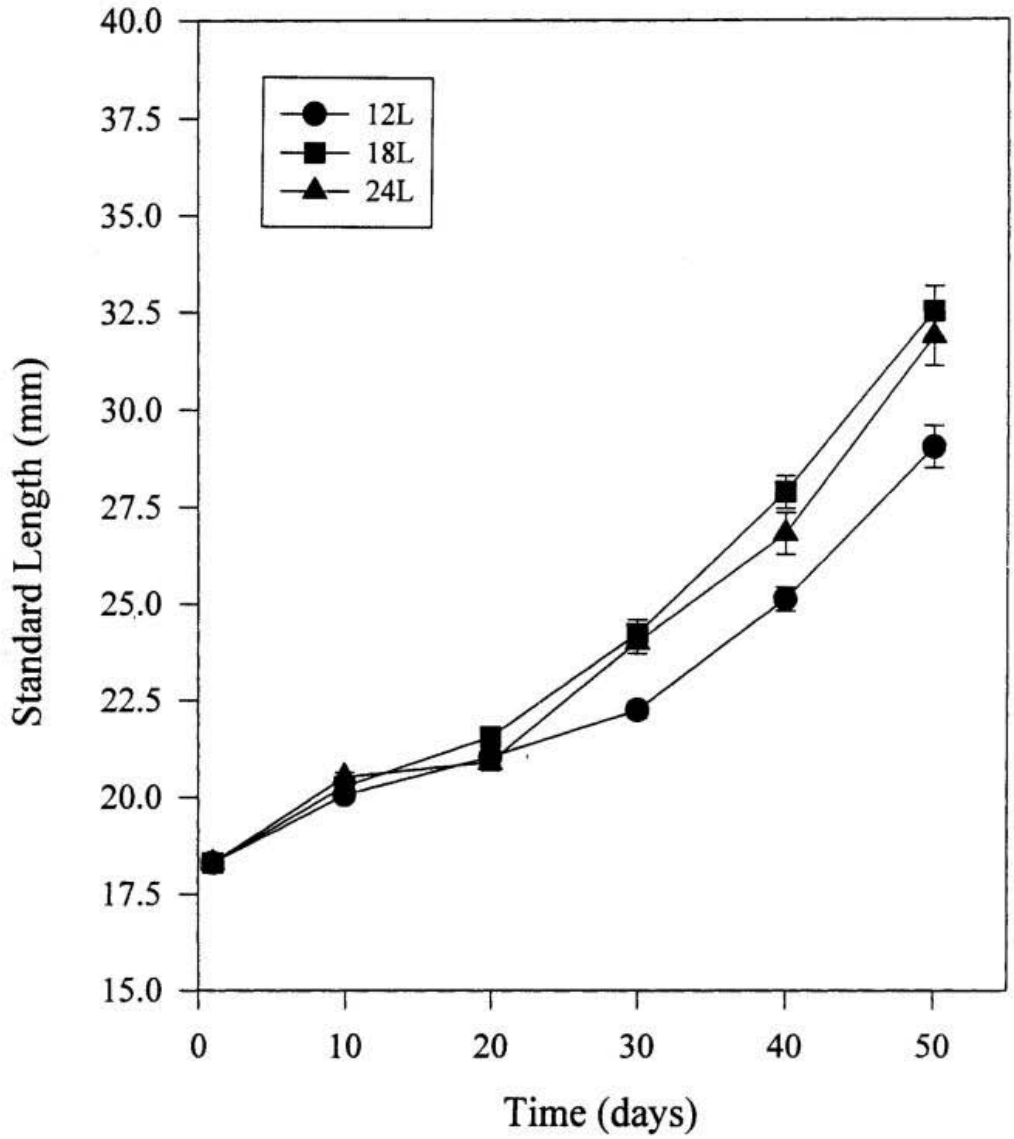

Figure 4: Mean standard length (mm) of larval wolffish over time (days) for photoperiod experiment (vertical bars represent standard error, n=30 for each data point).

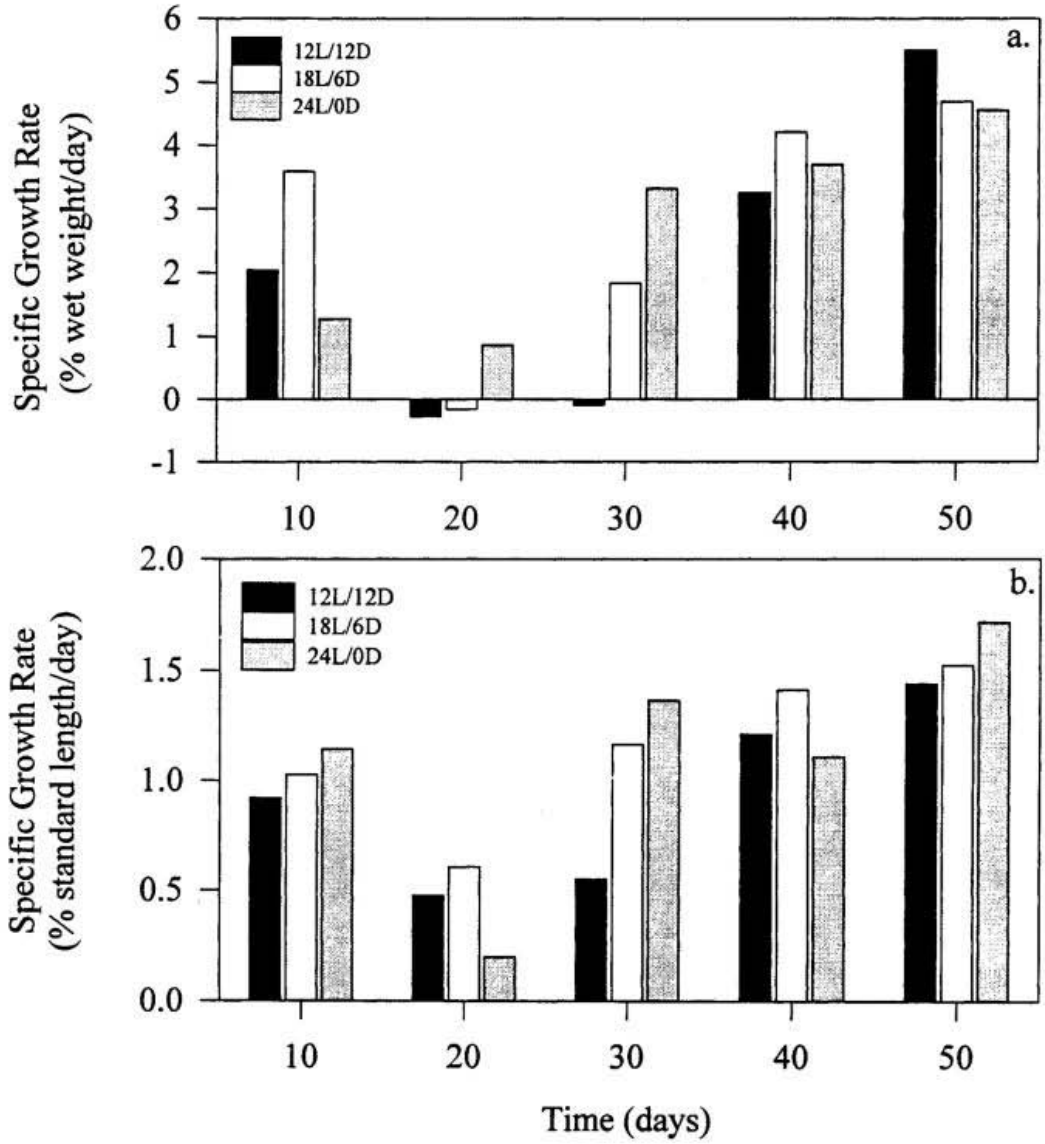

Figure 5: The mean wet weight specific growth rate (fig. a) and the mean standard length specific growth rate (fig. b) over time (days) for larval wolffish for the photoperiod experiment (n=30 for each data point).

Table 4: Results of Two Way Analysis of Variance (ANOVA) on the specific growth rates (% wet weight/day, % standard length/day) of larval wolffish for the photoperiod experiment (significance level $p<0.05$, * denotes significant difference).

VARIABLE	SOURCE	DF	F-VALUES	p-VALUES
Specific Growth Rate (% wet weight/day)	Treatment	2	2.48	0.1005
	Day	4	32.83	0.0001*
	Treatment*Day	8	3.27	0.0085*
Specific Growth Rate (% standard length/day)	Treatment	2	3.97	0.0295*
	Day	4	28.17	0.0001*
	Treatment*Day	8	2.84	0.0180*

Table 5: Results of Tukey's Studentized Range Test on the specific growth rates (% wet weight/day, and % standard length/day) of larval wolffish, for each sampling day separately, for the photoperiod experiment (significance level $p<0.05$, * denotes significant difference).

VARIABLE TESTED	DAY	DF	F-VALUES	p-VALUES
Specific Growth Rate (% wet weight/day)	10	2	4.02	0.0779
	20	2	1.13	0.3836
	30	2	16.83	0.0035*
	40	2	1.03	0.4137
	50	2	0.89	0.4572
Specific Growth Rate (% standard length/day)	10	2	1.82	0.2406
	20	2	2.89	0.1322
	30	2	18.92	0.0026*
	40	2	1.06	0.4038
	50	2	0.54	0.6101

Table 6: Tukey's groupings for the specific growth rates (% wet weight/day, % standard length/day) for the photoperiod experiment (Note: means with the same letter, for each age, are not significantly different, $p<0.05$).

Time (days)	Tukey's groupings (means) for each sample day		
	Treatment	% Wet Weight/Day	% Standard Length/Day
10	12L/12D	2.29^{A}	0.92^{A}
	18L/6D	3.57^{A}	1.03^{A}
	24L/0D	1.26^{A}	1.14^{A}
20	12L/12D	-0.86^{A}	0.48^{A}
	18L/6D	-0.22^{A}	0.61^{A}
	24L/0D	0.81^{A}	0.20^{A}
30	12L/12D	0.18^{B}	0.55^{B}
	18L/6D	1.90^{A}	1.16^{A}
	24L/0D	3.39^{A}	1.37^{A}
40	12L/12D	3.28^{A}	1.21^{A}
	18L/6D	4.23^{A}	1.41^{A}
	24L/0D	3.68^{A}	1.11^{A}
50	12L/12D	5.52^{A}	1.44^{A}
	18L/6D	4.64^{A}	1.53^{A}
	24L/0D	4.56^{A}	1.72^{A}

statistically significant differences in wet weight-SGR between the 18L and 24L treatments (Table 6). For days 40 and 50, the SGR of the 12L treatment was statistically similar to the other treatments indicating growth for this treatment was not compromised beyond day 30.

Specific Growth Rate (% standard length/day)

Results for the standard length-SGR's show similar trends as the wet weight-SGR's (Fig 5., Table 4). Day 30, was the only day on which significant differences were found among treatments. Differences in the SGR between the 18L (1.16%) and 24L (1.37%) treatments were not significant but both had significantly higher SGR's than the 12L (0.55%) treatment (Tables 5, 6).

3.4 Discussion

The results of this experiment demonstrated that photoperiod had an effect on larval wolffish survival and growth. During the first 20 days, the effects of photoperiod on these parameters, were not significant. Although a slight statistical difference does occur for both survival and weight during this period, the changes in growth and survival were minimal and were directly attributable to human error. During cleaning several larvae were killed when they were accidently siphoned.

Photoperiod effect on larval survival and growth was most evident during days 20-40 post-hatching. During this period, the trend for both survival and growth was statistically significant and consistent. Although no significant differences were recorded

between the 18L and 24L treatments, both treatments did differ significantly from the 12L treatment. The mortality rate of larvae in the 12L treatment nearly doubled that of the 18L treatment during this period indicating that the 12L photoperiod had a deleterious effect on larval wolffish survival from day 20 onwards. However, beyond day 40 the changes in survival and growth become less pronounced implying that the photoperiod requirement changed as the larvae approached the juvenile phase.

The trend in growth rates (mean wet weight, mean standard length) was similar to those for survival, as the 18L and 24L treatments produced the longest and heaviest larvae while the 12L treatment produced the smallest fish. Consistent with survival, growth for the 12L photoperiod showed the largest statistical differences beyond day 20 post-hatch.

The results suggest that days 20-40 post-hatch are a critical period for ensuring larval wolffish growth and survival. It is apparent that a significant biological phenomenon occurred during this period and was reflected in the variations or differences in survival rates, growth rates (length and weight) and specific growth rates for the three treatments. During this critical period, the reduced survival and growth rates of the 12L treatment indicate that the reduced photoperiod is inadequate for ensuring maximum survival or growth of larval wolffish during this time. Several studies conducted on larval wolffish suggest that the critical biological phenomenon which occurs during days 20-40, is the switch from endogenous to exogenous feeding and that this feeding transition may coincide with weight losses and/or significant mortalities (Blanchard, 1994; Strand *et al.*, 1995; Wiseman, 1997).

Despite a weight loss (negative specific growth rates) occurring in both the12L and 18L treatments on day 20, the 18L treatment (day 30) was able to attain a much higher SGR than the 12L (approximately 10 times higher) suggesting that the additional 6 hours of photoperiod contributed to the improved survival and growth during this transition to exogenous feeding.

Negative growth rates during the transitional feeding stage have been reported in previous wolffish studies (Strand *et al.*, 1995; Wiseman, 1997) and in a larval rabbitfish *Siganus guttanus* study (Duray and Kohno, 1988). Studies conducted on the starvation of larval wolffish determined the mortality rate for unfed larvae commenced around the third week (day 21post-hatch) with 100% mortality occurring by week five (day 35 post-hatch; Wiseman, 1997). Several authors suggest that high mortalities during this period may be caused by either a reduced feeding opportunity (resulting in a deficiency in nutrients and energy) or an inappropriate feeding adaptation (Moksness *et al.*, 1989; Blanchard, 1994; Strand *et al.*, 1995; and Wiseman, 1997).

It seems that 18L and 24L photoperiods in this study provided a longer feeding opportunity compared to the 12L due to the greater duration in which the commercial diet was present in the rearing tanks. Personal observations revealed that wolffish do not appear to feed actively during periods of darkness and larvae remain stationary on the bottom of the tanks during this period. Initially it was perceived that since live food was only presented during the light hours and that flushing rates for the tanks were similar for all treatments that all larvae had an equal period of time to forage. However, the dry food which sank to the bottom of the tanks, was not subjected to the same flushing rate as the

live food. Consequently, the 18L and the 24 L larvae would be able to take advantage of the additional nutrition derived from the dry supplemental pellets during the increased light hours. The 12L larvae however, would only have a couple of hours to feed on the supplemental feeding before the food was siphoned from the tanks in the morning. When considering the results of Wiseman's behavioural studies (1997) the larvae's ability to actively feed/and wean themselves onto the nutritional advantageous commercial pellet most likely contributed to the greater growth and survival of the 18L and 24L treatments. The additional hours of light increased the feeding period on the nutritionally complete diets during the transition period from endogenous to exogenous feeding.

Although reduced growth and survival, due to the inability to establish appropriate feeding behaviour may be one explanation for the poor performance of the 12L treatment, there are other factors which may have compounded the results seen in this treatment.

One possible explanation for the poor performance could be the swimming activity of the fish. Although larval wolffish are most active in the water column when they are feeding or searching for food, they have been observed to sit or "rest" on the bottom of rearing tanks (Moksness *et al*, 1989; Strand *et al.*, 1995; and Wiseman, 1997). Darkness in particular, is a cue which initiates settling on the bottom of tanks (Moret, personal observation; Pavlov, 1995; and Strand *et al.*, 1995). From personal observations, fish in the 12L treatment appeared to spend more time resting on the bottom of the tanks than the other two treatments. As most of the dead food, feces, and other wastes accumulate on the bottom of the tanks, the 12L larvae were resting in the detritus for extended periods of time. Examination of fish mortalities from the 12L tanks showed

that some larvae did have an accumulation of detritus around the gills. It is possible that gill irritation or bacterial infection may have contributed to some of the mortalities seen in this treatment.

Another possible explanation for some of the mortalities seen in this study, particularly for the 12L treatment, is temperature. Although efforts were made to maintain a rearing temperature between 6-8°C, as suggested by Wiseman (1997), due to the seasonal variation of the ambient water source, temperatures during the initial weeks of the experiment were as low as ~1.8°C and reached up to 12.0°C towards the end of the study. Consequently, the temperatures during the early part of the experiment were much lower then the optimal rearing temperature determined for larval wolffish (Wiseman, 1997). Maintaining an appropriate temperature is critical for feeding rate, metabolic function, and growth and survival of larval fish. Providing a lower than required temperature may have contributed to increased mortality and suppressed growth rate in this study. Furthermore, as Zeutzius and Rahmann (1984) and Rager (1982) suggest, single environmental factors (light, temperature, social conditions, etc.) can have differential influences on different parts of the sensory systems but in combination these effects can be severe. The combined effects of light and temperature on larval fish were addressed by Boehlert (1981) on splitnose rockfish (*Sebastes diploproa*) and by Solberg and Tilseth (1987) using cod (*Gadus morhua*) with each report concluding that the combination of both factors can seriously impede or enhance fish performance.

Although there was no statistical significant difference in growth or survival of larvae in the 18L and 24L hour treatments, the larvae in the 18L treatment had a

consistently higher trend in survival and growth compared to the 24L treatment. This contrasts with many larval fish studies which report a photoperiod of 24L gives the best performance. For example, increased survival and growth with a 24 hour photoperiod has been reported for a variety of species such as gilthead seabream, *Sparus aurata* (Tandler and Helps, 1985), sailfin sculpin, *Nautiichtys oculofasciatus*, (Marliave, 1977) Atlantic cod, *Gadus morhua* (Puvanendran and Brown, 2002) and larval rabbitfish, *Siganus guttanus* (Duray and Kohno, 1988). Increased growth with longer photoperiods has also been reported and was observed in green sunfish, *Lepomis canailles* (Gross *et al.*, 1965), plaice, *Pleuronectes platessa*, and sole, *Solea sole* (Fonds, 1979).

The results from my experiment are consistent with those found by Dowd and Houde (1980) in their larval sea bream study where the photoperiod which produced the highest survival rate also produced the greatest growth rate. For larval sea bream, a 13L photoperiod resulted in the highest survival and growth up to day 16 post-hatch. In my study, although no statistically significant differences occurred between the 18L and 24L treatments, the trend observed was that the 18L treatment consistently yielded the highest survival rate and produced the largest fish when compared to the other treatments.

These findings contrast with many of the previous photoperiod studies conducted where one photoperiod did not yield both the best survival and best growth rate. For example, Fuchs (1978) found improved growth but no difference in survival of larval sole (*Solea solea*) exposed to photoperiods of 18-24 hours light versus 12 hours light. In larval sea bass (*Dicentrarchus labrax*) study, Barahona-Fernandes (1979) found that a 12 hour photoperiod produced the highest survival whereas growth was best with an 18 hour

photoperiod. Barlow *et al* (1995) found that 24 hours of light resulted in higher growth rates for larval barramundi, but no difference in survival was found for either 8, 16 or 24 hours of light.

