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Improving Larval Culture and Rearing Techniques on Common Snook (*Centropomus undecimalis*)

Carlos Yanes Roca¹ and Kevan L. Main²

¹*Stirling University*

²*Mote Marine Laboratory*

¹UK

²USA

1. Introduction

The common snook or *Centropomus undecimalis* (Bloch) is a diadromus, stenothermic, euryhaline, estuarine-dependent species found in the tropical and sub-tropical western Atlantic Ocean from about 34° N to about 25° S latitude (Howells *et al.*, 1990). The snook physiology is characterized by a distinct lateral line, high divided dorsal fin, sloping forehead, a large mouth, a protruding lower jaw and a yellow pelvic fin (Fore & Schmidt, 1973).

Partial genetic isolation occurs between Florida's Atlantic and Gulf Coast stocks (Tringali & Bert, 1996). Snook are protandric hermaphrodites: some males develop into females between 1 and 7 years of age, having a maximum 20-year lifespan. Females are generally larger than males of the same age, at the same time it is unusual to find females smaller than 500 mm in fork length. Snook growth rates are highly variable. For instance, Atlantic Coast fish grow more quickly and to a larger size than do fish on the Gulf Coast (Taylor *et al.* 2000).

Common snook form the basis of important fisheries throughout their range due to their sporting and culinary attributes (Tucker *et al.*, 1985; Matlock & Osburn, 1987). Numbers of common snook have declined over recent years due to shoreline development, fishing pressure, and loss of coastal habitats. As a result, common snook were designated as a game fish restricted to recreational harvest only. Depletion of some Florida stocks during the late 1970's and the early 1980's (Bruger & Haddad, 1986) resulted in common snook being declared a species of special concern, and they are now protected by strict regulations enacted by the Florida legislature.

The ultimate objective of hatchery-based production of common snook in Florida is the supply of high quality animals to restore declining stocks and enhance local populations. The quality of the juveniles, environmental conditions and releasing techniques are all involved in the success of restocking programs (Tsukamoto 1993). The objective of larval rearing is to mass-produce high-quality and healthy juvenile fish. The management of both the rearing environment and feeding regime are the most important aspects of this activity.

To improve larval rearing techniques, a good understanding of larval morphology, behaviour, live food and artificial diet requirements, and environmental conditions is fundamental (Liao *et al.*, 2001).

Collection of data on the conditions required for spawning, larval rearing, and release of common snook into marine and freshwater systems began in 1974 at the Florida Game and Fresh Water Fish Commission (Ager *et al.*, 1978; Shafland and Koehl, 1980, Chapman *et al.*, 1978). Information on the development of laboratory reared larvae and juveniles (Lau and Shafland, 1982) and on the lower lethal temperature (15°C) for juveniles (Howells *et al.*, 1990) was also documented. These studies described the basic common snook biology and the principles for captive rearing. Although research on this species in Florida and Texas was carried out during the 1970's, 1980's and 1990's, there are still a number of gaps in our understanding of the requirements for successful larval rearing and broodstock management.

1.1 Snook larval culture

Techniques of larviculture have gradually been developed from simply collecting the stocking material in the wild to using modern, advanced facilities for complete larviculture practices (Liao *et al.*, 2001). Common snook culture research in Florida, Texas, Mexico and Brazil has primarily relied on collection of fertilized eggs from mature wild fish and more recently at Mote Marine Laboratory on captive broodstock production. At this time, snook larviculture practices are still under-development when compared to many other marine fish species, such as red drum and cobia. This paper is focused on larval rearing during the first 14 days after hatching using wild, strip spawned eggs. The techniques investigated include the design of a larviculture system, diet requirements of larvae, and system management.

1.1.1 Importance of temperature on embryonic and larval development

Nearly every aspect of early fish development is affected by temperature (i.e., fertilization, hatching, first feeding) (Alderdice and Velsen, 1978; Heggberget and Wallace, 1984, Brännäns, 1987; Crisp, 1988; Kane, 1988; Jensen *et al.*, 1989; Beacham and Murray, 1990; Blaxter, 1992). Other aspects affected by temperature are the yolk conversion efficiency as demonstrated in salmonid embryos (Heming, 1982; Heming and Buddington, 1988; Marr, 1996; Peterson & Martin-Robichaud, 1995) and in striped bass (Peterson *et al.*, 1996). Also larval size and fitness at the end of the endogenous feeding period are directly affected by temperature (Peterson *et al.*, 1977, 1996, Baynes and Howell, 1996). Therefore, temperature has a key controlling effect on metabolic processes through thermal dependence on enzymatic activity (Brett, 1970; Rombough, 1988; Blaxter, 1992).

1.1.2 Larval stocking densities

One of the key aspects of successful large-scale production is determining the optimum larval stocking densities. For several species of fish, such as sea bass (*Dicentrarchus labrax*) or sea-bream (*Sparus aurata*), optimum culture densities are well known. The optimal stocking density varies between species depending on the behavioural and physical characteristics (Tagawa *et al.*, 1997, 2004; Kaji *et al.*, 1999; Hernandez-Cruz *et al.*, 1994). Larval density

studies for common snook have not been conducted, although some work has been done on fat snook (*Centropomus parallelus*) evaluating the effect of larval and juvenile densities on growth (Cerqueira *et al.*, 1995).

1.1.3 Prey density

Prey density (Werner and Blaxter, 1981) is one of the factors affecting feeding efficiency and consequently larval growth and survival under culture conditions. Enhancing feeding efficiency, at first feeding, can reduce the risk of starvation during the first days of development (Peña *et al.*, 2004). It has also been shown that foraging success increases with prey density (Wyatt, 1972; Laurence, 1974, 1978; Houde and Schekter, 1980; Munk and Kiørboe, 1985) until an asymptote is reached (Houde and Schekter, 1980; Klumpp and Von Westernhagen, 1986). Feeding levels (e.g. rotifer densities) must be tailored to the needs and consumption rates of the larvae at different ages so that food is not wasted, larvae are not underfed, and rearing water is not fouled. The usefulness of food to larvae at particular stages may be measured by food intake, growth and survival (Duray, *et al.*, 1996).

1.2 First feeding

One of the key restrictions in larval rearing is first feeding at early stages of development. This is a major bottleneck for larval culture, due primarily to their small size and often poorly developed digestive system (Person Le Ruyet, *et al.*, 1993). Many marine fish larvae require motile prey organisms (Pedersen *et al.*, 1987; Pedersen and Hjelmeland, 1988). Visual skill is not only important for feeding but also for orientation, schooling and eluding predators (Blaxter, 1986, Batty 1987). Larval survival clearly depends on their ability to feed successfully (Heath, 1992). During the endo-exotrophic phase (Mani-Ponset *et al.*, 1996), larvae utilize nutrients from both yolk sac and their surrounding environment. This phase starts soon after hatching, especially in larvae with a small yolk sac (Calzada *et al.*, 1998). This first feeding phase is critical for larval survival; therefore, successful synchronization between exhaustion of endogenous reserves and first feeding must occur.

Larval mouth size at first feeding is also an important factor for larval survival. The mouth size of first-feeding larvae mechanically restricts the size of the food particles that can be ingested. In general, mouth size is correlated with body size, which in turn is influenced by egg diameter and the period of endogenous feeding (i.e., yolk sac consumption period). For example, Atlantic salmon eggs are usually at least four times larger than Gilthead sea bream eggs and consequently on hatching yield large salmon larvae with large yolk sac supplies (i.e., sufficient endogenous feed reserves for the first three weeks of their development). Whereas first-feeding Gilthead sea bream larvae are very small with limited yolk sac reserves, and consequently can only feed endogenously for about three days (Jones & Houde, 1981).

1.2.1 Background phytoplankton ('Green water')

Most marine fish larvae are visual feeders and feeding success of larvae at various developmental stages depends on the provision of suitable food, the rearing environment, and on the visibility and adequate density of the prey (Ina *et al.*, 1979, Hunter, 1980). Publications on the rearing of marine fish larvae indicate that phytoplankton cultures

enhance survival rates (May, 1971; Al-Abdul-Elah, 1984; Hernandez-Cruz, *et al.*, 1994; Marliave, 1994). Furthermore, several papers have discussed the beneficial effect of adding microalgae to larval rearing tanks in order to improve larval growth and survival (Howell 1979; Scott & Middleton 1979; Jones & Houde, 1981; Bromley & Howell 1983; Vasquez-Yeomans, *et al.*, 1990; Naas, *et al.*, 1992; Hernandez-Cruz *et al.*, 1994; Marliave 1994; Tamaru, *et al.*, 1994). These papers discuss the effect of micro-algae on the nutritional and behavioural aspects of fish larvae. Some fish larvae take up substantial amounts of micro-algae during the initial days after hatching (Van der Meeren, 1991; Reitan, *et al.*, 1991) which maybe used as a food source. In recent years, the benefits of culturing larvae in 'green water' is considered to be optical rather than nutritional to fish larvae (Marliave 1994).

1.2.2 Rotifers and their nutritional value

Live food organisms are an important food source for the first feeding of early larval stages. The most widely used starter live-food organism in fish larviculture is the marine rotifer *Brachionus plicatilis*. The successful development of commercial fish farms in the Mediterranean has been made possible by several improvements in production techniques for rotifers (Candrea *et al.*, 1996; Dehasque *et al.*, 1998). Rotifers are an ideal link in the food chain for different stages of fish and shrimp larvae. Rotatoria (=Rotifera) belong to the smallest metazoan of which over 1000 species have been described, 90% of which inhabit freshwater habitats. They seldom reach 2 mm in body length. Males have reduced sizes and are less developed than females; some measuring only 60 µm. The body of all species consists of a constant number of cells, with various *Brachionus* species containing approximately 1000 cells, which should not be considered as single identities, but as a plasma area. Growth of the animal is achieved by plasma volume increase and not by cell division. The epidermis contains a densely packed layer of keratin-like proteins and is called the lorica. The shape of the lorica and the profile of the spines and ornaments allow determination of different species and morphotypes. A rotifer body is differentiated into three distinct parts consisting of the head, trunk and foot. The head carries the rotatory organ or corona, which is easily recognized by its annular ciliation and is the characteristic that led to the name Rotatoria (bearing wheels). The retractable corona assures locomotion and a whirling water movement, for the uptake of small food particles (mainly algae and detritus). The trunk contains the digestive tract, the excretory system and the genital organs. A characteristic organ of rotifers is the mastax (a calcified apparatus in the mouth region) that is very effective in grinding ingested particles. The foot is a ring-type retractable structure without segmentation ending in one or four toes. *B. plicatilis*, a cosmopolitan inhabitant of inland saline and coastal brackish waters. It has a lorica length of 100 to 340 µm, with the lorica ending in 6 occipital spines (Fukusho, 1989).

