THE EFFECTS OF EARLY WEANING ON THE BEHAVIOUR, GROWTH AND SURVIVAL OF ATLANTIC COD (Gadus morhua) AND FAT SNOOK (Centropomus parallelus) LARVAE

CENTRE FOR NEWFOUNDLAND STUDIES

TOTAL OF 10 PAGES ONLY
MAY BE XEROXED

(Without Author's Permission)

TARCISIO T. ALVES JR.

The effects of early weaning on the behaviour, growth and survival of Atlantic cod (*Gadus morhua*) and fat snook (*Centropomus parallelus*) larvae

by

Tarcisio T. Alves Jr.

A thesis submitted to the School of Graduate Studies
in partial fulfilment of the requirements for the
degree of Master of Science in Aquaculture

MSc Aquaculture Programme
Ocean Sciences Centre
Memorial University of Newfoundland

St. John's, Newfoundland

2003

Library and Archives Canada

Published Heritage Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et Archives Canada

Direction du Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 0-612-99047-8
Our file Notre référence
ISBN: 0-612-99047-8

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

ABSTRACT

Live food production is one of the most costly activities during the intensive culture of the larval stages of marine fish. But the culture of marine fish larvae requires extensive use of live food to produce healthy larvae. However, the process of weaning larvae from a live to a dry diet results in loss of growth and increased in mortality. Combining live feed and manufactured diets (co-feeding) at an early developmental stage has been shown to improve growth and survival of larvae compared to the use of live feed only. Moreover, it helps to condition the larvae to more easily accept the manufactured diet when live feed is withdrawn, resulting in a shorter weaning period.

In this thesis, I conducted weaning trials to improve the larviculture protocols of two marine species, the Atlantic Cod (*Gadus morhua*), a temperate marine species, and Fat Snook (*Centropomus parallelus*), a tropical marine fish. Several combinations of live and inert food were examined as feeding protocols, to determine the earliest point at which larvae of these species can be successfully weaned from *Artemia* onto a dry diet.

Two weaning experiments were conducted with Atlantic cod larvae. In the first experiment, 25 day post hatch (DPH) larvae were assigned to one of four treatments using *Artemia* as live food for 3, 5, or 10 days and a treatment without *Artemia* (larvae co-fed with rotifers for 7 days). By 45 DPH, larvae reared with *Artemia* for 10 days had significantly ($P<0.05$) better growth, had the highest survival and ingested more dry food pellets compared to all other treatments. In the second experiment, three treatments were developed in which larvae were co-fed with dry diet and *Artemia* on 25, 30 and 35 DPH

for 10 days. A fourth treatment was assigned as a control in which larvae were fed only *Artemia*. Weaning age did not significantly ($P>0.05$) affect either growth or survival of larvae. However, larvae in the control treatment were larger and had a significantly ($P<0.05$) higher frequency of *Artemia* ingestion. Results from both experiments indicate that Atlantic cod larvae can be completely weaned by 35 DPH.

Feeding trials were conducted with fat snook larvae. Fat snook larvae of 30 DPH were assigned to five feeding trials in which larvae received *Artemia* for 5, 10 and 15 days (co-fed with a locally prepared dry diet), *Artemia* for 10 days (co-fed with a commercial dry diet), and only *Artemia* (control treatment). Larval survival was not significantly affected ($P>0.05$) by treatment. Fat snook larvae were successfully weaned by 35 DPH, but larvae weaned by 40 DPH displayed higher growth rates and were significantly ($P<0.05$) larger by the end of the experiment. There were no significant differences in any growth parameter between larvae weaned by 40 and 45 DPH. However, comparisons between treatments using different dry diets revealed a significant difference ($P<0.05$) in the frequency of dry diet ingestion, although it did not influence the growth of larvae.

The results of the present study will benefit the intensive culture of both species, sharpening the current larviculture protocols and lowering costs on its large scale production.

DEDICATION

I dedicate this thesis to my parents, Tarcisio and Ivone Alves (in memoriam), to whom I am deeply grateful for all their love, encouragement and efforts for providing everything possible for my education.

ACKNOWLEDGEMENTS

I would like to express my largest gratitude to my supervisor, Dr. Joe Brown, for his continuous encouragement and guidance throughout this study. He is the most supportive and understanding supervisor one could have.

Special thanks to Aline ('bruxinha') and my sisters Leila and Lilian, who have been a great source of strength all through this work. Although away physically for a while, they were always present spiritually and the most supportive people I would need during my studies in Canada.

Thanks to everybody in the OSC. I thank Dr. V. Puvanendran, for his advise and providential tips in aquaculture design, due to his expertise in marine finfish hatcheries. Thanks to Danny and Darryl, who were always willing to provide any means to carry my experiments. Thanks a lot to Karen for her friendship, and for helping me whenever I needed throughout my stay in St. John's and Florianópolis. Thanks to you too Kelly. Many thanks for the people in the ARDF/OSC: Lori, Louise, Maria, Francine, Jennifer and Amanda. Thanks to both Trevors (Avery and Snow) for their help in all computer problem solving.

I am very grateful to Dr. Vinicius Cerqueira for his welcoming in Florianópolis, his scientific guidance and for providing all conditions to carry out the snook experiments at the LAPMAR, Brazil. I would also like to thank everyone at LAPMAR: Israel, Sayão, Jaqueline, Alexandre, Camila and Tiago, for their technical help, friendship and comic relief when things started to get crazy.

Thanks to Marcelo Miranda, for his friendship and solidarity in sharing wishes to go surfing on chilly waves during our stay in St. John's.

I am grateful to the Canadian International Development Agency/CIDA and Dr. Anthony Dickinson for the financial support of my studies through a Marine Science Scholarship and research funds. I thank to Colleen for her advise and for everything else she has done for me.

Thanks also to AquaNet for its financial support to attend conferences and lab work in Brazil.

TABLE OF CONTENTS	Page number

TABLE OF CONTENTS – Continued	Page number

List of Tables	Page number

List of Tables – Continued	Page number

List of Figures	Page number

List of Figures – Continued	Page number

List of Figures – Continued	Page number

Chapter 1

General introduction

The larviculture of many marine finfish species is still dependent on live feed, such as the rotifer (*Brachionus* spp.) and brine shrimp (*Artemia* sp.) until metamorphosis (Tucker and Jory, 1991; Sorgeloos *et al.*, 2001). Although rotifers and *Artemia* are not encountered in the natural environment of most cultured larvae, these organisms are currently the most utilized as live prey in commercial hatcheries. Even though the cultivation methods of prey organisms for marine fish larvae are relatively simple, they are also expensive and often unreliable (Person Le Ruyet *et al.*, 1993).

To ensure a reliable, continuous supply of plankton year-round, cultures of algae and zooplankton have to be established. Live feed supply is generally required past metamorphosis when most fish species are then weaned onto dry formulated diets. Although the organisms employed as larval feed have contributed to the success and the increase in production of many finfish species, they present many problems. These include large investments in establishing the 'food chain', which is often unreliable and several technical difficulties in preparing them to meet the nutritional requirements of larvae (Kanazawa *et al.*, 1989; Kolkovski, 2001). In calculating the costs involved with feeding live food to seabass (*Dicentrarchus labrax*), Person Le Ruyet *et al.* (1993) found that the live prey (*Artemia*) accounted for about 80% of the production cost for juvenile up to 45 days old. Thus, the reduction in its use in late stages of larvae would help reduce production costs.

Artemia is considered an ideal choice among marine fish farmers, due to its availability in diverse forms and their wide size range. However, the use of *Artemia* is often considered of questionable nutritional value as about 30% of the nutritional value of the *Artemia* nauplii is lost as they get older (Watanabe and Kiron, 1994). Since *Artemia* nauplii are non-selective particle feeders, some methods have been developed to incorporate different products into the *Artemia* prior to feeding to fish larvae. This method of *Artemia* enrichment or boosting, is widely applied in marine fish hatcheries for enhancing the nutritional value of *Artemia* with essential fatty acids (Highly Unsaturated Fatty Acids-HUFAs) (Sorgeloos *et al.*, 2001). Additionally, they are often used as a carrier to deliver essential nutrients, prophylatics and therapeutics to fish larvae (Leger *et al.*, 1987).

The enhanced digestion of *Artemia* by the fish larvae may actually be attributed to the autolytic action of the endogenous enzymes (amylase and trypsin) present in *Artemia* (Watanabe and Kiron, 1994). Kolkovski *et al.* (1997a) suggested that feeding prey organisms, such as *Artemia* nauplii, a short time before dry diets are offered may increase digestive activity and improve the ability of larvae to digest the dry diets. Live food organisms consumed by the larvae assist the digestion process by 'donating' their digestive enzymes, thus activating the larval endogenous digestive enzymes. Live food organisms also contain gut neuropeptides and nutritional 'growth' factors which enhance digestion (Kolkovski, 2001).

During recent years, intensive research has been conducted to find full or partial replacements for live food organisms (Kolkovski and Tandler, 1995; Fernandez-Diaz and

Yufera, 1997; Rosenlund *et al.*, 1997; Cahu *et al.*, 1998; Southgate and Partridge, 1998). In spite of considerable advances in the formulation of artificial feed for fish larvae, it is not possible yet to completely substitute for *Artemia* in the rearing protocols of many marine species, and this prey still remains essential in commercial hatchery operations (Sorgeloos *et al.*, 2001).

An ideal dry diet is often considered the missing link in the marine fish larviculture cycle and efforts to formulate dry diets require an extensive understanding of the nutrient needs of the developing fish (Watanabe and Kiron, 1994; Yufera *et al.*, 1999; Cahu and Zambonino Infante, 2001).

Early introduction of dry formulated diets is often reported as providing an easier weaning onto dry food. However, weaning small sized marine fish larvae at first feeding directly onto compound pellets is difficult, and up to now dry diets alone are unable to replace completely live food (Person Le Ruyet, 1991; Holt, 1993; Lazo *et al.*, 2000a and 2000b). Kestemont *et al.* (1995) suggested that the size and weaning age are directly linked to the ontogenesis and development of digestive tract of a given species.

Gennari *et al.*(1992) pointed out that weaning marine fish too early may result in lower juvenile growth and survival, therefore increasing production costs. There is physiological support that the digestive enzymes capable of metabolizing artificial diets are present in some marine fish only when larvae reach late stages of development (Koven *et al.*, 2001). Besides, fish showing delayed weaning are reportedly growth-retarded and, for carnivorous species, will likely become victims of cannibalistic behaviour (Lee and Ostrowski, 2001).

Several authors have reported that larvae of the majority of marine fish species refuse to ingest dry formulated diets from the early stages (Kanazawa and Teshima, 1988; Tandler and Kolkovski, 1991; Kolkovski and Tandler, 1995). This is generally explained as being due to the unsuitable composition, low palatability, or the physical characteristics of currently available dry feed (Appelbaum, 1985; Walford *et al.*, 1991; Kolkovski and Tandler, 1995), rather than an intrinsic incapacity of the early larva of marine fish to assimilate inert diets (Yufera *et al.*, 1999).

The problems encountered with prepared food are related to the intrinsic nature of the dry particles (size, texture, digestibility, leaching of nutrients), to the methods of administering them (i.e. optimal food switch timing), and to insufficient knowledge of the feeding physiology, nutritional requirements and behaviour of the larvae in the presence of such food (Fernandez-Diaz *et al.*, 1994). Particularly small particles cannot be easily detected by larvae, whereas large ones can be difficult to ingest and may even promote a blockage of the digestive tract (Walford *et al.*, 1991; Fernandez-Diaz *et al.*, 1994).

Conversely, many authors have reported successful early weaning of several species with reasonable survival and growth rates [i.e., Common sole at 10 day post-hatch (DPH), Appelbaum, 1985; European sea bass at 20 DPH, Person Le Ruyet *et al.*, 1993; Cahu and Zambonino Infante, 1994; Red drum at 8 DPH, Holt, 1993; Gilthead sea bream from mouth opening, Fernandez-Diaz and Yufera, 1997; Red sea bream from mouth opening, Takeushi *et al.*, 1998].

Therefore, by improving the acceptance of dry diets for marine fish larvae and the formulation of a more digestible diet still remains an important step. Before this is

achieved, live food (phyto- and zooplankton) will remain an important food source for the start feeding of early larval stages (Dhert *et al.*, 2001). Recent progress has been made in formulating and administering artificial feed to larvae of several marine fish species (Rosenlund *et al.*, 1997; Canavate and Fernandez-Diaz, 1999; Cahu and Zambonino Infante, 2001), while replacing or reducing live feed dependence. This was achieved by co-feeding of live and dry feed (Walford *et al.*, 1991; Person Le Ruyet *et al.*, 1993; Kolkovski and Tandler, 1995; Lavens *et al.*, 1995; Rosenlund *et al.*, 1997; Tucker, 1998).

Weaning marine fish larvae to dry diets is done gradually, starting with co-feeding and reducing the amount of live feed, usually *Artemia*, until replacement with dry diets is completed (Jones *et al.*, 1993; Kolkovski *et al.*, 1997b,c).

Larvae of the majority of cultured marine fish will eat dry feed and will thrive on it if they are weaned during or even before metamorphosis. The quality of the dry diet and the presence of attractants are considered other important factors determining weaning success among several species currently cultured (Lee *et al.*, 1996; Daniels and Hodson, 1999). Combining live feed and dry formulated diets (co-feeding) from an early developmental stage has been shown to improve growth and survival of marine fish larvae compared to the use of live feed only (Ehrlich *et al.*, 1989; Person Le Ruyet *et al.*, 1993; Walford and Lam, 1993; Lavens *et al.*, 1995; Rosenlund *et.al.*, 1997; Canavate and Fernandez-Diaz, 1999). Furthermore, it seems that co-feeding improves and stabilizes the nutritional condition of the larvae and it helps to condition the larvae to more easily accept the dry diet when live feed is withdrawn, resulting in a shorter weaning period (Rosenlund *et.al.*, 1997).

In this thesis, I developed weaning trials applicable for the different larviculture protocols used to culture two distinct marine species, Atlantic Cod (*Gadus morhua*), a temperate marine species, and Fat Snook (*Centropomus parallelus*), a tropical marine fish. Different feeding treatments including several combinations of live and dry food, were performed, aimed at determining the earliest point at which larvae of these species can be successfully weaned from *Artemia* onto a dry diet. Both species are of commercial importance in Canada and Brazil, respectively.

Chapter 2

The effects of early weaning on the behavior, growth and survival of Atlantic cod (*Gadus morhua*, Linnaeus 1758) larvae.

2.1 Introduction

Atlantic cod, *Gadus morhua*, is a marine fish that can be found in cool waters anywhere from the surface to depths of 500 or 600 m in the northern hemisphere of Atlantic Ocean (Lear, 1993). They tend to migrate in large schools from inshore waters to the edge of the continental shelf. Atlantic cod is a batch spawner. In Labrador and northern Newfoundland, spawning occurs from March to May, while on the south coast of Newfoundland, spawning tends to begin in May (Lear, 1993).

Atlantic cod has been an important food fish for centuries and the cod fishery is considered one of the primary reasons for the development of countries around the North Atlantic (Hutchings and Myers, 1994; Kurlansky, 1997). However, the Northern cod stock (off northeast Newfoundland and Labrador) has experienced substantial declines in abundance during the last several decades from over-exploitation and is facing commercial extinction (Cook *et al.*, 1997). Despite an ongoing moratorium on fishing the Northern cod stock since 1994, this stock has not showed signals of recovering (Fogarty *et al.*, 2001).

In the past, most research on cod dealt with older fish, which was the focus of the fishery. Much of the research into the early life of Atlantic cod has only been carried out in the last decade (Lear, 1993). Meanwhile, advances have been made towards the

establishment of cod aquaculture, and there is a renewed interest in the intensive culturing of Atlantic cod in northern Europe as an alternative to cage-farmed salmon (Shields, 2001).

In Canada, several recent studies carried out at the Ocean Sciences Centre have yielded crucial knowledge regarding Atlantic cod larviculture thus enabling the development of a reliable rearing technology for this species. Important aspects of intensive culturing environment such as light intensity, larval density and live food requirements for rearing Atlantic cod larvae were investigated recently (Puvanendran and Brown, 1999, 2002; Puvanendran *et al.*, 2002). Although considerable improvements in Atlantic cod production protocols were achieved, further studies aimed at lowering production costs in intensive rearing systems would greatly benefit commercial hatcheries.

Production of juveniles of all major marine fish species is still dependent on the supply of cultured live prey, such as rotifers, copepods and *Artemia* (Cahu and Zambonino-Infante, 2001). The search for alternative food substitutes (i.e. dry formulated diets) has been extensive and is still ongoing. Live food production is the most costly activity regarding the intensive culture of the early larval stages of marine fish (Person Le Ruyet *et al.*, 1993; Koven *et al.*, 2001). Moreover, the nutritional quality of the currently produced live feed used in cod rearing is still a concern, and the need for early weaning is being emphasized (Olsen, 1997).

However, few studies have addressed the importance of the elimination or partial substitution of live food in cod larval feeding schemes. Although Atlantic cod is weaned

after metamorphosis (Rosenlund *et al.*, 1993; Olsen, 1997), Tucker (1998) emphasized that prolongation of live foods specifically for this species could make weaning more difficult. On the other hand, insufficient amounts of live food may seriously compromise larval growth and survival depending on the degree of prey depletion (van der Meeren and Naas, 1997).

Even though some authors have reported negative effects on survival and growth of Atlantic cod when dry formulated diets were administered in early larval stages (Ringo *et al.* 1991), the reason for this may be lower water quality due to fouling caused by some of the dry diets (Rosenlund *et al.*, 1993). The performance of dry diets for marine fish larvae is frequently improved when they are co-fed with *Artemia* (Kolkovski *et al.*, 1997b; Rosenlund *et al.*, 1997; Koven *et al.*, 2001). Koven *et al.* (2001) suggested that nutritional factors in the live food could positively influence the ingestion, digestion and assimilation of the dry diets by larvae.

During the 1990s the pre-weaning period was greatly reduced in some species due to research results. These species include: European sea bass, *D. labrax*, (Person Le Ruyet *et al.*, 1993; Zambonino-Infante *et al.*, 1997; Cahu *et al.*, 1998) and Gilthead sea bream, *S. aurata* (Fernandez-Diaz and Yufera, 1997). Recently, Baskerville-Bridges and Kling (2000) investigated the effects of early weaning on laboratory reared Atlantic cod larvae. They achieved satisfactory survival rates weaning cod larvae from rotifers directly to dry diet by 22 days post-hatch (DPH). Although they found that cod larvae could be cultured through metamorphosis using only rotifers as live prey, a significant growth enhancement was attained when *Artemia* were co-fed to larvae, resulting in a 150%

increase in body weight, compared to larvae reared without *Artemia*. However, they only ran one trial using *Artemia*. Given the better performance of the larvae on this food, I decided to expand on their study and test the effect of using *Artemia* for various time periods during weaning.

I intend to evaluate the behaviour, growth and survival of Atlantic cod larvae reared under different feeding schemes using *Artemia* during early weaning trials. Two experiments were performed in order to determine when and how to effectively wean Atlantic cod larvae. My hypothesis is that cod larvae would show satisfactory growth performance when dry diets were administered along with *Artemia* during early weaning. In the first experiment I attempted to determine the most reasonable co-feeding period with live food (rotifers and *Artemia*). I also included a treatment where cod larvae were weaned onto dry diet directly from rotifers, such as the treatment employed by Baskerville-Bridges and Kling (2000).

In the second experiment, different weaning ages were studied to determine when it is most suitable to start co-feeding with *Artemia*.

2.2 Materials and methods

The experiments were conducted during the spring of 2001 and spring/summer of 2002 at the Ocean Sciences Centre (OSC), Memorial University of Newfoundland.

2.2.1 *Experiment I*

2.2.1.1 Broodstock handling, egg incubation and husbandry procedures

Fertilized eggs were obtained through naturally spawning of mature Atlantic cod broodstock transferred from the Bay Bulls cage site (Newfoundland Aqua Ventures) to the Aquaculture Research Development Facility (ARDF/OSC).

Eggs were disinfected with Zep Perosan® (hydrogen peroxide + peroxyacetic acid + acidic acid), incubated at 5-7°C and hatched at about 100.6 degree-days. Once hatched, approximately 22,500 larvae were transferred to each of three cylindrical incubator tanks of 280 litres. They did not receive any food until 2 days post-hatch (DPH).

From 3 day post-hatch (DPH) onwards, rotifers (*Brachionus plicatilis*) raised on microalgae *Isochrysis sp.* (T-ISO; Tahitian strain) were fed to larvae until 9 DPH. Throughout the next two days larvae were fed on a mixture of rotifers enriched with T-ISO and Algamac 2000® (1:1) and by 12 DPH onwards, only rotifers enriched with Algamac 2000® were fed to larvae until 25 DPH.

A prey concentration of 2000 rotifers/litre was initially employed (up to 6 DPH) followed by a subsequent higher concentration of 4000 rotifers/litre (Puvanendran and Brown, 1999). Live food was added to rearing tanks three times a day (10:00, 16:00 and 22:00). Rearing tanks were greened daily with microalgae *Isochrysis* sp. The seawater

temperature in tanks was maintained between 10-12°C, set up in a flow-through system (at 1.2 litre/min) and tanks were kept under continuous light 24 hours a day (approximately 1000 lux).

2.2.1.2 Experimental set up

On day 23 post-hatch (234.2 degree-days), batches of 300 larvae (mean standard length of 7.8mm; S.D. =0.7) were transferred from the rearing tanks and stocked in each of twelve, black rectangular experimental aquaria of 30 litres capacity. This larval density was chosen from previous studies which predicted a survival of about 25% from a hypothetical initial stocking density of 40 larvae/litre (Puvanendran and Brown, 1999).

Experiment I started at 25 DPH (256.5 degree-days) after 2 days of acclimation in experimental aquaria. Each treatment (four feeding trials; Figure 2.1) had three replicates, completely randomized and were assigned as follows:

Treatment 1: Control treatment, larvae received no *Artemia* and were weaned from rotifers directly to dry food. A co-feeding procedure with enriched rotifers (Algamac 2000®) was done for seven days, at a concentration of 4000prey/litre, decreasing to 2000prey/litre on the two last days of co-feeding (Figure 2.1).

Figure 2.1 Feeding trials (T) used for weaning Atlantic cod larvae in **Experiment I**. Nutra Marine® dry food was employed in this experiment. (DPH: Days post-hatch).

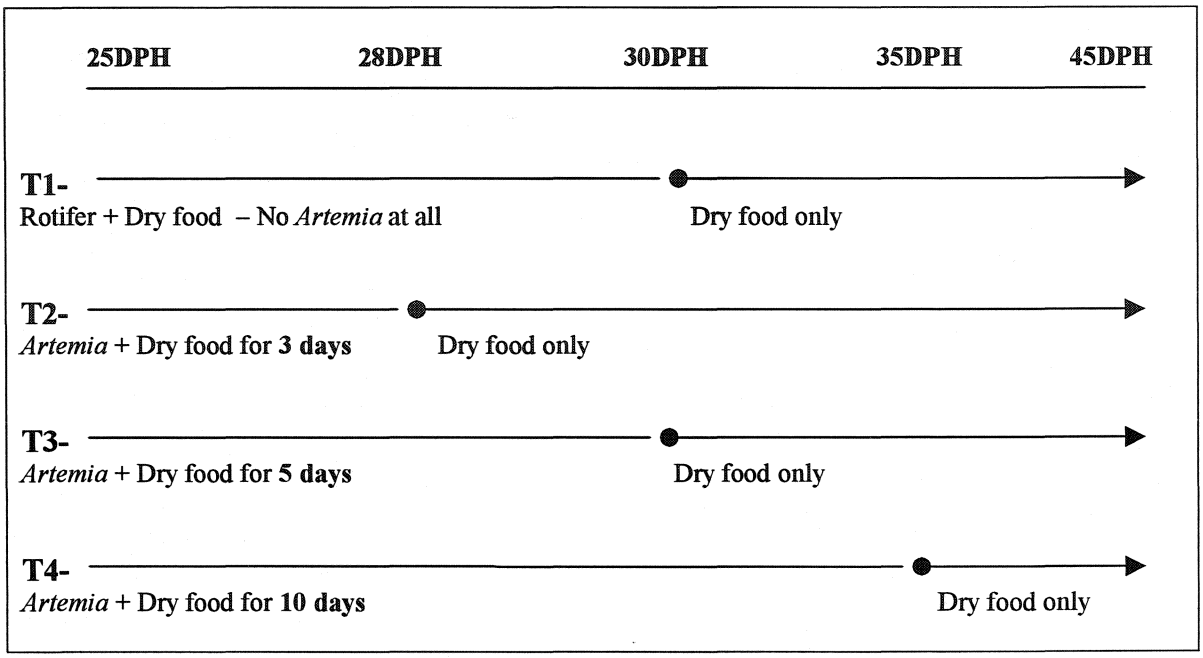

Table 2.1 Nutritional composition of Nutra Marine®

(Perla Larva*) dry diet utilized in **Experiment I**.

Chemical analysis	%
Protein	62
Oil	11
N.F.E.	9
Moisture	8
Ash	10

* - Ingredients: LT Fishmeal, Wheat products, Fish protein concentrate, Fish oil, Crustacean Meal, Vitamins, Minerals.
Source: Nutra Marine®.

Treatments 2, 3 & 4: Enriched *Artemia* (Great Salt Lake strain) was fed to larvae over 3, 5 and 10 days respectively. In these trials, a mixture of enriched rotifers (administered only at morning feeding) and *Artemia* nauplii (provided during the afternoon and evening feedings) were fed to larvae during the two first days for each feeding protocol, in order to gradually switch larvae to *Artemia*. Enriched *Artemia* (Algamac 3010®, DC DHA Selco® and Hydrolyzed krill protein were used as enrichments and were used on alternate days) was the only live prey provided for the remaining days of each co-feeding trial. *Artemia* were introduced in tanks initially at 2000prey/litre decreasing to 1000 and 500prey/litre on the two last days of co-feeding respectively (Figure 2.1).