From my results it is obvious that a photoperiod of 12L is insufficient for maintaining good growth and survival of hatchery reared larval wolffish. A photoperiod of 18L should be used in throughout the entire larval stage in order to achieve the highest survival and growth rate. As the larvae enters the juvenile stage another assessment of the "ideal" photoperiod for this life stage is necessary.

Chapter 4: The Effect of Light Intensity on Larval Wolffish Growth and Survival

4.1 Introduction

The current successes achieved in the culture of striped wolffish have been a result of determining specific biological and environmental needs of the animal.

Like temperature, light is considered an important environmental factor in fish culture and is believed to influence each stage of development from egg to sexually mature adults (Downing and Litvak, 1999). Light can influence the behaviour of animals through its variation in intensity, wavelength, polarization, photoperiod, and seasonal variability (Munz, 1975; McFarland, 1986; Puvanendran and Brown, 2002).

In Chapter Three, the effects of photoperiod on larval wolffish growth and survival were examined and it was determined that photoperiod plays a significant role in the performance of larval wolffish. A photoperiod of 18 hours or continuous of light maximized performance. However, unlike many other larval marine species, continuous light (24 hours light) did not offer any benefit to wolffish production (growth or survival) beyond what 18 hours of light offered. Instead, this could be considered an economic loss as performance values were not improved despite higher utility costs for maintaining continuous light.

Numerous studies have been conducted on the diverse effects of light intensity on larval fish. The effects of light intensity on embryonic and morphological development are predominantly species specific. For Atlantic salmon (*Salmo salar*) and Japanese medaka (*Oryzias latipes*) darkness delays hatching, but for walleye pollock (*Theragra*

chalcogramma) and Atlantic halibut (*Hippoglossus hippoglossus*) eggs held in darkness hatched sooner than those held under diel or continuous light (Brannas, 1987; Yamagami, 1988; Helvik and Walther, 1992; Olla and Davis, 1993).

During larval development, Bolla and Holmefjord (1988) observed that total darkness (0 lux) gave a significantly higher percentage of normal Atlantic halibut larvae in comparison to those subjected to light intensities of 3, 30, or 300 lux. Similarly, a study conducted on summer flounder by Watanabe *et al.* (1998), demonstrated that embryonic development in summer flounder appeared to be faster at higher light intensities. However, a comparison of the first feeding larvae in that study, indicated that those which hatched under 500 lux (salinity 36 g/L) showed maximum values whereas those which were raised at 2000 lux had shorter notocord lengths (compared to 0-1000 lux) suggesting that faster development under high light intensity may have its limitations.

From a sensory perspective, the availability of light during the early stages of fishes has also been observed to affect the normal development of the eye. In the cichlid (*Haplochromis burtoni*; Zeutzius and Rahmann, 1984) and rainbow trout (*Oncorhynchus mykiss*, Rahmann *et al.*, 1979), light deprivation during the early larval stages compromised the normal development of the eye resulting in reduced visual acuity.

Behaviourally, light intensity has been demonstrated to have an effect on the schooling activity of many larval fish species as determined by nearest neighbour distance. Typically, a reduction in cohesion occurs with the onset of darkness or a decrease in light intensity (Brett and Groot, 1963; Azuma and Iwata, 1994). In addition

to schooling, light intensity has been well documented to affect diel migration (phototaxis) of larval fish. As an example, light is known to play a role in the control of vertical migration of herring (Wales, 1984) and larval and juvenile sole (Champalbert *et al.*, 1992). Research conducted by Blaxter (1973) with herring and plaice larvae demonstrated the sensitivity of larval movement to changes in light intensity. It was noted that large changes in light intensities were irrelevant until dusk/dawn intensity levels were reached and larvae migrated to the surface and increased activity. As light levels increased, they moved downwards. The phototactic response to light intensity may also vary with the stage of development. For walleye, larvae and juveniles (1-8 weeks) were attracted to the highest light intensity (7800 lux), however when the fish were older than 8 weeks, they aggregated at the lowest light intensities (Bulkowski and Meade, 1983).

As with the effects of light intensity on the development of larvae, light intensity effects on larval performance (growth and survival) are also species specific. Larval Atlantic cod (*Gadus morhua*), black porgy (*Mylio macrocephalus*), and haddock (*Melanogrammus aeglefinus*) all showed better performance under high light intensities, whereas larval lingcod (*Ophiodon elongatus*) showed better performance under low light intensities or darkness (Kiyono and Hirano, 1981; Appelbaum *et al.*, 1995; Downing and Litvak, 1999; Puvanendran and Brown, 2002). Downing and Litvak (1999) and Puvanendran and Brown (2002) attribute the increased performance under higher light intensity to increased feeding success or foraging ability. They observed that feeding/foraging is enhanced due to improved prey recognition, prey/background

contrast, and foraging encounters under the higher light intensities.

Despite the numerous studies on the effects of light intensity on the performance of marine larvae, the effects of light intensity on larval wolffish have not been addressed. As shown in Chapter Three, photoperiod was observed to have a significant effect on larval wolffish growth and survival. Barahona-Fernandes (1979) suggested that the interaction between light intensity and photoperiod is complex and that both factors (and their interaction) should be considered when addressing larval performance. Based upon this information, the objective of this study, using the 18-hour light photoperiod, was to determine the effects of light intensity on larval wolffish performance. The hypothesis that increased light intensity would result in increased larval performance was tested.

4.2 Materials and Methods

4.2.1 General Methodology for the Light Intensity Experiments

Egg collection and incubation, determination of performance criteria (survival, standard length, wet weight, and specific growth rates), and statistical analysis were performed as outlined in Chapter Three.

4.2.2 Experiment 1 Methodology

To determine the effects of light intensity on larval wolffish growth and survival, four light intensities (10, 40, 160 and 320 lux) were tested. This experiment was conducted at the Wesleyville Hatchery (May-June, 1995) and experimental light intensities represent levels available at the Hatchery at the time of the experiment.

Larvae were maintained in aerated, one cubic metre (1 m^3) circular, dark green tanks in a volume of approximately 35 litres. Three replicate tanks were used for each treatment.

Sea water was 70% ambient and 30% re-circulated water with a mean temperature 6.5 °C (temperature range: 4.0-9.0 °C), during the experiment. Since, rearing temperatures between 6-8°C had previously demonstrated good growth and survival for larval wolffish, water flows were adjusted seasonally to attain this temperature (Wiseman, 1997).

All experimental tanks were located within a single room at the hatchery. In order to maintain the experimental light levels (lux) throughout the experiment, Standard General Electric™ incandescent light bulbs were positioned centrally above each replicate tank (approximately 12 feet above tanks). A photoperiod of 18 hours light/6 hours dark was maintained by using an electronic timer. Light intensities (recorded in units of lux) were measured at the surface of the water using a SPER 840006 Scientific Light Metre.

Feedings occurred three times daily during the light hours. The diet consisted of a combination of *Artemia franciscana* nauplii (prey density: 1000 prey/litre) and a dry commercial pellet (Lansy 300-500 μm) which was fed to excess. *Artemia* decapsulation and enrichment were in accordance with *Artemia* Systems standard manual (Sorgeloos *et al.*, 1986). This feeding protocol had been successfully used in previous larval wolffish feeding studies (Brown *et al.*, 1997). Water flows were turned off for approximately 15-20 minutes during feeding in order to avoid flushing newly introduced food from the

tanks. This experiment was terminated at day 30 due to mechanical difficulties.

4.2.3 Experiment 2 Methodology

Based upon the results obtained from Experiment 1, a second light intensity study was conducted at the Ocean Sciences Centre (OSC) during May-June, 1996. Three light intensities (320, 750, and 1200 lux) were tested. The 1200 lux intensity represented the the maximal intensity able to be produced in the laboratory at the time of the study.

Newly hatched larvae (1-12 hours post-hatch) from 3 distinct egg masses were transferred from the Wesleyville Hatchery to the OSC. Immediately after arriving at the OSC, larvae were randomly distributed among the experimental tanks. Fish were acclimatized to the rearing tanks for an additional 24 hours prior to starting the experiment (25-48 hours post-hatch). Nine (3 replicate tanks/treatment) flow through, rectangular, glass aquaria (45 cm x 30 cm x 30 cm, 30 litre volume) were used as rearing tanks. Consistent with methods outlined in Chapter Three, all walls/sides of the glass aquariums were covered with black plastic to reduce outside disturbances and limit mirror reflections in the tanks (Pearce, 1991; Barlow *et al.*, 1995).

Mean ambient sea-water temperature during the experiment was 7.0°C (range from 4.5-9.2°C). To maintain a rearing temperature between 6-8°C, water flows were adjusted seasonally and all tanks were submerged in wet benches filled with ambient flow through, sea-water. Aerators and individual de-gassing units were placed inside the central header tank and each replicate tank in order to provide constant aeration and help eliminate super supersaturated gases from the tanks. Gas levels were monitored

throughout the experiment with an oxygen meter (once per week).

All experimental treatments were in the same light controlled room. Experimental light intensities were attained by positioning Standard General Electric™ incandescent bulbs (varying watt levels) approximately 3 feet above the treatment tanks. The experimental photoperiod consisted of 18 hours light and 6 hours dark. A 20 minute twilight period (approximately 40% of the experimental lux) was simulated before and after the main lights were turned on/off. Both photoperiod and twilight were controlled electronically with a timer. Light intensities (recorded in units of lux) were measured at the waters surface using a SPER 840006 Scientific Light Metre.

The feeding schedule, sampling methodology and recording of performance data were consistent with the first light intensity experiment . The experiment was terminated on day 50 when the larvae commenced juvenile-like behaviour (i.e. settling on the bottom of the tanks and establishing territories).

4.3 Results

4.3.1 Experiment 1

Survival Rate

Both light intensity and day had a significant effect on the survival of larval wolffish (Table 7). According to the Tukey's Test (Table 8), statistically significant differences in survival was seen among treatments for days 10 and 20 of the study but not for day 30 (Fig. 6). The Tukey's grouping (Table 9) for days 10 and 20 of the study show that the 320-lux treatment had the lowest mean survival rate among the treatments. No

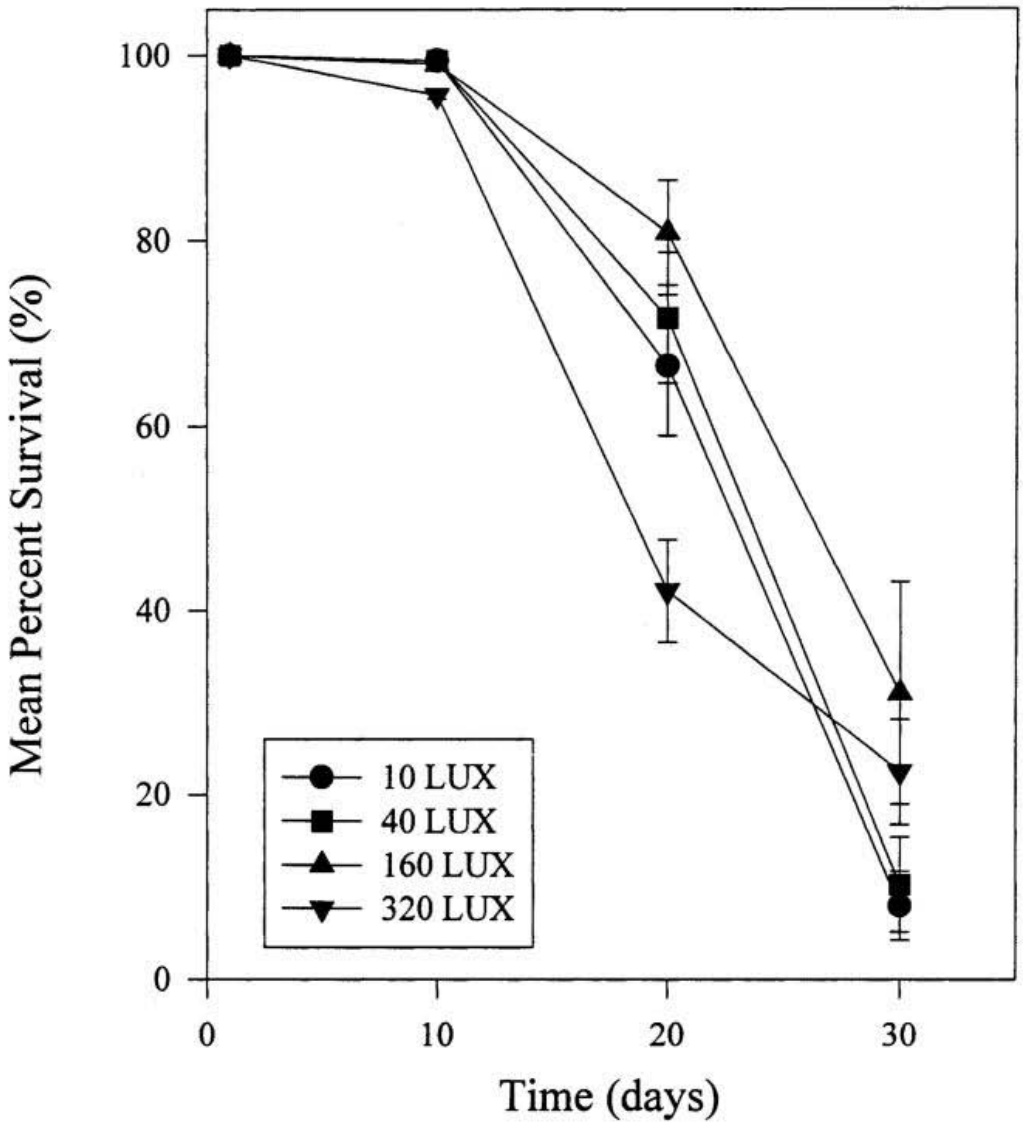

Figure 6: Mean percent survival (%) of larval wolffish over time (days) for Light Intensity Experiment 1 (vertical bars represent standard error, n=30 for each data point).

Table 7: Results of Two Way Analysis of Variance (ANOVA) on the mean percent survival, mean wet weight, and mean standard length of larval wolffish for Light Intensity Experiment 1 (significance level $p<0.05$, * denotes a significant difference).

VARIABLE	SOURCE	DF	F-VALUES	p-VALUES
Mean Percent Survival	Treatment	3	5.13	0.0069*
	Day	2	208.9	0.0001*
	Treatment*Day	6	2.87	0.0297*
Mean Wet Weight	Treatment	3	24.49	0.0001*
	Day	2	32.63	0.0001*
	Treatment*Day	6	0.84	0.5514
Mean Standard Length	Treatment	3	67.10	0.0001*
	Day	2	131.22	0.0001*
	Treatment*Day	6	1.82	0.1365*

Table 8: Results of Tukey's Studentized Range Test on the mean percent survival, mean wet weight, and mean standard length of larval wolffish for each sample day for Light Intensity Experiment 1 (significance level $p<0.05$, * denotes a significant difference).

VARIABLE TESTED	DAY	DF	F-VALUES	p-VALUES
Mean Percent Survival	10	3	5.63	0.0226*
	20	3	5.85	0.0204*
	30	3	1.93	0.2039
Mean Wet Weight	10	3	18.06	0.0006*
	20	3	18.75	0.0006*
	30	3	4.67	0.0362*
Mean Standard Length	10	3	41.56	0.0001*
	20	3	49.14	0.0001*
	30	3	12.03	0.0025*

Table 9: Tukey's groupings for the mean percent survival, mean wet weight, and mean standard length for Light Intensity Experiment 1 (significance level $p<0.05$, means with the same letter, for each age, are not significantly different).

Time (days)	Tukey's groupings (means) for each sample day			
	Treatment	Survival (%)	Wet Weight (g)	Standard Length (mm)
10	10 LUX	99.43 ^{A}	0.0725 ^{C}	19.00 ^{E}
	40 LUX	99.43 ^{A}	0.0750 ^{C}	19.20 ^{E}
	160 LUX	99.05 ^{AB}	0.0850 ^{C}	19.50 ^{E}
	320 LUX	95.62 ^{B}	0.1000 ^{D}	21.75 ^{F}
20	10 LUX	66.48 ^{AB}	0.0700 ^{C}	19.83 ^{E}
	40 LUX	71.62 ^{AB}	0.0673 ^{C}	19.73 ^{E}
	160 LUX	80.76 ^{A}	0.0790 ^{C}	20.07 ^{E}
	320 LUX	42.10 ^{B}	0.1147 ^{D}	23.22 ^{F}
30	10 LUX	8.00 ^{A}	0.1000 ^{C}	21.79 ^{E}
	40 LUX	10.29 ^{A}	0.1055 ^{C}	22.11 ^{E}
	160 LUX	31.05 ^{A}	0.1177 ^{CD}	22.23 ^{E}
	320 LUX	22.49 ^{A}	0.1557 ^{C}	24.80 ^{F}

statistical differences in survival were observed among treatments on day 30 of the study. The high mortality seen in the 320-lux treatment on day 20 and 30 of the study is attributable to a mechanical error in the rearing systems where petroleum was leaked into the rearing tanks. Final survival values (Fig. 6) for the light intensity treatments were:10-lux (8.00%), 40-lux (10.29%), 160-lux (31.05%), and 320-lux (22.49%).

Wet Weight

Light intensity and day had a significant effect on the overall weight wet of larval wolffish (Table 7, Fig. 7). Statistically significant differences (Table 8) in wet weight were observed among the light intensity treatments (10, 40, 160, 320-lux) throughout the entire study (days 10-30). According to the Tukey's groupings (Table 9) there were no statistically significant differences in wet weight among treatments 10-lux, 40-lux, or 160-lux on days 10 or 20 of the experiment. However, the wet weight of the 320-lux treatment was significantly greater than the other treatments on these days. On day 30 of the study, the 10 lux and 40 lux treatments had statistically lower wet weights than the 320-lux treatment. Although there was no statistically significant difference between the 160-lux and 320-lux treatments on day 30, the 320-lux had the greatest final mean wet weight. The final mean wet weights for the study were 10-lux (0.1000g), 40-lux (0.1055g), 160-lux (0.1177g), and 320-lux (0.1557g), showing a trend that wet weight increased as light intensity increased (Fig. 7).