The nutritional value of *B. plicatilis* is dependent on the nutritional value of its food source, which can influence its suitability as a starter feed for marine larvae, and is determined by the concentrations of highly unsaturated fatty acids ((n-3) HUFA), such as docosahexaenoic acid (DHA, 22:6(n-3)) and eicosapentaenoic acid (EPA, 20:5(n-3)). Low dietary HUFA levels can lead to high mortality in fish larviculture. Koven *et al.* (1990) suggested that HUFAs function as essential components of bio-membranes, and that their levels in the tissue phospholipid fraction are associated with larval growth. Rainuzzo *et al.* (1997) emphasized the importance of DHA in the development of neural tissues such as brain and retina,

considering that the larval head constitutes a significant part of the body mass, and that predatory fish larvae rely on vision to capture their food. Sorgeloos *et al.*, (1988) reported a strong correlation between dietary EPA content and survival, and between DHA and growth of Asian sea bass larvae. Watanabe (1993) concluded that DHA and EPA increased survival and growth of several marine fish larvae. At the same time, Kanazawa (1993) observed that high DHA levels increased the tolerance of red sea-bream larvae to various stressful conditions.

1.2.3 Copepods

The suitability of copepods as live prey for marine fish larvae is now well established, but their use in aquaculture remains sporadic. Although of lower nutritional value, the relative ease of production of rotifers and *Artemia* nauplii continues to ensure their predominance. Studies in the literature have highlighted differences in the levels and ratios of fatty acids, lipid classes and pigments between copepods and traditional live prey used in hatcheries. Such differences are important for fish larval nutrition, as previously mentioned. The consequences of poor nutrition during fish larval development can result in deformities or malpigmentation, and in some cases may be less obvious, such as effects on temperature tolerance or growth during later life stages. (Støttrup, 2000). Rearing the larvae of most marine fish species requires provision of live prey for variable periods from the onset of exogenous feeding. A common feature of these species is the production of small pelagic eggs. Larvae generally hatch at an early stage in their development of the digestive system as well as the development of organs critical for successful feeding, such as vision and motor development (Støttrup, 2000). Effects such as tolerance to low temperatures during the juvenile stage have been shown to be related to the larval diet (enriched vs non-enriched *Artemia*) (Howell, 1994), which were not detectable during or at the end of the larval stage. Several studies have shown that rearing marine fish on natural zooplankton can ameliorate these nutritional deficiencies (Nellen, 1981)

1.2.4 Artificial microparticulate diets

A number of studies were carried out to find satisfactory, formulated diets that would substitute for natural live food (rotifers, *Artemia* sp.) in larval rearing of various fish species (Lazo, et al., 2000; Yufera, et al., 1999; Dabrowski, et al., 2003; Takeuchi, et al., 2003). Feeds used as first food during fish larval development must be fine-grained, acceptable, digestible and utilized for body protein/lipid synthesis by the larvae (Ostaszewska, et al., 2005). They should also include the optimal composition of nutrients to achieve high survival and growth rate, and correct development (metamorphosis) of fish. Simultaneously with the efforts on feed formulation, studies of digestion physiology in the gastrointestinal system development in fish larvae must be performed (Ostaszewska, et al., 2005). Ontogenesis, differentiation and development of functions of all organs are genetically determined. However, fish larvae are able to adapt, within some limits, to variable environmental and feeding conditions (Webb, 1999).

1.2.5 Snook aquaculture

Collection of data on the conditions required for spawning, larval rearing, and release into marine and freshwater systems began in 1974 at the Florida Game and Fresh Water Fish

Commission (Ager *et al.*, 1978; Shafland and Koehl, 1980, Chapman *et al.*, 1978). These studies provided information on the lower lethal temperature (15°C) for juveniles (Howells *et al.*, 1990) and preliminary developmental results for laboratory reared larvae and juveniles (Lau and Shafland, 1982). These studies described basic common snook biology and the principles for captive rearing. The early studies on this species in Florida and Texas conducted in the 1970's, 1980's and 1990's, were unable to identify the appropriate culture requirements to support captive spawning and larval rearing of common snook.

1.2.6 Objectives

The objectives of the series of experiments reported here were to improve larval survival of common snook during the first 14 days after hatching.

The main aims were to:

1. Investigate the influence of temperature on hatch rate
2. Establish the effect of egg stocking density on larval survival and growth
3. Determine the influence of flow rate on larval survival
4. Investigate the effect of the green water technique on larval survival and growth
5. Determine the influence of rotifer density on larval survival
6. Investigate effect of alternative live food species on larval survival and growth
7. Evaluate the acceptance of micro-diet feeding by larval snook

2. Materials and methods

All the larval rearing experiments reported here took place at the Mote Marine Laboratory facilities, located in Sarasota, Florida. During the first two years experiments were carried out in aquaculture systems located at Mote Marine Laboratory. The studies carried out in the later two years were conducted in the new aquaculture systems located at Mote Aquaculture Research Park (MAP).

Artificial seawater (Instant Ocean ®) was used at both locations; however, conditions at Mote Marine Laboratory were not ideal because the systems were located under a building that had poor ventilation, limited lighting, and a lack of temperature control. At MAP, experiments were conducted in a variety of tank systems equipped with state-of-art filtration, and in isolated and temperature controlled experimental rooms.

2.1 Live culture

The suitability of three live food types (microalgae, rotifers, copepods) were investigated in parallel. Each live culture was maintained in separate rooms using water from a different reservoir, in order to avoid any possibility of cross-contamination.

2.1.1 Microalgae

The main microalgae used in the snook trials was *Nannochloropsis occulata*; this is non motile, green coloured cell with no flagella. It is a small, elliptical cell, 4-6 µm in diameter, with few distinguishing features. The chloroplast usually occupies much of the cell. Cells tend to float in culture and stay in suspension without aeration. This organism is placed in a separate

division from *Nannochloris* because of its lacks of chlorophyll b. These algae are a popular food source for rotifers and filter feeders.

N. oculata was used to feed the rotifer cultures and to create a green water environment in the larval systems. The procedure used for its culture was the classical batch culture method, which consists of inoculating culture tubes with low density of algae cells. After two weeks, test tube cultures were transferred into 250 ml flasks and later (1 week) into a larger 19 L carboy culture vessels. After a week, a 100-liter cone shape transparent tank was inoculated with a full carboy. The culture was kept running with four 200 L transparent fiberglass tanks during the experiment's duration to ensure reliable microalgae production. All cultures were exposed to 24 hour white light condition (1000 lux), water temperature was kept at 29 °C, and had constant aeration.

N. oculata paste was also obtained from Reed Mariculture. This paste is a highly concentrated media (68 billion/ml) of *N. oculata* that was kept frozen until the day before it was used. The paste was used to reduce the time involved in batch culture of live algae and to test the difference between live and frozen paste algae as a food source for rotifers and for creating a green water environment.

2.1.2 Rotifers

Four different types of rotifers were used in the snook larval rearing trials. *Brachionus rotundiformis* or small (S-type) rotifers and *B. plicatilis* or large (L-type) rotifers, which can be clearly distinguished by their morphological characteristics: the lorica length of the L-type ranges from 130 to 340 µm (average 239 µm), and in the S-type ranges from 100 to 210 µm (average 160 µm). Moreover, the lorica of the S-type has pointed spines, while the L-type has obtuse angled spines. Two other types of rotifers were used. The SS type rotifer (Super small rotifers) ranges between 100-120 µm, which are preferred for the first feeding of fish larvae with small mouth openings (rabbitfish, groupers, and other fish with mouth openings less than 100 µm at first feeding). Those rotifers, however, are not genetically isolated from S-strains, but are smaller than common S-strains (Person Le Ruyet, *et al.*, 1993). The last strain was an SS rotifers from the University of Ghent, Belgium, that were genetically modified to resist warmer temperatures (above 30 °C).

All the rotifers strains were cultured using a batch culture method. Batch cultivation, due to its simplicity, is probably the most common type of rotifer production in marine fish hatcheries (Fukusho, 1989; Nagata and Hirata, 1986; Snell, 1991). The culture strategy consists of either the maintenance of a constant culture volume with an increasing rotifer density or the maintenance of a constant rotifer density by increasing the culture volume. In batch culture, a total harvest of the rotifers is done with part of the rotifers being used as food for fish larvae and part used as inoculum for the next culture (Hirata, 1980; Lubzens, 1987). All the rotifers were fed *N. oculata*.

2.1.3 Copepods

The calanoid copepod *Acartia tonsa* (*Acanthacartia*) was cultured for some of the feeding experiments. This species was chosen due its small size (80-100µm), nutritional value and availability. The copepods were cultured at Florida State University (Tallahassee) and eggs were sent every two weeks on ice (4°C) in 100 ml flasks. Once in the lab, eggs were

refrigerated at 4°C until they were needed. Copepod eggs were taken out the 100 ml flask and placed in a 500 ml transparent flask with seawater at 35 ppt and 28°C under a 12 hours light:dark period. No aeration was needed during the 48 hours hatching period. After hatch, *A. tonsa* were fed to the snook larvae. Feeding densities varied depending on the experiment.

2.2 Larval rearing systems

All the experiments were conducted using two independent experimental systems: small and large microcosm systems.