Nutra Marine® dry food pellets (Perla Larva 6.0 ranging from 400-600μm; see Table 2.1 for nutritional composition) were sieved through nylon mesh of 300-500μm in order to obtain suitable pellet size for the larvae. At the start of the experiment, larvae were provided with the smallest sized pellet (300μm) in a higher proportion and the pellet size was increased gradually as the larvae grew. Dry diet was used along with live feed from the first experimental day.

2.2.2 *Experiment II*

2.2.2.1 Broodstock handling, egg incubation and husbandry procedures

Fertilized eggs were obtained from broodstock maintained as in Experiment I. Larvae hatched after about 105.4 degree-days, were transferred to 6,000 litre tanks at a stoking density of approximately 40 larvae/litre and were raised following the same rearing procedures described for Experiment I.

2.2.2.2 Experimental set up

On day 23 post-hatch (239.7 degree-days), batches of 300 larvae (mean standard length of 8.2mm; S.D. =0.4) were transferred from the 6000 rearing tanks and stocked in each of twelve, black 30 litres rectangular experimental aquaria. Experiment II started at 25 DPH (261.8 degree-days) after 2 days of acclimation in experimental aquaria where larvae received only enriched rotifers. Each treatment (four feeding trials; see Figure 2.2) had three replicates, as follows:

Treatment 1: Control treatment, larvae received only *Artemia* until the end of the experiment. No dry food was used.

Treatments 2, 3 & 4: Enriched *Artemia* (Great Salt Lake strain) was co-fed to larvae for a period of 10 days, starting at 25, 30 and 35 DPH respectively. The co-feeding period of 10 days was chosen based on the best results from Experiment I (treatment 4). In Experiment II, I used Dana feed® A/S (COD DAN-PEL 1656; see Table 2.2 for nutritional composition) dry diet instead of Nutra Marine®, since the first one was employed in the ARDF/OSC cod production protocol for the year of 2002.

Figure 2.2 Feeding trials (T) used for weaning Atlantic cod larvae in **Experiment II**. Dana Feed A/S® dry food was employed in this experiment. The co-feeding period of 10 days (*Artemia* + dry food) was employed to all treatments, except for treatment 1 (T1). (DPH: Days post-hatch).

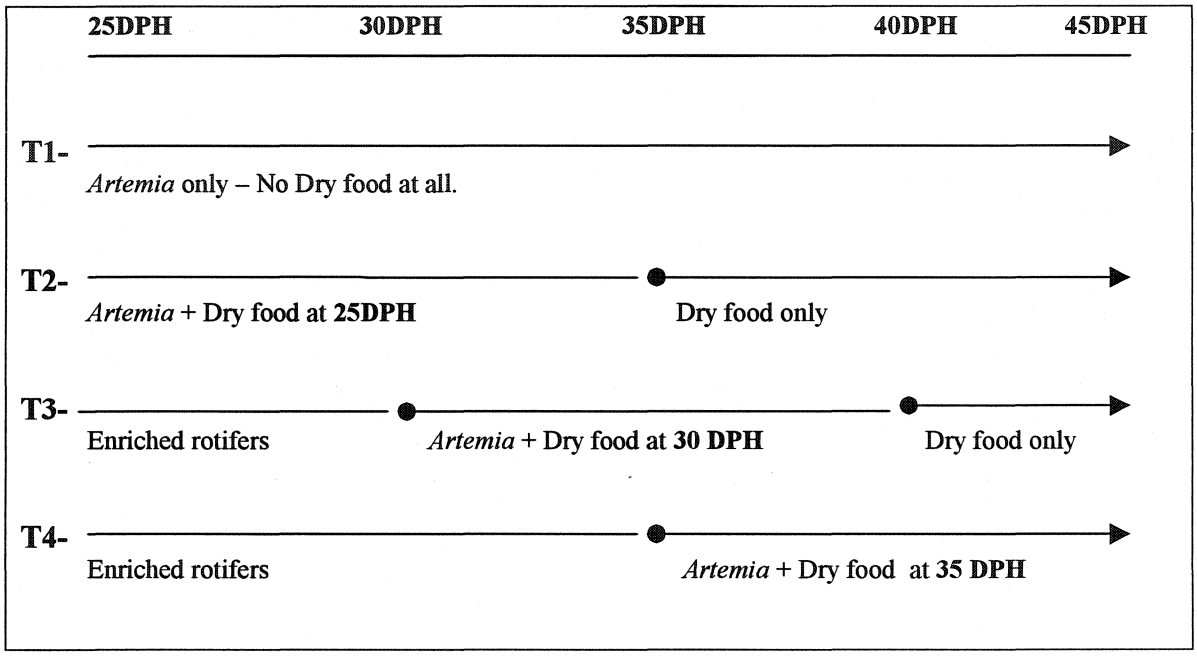

Table 2.2 Nutritional composition of Dana feed® A/S (COD DAN-PEL 1656*) utilized in **Experiment II**.

Chemical analysis	%	Energy calculation	
Crude protein (min)	56.0	Gross energy (kcal)	5172
Crude oils and fats (min)	16.0	Metabolisable energy (kcal)	4078
Carbohydrates	11.0	Metabolisable energy (MJ)	17.1
Fibre (max)	1.1		
Crude ash (max)	10.4		
Phosphorus (max)	1.5		

* - Ingredients: Fish products, cereal grains, oils and fats, pre-mix, vitamins and minerals. Source: Dana feed® A/S.

In this experiment the same feeding schedule as in treatments 2, 3 and 4 of Experiment I was used, where a mixture of rotifers and *Artemia* was used as live food for the first two days of the experimental period. *Artemia* was enriched and employed in the same manner as described for Experiment I. The Dana feed® A/S dry food pellets (ranging from 400-600μm) were sieved through nylon mesh of 300-500μm in order to obtain suitable pellet size for the mouth size of larvae. At the start of the experiment, larvae were given the smallest sized pellet (300μm) and the pellet size was increased gradually as the larvae grew.

In both experiments the dry diet was added to aquaria by hand, a half-hour before adding live prey. Automatic feeders (Lifeguard® automatic fish feeders, Rainbow Lifeguard Aquarium Products, CA, USA) were used 24 hours a day releasing pellets to aquaria approximately every 2 hours. Live food was added to experimental tanks three times daily (10:00, 16:00 and 22:00) and always co-fed along with dry feed.

Each experimental aquarium had aeration and was set up with a flow-through water system at a rate of 1.2 litre/min. The outflow was covered by 600 μm nylon mesh. A period of 24h of light (approximately 1000 lux) was employed throughout the experimental period. The water temperature remained around 10-13°C in Experiment I and around 10-14°C in Experiment II.

Aquaria were cleaned (siphoned) prior to the first daily feeding and mortality rates were estimated from number of dead larvae counted daily.

Both experiments lasted until 45 DPH, when larvae showed signs of reaching metamorphosis (development of distinct dorsal and ventral fins).

2.2.3 Data collection

At the beginning of both experiments, samples of 40 larvae were randomly taken from the common rearing tanks and slightly anaesthetized in 0.1% tricaine methanesulfonate (MS-222) for initial measurements. On 25, 29, 34, 39 and 45 days post-hatch (DPH), samples of 10 larvae from each experimental tank were taken for measurements.

Larvae were measured for standard length (mm; from the tip of upper jaw until the end of notochord) and myotome height (mm; depth of body posterior to the anus) by recording digital images of larvae with a camera attached to a computer (Pixera Viewfinder© Version 2.6, Pixera Corporation). Larvae were posteriorly measured to the nearest 0.1 mm using an image analysis software (Matrox Inspector© Version 3.0, Matrox Electronic System Ltd).

Behavioral observations of larvae were performed on 25, 26, 27, 28, 29, 31, 34, 38, 40 and 44 DPH in Experiment I, and on 25, 28, 30, 33, 34, 35, 38, 40, 42, 44 and 45 DPH in Experiment II. The observations were conducted using the software "The Observer" (Noldus Information Technology, Netherlands, 1990) installed on an event recorder (PSION Workabout®). On each day of observation, five larvae from each of two tanks (per treatment) were randomly chosen and observed for 120 seconds each using the focal animal technique as defined by Altman (1974).

The computer's keyboard was configured using "The Observer" so that each behavioral pattern to be observed was assigned to a distinct key. Six behavioral patterns (Puvanendran and Brown, 1999) were used (Table 2.3). Recorded data consisted of time spent swimming and time motionless and the frequency of feeding choices by Atlantic cod larvae. Survival was estimated by determining the difference from the initial number of larvae and the remaining larvae in each aquarium at the end of the experiment.

Table 2.3 Operational definition of behavioral activities observed during the cod weaning trials.

Activities	**Definition**
Swim	Forward movement of the larvae through water column
Motionless	Larvae is motionless
Rotifer	Larvae captures and ingests rotifer
Artemia	Larvae captures and ingests *Artemia*
Dry food	Larvae ingests dry food pellet
Reject	Larvae bites but rejects pellet

Adapted from Puvanendran and Brown (1999).

2.2.4 Data Analysis

2.2.4.1 Growth parameters

A *Condition index* using the ratio of body depth to body length was used to evaluate the condition of larvae for each treatment (Koslow *et al.*, 1985).

Condition index = myotome height / standard length

The growth rate of larvae was determined using the *Length-specific growth rate* (SGR) equation (Cowan and Houde, 1990):

$$SGR = (\ln(L_t) - \ln(L_0)) / t) \times 100$$

Where L_t is the final length of larvae at time t, L_0 is the initial length, and t is the period of time between L_t and L_0 in days.

Mortality was estimated by the instantaneous mortality rate ($Z\ .day^{-1}$) equation (Cowan and Houde, 1990):

$$Z = (\ln(Nt) - \ln(N_0)) / t \times 100$$

Where Nt is the number of larvae alive by the end of time t, N_0 is the initial number of larvae alive at the beginning of time t, and t is the period of time between N_t and N_0 in days.

2.2.4.2 Statistical analysis

Initially, data on growth parameters and behavioral observations were evaluated for normality using the Kolmogorov-Smirnov nonparametric test. The plot of residuals was analyzed to assure that assumptions of analysis of variance (ANOVA) were satisfied (Sokal and Rohlf, 1995). SGR, condition index and survival data were log transformed

before conducting analyses in order to meet the parametric requirements of ANOVA. The effects of treatments (as fixed factor) on growth parameters in the two experiments were analyzed using one-way ANOVA. Differences among treatments were considered significant at $P \leq 0.05$, and Tukey's HSD post hoc multiple range tests were subsequently performed to address any significant differences among treatment means for each sampling period (Sokal and Rohlf, 1995).

Data on frequency of feeding events (rotifer, *Artemia*, dry food and reject) for the two experiments could not be normalized by any transformation. Thus, a Kruskal-Wallis nonparametric test was applied to these variables (frequency for each chosen food item) while the Mann-Whitney test was performed whenever the comparison was between only two treatments (Sokal and Rholf, 1995).

In Experiment I, rotifer ingestion frequency data (from treatment 1) was pooled together with *Artemia* ingestion frequency (from all other treatments) and considered as one variable (live food ingestion frequency). In Experiment II, *Artemia* was used as live prey in all treatments, thus only its ingestion frequency data was used as the live food variable. Data on "swim" and "motionless" durations for the two experiments were normally distributed and were analyzed by analysis of variance. Analyses were performed using the SPSS PC + software package (SPSS, 1999).

2.3 Results

2.3.1 *Experiment I*

2.3.1.1 Growth

At the beginning of the experiment, larvae in all treatments measured 7.8mm in standard length (S.D. =0.7) and there were no significant differences among the groups (F= 1.651; df=3; P=0.0987).

Results from analysis of variance showed that standard length of cod larvae were significantly influenced by treatment (F=17.43, df=3, P=0.0002; Figure 2.3). However, no significant differences in standard length were detected among larvae in treatments up to 34 DPH (Table 2.4; Tukey's multiple range test; α=0.05). From this age onwards, larvae reared in treatment 1 (rotifer as live food) and 2 (*Artemia* for 3 days) were significantly smaller than those in the treatment 3 (*Artemia* for 5 days) and treatment 4 (*Artemia* for 10 days). Larvae in treatment 4 were significantly larger than larvae in all other treatments by the end of the experiment (Table 2.4; Figure 2.3).

Treatment had a significant influence on the condition of larvae (F=7.45, df=3, P=0.0025). By the end of the experiment larvae reared in treatment 4 had a significantly better condition than larvae reared in treatments 1 and 2 (Table 2.5; Figure 2.4).

Treatment also significantly influenced growth rate (SGR) of larvae (F=12.82, df=3, P=0.0005). During the first growth period (25-29 DPH), larvae in treatments 2, 3 and 4 had similar growth rates, but from the end of the second growth period (from 34 DPH onwards), larvae in treatment 4 showed better growth rate compared to larvae in all other treatments, while larvae in treatment 2 had the lowest growth rate (Table 2.6; Figure 2.5).

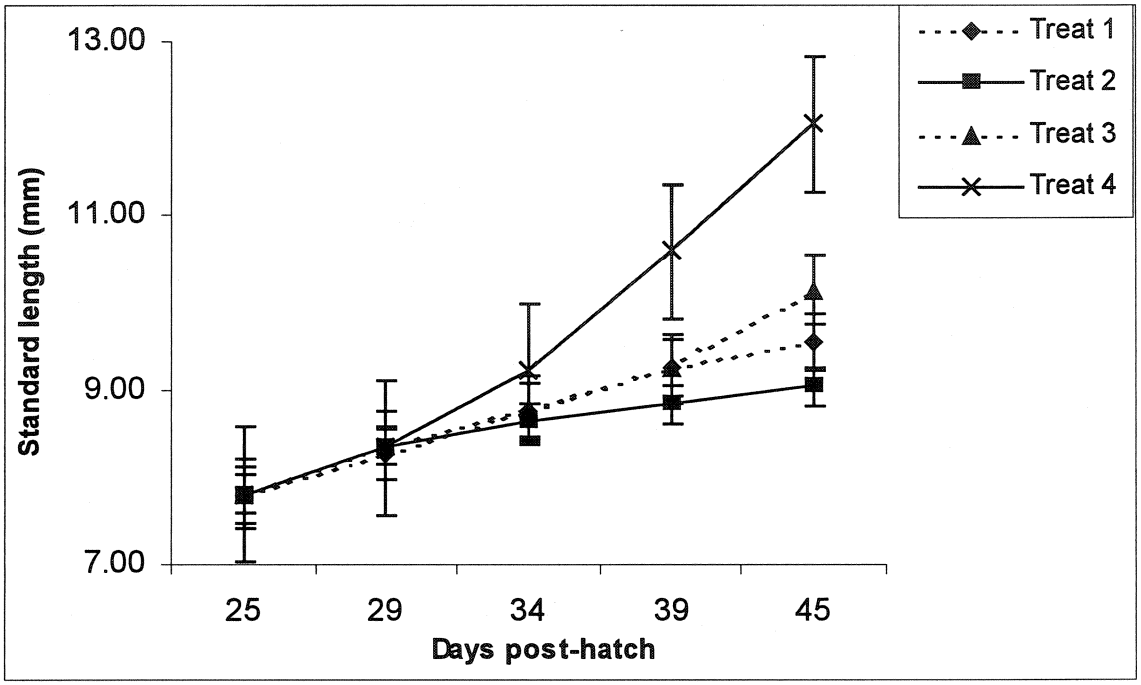

Figure 2.3 Mean **standard length** (mm) of Atlantic cod larvae in different weaning treatments over age (days post-hatch) in **Experiment I**. Each symbol represents mean of 30 larvae measured per treatment per sampling day. Vertical bars indicate SE. See Figure 2.1 for details of feeding trials.

Table 2.4 Results from Tukey's HSD analysis comparing **standard length** of Atlantic cod larvae in early weaning trials employed in **Experiment I**.

	Treatment comparison					
Age (DPH)	**1 – 2**	**1 – 3**	**1 – 4**	**2 – 3**	**2 – 4**	**3 – 4**
29	-0.081	-0.094	-0.07	-0.011	0.01	0.021
34	0.144	-0.016	-0.442	-0.157	-0.58	-0.435
39	0.421	0.011	-1.347*	-0.417	-1.762*	-1.352*
45	-0.513	-0.592	-2.514*	-1.17*	-3.124*	-1.928*

* - Significant difference at α=0.05

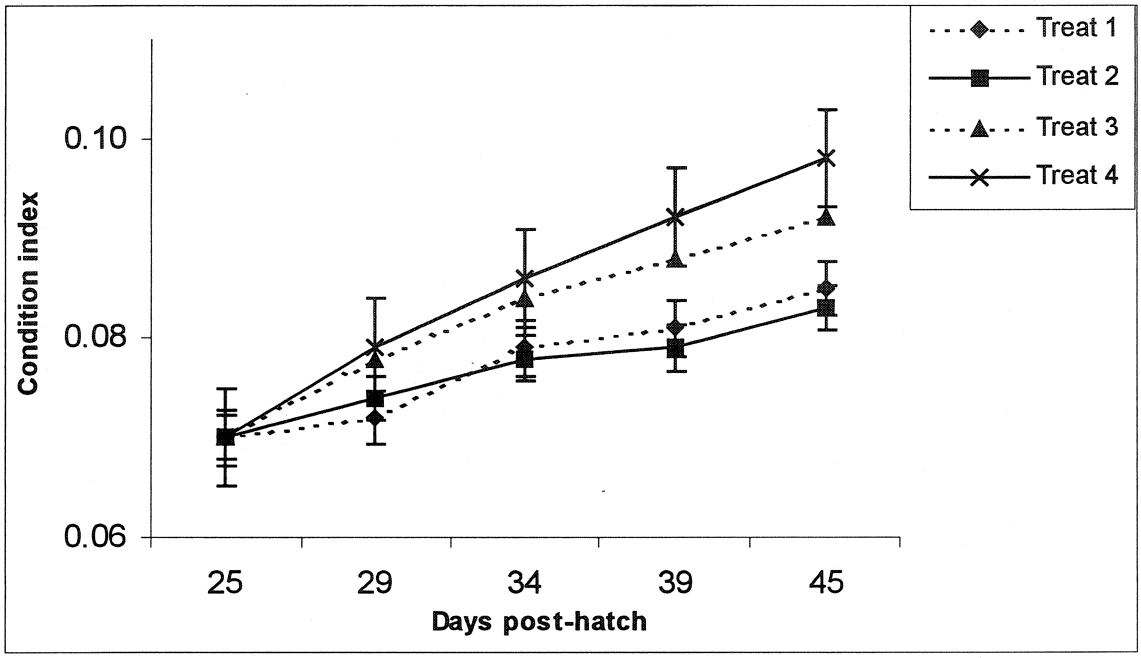

Figure 2.4 Mean **condition index** of Atlantic cod larvae in different feeding treatments over age (days post-hatch) in **Experiment I**. Each symbol represents mean of 30 larvae measured per sampling day. Vertical bars indicate SE. See text for details.

Table 2.5 Results from Tukey's HSD analysis comparing **condition index** of Atlantic cod larvae in early weaning trials employed in **Experiment I**.

	Treatment comparison					
Age (DPH)	**1 – 2**	**1 – 3**	**1 – 4**	**2 – 3**	**2 – 4**	**3 – 4**
29	-0.012	-0.035	-0.040	-0.023	-0.029	-0.005
34	0.005	-0.026	-0.036	-0.032	-0.042	-0.010
39	0.011	-0.036	-0.055	-0.046	-0.068 *	-0.019
45	0.010	-0.034	-0.067 *	-0.044	-0.074 *	-0.027

* - Significant difference at α=0.05

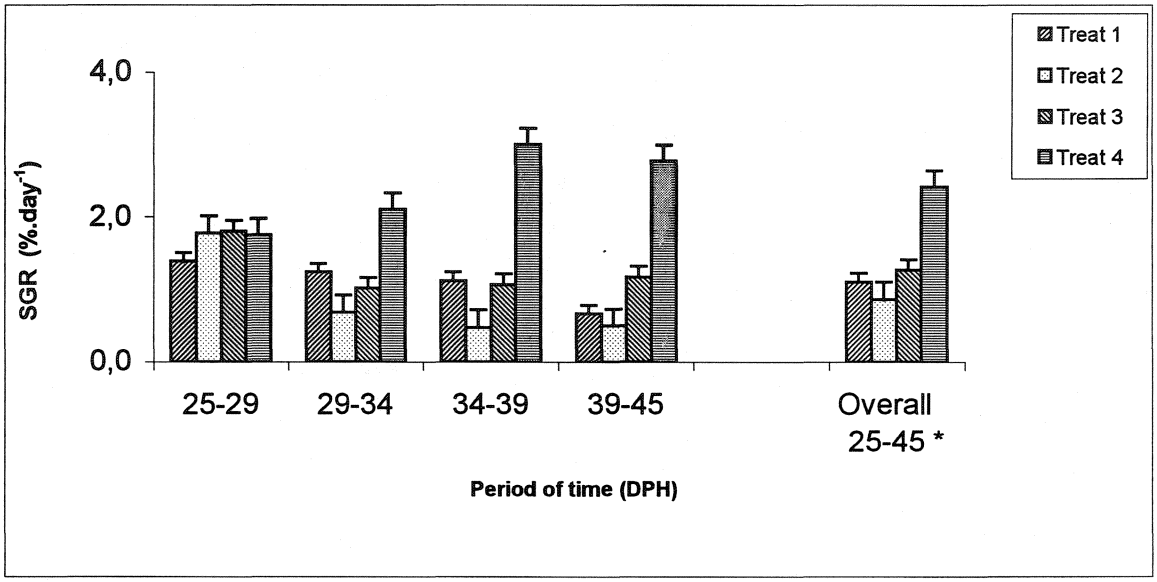

Figure 2.5 Means of **length-specific growth rate** (**SGR**; %.day^{-1}) of Atlantic cod larvae in weaning trials employed in **Experiment I**. Each bar represents the mean of SGR of 30 larvae measured per treatment measured at the end of each growth period. Vertical bars indicate SE. See text for details.
(* - Represents the overall SGR achieved by larvae in all treatments for the total experimental period).

Table 2.6 Results from Tukey's HSD analysis comparing **SGR** of Atlantic cod larvae in early weaning trials employed in **Experiment I**.

	Treatment comparison					
Age (DPH)	**1 – 2**	**1 – 3**	**1 – 4**	**2 – 3**	**2 – 4**	**3 – 4**
25-29	-0.108	-0.1154	-0.1032	-0.0073	0.0049	0.0122
29-34	0.2574	0.0856	-0.2323	-0.1718	-0.4897 *	-0.324 *
34-39	0.3732	0.0201	-0.4332	-0.3532	-0.8065 *	-0.4533*
39-45	0.1293	-0.2486	-0.6229*	-0.3780	-0.7523*	-0.3743*

* - Significant difference at α=0.05

By the end of the experiment, there were no significant differences in standard length, condition and SGR between treatments 1, 2 and 3, while larvae in treatment 4 were significantly larger than larvae in treatments 1 and 2, and had the highest SGR compared to all other treatments. The poorest growth in terms of standard length and length-standard growth rate occurred when larvae received *Artemia* for only 3 days. The average length-specific growth rate (SGR; $\%.day^{-1}$) for the experimental period ranged from 0.57 to 3.04 $\%.day^{-1}$ among all feeding trials.

2.3.1.2 Instantaneous mortality (Z) and Survival

Treatment had a significant effect on mortality rate (Z) of cod larvae (F=8.63, df=3, P=0.0016). On 29 DPH, there were no significant differences among treatments 2, 3 and 4 (all using *Artemia* as live food) but all had significantly higher mortality rates compared to treatment 1 (co-fed rotifer) (Table 2.7; Figure 2.6). However, from 34 DPH until the end of the experiment, treatment 4 had significantly less mortality than any other treatment (Table 2.7).

Survival of cod larvae was significantly affected by treatment (F=34.05, df= 3, P<0.0001) and was significantly higher when *Artemia* was used for 10 days (treatment 4; 25.7%) compared to any other treatment (Figure 2.7; Table 2.8; Tukey's multiple range test; α=0.05). By the end of the experiment, there was no significant difference among treatment 1 and treatment 3 (Table 2.8; Figure 2.7).

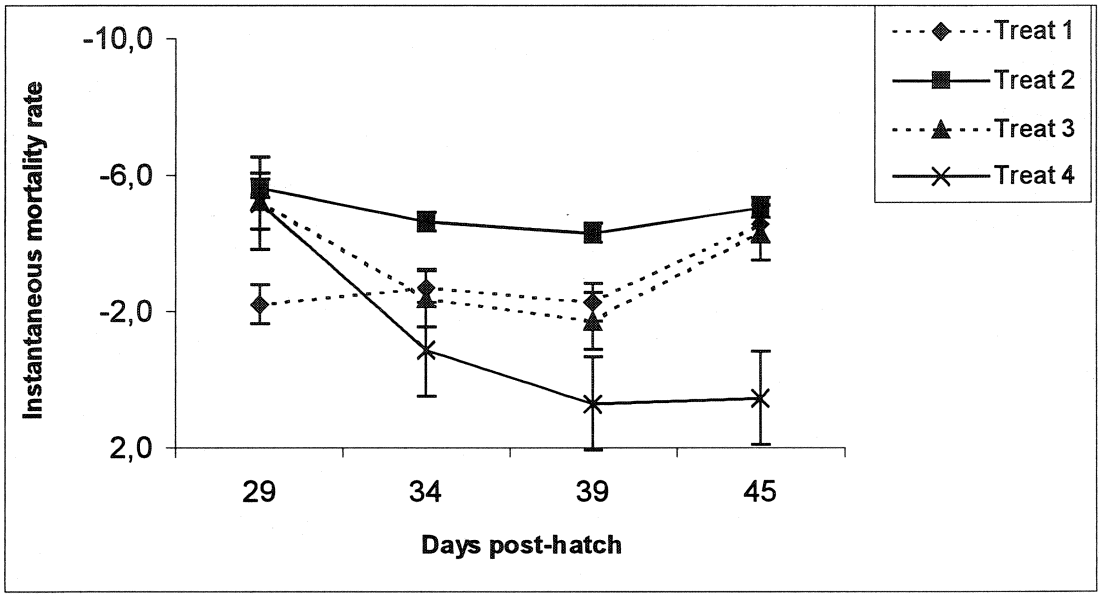

Figure 2.6 Mean **instantaneous mortality rate (Z)** of Atlantic cod larvae in different weaning treatments over age (days post-hatch) in **Experiment I**. Each symbol represents mean of Z calculated for the periods indicated in Figure 2.5. Vertical bars indicate SE.