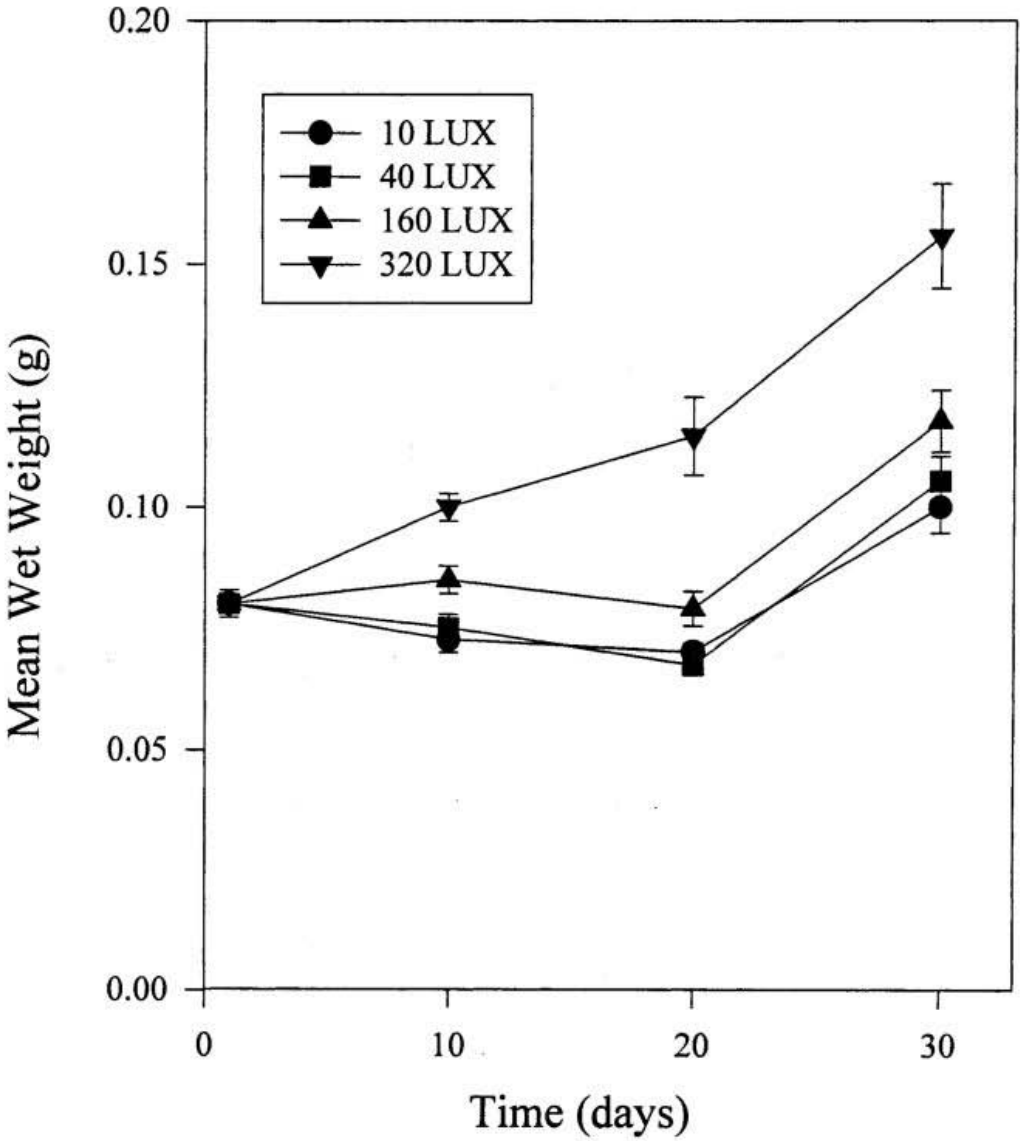

Figure 7: Mean wet weight (g) of larval wolffish over time (days) for Light Intensity Experiment 1 (vertical bars represent standard error, n=30 for each data point).

Standard Length

The mean standard length of larval wolffish was significantly affected by light intensity and day (Table 7). The Tukey's test (Table 8) shows statistically significant differences occurred between treatments for all days of the study (days 10, 20, 30). Tukey's groupings (Table 9) indicate the 320-lux treatment had a significantly greater mean standard length than the 10, 40, or 160-lux treatments for each sampling day. The final wet weights (Fig. 8) for the study were 10-lux were 10-lux (21.79 mm), 40-lux (22.11 mm), 160-lux (22.23 mm) and 320-lux (24.80 lux).

Specific Growth Rates (% wet weight/day)

The wet weight specific growth rate is affected by day (Table 10). Tukey's Test results and the Tukey's groupings (Tables 11 and 12, respectively) show that day 10 is the only day in which statistically significant differences in wet weight growth rates occur among treatments. On day 10 (Table 12, Figure 9), the 10-lux and 40-lux treatments experienced negative SGRs, where as the 160 lux and 320 lux treatments had positive SGRs. Although the 320-lux treatment was the only treatment to have a positive specific growth rate on day 20 of the study, there were no statistically significant differences observed among the four light intensity treatments at this time. Day 30 showed no significant differences in wet weight SGR among the treatments.

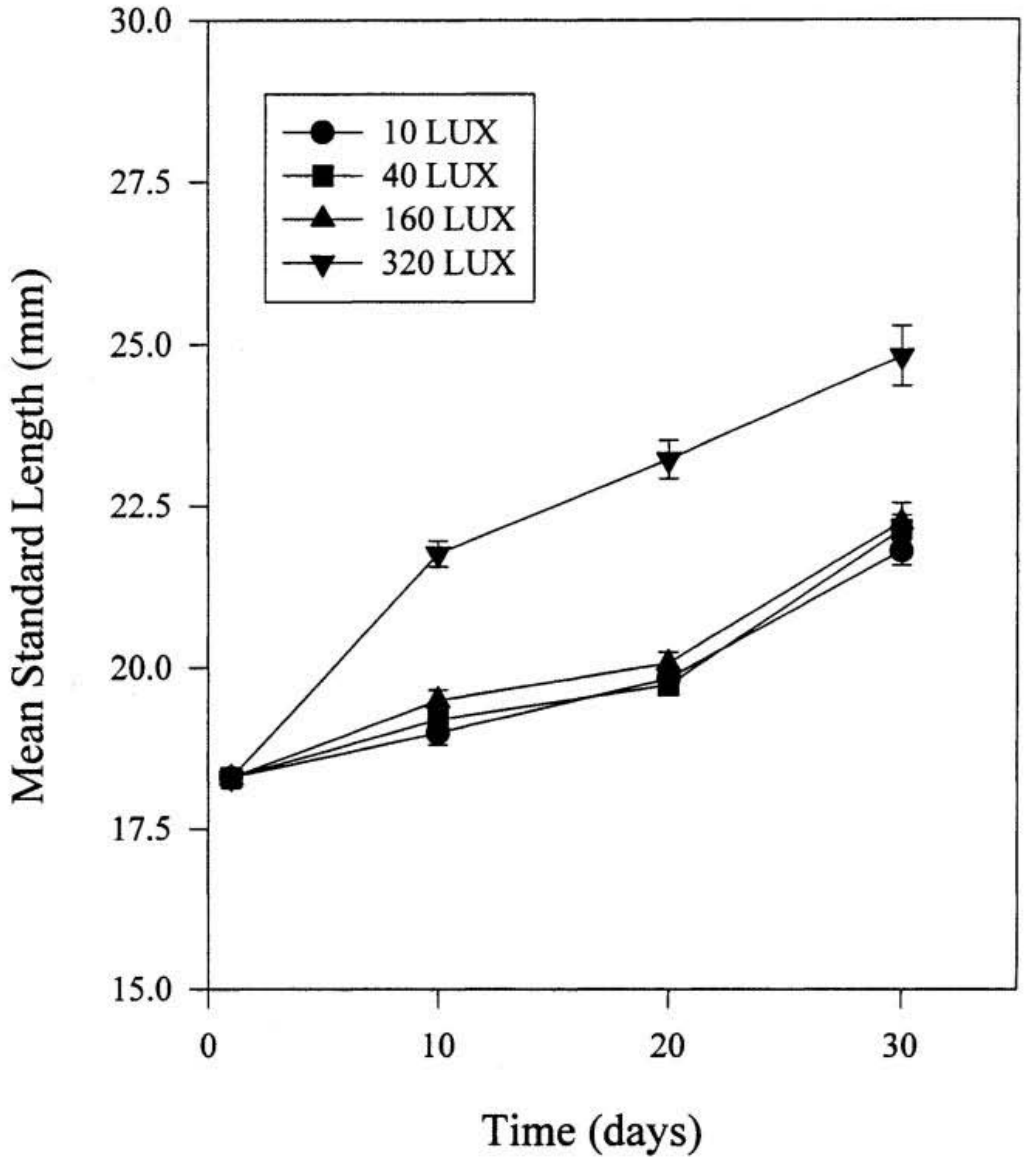

Figure 8: Mean standard length (mm) of larval wolffish over time (days) for Light Intensity Experiment 1 (vertical bars represent standard error, n=30 for each data point).

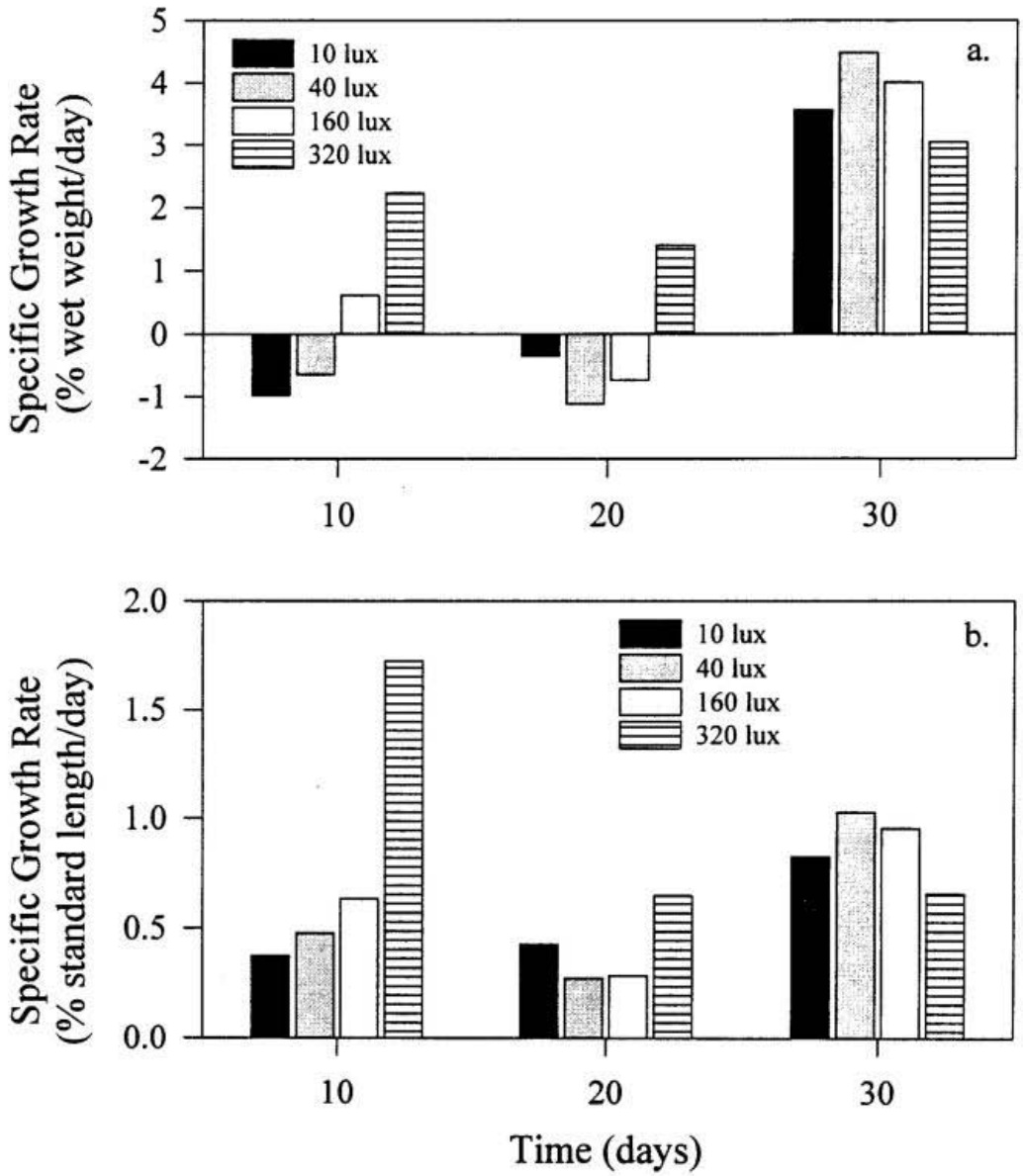

Figure 9: Mean wet weight specific growth rate (fig. a) and mean standard length specific growth rate (fig. b) of larval wolffish over time (days) for Light Intensity Experiment 1 (n=30 for each column).

Table 10: Results of Two Way Analysis of Variance (ANOVA) on the specific growth rates (% wet weight/day, % standard length/day) of larval wolffish for Light Intensity Experiment 1 (significance level $p<0.05$, * denotes a significant difference).

VARIABLE	SOURCE	DF	F-VALUES	p-VALUES
Specific Growth Rate (% wet weight/day)	Treatment	3	2.88	0.0567
	Day	2	20.40	0.0001*
	Treatment*Day	6	3.06	0.0228*
Specific Growth Rate (% standard length/day)	Treatment	3	6.15	0.0030*
	Day	2	19.53	0.0001*
	Treatment*Day	6	5.08	0.0017*

Table 11: Results of Tukey's Studentized Range Test on the specific growth rates (% wet weight/day, % standard length/day) of larval wolffish for each sample day for Light Intensity Experiment 1 (significance level $p<0.05$, * denotes a significant difference).

VARIABLE TESTED	DAY	DF	F-VALUES	p-VALUES
Specific Growth Rate (% wet weight/day)	10	3	6.53	0.0153*
	20	3	1.61	0.2615
	30	3	0.27	0.8447
Specific Growth Rate (% standard length/day)	10	3	32.18	0.0001*
	20	3	1.89	0.2090
	30	3	1.95	0.2009

Table 12: Tukey's groupings for the specific growth rates (% wet weight/day, % standard length/day) for Light Intensity Experiment 1 (significance level $p<0.05$, means with the same letter, for each age, are not significantly different).

Time (days)	Tukey's groupings (means) for each sample day		
	Treatment	% Wet Weight/Day	% Standard Length/Day
10	10 LUX	-0.98 ^{A}	0.38 ^{C}
	40 LUX	-0.65 ^{A}	0.48 $^{C D}$
	160 LUX	0.61 ^{B}	0.63 ^{D}
	320 LUX	2.23 $^{A B}$	1.73 ^{E}
20	10 LUX	-0.35 ^{A}	0.43 ^{C}
	40 LUX	-1.13 ^{A}	0.27 ^{C}
	160 LUX	-0.73 ^{A}	0.29 ^{C}
	320 LUX	1.40 ^{A}	0.65 ^{C}
30	10 LUX	3.56 ^{A}	0.83 ^{C}
	40 LUX	4.49 ^{A}	1.03 ^{C}
	160 LUX	4.01 ^{A}	0.96 ^{C}
	320 LUX	3.05 ^{A}	0.66 ^{C}

Specific Growth Rate (% standard length/day)

According to Table 10, the standard length-SGR is significantly affected by day and light intensity. The results from the Tukey's Test (Table 11) indicate that day 10 is the only day where a statistically significant difference in the standard length SGR occurred. On day 10 there was little difference in SGR between the 10-lux, 40-lux and 160-lux treatments, however, the standard length SGR for the 320-lux treatment was significantly greater than these three treatments. For days 20 and 30 there were no significant differences in standard length SGR among any of the treatments (Table 12, Figure 9).

4.3.2 Experiment 2

Survival

Both light intensity and day had a significant effect on larval wolffish survival (Table 13). There were no significant differences in survival among treatments during days 10-40 of the study, however a significant difference between treatments occurred on day 50 (Table 14). On day 50, the 320-lux treatment (Table 15) had a significantly lower larval survival rate than the other two treatments. At the end of the study the final survival rate for the treatments were 1200-lux (92.00%), 750-lux (92.44%), and 320-lux (81.11%).

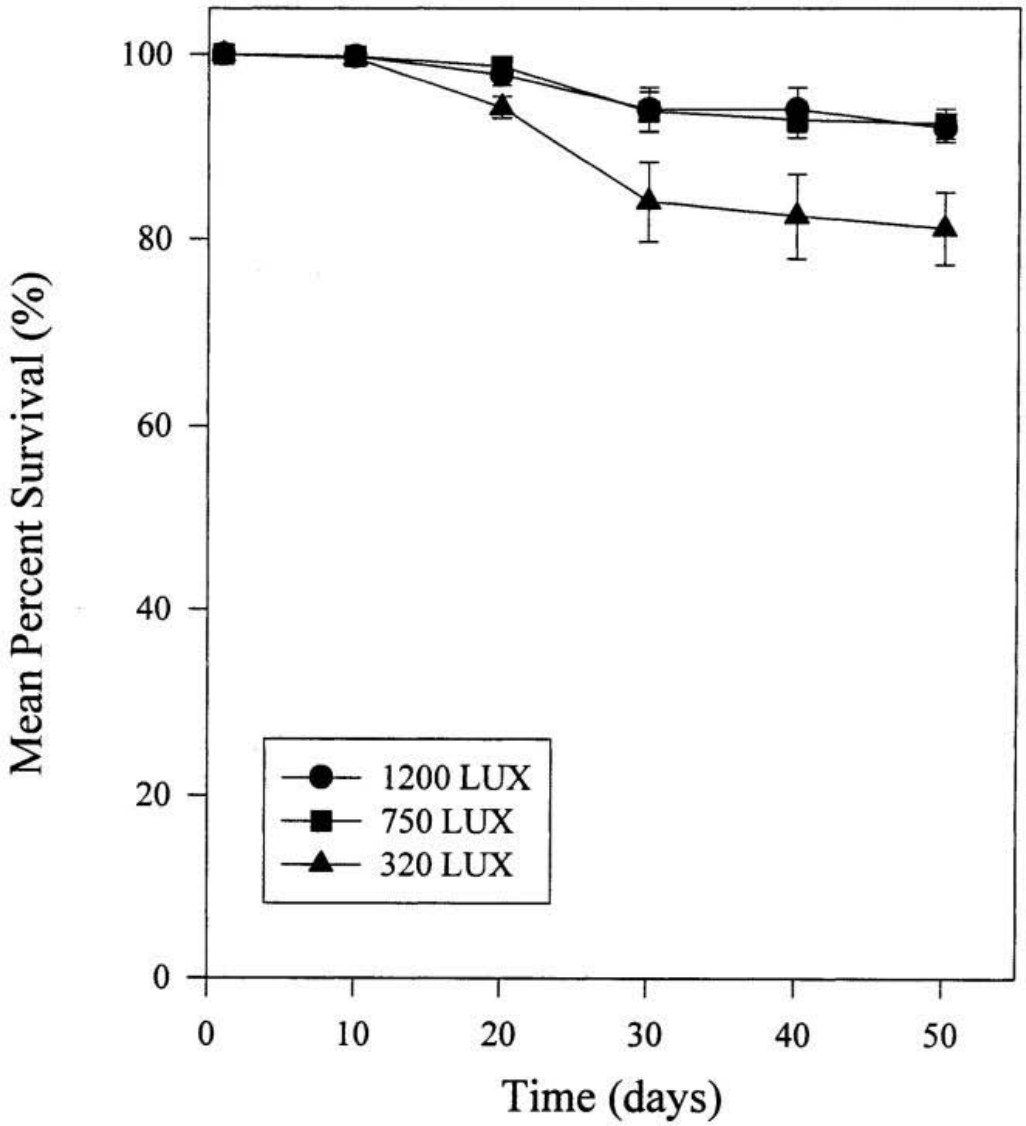

Figure 10: Mean percent survival (%) of larval wolffish over time (days) for Light Intensity Experiment 2 (vertical bars represent standard error, n=30 for each data point).