2.2.1 Small microcosm system

The small microcosm system (System A) was a self-contained recirculating system (Rana, 1986), which allowed several different experiments to be run at the same time, with the appropriate replication.. The system was made of transparent plexiglass, and contained 48 2-L tanks (Figure 1), The system dimensions were 1.25 m in length by 70 cm in width by 20 cm in height. The individual tank dimensions (Figure 1) were 12x10x20 cm, with an opening to allow water exchange 17.5 cm from the bottom, which was covered by a mesh screen (75 µm). In addition to the larval tanks, the system included two sumps, a 140 liter sump (filled with biofiltration beads) and a 120 liter sump (with a fluidized bed and a carbon filter). Air stones, were placed in both sump and a pure oxygen ceramic stone was also in the second sump, in order to keep the dissolved oxygen levels between 8 to 10 mg/L. Flow rates within the larval tanks was individually regulated through a drip valve. Flow rates varied between tanks depending on the experimental requirements. The tank recirculating system was based on an overflowing system, with a drip valve on the inflow and a 75 µm mesh rectangular opening for tank outflow. For the first 3 days after hatching, a transparent

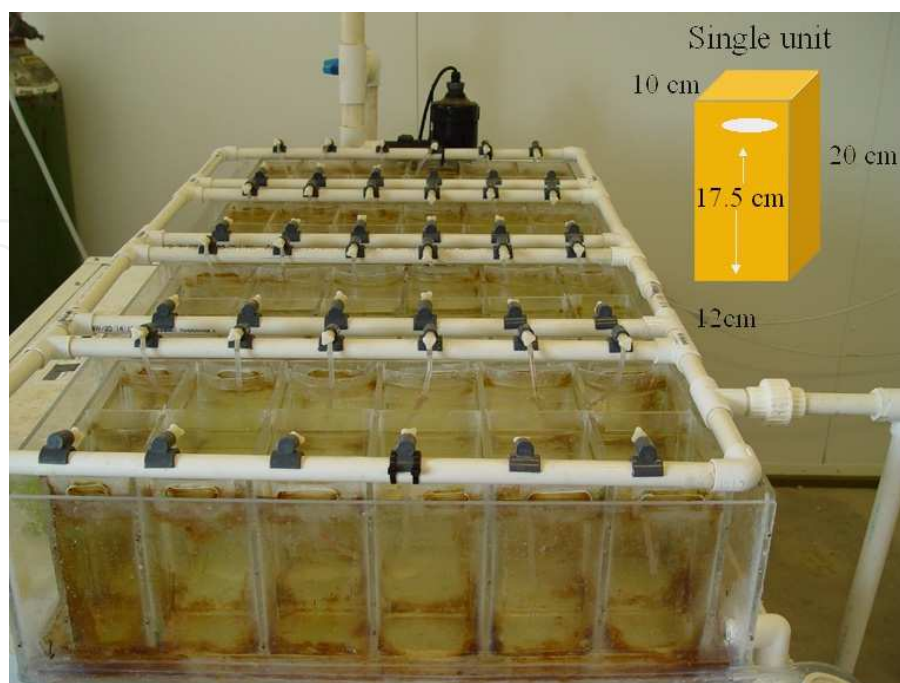


Fig. 1. System A (Microcosms) and individual tank dimensions

plastic separator was placed close to the outflow to avoid egg and larvae impingement on the mesh to reduce possible mortalities. All the tanks outflows drain to a common canal through the UV light filter and into the sumps.

Daily 10% water exchange was conducted. Water quality was checked three times a day (every five hours) for temperature, salinity, dissolved oxygen and pH. Nitrite, ammonia and nitrate were checked on a weekly basis. During the feeding experiments, residual prey counts were taken prior to feeding. Tanks were fed 1, 2, 3 and 4 times a day depending on the experiment.

2.2.2 Large microcosm system

The large microcosm system (System B) was built in 2005 to provide additional replicated experimental tanks. The water volume in the System B tanks (6L) was 3 times the water volume of the System A tanks (2L) and was used to conduct simultaneous trials to compare the influence of increased water volume on larval survival. System B tanks were placed in a green bottom fiberglass raceway, where individual tanks were maintained in a water bath (Figure 2). Twelve (6 l) tanks shared the same filtration system, which included a fluidized bed, a moving bed bioreactor, UV, and a protein skimmer. The system had two 300-litre sumps under the raceway, where the filtration system was set up. Air stones were placed in each tank to keep dissolved oxygen at desired levels. Water heaters were placed in the raceways to maintain constant temperature.



Fig. 2. Larval rearing System B. Full system and individual rearing tanks (left to right)

Inflow water was regulated individually per tank, and outflow water passed through a 75 μm mesh standpipe, draining into a common drain channel (Figure 2, middle picture) leading to the first sump. Slight aeration was also supplied to the individual tanks. A 25% water exchange was carried out every week. Like in the microcosms, water quality (temperature, salinity, dissolved oxygen and pH) was checked three times a day (every five hours). Nitrite, ammonia and nitrate were monitored weekly.

2.3 Experimental methodology

After collecting common snook eggs in the field, eggs were fertilized and transported to the laboratory. Eggs were stocked in experimental systems within 2 to 3 hours after fertilization. Eggs were stocked at a salinity of 35 ppt, temperature of 28°C (except for the temperature experiments), 9-10 mg/l of dissolved oxygen and pH of 7.9. During the first two years, larvae were fed 3 days after hatching; during the last two years, feeding started 2 days after

hatching. All specimens that survived past Day 14 after hatching were sacrificed, for total length and myomere height measurements.

2.3.1 Influence of temperature on hatch rate

Eggs were stocked in 2 L PVC floating containers, similar containers were at the same time floating in a 300 L raceway tank. Three raceway tanks were used, each one of them had a different temperature (23, 28 and 30°C). Each raceway tank had three 2 L PVC floating container and each container was stocked with 100 snook eggs per litre. Aeration was removed before stocking. Water quality parameters were maintained within acceptable values. Twenty hours after fertilization, containers were removed from the raceways and percent hatch was determined (see the formula below). This experiment was replicated four times to obtain reliable results.

$$\text{Hatch rate} = \frac{\text{Total number hatched larvae}}{\text{Total number of eggs stocked}} * 100$$

2.3.2 Effect of egg stocking density on larval survival and growth

Nine tanks were stocked with three different egg densities (three replicates per density). The densities used were: high density (375 eggs/L), medium density (200 eggs/L) and low density (200 eggs/L). All tanks were fed S type rotifers three times a day at a concentration of 30 rot/ml. The tanks were harvested fourteen days after hatch (DAH) and larvae were counted and measured (total length and myomere height). This experiment was run in both systems A and B.

2.3.3 Influence of flow rate on larval survival

Common snook eggs were stocked at a density of 200 eggs/L and exposed to three different flow rates (no flow, slow flow (10 ml/min) and high flow (30 ml/min)). Each flow treatment had 3 replicates per day. All tanks were fed three times a day with SS type rotifer at a concentration of 30 rotifers/ml. System A tanks (27 tanks) were stocked, nine per flow treatment. Larvae were harvested on 3, 6 and 10 DAH to determine survival.

2.3.4 Effect of background phytoplankton in the water (Green water technique) on larval survival and growth

Snook were stocked at 200 eggs/L in ten system A tanks and the flow rate was set at 15 ml/min. Five tanks were stocked with *Nannochloropsis oculata* at 1000/ml (green water) and five tanks without (clear water). All tanks were fed SS type rotifers three times a day with a concentration of 30 rot/ml. The experiment was terminated at 14 DAH and all larvae were counted to establish larval survival and measured (total length and myomere height) for growth.

2.3.5 Influence of rotifer density on larval survival

The effect of rotifer density on larval survival was evaluated in 15 System A and System B tanks. Three SS type rotifers densities were used with: 5 rotifers/ml, 15 rotifers/ml and 30 rotifers/ml. All the tanks were stocked at the same time with 200 eggs/L. Residual rotifer

counts were taken before every feeding and feeding amounts were adjusted, in order to maintain the same rotifer concentration throughout the experiment. All larvae were harvested 14 DAH and counted to calculate survival.

2.3.6 Effect of alternative live food species on larval survival and growth

To investigate the effect of alternative live food species on larval survival and growth, four diets were given to the larvae: a) 100% rotifers, b) 75% rotifers and 25% copepods, c) 50% rotifers and 50% copepods, and d) 75% copepods and 25% rotifers. All the tanks were stocked at the same time and fed 30 prey/ml; the number of each prey (rotifer or copepod) was determined by the above-mentioned percentages. Twelve tanks were used in System A and System B for this experiment and there were three tanks per experiment diet. Flow rate was maintained at 10 ml/min during the 14 day trial. Total length and myomere height were taken at 14 DAH and stomach contents were examined.

2.3.7 Acceptance of micro-diet feeding by larval snook

A total of 21 (2L PVC) floating tanks were placed in the raceway and each tank was stocked with 300 eggs/L. The experiment was run twice for a period of 7 days, from day 2 after hatching till day 8 after hatching. Seven tanks were fed one of the following three diets: a) a SS type strain (150 μ m) rotifer, at a density of 30 rot/ml, fed three times a day, b) a 100 μ m artificial micro-diet, fed twice a day, c) a 150 μ m artificial micro-diet fed twice a day. Each day three tanks were taken out and larvae stomach contents were examined under a microscope.

3. Results

3.1 Influence of temperature on hatch rate

The influence of incubation temperature on hatch rate was investigated to establish the optimal temperature for incubating snook eggs. Embryos exposed to 23°C water had the lowest mean hatch rate of 5.9% (range 0.8 to 11.4%) (Figure 3). The highest hatch rates of 23.5% occurred at 28°C (range 21.7% to 27%). No significant difference in hatching rate ($p > 0.05$) was found between the 28°C and 30°C treatment, but a significant difference ($p < 0.05$) was found when 28°C was compared to 26°C and 23°C.

3.2 Effect of egg stocking density on larval survival

The effect of egg stocking density on larval survival at 14 DAH is presented in (Figure 4). At a stocking density of 375 eggs/l (High density) per tank, the mean survival from three tanks was 0.6%. Tanks stocked with a medium density (200 eggs/l) had a similar mean survival percentage with 0.5%. The highest survival was obtained at the low density stocking (100 eggs/l) with a mean overall 1.2% survival by 14 DAH (Figure 4). No significant difference ($p > 0.05$) was obtained between the high and medium density treatments, but a significant difference ($p < 0.05$) was obtained between the low density treatment and the other two.

