Husbandry and larval rearing of common snook (*Centropomus undecimalis*)

UNIVERSITY OF STIRLING

A thesis submitted for the degree of Doctor on Philosophy

By

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This work has been conducted exclusively by my own research. Work in this thesis has neither been accepted nor is being submitted for any other degree. Work and analysis in this thesis has been conducted independently unless otherwise acknowledged.

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Acknowledgements

Abstract

Common snook (*Centropomus undecimalis*) is a relatively new species for aquaculture; considered as a recreational species and not commercial. The aim of this study was to develop common snook larval rearing techniques for stock enhancement. Common snook culture has two main bottlenecks, broodstock management and larval culture. High mortality during the first 6 days is the main limitation for successful larval survival. Broodstock management of common snook is still developing and the only source of common snook eggs is from wild broodstock. Securing a regular supply during the natural spawning was essential to reach the main objective. Finding the optimal spawning sites, as well as optimal spawning time was achieved.

Results showed Terra Ceia, Longboat and Cayo Costa to be the best sites for wild broodstock collection. The onset of spawning was triggered by a rise in water temperature. During the 4 years of this study spawning started at the end of May and finished in September. Total capture results and egg quality results, such as fertilization, hatching rate and lipid analysis, indicated June and July as the peak months during the spawning season. Common snook follow a lunar spawning cycle. Results showed that one to three days after the new and full moon were the peak spawning periods and therefore the best days to capture wild stock.

Common snook egg lipid composition fits the general marine fish fatty acid composition with saturated fatty acids predominating. On the other hand, the omega 3, omega 6 (n-3/n-6) ratio was lower than the typical marine fish and arachidonic acid values were significantly higher than other marine species. This egg fatty acid profile will be helpful in the future to compare it with captive spawned eggs for egg quality purposes.

Description of the common snook embryonic and larval development for the first 14 days was carried out. This has strengthened knowledge for this species' development, and should provide a helpful tool to identify common snook embryos and larvae in the wild.

Novel improvements to existing common snook larval culture protocols were implemented. Larval lipid analysis throughout development, and high mortality around day 6 post hatching, suggested that common snook larvae were dying of starvation. Gross morphological development and ultra-structure findings in the digestive and eye system development during the first three days indicated that day 2 post hatching larvae were capable of capturing and digesting food. Additionally, larval nutritional improvements were made, increasing the larval survival. The most significant ones were: finding a smaller and more nutritional prey (SS type rotifers and copepods), finding an optimal stocking and feeding density and the importance that green water technique has on larval survival.

Overall, larval success was improved from a zero percent survival during the first 14 days to a 2% survival rate.

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Chapter 1: General Introduction

1 General Introduction

1.1 *Taxonomy of Centropomus undecimalis*

The common snook or *Centropomus undecimalis* (Bloch) is a diadromous, 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), (Figure 1.1).

Partial genetic isolation occurs between Florida's Atlantic and Gulf Coast stocks (Tringali & Bert, 1996). Snook are protandrous hermaphrodites: some males develop into females when 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).
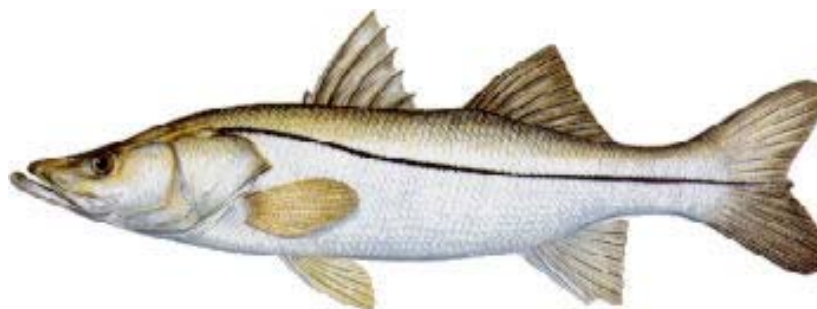
Figure 1.1.*Centropomus undecimalis*

1.1.1 Embryonic and larval development

Common snook adult physiology is well documented, in contrast, the early life history of this species remains comparatively unknown, due mainly to the difficulty of finding embryos and larvae in the wild, as well as the undeveloped captive rearing protocols, which still rely 100% on wild spawning for seed supply. Lau & Shafland (1982) described the larval development from day 14 post hatching, descriptions of earlier stages are not available, and larval or embryonic development descriptions have not been reported. Knowledge on the life history of any fish species is critical to develop successful captive breeding and rearing protocols, therefore a detailed description of embryonic and larval development from the first 14 days has been carried in the present study.

1.2 The Centropomus genus

Worldwide, 12 species of the genus *Centropomus* occur in the tropics and sub-tropics of North and South America; four of these species occur in Florida. Along with common snook, the other three species are sword-spined snook (*Centropomus ensiferus*), tarpon snook (*Centropomus pectinatus*) and fat snook (*Centropomus parallelus*), (Rivas, 1986). The latter three species tend to occupy riverine areas. The smallest of the four Florida species, the sword-spined snook is named for the length of the second anal fin spine (Rivas, 1986). Reaching only about 304 mm in length, this species is also the rarest and has been reported only in the freshwater canals and rivers of southeast Florida. Usually, neither the sword-spined nor the tarpon snook grows large enough to be legally caught by anglers (Marshall, 1958). The tarpon snook gets its name from its upturned tarpon-like snout. It has a more compressed body than the

other three species and an orange-yellow pelvic fin with a blackish tip. It may grow to a length of 508 mm and is most commonly found in shaded, brackish water pools (Rivas, 1986).

The fat snook is a rotund species with a deep body, and may reach 508 mm in length. This second-largest member of Florida's snook family is found from the Lake Okeechobee watershed south to the Florida Keys (Rivas, 1986). Fat snook is currently the only one of these four species that has been reared successfully in captivity. The largest of the four species, the common snook (*Centropomus undecimalis*) is also the most abundant, wide-ranging, and sought-after. (Marshall, 1958). Not surprisingly, then, it is the best studied of the four and the species used for this study.