Table 2.7 Results from Tukey's HSD analysis comparing **instantaneous mortality rates (Z)** of Atlantic cod larvae in early weaning trials employed in **Experiment I**.

Age (DPH)	Treatment comparison					
	1 – 2	1 – 3	1 – 4	2 – 3	2 – 4	3 – 4
29	3.381 *	3.002 *	2.943 *	-0.379	-0.438	-0.059
34	1.931	-0.0303	-1.803	-2.234 *	-3.734 *	-1.500
39	2.026	-0.545	-2.974 *	-2.571	-5.004 *	-2.229
45	0.491	-0.219	-5.078 *	-0.710	-5.569 *	-4.859 *

* - Significant difference at α=0.05.

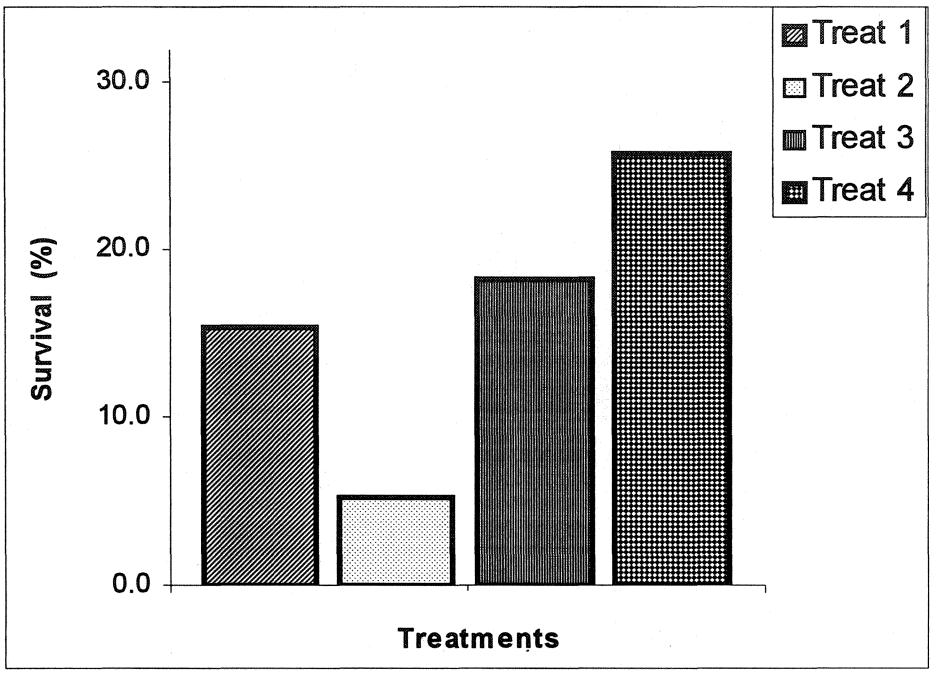

Figure 2.7 Overall **survival** of Atlantic cod larvae reared in different feeding treatments at the end of the **Experiment I**.

Table 2.8 Results from Tukey's HSD analysis comparing **survival** of Atlantic cod larvae in early weaning trials by the end of **Experiment I**.

Treatment comparison	Mean differences
1 – 2	0.475 *
1 – 3	-0.076
1 – 4	-0.223 *
2 – 3	-0.551 *
2 – 4	-0.698 *
3 - 4	-0.147 *

* - Significant difference at α=0.05

2.3.1.3 Behaviour

Atlantic cod larvae spent on average, 12.43% of their time swimming and were motionless most of the time (around 82.63%). Larvae spent approximately 4.94% of the time foraging.

The overall average swimming duration was 7.5 ± 0.61 sec/min among all treatments at the end of the experiment. Swimming duration was significantly affected by treatment (F= 23.25; df=3; P<0.0001; Figure 2.8). By 36 DPH, larvae in treatment 2 swam significantly more than larvae in treatments 3 and 4 (Figure 2.8; Table 2.9; Tukey's multiple range test; α=0.05). However, by the end of the experiment there were no significant differences between treatments. Contrary to expectations, the duration of motionless did not show any significant differences among treatments (F= 2.007; df=3; P=0.1384; Figure 2.9).

Larvae reared in all feeding trials did not show any significant difference in live food ingestion during 25 to 27 DPH (4 treatments, Kruskal-Wallis test; X^2=4.201, df=3, P=0.240), 25 to 29 DPH (3 treatments, Kruskal-Wallis test; X^2= 2.457, df=2, P=0.292), and 25 to 31 DPH (2 treatments, Mann-Whitney test; Z= -0.987, P=0.1618) (Figures 2.10 to 2.13). However, treatment significantly affected dry food ingestion of larvae (Kruskal-Wallis test; X^2=8.741, df=3, P=0.032; Figures 2.10 to 2.13). By the end of the experiment, larvae reared in treatment 4 had a significantly higher frequency of dry food ingestion than larvae in all other treatments (Table 2.10; Mann-Whitney test; Figures 2.10 to 2.13). There was no significant difference among treatments for reject frequency (Kruskal-Wallis test; X^2=4.254, df=3, P=0.235).

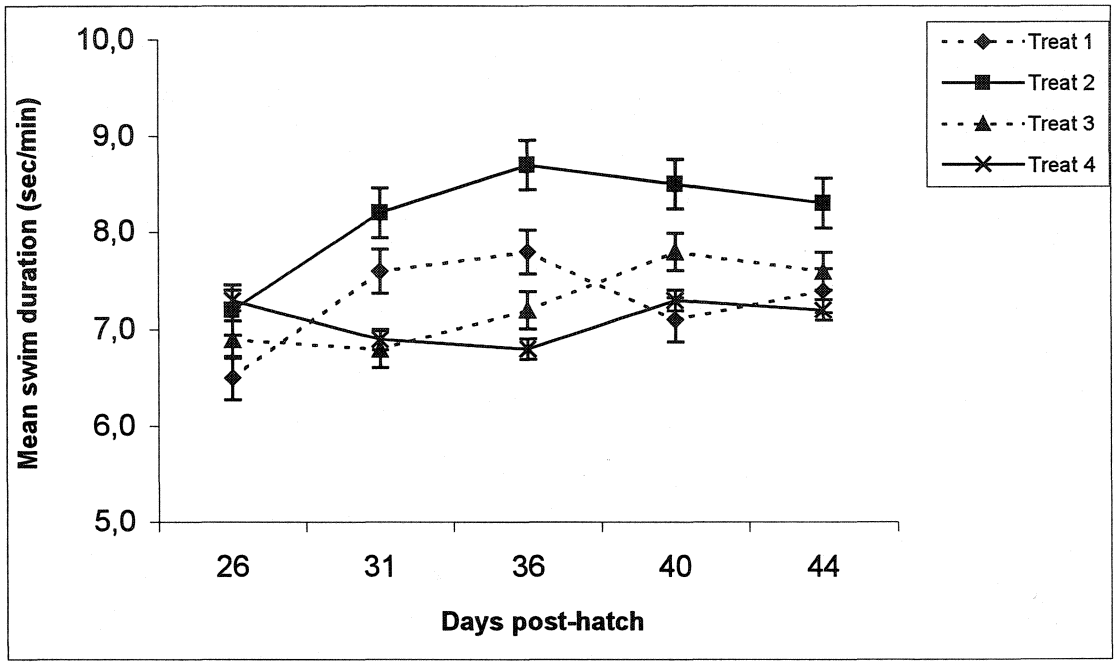

Figure 2.8 Mean **swim duration** of Atlantic cod larvae in different weaning treatments over age (days post-hatch) in **Experiment I**. Each symbol represents mean of 10 larvae observed per treatment per observational day. Vertical bars indicate S.E. See Figure 2.1 for details of feeding trials.

Table 2.9 Results from Tukey's HSD analysis comparing the mean **swimming** time spent by Atlantic cod larvae in early weaning trials employed in **Experiment I**.

	Treatment comparison					
Age (DPH)	**1 – 2**	**1 – 3**	**1 – 4**	**2 – 3**	**2 – 4**	**3 – 4**
26	-0.7	-0.4	-0.8	0.3	-0.1	-0.4
31	-0.6	0.8	0.7	1.4	1.3	-0.1
36	-0.9	0.6	1.0	1.5 *	1.9 *	0.4
40	-1.4	-0.7	-0.2	0.7	1.2	0.5
44	-0.9	-0.2	0.2	0.7	1.1	0.4

* - Significant difference at α=0.05

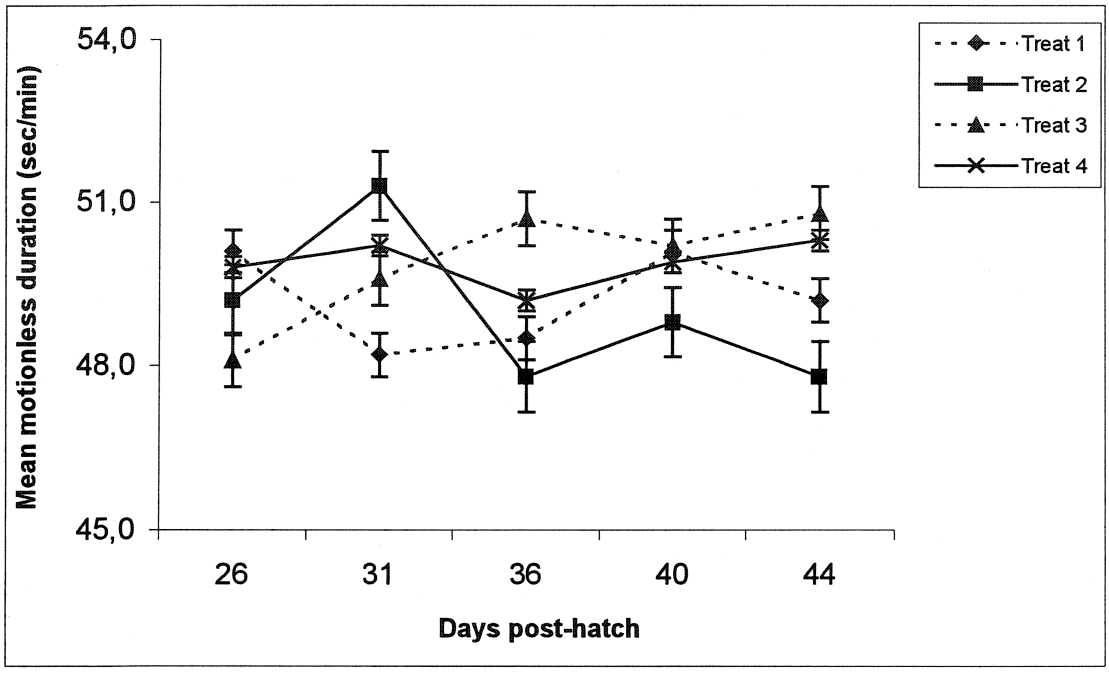

Figure 2.9 Mean **motionless duration** of Atlantic cod larvae reared at different feeding treatments over age (days post-hatch) in **Experiment I**. Each point represents mean of 10 larvae observed per treatment per observational day. Vertical bars indicate S.E. See Figure 2.1 for details of feeding trials.

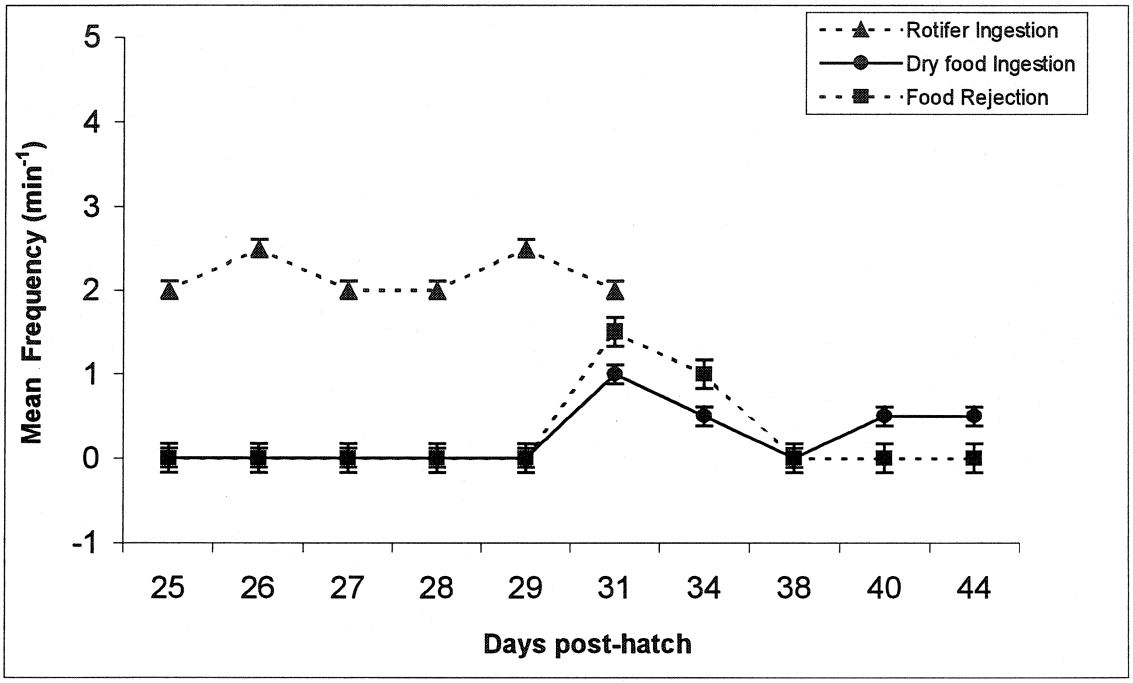

Figure 2.10 Mean frequency of ingestion and rejection of different food items by Atlantic cod larvae reared in **Treatment 1** over age (days post-hatch) in **Experiment I**. Each point represents mean of 10 larvae observed treatment per observational day. Vertical bars indicate S.E. See Figure 2.1 for details of feeding trials.

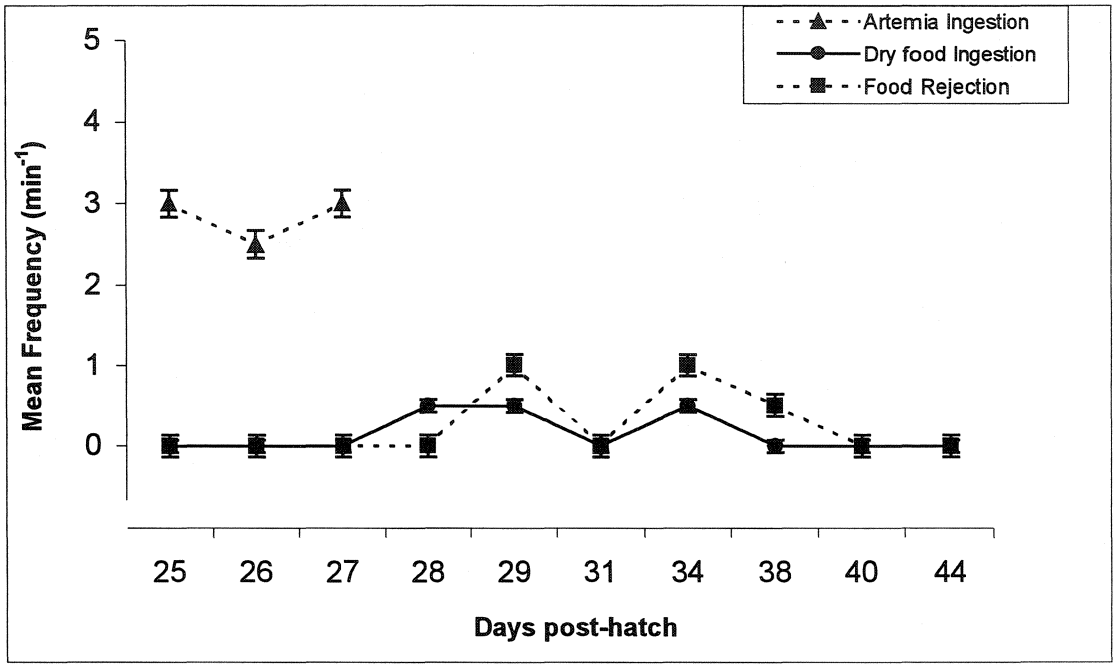

Figure 2.11 Mean frequency of ingestion and rejection of different food items by Atlantic cod larvae in **Treatment 2** over age (days post-hatch) in **Experiment I**. Each symbol represents mean of 10 larvae observed treatment per observational day. Vertical bars indicate S.E.

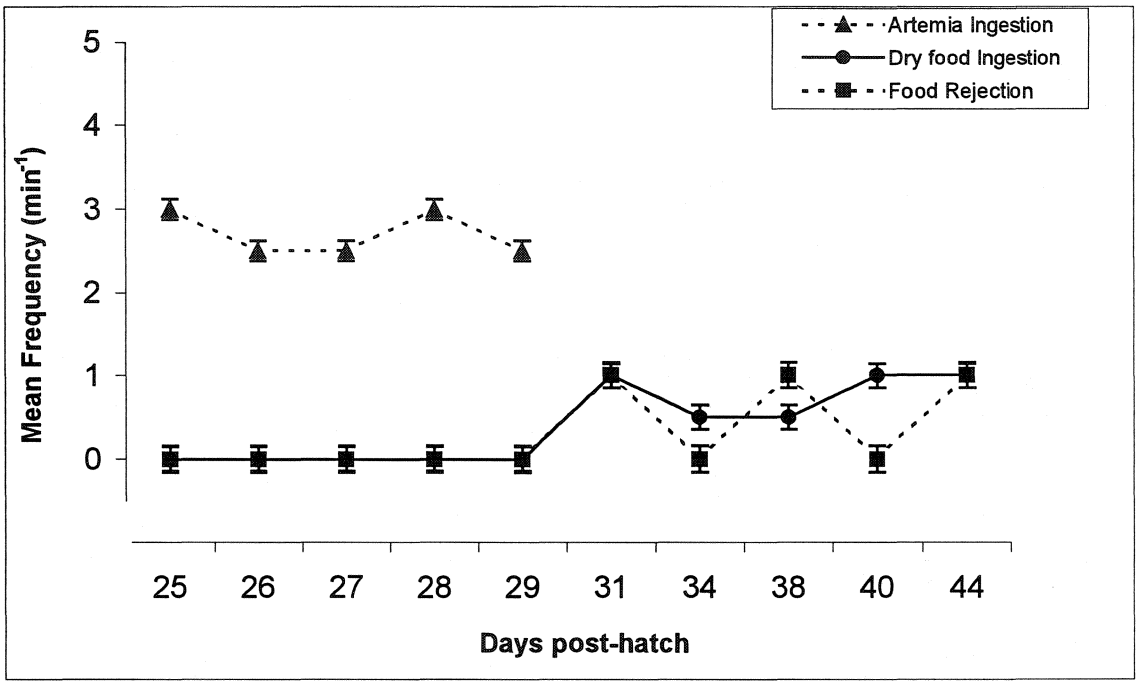

Figure 2.12 Mean frequency of ingestion and rejection of different food items by Atlantic cod larvae in **Treatment 3** over age (days post-hatch) in **Experiment I**. Each symbol represents mean of 10 larvae observed treatment per observational day. Vertical bars indicate S.E.

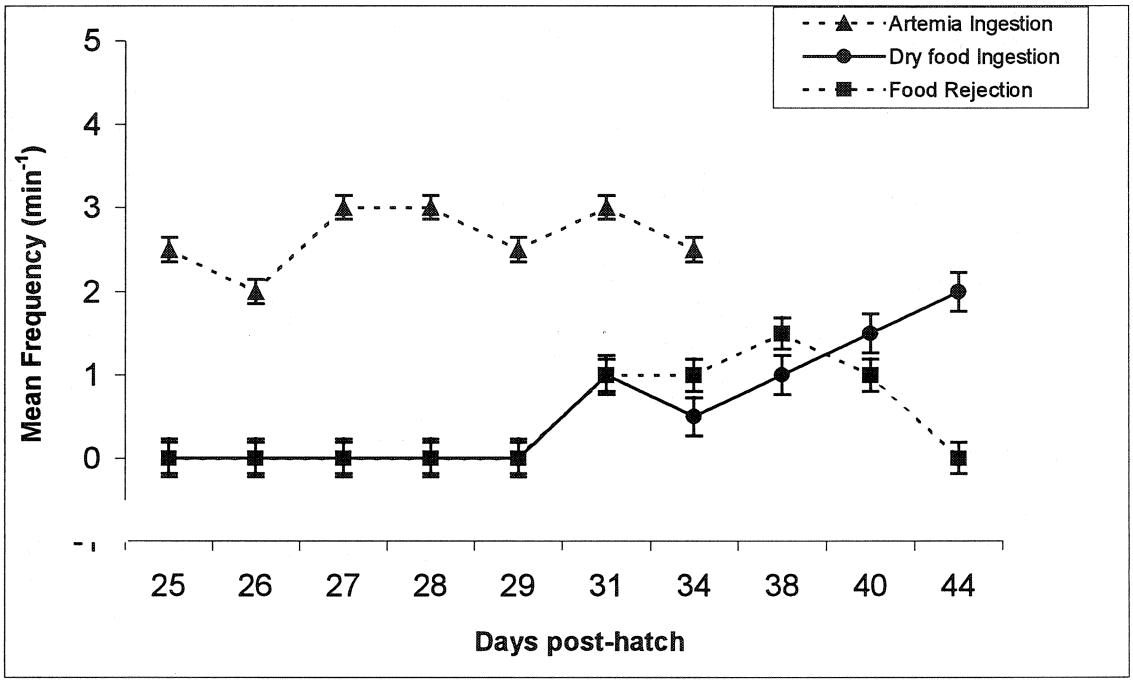

Figure 2.13 Mean frequency of ingestion and rejection of different food items by Atlantic cod larvae in **Treatment 4** over age (days post-hatch) in **Experiment I**. Each symbol represents mean of 10 larvae observed treatment per observational day. Vertical bars indicate S.E.

Table 2.10 Results from Mann-Whitney test, comparing the **dry food ingestion** frequency by Atlantic cod larvae in early weaning trials employed in **Experiment I**.

Treatment comparison	Mann-Whitney test results
1 – 2	Z= -1.432; P= 0.638
1 – 3	Z= -1.654; P= 0.098
1 – 4	Z= -2.985; P= 0.002 *
2 – 3	Z= -1.347; P= 0.178
2 – 4	Z= -3.052; P= 0.002 *
3 – 4	Z= -2.652; P= 0.008 *

* - Significant difference at $P<0.05$

2.3.2 *Experiment II*

2.3.2.1 Growth

Larvae averaged 8.2mm (standard length; S.D.= 0.4). There were no significant differences among larvae in all treatments prior to the start of the weaning trials in Experiment II (ANOVA; F= 2.025; df=3; P=0.068).

Treatment did not have any significant effect either on standard length (F= 1.882; df=3; P=0.0782; Figure 2.14), condition (F= 1.671; df=3; P=0.0967; Figure 2.15), or SGR (F= 2.039; df=3; P=0.067; Figure 2.16) of cod larvae in Experiment II. By the end of the experiment, larvae reared in treatment 1 (*Artemia* only) attained a larger size than larvae in all other treatments (Figure 2.14). Larvae in treatment 1 also had the higher overall SGR (%day^{-1}), although during the period of 39 to 45 DPH, larvae in treatments 3 and 4 had better growth rates, compared to all treatments (Figure 2.16). The overall SGR by the end of the experiment ranged from 2.62 to 3.10 %day^{-1} among all treatments.

2.3.2.2 Instantaneous mortality (Z) and Survival

Instantaneous mortality rates were not significantly influenced by treatment (F= 1.813; df=3; P=0.0837; Figure 2.17) in experiment II. After an initial high mortality in all treatments, by 39 DPH it had lowered and remained similar until the end of the experiment (Figure 2.17). By the end of the experiment, there were no significant differences in survival between all treatments (F= 0.784; df=3; P=0.245; Figure 2.18), while treatment 3 achieved the highest survival rate (27.1%) of Experiment II.

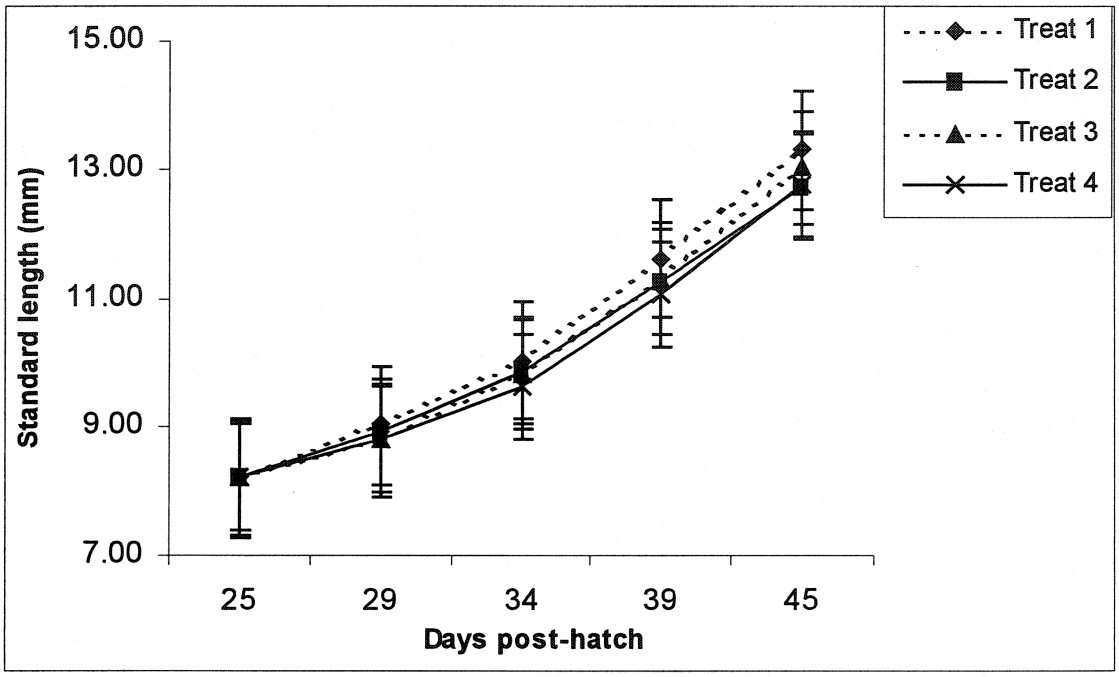

Figure 2.14 Mean **standard length** (mm) of Atlantic cod larvae in different weaning treatments over age (days post-hatch) in **Experiment II**. Each symbol represents mean of 30 larvae measured per treatment per sampling day. Vertical bars indicate SE. See Figure 2.2 for details of feeding trials.