3.3 Influence of flow rate on larval survival

Snook larvae were exposed to three different flow rates. Those fish exposed to no flow during the first 10 days (Figure 5) had a mean survival rate of 45% by 3 DAH, survival then

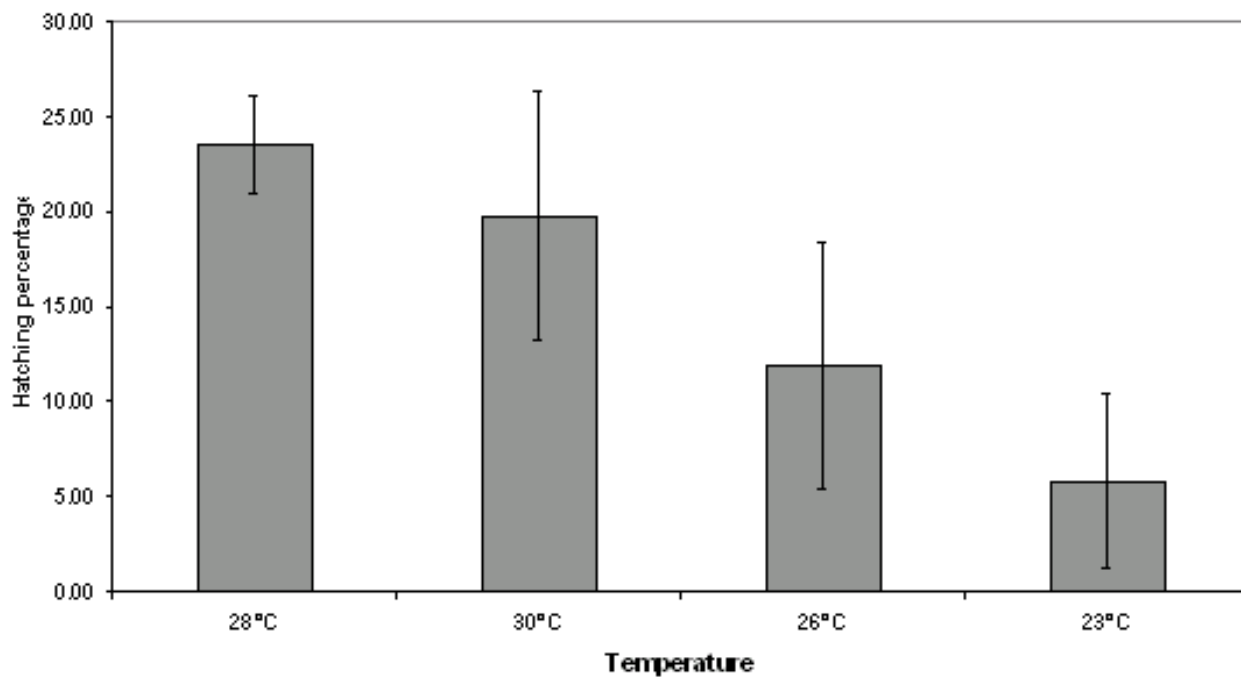


Fig. 3. Influence of temperature on percent hatch in snook larvae.

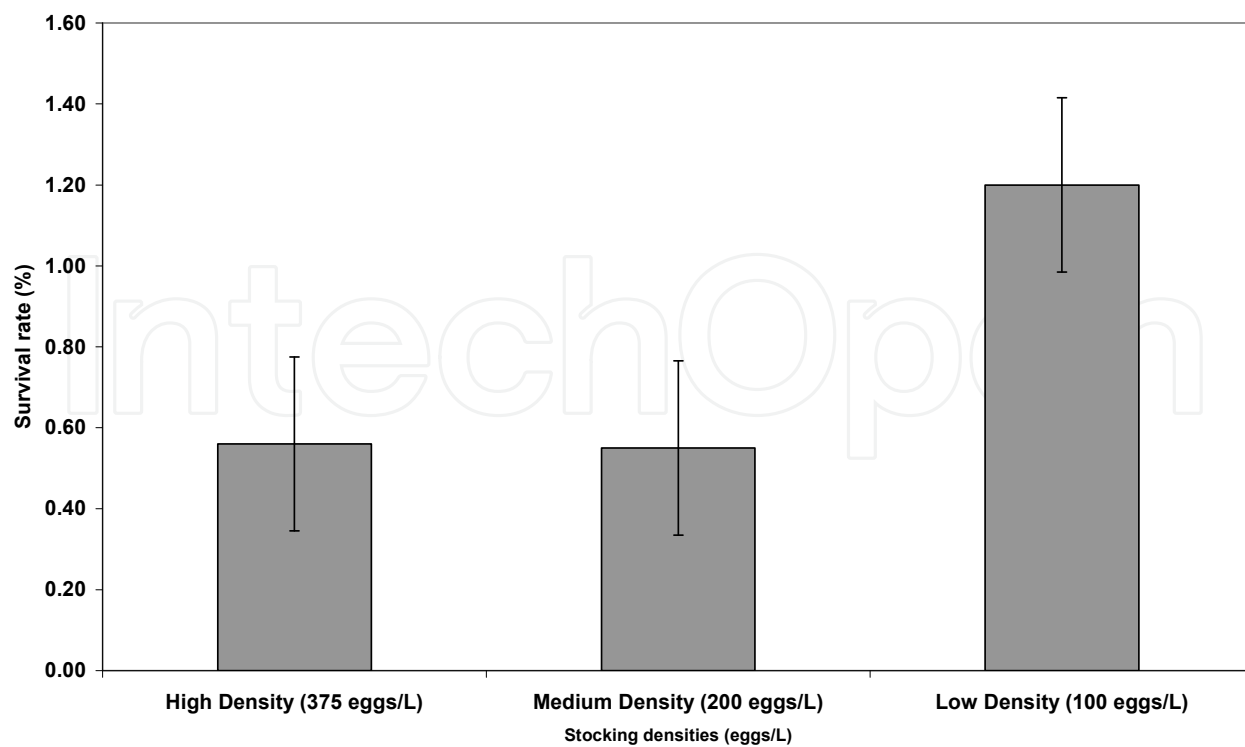


Fig. 4. Larval survival at three stocking densities at 14 DAH.

decreased to 19% by day 6. At 10 DAH, larval mean survival was only 5% from the initial stocking density. The slow flow (10 ml/min) treatment had a mean survival of 40% at 3 DAH, decreasing 3 days later to a 32% survival. Finally a mean survival of 21% was observed by 10 DAH (Figure 5). The third experiment had the lowest values in terms of survival. At high flow (30 ml/min), survival at 3 DAH was 16%, decreasing on Day 6 to a mean of 10% survival and by day 10 after hatch larval mean survival was 9% (Figure 5). No significant difference ($p > 0.05$) was observed between the no flow and slow flow treatment at 3 DAH, but a significant difference ($p < 0.05$) in survival was found between all the treatments 6 and 10 DAH.

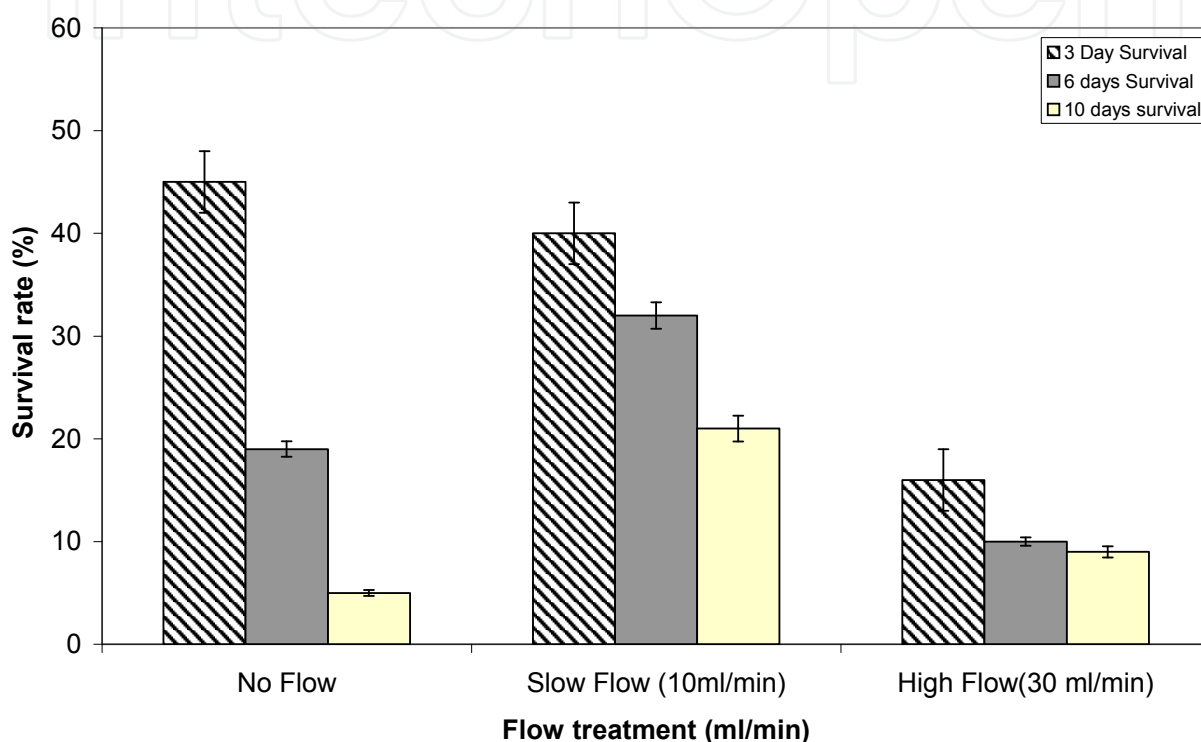


Fig. 5. Snook larval survival under three types of flow conditions.