1.3 Common snook habitat

In Florida, mangrove shoreline is the principal habitat of common snook. Both snook and mangroves are limited to the area south of the frost line that connects Cape Canaveral on the Atlantic Coast and Tarpon Springs on the Gulf Coast (Figure 1.2). This northern limit approximates to the 15°C water isotherm that occurs in winter (Marshall, 1958; Gilmore *et al.*, 1983; Rivas, 1986). Early-juvenile (up to a year old) snook occupy moderately sloping banks found under overhanging vegetation within estuarine waters (Peters *et al.*, 1998). As juveniles grow they can be found in a wide range of estuarine habitats.
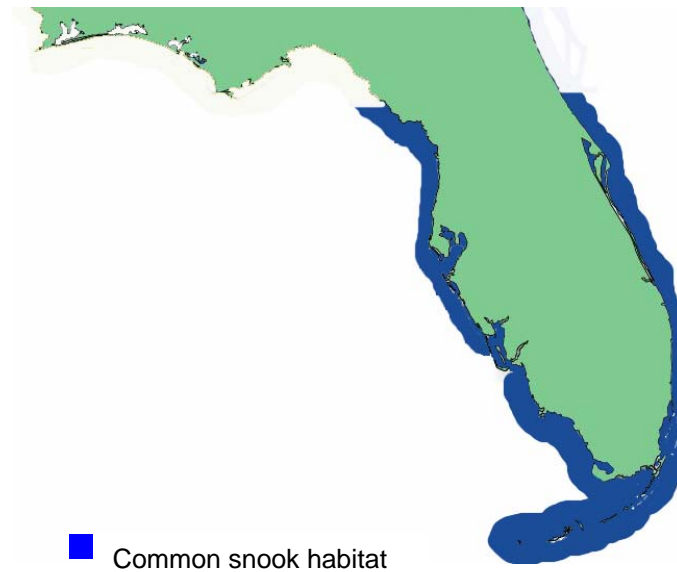
Figure 1.2. Common Snook habitat distribution in Florida, USA

Adult common snook are mainly found during most of the year in mangrove habitats, upstream in brackish water rivers and creeks, and only during the spawning season when the adults migrate to marine coastal waters along beaches and the mouths of estuaries (Marshall, 1958; Chavez, 1963; Rojas, 1975; Frazer, 1977*)*. Although the general spawning migratory pattern is well known (Taylor et al, 2000), the need for wild broodstock to obtain eggs for captive rearing requires more detailed local knowledge from the locations of spawning grounds in our study area (Sarasota Bay). At the same time, the topography of the locations chosen needs to fit in with the sampling gear limitations so that the sampling efficiency is maximised.

1.4 The Common snook reproductive biology

Spawning of snook has been studied for the last 55 years, but despite the importance of common snook as a popular game fish, the description of its reproductive biology is incomplete. Common snook in Florida are shown to have a daily spawning cycle in which spawning episodes occur during the late afternoon and the early evening hours during the lunar phases and during all tidal stages (Taylor *et al.*, 1998).

Variation in the spawning season can be observed at different locations. Within the study area (Tampa and Sarasota Bay), the spawning season has been estimated to take place during May-December with peak activity occurring during July-September (McMichael *et al.*, 1989). In order to secure a regular supply of eggs for larval rearing and to interfere as little as possible with the wild spawning, the determination of the months in which the spawning activity is the highest was investigated. Egg quality and larval survival are directly influenced by the parental reproductive performance and the physical conditions to which they are exposed *(*Sargent *et al.,* 1999b)*,* therefore egg quality and larval survival were used as parameters to identify the optimal time during the spawning cycle when the best quality eggs could be obtained.

1.5   Common snook feeding habits

Larval snook feed primarily on copepod eggs and larvae, other invertebrates' eggs, algae, and plant tissues (Harrington & Harrington, 1961). Juvenile snook are reported to feed on bay anchovy, pinfish, mosquitofish, grass shrimp, killifishes, and insects (Harrington & Harrington 1961, Gilmore *et al.,* 1983).

Adults feed mostly on fish, crabs, shrimp, and some plant tissues (Fore & Schmidt, 1973). Reported important fish species consumed by snook are menhaden, mojarras, mullet, pinfish, anchovies, pigfish, and sailfin mollies (Fore and Schmidt 1973). In contrast, no information can be found on common snook larval (younger than 12 days) feeding habits. It has been suggested that copepods are their main diet in the wild, but since no wild larvae, younger than 12 days old, nor eggs have been found in the wild such affirmation is uncertain. More information on the larval diet (first feeding) for captive rearing is a challenge since no reference can be found in the wild (till now), basic investigation on larval rearing has been done during this study, prey type, prey density, stocking density and tank type has been one of the many topics investigated, in order to improve larval survival.

1.6 The Common snook fishery

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 1970s and the early 1980s (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.

Several measures have been introduced (Dec. 1998) to protect the common snook stocks, currently the snook regulations for Florida state a minimum capture size of 660 mm and maximum size of 863mm. Snook fishing is banned from December 15-January 31 and between June to August. The bag limit was set at two per day, but new regulations limit it to 1 per day, as well as expanding the closure of fishing from May to August (Florida Fish and Wildlife Conservation Commission, 2005).

Common snook is now a game fish, so there are only recreational landings. In 2003, anglers landed a total of 81,429 fish or 255,633 kilograms statewide. Landings by coast were similar though anglers landed slightly more fish on the Gulf Coast (51% by weight and 55% by number).
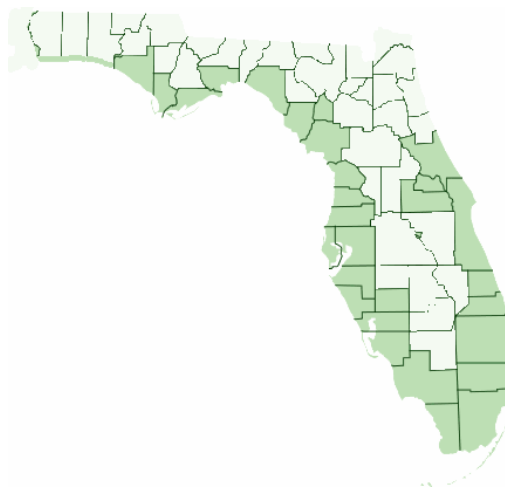
Figure 1.3. Florida's geographic distribution of recreational landings of common snook during 2003