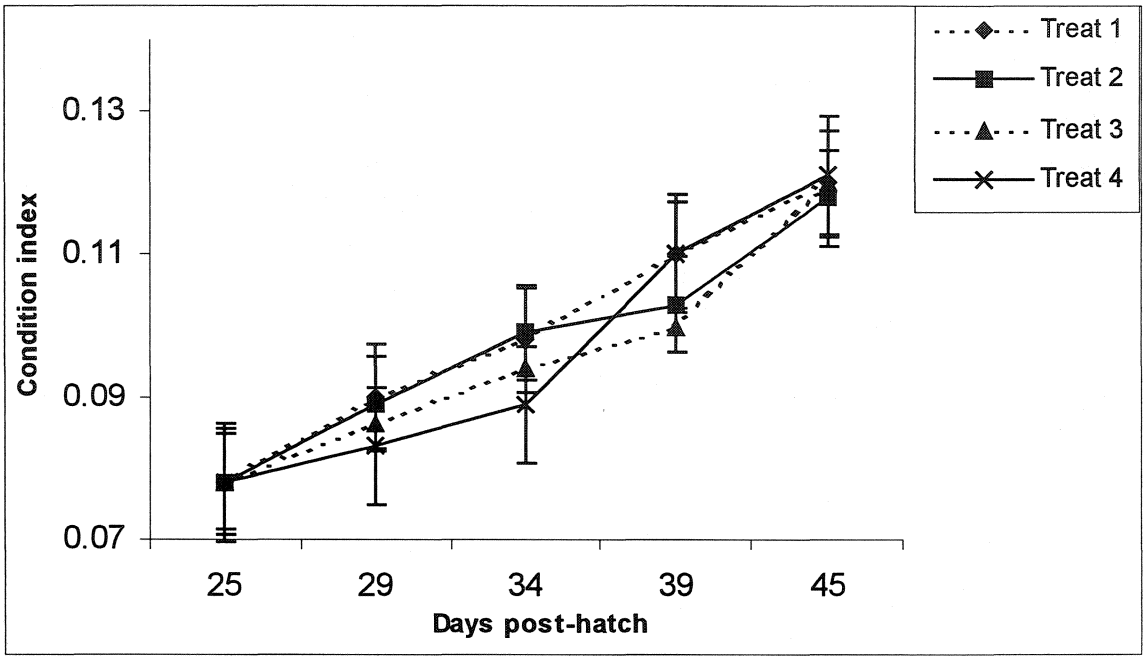

Figure 2.15 Mean **condition index** of Atlantic cod larvae in different feeding treatments over age (days post-hatch) in **Experiment II**. Each symbol represents mean of 30 larvae measured per sampling day. Vertical bars indicate SE. See text for details.

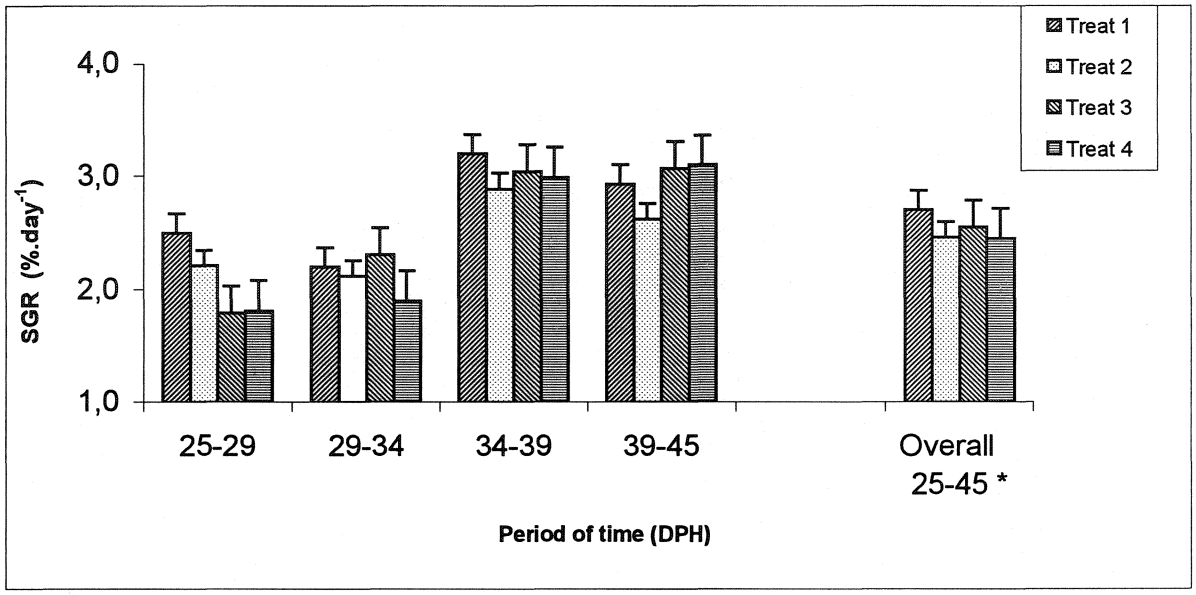

Figure 2.16 Means of **length-specific growth rate** (**SGR**; $\%.day^{-1}$) of Atlantic cod larvae in weaning trials employed in **Experiment II**. Each bar represents the mean of SGR of 30 larvae measured per treatment measured at the end of each growth period. Vertical bars indicate SE. See text for details.
(* - Represents the overall SGR achieved by larvae in all treatments for the total experimental period).

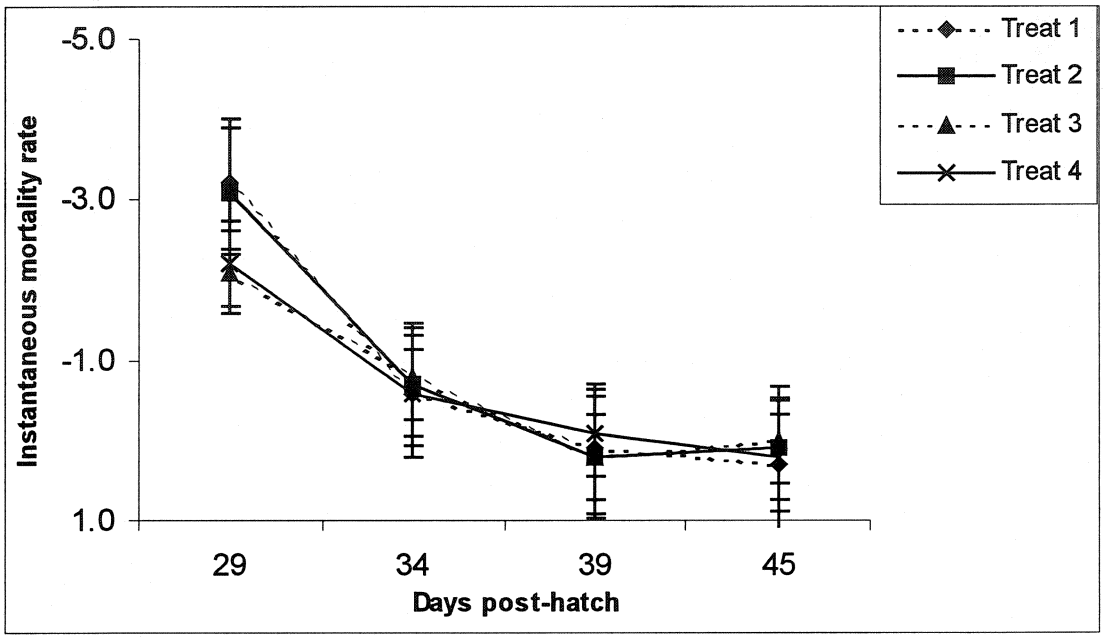

Figure 2.17 Mean **instantaneous mortality rate (Z)** of Atlantic cod larvae in different weaning treatments over age (days post-hatch) in **Experiment II**. Each symbol represents mean of Z calculated for the periods indicated in Figure 2.16. Vertical bars indicate SE.

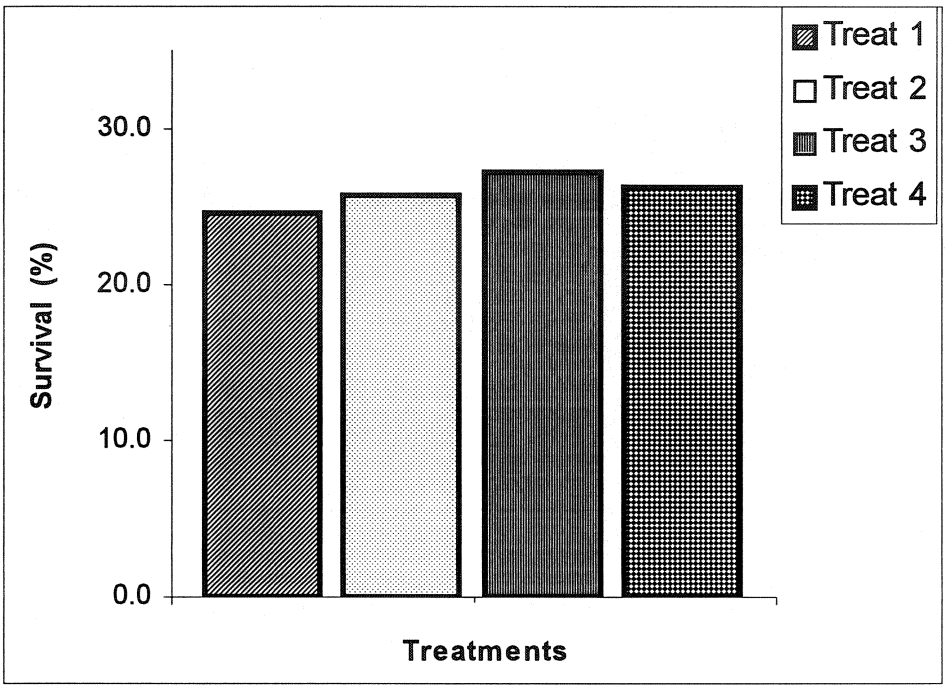

Figure 2.18 Overall **survival** of Atlantic cod larvae reared at different feeding treatments at the end of the **Experiment II**.

2.3.2.3 Behaviour

In Experiment II, Atlantic cod larvae spent on average, 12.10% of their time swimming and were motionless most of the time (around 83.27%). Larvae spent approximately 4.63% of the time foraging.

The overall average swimming time was 7.2 ± 0.43 sec/min among all treatments at the end of the experiment. Treatment did not significantly affected swimming (F= 0.712; df=3; P=0.339; Figure 2.19) or motionless (F= 1.414; df=3; P=0.1261; Figure 2.20) time spent by Atlantic cod larvae in Experiment II.

Comparisons between treatments when *Artemia* was administered indicated that treatment did not have any effect on *Artemia* ingestion by cod larvae, at least until 40 DPH (25 to 30 DPH, 2 treatments, Mann-Whitney test; Z=1.147, P=0.1257; 30 to 35 DPH, 3 treatments, Kruskal-Wallis test; X^2=4.532, df=2, P=0.103 and 35 to 40 DPH, 3 treatments, X^2=4.946, df=2, P=0.0843; Figures 2.21 to 2.24). However, a comparison between treatments 1 and 4 during the last period of co-feeding with *Artemia* (35 to 45 DPH) revealed that larvae in treatment 1 had a significantly higher frequency of *Artemia* ingestion (Mann-Whitney test; Z=-2.641, P= 0.041; Figures 2.21 and 2.24). Treatment did not have a significant effect on frequency of dry food ingestion by Atlantic cod larvae for any experimental period in which this food item was used simultaneously (30 to 35 DPH, 2 treatments, Mann-Whitney test; Z=0.362, P= 0.3587; and 35 to 45 DPH, 3 treatments, Kruskal-Wallis test; X^2=3.89, df=2, P=0.143; Figures 2.22 to 2.24). No significant difference was detected among treatments for rejection frequency (Kruskal-Wallis test; X^2=1.472, df=2, P=0.479; Figures 2.22 to 2.24).

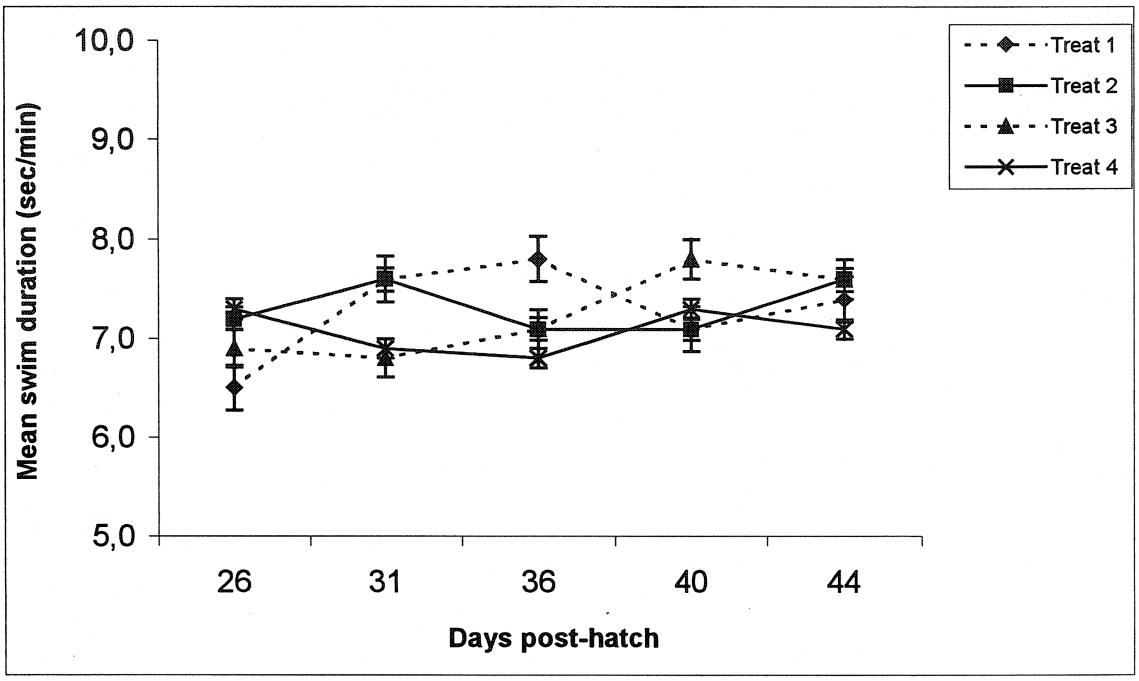

Figure 2.19 Mean **swim duration** of Atlantic cod larvae in different weaning treatments over age (days post-hatch) in **Experiment II**. Each symbol represents mean of 10 larvae observed per treatment per observational day. Vertical bars indicate S.E. See Figure 2.2 for details of feeding trials.

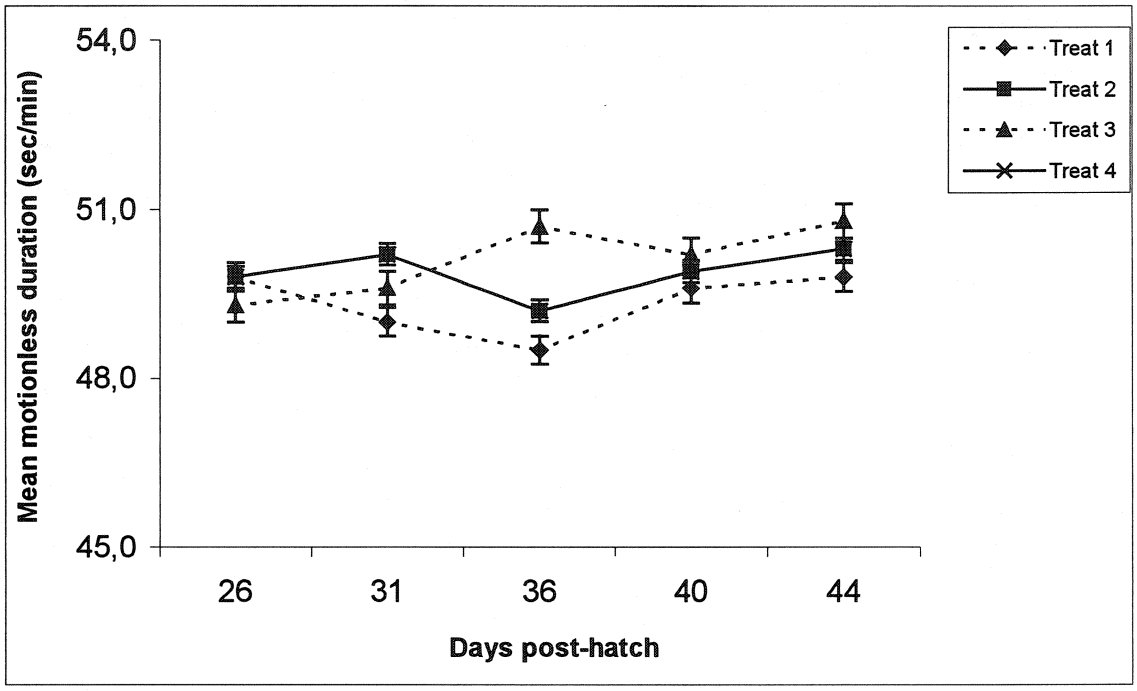

Figure 2.20 Mean **motionless duration** of Atlantic cod larvae reared at different feeding treatments over age (days post-hatch) in the **Experiment II**. Each symbol represents mean of 10 larvae observed per treatment per observational day. Vertical bars indicate S.E. See Figure 2.2 for details of feeding trials.

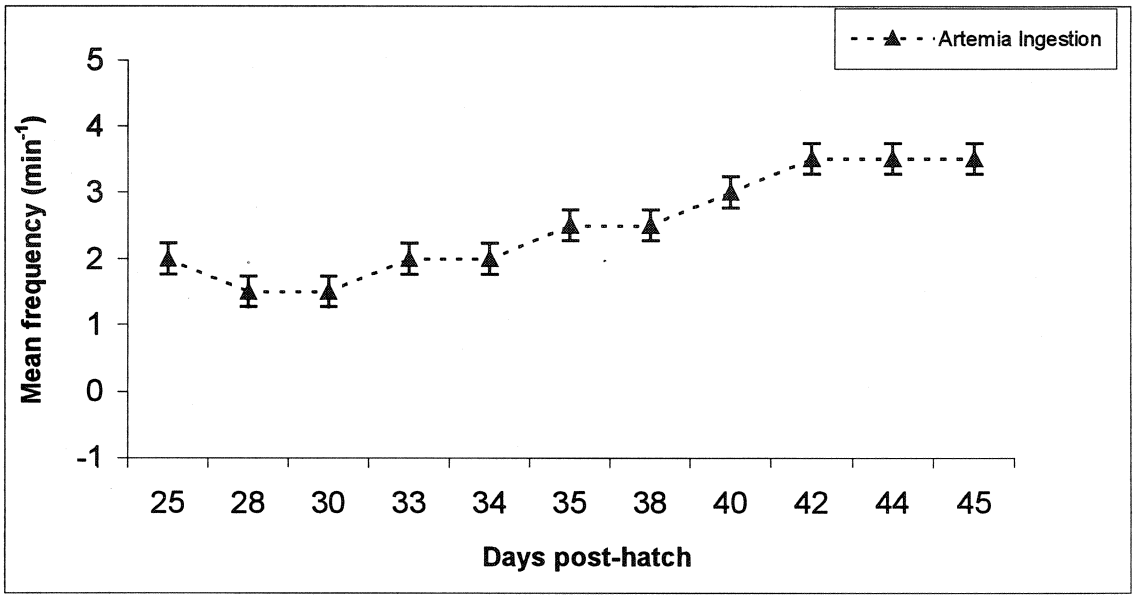

Figure 2.21 Mean frequency of ingestion of ***Artemia*** by Atlantic cod larvae reared in **Treatment 1** over age (days post-hatch) in **Experiment II**. Each symbol represents mean of 10 larvae observed treatment per observational day. Vertical bars indicate S.E. See Figure 2.2 for details of feeding trials.

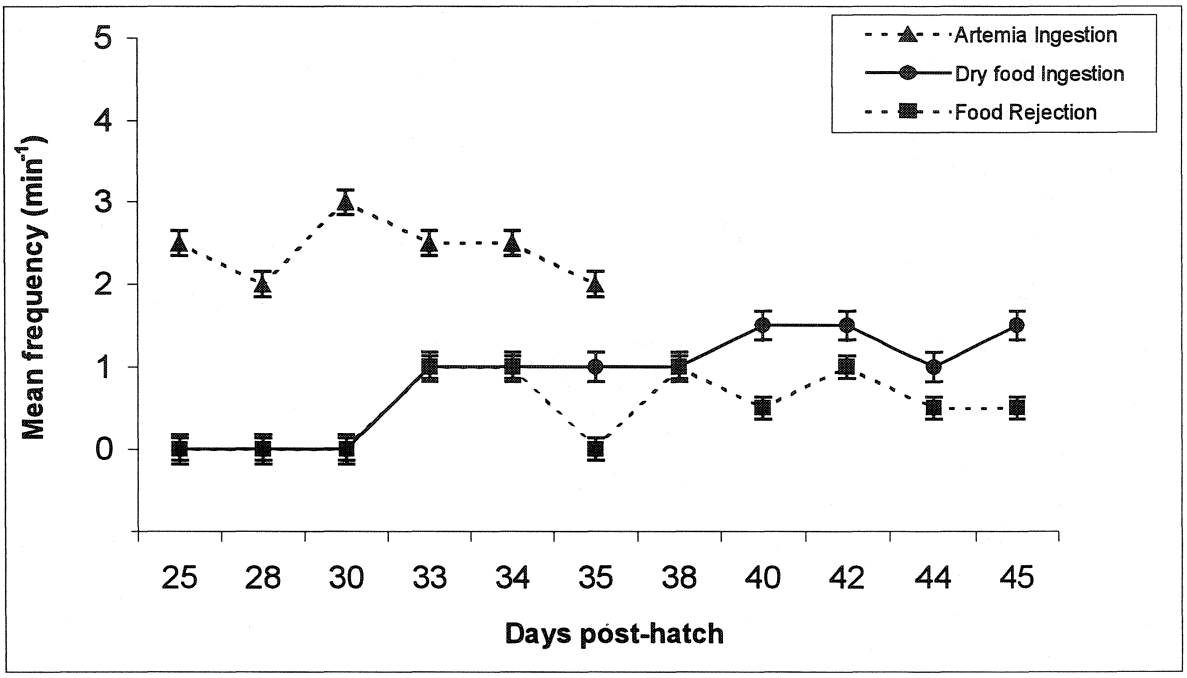

Figure 2.22 Mean frequency of ingestion and rejection of different food items by Atlantic cod larvae in **Treatment 2** over age (days post-hatch) in **Experiment II**. Each symbol represents mean of 10 larvae observed treatment per observational day. Vertical bars indicate S.E.

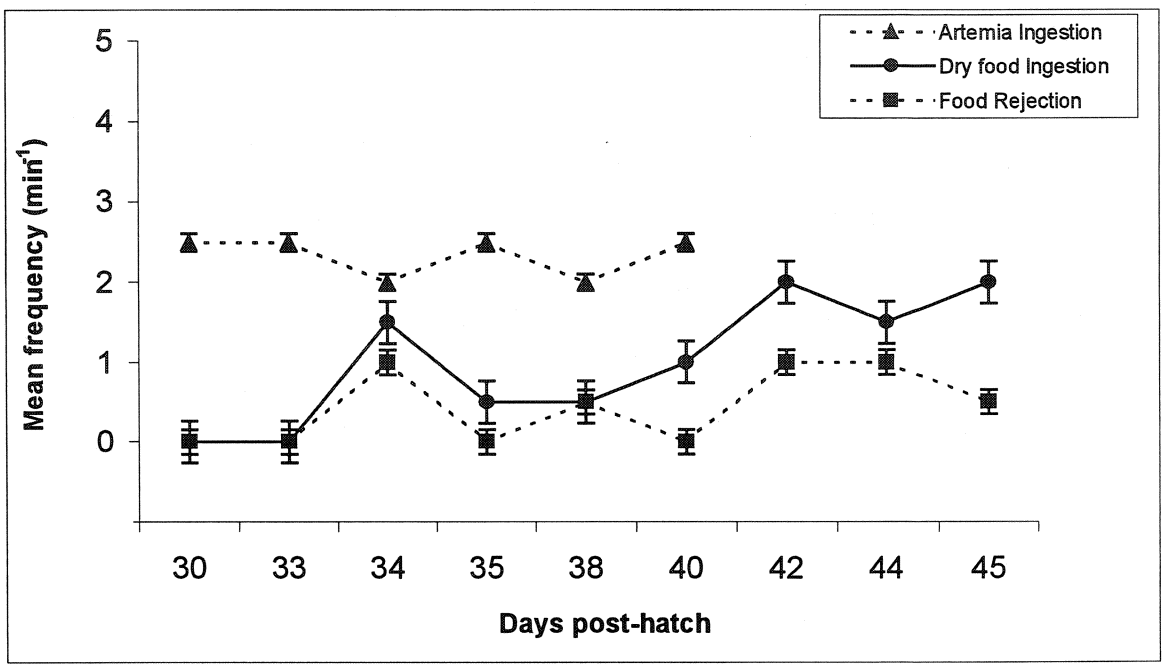

Figure 2.23 Mean frequency of ingestion and rejection of different food items by Atlantic cod larvae in **Treatment 3** over age (days post-hatch) in **Experiment II**. Each symbol represents mean of 10 larvae observed treatment per observational day. Vertical bars indicate S.E.