3.4 Effect of background phytoplankton in the water (Green water technique) on larval survival and growth

Larvae were stocked in water with no algae and in water with algae (*N. occulata*) at a concentration of 1000/ml. Larvae tanks without algae are referred to as 'clear water' (Figure 6). The survival of larvae was significantly ($p < 0.05$) influenced by the presence of *N. occulata* in the rearing water. The mean survival at 14 days post hatching in clear water was significantly lower at 0.17%, compared with 0.55% survival in green water tanks (Figure 6).

Length and myomere height in larvae from the clear water tanks had an average standard length (SL) of 3.20 mm and average myomere height of 0.70 mm (Figure 7); on the other hand, larvae in tanks subjected to green water technique had an average length of 3.34 mm and average myomere height of 0.73 mm. Although standard length and myomere height in the green water tanks were higher, no significant difference was found ($p = 0.053$).

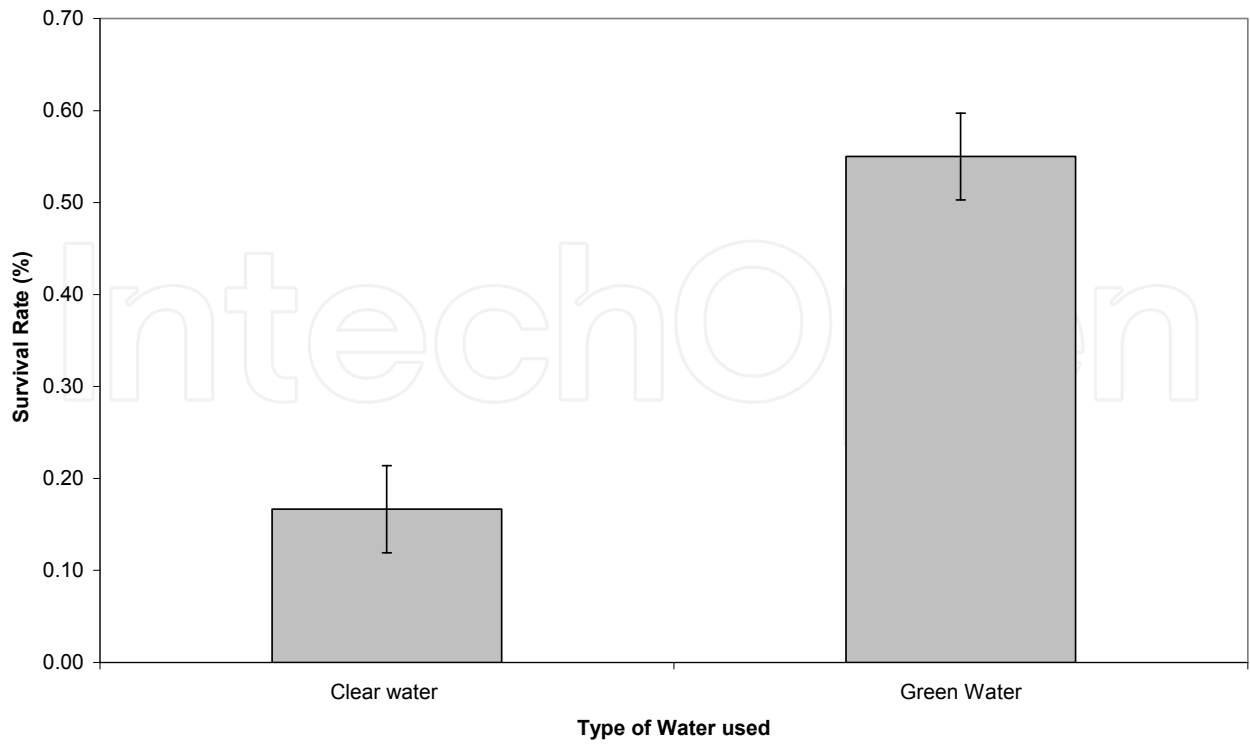


Fig. 6. 14 DAH snook larval survival from tanks with *N. oculata* (Green water) and without phytoplankton (clear water)

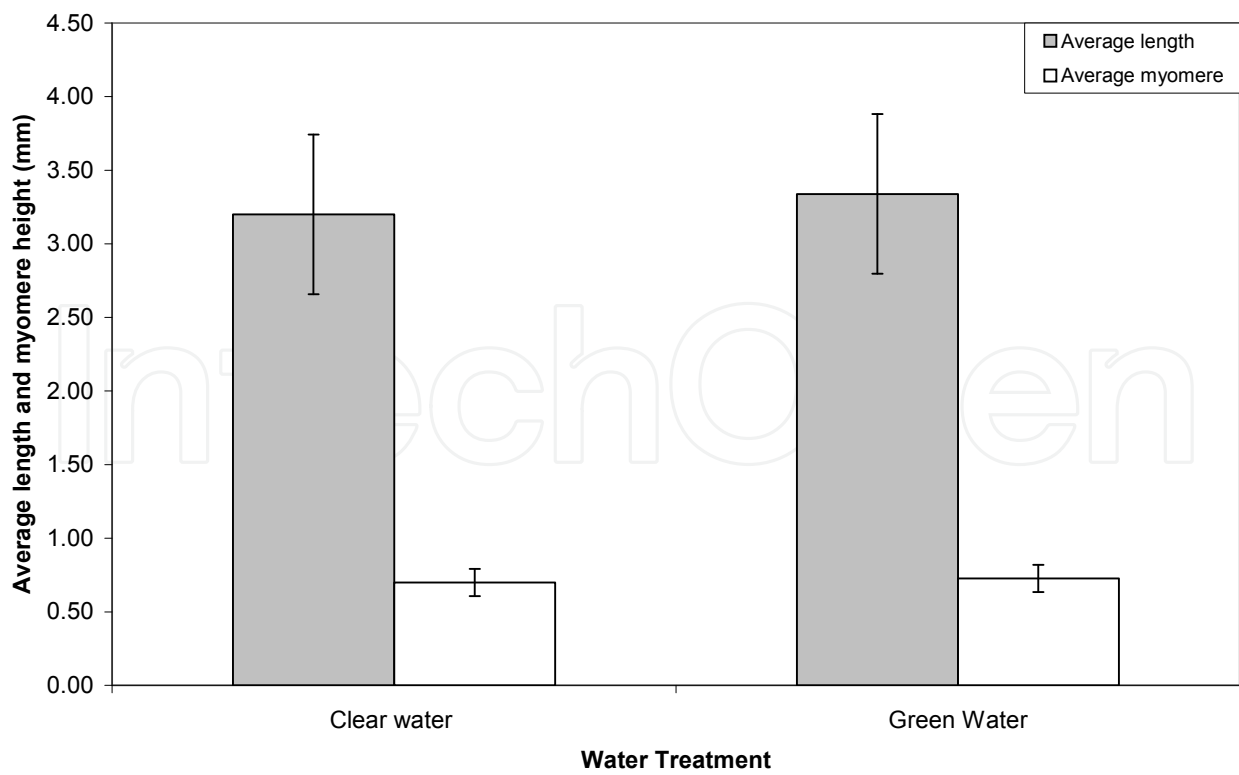


Fig. 7. Average snook larval length (SL) and myomere height from green water and clear water tank

3.5 Influence of rotifer density on larval survival

Three different rotifers densities were evaluated to determine the effect on growth and survival of common snook larvae (Figure 8). After 14 days, larvae fed 5 rotifer/ml diet had an average survival rate of 0.86% and only 13% of all tanks stocked had live larvae. All the tanks where common snook larvae were fed 15 rotifers/ml had a mean larvae survival of 2.5%, and 20% of tank of all tanks stocked had live larvae. In the third treatment with 30 rotifers/ml, 20% of the tanks had live larvae and a total average of 3.67% larvae survival. No significant differences was found in larval survival between tanks fed 15 and 30 rotifer/ml ($p=0.053$).

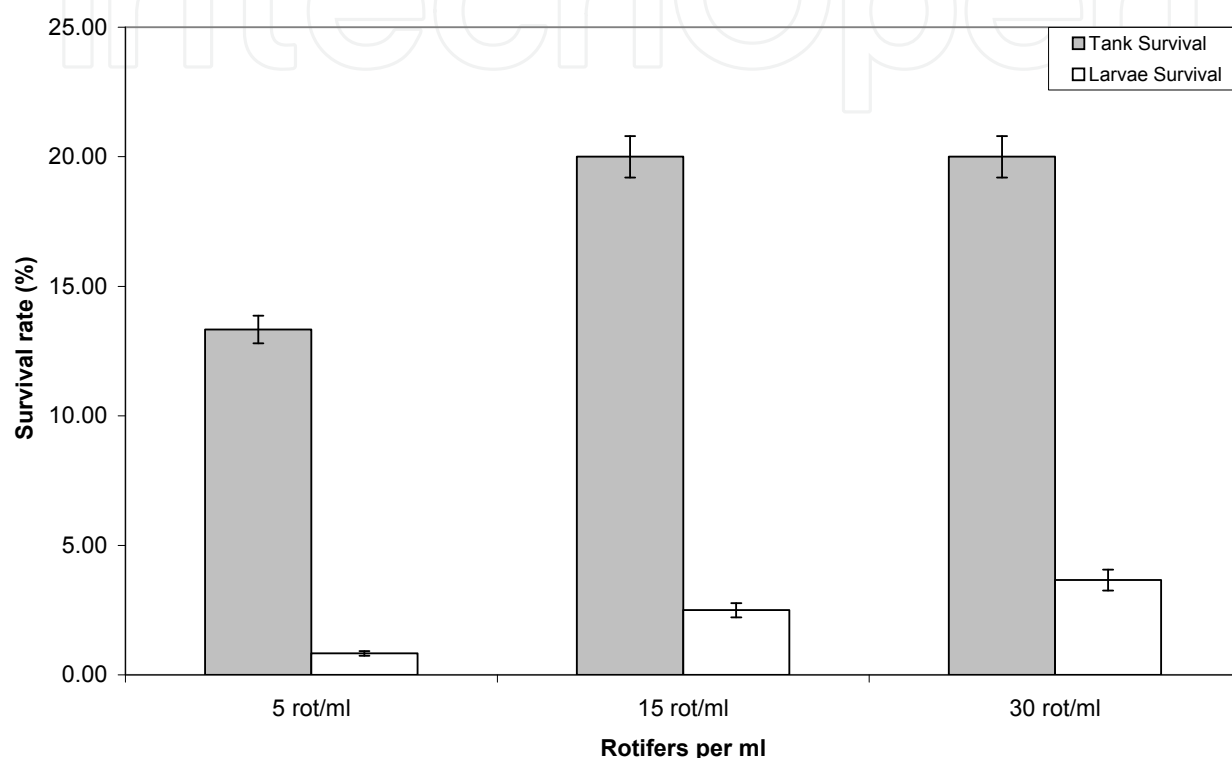


Fig. 8. Effect of 3 different rotifer densities on larval survival

Increasing rotifer concentrations from 5 to 30 rotifers/ml did not significantly ($p>0.05$) affect the larval length or myomere height (Figure 9). Larvae fed with 15 rotifers per ml had the highest mean length (SL) at 3.58 mm, followed by the other two treatments (3.48 and 3.49 mm in average respectively). Mean myomere height results were 0.74, 0.78 and 0.69 mm respectively.