The 2003 total landings (by weight) of snook were 7% lower than the average landings in the previous five years (1998–2002) and were 3% higher than the 1982–2003 historical average landings. From 1990 through 1997, numbers of snook landed on the Atlantic Coast generally increased, eventually reaching about 74,000 fish in 1997. Atlantic Coast landings have remained stable since

1998, averaging about 33,000 snook (112,950 kilograms) landed each year. Landings on the Gulf Coast reached peaks in 1992, 1993, and 1997, averaging 253,800 kilograms (about 81,000 fish) landed in each of those years (Figure 1.4). During the period 2000-2003 snook landings on the Gulf Coast have averaged about 43,000 fish or 308,000 pounds each year (Florida Fish and Wildlife Conserv. Comm, 2005)

Common snook availability on both coasts, as measured by standardized total-catch rates, increased from the early 1980s through the mid-1990s. On the Atlantic Coast, catch rates fluctuated without trend during 1994-2000 before increasing in 2002 and 2003. Gulf Coast total-catch rates for anglers follow the same trends as seen on the Atlantic Coast during the period between the early 1980s and mid 1990s. This was followed by fluctuations in catch rates with a slightly increasing trend between 1995 and 2003.
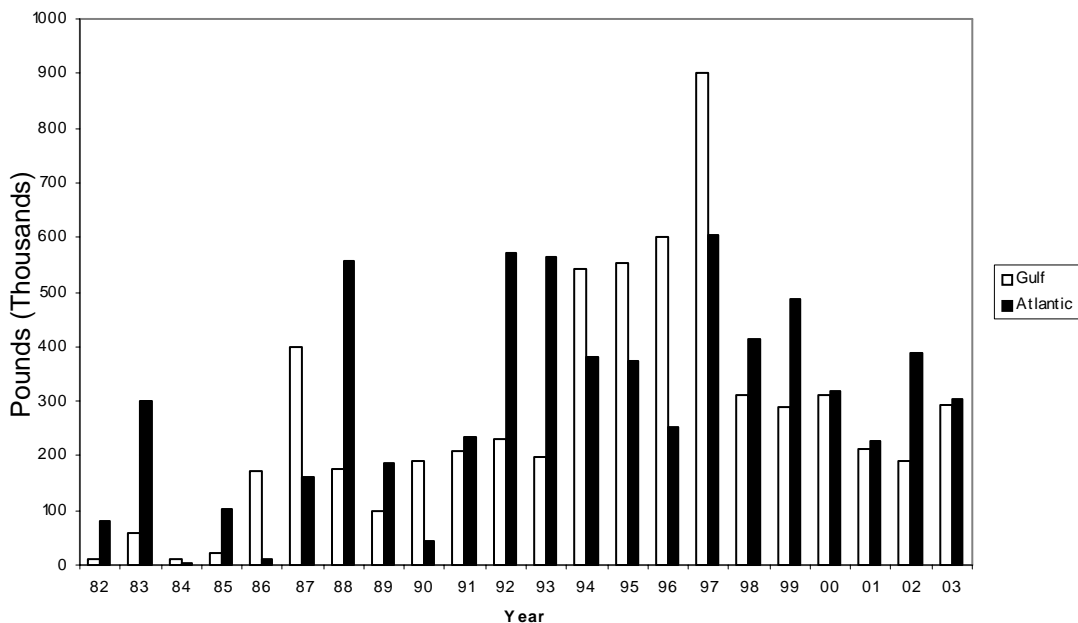
Figure 1.4. Total recreational annual landings of common snook off the Atlantic and Gulf Coasts of Florida, 1982–2003 (Florida Fish and Wildlife Conserv. Comm., 2005).

Relatively strong year classes were evident from fishery-independent data in 1997–1998 (Atlantic Coast) and 1999 (Gulf Coast). Relative abundance declined from 1997–2000 on the Atlantic Coast, followed by an increase through 2003, while numbers on the Gulf Coast fluctuated without trend (Florida Fish and Wildlife Conserv. Comm., 2005)

The Commission's management objective for snook is to maintain the transitional spawning potential ratio [1] (SPR) at, or above, 40%. The 2002 assessment was the first assessment after the implementation of the 660-863mm capture size limits in December 1998. The 2002 assessment indicated that the 2001 estimate of the Atlantic Coast average fishing mortality for ages 6–10, was 0.17 per year, similar to that from 1999 and lower than the average from 1997. On the Gulf Coast in 2001, the average fishing mortality for the same ages was higher, 0.34 per year, than on the Atlantic Coast; however, it has decreased from the 1997 high value of 1.08 per year. The spawning biomass of snook in 2001 continued to decrease on the Atlantic Coast and increase on the Gulf Coast. The transitional SPR on the Atlantic Coast was estimated at 30%; it was 26% on the Gulf Coast. Both figures were below the Commission's objective. The static SPR values indicate that, if fishing mortality rates continue at 2001 levels, snook will remain over-fished on both coasts. However, the commission implemented additional regulations on the Gulf Coast; the additional regulations are projected to reduce fishing mortality sufficiently to achieve the commission's goal (Florida Fish and Wildlife Conserv. Comm., 2005)

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[1]Spawning Potential Ratio (SPR): the ratio of spawning potential per recruit under a given fishing regime relative to spawning potential per recruit with no fishing. This also known as %MSP, where MSP stand for Maximum Spawning Potential.

1.7 Mote Marine Laboratory

The present study was conducted at Mote Aquaculture (Figure 1.5), which was formed in January in 1996, as a division of Mote Marine Laboratory, to develop methods for breeding and raising local species of marine fish and has established a programme for fingerling production of common snook for restocking purposes.

Common Snook successfully spawn in high numbers in the wild during the spawning season. Eggs are stripped and fertilized in the field and brought to the larval rearing tanks. Common snook juveniles' sizes currently range from 8 to 14 cm. Snook fingerlings are raised through winter for stocking during spring. A portion is raised in tanks to adult size to determine optimal conditions for growth and survival. Captive breeding is currently under development, therefore larval rearing relies 100% on the wild stocks.

Figure 1.5. Inland Mote marine aquaculture park

1.8 Background to the present study.

Although common snook research has been conducted for over two decades, such efforts have been sporadic and less intense than those spent on other similar species such as barramundi (*Lates calcarifer*). Thus far, barramundi (*Lates calcarifer*) and fat snook (*Centropomus parallelus*) have been spawned voluntary and hormonally induced (Alvarez-Lajonchere *et al.*, 2002).