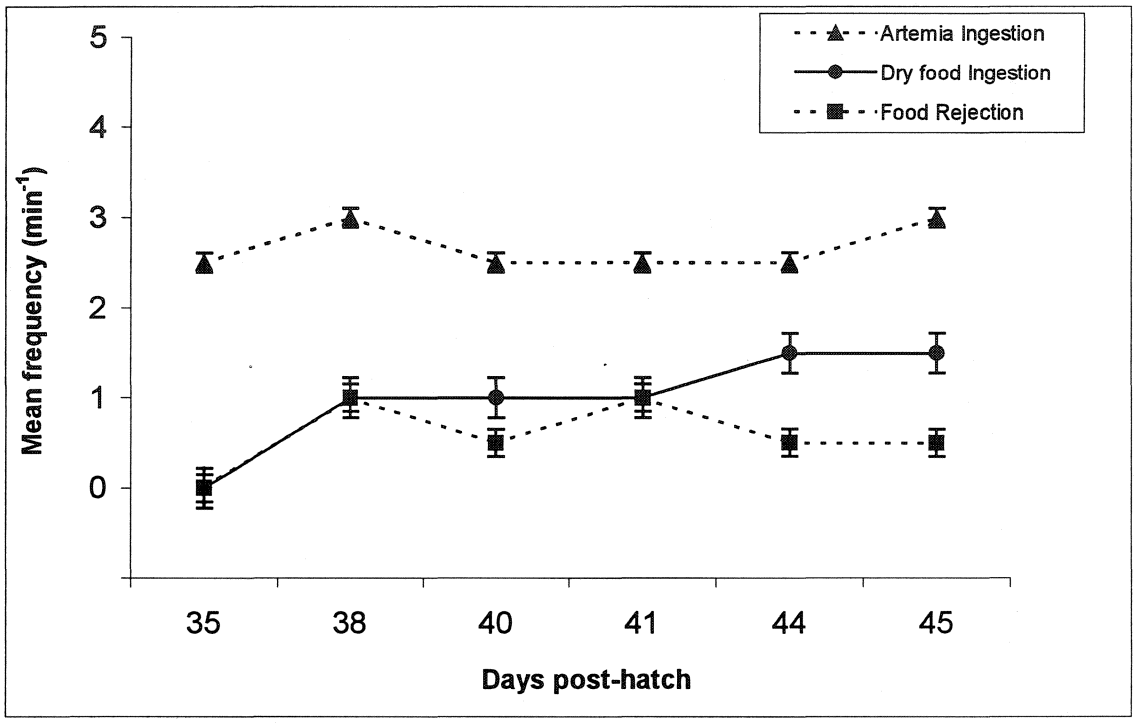

Figure 2.24 Mean frequency of ingestion and rejection of different food items by Atlantic cod larvae in **Treatment 4** over age (days post-hatch) in **Experiment II**. Each symbol represents mean of 10 larvae observed treatment per observational day. Vertical bars indicate S.E.

2.4 Discussion

At the beginning of the Experiment I, the larvae seemed prefer *Artemia* over rotifers in the second feeding in treatments 2, 3 and 4. In all treatments, during the co-feeding period when live prey was administered in high concentration, only a few larvae ingested dry feed. Cod larvae choose live prey and only when live prey was lowered to half ration (during the last two days of co-feeding in each treatment) were larvae observed to choose dry pellets. However, during the initial period of the Experiment I (from 25 to 31 DPH), larvae ignored the dry feed and did not identify it as potential food. On 31 DPH, larvae in treatments 3 and 4 started to accept the dry food, and the presence of dry food (distinct coloration) in the stomach of larvae could be clearly detected. Larvae approached dry diet pellets similar to live feed, as described by Puvanendran and Brown (1999). I did not observe any rejection of *Artemia*.

Until 34 DPH no differences in larval performance were detected between treatments 3 and 4. By the end of Experiment I, larvae in treatment 4 had the best growth rate and were the largest among all treatments.

No significant difference in condition was detected in larvae from treatments 3 and 4 by the end of the Experiment I. However, the difference in growth rate of larvae observed between these treatments and treatments 1 and 2 may be explained by the shorter co-feeding period to which the latter two treatments were subjected.

Treatment 2, in which larvae were co-fed *Artemia* for only 3 days, demonstrated that its use for such a short period did not promote satisfactory growth before metamorphosis. In treatment 1, using only rotifers for co-feeding with dry feed, larvae

had a similar growth performance as larvae in treatment 2 and this confirmed the inefficiency of using *Artemia* for a short time (3 days).

During the initial days of the Experiment I, high mortalities were observed in all treatments using *Artemia*, while it was not observed in treatment 1, where larvae continued to receive the same live food (rotifers) that had been provided prior to the start of the experiment.

From 31 DPH until the end of the Experiment I, larvae reared in treatment 2 swam significantly more than larvae in any other treatment, but the duration of motionless periods for larvae in this treatment was not significantly different compared to other treatments. Since larvae had been fed only live prey prior to starting the experiment, there may have been a tendency for larvae to search for this type of food. Thus, the higher swimming activity of larvae in treatment 2 throughout the experiment might be due to larvae searching for live prey and not recognizing dry food.

Several authors have demonstrated that poor feeding conditions can compromise cod larval condition and hinder the larvae's foraging ability. This suggests that an efficient foraging behaviour is strongly dependent on the correct development of foraging ability (Yin and Blaxter, 1987; Puvanendran *et al.*, 2002). Since feeding by larvae requires certain foraging skills, a period of adaptation, such as 3 or even 5 days of co-feeding at this age (25 DPH; treatments 2 and 3, respectively) appeared to be insufficient for larvae to "learn" to identify and switch to the new type of food (live prey to dry pellets).

Baskerville-Bridges and Kling (2000) suggested that Atlantic cod larvae could be weaned onto a microparticulate diet long before metamorphosis (around 22 DPH) using only rotifers as live prey during a co-feeding procedure. They found, however, that cod larvae still required *Artemia* for larval development, since larvae co-fed fed with *Artemia* had higher growth rate and reached longer size than larvae without *Artemia* as a component of their diet. These authors also observed that cod larvae increased the inert food ingestion in 120% when co-feeding with *Artemia*.

In my first experiment, I used rotifers as the only live prey in co-feeding during seven days (treatment 1), a similar trial to the employed by Baskerville-Bridges and Kling (2000). Larvae in treatment 1 achieved around 10mm length by 45 DPH, and had low survival rate (15.4%). However, when *Artemia* were used for 10 days (treatment 4), larvae were longer (~l2mm) and achieved a higher survival rate (25.7%) by the end of the experiment. This, together with the behavioral observations, lead me to employ only feeding schemes using *Artemia* in my second experiment.

Cod larvae could be weaned onto dry diet directly from rotifers in Experiment I, but had a higher performance being fed on *Artemia* during weaning. Therefore, the results form my first experiment did not support conclusions that larvae could rely only on rotifers as live prey during weaning protocols, as suggested by previous studies (Baskerville-Bridges and Kling, 2000).

Although efforts were made to keep the experimental conditions (i.e. water quality, pH, temperature, stocking density, etc.) equivalent to these encountered by larvae in commercial rearing tanks, high mortalities were observed in all treatments just after the

start of the experiment. For all treatments some mortality would be attributed to mechanical damage, which occurred during transfer of the larvae from the rearing tank to the experimental aquaria. High mortalities are often reported during (and immediately after) handling and moving of young cod larvae such as those utilized in my study. However, larvae in treatment 1, which were co-fed rotifers, seemed to handle this transfer better as they had the lowest mortality rate during the initial experimental period (up to 29 DPH). Moreover, the higher mortality rates observed during the initial experimental period in all treatments using *Artemia* as live food compared to those in treatment 1 (only rotifer) might also be attributed to the different live prey (*Artemia*) offered to larvae. It seems that some larvae could not make the switch to the new food item (*Artemia*).

Although the inability to ingest *Artemia* has to be considered when taking into account the reasons for the initial mortalities, the majority of growing larvae, such as those of 25 days old reared in the weaning trials, are able to catch and eat the *Artemia* nauplii (~ 280μm length) employed at the onset of the feeding schedule. Based on larval performance in all treatments in Experiment I, co-feeding with *Artemia* and dry feed for 10 days (treatment 4) appeared to be the best strategy. Larvae in this treatment showed the best growth rate and had the highest survival compared to all other treatments. This period was chosen to be the fixed co-feeding period in Experiment II where different weaning ages accessed.

In Experiment II, there were no significant differences in growth of larvae (SL, condition and SGR) among all treatments. However, although not significant larvae fed only *Artemia* (treatment 1) showed a higher mean final standard length (~13.3mm)

compared to larvae which had been co-fed with dry food (treatment 4; ~12.76mm). This growth performance of larvae is reasonable since larvae in treatment 4 started to fed on *Artemia* only on 35 DPH, while larvae in treatment 1 received *Artemia* continuously throughout the experiment.

The behavioral observations support this, since larvae in treatment 4 had as high frequency of dry food ingestion as in treatments 2 and 3, which would improve the overall condition and growth rate of larvae.

In accessing the different weaning ages for common perch (*Perca fluviatilis*), Cahu and Zambonino-Infante (1994) attributed the inability of young larvae to grow satisfactorily fed only with formulated diet to the larvae's immaturity or lack of a functional enzymatic system. Kestemont *et al.* (1995) suggested that larval weaning should occur at the onset of the stomach function; i.e., after switching its intestinal digestion to an essential digestion at the stomach level. For the majority of fish species this occurs just after the start of metamorphosis. Nonetheless, it has been suggested by Youson (1988) that the threshold for the onset of anatomical and physiological changes during metamorphosis of larvae is highly variable, and depends on the species.

In my second experiment, weaning age did not influence larval growth or survival of cod larvae. A comparison between treatments 1 (only *Artemia*) and 4 (*Artemia* and dry feed by 40 DPH), showed that larvae in treatment 1 had a higher *Artemia* ingestion frequency, while in treatment 4 larvae leveled off their feeding rate with the additional dry food intake. This demonstrates a clear trend to increase overall food intake (*Artemia* in treatment 1 and both items in treatment 4) by larvae around 40-45 DPH. In fact, larvae

from all other treatments also had ingested dry food prior to 40 DPH suggesting that the earlier larvae are fed on a dry diet the easier they can switch from live prey.

As observed in Experiment I, some mortality was also noted in Experiment II when *Artemia* were firstly administered to larvae. It was observed that most mortality arose when *Artemia* was provided for the first time in treatments 3 and 4 (30 DPH and 35 DPH, respectively).

Swimming activity did not change among larvae in all treatments in Experiment II, and even where *Artemia* was withdrawn early (i.e., treatment 2), larvae continued to show a constant swimming pattern. This observation and the observation that larvae had similar growth performance in all treatments, suggests that swimming did not increase energy use.

Although the extreme feeding conditions employed during Experiment I could induce cannibalism behaviour among larvae, it was not observed in any treatment. This may have been due to the high homogeneity in larval size from the beginning of experiments. In fact, according to other observations (L. Thorne, personal communication) cod larvae begin to exhibit cannibalistic and aggressive behavior around 50 days post-hatch (when they achieve about 15 mm), and this is more frequently observed during the juvenile stage.

Larvae in treatment 2 of the Experiment II attained a larger size (45 DPH; SL ~12.73mm) and had a better growth rate (2.46%day^{-1}) compared to larvae in treatment 4 from Experiment I (45 DPH; SL ~12.05mm; SGR of 2.04%day^{-1}), which were raised under identical rearing and feeding conditions. This may be explained as being due to the

higher temperature and the different dry food employed in the Experiment II. The Dana® feed, which had been sieved to get shorter pellets, showed better granulation, promoted more buoyancy and therefore was more easily diffused in water. This made it more available to the larvae in the water column. Although both diets employed in Experiment I and II had similar nutritional composition, the formulated diet used in Experiment II seemed to be more stable in water, thus providing higher integrity until caught by the larvae.

In Experiment II, the similarity in growth and survival rates between all treatments, suggests that 35 day-old larvae co-fed with *Artemia* for 10 days are able to be weaned onto dry diet. No morphological signs of nutrient deficiency were found, and larvae looked healthy and showed normal shoaling behaviour.

Weaning cod early, through the strategy suggested here, allows for reduced levels of *Artemia* to be fed over the weaning process. Cod larvae could be weaned early without affecting growth rates, larval condition or survival. However, further studies are suggested to improve performance of currently used artificial diets, as well as towards reducing the quantity of *Artemia* required using this feeding strategy.

The results of the experiments carried out in my study provide some fine tuning to the cod production protocols currently employed at ARDF/OSC. I hope that these findings can bring new approaches to the cod larvae feeding procedures, while enhancing the yield of healthy juveniles and at the same time lowering the overall production costs.

Chapter 3

The effects of early weaning on the behavior, growth and survival of fat snook (*Centropomus parallelus*, Poey 1864) larvae

3.1 Introduction

The snooks are catadromous marine fish of the family Centropomidae with a worldwide tropical distribution. The most widely studied snook species is the Asian sea bass, *Lates calcarifer* (Bloch), in the Indo-Pacific region and those of the genus Centropomus, inhabiting the Pacific and Atlantic coasts of America (Seaman and Collins, 1983; Rivas, 1986). Centropomids are considered important to commercial and recreational fisheries, as well as for aquaculture. Farming of centropomids is significant and in 1998 commercial scale operations produced about 19,600 metric tons of the Asian snook *L. calcarifer* (FAO, 1999).

Snooks have been reported among the most suitable marine species for on growing in cages, pens, intertidal ponds and "raceways" in Latin America (Vasquez, 1988; Roberts, 1990). This is mainly due to their capacity to deal with high stocking density, their fast growth, broad salinity tolerance and also their high market acceptance (Shafland, 1979; Lau and Shafland, 1982; Tucker *et al.*, 1985;Tucker and Jory, 1991; Alvarez-Lajonchere, 2001).

Artificial propagation techniques of the common snook, *Centropomus undecimalis*, have been developed in Florida for several years (Ager *et al.*, 1978; Chapman, 1982; Tucker and Kennedy, 2001) and it has been proven that a high rate of viable eggs can be attained if timing of spawning is adequately controlled. Studies on rearing techniques of early stages of common snook (Shafland and Koehl, 1980; Tucker,

1987; Serfling, 1998) and larval development has been described in detail (Lau and Shafland, 1982). The fat snook, *Centropomus parallelus* (Poey 1864), is widely distributed within the American Atlantic and Pacific tropics and subtropics ranging from southern Florida (USA) and the Mexican Gulf coast to Florianopolis, Brazil (Fraser, 1978; Rivas, 1986).

Adult snook (*Centropomus* spp.) occur in a wide range of salinities but are most abundant in brackish estuaries, particularly in mangrove areas. They can also be found in a variety of other habitats, such as nearshore reefs, sandy beaches, and other shorelines in fresh-, brackish, and marine waters. Except for the usually short movement to spawning areas, snook are nonmigratory. They are unable to spawn in freshwater, however, since their sperm is activated only by saltwater (Seaman and Collins, 1983). The spawning season for the Southern Brazilian coast range from spring through early fall, from approximately late November to April (Mioso, 1995).

Fat snook, also commonly named "robalo-peva" in Brazil, were firstly proposed for aquaculture by Patrona (1984 cited by Tucker, 1987). In the early 1990s investigations were undertaken at the Marine Fish Culture Laboratory (Laboratorio de Piscicultura Marinha-LAPMAR/UFSC, Santa Catarina, Brazil) to determine adequate procedures for rearing fat snook. Reliable techniques in broodstock handling and larviculture were attained and culminated in the first successful juvenile production of this species in Brazil in 1992 (Cerqueira *et al.*, 1992; Cerqueira, 1995).

Since then several studies were carried out at LAPMAR aimed at improving reproduction (Mioso and Cerqueira, 1994; Mioso *et al.*, 1994ab; Mioso, 1995),

larviculture (Cerqueira *et al.*, 1992; Alvarez-Lajonchere, 2001; Temple *et al.*, in press) and aspects of feeding in fat snook culture (Honczaryk and Cerqueira, 1994; Cerqueira and Bernardini, 1995; Borba, 1997) to provide basic rearing protocols for this species.

Although considerable advances have been accomplished in snook culture, it is still necessary to have more accurate information concerning larviculture techniques to establish reliable juvenile production either for local farming and or for restocking programs. Tucker (1987) suggested that broodstock handling and adequate feeding through the early stages of larvae are the main factors in the successful development of centropomid aquaculture. The weaning process is considered as one of the most critical stages in the rearing of marine fish larvae and has to be carefully planned. Successful weaning depends on both the morphological and physiological development of larvae for each species as well as the quality of formulated diets (Devresse *et al.*, 1991).

Fish may detect food either visually or chemically, but taste is used to make the decision of whether to ingest or reject the food (Atema, 1980). Appelbaum (1985) pointed out that acceptance by fish larvae is strongly influenced by the aroma of food and suggested that conspicuous differences are found among different fish species in this respect.

In a study with European sea bass, *Dicentrarchus labrax*, Person Le Ruyet *et al.* (1993) attained good larval growth and survival using a formulated diet from 40 days post-hatch (DPH), even though the weaning of this species was usually conducted at day 55 post hatch. Genari *et al.* (1994) suggested that this species could be weaned onto artificial diet even earlier using a specially formulated dry diet. Feeding *D. labrax* larvae

with only formulated diet from 20 DPH, Zambonino-Infante *et al.* (1997) obtained significant growth and a good survival rate, compared to performance of larvae reared with live zooplankton alone.

Honkzaric and Cerqueira (1994) performed feeding trials testing natural and chemical ingredients in experimental diets in order to improve the attractiveness of dry diets for fat snook, *C. parallelus*, larvae. They found good acceptance by larvae using a combination of a shark-based diet and synthetic free amino acids, however larvae had a low survival during weaning, probably due to the use of only *Artemia* nauplii without any enrichment, during the co-feeding period.

Cerqueira and Bernardini (1995) compared the responses of fat snook juveniles during late weaning trials using an experimental prepared diet with the same compounds used by Honczarik and Cerqueira (1994), and a commercially available dry diet formulated for temperate species. They found that although the shark-based diet satisfactorily stimulated feeding behaviour, it compromised fish health later in development due to the high urea levels present in this diet. In their experiment they did not use any co-feeding period with *Artemia*, and frozen *Artemia* was used to feed fish before the experiment.

In another study on larval fat snook feeding, Borba (1997) assessed different weaning ages using a formulated diet based on high quality fish meal as the protein source with a mixture of synthetic attractants (including free amino acids and betaine). Although this resulted in satisfactory survival rates for 37 DPH weaned larvae, better

growth results were obtained for larvae weaned by 57 DPH. Borba (1997) used a co-feeding period of 3 days using live *Artemia* (enriched with SELCO) as live prey.

Recently, the Brazilian government imposed rigid conditions for the importation of commercial live food enrichment emulsions and commercial starter dry diets and forcing the aquaculture operation to develop their own products in order to improve the nutritional quality of feed and at the same time keeping research affordable.

During the past four years, the LAPMAR altered the research approach of fat snook rearing protocols with improvements in microalgae production, manufacture of an enrichment emulsion containing high PUFAs for live feed, and changed the feeding regimen during the early larviculture stages (Alvarez-Lajonchere, 2001). These changes enhanced the quantity and quality of juvenile produced.

However, the optimal age for switching from live prey to artificial food during *C. parallelus* larval rearing still remained uncertain. The length of the feeding period on live food may be crucial if larvae are to maximize growth and achieve high survival. Thus, it was considered important to study co-feeding periods as well as to carry-out comparisons of larval performance reared with the available manufactured diets during the weaning process.

In this chapter, I carried out evaluations of growth and survival and feeding behaviour of fat snook larvae during early weaning. The effect of enriched *Artemia* as live prey and different dry diets were investigated. It was hypothesized that longer period of co-feeding would improve larvae performance throughout the weaning process of fat snook larvae.

3.2 Materials and methods

The experiment was conducted from January until March/2002 at the Marine Fish Culture Laboratory (LAPMAR - Laboratorio de Piscicultura Marinha - UFSC, Florianopolis, Santa Catarina, Brazil) installations, situated at 27°37'S and 48°27'W.

3.2.1 Induced spawning and husbandry procedures

Adult cultured fat snook broodstock were kept in $10m^3$ concrete indoor tanks under controlled environmental conditions to facilitate maturation. The ovarian maturity of broodstock was assessed by biopsy. A polyethylene cannula was inserted into the oviduct of an anaesthetized female and a sample of gonadal tissue was removed by suction as the cannula was withdrawn (Alvarez-Lajonchere *et al.*, 2001). The developmental stage of oocytes was assessed from microscopic appearance of vitellogenic stage of oocytes (Bromage, 1995). In males, maturity was assessed by the presence or absence of milt when gentle pressure was applied to the abdomen. Mature fish were hormonally induced using intramuscular injections of sustained-release pellets composed of 50μg/kg of LHRHa (Luteinizant Hormone Releasing Hormone analog) for females, and a dose of 25μg/kg for males (Cerqueira *et al.*, 1992; Zohar, 1996).

Mature induced fish were then transferred to 1,000 litres fiberglass spawning tanks at a sex ratio of 2:1 for males and females respectively. Viable fertilized eggs (buoyant) were obtained approximately 36 hours later (at 24 ± 1°C) from spawning tanks and put in incubators.

Egg stocking density and incubation procedures were carried out in accordance with methodology described by Mioso (1995). Larvae hatched approximately 20 hours later (at 25 ± 1°C) and were transferred to 5,000 liter circular indoor larviculture tanks. Larvae were raised in these tanks following the standard LAPMAR rearing protocol.

Tanks were maintained with slight aeration, keeping a static system (no water flow) until 3 days post-hatch (DPH). From 3 DPH onwards a flow-through water system was employed and maintained at approximately 26°C, using heaters. Rotifers (*Brachionus plicatilis*) raised on a local strain of *Nannocloropsis* spp. were fed to larvae twice daily using a prey density of about 30,000 prey/liter from 3 DPH until 15 DPH. At this point, a mixture of rotifers and enriched *Artemia* (Macau-Brazil cysts) was used at about 5,000 prey/liter, twice daily until 25 DPH. During the first three days (15-18 DPH), *Artemia* nauplii was employed followed by enriched *Artemia*.

By 25 DPH, only enriched *Artemia* was used. *Artemia* was enriched with a locally made emulsion 24h prior to feeding larvae. The ingredients and proportions utilized to manufacture this emulsion are shown in Table 3.1. Rearing tanks were greened daily with microalgae *Nannochloropsis* spp.

Table 3.1 Ingredients used by LAPMAR to prepare the *Artemia* enrichment emulsion.

Ingredients	%
Filtered water (ml)	48.0
Xantanin starch (g)	8.0
Squid extract (g)	12.0
Casein (g)	6.0
Choline chloride (g)	2.0
Vitamin C (g)	8.0
Beta Carotene (g)	0.2
Cod liver oil (ml)	12.0
Papric (g)	4.0

Source: LAPMAR.

Table 3.2 Diet formulations used by LAPMAR to manufacture the starter dry diet.

Ingredients	%	Final estimated composition*	
Fish meal	50.3	Gross protein (%)	50.65
Squid meal	27.0	Gross lipids (%)	19.51
Gel starch	10.7	Carbohydrate (%)	16.61
Incromega FD3322	5.0	Ash content (%)	6.2
Canola oil	1.0	Gross energy (Kcal/Kg)	4387
Lecithin (Soya)	1.0	Protein/Energy ratio (mg protein/kcal)	115
Premix vitamin/mineral (Nutron®)	3.0	Σ (n-3) HUFA (minimum %)	37.6
Bicalcic phosphatic	0.5	DHA (minimum %)	12.3
Carboxi Methyl Celulose – CMC	1.0	EPA (minimum %)	17.3
Vitamin C	0.5	DHA:EPA	0.7

* - Estimation based on dry matter.
Source: LAPMAR.

3.2.2 Experimental design

On day 28 post hatch (714 degree-days), batches of 240 larvae (mean of 9.6mm; S.D. =1.4) were transferred from the main rearing tank and stocked in each of fifteen, black circular 120 liter experimental tanks. The volume of seawater in each tank was maintained at 80 liter in order to maintain a desired larval density of 3 larvae/liter. During two days of acclimation only enriched *Artemia* were fed to larvae, and the experiment started at 30 DPH (766 degree-days). Each treatment (five feeding trials; Figure 3.1) had three replicates.

Treatment 1: Control treatment, fish fed only enriched *Artemia*. No dry food was used.

Treatments 2, 3 and 4: *Artemia* introduced for 5, 10 and 15 days and co-fed with a LAPMAR starter dry diet. The ingredients and basal composition of this diet are shown in Table 3.2.

Treatment 5: *Artemia* introduced for10 days and co-feeding was done using Nutra Marine® (Perla Larva 6.0) dry food. The nutritional composition of the Nutra Marine® dry diet can be seen in Table 3.3. Both dry food particles used in the treatments (the LAPMAR prepared diet, ranging from 500-1000μm and the Nutra marine®, ranging from 400-600μm were sieved through nylon meshes ranging from 300-500-600μm in order to assure a suitable pellet size for the mouth size of the larvae. This procedure also provided a standardized pellet size for all treatments. At the start of the experiment larvae were given the smallest sized pellet (300μm) in a higher proportion and the pellet size was increased gradually as the larvae grew.

Figure 3.1. Feeding trials used for weaning fat snook larvae. In treatments 2, 3 and 4 a prepared dry diet by LAPMAR was used. In treatment 5 the Nutra marine® dry diet was used. DPH (Days post-hatch).