3.6 Effect of alternative live food species on larval survival and growth

Four different diets were used in this experiment: 3 using copepods (*Acartia tonsa*) and SS type rotifers, one with 100% rotifers. The diet with 75% copepods and 25% rotifers (Figure 10) had the highest mean survival (1.44%), the second highest average larval survival occurred with the diet that had 100% rotifers (0.83%). The other two diets (50% rotifers and 50% copepods, and 25% copepods and 75% rotifers) had similar results with 0.61% mean survival. A significant difference was found between the 75/25 copepod/rotifer diet and the other three diets ($p> 0.05$). No significant difference was found between the 50/50 and the 25/75 diets ($p<0.05$).

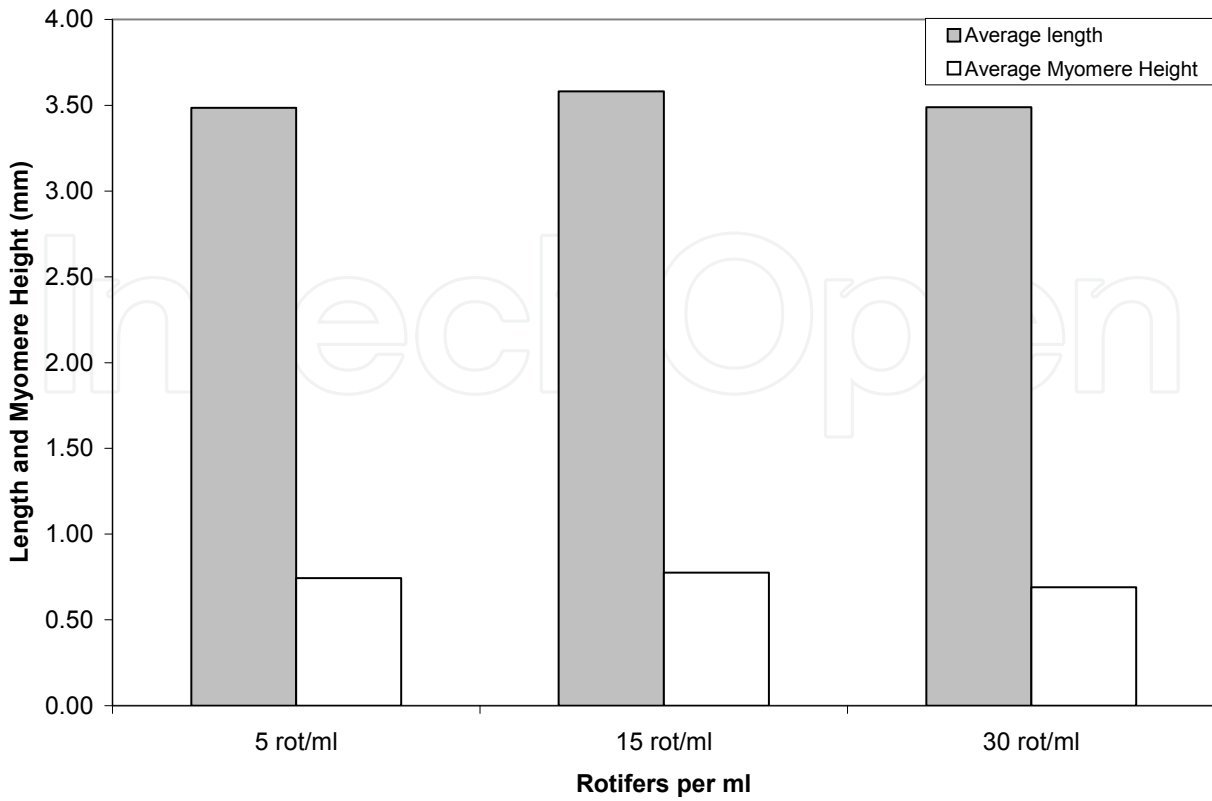


Fig. 9. Snook larval length (SL) and myomere height at three rotifers densities.

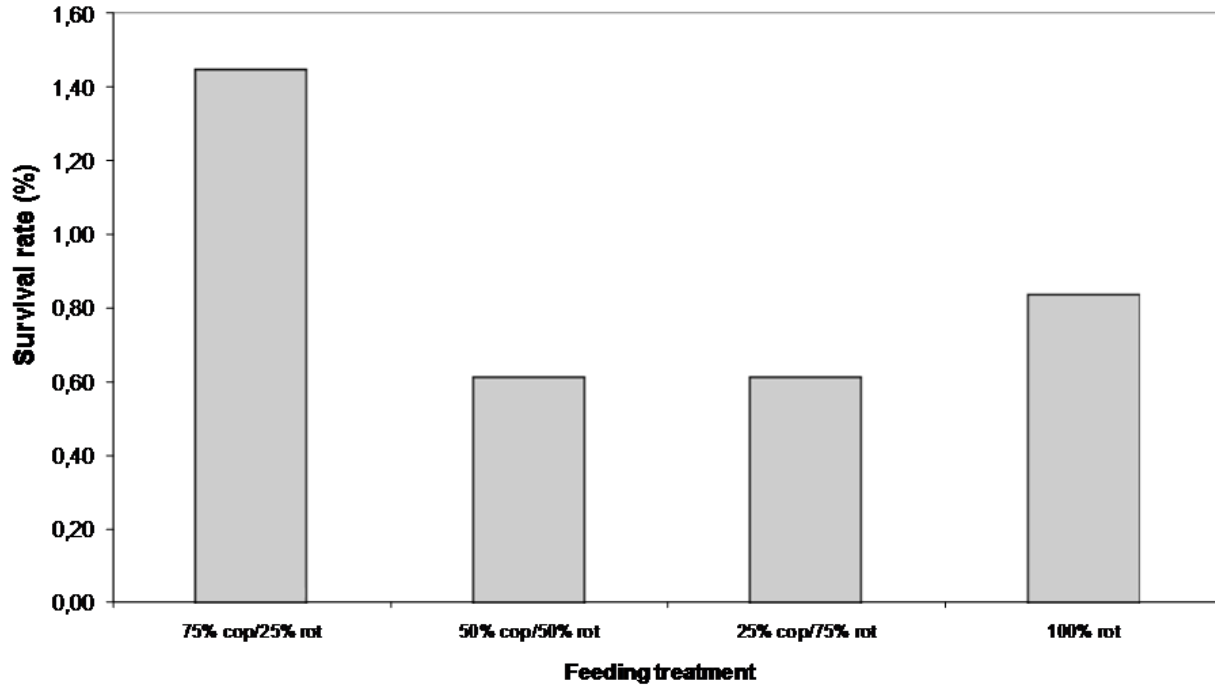


Fig. 10. Snook larval survival after 14 days associated with four diets that combined copepods and rotifers at different percentages. Feeding treatment 75/25 consisted of 75% copepods and 25% rotifers, 50/50 consisted of 50% copepods and 50% rotifers, and the 25/75 consisted of 25% copepods and a 75% rotifers.

The influence of live food combinations on larval growth is presented in Figure 11. Larvae at the 75/25 (copepod/rotifer) and the 50/50 tanks had the average highest length (4.70 mm), followed by the 25/75 (copepod/rotifer) with an average length (SL) of 4.32 mm, which was very similar to the 100% rotifer diet (average length (SL) of 4.28 mm). No significant difference was found between the first two treatments ($p > 0.05$) or between the other two (25/75 and 100 rot), but a significant difference was found between the first two and the last two ($p < 0.005$). Myomere height was similar on all the diets ranging from 1 mm to 1.04 mm.

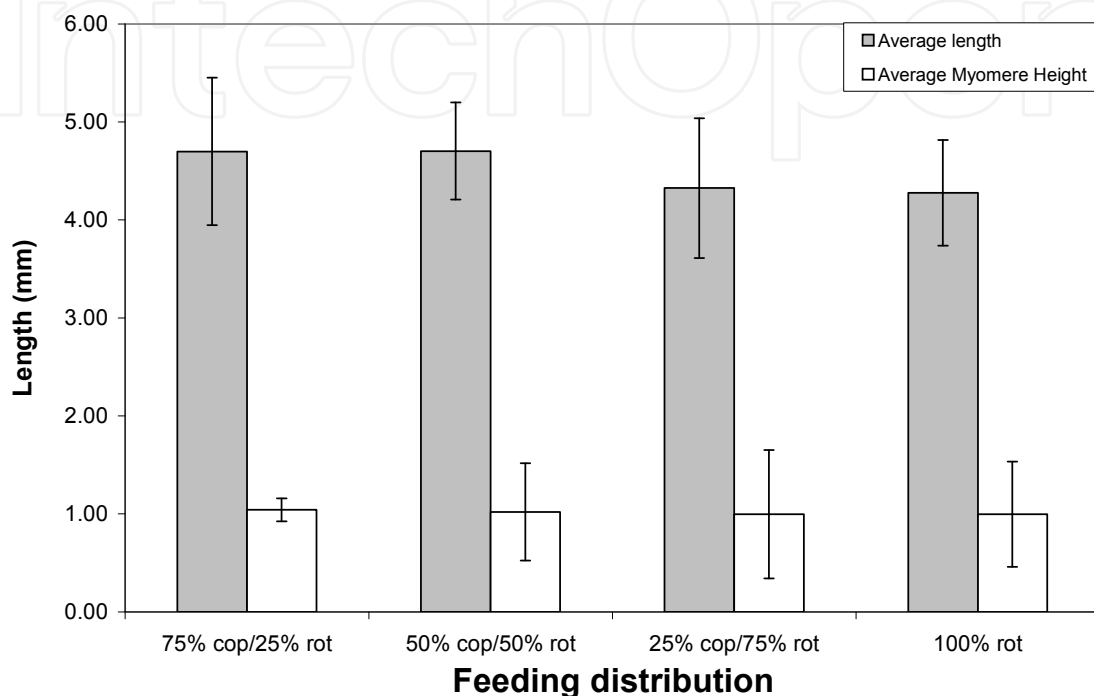


Fig. 11. Average length (SL) and myomere height from larvae exposed to four diets.