Snook are gregarious and tolerate crowding (Fore & Schmidt, 1973). Optimal temperature is 27-28 °C, but they can survive in the range 10-35 °C (Volpe, 1959). Snook can survive transfer to fresh water 15 days after hatching, and juvenile growth is at least as fast as in salt water (Chapman *et al.*, 1978). They can also survive very low water quality (e.g., dissolved oxygen down to 0.9-1 mg/L, high turbidity), (Chapman *et al.*, 1978). Induced spawning of newly caught animals has been carried out which resulted in poor egg quality; (Neidig *et al.*, 2000). Normally, fish are stripped in the wild but some eggs were released in the holding tank. Even when egg quality seems high, high mortality is observed with yolk exhaustion (5 days post hatching[2]), (Tucker, 1987). This is probably related to food deficiencies (e.g., prey too large) or bacterial infection (Tucker, 1987). At about 10-12 dph, mortality from essential fatty acid deficiency is possible. Egg quality seem to be one of the first key issues for successful common snook larval rearing; and the present study has focused on this issue and in order to quantified accurately egg quality, key factors were investigated. Lipids levels in both eggs and larvae have been investigated and the influence that they have on egg quality and larval survival. At the same time the

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[2] Days post hatching, dph

determination of the lipid levels through the larval development has allowed us to identify when the larvae were dying and why and consequently investigated the possible solutions. The determination of the lipid level fluctuations in unfertilized eggs has also allowed determination of the months when the egg quality is optimal.

The obstacle for snook farming is the need for refining larval culture techniques to increase survival, especially during the first week. Another problem is cannibalism, which, if controlled, could increase larval survival by at least 10-15% (Tucker, 1987)

1.9 Aims of this thesis

The objective of this study was to increase the snook juvenile production for stock enhancement. In order to reach this objective, several aspects of the common snook life cycle were investigated:

  I.   To determine wild spawning locations for egg collection
 II.   To investigate the influence of lunar cycles on wild spawning and egg quality
III.   To evaluate the wild spawning timeframe and identify the months when spawning activity is highest.
IV.   To determine the optimal time during the spawning cycle with respect to egg quality and larval survival.
 V.   To describe the wild common snook eggs' fatty acid composition
VI.   To produce an annual quantification of egg fatty acid composition

VII. To monitor the egg fatty acid composition through the spawning season

VIII. To investigate the influence of the unfertilized eggs' docosapentaenoic acid (DHA) levels on egg fertilization.

IX. To evaluate the effect of docosapentaenoic acid (DHA) levels in eggs on larval hatching rates.

X. To determine the influence of docosapentaenoic acid (DHA) levels in eggs on larval survival rates.

XI. To analyse the levels of larval docosapentaenoic acid (DHA) through the development cycle.

XII. To evaluate the effects of temperature during egg incubation.

XIII. To investigate feeding techniques in an attempt to improve larval survival and growth.

XIV. To monitor the effect of different rearing systems on larval survival.

XV. To describe the embryonic and early larval development cycle.

XVI. To investigate early larval development and ultra-structural changes during the first three days.

Chapter 2: General Materials & Methods

2 General Materials and Methods

2.1 Sampling sites

The capture of wild stock for gamete and milt collection took place over the course of 4 years (Table 2.1). A total of 96 field trips were made to 7 locations spread along Sarasota County, Manatee County and Port Charlotte (Florida, USA) (Figure 2.1). The sites were located at passes and estuaries, characterized by their seagrass and sandy bottoms. Broodstock were collected during the outgoing tides between the 15:00-20:00 hours, at the different lunar phases (i.e. full moon)
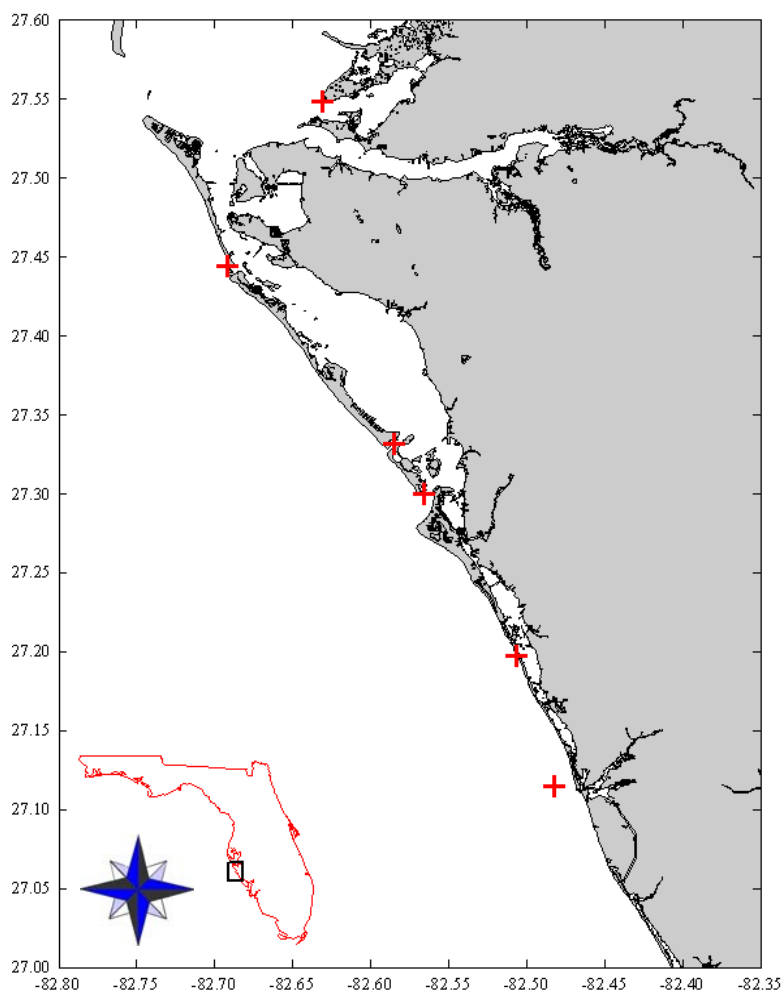
Figure 2.1. Sampling site map, Central West Florida. Red crosses marked sampling sites