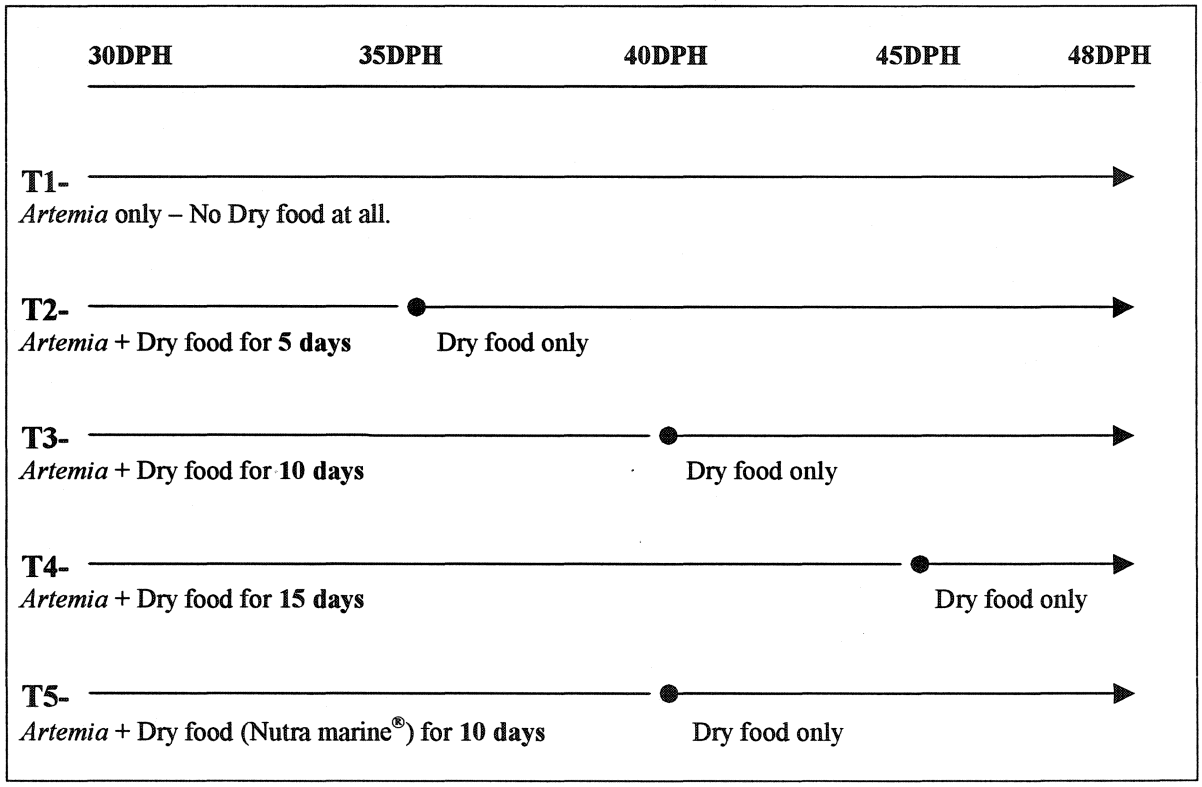

Table 3.3 Chemical analysis of Nutra Marine® (Perla Larva*) dry diet.

Chemical analysis	%
Protein	62.0
Oil	11.0
N.F.E.	9.0
Moisture	8.0
Ash	10.0

* - Ingredients: LT Fishmeal, Wheat products, Fish protein concentrate, Fish oil, Crustacean Meal, Vitamins, Minerals.
Source: Nutra Marine®.

Based on observations conducted in the first two days of my experiment, I changed to pellet size 2:1 (500:300μm) in order to attain an appropriate distribution of food particles size for fat snook larvae. By 38 DPH, it was observed that the majority of larvae in treatment 5 were ingesting the larger particles, thus most of the pellets offered in the treatment were increased to 500μm.

The dry diet was added to tanks by hand, a half-hour before adding *Artemia*. Automatic feeders (Lifeguard® automatic fish feeders, Rainbow Lifeguard Aquarium Products, CA, USA.) were used during the day. Live food was administered to experimental tanks twice daily (09:00 and 15:00) and always co-fed with dry feed. Initially 2,500prey/litre were added which decreased to 1,000 and 500prey/litre the last two days of co-feeding.

Each experimental tank had aeration and was set up with a flow-through water system at a rate of 800ml/min. The outflow was covered by 600μm nylon mesh. The water temperature in the tanks was kept around 25 ± 2.6°C. During the study period experimental tanks were exposed to 12h of light (ranging from 450 to 900 lux) and 12h dark. This photoperiod was chosen based on previous observations by LAPMAR researchers that this photoperiod would reduce cannibalism. Dissolved oxygen (mg/ l) was measured with a YSI meter, three times a week and all tanks were cleaned daily prior to the first daily feeding. Mortality rate was estimated from the number of dead larvae counted after cleaning tanks. The experiment lasted until 48DPH, totaling 19 days for the experiment.

3.2.3 Data collection

At the beginning of the experimental period, a sample of 30 larvae were randomly taken from each tank and sedated with ethylenoglycol-monophenylether (at 0.01%) for measurements. On 30, 36, 42 and 48 DPH, samples of 10 larvae from each tank of each treatment were taken for measurements. Larvae were measured for standard length (mm; from the tip of upper jaw to the end of notochord) and myotome height (mm; depth of body posterior to the anus) using a dissecting microscope equipped with micrometer ocular (calibrated to 0.1mm). Survival was estimated by determining the difference from the initial number of larvae and the larvae remaining in each tank at the end of the experiment.

Behavioral observations were carried out on days 30, 35, 40, 45 and 48 DPH using "The Observer" software (Noldus Information Technology 1990) installed on a lap top computer. On each day of observation, five larvae from each of three tanks (per treatment) were randomly chosen and observed for 120 seconds using the focal animal technique as defined by Altman (1974).

The computer keyboard was configured using "The Observer" in such way that each behavioral pattern to be observed was assigned to a distinct key. Five behavioral patterns (Puvanendran and Brown, 1999) were quantified (Table 3.4). Recorded data consisted of time spent swimming and motionless and the frequency of feeding choices (*Artemia* or dry food) by fat snook larvae.

Table 3.4 Operational definition of behavioral activities observed during the fat snook weaning trials.

Activities	Definition
Swim	Forward movement of the larvae through water column
Motionless	Larvae is motionless
Artemia	Larvae captures and ingests *Artemia*
Dry food	Larvae ingests dry food pellet
Reject	Larvae bites but rejects pellet

Adapted from Puvanendran and Brown (1999).

3.2.4 Data analysis

3.2.4.1 Growth parameters

A *Condition index* using the ratio of body depth to body length was used to evaluate the condition of larvae for each treatment (Koslow *et al.*, 1985).

Condition index = myotome height / standard length

The growth rate of larvae was determined using the *Length-specific growth rate* (SGR) equation (Cowan and Houde, 1990);

$$SGR = (\ln(L_t) - \ln(L_0)) / t) \times 100$$

Where L_t is the final length of larvae at time t, L_0 is the initial length, and t is the period of time between L_t and L_0 in days.

3.2.4.2 Statistical analysis

Data on growth parameters were statistically analyzed using one-way analysis of variance (ANOVA). Prior to the analyses, the data were evaluated for normality using Kolmogorov-Smirnov nonparametric test and plot of residuals analyzed to ensure that assumptions of ANOVA were satisfied (Sokal and Rohlf, 1995). Survival, SGR and

Condition index were log transformed before conducting analyses in order to meet the ANOVA requirements. Differences among treatments were considered significant at $P \leq 0.05$, and Tukey's HSD post hoc multiple range tests were performed to determine which treatment are different from each other (Sokal and Rohlf, 1995).

Data on swim and motionless duration were normally distributed while data on frequency of feeding events (*Artemia*, dry food and reject) could not be normalized by any transformation. Thus, a Kruskal-Wallis nonparametric test was applied to these variables and the Mann-Whitney test was performed whenever the comparison was between only two treatments (Sokal and Rholf, 1995).

Data on frequency of dry food ingestion were subsequently analyzed by Mann-Whitney test in order to carry comparisons between each of two treatments.

Analyses were performed using the SPSS PC + software package (SPSS, 1999).

3.3 Results

Dissolved oxygen ranged from 4.1 to 5.8mg.l^{-1} and salinity remained around 35ppm during the experimental period.

3.3.1 Growth

The average length of larvae was 9.6 mm ± 1.2 (S.D.) at the start of the experiment and there were no significant differences among treatments (one-way ANOVA; F=1.231; df=4; P=0.143).

The time of co-feeding with *Artemia* had a significant effect on mean final standard length (one-way ANOVA; F=2.650, df= 4, P=0.028; Figure 3.2) of fat snook larvae. Larvae reared with *Artemia* for 5 days (treatment 2) were significantly smaller than larvae in all other treatments. However, there were no significant differences in standard length between larvae in treatments 1, 3, 4 and 5 (Table 3.5; Tukey's multiple range test; α=0.05).

Length-specific growth rate (SGR; %day^{-1}) was significantly different among treatments (one-way ANOVA; F=3.376, df=4, P=0.013; Figure 3.3). Larvae in treatment 2, after 42 DPH, had a significantly lower SGR than in all other treatments, but there were no significant differences in SGR between larvae in treatments 3, 4, 5 and the *Artemia* only treatment (Table 3.6; Tukey's multiple range test; α=0.05).

The average SGR over the entire experimental period ranged from 5.53 to 7.40% day^{-1} among all feeding trials (Table 3.7).

Treatment did not have a significant effect on the condition of larvae (one-way ANOVA; F=1.654, df=4, P=0.086), but a trend was observed in that larvae in treatment 2 had the lower condition at the end of the experiment (Figure 3.4). The poorest growth in terms of standard length and length standard growth rate occurred when larvae received *Artemia* for only 5 days (treatment 2).

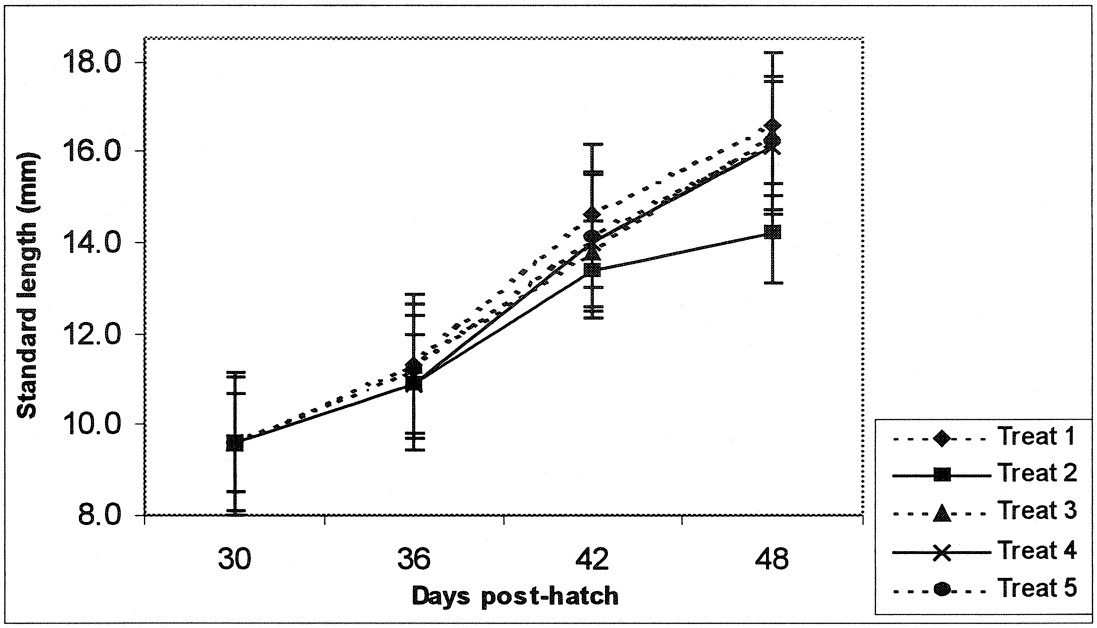

Figure 3.2 Mean **standard length** (mm) of fat snook larvae reared at different feeding treatments over age (days post-hatch). Each plot represents mean of 10 larvae measured per treatment per sampling day. Vertical bars indicate SE. See Figure 3.1 for details of feeding trials.

Table 3.5 Results of the Tukey's multiple range test of the final mean **standard length** (SL; means ± S.D.) with respective coefficients of variation (CV) and survival of fat snook larvae for each feeding trial at the end of the experiment.

Feeding Treatments	**SL (mm)**	**CV (%)**	**Survival (%)**
T1 – *Artemia* only	16.6 ± 1.30^{a}	4.11	99.5^{a}
T2 – *Artemia* for 5 days, using LAPMAR dry diet	14.2 ± 1.12^{b}	3.54	91.8^{a}
T3 – *Artemia* for 10 days, using LAPMAR dry diet	16.4 ± 1.33^{a}	3.97	98.7^{a}
T4 – *Artemia* for 15 days, using LAPMAR dry diet	16.1 ± 1.28^{a}	4.57	99.7^{a}
T5 – *Artemia* for 10 days, using Nutra Marine® dry feed	16.2 ± 1.25^{a}	4.24	99.3^{a}

See Figure 3.1 for details of feeding trials until 48 days post-hatch (DPH).
All larvae were fed dry feed, except those on treatment 1 (control).
Values with the same superscript are not significantly different.
(Tukey's multiple range tested at α=0.05).

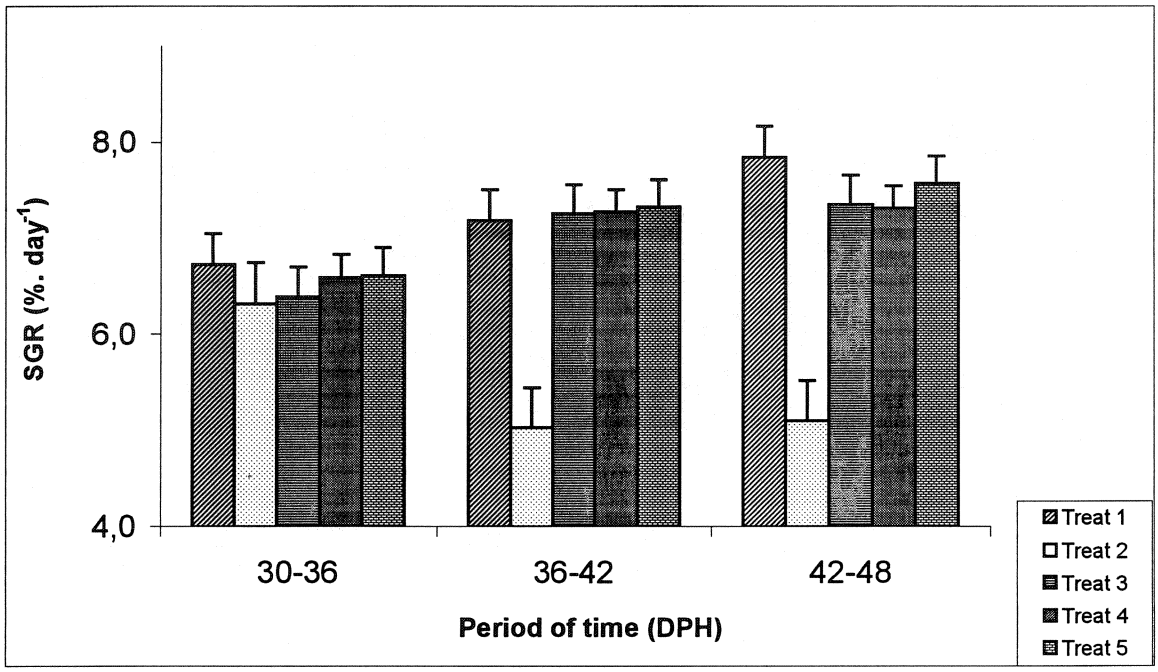

Figure 3.3 Means of **length-specific growth rate** (**SGR**; %.day^{-1}), of fat snook larvae reared in different feeding trials. Each bar represents the mean of SGR of 10 larvae measured per treatment measured at the end of each growth period. Vertical bars indicate SE. See text for details.

Table 3.6 Results of the Tukey's multiple range test on the Influence of co-feeding period with *Artemia* on **length-specific growth rate** (SGR*; means ± S.D.) of fat snook larvae from 30 to 48 DPH.

Feeding Treatments	**SGR 30-36 DPH**	**SGR 36-42 DPH**	**SGR 42-48 DPH**
T1 – *Artemia* only	6.72 ± 1.34^{a}	7.18 ± 0.76^{a}	7.84 ± 0.55^{a}
T2 – *Artemia* for 5 days, using LAPMAR dry diet	6.32 ± 0.82^{a}	5.02 ± 0.52^{b}	5.09 ± 0.43^{b}
T3 – *Artemia* for 10 days, using LAPMAR dry diet	6.39 ± 0.65^{a}	7.25 ± 0.22^{a}	7.35 ± 0.51^{a}
T4 – *Artemia* for 15 days, using LAPMAR dry diet	6.59 ± 1.22^{a}	7.27 ± 0.78^{a}	7.31 ± 0.58^{a}
T5 – *Artemia* for 10 days, using Nutra Marine® dry feed	6.61 ± 0.69^{a}	7.32 ± 0.45^{a}	7.57 ± 0.27^{a}

* - Averaged value of SGR (%.day $^{-1}$) for the period.
Values with the same superscript are not significantly different (Tukey's multiple range tested at α=0.05).

Table 3.7 Results of the Tukey's multiple range test of the final mean **SGR, condition index** and respective **coefficients of variation** (CV) of fat snook larvae for each feeding trial.

Feeding Treatments	**SGR (% day $^{-1}$)**	**CV (%)**	**Condition index**	**CV (%)**
T1 – *Artemia* only	7.40 [a]	2.91	0.24 [a]	1.87
T2 – *Artemia* for 5 days, using LAPMAR dry diet	5.53 [b]	1.25	0.24 [a]	1.22
T3 – *Artemia* for 10 days, using LAPMAR dry diet	7.06 [a]	2.85	0.25 [a]	2.01
T4 – *Artemia* for 15 days, using LAPMAR dry diet	7.12 [a]	2.56	0.25 [a]	1.86
T5 – *Artemia* for 10 days, using Nutra Marine® dry feed	7.21 [a]	3.07	0.25 [a]	1.74

In each column, values with the same superscript are not significantly different (Tukey's multiple range tested at α=0.05).

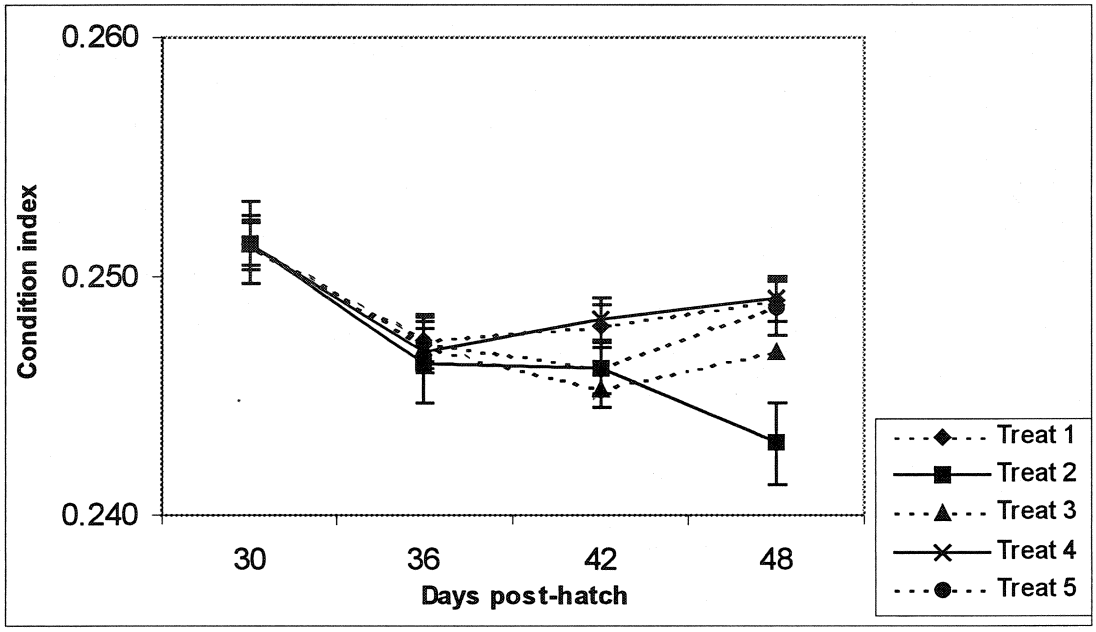

Figure 3.4 Mean **condition index** of fat snook larvae reared at different feeding treatments over age (days post-hatch). Each plot represents mean of 10 larvae per treatment per sampling day. Vertical bars indicate SE.

3.3.2 Survival

Fat snook larvae seem to be quite robust during the larval stage, as few mortalities occurred. Larvae had over a 91% survival rate up to 48 days post hatch, the end of the experiment, and there were no significant differences in survival rate among the feeding trials ($P>0.05$; Table 3.5). However, a high mortality occurred in one replicate tank from treatment 2, lowering the overall survival of this treatment. The reason for this was an algae "string" that appeared overnight entangling the larvae. Thus, the survival data for this tank was not considered in the analysis and only two replicate tanks for this treatment were considered. This treatment still had the lowest overall survival (91.8%) among all treatments.

3.3.3 Behaviour

Fat snook larvae spent on average approximately 27% of their time swimming and were motionless most of the time (around 65%). Larvae spent approximately 7% of the time foraging.

The overall average swimming time was 15.9 ± 0.50 sec/min among all treatments at the end of the experiment. Neither swimming (one-way ANOVA; $F=1.042$, $df=4$, $P=0.1781$; Figure 3.5) nor motionless (one-way ANOVA; $F=1.547$, $df=4$, $P=0.098$; Figure 3.6) duration were significantly different among treatments.

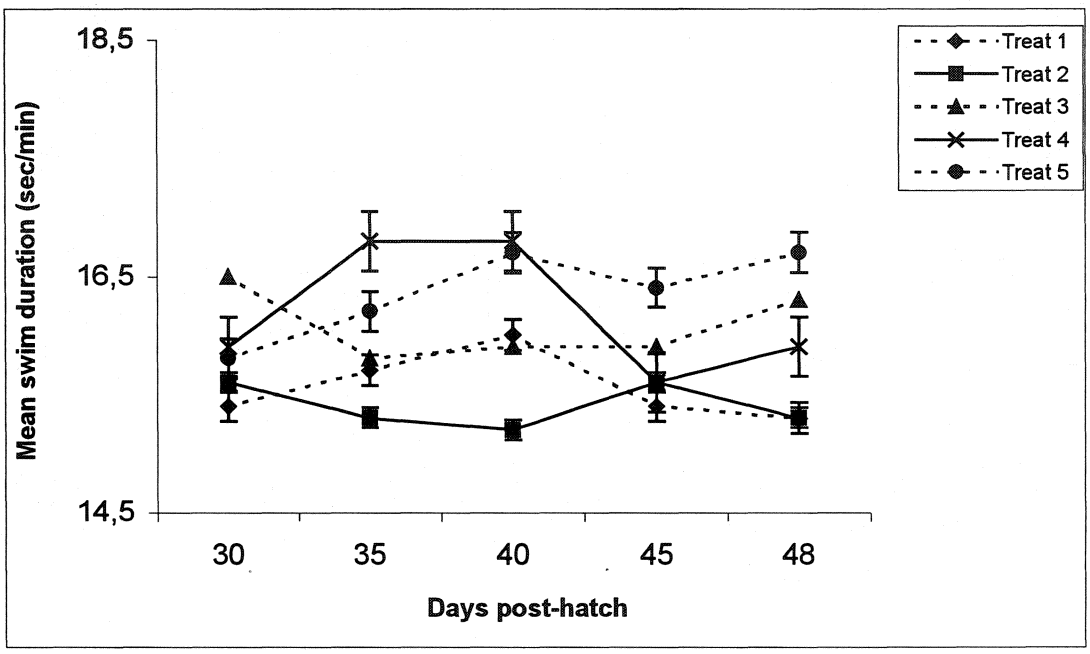

Figure 3.5 Mean **swim duration** of fat snook larvae reared at different feeding treatments over age (days post-hatch). Each point represents 15 larvae observed per observational day. Vertical bars indicate SE. See Figure 3.1 for details of feeding trials.

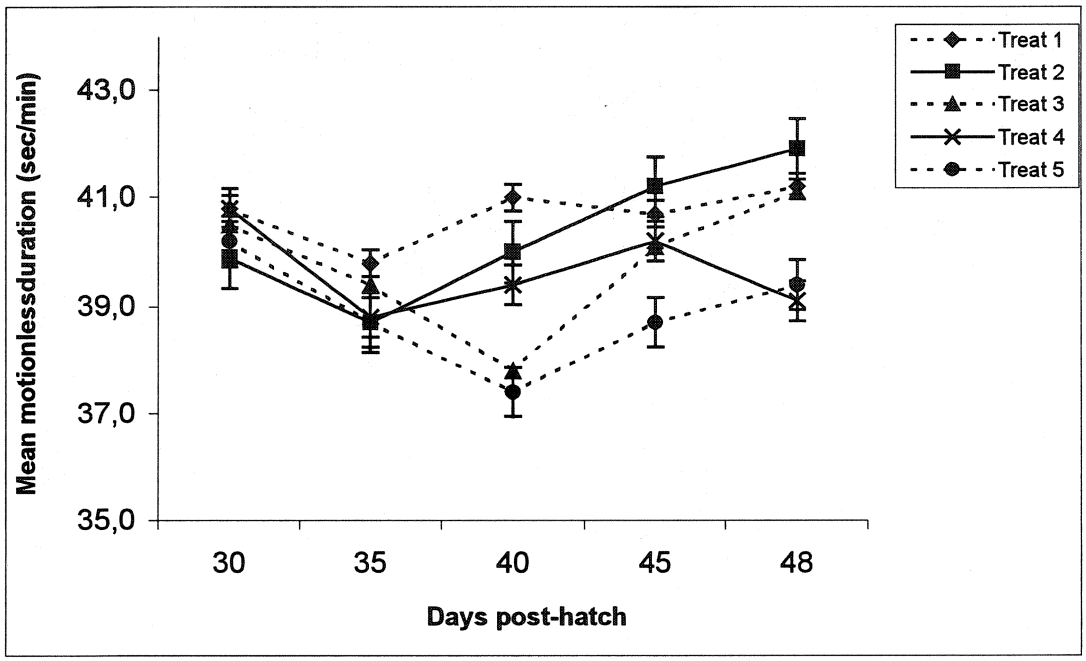

Figure 3.6 Mean **motionless duration** of fat snook larvae reared at different feeding treatments over age (days post-hatch). Each point represents 15 larvae observed per observational day. Vertical bars indicate S.E. See Figure 3.1 for details of feeding trials.