Table 13: Results of Two Way Analysis of Variance (ANOVA) on the mean percent survival, mean wet weight, and mean standard length of larval wolffish for Light Intensity Experiment 2 (significance level $p<0.05$, * denotes a significant difference).

VARIABLE	SOURCE	DF	F-VALUE	p-VALUES
Mean Percent Survival	Treatment	2	15.41	0.0001*
	Day	4	25.37	0.0001*
	Treatment*Day	8	0.72	0.6720
Mean Wet Weight	Treatment	2	46.56	0.0001*
	Day	4	617.76	0.0001*
	Treatment*Day	8	1.96	0.0867
Mean Standard Length	Treatment	2	25.66	0.0001*
	Day	4	142.07	0.0001*
	Treatment*Day	8	1.72	0.1353

Table 14: Results of Tukey's Studentized Range Test on the mean percent survival, mean wet weight, and mean standard length of larval wolffish for each sample day for Light Intensity Experiment 2 (significance level $p<0.05$, * denotes a significant difference).

VARIABLE TESTED	DAY	DF	F-VALUES	p-VALUES
Mean Percent Survival	10	2	0.33	0.7290
	20	2	3.19	0.1138
	30	2	3.13	0.1175
	40	2	3.85	0.0841
	50	2	6.82	0.0286*
Mean Wet Weight	10	2	3.61	0.0936
	20	2	5.75	0.0403*
	30	2	14.41	0.0051*
	40	2	8.17	0.0194*
	50	2	19.40	0.0024*
Mean Standard Length	10	2	13.17	0.0064*
	20	2	11.38	0.0091*
	30	2	8.88	0.0161*
	40	2	3.79	0.0864
	50	2	52.53	0.0002*

Table 15: Tukey's groupings for the mean percent survival, mean wet weight, and mean standard length for Light Intensity Experiment 2 (significance level $p<0.05$, means with the same letter, for each age, are not significantly different).

Time (days)	Tukey's groupings (means) for each sample day			
	Treatment	Survival (%)	Wet Weight (g)	Standard Length (mm)
10	1200 LUX	97.78 ^{A}	0.122 ^{C}	21.92 ^{E}
	750 LUX	99.78 ^{A}	0.118 ^{C}	21.72 ^{E}
	320 LUX	99.56 ^{A}	0.112 ^{C}	21.20 ^{F}
20	1200 LUX	97.78 ^{A}	0.183 ^{C}	24.92 ^{E}
	750 LUX	98.67 ^{A}	0.163 ^{CD}	24.05 ^{E}
	320 LUX	94.22 ^{A}	0.145 ^{D}	22.72 ^{F}
30	1200 LUX	94.00 ^{A}	0.275 ^{C}	28.32 ^{E}
	750 LUX	93.78 ^{A}	0.217 ^{D}	25.92 ^{EF}
	320 LUX	84.00 ^{A}	0.193 ^{D}	24.78 ^{F}
40	1200 LUX	94.00 ^{A}	0.399 ^{C}	32.12 ^{E}
	750 LUX	92.89 ^{A}	0.337 ^{CD}	30.10 ^{E}
	320 LUX	82.44 ^{A}	0.282 ^{D}	29.30 ^{E}
50	1200 LUX	92.00 ^{A}	0.629 ^{C}	38.97 ^{E}
	750 LUX	92.44 ^{A}	0.548 ^{C}	37.00 ^{E}
	320 LUX	81.11 ^{B}	0.495 ^{D}	32.67 ^{F}

Wet Weight

Both treatment and day showed significant effects on the wet weight of larval wolffish. Although there were no significant differences among larvae in the treatments on day 10, significant differences occurred between treatments from days 20-50 (Table 13). The trend in larval wet weight was consistent for each sample day of the study (Fig.11, Table 14) with the larvae in the 1200-lux treatment having a greater wet weight than the 320-lux treatment. The final larval wet weights at the end of the study were 1200-lux (0.629 g), 750-lux (0.548 g), and 320-lux (0.495 g).

Standard length

Standard length was significantly affected by both treatment ($F=25.66$, $p=0.0001$, $df=2,30$; Table 13) and day ($F=142.07$, $p=0.0001$, $df=4,30$; Table 13). Significant differences in larval length occurred between treatments for all days except day 40 (Figure 12, Table 14). The trend for standard length is the same as the trend for wet weight (Fig. 11, Table 15) with the 1200-lux treatment having a greater standard length than the 320-lux. At the end of the study, the final larval standard lengths were: 1200-lux (38.97 mm), 750-lux (37.00 mm), and 320-lux (32.67 mm).

Specific Growth Rate (% wet weight/day)

The results from the ANOVA indicated that treatment did not have a significant effect on the wet weight specific growth rate, whereas day did have a significant effect on the wet weight specific growth rate (Table 16). Since there was no significant treatment

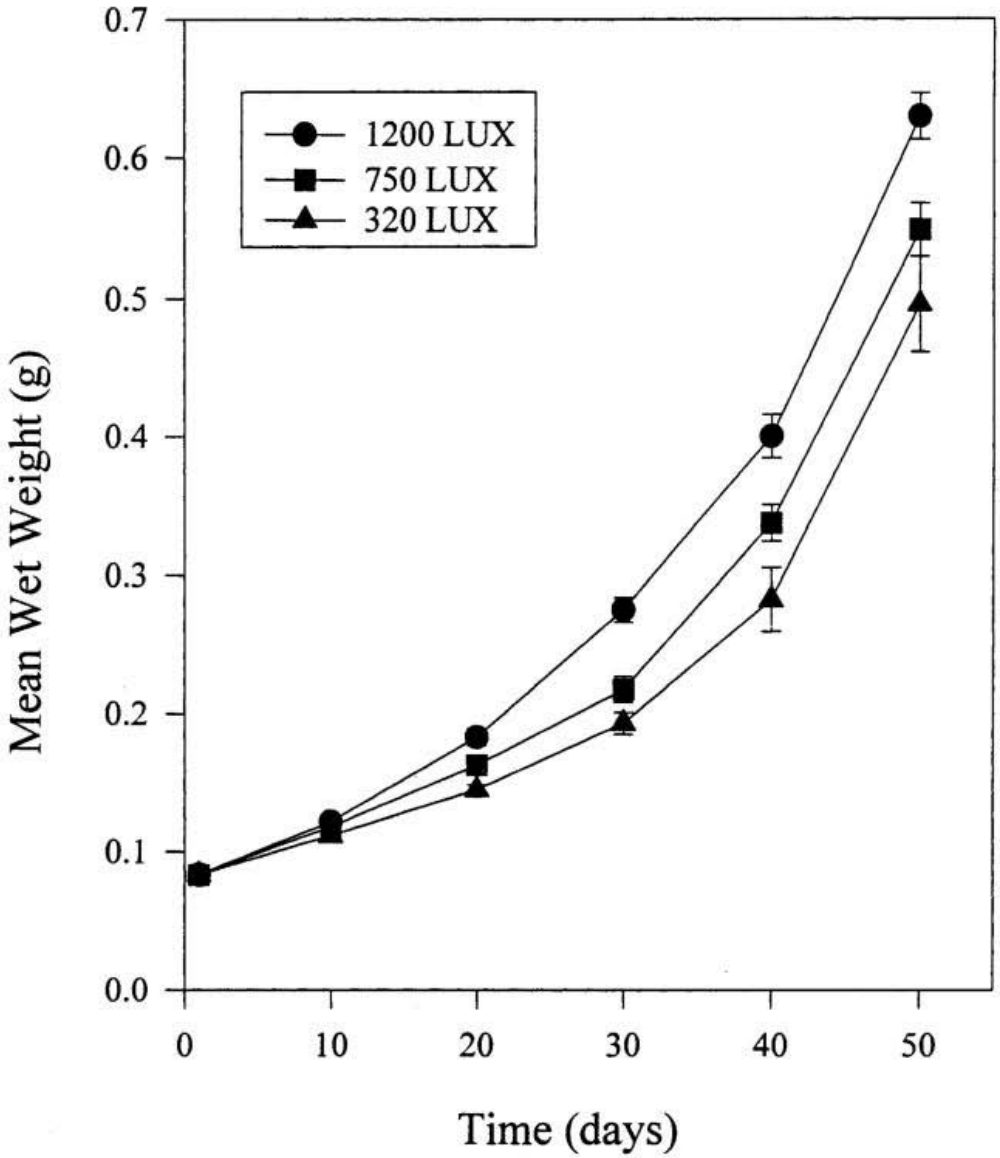

Figure 11: Mean wet weight (g) of larval wolffish over time (days) for Light Intensity Experiment 2, (vertical bars represent standard error, n=30 for each data point).

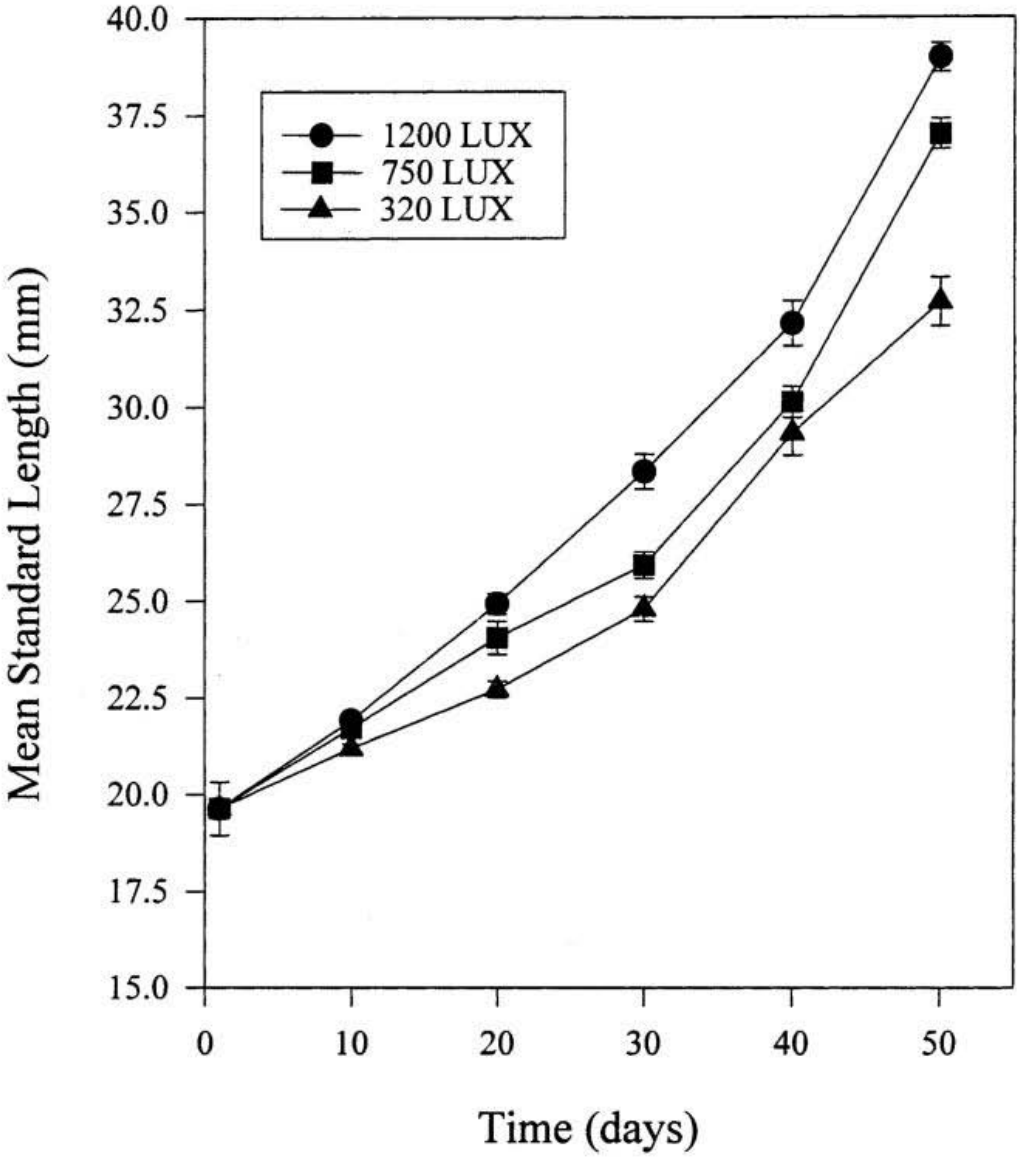

Figure 12: Mean standard length (mm) of larval wolffish over time (days) for Light Intensity Experiment 2 (vertical bars represent standard error, n=30 for each data point).

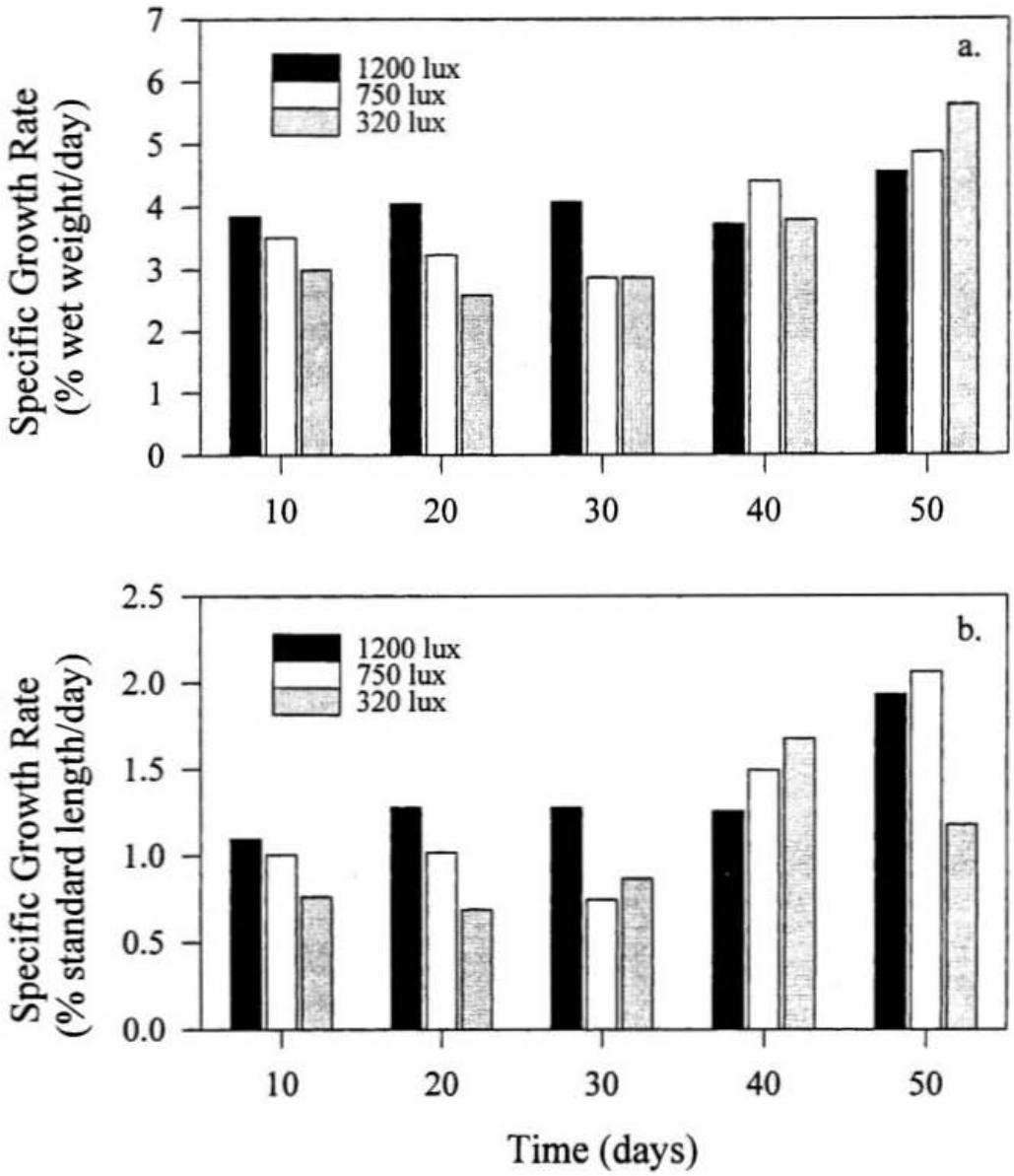

Figure 13: Mean wet weigth specific growth rate (fig. a) and the mean standard length specific growth rate (fig. b) of larval wolffish over time (days) for Light Intensity Experiment 2 (n= 30 for each column).

Table 16: Results of Two Way Analysis of Variance (ANOVA) on the specific growth rates (% wet weight/day, % standard length/day) of larval wolffish for Light Intensity Experiment 2 (significance level $p<0.05$, * denotes a significant difference).

VARIABLE	SOURCE	DF	F-VALUES	p-VALUES
Specific Growth Rate (% wet weight/day)	Treatment	2	1.95	0.1605
	Day	4	3.36	0.0219*
	Treatment*Day	8	1.27	0.2932
Specific Growth Rate (% standard length/day)	Treatment	2	3.73	0.0359*
	Day	4	10.08	0.0001*
	Treatment*Day	8	2.47	0.0345*

Table 17: Results of Tukey's Studentized Range Test on the specific growth rates (% wet weight/day, and % standard length/day) of larval wolffish, for each sample day for Light Intensity Experiment 2 (significance level $p<0.05$, * denotes a significant difference).

VARIABLE TESTED	DAY	DF	F-VALUES	p-VALUES
Specific Growth Rate (%wet weight/day)	10	2	3.81	0.0853
	20	2	1.83	0.2403
	30	2	2.32	0.1790
	40	2	1.13	0.3847
	50	2	0.61	0.5733
Specific Growth Rate (%standard length/day)	10	2	17.36	0.0032*
	20	2	6.12	0.0356*
	30	2	2.76	0.1412
	40	2	1.27	0.3475
	50	2	2.34	0.1775

Table 18: Tukey's groupings for the specific growth rates (% wet weight/day, % standard length/day) for Light Intensity Experiment 2 (significance level $p<0.05$, means with the same letter, for each age, are not significantly different).