Table 2.1. Wild stock sampling time framework

Sampling Time framework
From the 29th of May until the 9th of September 2002
From the 18th of April 2003 until the 29th of August 2003
From the 21st of June 2004 until the 19th of August 2004*[**]
From the 25th of May until the 8th August 2005

2.2 Sampling procedures for gamete collection in the field

2.2.1 Capture of wild stocks

An egg collection team consists of a minimum of 6 people and one vessel capable of carrying the team and the required equipment. The natural spawning sites were reached one or two hours prior to the outgoing tide. After arriving at the collecting site, the spawning schools were identified using the rolling behaviour of the fish known as 'spawning balls'. Once the spawning balls were identified, a 91 m by 2.1 m seine net, equipped with a purse at the centre, was deployed from the boat, enclosing the spawning ball within the net and the embankment. The collection team, excluding the boat captain, then helped to pull the net onto the shore and collect any fish trapped. During this process, one person snorkelled around the seine net to make sure that the net was clear of any obstructions on the sea bottom.

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[*] Spawning season sampling delayed due to a delay on the stock enhancement program.

The snook were then sexed and placed in designated male and female hapa nets (1.2 m by 12 m). Male snook were maintained in shallow water and identified by their pin-like genital pore, while female snook were identified by their larger, swollen, genital pore. The female genital pore also had a slit perpendicular to its body axis. Both males and females were checked for signs of spawning readiness. If the males were not releasing milt readily, they were instantly released. However, all females and flowing males were held in the hapa until broodstock were manually stripped. If females were not releasing eggs readily, they were returned to the hapa for inspection twenty minutes later.

2.2.2 Gamete extraction

Eggs from the female snook were collected in a dry, clear graduated 1000 ml plastic container with a lid. Eggs were collected by gently massaging the snook's abdominal area (from pelvic fins to vent) by hand. If eggs were released, they were checked for colour, signifying viability. If eggs were dark yellow, they may be too old and not viable (Neidig *et al.*, 2000). Also, if eggs had any blood in them, they were also not considered viable. A pale yellow to creamy-white coloured egg is desirable. A minimum of 5 and a maximum of 20 females fish were stripped during each spawning trip. Once the eggs were collected two samples of 1 ml of eggs were taken, fixed in BHT 2:1 Chloroform-Methanol and stored at -30 C° for later lipid analysis.

2.2.3 Sperm Extraction

Milt from the male snook was collected using a method similar to the one used to collect the eggs from the females. One person held the snook and gently, yet firmly, massaged the fish genital area and another person wiped the genital pore with a dry cloth (in order not to activate the sperm). The milt was collected in graduated, dry plastic syringes and was chilled with ice packs until needed for fertilization. The ratio of males contributing milt to females contributing eggs was 5:1 (5 males to 1 female), in order to increase genetic diversity.

2.2.4 Fertilization

After obtaining approximately 300 ml of eggs (approximately 700,000-840,000 eggs) assuming approximately 2800 eggs in 1 ml, (Neidig *et al* 2000), 5 drops of milt were added to the container holding the eggs. This volume of eggs was measured and recorded for later dilution calculations. After mixing the eggs and milt thoroughly, approximately 200-300ml of filtered seawater was added to activate the sperm. The sea water used for sperm activation was made prior to the collection trip using filtered and dechlorinated freshwater and Instant Ocean ® salt mixed to salinity of 35 ppt. This water was held at the same temperature as the sampling site water until it was needed. The activated egg and sperm mixture was held for 2 minutes and then the eggs were rinsed in a 100 μm sieve with filtered instant ocean.

2.2.5 Gamete transportation

The eggs were then rinsed into a plastic bag with 6 L of filtered instant ocean. This addition of water to eggs was also measured and recorded for later dilution calculations. The bags were filled with pure oxygen, sealed with rubber bands and placed in coolers for transport. Transportation time varied depending on the sampling site location with respect to the laboratory (1 to 2 and a half hours).

2.3 Rearing systems and stocking protocols

Artificial water was used throughout this study consisting of filtered and de-chlorinated freshwater mixed with Instant Ocean ®, in order to make saline water at 35 ppt. For the first two years, although the experimental systems were next to the shore, no bay water was used in order to avoid red tide cells (*Karenia brevis)* entering the system, as well as undesirable organisms such as jelly fish larvae etc…
The following two years the experimental systems were moved 20 miles inland, using the local well water as the main water source to make the saline water for the systems. Prior to being used in the systems, sea water was made in a 1500 L water reservoir where it was aerated and re-circulated though a filtration linked to a UV filter and a carton filter (Figure 2.2).

Figure 2.2. System water reservoir

2.3.1 Experimental systems

Three systems were used during the course of this study; two experimental systems, and a production system. The experimental systems were built at Mote Marine Laboratory in the aquaculture facilities.

The experimental system "A" (also named "Microcosmos") consisted of a re-circulating system of 48x2L compartments made of Plexiglas, with an independent filtration system, a UV filter, a fluidised bed, a carbon filter and bio-filtration beads. A 10% daily water change was carried out for the duration of the experiment. After every experiment (14 days) the system was drained and cleaned. Water quality parameters were measured three times a day, except for ammonia, nitrites and nitrates, which were taken on a weekly basis. External conditions were controlled maintaining the water temperature at 28°C and a fourteen hours daylight (14:10) photoperiod. More detailed information about system A can be found in chapter 5.

The experimental system "B" consisted of a re-circulating system containing 24x6L tanks made of transparent plastic with a mesh drain pipe in the middle, each placed in a fibreglass raceway. An independent filtration system for every 12 tanks was set up, with a UV filter, a protein skimmer and bio-filtration beads. A 25% water change was carried out on a weekly basis. Water quality parameters were measured three times a day except for ammonia, nitrites and nitrates, which were measured weekly. External conditions were controlled in a similar manner to System A, maintaining the water temperature at 28°C and a 14:10 photoperiod. More detailed information about system B can be found in chapter 5.

2.3.2 Production System for mass rearing of snook larvae

The production system was based on three 3300 L, black fibreglass tanks, part of a re-circulation system with an independent filtration system formed by a UV filter, fluidised bed, protein skimmer and bio-filter beads. The water temperature was reasonably stable at around 28°C, although external conditions were not controlled. Water quality parameters were measured three times a day except for ammonia, nitrites and nitrates, which were taken on a weekly basis. More detailed information about the production system can be found in chapter 5.