The stomach contents of 14 day old larvae were examined for food items (Figure 12). In the 75/25 treatment, 30.8% of the larvae had both rotifers and copepods in the guts, in the 50/50 diet, 30% of the larvae also had both rotifers and copepods in the guts, and in the 25/75 diet 18 % of the larvae had both rotifers and copepods in the guts. The rest of the larvae only had rotifers.

3.7 Acceptance of micro-diet feeding by larval snook

The stomach contents from larvae fed micro-diets were examined in this experiment. In tanks fed with rotifers, the percent of larvae with rotifers in the stomach after 7 days ranged from 10 to 37 (Figure 13). In tanks where 100 μ m micro-diet food was offered, percent larvae with dry diet ranged from 6 to 28%. Finally, in tanks where the 150 μ m micro-diet was offered the percent of larvae with dry food ranged from 7 to 27%.

4. Discussion

4.1 Importance of temperature during incubation

Fish are affected by many intrinsic and extrinsic factors. These factors can affect developmental controls resulting in phenotype alterations (Johnston *et al.*,1996; Adriaens &

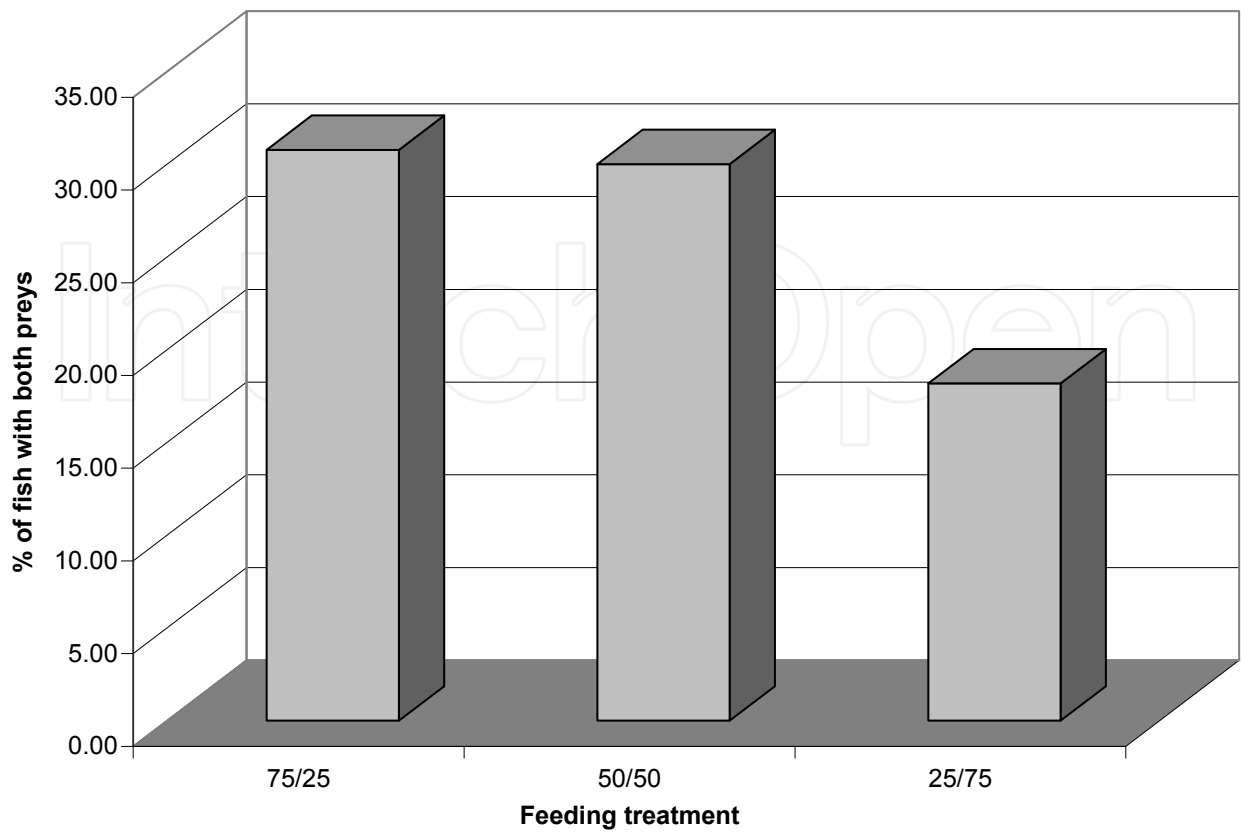


Fig. 12. Percent of rotifers and copepods found in the snook larvae stomach contents from four diets.

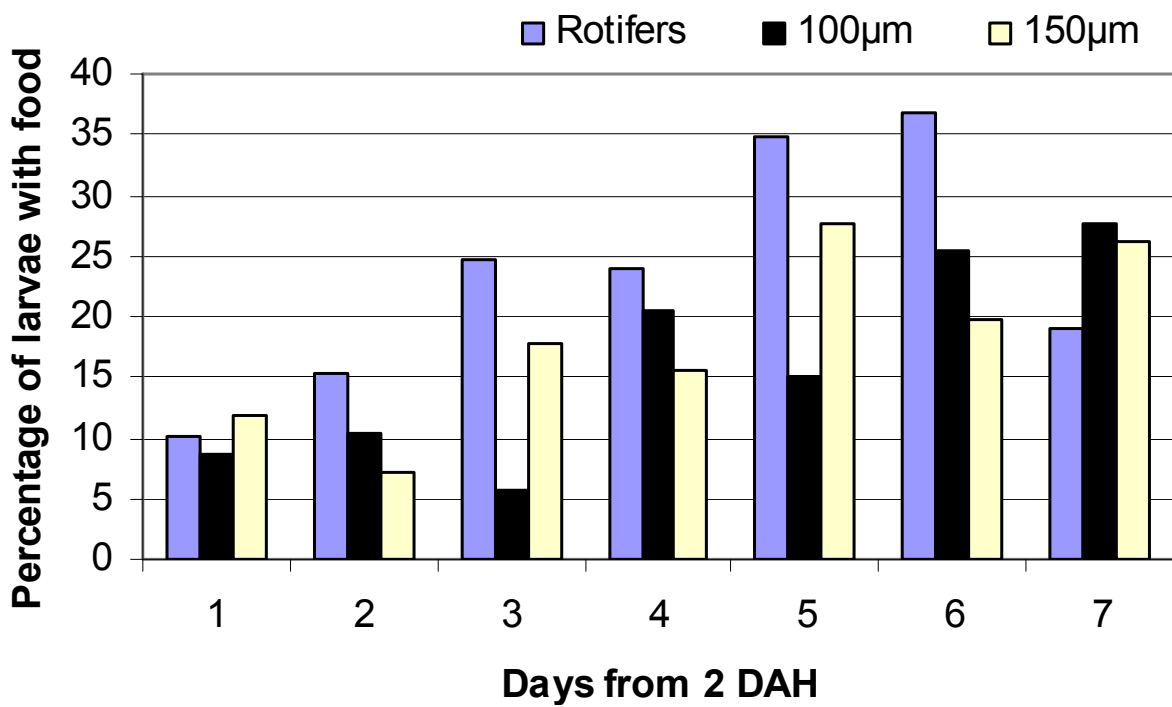


Fig. 13. Percent of larvae with food in the digestive system from the three diets (SS type rotifers, 100 and 150µm micro-diets).

Verraes, 2002). Among the abiotic factors, temperature has the most significant effect on development and growth (Blaxter, 1992; Kamler, 1992a; Hochachka & Somero, 2001), influencing developmental timing and formation and function of key tissues and structures (Kamler, 1992b; Fuiman *et al.*, 1998; Koumoundouros *et al.*, 1999) and the synchronization of these continuous developmental paths (Kovac, 2002). Temperature can also have a direct effect on physiology through its effects on enzyme reaction rates (Hochachka and Somero 2001).

Hatching rate of common snook varied with temperature in this study. Eggs incubated at 28°C showed the best hatching rates; however, no growth trials were conducted. These results confirm Rideout *et al.*, (2004) findings of a direct influence of temperature during the incubation period on larval growth, which also has a direct effect on hatch rates (Pepin *et al.*, 1997). It has also been argued that survival may be proportional to larval size, since larval mortality rates have been shown to be inversely proportional to growth rates (Pepin, 1991).

4.2 The effect of stocking density on larval survival

Egg stocking density is a key factor in larviculture; without an optimal stocking density, overall survival can be affected. The common snook stocking density experiments results showed the lower stocking density (100 eggs/l) to be the one with the higher survival. This finding agrees with Hernandez-Cruz *et al.*, (1999), who obtained low survival in red porgy when eggs were stocked in high densities. On the other hand, Tagawa *et al.*, (1997) found higher survival at higher rearing densities in Japanese flounder and this finding was confirmed in three other marine teleosts (Kaji *et al.*, 1999). Tagawa *et al.*, (2004). The higher survival at high densities was attributed to substances (proteins) secreted by larvae that were beneficial for their survival. Although more experiments need to be done on common snook density, the results clearly showed low survival at high densities, which might be due to the a high level of competition for prey or over-crowding the environment and reduced water quality conditions.