Time (days)	Tukey's groupings (means) for each sample day		
	Treatment	% Wet Weight/Day	% Standard Length/Day
10	1200 LUX	3.85 ^{A}	1.10 ^{A}
	750 LUX	3.52 ^{A}	1.06 ^{A}
	320 LUX	3.00 ^{A}	0.76 ^{B}
20	1200 LUX	4.05 ^{A}	1.28 ^{A}
	750 LUX	3.23 ^{A}	1.02 ^{AB}
	320 LUX	2.58 ^{A}	0.69 ^{B}
30	1200 LUX	4.07 ^{A}	1.28 ^{A}
	750 LUX	2.86 ^{A}	0.75 ^{A}
	320 LUX	2.86 ^{A}	0.87 ^{A}
40	1200 LUX	3.72 ^{A}	1.26 ^{A}
	750 LUX	4.40 ^{A}	1.49 ^{A}
	320 LUX	3.79 ^{A}	1.67 ^{A}
50	1200 LUX	4.55 ^{A}	1.93 ^{A}
	750 LUX	4.86 ^{A}	2.06 ^{A}
	320 LUX	5.62 ^{A}	1.18 ^{A}

effect, the Tukey's Test (Table 17) results and the Tukey's groupings (Table 18) did not show significant differences among treatments for each of the sampling days.

Specific Growth Rate (% standard length/day)

The specific growth rate for standard length of larvae was significantly affected by both treatment (Table 16) and day (Table 16). Tukey's Test results (Table 17) indicate significant differences in growth of larvae occur on days 10, and 20 but no significant differences in growth were seen during days 30-50. For days 10-20, the SGR (standard length) for the 1200- lux treatment was greater than the 320- lux treatment (Table18, Figure 13).

4.4 Discussion

The results from the preliminary study, Experiment-1 indicate that light intensity has a significant effect on larval wolffish growth and survival. Initial survival rates for the study (day 10) showed that survival rate was highest among the lower light intensity treatments (10 and 40 lux, 99.43%). However, for day30 the reverse trend was noticed with mean percent survival increasing with increasing light intensity. It is difficult to assess the survival rate for the 320 lux treatment because the rearing tanks experienced technical difficulties (petroleum leaking into the system from the re-circulation pump) which may have contributed to the mortalities observed in this system.

However, when comparing the growth parameters (mean standard length and mean wet weight) the trend was relatively consistent among the treatments over time.

Both wet weight and standard length increased with increasing light intensity for each sample day. The 320 lux treatment had significantly greater wet weights and lengths compared to all other higher intensity treatments.

When comparing the survival results of Experiment-1 to other larval wolffish studies, the survival rates in this study were much lower than the >90% survival rates reported by the other studies despite similar rearing protocols (Brown *et al.*, 1997; Wiseman, 1997; Hendry and Halfyard, 1998; Halfyard *et al.*, 2001). It was evident that low light intensity compromised larval performance.

Due to the technical difficulties experienced by the 320 lux treatment in Light Experiment-1, the "potential" for survival for this treatment was undetermined. The growth rates (mean standard length and mean wet weight) were comparable to results reported by other authors (Wiseman, 1997; Strand *et al.*, 1995) and exceeded those of the lower intensity treatments (10, 40, 160 lux) suggesting that if technical difficulties had not occurred then survival rates may have approached rates found in other larval wolffish papers. Unfortunately, the light intensity treatments tested during this experiment represented those which were attainable (in terms of lux levels, and number of experimental tanks) in the hatchery at that time. In order to explore what the potential impact of higher light intensities (320 lux or greater) on wolffish growth or survival it was essential that an additional experimental trial would be conducted.

Since the growth results from the 320 lux treatment were comparable to previously reported values, this served as the minimum intensity for Experiment 2.

Results from Experiment 2 demonstrated that both growth and survival were enhanced by using higher light intensities. For days 10 and 20, little difference was seen among treatments in terms of larval survival. However, by day 30 the 320 lux treatment showed evidence of increasing larval mortality in comparison to the 750 and 1200 lux treatments. By day 50, the 320 lux treatment had a significant lower larval survival rate (81.11%) compared to the other treatments (750 lux-92.44%; 1200 lux-92.00%).

However for the growth rates (mean wet weight, mean standard length), a consistent trend was observed. Growth rates increased with increasing light intensity for each sampling day.

A comparison of the wet weight specific growth rates from both light intensity experiments (1 and 2) revealed that no negative SGR's were observed among treatments having a light intensity of 320 lux or greater. Negative specific growth rates are typically associated with the total absorbtion of the yolk sac and the transitional switch from endogenous to exogenous feeding. Negative SGR's have been reported in other larval wolffish studies, and typically occur between days 20-40 post-hatch (Strand *et al.*, 1995; Wiseman, 1997). On day 10, there was little difference among any of the light intensities (10 to 1200 lux) tested as 99% survival occurred in all light intensities treatments. One could argue that the larvae were still living on the energy reserves of the yolk sac and not yet dependent on the external environment for food or energy reserves. However, as the transition from endogenous to exogenous feeding is made (days 20 onwards) the significance of light intensity, and its potential impact on feeding, growth, and survival is more apparent. The marked increase in mortality among the lower light levels (10, 40,

160 lux) during the period of yolk sac depletion and the switch to external food reinforces the importance of light intensity in the early larval rearing environment, particularly with respect to a larva's successful adaptation to first feeding during the critical switching phase. Any "advantage" which can improve the growth and survival through this period should be utilized. It appears, based upon the results of this experiment, that increased light intensity may be a technique to improve survival and growth.

Better growth and survival rates under higher light intensities have been reported in a number of other studies. For example, research conducted on larval Atlantic cod demonstrated that cod exposed to intensities of 300, 600, 1200, and 2400 lux, had better growth, survival, and condition indices at the 2400 lux intensity at any given age (Puvanendran, 1999a; Puvanendran and Brown, 2002). Similar results were noted by Kiyono and Hirano (1981) who found that growth and survival of larval black porgy increased with increasing light intensity, and by Downing and Litvak (1999) who found that larval haddock had greater growth and survival at a light intensity of 110 lux compared to 5 lux.

However, for many other species, increased light may offer no benefit or may serve as an impediment to production. As an example, larvae of the southern flounder (*Paralichthys lethostigma*) raised at high light (1362 lux) did not show any significant differences in growth or survival when compared to those raised at low light (457 lux) intensity (Denson and Smith, 1997). The same scenario is true for larval lingcod (*Ophiodon elongatus*) where optimal survival during the larval stage can be improved by keeping the larvae in darkness or at low light intensity (Appelbaum *et. al.*, 1995).

Negative effects of increased light intensity during the larval phase have also been described by Bolla and Holmefjord (1988) who found that total darkness (0 lux) gave a significantly higher percentage or normal halibut larvae compared to those reared under higher light intensities. Similarly, Watanabe *et al.*, 1998) found that summer flounder larvae (*Paralichthys dentatus*) reared under 500 lux (compared to 0, 1000, 2000 lux) when combined with high salinity showed maximal growth values, and did not have the shorter notocords found in the higher light (2000 lux) treatment.

The response of larval wolffish to light intensity, yielded results consistent with that of the photoperiod trial (Chapter Three) where the light intensity which produced the highest survival rate also produced the best growth rate. However, this consistency is not noted among all species. As an example, a study conducted on Atlantic salmon (*Salmo salar*) by Wallace *et al.* (1988) found that the best growth rates occurred at 700 lux but the highest mortalities also occurred at this level. Dark reared larvae (0 lux), however, reported 0% mortality rate. The authors attribute increased feeding rates under the high light intensity to the better growth but claim that a high stress response also occurred at this level which contributed to the mortality rate. Similarly, sea bass larvae (*Dicentrarchus labrax*) showed better growth but poorer survival at high light intensities (Barahona-Fernandes, 1979). These authors claim that exposure to a strong light intensity before the pigmentation of the larvae is lethal to the larvae. Since wolffish larvae hatch at an advanced state with pigmentation in the skin and eyes, the higher light intensities used in this study clearly did not have the same negative impact as was seen for the more "primitive" sea bass larvae.

One rationale for why there is a species specific response to light intensity is the natural ecology of the species. This concept is well demonstrated with a study conducted by Puvanendran and Brown (1998) where growth and survival rates of two stocks (Newfoundland and Scotian Shelf) of Atlantic cod were compared under low (8.5 lux) and high (650 lux) light intensities. For Newfoundland cod, larvae had better growth and survival under 650 lux, whereas the Scotian shelf cod performed better under the lowest (8.5 lux) intensity. The authors stated that the results were most likely due to the variable spawning season of each stock. Scotian shelf cod spawned between November to January when light levels were between 13-31 lux at depths of 20-45 metres. These values are closer to the 8.5 lux intensity in which the Scotian shelf cod had the best performance. In contrast, Newfoundland cod which spawn from April to July, are subjected to light levels in the range of 13000-20000 lux at the surface (Puvanendran and Brown, 1998). The authors speculated that a species, and even more specifically a stock, would select a light intensity that best mimics that of its natural environment.

For the larval wolffish used in this study, hatching typically occurs between April to May (Watkins, 1995. pers. comm.), comparable to that of the Newfoundland cod studied by Puvanendran and Brown (1998). It is therefore safe to assume that under natural conditions, light intensities experienced by larval wolffish would be in a range of 13000- 20000 lux. Consequently, the better performance observed by the larval wolffish raised under the high light intensity (1200 lux) may be due to the similar light levels to its natural environment.

The results from this study indicate that higher light intensities in the range of 750 lux to 1200 lux are essential in order to maximize growth and survival of larval wolffish. However, the mechanism for this enhanced growth and survival is unclear. The results seen may be due to ecological compatibility (genetic pre-disposition), physiology, or behavioural (i.e. foraging activity).

As a result, it was considered advantageous for culture purposes to conduct a behavioural assessment of light intensity on the foraging ability of larval wolffish (Chapter Five). The objective of the study was to determine whether light intensity impacts the foraging ability (in addition to the performance, as determined in this study) of larval wolffish and to develop a protocol or "light schedule" which could be implemented as part of standard husbandry.

Chapter 5. The Effects of Light Intensity on Larval Wolffish Feeding Behaviour

5.1 Introduction

The impact of light (photoperiod and light intensity) on the growth and survival of larval wolffish has been addressed in this thesis. Results from the photoperiod experiment, determined that a photoperiod of 18 hours maximized larval performance. Increasing daylight hours beyond this appeared to offer no advantage to larval performance, whereas, a decrease in daylight hours (12 hours light) compromised or reduced both growth and survival. In the studies in Chapter Four on the effects of light intensity on larval wolffish growth and survival, it was determined that the intensity of light available during the larval phase has highly significant effects on both growth and survival. For example, light intensities of 750 or 1200 lux yielded approximately 92.00% survival after 50 days of experimentation whereas light intensities of 10, 40, and 160 lux yielded survival rates of 8.00%, 10.29%, and 31.05%, respectively, after 30 days of experimentation.

The mechanism for the poor survival and growth of larval wolffish under low light intensities is unknown. The response may be caused by stress or reduced foraging ability. Conversely, the success of larvae under higher light intensity may be a simple adaptation to the light intensity levels present in its natural environment. The success may also be due to increased foraging ability caused by improved vision and prey recognition under higher light levels.

According to Brown *et al.* (1997) behavioural observation, in conjunction with growth and survival information is a powerful tool for understanding the behavioural adaptations of larvae. This is particularly important when growth or survival data are the only variables measured and the underlying reason for the differences in performance is unclear. Brown *et al.* (1997) further explain, that this concept is especially true for feeding which is an area of critical importance in larval production. The results from Chapter Four support this idea as the mechanism for the success under higher light is unclear, but speculatively may be attributed to improved foraging success.

Foraging success is dependent on a series of events involving the encounter, attack, and capture of prey (O'Brien, 1979; Wanzenböck and Schiemer, 1989; Downing and Litvak, 1999). Various methods used to enhance foraging include manipulation of light intensity (Batty, 1987; Britz and Piennar, 1992; Downing and Litvak, 1999; Puvanendran, 1999a), tank wall colour (Tamazouzt *et al.*, 2000), spectral composition (Gehrke, 1994), green water technique, turbidity (MacKenzie and Kiørbe, 1993), the positioning of lights (e.g. internal or external overhead lighting) in the rearing environment (Malison and Held, 1992; Gulbrandsen *et al.*, 1996), pulse feeding (Rabe and Brown, 2000), and increasing prey densities (Munk and Kiørbe, 1985; Puvanendran and Brown, 2002).

In theory, by increasing the amount of available light for larva to see/recognise prey, the larva's probability of encounter and successful attack should also be improved. Feeding behavioural trials conducted by Wiseman (1997) assessing optimal prey densities for feeding and weaning, demonstrate that wolffish are active, visual feeders,

and exercise distinct prey preferences during larval development. Therefore, the role of vision appears to play a role in the successful foraging ability of this species. As stated in Chapter Two, larval wolffish hatch at an advanced stage of development, with large pigmented eyes and a reduced yolk sac. When considering the role of vision in the foraging of larval fish, Blaxter (1986) stated that there is a minimum light intensity threshold (0.1 lux) required in order for larval fish to feed. It is understood in larviculture, that providing fish with light intensities above this threshold enhances feeding ability (Puvanendran, 1999a). For species such as cod and haddock, which have less developed sensory systems at hatch, coupled with poorly developed, unpigmented eyes, higher light intensities may serve to enhance the prey recognition and foraging ability. Due to the advanced eye development in wolffish the degree to which increased light intensities improve prey recognition and successful foraging is unclear.

In larviculture, behavioural studies in larval foraging in combination with performance data, have yielded valuable information which can be translated into production or hatchery protocols. Numerous examples exist in literature supporting the concept of using behavioural observations during the larval stages, especially during the critical first feeding stage (Skiftesvik, 1992; MacKenzie and Kiørbe, 1993; Brown *et al.*, 1997).

The use of behavioural assessments in larval wolffish production has already occurred. Feeding behaviour in conjunction with performance studies, determined that prey densities of 900 *Artemia*/litre when combined with dry feed yielded better growth rates than a diet of 100Artemia/litre combined with dry feed, and that the larvae showed

selectivity for prey types (Wiseman, 1997). A simple behavioural assessment of the feeding preferences of Atlantic wolffish larvae (*Anarhichas lupus*) allowed for a weaning protocol to be developed for this species (Wiseman, 1997; Brown *et al.*, 1997). Unlike a study conducted on larval sea bass (*Dicentrarchus labrax*) by Barnabé and Guissi (1994) which actively tried to manipulate dietary preferences and food selection in order to overcome the problem of weaning, the study on Atlantic wolffish (*Anarhichas lupus*) larvae simply consisted of offering larvae a combination of live food (*Artemia* sp.) in conjunction with a commercial pellet (from the onset of first feeding), and quantifying the food selection of the larvae over time. Initially, larvae ingested mostly live prey with a smaller amount of commercial food being consumed. However, from week 6 onwards the larvae were feeding almost exclusively on dry food. The authors say that the shift to almost exclusively dry food may be related to the development of digestive capability or to the changing energy requirements of the larvae as they grow. This non-manipulative study on the weaning of larval wolffish, clearly dictates to producers the time frames in which live food can be successfully eliminated or weaned from the diet. These behavioural observations have become an important technique in eliminating the live food stage from production.

Based upon the experimental results from the light intensity study (Chapter Four) as well as the results from Wiseman's feeding study described above (1997), it was determined that a behavioural assessment of light intensity on the foraging ability of larval wolffish would be beneficial for production purposes. The objective of the study was to determine whether light intensity impacts the foraging ability (in addition to the

performance, as determined in Chapter 4) of larval wolffish and to develop a protocol or "light schedule" which could be implemented as part of standard husbandry.

5.2 Materials and Methods

This experiment was conducted in conjunction with Light Intensity Experiment 2 (Chapter 4) therefore standard rearing protocols (feeding, photoperiod, light intensities) for this trial are outlined in Chapter 4. Observations on wolffish feeding behaviour started on day four and continued until the experiment was terminated on day 50. For each 10 day interval, two behavioural observations were conducted, totalling 10 observations for each light intensity treatment for the 50 day experiment. All behavioural observations were made by an observer positioned in front of the experimental tanks. Observations started approximately one to two minutes after the introduction of the first daily food ration (*Artemia* species and dry pellets) into the tanks. For each observation session, the focal animal technique (Altman, 1974) was used to observe five randomly selected larvae in each treatment tank, totalling thirty larvae per light intensity treatment for each 10 day interval. Each larva was observed for one minute and the frequency of occurrence of each feeding modal action pattern (MAP; Table 19) was recorded. For the activity pattern (swimming) both the duration and frequency of the activity were recorded.

In accordance with Barlow (1977), a MAP is a spatiotemporal pattern of coordinated movement, which clusters around some mode, making the behaviour recognizable. The feeding MAPs observed and recorded in this experiment include:

orient, fixate, lunge, bite, capture, and escape. The action pattern (AP) recorded during the study was swimming behaviour.

Table 19: Operational Descriptions of the Modal Action Patterns (MAPs) for Striped Wolffish Larvae.

MAPs	*Behavioural Description*
Orient	Response of the larva to prey item which involves moving the body/trunk so that the larva faces the prey.
Fixate	Pause between orientation and attempted prey capture. The head of the larva remains in a fixed position and the larva focuses on the particular prey item in front of it.
Lunge	An attempted prey capture response in which the larva assumes an S-shaped position and then rapidly moves at least 1/4 of its body forward towards the prey item.
Bite	Involves quickly opening and closing the mouth in response to a prey item. Bites do not always proceed lunging or involve a forward motion.
Capture	Bite attempt is successful, larva capture the prey item.
Miss	Bite attempt is unsuccessful, larva does not capture the prey item.

Table 20: Operational Definition of the Behavioural Action Pattern for Striped Wolffish Larvae.

Action Pattern	Description
Swimming	Larva is moving through the water column in either a horizontal or vertical pattern

Definitions for feeding activity were in accordance with the following equations and were based upon the feeding definitions (Table 19):

Forage = (Orients + Fixates + Lunges + Bites)

Successful Foraging = Captures/(Lunges+Bites) * 100

Unsuccessful Foraging = Miss/(Lunges+Bites)* 100

Feeding Effort = (Lunges+Bites)/(Orients+Fixates) * 100

For the feeding activity, the frequency of the activity was recorded. For successful foraging, unsuccessful foraging, and feeding effort, the percent frequency of the feeding activity was recorded. For the swimming activity both the frequency and duration of the activity were recorded. All data collected was recorded on a pre-programmed, computerized Tandy 102 Event Recorder.

Statistical Analysis

A two-way ANOVA (SAS version 6.1, Cary, NC) was used for all comparisons. Data which were not normally distributed were arcsin transformed to meet the assumptions of the test. Where significant day*treatment interactions occurred , a Tukey's Test for Multiple comparisons was performed among treatments for each sampling day. Level of significance was set at $\propto$=0.05.