2.3.3 Stocking of fertilized common snook eggs

After transportation from the sampling site to the laboratory, 3 samples containing I ml of fertilized eggs were examined under a light microscope to asses their fertilization rate, egg size and morphology. Also, volumetric eggs counts were taken to calculate the quantity of eggs collected. Other samples of fertilized eggs were collected and placed in floating hatching cells (Figure 2.3) that were, themselves, placed in the production tanks and collected after embryos hatched the following day. These hatching cells consisted of a 5 cm PVC piece of tubing open on the top end, and 100µm mesh on the bottom end, that allowed for water circulation inside the cylinder. Floats were attached to the outside of the cylinders and located at a height that allowed the cylinder to have a total volume of water of 2 L. Hatching cells were stocked with approximately 200 eggs.

All fertilized eggs were disinfected with hydrogen peroxide (2ml/L) during 1 minute prior to stocking in tanks, in order to avoid unwanted organisms (such as jellyfish larvae) entering the tanks. Depending on the amount of gametes obtained in the field, different tanks were stocked following the stocking protocols chosen to fit the various experiments (See Chapter 5). Water temperatures in all tanks were between 28-29 °C, at 35 ppt and 6-8 mg/l dissolved oxygen.

Prior to stocking, production tanks were set up with very light aeration and no water circulation in order to reduce any water movement to the minimum.
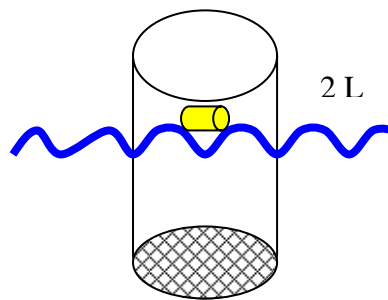
Figure 2.3. PVC hatching tank

2.4 Gamete Quality Assessment techniques

2.4.1 Egg fertilization assessment

Three samples were taken with a 1 ml syringe from a lightly stirred plastic bag that contained 6 L of water plus the collected eggs (~ 400 ml of pure eggs). Each sample was place in a Sedgwick Rafter® cell under the light microscope. Eggs were then observed and classified into two groups: Fertilized and unfertilized.

Eggs where the cell development was past the Blastula stage were considered to be fertilized, and those that did not show any type of development, or which were not past the blastula stage, were considered unfertilized.

Once the counts were made the following calculation was used to obtain the fertilization rate:

Fertilization rate = Fertilized eggs * 100 / (Total number of eggs)

An average from the three 1ml samples was calculated, which was used as the total fertilization rate for that day.

2.4.2 Eggs hatching percentage assessment

Three samples of 1 ml of eggs and water (~200 eggs) were placed in three hatching tanks (Figure 2.3) in standard water conditions (28°C, 35 ppt, 8 mg/L DO). After hatching (~16 hours after fertilization), contents in the tank were placed in a dish and counted under the light microscope. Hatching percentage was calculated by the following formula:

Hatching percentage = Larvae *100 / (Total number of eggs)

2.4.3 Egg diameter examination

During the 2002 and 2003 egg diameters were measured from 28 trips. After each sample collection, 100 eggs were randomly sampled and placed under

the light microscope in order to record individual diameter measurements (μm).

2.5 Samples Analysis

2.5.1 Samples and fixation

Eggs samples for lipid analysis were taken from spawing females on the field. Unfertilized eggs were taken from two random females in each trip; 1 ml from each female of unfertilized eggs were placed in a solution of 2 ml of (2:1, v/v) chloroform/methanol + 0.01% butylated hydroxy toluene (BHT) and stored at –30°C, until analyzed.

Forty-eight samples of unfertilized eggs in total were collected and analyzed during the study from the 24 successful spawning trips.

Fourteen larval samples from the production tanks were also collected and analyzed. The first sample was collected 4 days post hatching collecting samples every two days from day 4 until day 14 (five larvae per sample) and every 15 days from day 30 until day 80 (1 larva per sample). Another set of three samples of 30 days old larvae (first grading) were collected, as well as three samples from day 80 larvae. All the larval samples were also fixed in (2:1, v/v) Chloroform/methanol + 0.01% butylated hydroxy toluene (BHT) and stored at –30°C until analyzed.

2.5.2 Lipids analysis

2.5.2.1 Lipid extraction

Total lipid from unfertilised snook eggs, and from snook larvae, was extracted according to the method of Folch *et al.* (1957). This method extracts all lipid classes except polyphosphoinositides. Eggs and larvae samples (1 gr weighed to five decimal places) were placed directly in large, glass, stoppered, 20 ml tubes together with 10 ml of chloroform:methanol (C:M)(2:1, v/v).

Eggs and larvae samples were homogenised for 2 minutes using an Ultraturrax^{TM} homogeniser. This was carried out carefully to avoid splashing the mixture and to prevent excess heat formation and vaporisation of the solvents. After the homogenisation of each sample, the Ultraturrax^{TM} probe was rinsed with C:M (2:1, v/v) in order to avoid lipid contamination between the different samples. After homogenisation, the stoppered tubes were allowed to stand in ice for approximately 60 minutes to ensure that extraction of all the lipids occurred. Then, 2.50 ml (0.25 volumes) of 0.88% (w/v) potassium chloride (KCl) in distilled water was added to the homogenate of eggs and larvae samples, respectively. The mixture was shaken, vortexed and the stoppered tubes were left to stand on ice for 5 minutes. The solvent and aqueous layers separate to give a lower chloroform-rich phase containing the lipids and an upper aqueous methanol-phase containing water soluble components. The organic layer that contains the purified lipid was separated from the aqueous layer containing the non-lipid contaminants by centrifugation (Jouan C 412 bench centrifuge) of the tubes for 5 minutes at 1500 rpm. The

organic layer is the lower phase, which comprises about 60 per cent of the total volume. The upper aqueous layer was carefully aspirated and discarded and the lower organic phase was filtered through a clean pre-washed (in C:M 2:1 v/v) filter paper (Whatman No.1) into a new 10 ml test tube. The homogeniser tubes and the filter papers were rinsed with a small amount of C:M (2:1) and the rinses were added to the extract in order to minimise losses of the total lipids through this process.