Differences in frequency of *Artemia* ingestion were examined for the periods where this food item was administered. For all treatments no significant differences were found over the period 30 to35 DPH (5 treatments, Kruskal-Wallis test; X^2= 6.381, *df*=4, *P*=0.172), 35 to 40 DPH (4 treatments, Kruskal-Wallis test; X^2=2.478, *df*=3, *P*=0.479) or from 40 to 45 DPH (2 treatments, Mann-Whitney test; *Z*=-1.4724, *P*=0.070) (Figures 3.7 to 3.11).

The dry food ingestion frequency by fat snook larvae was significantly affected by treatments (4 treatments; Kruskal-Wallis test; X^2=9.036, *df*=3, *P*=0.028; Figures 3.8 to 3.11). By the end of the experiment, larvae in treatment 5 showed a significantly higher dry food ingestion frequency compared to the larvae in all other treatments (Table 3.8; Mann-Whitney test; Figures 3.8 to 3.11). However, there was no significant difference among treatments for the frequency of reject (4 treatments; Kruskal-Wallis test; X^2=5.875, *df*=3, *P*=0.1179).

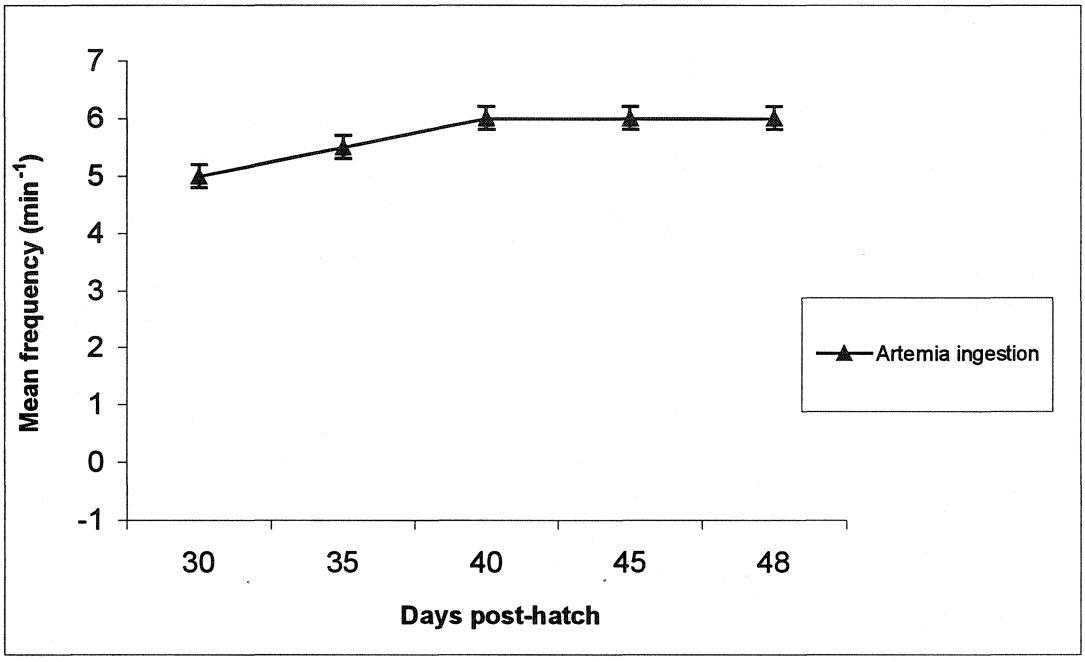

Figure 3.7 Mean frequency of ingestion of ***Artemia*** by fat snook larvae in **Treatment 1** over age (days post-hatch). Each point represents 15 larvae observed per observational day. Vertical bars indicate S.E. See Figure 3.1 for details of feeding trials.

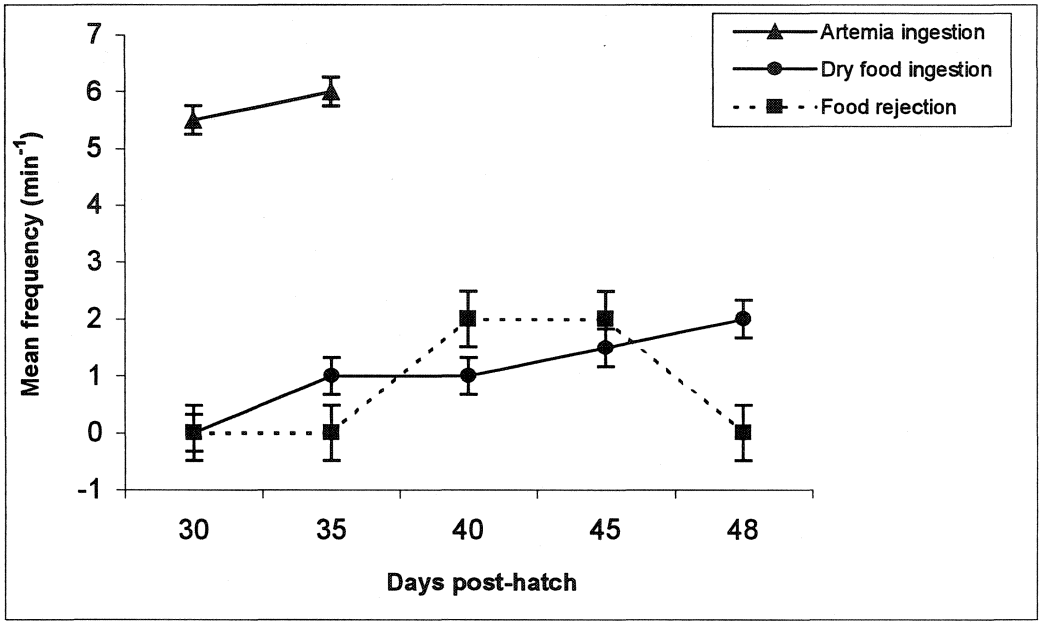

Figure 3.8 Mean frequency of ingestion and rejection of different **food items** by fat snook larvae in **Treatment 2** over age (days post-hatch). Each point represents 15 larvae observed per observational day. Vertical bars indicate S.E. See Figure 3.1 for details of feeding trials.

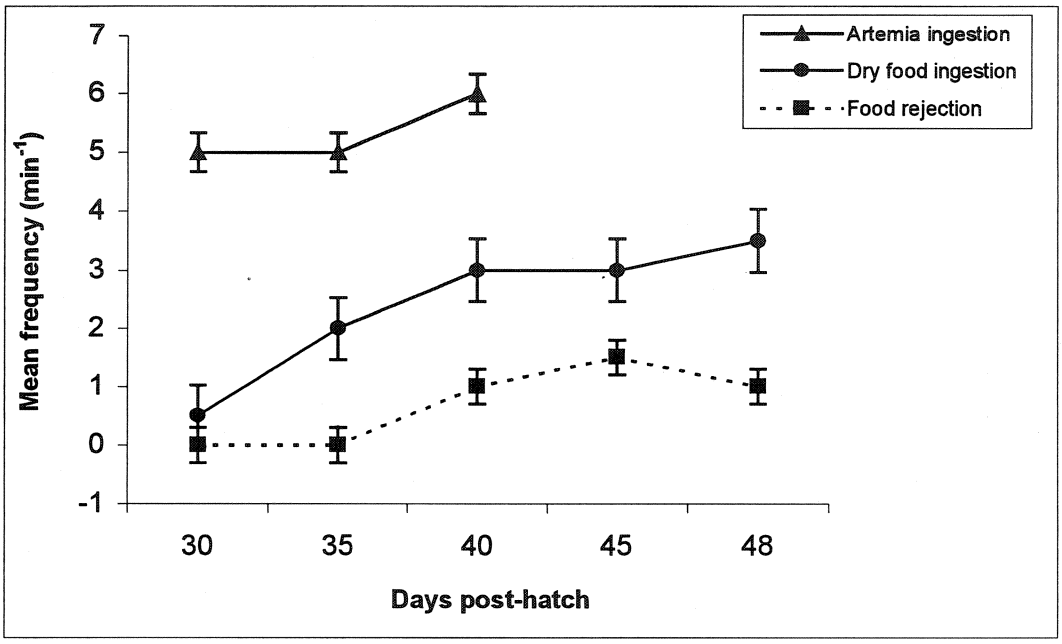

Figure 3.9 Mean frequency of ingestion and rejection of different **food items** by fat snook larvae in **Treatment 3** over age (days post-hatch). Each point represents 15 larvae observed per observational day. Vertical bars indicate S.E. See Figure 3.1 for details of feeding trials.

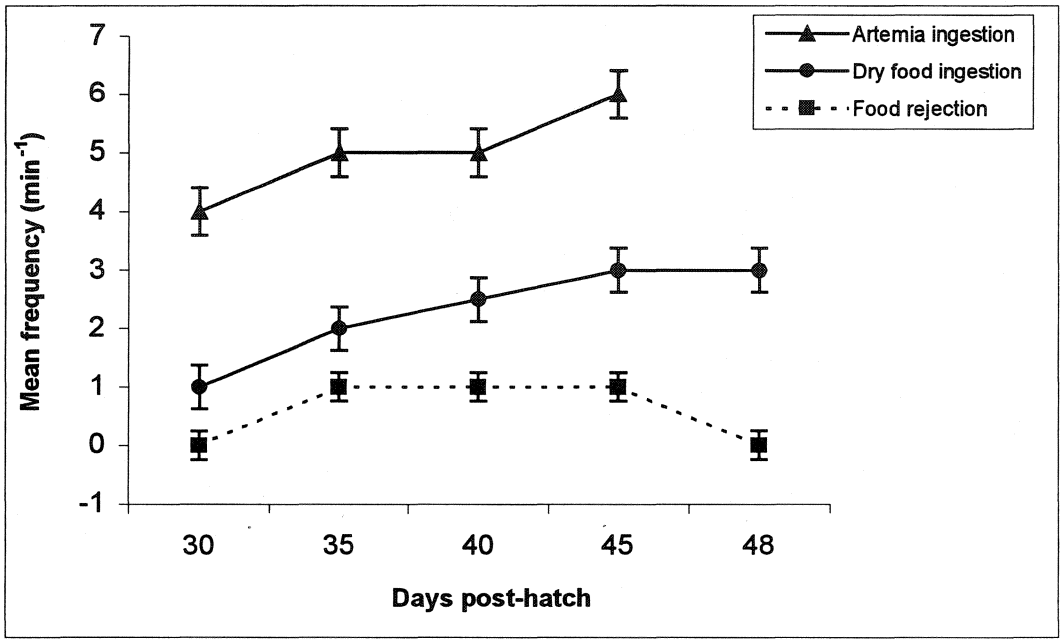

Figure 3.10 Mean frequency of ingestion and rejection of different **food items** by fat snook larvae in **Treatment 4** over age (days post-hatch). Each point represents 15 larvae observed per observational day. Vertical bars indicate S.E. See Figure 3.1 for details of feeding trials.

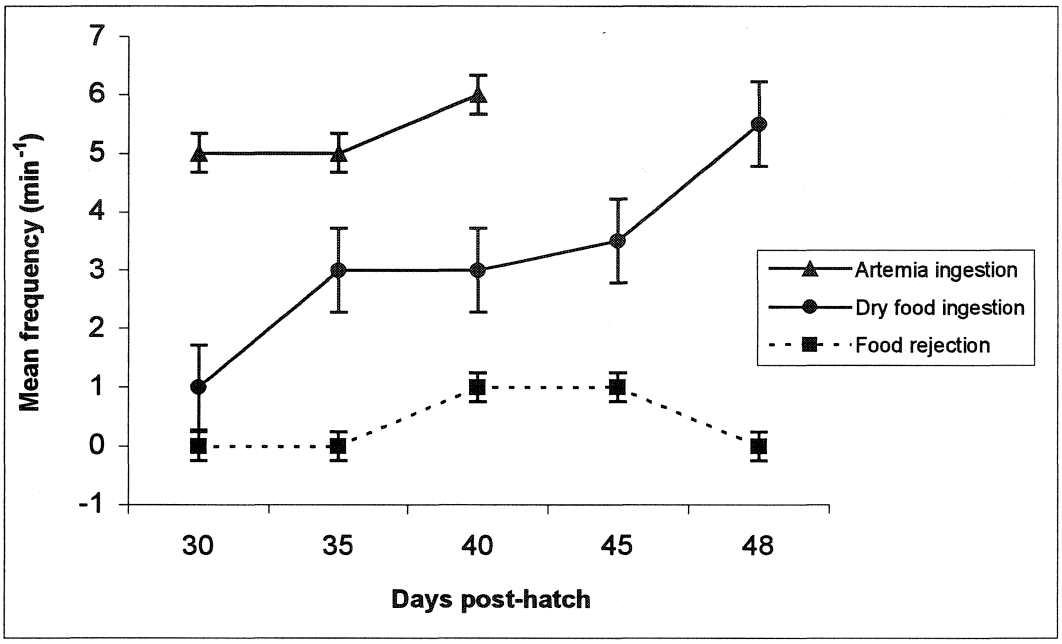

Figure 3.11 Mean frequency of ingestion and rejection of different **food items** by fat snook larvae reared in **Treatment 5** over age (days post-hatch). Each point represents 15 larvae observed per observational day. Vertical bars indicate S.E. See Figure 3.1 for details of feeding trials.

Table 3.8 Results from Mann-Whitney test, comparing **dry food ingestion** frequency by Fat snook larvae in early weaning trials.

Treatment comparison	Mann-Whitney test results
2 – 3	Z= -1.4523; P= 0.0732
2 – 4	Z= -3.2251; P= 0.0006 *
2 – 5	Z= -3.7125; P= 0.0001 *
3 – 4	Z= -1.3141; P= 0.0941
3 – 5	Z= -2.8621; P= 0.0022 *
4 – 5	Z= -2.4732; P= 0.0067 *

* - Significant difference at $P<0.05$

3.4 Discussion

From the start of the experimental period when dry food was offered to fat snook larvae, they approached the smallest pellet (300μm) and were fully weaned to the artificial diet by 35 DPH. The daily amount distributed to tanks was estimated to be 3g in each experimental tank. By the end of the experiment, larvae reared with *Artemia* for 5 days (treatment 2) were significantly smaller and had lower growth rate than larvae in all other treatments. There were no significant differences in standard length and SGR between treatments 1, 3, 4 and 5. However, larvae in this last treatment were the most efficient in catching and ingesting the dry food pellets.

A relatively low variation in larvae size was found among feeding trials. In treatment 2 larvae showed the lowest coefficient of variation for SGR. This conforms to the observations of Borba (1997) where larvae were weaned by 37 DPH and homogeneity in several growth performance variables was also observed. Borba (1997) suggested that this might be caused by cannibalism, which would result in homogeneity of size of larvae.

During the behavioral observations, fat snook larvae showed short and fast swimming activity followed by long periods spent motionless. Upon adding live prey (*Artemia*) in tanks, larvae changed this pattern and after a short period of motionless larvae increased their swimming speed and captured prey quickly. Fat snook larvae of the age studied here did not display a "s" shape pattern described for younger larvae of this species (Temple *et al.*, in press) or even observed for larvae of other marine species (Ellertsen *et al.*, 1980).

On the first two days of administration of dry diets, larvae still did not respond to the presence of pellets. Nevertheless, by 35 DPH larvae seemed to "learn" when dry food pellets were introduced and larvae began to catch the inert food more efficiently. It was most evident in treatment 5 (using Nutra marine® diet).

Aggressive behaviour was occasionally observed in my study, as one larvae chasing another larvae, prior to the first daily feeding. No mortality in my study could be attributed to cannibalism. The initial low size variability among larvae and low stocking density, as well as the constant presence of dry food in water seemed to provide optimal rearing conditions and cannibalism was avoided.

According to previous research in LAPMAR (J. Araujo, personal communication), the 12 hours dark period employed in my study also helped to reduce cannibalism, although no food was present during that period.

Several authors have emphasized that attention should be paid to the dimension and composition of dry food offered to larvae. Diet must be appropriate to the size of the larval mouth. Cahu and Zambonino-Infante (2001) successfully used dry food pellets of around 200-400μm in experiments with 40 day-old sea bass (*D. labrax*) larvae, while in commercial hatcheries 400-600μm pellets are used. However, they pointed out that small particles (< 100μm) cannot be detected by the majority of larvae, while large pellets are difficult to ingest or can cause a blockage of the digestive tract (Walford *et al.*, 1991).

Fernandez Diaz *et al.* (1994) observed that seabream (*Sparus aurata*) larvae select the size of microparticles in relation to their size and mouth width. Sea bream larvae from 6mm are reportedly able to ingest particles larger than 250μm. It was suggested by

Tucker (1987) that because snook have relatively large mouths and stomachs, acceptable feed size should increase quickly.

Although fat snook larvae started to approach dry food pellets on the first day of its availability (30 DPH), few larvae ingested them. As the larvae grew, from 35 DPH onwards, an increased trend in the frequency of ingestion of both live prey and dry food was noted in treatments 3, 4 and 5. This occurred at a point when live food was decreased to half ration (two days prior the end of live prey period), and a slight increase in dry food ingestion was observed, except for treatment 2. In this treatment larvae did not accept dry food and larvae showed considerable more rejection of dry food compared to all other treatments.

In a study with wolfish (*Anarhichas lupus*), a cold water species, Brown *et al.* (1997) observed a similar trend of feeding where larvae initially chose live prey (*Artemia*) and by the 6th week after hatch larvae started to prefer dry food. It was also observed by these authors that higher survival rates were obtained when larvae were fed a combination of live and dry feed.

It is frequently observed that fish larvae preferentially choose live prey over dry food when both foods are offered (Dutton, 1992; Fernandez-Diaz *et al.*, 1994; Yufera *et al.*, 1995; Canavate and Fernandez-Diaz, 1999) even though it is also observed that indirect higher ingestion rates of dry food occurs (Kolkovski *et al.*, 1997b).

Webster and Lowell (1990) observed a much higher presence of microdiet pellets in the gut of striped bass (*Morone saxatilis*) larvae when live *Artemia* was used instead of frozen *Artemia*. These authors attributed this to the lack of mobility and short retention

time that non-living feeding particles remain in the water column. In an attempt to wean fat snook larvae, Cerqueira *et al.* (1992) performed feeding trials where live feed was changed to frozen *Artemia*, and by 50 DPH juveniles were first introduced to dry food. Honczaryk and Cerqueira (1994) attained better results by using different natural ingredients and synthetic attractants in the dry feed. They observed that fat snook larvae seemed to accept the experimental diets as they captured the microdiet pellets early in development but still rejected them in some feeding trials. In their study, low larval growth and survival observed in some treatments was associated with the use of unenriched *Artemia* nauplii as live prey and also a prolonged period of using frozen *Artemia*. Several other studies on fat snook larval feeding followed utilizing frozen *Artemia* as the natural food prior to the experimental weaning periods (Cerqueira and Bernardini, 1995; Borba, 1997).

In a study carried out by Borba (1997), weaning at different ages was studied and fat snook larvae were weaned by 37 DPH. However, larvae decreased 25% in weight compared to control (only *Artemia*) and 29.7% in relation to larvae weaned by 55 DPH. Borba (1997) also found that fat snook larvae fed only *Artemia* did not show a better growth than larvae co-fed on dry diets, and they had the lowest condition among treatments. This is similar to my study of fat snook where the control treatment showed similar growth and condition as the other treatments (no significant differences except for treatment 2). Rosenlund *et al.* (1997) pointed out that acceptable growth rates cannot be maintained using live feed exclusively due to the low nutrient content and restricted feed intake.

Although centropomid larvae are reported to thrive on microdiets, several authors have emphasized the importance of using a combination of live prey and inert diets (co-feeding) to achieve satisfactory survival and growth rates (Edwards and Henderson, 1987; Juario *et al.*, 1991; Walford *et al.*, 1991; Walford and Lam, 1993).

Tucker (1987) suggested that prolonging the weaning process of common snook, *C. undecimalis*, by supplementing the artificial diet with live prey could increase the difficulty of weaning the fish to dry food. However, in my study, in treatment 2, the short co-feeding period with live prey (5 days), larvae could not be weaned by 35 DPH. Although larvae in this treatment had similar survival to all other treatments, larvae in treatment 2 showed significant lower growth rates from the middle of the experimental period onwards.

In an investigation on white seabass (*Atractoscion nobilis*), Barnabe and Guissi (1994) pointed out that it is during specific periods of development that fish larvae are able to ingest prepared diets. This is related to their behavioral and physiological development. The nutritional content of both live and artificial diets is also very important for larval growth, survival and weaning success (Person Le Ruyet *et al.*, 1993). It is well known that marine fish larvae have a limited ability to assimilate protein and lipid (Hamre *et al.*, 2001). The problems that fish larvae experience when fed prepared food may result from the physiological and morphological immaturity of the digestive system (Lauff and Hofer, 1984; Lam, 1991), the absence of proteolytic enzymes in live food or both (Dabrowski and Glogowski, 1977; Kolkovski *et al.*, 1997b; Garcia-Ortega *et al.*, 1998).

Previous studies have shown that the SGR of fat snook is highly variable. Honczaryk and Cerqueira (1994) observed that a fixed co-feeding period of 8 days, resulted in a variable SGR (around 5% among treatments). Moreover, these authors verified that the best results for SGR ($10.22\%day^{-1}$) of larvae fed inert diet (shark based) did not significantly differ from control treatment (fed only *Artemia*). Although the SGR in the present study was lower than those obtained in previous feeding experiments of fat snook, the SGR obtained in my study was calculated using standard length increments, while other authors (Honczaryk and Cerqueira, 1994; Borba, 1997) utilized body weight as the variable to evaluate larval growth. However, SGR variation in my experiment showed reasonable similarity (in increment rates) among feeding trials when compared to previous studies.

In their study, Cerqueira and Bernardini (1995) investigated late weaning of 50 day-old juvenile fat snook using an experimental and a commercial dry diet formulated for cold water fish. They found that the shark-based diet (experimental) contained natural attractants stimulating fat snook feeding behaviour. However, larvae were stimulated by this diet only during a short period at the beginning of the experimental period, and larvae did not have good growth. Furthermore, they observed a higher FCR (Food Conversion Ratio) for this diet compared to the commercial diet (INVE®).

In the present study I observed a similar result. The LAPMAR prepared diet seems to be lighter than the Nutra marine® dry food, and remained in the water column longer, thus it was more available to the larvae. In contrast, the Nutra marine® dry diet showed more attractiveness as larvae in treatment 5 had a significant higher ingestion

frequency of dry pellets compared to larvae in treatment 3 (LAPMAR dry diet), while both had the same co-feeding period with *Artemia*. However, there was no significant difference in either frequency of *Artemia* ingestion, growth rate (SGR) or condition between larvae reared in these treatments. Furthermore, the comparable performance of larvae in treatments 3 and 5 (both co-fed *Artemia* for 10 days) and treatment 4 (using *Artemia* for 15 days), may also be a result of overall food intake, food digestibility, and better assimilation of both dry diets, rather than the nutritional value of *Artemia*. Moreover, no significant difference in larval growth performance was found between these treatments and the control treatment.

Although larval growth rates (SGR) were similar among treatments (except for treatment 2), larvae from treatment 5 attained the highest mean final standard length among treatments using dry diet. The dry diet used in this latter treatment was originally formulated for cold water species, but it displayed a reasonable suitability for fat snook, a tropical species.

Even though larval performance was not showed significant different among the feeding trials using identical co-feeding periods (treatments 3 and 5), the behavioral observations provided reasons for slightly better growth of larvae fed on Nutra marine® dry diet over larvae reared with the LAPMAR prepared diet.

The current LAPMAR fat snook rearing protocol uses *Artemia* as live prey from 20 DPH, until approximately 50 DPH when larvae are weaned onto formulated diet. A co-feeding period of 20 days with live prey is usually employed, and dry food is first given to larvae around the 40^{th} day post-hatch. The findings of my study could result in

improvements in both growth and survival rates of fat snook larvae when larvae are weaned earlier, by 35 DPH. However significantly better results can be obtained when larvae are weaned by 40 DPH and co-fed *Artemia* for 10 days along with the LAPMAR diet.

Reducing the size of dry food pellets manufactured by LAPMAR from the pellet size currently employed, can replace live feeds at earlier larval stages. However, in order to achieve the best larval response, this procedure requires a protocol where *Artemia* is gradually withdrawn over a period of 10 days.

Chapter 4

Summary and further directions

Marine fish culture throughout the world is hampered by the lack of reliable rearing systems and practical diets for larvae. In most cases, high yields of marine species depend upon feeding the larvae with live prey up to several weeks after hatching. The production of live food is costly, unreliable and high mortalities are noticed when larvae are weaned to artificial diets, therefore fish hatcheries often do not achieve high productivity.

In my study, the main objectives were to evaluate if early weaning of Atlantic cod and fat snook is technically possible, in spite of the fact that an extended weaning period of late larval stages is already in current practice. Although important information regarding Atlantic cod larviculture has been gathered so far, crucial stages, such as weaning, still remain as one of the most critical steps for reliable juvenile production.

The results from both experiments in Chapter 2 with Atlantic cod larvae supported an overall evaluation of larvae performance during extreme weaning conditions. The period of using *Artemia* in weaning could be kept to around 10 days in most commercial hatcheries. Feeding cod larvae with *Artemia* and dry food earlier in my experiments resulted in similar growth rates and survival to larvae that had been fed *Artemia* later in larval development. The ingestion rate of inert food by younger larvae also suggests that larvae are able to feed on such a diet long before metamorphosis.

Using dry food pellets of smaller dimensions also brought improvements of dry diet intake rates as cod larvae accepted more readily the formulated diet used in both experiments. However, Atlantic cod larvae still require *Artemia* to act as a nutritional enrichment-carrier and as a prey item between rotifers and formulated diet. On the other hand, in order to get the best larval performance, the artificial diet employed for younger larvae needs to be ingested at a similar rate to live prey and be of appropriate nutritional composition. One of the major potential advantages of artificial diets is the ability to adjust the nutritional composition to suit the requirements of larvae. The balance between leakage of feed attractants and loss of nutrients must be well controlled. The smaller the pellet size, the greater relative leakage of vitamins and nutritive substances to the water, and thus the efficiency of the artificial diet relies on the manufacturing process of the feed.