5.3 Results

5.3.1 Modal Action Patterns-Frequency

Orient-Fixate

Light intensity and day had a significant effect on the frequency of orient-fixate (Table 21, Figure 14). Day 30 was the only day which showed a significant difference among treatments (Table 22) with the lowest light intensity (320 lux) having a statistically significant lower frequency (4.97 occurrences per minute) of the orient-fixate

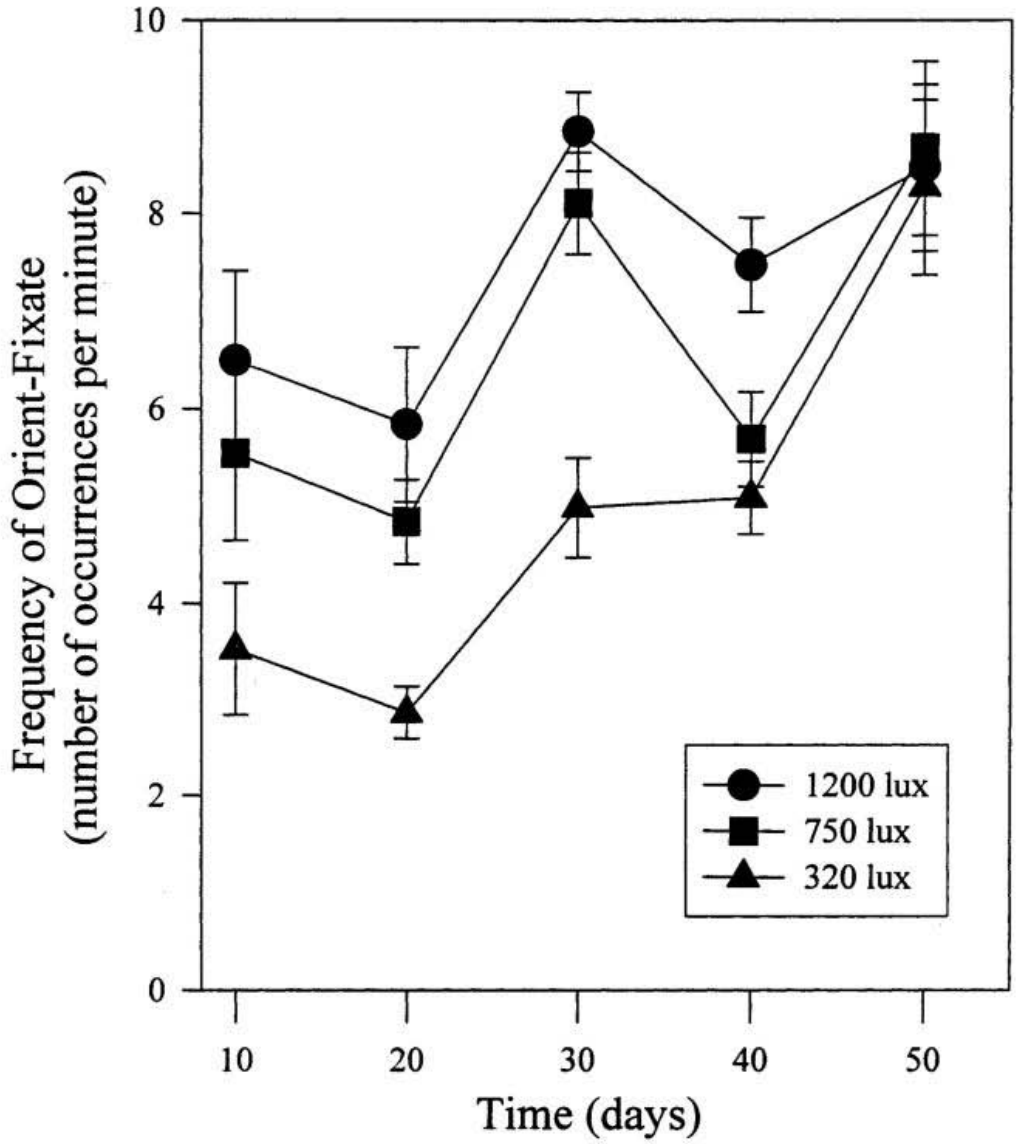

Figure 14: The frequency (number of occurrences per minute) of orient-fixate over time (days) for larval wolffish feeding under varying light intensities (n=30 for each data point, vertical bars represent standard error).

Table 21: Results of Two Way Analysis of Variance (ANOVA) on the frequency (number of occurrences per minute) of the MAPs (modal action patterns: orient-fixate, lunge-bite, captures, miss) for larval wolffish feeding under varying light intensities (significance level $p<0.05$; * denotes significant differences).

MAP	SOURCE	DF	FREQUENCY	
			F-Value	p-Value
Orient-Fixate	Treatment	2	18.26	0.0001*
	Day	4	16.47	0.0001*
	Treatment*Day	8	1.57	0.1769
Lunge-Bite	Treatment	2	13.07	0.0001*
	Day	4	32.55	0.0001*
	Treatment*Day	8	1.30	0.2789
Capture	Treatment	2	17.38	0.0001*
	Day	4	42.45	0.0001*
	Treatment*Day	8	1.47	0.2114
Miss	Treatment	2	1.03	0.3682
	Day	4	0.71	0.5914
	Treatment*Day	8	1.01	0.4506

Table 22: Results of Tukey's Studentized Range Test on the frequency (number of occurrences per minute) of the MAPs (orient-fixate, lunge-bite, captures, miss) for each observation day for larval wolffish feeding under varying light intensities (significance level $p<0.05$; * denotes significant differences).

MAP	DAY	DF	FREQUENCY	
			F-Value	p-Value
Orient-Fixate	10	2	2.90	0.1318
	20	2	4.52	0.0636
	30	2	10.71	0.0105*
	40	2	3.99	0.0791
	50	2	0.17	0.8477
Lunge-Bite	10	2	1.35	0.3290
	20	2	2.67	0.1480
	30	2	8.49	0.0178*
	40	2	5.28	0.0476*
	50	2	0.05	0.9532
Capture	10	2	2.81	0.1374
	20	2	2.76	0.1411
	30	2	12.48	0.0073*
	40	2	5.55	0.0431*
	50	2	0.19	0.8337
Miss	10	2	2.72	0.1440
	20	2	0.51	0.6223
	30	2	2.36	0.1750
	40	2	1.00	0.4219
	50	2	1.00	0.4219

Table 23: Tukey's groupings for the mean frequency of the MAPs (orient-fixate, lunge-bite, captures, miss) for larval wolffish feeding under varying light intensities (Note: means with the same letter, for each age, are not significantly different, $p<0.05$).

Time (days)	Treatment (Light Intensity)	Modal Action Patterns (MAPs) Orient-Fixate	Lunge-Bite	Captures	Miss
10	1200 lux	6.50^{A}	3.70^{C}	3.50^{E}	0.20^{G}
	750 lux	5.53^{A}	3.67^{C}	2.60^{E}	0.87^{G}
	320 lux	3.53^{A}	2.27^{C}	1.60^{E}	0.67^{G}
20	1200 lux	5.83^{A}	4.83^{C}	4.70^{E}	0.67^{G}
	750 lux	4.83^{A}	3.63^{C}	3.27^{E}	0.50^{G}
	320 lux	2.87^{A}	2.17^{C}	1.93^{E}	0.23^{G}
30	1200 lux	8.84^{A}	8.12^{C}	8.50^{E}	0.00^{G}
	750 lux	8.10^{AB}	7.60^{CD}	7.47^{E}	0.03^{G}
	320 lux	4.97^{B}	4.70^{D}	4.60^{F}	0.30^{G}
40	1200 lux	7.47^{A}	6.93^{C}	6.87^{E}	0.00^{G}
	750 lux	5.67^{A}	5.27^{CD}	5.13^{EF}	0.00^{G}
	320 lux	5.07^{A}	4.40^{D}	4.27^{F}	0.07^{G}
50	1200 lux	8.47^{A}	8.07^{C}	8.00^{E}	0.00^{G}
	750 lux	8.67^{A}	8.20^{C}	8.20^{E}	0.00^{G}
	320 lux	8.27^{A}	8.00^{C}	7.80^{E}	0.00^{G}

MAPs than the highest light intensity (1200 lux) treatment (8.84 occurrences per minute; Table 23). For all other days, there were no significant differences among the three light intensities, however, the trend from day 10-50 was consistent with the 320 lux light intensity having a lower frequency of orient-fixate MAPs than the higher light levels (Table23, Figure 14).

Lunge-Bite

Lunge-bite was significantly affected by both light intensity and day (Table 21, Figure 15). According to the Tukey's Test (Table 22), there were significant differences in the frequency of the lunge-bite MAPs between light intensity treatments on days 30 and 40. For each of these days, the Tukey's groupings (Table 23) reveal that the 1200 lux treatment had a significantly higher frequency of the lunge-bite MAPs than the lowest light intensity (320 lux). A trend observed during days 10-40, was the 1200 lux intensity had a higher frequency of the MAP, followed by the 750 lux, and finally the 320 lux, which had the lowest frequency (Table 23, Figure 15).

Capture

Both light intensity and day had a significant effect on the frequency of the MAP capture (Table 21; Figure 16). The results according to the Tukey's test (Table 22) indicate significant differences occurred among treatments on days 30 and 40. Consistent with the Tukey's groupings for lunge-bite, the highest light intensity treatment had a greater frequency of capture than the 320 lux treatment (Table 22) for days 30 and 40.

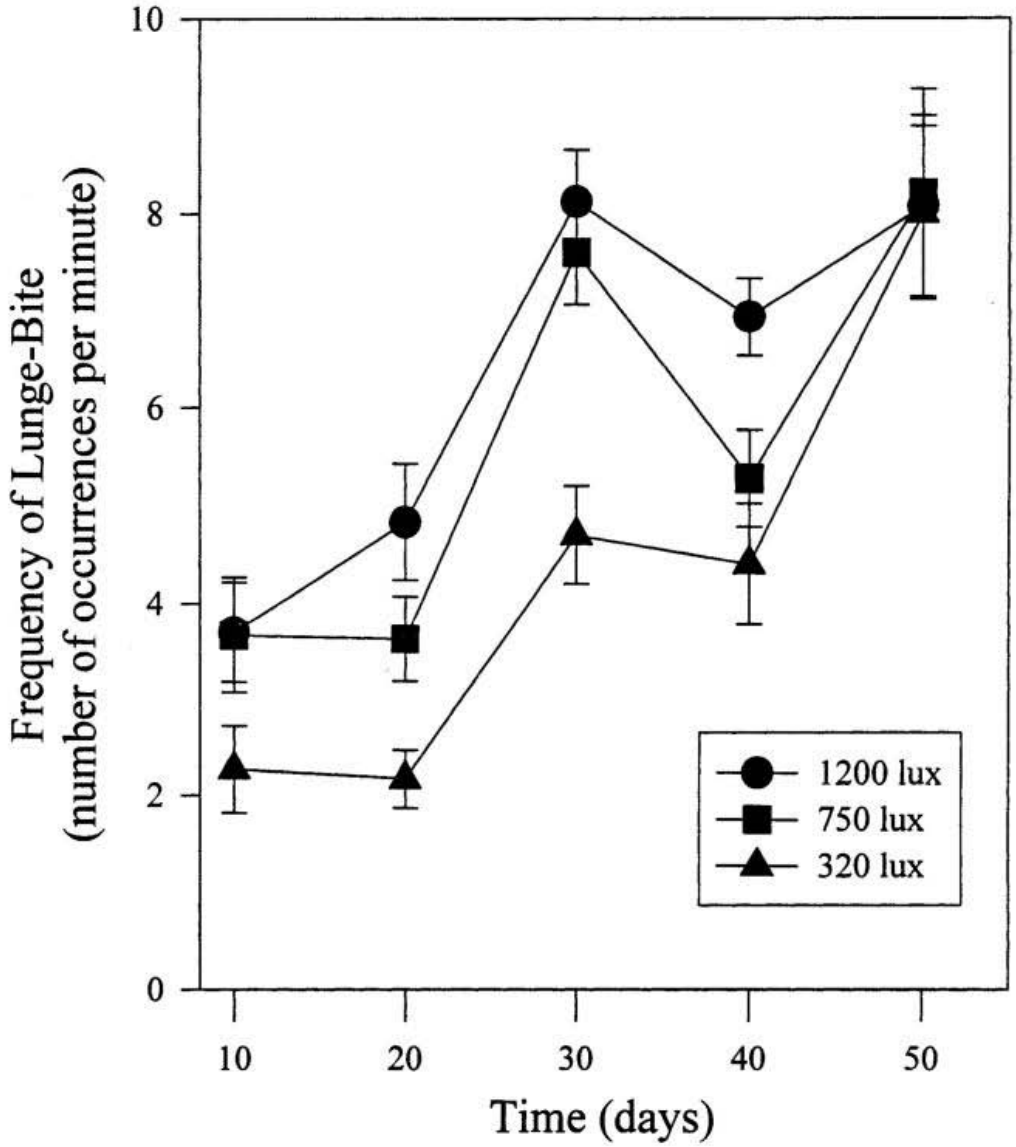

Figure 15: The frequency (number of occurrences per minute) of lunge-bite over time (days) for larval wolffish feeding under varying light intensities (n=30 for each data point, vertical bars represent standard error).

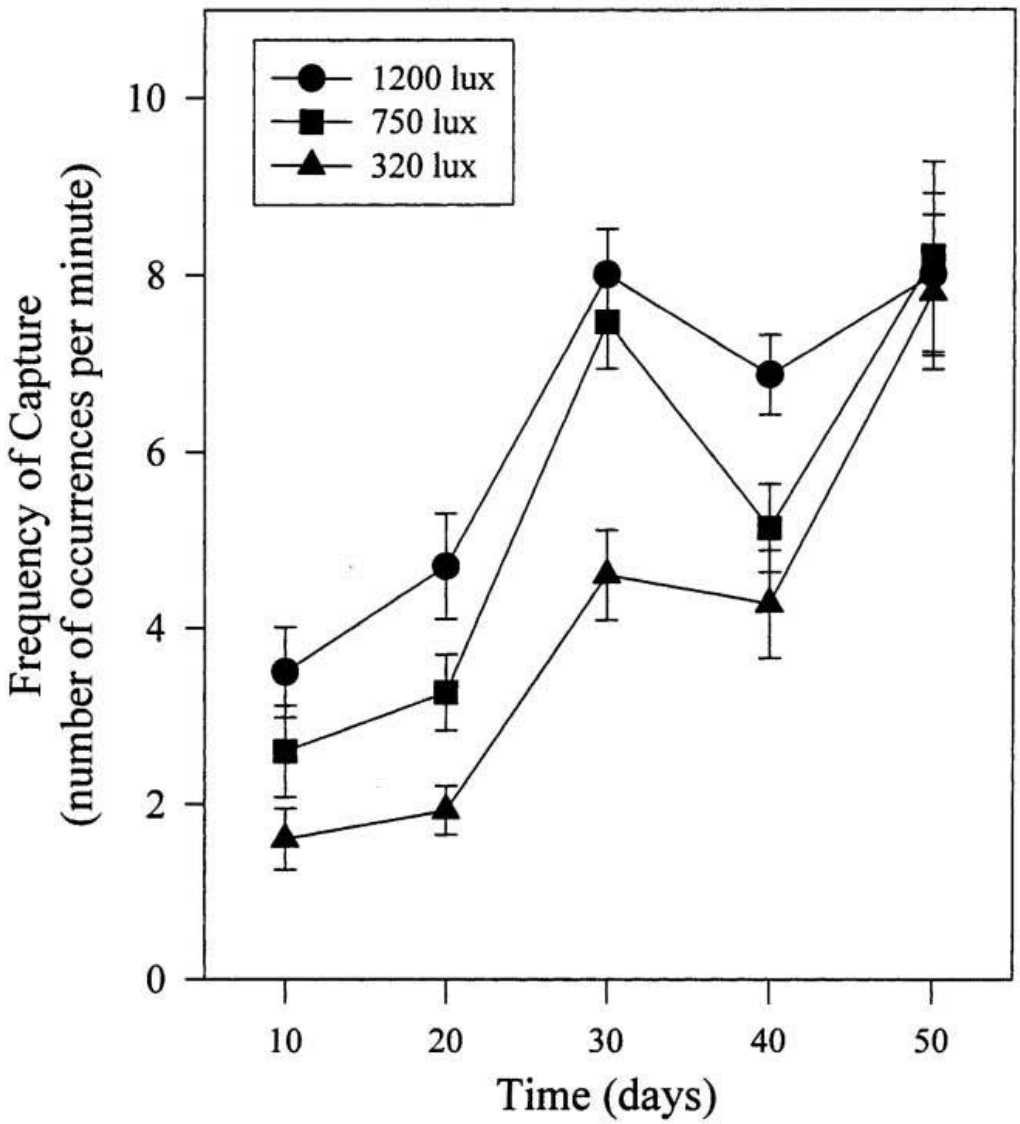

Figure 16: The frequency (number of occurrences per minute) of capture over time (days) for larval wolffish feeding under varying light intensities (n=30 for each data point, vertical bars represent standard error).