4.3 Influence of 'green water' on larval survival

Food availability is a key factor during first feeding and the consumption rate is dependent on availability. Additionally, the developmental stage of the individual affects the consumption rate (Houde and Schekter, 1980; Kentouri, 1985). Based on the previous findings, this study examined survival of larvae in green water and in clear systems. Final results showed a significant difference in survival between the two treatments, where larvae grown in a green water (*N. occulata* had ingested the algae and had higher survival than those reared in clear water. This result agrees with previously published data (Papandroulakis *et al.*, 2002; Divanach *et al.*, 1998; Oie *et al.*, 1997; Holmejford *et al.*, 1993). Also, it has been reported in cod (Van der Meeren, 1991) and halibut (Reitan, *et al.*, 1991). This may support the idea that microalgae are used as a direct food source at the start of feeding confirming the important role that phytoplankton has during the early stages of several species. Another explanation for the increased survival in green water is the role of phytoplankton in stabilization and improvement of the rearing medium and its direct (Moffatt, 1981; Reitan *et al.*, 1993; Van der Meeren, 1991) or indirect nutritional effect (Tamaru *et al.*, 1993). Phytoplankton has been reported as being a protective agent, antagonistic towards pathogenic bacteria (Kennedy *et al.*, 1998; Støttrup *et al.*, 1995). Skjermo & Vadstein (1999) noticed that microflora in rearing tanks of *Hippoglossus hippoglossus* were

more stable in the presence of phytoplankton, increasing the total bacteria population by about 45%. The same authors noticed also that bacteria in larval gut were similar to the ones in rearing water, being mostly species with low growth rate. In addition, Nicolas *et al.*, (1989) showed that stomach microflora affects survival during early stages of marine larvae. Other studies have hypothesized that green water produces a background effect that allows the fish to better locate its prey (Marliave, 1994). This effect has been documented to improved the larval rearing in a number of fish species, including dolphinfish *Coryphaena hippurus* (Ostrowski, 1989), yellow perch *Perca flavescens* (Hinshaw, 1985), walleye *Stizostedion vitreum* (Corraza & Nickum, 1981; Colesante, 1989), white bass *Morone chryops* (Denson & Smith, 1996), grouper *Epinephelus suillus* (Duray *et al.*, 1996), Dover sole *Solea solea* (Dendrinis *et al.* 1984), barramundi *Lates calcarifer* (Pearce 1991); and red porgy *Pagrus pagrus* (Rotllant *et al.* 2003).

4.4 Importance of rotifer density in common snook larviculture

Prey density is an important factor in successful larval rearing (Fushimi, 1983). Low density can cause larval starvation or nutritional deficiencies leading to high mortalities. High densities can deteriorate water quality and lead to system fouling, decreased oxygen levels, and increased ammonia levels. Finding the right prey density is crucial to avoid these problems and also to reduce costs associated with live food production.

During the rotifer density experiments, both 15 and 30 rotifers/ml treatment resulted in high survival. Although 30 rotifers/ml had the highest larval survival, the difference between the two treatments was not significant; therefore, the higher cost to produce 30 rot/ml treatment indicates that 15 rot/ml is the optimal density for the 2 and 6 L tank systems. Recent attempts to calculate optimal prey density for cod larvae (*Gadus morhua*) found that survival reaches a maximum level and then begins to decrease if prey densities are further increased (Puvanendran and Brown, 1999). The decrease in larval survival when higher prey densities are used may be a result of poor water quality due to the release of metabolites by the prey (Houde, 1975) or it may be related to a reduction in the ability of the larvae to capture prey; what Laurel *et al.* (2001) term a "confusion effect". Optimal rotifers densities differ between species, for instance the black sea bream needs 1-3 rotifers/ml (Kafuku & Ikenoue, 1983), and the red sea bream needs between 3-10 rotifers/ml (Fushimi, 1983). In the case of common snook, results showed that 5 rotifers/ml was inadequate to meet their food demand. Further experiments need to be carried out to find out the optimal density, this time focusing between 10 to 20 rotifers per ml.

4.5 Alternative prey for common snook larvae

The suitable size of prey for fish larvae varies with larval mouth size (Shirota, 1970), and fish larvae select larger prey size as they grow (Ivlev, 1961). Although many researchers have reported larval rearing trials with marine fishes, only a few studies have been conducted to compare the appropriate rotifer size among fish species and among different growth stages (Oozeki *et al.*, 1992, in Hagiwara *et al.*, 2001). Four strains of rotifers and one copepod species were tested to find the optimal prey type for common snook larvae; prey that will suit the physical needs as the larvae develops and grows in size.

Previous work done on snook larval culture used L type rotifers and had little success. The experiments run with the L type rotifers in these experimental trials, showed extremely low

survival and DHA analysis of larvae showed a steep decrease in DHA from 1 to 6 DAH. DHA values in 6 DAH larvae were below 1% of total lipid concentration (Yanes-Roca et al. 2009). In both experimental and production systems mass mortality regularly occurred between 5-6 DAH (Yanes-Roca et al. 2009) and 75-85% of all stocked tanks did not have live larvae after 6 DAH. These results along with the finding that only 5% of the larvae fed L type rotifers had food in their stomachs lead to the conclusion that the snook larvae were dying of starvation. This is likely due to the L type rotifer prey size, which was larger than the snook larvae mouth gape. Common snook larval rearing was successful when the larvae were fed copepods grown naturally in outdoor ponds (Lau & Shafland 1982), confirming the prey size hypothesis. Experimental trials comparing survival and growth with several strains of rotifers and copepods (*Acartia tonsa*) also showed an increase in snook larval survival. These findings agree with Doi et al., (1997a,b) and Toledo et al., (1997) found nauplii of copepods to be effective when fed to red spotted grouper, *Epinephelus coioides* and with Støttrup et al., (1997) who found an increase in larval survival when rotifer feeding was supplemented with *Tisbe* sp copepod.

4.6 Acceptance of micro-diets by larval marine fish

Production of marine fish juveniles in commercial hatcheries still depends on the supply of live prey, such as rotifers and *Artemia*. Artificial micro-diet substitution for live prey is crucial to lower production costs and sustain production of high and constant quality juveniles (Cahu & Zambino-Infante, 2001). The use of micro-diets for common snook larvae during the pre-weaning period was also tested. Since the first rearing of plaice (*Pleuronectes platessa*) larvae to metamorphosis using an artificial diets (Adron et al., 1974), many trials have been conducted, with different degrees of success, to utilize artificial diets in larval rearing of species, such as seabass *Dicentrarchus labrax* (Gatesoupe et al., 1977; Cahu & Infante, 1994; Kolkovski et al., 1997), sole *Solea vulgaris* (Gatesoupe et al., 1977), Atlantic silverside *Menidia menidia* (Seidel et al., 1980), red seabream *Chrysophrys major* and Ayu *Plecoglossus altivelis* (Kanazawa et al., 1982). In all cases, poor results were obtained when live food was replaced completely by micro-diets. However, during the last decade, the pre-weaning period has been greatly reduced in many species, such as European sea bass *Dicentrarchus labrax* (Person Le Ruyet et al., 1993, Zambonino-Infante et al. 1997). Cahu et al., (1998) reported that 35% of European sea bass larvae were fed exclusively compound diet from mouth opening. In other marine species, some survival was obtained when fed compound diet from mouth opening, such as sea bream *Sparus aurata* (Fernandez-Diaz & Yufera, 1997) and red sea-bream *Pagrus major* (Takeuchi et al., 1998).

Common snook larvae were offered two sizes of micro-diets (100µm, 150µm) and rotifers for 7 days, from 2 to 9 DAH. Although there was higher percentage of larvae with food in their stomachs in tanks fed SS type rotifers, a significant number of larvae offered micro-diet had food in their stomachs. These results were observed in pike-perch (Ostaszewska et al., 2005), where micro-diets were readily accepted, digested and absorbed as well as in the Japanese eel (Pedersen et al., 2003), and the gilthead sea-bream (Salhi et al., 1997). Earlier studies suggested that co-feeding with live food improved yellow perch growth and assimilation of artificial diets (Kolkovski et al., 1997), a method that could be applied to common snook, assuming that digestive enzymes of live food organisms supported digestive processes in fish larvae (Boulhic & Gabaudan 1992; Jones et al., 1993). However, some publications have reported contradictory results (Cahu & Zambino-Infante, 1997; Kolkovski et al., 1993).

Future research on micro-diets is needed to evaluate the effect on survival or growth. Results indicate that snook larvae seem to accept the artificial diet and based on the already mentioned literature findings on several marine larval species survival and growth, it appears that the common snook larvae pre-weaning period could be reduced.

5. Conclusions

Although common snook larval survival has improved, mortality is still high and more improvements in rearing techniques are needed to increase survival. Many factors could be responsible for the low survival rates observed, including the introduction of bacteria along with the live food (i.e., rotifers). Rotifers are major carriers of bacteria (Muroga and Yasunobu, 1987; Munro *et al.*, 1993, 1994). Studies to evaluate the effect of bacteria on snook larval culture may help the overall survival. In these trials, two different experimental tank sizes were used (2 L and 6 L tanks) and survival was better in the larger tanks. More research is needed to determine the optimal tank size to examine growth and survival in larval snook. After four years of research on the snook larval rearing techniques, positive improvements have been made and the critical bottlenecks and potential solutions have been identified. Findings such as optimal rearing temperature (28°C), appropriate flow and water management (green technique) are basic for future research. Other critical results were: finding appropriate prey size (SS rotifers and copepods), optimal stocking and prey densities and the acceptance of microdiets prior to weaning.

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This book provides an understanding on a large variety of aquaculture related topics. The book is organized in four sections. The first section discusses fish nutrition second section is considers the application of genetic in aquaculture; section three takes a look at current techniques for controlling lipid oxidation and melanosis in Aquaculture products. The last section is focused on culture techniques and management, ,which is the larger part of the book. The book chapters are written by leading experts in their respective areas. Therefore, I am quite confident that this book will be equally useful for students and professionals in aquaculture and biotechnology.

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51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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