Contrary to the Atlantic cod aquaculture, fat snook culture is still in its initial stages and much research has to be done on the nutritional requirements of larvae.

Fat snook aquaculture in Brazil is currently at a pilot scale. In Chapter 3, the weaning trials demonstrated that fat snook larvae displayed a satisfactory ability to identify, capture and ingest dry food pellets much earlier than currently done at LAPMAR. Better results were obtained when a smaller dry food was used. By 35 DPH fat snook larvae fed successfully on artificial diet only, employing a co-feeding period with *Artemia* of 5 days.

The artificial starter diet formulated by LAPMAR for weaning seems to be adequate for fat snook larvae, although larvae showed higher preference to the other

commercial diet. Further studies are still necessary to evaluate any growth enhancements in juveniles due to the higher food intake during the weaning by using a different artificial diet than that formulated by LAPMAR.

The continuous feeding (dry feed) using automatic feeders would also benefit the weaning process and may improve larval growth performance. As well, employing a feeding schedule with an artificial diet of 24 hours (releasing pellets at every 2 hours) should be done to determinate if this results in improvements in dry food acceptance during weaning.

The results of the experiments in my thesis represent improvements from the current larviculture protocols of both Atlantic cod and fat snook, allowing for a significant reduction in the daily supply of live food. It was also demonstrated that larvae of both species accepted dry food earlier if a suitable co-feeding period was employed.

Therefore, the profitability of larval production may be increased by reducing the requirement for live feed organisms through partial replacement of live foods. While live foods cannot be completely replaced in larval feeding protocols, this procedure might result in considerable cost savings. More research is required to establish the degree to which live feeds can be replaced with existing artificial diets.

References

Ager, L.A., Hammond, D.E. and Ware, F. 1978. Artificial spawning of snook. Proceedings of the Annual Conference Southeastern Association of Game and Fish Commissioners 30: 158-166.

Altman, J. 1974. Observational study of behaviour: sampling methods. Behaviour 91: 449-459.

Alvarez-Lajonchere, L. 2001. Fat snook breakthrough in Brazil. World Aquaculture Society 32: 23-25.

Alvarez-Lajonchere, L., Guerrero-Tortolero, D. and Perez-Urbiola, J.C. 2001. Validation of an ovarian biopsy method in a sea bass, *Centropomus medius* Guenter. Aquaculture Research 32: 379-384.

Appelbaum, S. 1985. Rearing of Dover sole, *Solea solea*, through its larval stages using artificial diets. Aquaculture 49: 209-221.

Atema, J. 1980. Chemical senses, chemical signals, and feeding behavior in fishes. In J. E. Bardach, J. J. Magnuson, R.C. May and J.M. Reinhart (Ed.). Fish behavior and its use in the capture and culture of fishes. ICLARM Conf. Proc. 5, 512p. Manila, Phillipines, pp. 57-101.

Barnabe G. and Guissi, A. 1994 Adaptations of the feeding behaviour of larvae of the sea bass, *Dicentrarchus labrax* (L.), to an alternating live-food/compound-food feeding regime. Aquaculture and Fisheries Management 25: 537-546.

Baskerville-Bridges, B. and Kling, L.J. 2000. Early weaning of Atlantic cod (*Gadus morhua*) larvae onto a microparticulate diet. Aquaculture 189: 109-117.

Borba, M.R. 1997. Efeito da idade e da utilização de compostos sintéticos como atrativos na adaptação da larva do robalo (*Centropomus parallelus* Poey, 1860) ao alimento formulado. MSc Thesis. UFSC/Santa Catarina, Brasil.

Borba, M.R., Cerqueira, V.R., Nolli, N.L. and Macchiavello, J.A.G. 1994. Utilização de diferentes fontes protéicas na dieta artificial de juvenis de robalo *Centropomus parallelus* em cultivo experimental. In: Simpósio Brasileiro de Aquicultura, 8, 1994, Piracicaba. Resumos dos trabalhos apresentados...Piracicaba: ABRAQ, 1994. p.43.

Bromage, N.R. 1995. Broodstock management and seed quality – general considerations. In N.R. Bromage and R.J. Roberts, editors. Broodstock management and egg and larval quality. Blackwell Science Ltd., Oxford/London/Edinburgh/Cambridge.

Brown, J.A., Wiseman, D. and Kean, P. 1997. The use of behavioural observations in the larviculture of cold-water marine fish. Aquaculture 155: 297-306.

Cahu, C. and Zambonino-Infante, J.L. 1994. Early weaning of sea bass (*Dicentrarchus labrax*) larvae with a compound diet: effect on digestive enzymes. Comparative Biochemistry and Physiology 109: 213-222.

Cahu, C. and Zambonino-Infante, J.L. 2001. Substitution of live food by formulated diets in marine fish larvae. Aquaculture 200: 161–180.

Cahu, C., Zambonino-Infante, J.L., Escaffre, A.M., Bergot, P. and Kaushik, S. 1998. Preliminary results on sea bass *Dicentrarchus labrax* larvae rearing with compound diet from first feeding, comparison with carp *Cyprinus carpio* larvae. Aquaculture 169: 1–7.

Canavate, J.P. and Fernadez-Diaz, C. 1999. Influence of co-feeding larvae with live and inert diets on weaning the sole *Solea senegalensis* onto commercial dry feeds. Aquaculture 174: 255-263.

Cerqueira, V.R. 1995. Testes de indução à desova do robalo *Centropomus parallelus*, do litoral da ilha de Santa Catarina com Gonadotrofina Coriônica Humana (HCG). In: Proceedings of Congresso Brasileiro de Engenharia de Pesca, 7, 1991, Santos: 95-101.

Cerqueira, V.R., Macchiavello, J.A.G. and Brugger, A.M. 1992. Produção de alevinos de robalo *Centropomus parallelus* Poey, 1860, através de larvicultura intensiva em laboratório. In: Proceedings of Simpósio Brasileiro de Aquicultura, 7, 1992, Peruíbe: 191-197.

Cerqueira, V. R. and Bernardini, M. E. 1995. The weaning of the fat snook *Centropomus parallelus* larvae with experimental and commercial artificial diets. European Aquaculture Society Special Publication. Larvi '95 Gent: v.24:272 – 275.

Chapman, P. 1982. Final report for sportfish introductions project. Study 1: artificial culture of snook. Florida Game and Fresh Water Fish Commission, Tallahassee.

Cook, R.M., Sinclair, A., and Stefansson, G. 1997. Potential collapse of North Sea cod stocks. Nature 385: 521–522.

Cowan , J.H. and Houde, E.D. 1990. Growth and survival of bay anchovy *Anchoa mitchilli* in mesocosm enclosures. Marine Ecology Progress Series 68: 47-57.

Dabrowski, K. 1984. The feeding of fish larvae: present "state of the art" and perspectives. Reproduction, Nutrition and Development 24: 807–833.

Dabrowski, K. and Glogowski, J. 1977. Studies on the role of exogenous proteolytic enzymes in digestion process in fish. Hydrobiologia 54: 129–134.

Daniels, H.V. and Hodson, R.G. 1999. Weaning success of southern flounder juveniles: effects of changeover period and diet type on growth and survival. North American Journal of Aquaculture 61: 47–50.

Devresse, B., Candreva, P., Leger, P. and Sorgeloos, P. 1991. A new artificial diet for the early weaning of sea bass (*Dicentrarchus labrax*) larvae. In: P. Lavens, P. Sorgeloos, E. Jaspers, F. Ollevier (Editors), Larvi'91 – Fish and Crustacean Larviculture Symposium, European Aquaculture Society. Spec. Pub. 15: 178-182. Gent, Belgium.

Dhert, P., Rombaut, G., Suantika, G. and Sorgeloos, P. 2001. Advancement of rotifer culture and manipulation techniques in Europe. Aquaculture 200: 129–146.

Dutton, P. 1992. Effects of experience on feeding success by larval white seabass *Atractoscion nobilis*. Journal of Fish Biology 41: 765-773.

Edwards, R.E. and Henderson, B.D. 1987. An experimental hatchery project: studies of propagation, culture and biology of snook (*Centropomus undecimalis*). Annual Gulf and Caribbean Fish Institute, 38. Florida. F. Williams (Ed). p.211-221.

Ehrlich, K., Cantin, M. and Rust, M. 1989. Growth and survival of larval and post larval smallmouth bass fed a commercially prepared dry feed and/or *Artemia* nauplii. Journal of the World Aquaculture Society 20: 1–11.

Ellertsen, B., Monkness, E. Solemdal, P. Strømme, T., Tilseth, S., Westgard, T. and Øiestad, V. 1980. Some biological aspects of cod larvae (*Gadus morhua* L.). Fishkeridirektoratets Skrifter Serie Havundersokelser 17:29-47.

Fernandez-Diaz, C. and Yufera, M. 1997. Detecting growth in gilthead seabream *Sparus aurata* larvae fed microcapsules. Aquaculture 153: 93–102.

Fernandez-Diaz, C., Pascual, E. and Yufera, M. 1994. Feeding behaviour and prey size selection of gilthead seabream, *Sparus aurata*, larvae fed on inert and live food. Marine Biology 118: 323-328.

Fogarty, M.J., Myers, R.A. and Bowen, K.G. 2001. Recruitment of cod and haddock in the North Atlantic: a comparative analysis. Journal of Marine Science 58: 952–961.

Food and Agriculture Organization (FAO). 1999. Aquaculture production statistics 1988-1997. FAO Fisheries Circular (815) Rev. 11, 203pp.

Foscarini, R. 1988. A review: intensive farming for red sea bream *Pagrus major* in Japan. Aquaculture 72: 191–246.

Fraser, T.H. 1978. Centropomidae. In W. Fischer (ed.) FAO species identification sheets for fishery purposes. West Atlantic (Fishing Area 31). FAO, Rome. Vol. 1-2.

Garcia-Ortega, A., Verreth, J.A.J., Coutteau, P., Segner, H., Huisman, E.A. and Sorgeloos, P. 1998. Biochemical and enzymatic characteristics of decapsulated cysts and nauplii of the brine shrimp *Artemia* at different developmental stage. Aquaculture 161: 501–514.

Gennari, L., Roncarati, A., Melotti, P. and Mordenti, O. 1992. Effects of early weaning on growth and survival of sea bass (*Dicentrarchus labrax* L.). Rivista Italiana di Acquacoltura 27: 133-143.

Gennari, L., Roncarati, A., Melotti, P. and Loro, F. 1994. Early weaning trials of sea bass (*Dicentrarchus labrax* L.) using different artificial diets. Aquaculture 124: 283-284.

Hamre, K., Næss, T., Espe, M., Holm, J.C. and Lie, Ø. 2001. A formulated diet for atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae. Aquaculture Nutrition 7: 123-132.

Holt, G.J. 1993. Feeding larval red drum on microparticulate diets in a closed recirculating water system. Journal of the World Aquaculture Society 24: 225-230.

Honczaryk, A. and Cerqueira, V.R. 1994. Adaptation of fat snook *Centropomus parallelus* (Poey) larvae to artificial diets. High Performance Fish: Proceedings of an international fish physiology symposium at the University of British Columbia in Vancouver, Canada, July 16-21 1994., Fish Physiology Association, Vancouver, BC, Canada , 1994, pp. 383-388.

Hutchings, J.A. and Myers, R.A. 1994. What can be learned from the collapse of a renewable resource? Atlantic cod (*Gadus morhua*) of Newfoundland and Labrador. Canadian Journal of Fisheries and Aquatic Sciences 51: 2126–2146.

Jones, D.A., Kamurudin, M.S. and Levay, L. 1993. The potential for replacement of live feeds in larval culture. Journal of the World Aquaculture Society 24: 191–210.

Juario, J.V., M.N. Duray and Fuchs, J. 1991. Weaning of seabass *Lates calcarifer* larvae to artificial diet. Larvi' 91, Fish and Crustacean Larviculture Symposium. European Aquaculture Society. Spec. Publ. 115, p. 183, Gent, Belgium.

Kanazawa, A. and Teshima, S. 1988. Microencapsulate diets for fish larvae. NOAA Tech. Rep. NMFS 70. National Marine Fisheries Service. Seattle, WA, pp. 57-62.

Kanazawa, A., Koshio, S. and Teshima, S. 1989. Growth and survival of larval red sea bream *Pagrus major* and japanese flounder *Paralichtys olivaceus* fed microbound diets. Journal of the World Aquaculture Society 20: 31-37.

Kestemont, P., Fogbé, E.D., Parfait, O., Micha, J.C. and Mélard, C. 1995. Relationship between weaning age, growth, survival and cannibalism in the common perch larvae *Perca fluviatilis*: preliminary data. In: Larvi'95 – Fish and Crustacean Larviculture Symposium, European Aquaculture Society. Spec. Pub., 24: 285-288. Gent, Belgium.

Kolkovski, S. 2001. Digestive enzymes in fish larvae and juveniles—implications and applications to formulated diets. Aquaculture 200: 181–201.

Kolkovski, S. and Dabrowski, D. 1999. Diets for fish larvae—present state of art. World Aquaculture '99. Sydney, Australia. World Aquaculture Society, p. 406.

Kolkovski, S. and Tandler, A. 1995. Why microdiets are still inadequate as a viable alternative to live zooplankters for developing marine fish larvae. In: Lavens, P., Jaspers, E., Roelants, I. Eds. , LARVI '95- Fish and Shellfish Symposium. Gent, Belgium. European Aquaculture Society. Spec. Publ. 24, 265–267.

Kolkovski, S., Arieli, A. and Tandler, A. 1997a. Visual and chemical cues stimulate microdiet ingestion in gilthead seabream, *Sparus aurata*, larvae. Aquaculture International 5: 527–536.

Kolkovski, S., Koven, W.M. and Tandler, A. 1997b. The mode of action of *Artemia* in enhancing utilization of microdiet by gilthead seabream *Sparus aurata* larvae. Aquaculture 155: 193–205.

Kolkovski, S., Tandler, A. and Izquierdo, M.S. 1997c. Effects of live food, dietary digestive enzymes on the efficiency of microdiets for seabass *Dicentrarchus labrax* larvae. Aquaculture 148: 313–322.

Koslow, J.A., Brault, S., Foirnier, R.O. and Hughes, P. 1985. Condition of larval cod (*Gadus morhua*) off southwest Nova Scotia in 1983 in relation to plankton abundance and temperature. Marine Biology 86: 113-121.

Koven, W., Kolkovski, S., Hadas, E., Gamsiz, K. and Tandler, A. 2001.Advances in the development of microdiets for gilthead seabream, *Sparus aurata*: a review. Aquaculture 194: 107–121.

Kurlansky, M. 1997. Cod: A biography of the fish that changed the world. Walker and Co., New York.

Lam, T.J. 1991. Fish larval physiology and its implications for larval rearing in aquaculture. Program and Abstracts. In: Fenical, W., Greenberg, M., Halvorson, H.O., Hunter-Cevera, J.C. Eds. , Second International Marine Biotechnology Conference IMBC'91 1991, 13–16 October 1991. 58 pp.

Lau, S.R. and Shafland, P.L. 1982. Larval development of snook, *Centropomus undecimalis* (Pisces: Centropomidae). Copeia 3: 618-627.

Lauff, M. and Hofer, R. 1984. Proteolytic enzymes in fish development and the importance of dietary enzymes. Aquaculture 37: 335–346.

Lavens, P., Sorgeloos, P., Dhert, P. and Devresse, B. 1995. Larval food. In: Bromage, N.R., Roberts, R.J. Eds. Broodstock Management and Egg and Larval Quality. University Press, Cambridge, UK, pp. 373–398.

Lazo, J.P., Holt, G.J. and Arnold, C.R. 2000a. Ontogeny of pancreatic enzymes in larval red drum (*Sciaenops ocellatus*). Aquaculture Nutrition 6: 183-192.

Lazo, J.P., Dinis, M.T., Holt, G.J., Faulk, C. and Arnold, C.R. 2000b. Co-feeding microparticulate diets with algae: toward eliminating the need of zooplankton at first feeding in larval red drum (*Sciaenops ocellatus*). Aquaculture 188: 339–351.

Lear, W.H. 1993. Atlantic Cod. In Underwater World. Department of Fisheries and Oceans.

Lee, C.S. and Ostrowski, A.C. 2001. Current status of marine finfish larviculture in the United States. Aquaculture 200: 89-109.

Lee, P.S., Southgate, P.C. and Fielder, D.S. 1996. Assessment of two microbound artificial diets for weaning Asian sea bass (*Lates calcarifer*, Bloch). Asian Fisheries Science 9, 115–120.

Leger, P., Bengtson, D.A., Sorgeloos, P., Simpson, K.L. and Beck, A.D. 1987. The nutritional value of *Artemia*: a review. In: Sorgeloos, P., Bengtson, D.A., Decleir, W., Jaspers, E. (Eds). *Artemia* research and its applications. Ecology, Culturing, use in Aquaculture, vol. 3. Universal Press, Wetteren, pp. 357–372.

Mioso, R. and Cerqueira, V.R. 1994. Incubação de ovos de robalo *Centropomus parallelus* Poey, 1860. In: Proceedings of Simpósio Brasileiro de Aquicultura, 8, 1994, Piracicaba, p. 100.

Mioso, R., Cerqueira, V.R. and Macchiavello, J.A.G. 1994a. Indução a ovulação com HCG e desova natural do robalo *Centropomus parallelus* Poey, 1860. In: Proceedings of Simpósio Brasileiro de Aquicultura, 8, 1994, Piracicaba, p.98.

Mioso, R., Cerqueira, V.R. and Macchiavello, J.A.G. 1994b. Indução a ovulação com HCG e fertilização artificial do robalo *Centropomus parallelus* Poey, 1860. In: Proceedings of Simpósio Brasileiro de Aquicultura, 8, 1994, Piracicaba, p.99.

Mioso, R. 1995. Indução a reprodução e incubação de ovos do robalo *Centropomus parallelus* Poey, 1860 (Pisces, Centropomidae) MSc Thesis. UFSC/Santa Catarina, Brasil.

Noldus Information Technology. 1990. The Observer. Version 2.0 (c). University Business & Technology Center, Wageningen, Netherlands.

Olsen, Y. 1997. Larval rearing technology of marine species in Norway. Hydrobiologia 358: 27-36.

Person Le Ruyet, J. 1991. Feeding of marine fish larvae: microdiets or live preys? In: P. Lavens, P. Sorgeloos, E. Jaspers, F. Ollevier (Editors), Larvi'91 – Fish and Crustacean Larviculture Symposium. European Aquaculture Society. Spec. Publ. 15, 168-169. Gent, Belgium.

Person-Le Ruyet, J., Alexandre, J.C., Thebaud, U. and Mugnier, C. 1993. Marine fish larvae feeding: formulated diets or live prey? Journal of the World Aquaculture Society 24: 211–224.

Puvanendran, V. and Brown, J.A. 1999. Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. Aquaculture 175: 77-92.

Puvanendran, V. and Brown, J.A. 2002. Foraging, growth and survival of Atlantic cod larvae reared in different light intensities and photoperiods. Aquaculture 214: 131-151.

Puvanendran, V, Leader, L.L. and Brown, J.A. 2002. Effect of prey concentration on the foraging behaviour of larval Atlantic cod from hatching to metamorphosis. Canadian Journal of Zoology 80: 689-699.

Ringo, E., Johansen, L. and Raa, J. 1991. Feeding of cod, *Gadus morhua* L., larvae on an artificial diet. Preliminary results. Fisheries Research 11: 191–193.

Rivas, L.R. 1986. Systematic review of the perciform fishes of the genus *Centropomus*. Copeia 3: 579-611.

Rosenlund, G., Meslo, I., Rodsjo, R. and Torp, H. 1993. Large scale production of cod. In: Reinertsen, H., Dahle, L.A., Jorgensen, L., Tvinnereim, K. Eds. , Proceedings of the First International Conference on Fish Farming Technology, Trondheim, Norway, 9–12 August. Balkema, Rotterdam, pp. 141–146.

Rosenlund, G., Stoss, J. and Talbot, C. 1997. Co-feeding marine fish larvae with inert and live diets. Aquaculture 155: 187-196.

Roberts, D.E. Jr. 1990. Snook (Centropomidae) and grouper (Serranidae) mariculture in the Gulf of Mexico and Caribbean Basin. Advances in Tropical Aquaculture, Tahiti, 20Feb - 4 Mar 1989. Ifremer/SDP, p.485.

Seaman, W.Jr., and Collins, M. 1983. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Florida) -- snook. U.S. Fish Wildl Serv. FWS/OBS-82/11.16. U.S. Army Corps of Engineers, TR EL-82-4. 16pp.

Serfling, S.A. 1998. Breeding and culture of snook, *Centropomus undecimalis*, in a closed-cycle, controlled environment culture system. p 482, In Abstracts, Aquaculture'98, World Aquaculture Society, Las Vegas (USA), February 15-19, 1998.

Shafland,P.L. 1979. Self-contained upwelling system for rearing larval fishes in the Laboratory. The Progressive Fish-Culturist 41: 10-13.

Shafland,P.L. and Koehl, D.H. 1980. Laboratory rearing of the common snook. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 33: 425-431.

Shields, R.J. 2001. Larviculture of marine finfish in Europe. Aquaculture 200: 55–88.

Sokal, R.R. and Rohlf, F.J. 1995. Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freeman company, NY, USA. 887 pp.

Sorgeloos, P., Dhert, P. and Candreva, P. 2001. Use of brine shrimp, *Artemia* spp., in marine fish larviculture. Aquaculture 200: 147-159.

Southgate, P.C. and Partridge, G.J. 1998. Development of artificial diets for marine finfish larvae: problems and prospects. In: De Silva, S.S. Ed., Tropical Mariculture. Academic Press, San Diego, California, USA, pp. 151–171.

SPSS Inc. 1999. SPSS for Windows, version 10.X Base User's Guide. SPSS Inc. Chicago, IL, USA.

Takeuchi, T., Ohkuma, N., Ishida, S. Ishizuka, W. Tomita, M. Hayasawa, H. and Miyakawa, H. 1998. Development of microparticle diet for marine fish larvae. Abstract only. 8th International Symposium on Nutrition and Feeding of Fish, 1-4 June 1998, Las Palmas de Gran Canaria, Canary Islands, Spain, p. 193.

Tandler, A. and Kolkovski, S. 1991. Rates of ingestion and digestibility as limiting factors in the successful use of microdiet in *Sparus aurata* larvae. In: Lavens, P., Sorgeloos, P., Jaspers, E., Ollevier, F. Eds. , Larvi '91—Fish and Crustacean Larviculture Symp. European Aquaculture Society. Spec. Publ. 15, 169–171. Gent, Belgium.

Temple, S., Cerqueira, V.R. and Brown, J.A. (in press). Defining prey density requirements for the intensive culture of larval fat snook (*Centropomus parallelus* Poey, 1860). Aquaculture.

Tucker, J.W. Jr. 1987. Snook and Tarpon Snook culture and preliminary evaluation for commercial farming. The Progressive Fish-Culturist 49: 49-57.

Tucker, J.W. Jr. 1998. Marine Fish Culture. Ed. Kluwer Academic Publishers. 731p. Boston, USA.

Tucker, J.W. Jr and Jory, D.E. 1991. Marine fish culture in the Caribbean region. World Aquaculture 22: 10-27.

Tucker, J.W. Jr., Landau, M.P. and Faulkner, B.E. 1985. Culinary value and composition of wild and captive common snook *Centropomus undecimalis*. Florida Scientist 48: 196-200.

Tucker, J.W. Jr. and Kennedy, S.B. 2001. Snook culture. Aquaculture 2001: Book of Abstracts, World Aquaculture Society, 143.

Van der Meeren, T and Nass, K.E. 1997. Development of rearing techniques using large enclosed ecosystems in the mass production of marine fish fry. Reviews in Fisheries Science 5: 367-390

Vasquez, O. 1988. A review of potentially cultivable marine species in Latin American waters. In: Production de Larvas y Juveniles de Especies Marinas, 1988, Coquimbo. Proceedings...Coquimbo: 1988, p. 31-37.

Walford, J. and Lam, T.J. 1993. Development of digestive tract and proteolytic enzyme activity in sea bass *Lates calcarifer* larvae and juveniles. Aquaculture 109: 187–205.

Walford, J., Lim, T.M. and Lam, T.J. 1991. Replacing live foods with microencapsulated diets in the rearing of seabass *Lates calcarifer* larvae: do the larvae ingest and digest protein-membrane microcapsules? Aquaculture 92: 225–235.

Watanabe, T. and Kiron, V. 1994. Prospects in larval fish dietetics. Aquaculture 124: 223-251.

Webster, C.D. and Lowell, R.T. 1990. Comparison of live brine shrimp nauplii and nonliving diets as first food for stripped bass larvae. Progressive Fish-Culturist 52: 171-175.

Yin, M.C. and Blaxter, J.H.S. 1987. Feeding ability and survival during starvation of marine fish larvae reared in the laboratory. Journal of Experimental Marine Biology and Ecology 105: 73-83.

Youson, J.H. 1988. First metamorphosis. In: Fish physiology: The physiology of developing fish. Hoar, W.S. and Randall, D.J. (Eds). 1988. London: Academic Press, Vol. 12.

Yufera, M., Fernandez-Diaz, C. and Pascual, E. 1995. Feeding rates of gilthead seabream *Sparus aurata* larvae on microcapsules. Aquaculture 134: 257–268.

Yufera, M., Pascual, E. and Fernandez-Diaz, C. 1999. A highly efficient microencapsulated food for rearing early larvae of marine fish. Aquaculture 177: 249-256.

Zambonino-Infante, J.L., Cahu, C.L. and Perez, A. 1997. Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval development. The Journal of Nutrition 127: 608-614.

Zohar, Y. 1996. New approaches for the manipulation of ovulation and spawning in farmed fish. Bulletin of National Research Institute of Aquaculture, Supplement 2: 43–48.