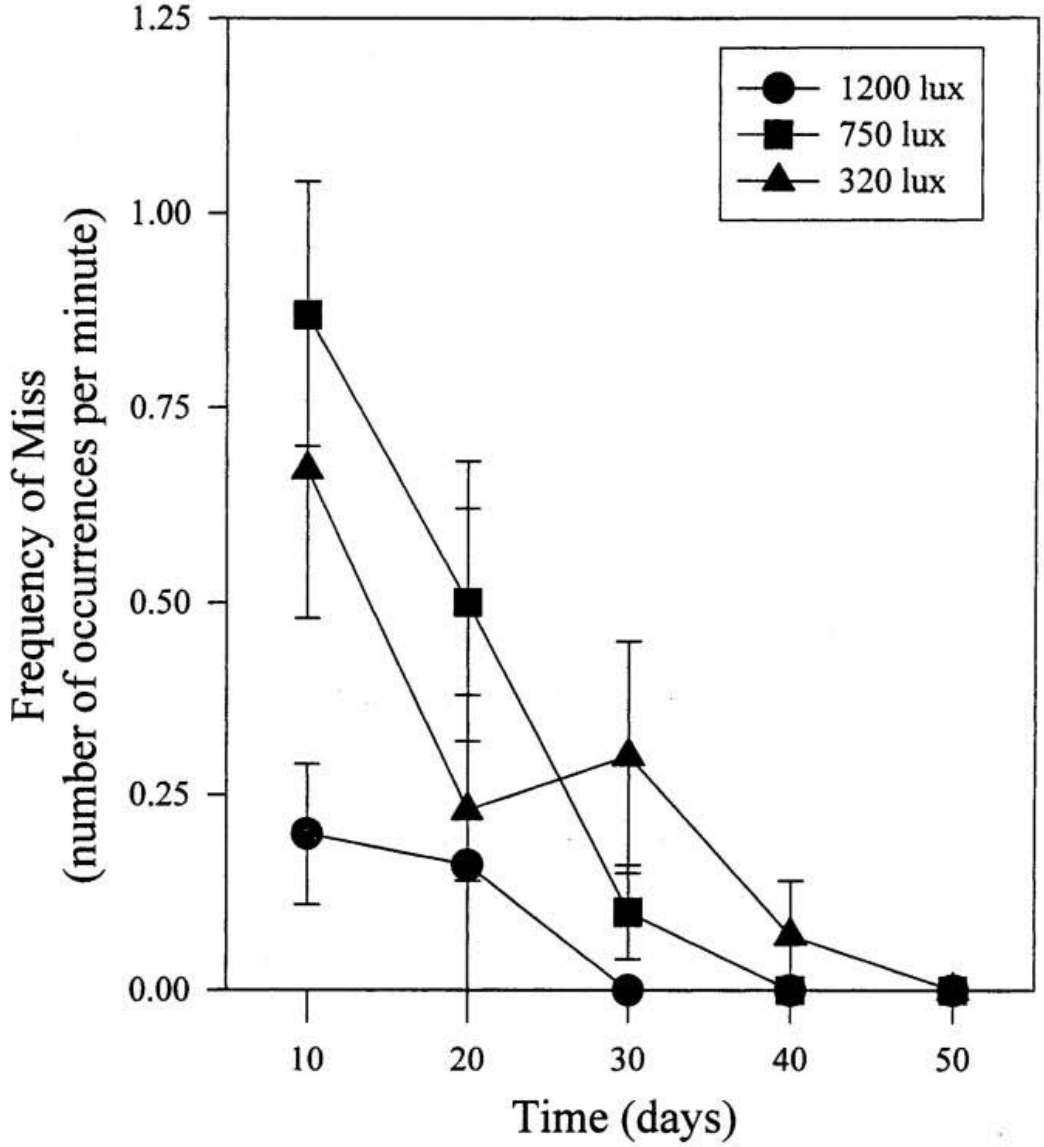

Figure 17: The frequency (number of occurrences per minute) of miss over time (days) for larval wolffish feeding under varying light intensities (n=30 for each data point, vertical bars represent standard error).

Miss

According to the ANOVA results (Table 21, Figure 17), there were no significant treatment or day effects for the frequency of the MAP miss.

5.3.2 Feeding Activities-Frequency

Foraging

According to the ANOVA results (Table 24) both light intensity and day had a significant effect on the foraging frequency of larval wolffish. The Tukey's results (Table 25) indicated that day 30 was the only day on which significant differences in foraging frequency occurred among treatments (F=10.32, p=0.0114). The Tukey's grouping for this day (Table 23) show that the 1200 lux treatment foraged at a greater rate (18.57 occurrences per minute) than the 320 lux treatment (10.00 occurrences per minute). Although not statistically significant, a consistent trend was observed during the study (Table 24, Figure 18) as the1200 lux treatment had the highest foraging frequency, followed by the 750 lux treatment, and finally the 320 lux treatment.

Successful Foraging

The larval wolffish's ability to successfully forage was affected by both treatment (Table 24) and day (Table 24). Day10, according to the Tukey's test (Table 25), was the only day on which a significant difference occurred among treatments. Results for this day showed that the 1200 lux treatment had a significantly higher successful foraging rate (94.64%) than the 750 lux treatment (73.76%) or the 320 lux treatment (71.43%; Table

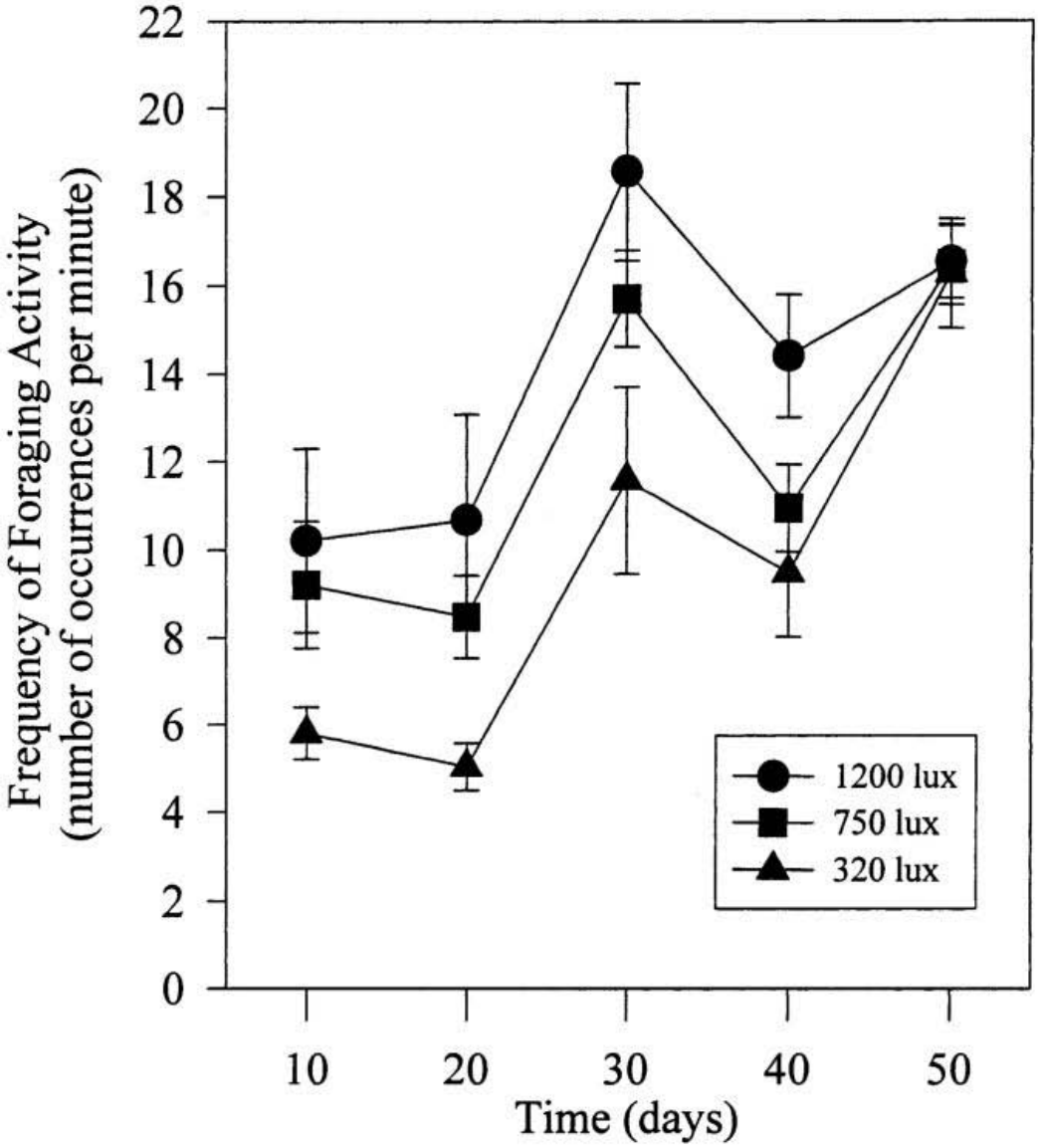

Figure 18: The frequency (number of occurrences per minute) of foraging activity over time (days) for larval wolffish feeding under varying light intensities (n=30 for each data point, vertical bars represent standard error).

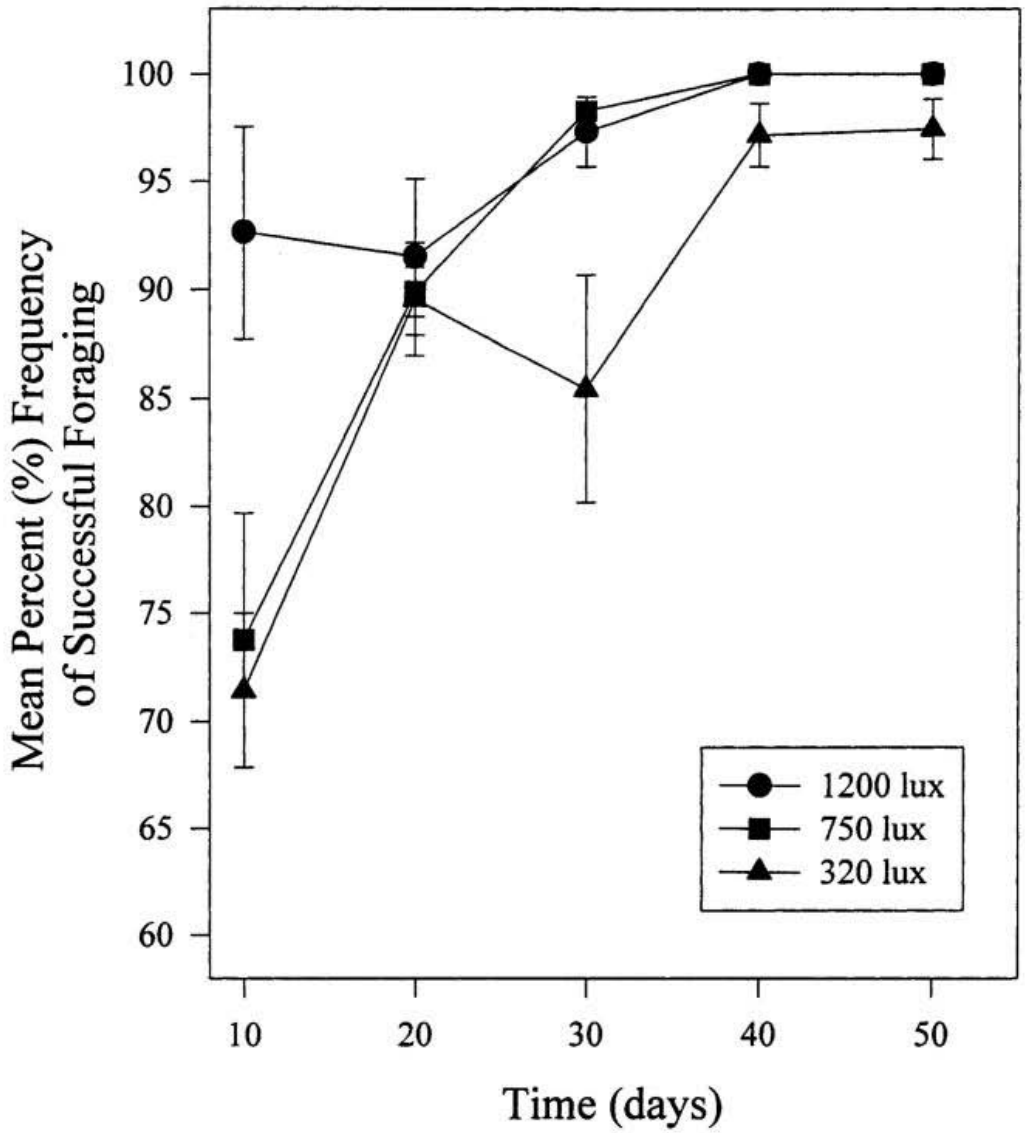

Figure 19: The mean percent (%) frequency of successful foraging over time (days) for larval wolffish feeding under varying light intensities (n=30 for each data point, vertical bars represent standard error).

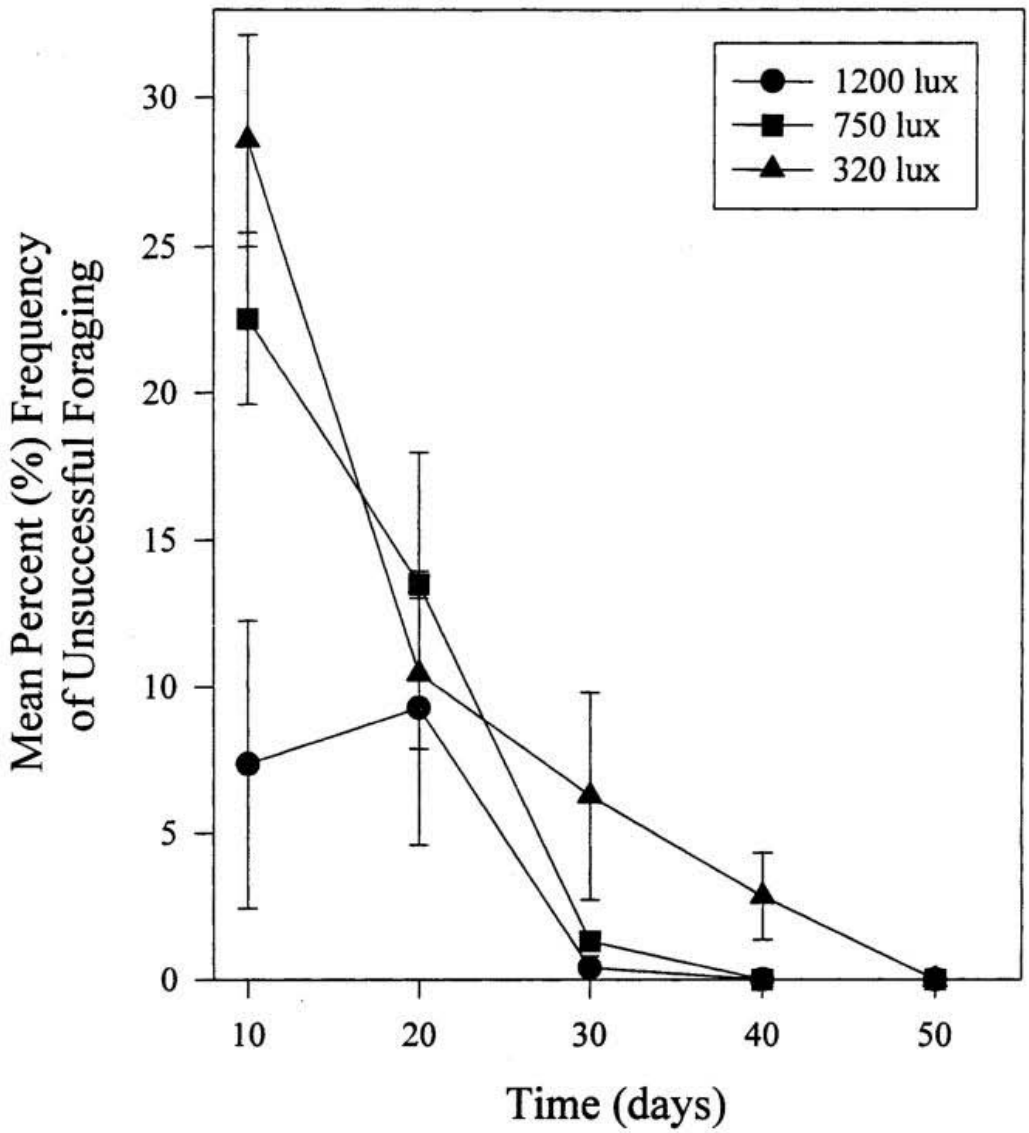

Figure 20: The mean percent (%) frequency of unsuccessful foraging over time (days) for larval wolffish feeding under varying light intensities (n=30 for each data point, vertical bars represent standard error).

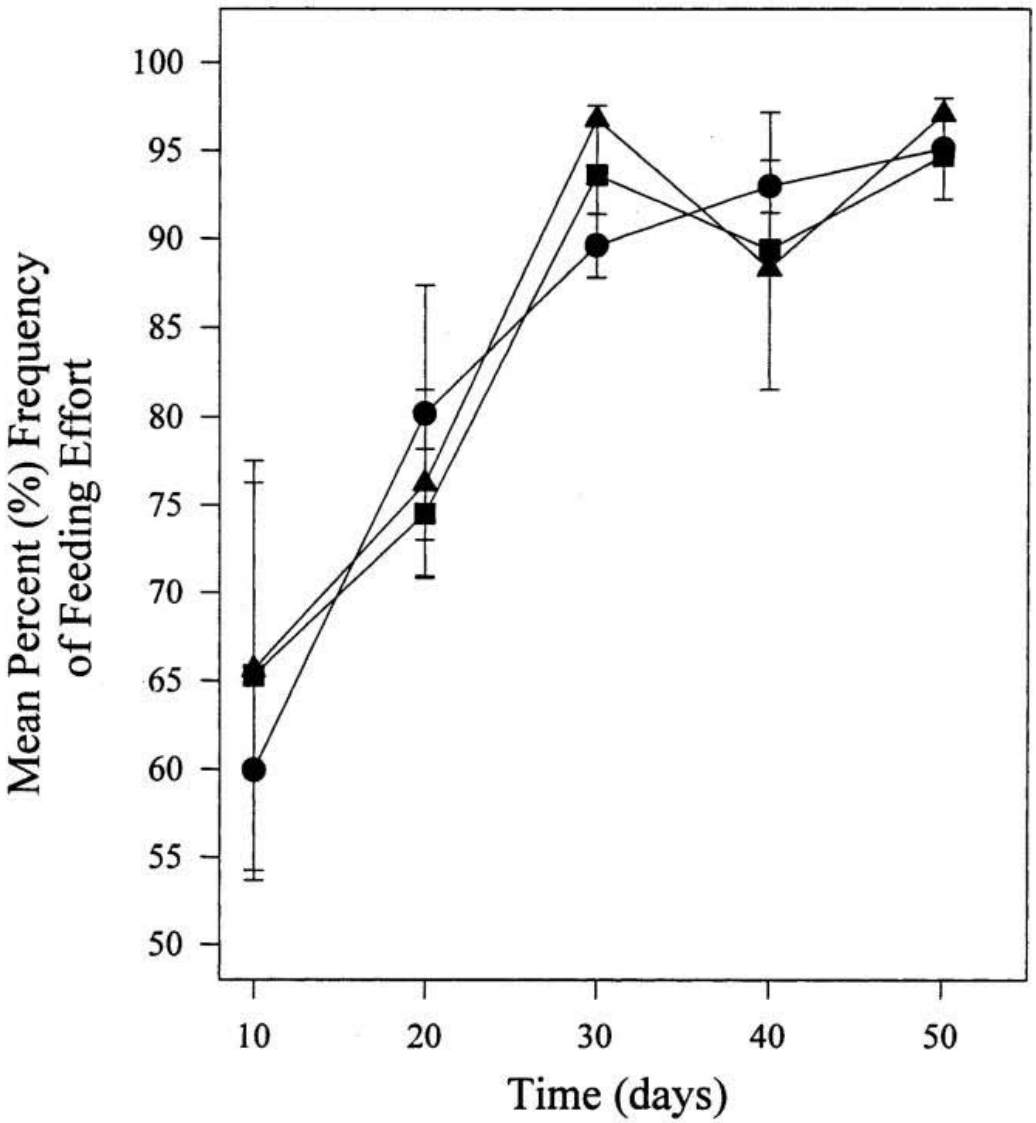

Figure 21: The mean percent (%) frequency of feeding effort over time (days) for larval wolffish feeding feeding under varying light intensities (n=30 for each data point, vertical bars represent standard error).