The solvent was evaporated under a stream of oxygen-free nitrogen in a fume cupboard. After evaporating the solvent in the samples, 1ml C:M (2:1 v/v) was added into the test tubes and vortexed. Test tube contents were then extracted and transferred into pre-weighed 2 ml glass vials. Glass vials were place on the nitrogen evaporator to evaporate the solvent. Glass vials were desiccated overnight under vacuum and the dried total lipid weight was determined gravimetrically the next morning. The lipid extract was then dissolved in a volume of C:M (2:1 v/v) containing 0.01% butylated hydroxy toluene (BHT 0.01% w/v) determined by the lipid weight, to give a standard concentration of 10 mg of lipid/ml solution. BHT was added to protect the total lipid against oxidation of the polyunsaturated fatty acids, the vial was flushed with oxygen-free nitrogen and stored at -20 °C until further analysis.

2.5.3 Fatty acid analysis

Fatty acids of the total lipid samples were converted to methyl esters by acid-catalysed trans-esterification, as described by Christie (2003). The preparation and purification of the fatty acid methyl esters (FAME) were performed as follows.

For each sample 1 mg of total lipid (equal to 100 μl of 10 mg/ml stock solution with C:M 2:1 v/v) was placed in a quickfit test tube. Then, 0.1 mg (equal to 100 μl of 1 mg/ml stock solution with C:M 2:1 v/v) of purified heptadecanoic acid (17:0) was added as an internal standard (internal standard represented 1/10 by weight of the total lipid). The solution was mixed and evaporated under oxygen-free nitrogen in order to remove the organic solvent. After that, 2 ml of methanol-sulphuric acid methylating reagent (1% sulphuric acid in methanol v/v) was added to the tube, together with 1 ml of toluene, which helped dissolve the neutral lipids. The stoppered tube was shaken to mix the solvents, flushed with nitrogen and incubated overnight (16 hr) at 50 °C in a dry-heating block. The tube was stoppered with a piece of paper to prevent the stopper blowing out when the tube is heated.

The tube was then removed from the heating block, allowed to cool at room temperature and 2 ml of 2% (w/v) $KHCO_3$, 1 ml of isohexane:diethyl ether (1:1 v/v) containing 0.01% (w/v) butylated hydroxy toluene (BHT) and 4 ml of isohexane:diethyl ether (1:1 v/v) were added. The tube was shaken and vortexed, then centrifuged at 1500 rpm for 2 minutes. The upper organic layer was transferred to a clean tube, while a further 5 ml of isohexane:diethyl ether (1:1 v/v) was added to the original tube, which again was shaken, vortexed

and centrifuged as before. The upper layer was added to the other tube, as before, and evaporated under oxygen-free nitrogen to remove the solvent. The dry extract in each tube comprised the crude fatty acid methyl ester (FAME) derivatives of the total lipid.

The FAME was re-dissolved in 100 μl of isohexane and vortexed. Purification of methyl esters was carried out by TLC on 20×20 cm glass plates pre-coated with silica gel G (Merck silica gel 60). The methyl esters of a sample were loaded on a 2 cm origin marked with a pencil at 1.8 cm above the bottom edge of the plate. The 20×20 cm plate was marked with four origins separated from each other by 1.5 cm with a 2 cm margin from the side edge of the plate. Methyl esters of 4 different samples were loaded on each TLC plate by means of a Hamilton syringe. The plate was then run in 100 ml of isohexane: diethyl ether: glacial acetic acid (90:10:1 v/v) to 1 cm from the top edge of the plate. The plate was then removed from the glass tank and the solvent was allowed to evaporate off in the fume cupboard.

Fatty acid methyl esters were visualised by spraying the edge of the plate with 1% iodine in $CHCl_3$ (w/v). Spraying was done only on the 2 origins located at each side of the plate by masking most of the origin off with a blank glass plate, so that only the very edge of the origin was exposed for spraying. That section was then sprayed lightly with the iodine solution. Thus, the FAME band was clearly visible and marked with a pencil. The FAME chromatograph is a doublet band, with saturated and monounsaturated fatty acids forming the upper band and PUFA the lower band. The FAME bands were all marked accordingly and then were scraped from the TLC plate using a razor blade. They were scraped into test tubes and FAME eluted with 5 ml of isohexane:

diethyl ether (1:1 v/v) plus 1 ml of isohexane: diethyl ether (1:1 v/v + BHT). Then, the tubes were centrifuged and the solvent was transferred carefully, without touching the lower silica layer, through a pre-washed filter paper (Whatman No.1) into a clean tube. The solvent was evaporated off under oxygen-free nitrogen and the purified FAMEs were transferred to small glass vials in 1 ml isohexane (+ BHT). The labelled vials were flushed with oxygen-free nitrogen and stored at -20 °C in the freezer until gas liquid chromatography analysis.

2.5.4 Gas liquid chromatography (GLC) analysis of FAMEs

The fatty acid composition of the total lipid of each sample was determined by gas-liquid chromatography of its methyl ester derivatives, prepared by transesterification as described above. Gas-liquid chromatography (GLC) is a form of partition chromatography in which the compounds to be separated are volatised and passed in a stream of inert gas (the mobile phase) through a column in which a high boiling-point liquid (the stationary phase) is coated onto a solid supporting material. The substances are separated according to their partition coefficients, which are dependent on their volatilities and on their relative solubility in the liquid phase. They emerge from the column as peaks of concentration, which are detected by some means that converts the concentration of the component in the gas phase into an electrical signal. This is amplified and passed to a continuous recorder so that a tracing is obtained with an individual peak for each component as it is eluted. With a suitable

detector, the areas under the peaks bear a direct linear relationship to the mass of the components present. The GLC used for our FAMEs was fitted with a flame ionisation detector (FID).