26, Figure 19) . For the remainder of the study, there were no significant differences in the successful foraging frequency among treatments.

Unsuccessful Foraging

According to the ANOVA results (Table 24) day had an effect on the frequency of unsuccessful foraging by larval wolffish whereas treatment had no effect. The Tukey's table (Table 25) indicated day 10 was the only day which showed a difference in the frequency of unsuccessful foraging. Tukey's grouping for this day show that the 1200 lux treatment had a significantly lower frequency (7.63%) of unsuccessful foraging in comparison to the lowest light intensity treatment, 320 lux (28.57%).

Feeding Effort

The ANOVA results (Table 24) indicate that there were no significant treatment or day effects on the larval wolffish's feeding effort. Results indicate that there was no clear trend in feeding effort (Table 26, Figure21).

5.3.3 Action Pattern-Frequency and Duration

Swimming

The frequency of swimming was significantly affected by both treatment and day (Table 26, Figure 22). According to the Tukey's test results (Table 26), day 30 was the only day on which significant differences occur among treatments ($F=11.13$, $p=0.001$). On this day, the 1200 lux treatment had a significantly higher swimming frequency (8.67

Table 24: Results of Two Way Analysis of Variance (ANOVA) on the frequency (number of occurrences per minute) of the feeding activities (foraging activity, successful foraging, unsuccessful foraging, feeding effort) for larval wolffish feeding under varying light intensities (significance level $p<0.05$; * denotes significant differences).

MAP	SOURCE	DF	FREQUENCY	
			F-Value	p-Value
Foraging Activity	Treatment	2	16.86	0.0001*
(frequency)	Day	4	25.00	0.0001*
	Treatment*Day	8	1.48	0.2069
Successful Foraging	Treatment	2	14.49	0.0001*
(mean % frequency)	Day	4	22.38	0.0001*
	Treatment*Day	8	1.72	0.1356
Unsuccessful Foraging	Treatment	2	2.28	0.1201
(mean % frequency)	Day	4	18.06	0.0001*
	Treatment*Day	8	2.24	0.0521
Feeding Effort	Treatment	2	0.14	0.8695
(mean % frequency)	Day	4	1.48	0.3003
	Treatment*Day	8	0.37	0.9277

Table 25: Results of Tukey's Studentized Range Test on the frequency (number of occurrences per minute) of the feeding activities (foraging activity, successful foraging, unsuccessful foraging, feeding effort) for each observation day for larval wolffish feeding under varying light intensities (significance level $p<0.05$; * denotes significant differences).

MAP	DAY	DF	FREQUENCY	
			F-Value	p-Value
Foraging Activity	10	2	2.36	0.1757
(frequency)	20	2	3.48	0.0991
	30	2	10.32	0.0114*
	40	2	5.11	0.0500
	50	2	0.10	0.9030
Successful Foraging	10	2	5.22	0.0486*
(mean % frequency)	20	2	2.38	0.1733
	30	2	4.85	0.0558
	40	2	3.92	0.0816
	50	2	3.80	0.0858
Unsuccessful	10	2	7.87	0.0210*
Foraging	20	2	0.22	0.8119
(mean % frequency)	30	2	2.36	0.1754
	40	2	3.71	0.0896
	50	2	0.00	0.0000
Feeding Effort	10	2	0.27	0.7695
(mean % frequency)	20	2	0.27	0.7712
	30	2	1.79	0.2462
	40	2	0.18	0.8410
	50	2	0.42	0.6761

Table 26: Tukey's groupings for the mean frequency of the feeding activities (foraging activity, successful foraging, unsuccessful foraging, feeding effort) for larval wolffish feeding under varying light intensities (Note: means with the same letter, for each age, are not significantly different, $p<0.05$).

		Modal Action Patterns (MAPs)			
Time (days)	Treatment (Light Intensity)	Foraging Activity (frequency)	Successful Foraging (mean % frequency)	Unsuccessful Foraging (mean % frequency)	Feeding Effort (mean % frequency)
10	1200 lux	10.20^{A}	92.64^{C}	7.63^{E}	59.96^{G}
	750 lux	9.20^{A}	73.76^{D}	22.53^{EF}	65.28^{G}
	320 lux	5.80^{A}	71.43^{D}	28.57^{F}	65.61^{G}
20	1200 lux	10.67^{A}	91.49^{C}	9.28^{E}	80.17^{G}
	750 lux	8.47^{A}	89.88^{C}	13.51^{E}	74.50^{G}
	320 lux	5.03^{A}	89.54^{C}	10.46^{E}	76.23^{G}
30	1200 lux	18.57^{A}	97.80^{C}	0.41^{E}	89.58^{G}
	750 lux	15.70^{AB}	98.27^{C}	1.32^{E}	93.59^{G}
	320 lux	11.57^{B}	85.40^{C}	6.28^{E}	96.77^{G}
40	1200 lux	14.40^{A}	1.06^{C}	0.00^{E}	92.94^{G}
	750 lux	10.93^{A}	0.81^{C}	0.00^{E}	89.34^{G}
	320 lux	9.47^{A}	0.77^{C}	2.86^{E}	88.28^{G}
50	1200 lux	16.53^{A}	8.07^{C}	0.00^{E}	95.08^{G}
	750 lux	16.47^{A}	8.20^{C}	0.00^{E}	94.66^{G}
	320 lux	16.27^{A}	8.00^{C}	0.00^{E}	97.08^{G}

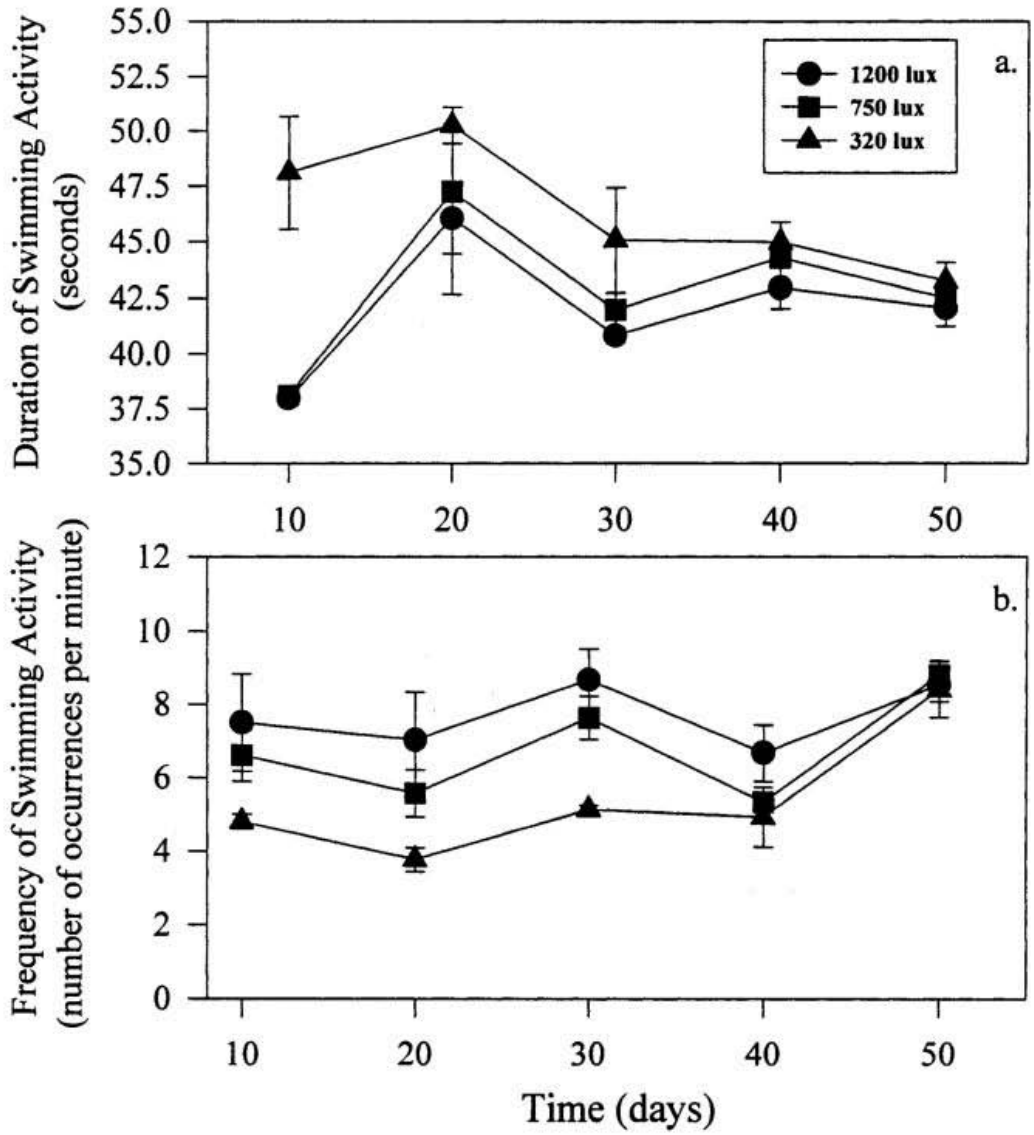

Figure 22: The duration (seconds; fig. a) and frequency (occurrences per minute; fig. b) of swimming activity over time (days) for the larval wolffish feeding under varying light intensities (n=30 for each data point, vertical bars represent standard error).

Table 27: Results of Two Way Analysis of Variance (ANOVA) on frequency (number of occurrences per minute) and the duration (seconds) of swimming activity for larval wolffish feeding under varying light intensities (significance level $p<0.05$; * denotes significant differences).

MAP	SOURCE	DF	FREQUENCY		DURATION	
			F-Value	p-Value	F-Value	p-Value
Swimming	Treatment	2	12.59	0.0001*	11.00	0.0003*
	Day	4	9.39	0.0001*	7.73	0.0002*
	Treatment*Day	8	1.04	0.4271	1.84	0.1082

Table 28: Results of Tukey's Studentized Range Test on the frequency (number of occurrences per minute) and duration (seconds) of swimming activity for each observation day of larval wolffish feeding under varying light intensities (significance level $p<0.05$; * denotes significant differences).

			TUKEY'S RESULTS			
MAP	DAY	DF	FREQUENCY		DURATION	
			F-Value	p-Value	F-Value	p-Value
Swimming	10	2	2.47	0.1455	15.31	0.0044*
	20	2	3.59	0.0945	0.70	0.5315
	30	2	9.22	0.0148*	2.33	0.1788
	40	2	1.97	0.2197	1.75	0.2515
	50	2	0.13	0.8798	0.61	0.5737

Table 29: Tukey's groupings for the mean frequency (occurrences per minute) and duration (seconds) of swimming activity for larval wolffish feeding under varying light intensities (Note: means with the same letter, for each age, are not significantly different, $p<0.05$).

Time (days)	Treatment (Light Intensity)	Swimming Action Pattern	
		Frequency	Duration
10	1200 lux	7.50^{A}	37.97^{E}
	750 lux	6.60^{A}	38.13^{E}
	320 lux	4.80^{A}	48.11^{F}
20	1200 lux	7.03^{A}	46.05^{E}
	750 lux	5.57^{A}	47.25^{E}
	320 lux	3.77^{A}	50.26^{E}
30	1200 lux	8.67^{A}	40.81^{E}
	750 lux	7.63^{AB}	41.95^{E}
	320 lux	5.13^{B}	45.07^{E}
40	1200 lux	6.67^{A}	42.94^{E}
	750 lux	5.33^{A}	44.28^{E}
	320 lux	4.93^{A}	43.25^{E}
50	1200 lux	8.53^{A}	42.04^{E}
	750 lux	8.80^{A}	42.47^{E}
	320 lux	8.40^{A}	43.25^{E}

occurrences per minute) in comparison to the 320 lux treatment which had a lower swimming frequency (5.13 occurrences per minute). A consistent trend among the treatments for each sampling day was the larvae in the 1200 lux treatment had the highest swimming frequency, followed by the 750 lux treatment, and finally the 320 lux treatment (Figure 22).

The ANOVA results for the duration of swimming (Table 25) show that both day and treatment significantly affected the duration of the larval swimming. Unlike frequency, Tukey's results indicate that day 10 is the only day on which significant differences occur among treatment ($F=15.31$, $p=0.0001$; Table 26). The Tukey's grouping for this day (Table 27) show that the larvae in the 1200 lux treatment had a significantly shorter duration of swimming (37.97 seconds) than either the 750 lux treatment (38.13 seconds) or the 320 lux treatment (48.11 seconds).

5.4 Discussion

Results indicate that light intensity (320 lux, 750 lux, 1200 lux) does have an effect on the feeding and activity of larval wolffish. For the feeding MAPs (orient-fixate, lunge-bite, capture) the frequency of occurrence of these MAPs increased with increasing light intensity. Although there was a relationship between MAP frequency and increasing light intensity, there does not appear to be a relationship between frequency of occurrence with increasing size or age. The most significant effect of light intensity on the MAPs occurred during days 30-40 of the experiment. This period corresponds to the switch from endogenous to exogenous feeding in larval wolffish. It is crucial for larval survival

that appropriate feeding behaviours are established at this time. During this period, the larvae in the highest light intensity treatment (1200 lux) had significantly greater frequencies of the MAPs in comparison to the lowest light intensity treatment (320 lux). An exception to this was the frequency of occurrence of misses, where light intensity did not appear to have any effect on the frequency of the MAP. Further there was no correlation between size or age with the frequency of occurrence of this MAP.

For the feeding activities (foraging and successful foraging), the frequency of each of these behaviours increased with increasing light intensity. For foraging activity, significant differences were observed during days 30-40 of the study, with a peak in total foraging activity occurring on day 30. It appears that larvae in the highest light intensity treatment are the most active foragers during this critical period. For newly hatched larvae, light does appear to play a role in the frequency of successful prey capture. On day 10 of the study, larvae in the 1200 lux treatment were 92.64% successful at capturing prey in comparison to larvae in the lowest light intensity treatment which were only 71.43% effective. This trend decreases as the larvae increase in age and size (see data, Chapter Four). Similarly, unsuccessful foraging demonstrated significant differences among treatments on day 10. Larvae in the 320 lux treatment had a higher failure rate (28.57%) compared to the 1200 lux treatment (7.63%). By the end of the study (day 50), there was no difference observed between treatments in terms of successful or unsuccessful foraging. Larval wolffish at this age/size were 100% effective in capturing prey. This suggests that the role of light intensity becomes less significant in terms of foraging success, as the larvae grow.

When comparing feeding effort to the other feeding activities, light intensity does not appear to play a role in the frequency of feeding effort. Essentially, if wolffish are able to perceive prey, they make an attempt at consuming it. However, the data on successful and unsuccessful feeding indicate that although the larvae are making an effort to feed, the probability of success is greater in the higher light treatments. For all treatments, feeding effort improves with time.

There was a positive relationship between the frequency of swimming and increasing light intensity. Swimming duration was negatively correlated to light intensity suggesting that larvae under low light intensity have to search greater volumes of water (for a longer period of time) in order to find food. This difference in duration becomes less with time, suggesting that light intensity offers less benefit to the searching behaviour of larval wolffish as the fish increases in size/age.

The results from this study indicate that foraging activity and MAPs increase with increasing light intensity. Similar results have been reported for largemouth bass, *Micropterus salmoides* (McMahon and Holanov, 1995), longnose dace *Rhinichthys cataractae* (Beers and Culp, 1990), greenback flounder, *Rhombosolea tapirina*, (Cox and Pankhurst, 2000) and larval cod , *Gadus morhua* (Puvanendran and Brown, 2002). The authors suggest that light improves foraging by increasing prey encounter rates, decreasing search time, and increasing attack efficiency and reaction distance.

Studies on the foraging behaviour of larval cod revealed that cod larvae foraged most frequently and successfully at light intensities which most closely resembled those of their natural environment (Puvanendran, 1999a). This is also supported in a study

conducted by Huse (1994) on three marine larval fish species: cod (*Gadus morhua*), plaice (*Pleuronectes platessa*), and turbot (*Scophthalmus maximus*). For each of the species examined the optimal illumination level varied in accordance to feeding strategy, habitat, and prey types.

The results from this study support this idea as the wolffish larvae were more active foragers at light intensities which best matched the light intensities found in their natural environment during the time of first-feeding (see Chapter Four).

The behavioural results, as well as the growth and survival data, suggest that despite the potential impact of lower light intensity on foraging, larvae in the low light treatments were able to grow and obtain survival rates of approximately 81% at the end of 50 days. This suggests that other factors may contribute to the foraging behaviour of the larvae. A study by Knutsen (1992) on the north sea turbot (*Scophthalmus maximus*) and dover sole (*Solea solea*) indicates that chemosensory processes at an early age may assist larvae in finding prey.

Swimming activity may be considered an indication of first feeding (Skiftesvik, 1992), and is commonly associated with foraging activity or the search for food (Munk and Kiørboe, 1985; Batty *et al.*, 1990). Studies on species such as cod (Puvanendran, 1999a) and herring (Batty *et al.*, 1990) have reported increases in swimming activity with the search for food. The results from my study indicate that swimming activity frequency was positively correlated to light intensity, suggesting that at higher light levels, larvae were more likely to actively search or forage. The foraging data supports this result. Corresponding to the foraging data, the most significant effects of light intensity on

swimming activity occurred during day 30. Thus it appears, that as larvae switch to exogenous food reserves, they increase their swimming and foraging behaviours.

The results for the swimming duration show it is higher at lower light intensity treatments, suggesting that the larvae take a longer period of time to find prey. The larvae spend more time searching for prey under reduced light levels, likely because the visual perception of the prey is reduced.

In summary, light intensity plays a role in larval wolffish foraging with improvements in foraging observed at increasing light intensities. It is suggested that light levels be maintained at high intensity (1200 lux) from hatching until the critical switch from endogenous to exogenous feeding, as it greatly enhances the foraging rate, growth, and survival rates for larval wolffish.

Chapter 6. Conclusions

The role of light is an important factor in the culture of larval wolffish. Experiments on the effects of photoperiod and light intensity on survival and growth suggest that there are critical values which should be provided to the larvae in order to ensure maximum growth and survival.

It is therefore recommended that a photoperiod of at least 18L be provided during the first 50 days of larval wolffish production. Photoperiods below this level (12L) compromise growth and survival, whereas values above this (24L) offer no benefit to production and can be considered as an economic loss. A photoperiod of 24L would increase hatchery utility cost, but does not offer any advantage to growth or survival beyond the 18L treatment.

For light intensity, an intensity of approximately 1200 lux improves growth and survival rates. Further, a light intensity of 1200 lux maximized foraging rate during the critical switch from endogenous to exogenous feeding.

For all parameters studied, the significance of these variable on wolffish production appears to decrease as the larvae wolffish approach the juvenile phase (day 50). It is recommended that light requirements for wolffish be re-examined at this time.

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