Fatty acid methyl esters were separated and quantified by gas-liquid chromatography (GLC, Fisons GC8000, Fisons Ltd.) using a fused silica capillary column (30 m × 0.32 mm i.d.) coated with the stationary phase CP wax 52 CB (Chrompak Ltd., London, United Kingdom). Hydrogen was used as carrier gas at a flow rate of 2.5 ml/min. Samples were loaded by injecting 1 μg of FAME (equal to 1 μl of 1 mg FAME/ml isohexane) with a syringe directly into the packing material of the column ("on-column injection"), so that the flow of gas was not interrupted. The oven temperature was programmed to rise from the injection temperature of 50 °C to 150 °C at 40 °C/min and from 150 °C to 225 °C at a rate of 2 °C/min and the final temperature of 225 °C was maintained for 5 minutes. Individual methyl esters were identified by comparison to known standards (marine fish oil) and by reference to published data (Ackman, 1980). Peak areas of fatty acids were quantified with reference to the peak area of 17:0 internal standard (Heptadecanoic acid, Sigma Chamical Company, St. Louis, USA) and processed using a software package (Chromcard for Windows, ThermoQuest, Milan, Italy).

2.5.5 Data analysis

Two main statistical software packages were used to analyse the data collected for this chapter, Excel 2000 and SPSS 12th edition. Most of the data was analysed using a uni-variate ANOVA method with a polinomic contrast. The other analytical method used was a bivariate correlation with a Spearman correlation factor due to the data normality. The Excel software package was mainly used for the graph making as well as the SPSS 12th.

2.5.6 Larval development analysis

During the 2003 season, five larvae were randomly sampled, on a daily basis, from the production tanks (from day zero DPH until day 15 DPH). A detailed description of the snook gross morphology for the first 15 days of life was carried out mainly under light microscopy photography as well as under the SEM (3 days time period). After day 15, 5 samples were taken every 15 days until day 80. More detailed description of protocols and materials can be found on materials and methods in chapter 6.

Study of the vision and digestive system development on the first 3 days post hatching was carried out at the Institute of Aquaculture (Stirling University, UK), with samples previously preserved at Mote Marine Laboratory, (Sarasota, FL). Samples were prepared for ultra-structure analysis to later be viewed and documented under a light microscope and a transmission electron microscope (TEM). More detailed description of techniques and protocols can be found at the materials and methods in chapter 6.

Chapter 3:

Environmental and topographical factors influencing

spawning and egg quality

3 Environmental and topographical factors influencing spawning and egg quality

3.1 Introduction

The common snook reproductive cycle has not been closed in captivity as yet, although the cycles for closely related species such as barramundi (*Lates calcarifer*) and fat snook (*Centropomus parallelus*) have been closed, producing viable F1 in captivity from a captive broodstock (Alvarez-Lajonchere et al., 2002). Therefore the first aspects that needed to be addressed in order secure common snook eggs focused on determining the wild common snook spawning period. A better understanding of the best locations and time frame to obtain spawning females was required. The study area was located on the west central coast of Florida based around Sarasota county (Figure 2.1).

3.1.1 Natural spawning period

In Florida, spawning occurs from May to mid-November with peak spawning occurring between June-July along the southeast and southwest coasts, and in August along the east central coast of Florida. In Texas, the primary spawning period is between June-August (Matlock and Osburn 1987). These peaks may vary among locations (Roberts *et al.*1999).

Roberts *et al.,* (1999) stated that the gonadosomatic index of adult snook, and the catch per unit effort (CPUE) of larvae, were highest during the new moon period in June and July. Eggs were most abundant during late evening and in the early hours of the morning. Some spawning may occur all year round in the warmer parts of their range (Marshall 1958, Volpe 1959, Ager *et al* 1978, Tucker 1987).

Common snook can spawn repeatedly during a single season (Fore and Schmidt 1973, Collins & Finucane, 1984) and are considered batch-synchronous, i.e., they can spawn once every 3 to 4 days for about 152 days from mid April to mid September in Florida waters. Fish that are ready to spawn congregate in schools in saline shallow areas near river mouths, estuarine passes, and along open beaches in the vicinity of inlets. Actual spawning is most likely to occur in the shallow near shore waters (Marshall 1958, Volpe 1959, Ager *et al.* 1978, Bruger, 1981, Gilmore et al. 1983). Salinities higher than 20ppt are necessary to activate sperm for successful spawning (Ager *et al.* 1978, Shafland and Koehl, 1980).

3.1.2 Location and selection of spawning sites

Earlier studies (Marshall 1958, Volpe 1959, Ager *et al.* 1978, Bruger 1981, Gilmore *et al.* 1983) and discussions with the local professional fishermen, had revealed the location of snook spawning sites. A series of locations along Sarasota County, Manatee County and Port Charlotte (Figure 2.1) were chosen for egg collection. Although the spawning sites were known, catching

success of common snook is determined by the site-specific topographic characteristics, since catching methods are based on using seine nets (91.5 m by 2.1 m) during outgoing tides when current speeds are higher. Shallower and slower outgoing water speeds are preferable for more effective capture. Other factors for choosing collection sites include, proximity to the laboratory, and isolation from recreational fishermen and accessibility.

3.1.3 Influence of lunar phase on spawning activities

The lunar cycle provides a strong, predictable set of environmental cues for marine species. Environmental cycles (e.g., tidal water movement, moonlight) entrain endogenous reproductive cycles, synchronizing gamete release under favourable conditions (Taylor 1984, Omori 1995). Lunar synchronized spawning, for example, is commonly documented for species of shallow waters with large tidal fluctuations (Korringa 1947, Taylor 1984). Reef fishes often mass in spawning aggregations on a specific lunar and seasonal cycle (Johannes 1981, Robertson et al. 1990).

Such aggregations are best documented for commercially important fishes such as groupers, Serranidae, including Nassau grouper (*Epinephelus striatus*), red hind (*E. guttatus*), and tiger grouper (*Mycteroperca tigris*) in the Caribbean (see review in Domeier and Colin, 1997). Eklund *et al.*, (2000) observed black grouper (*M. bonaci*) aggregating during their spawning season just outside no-take zones along the Florida Keys reef tract. Samoilys &

Squire (1994) and Samoilys (1997) documented spawning aggregations of coral trout (*Plectropomus leopardus*) from the Great Barrier Reef, and Johannes & Squire (1988) described the aggregating behaviour of squaretail coral grouper (*P. areolatus*) from the Solomon Islands. Most recently, Sala et al. (2000) observed aggregating behaviour in two species of serranids, the sawtail grouper (*M. prionura*) and the leopard grouper (*M. rosacea*) from the Gulf of California.

In addition, lunar cycles have been implicated in spawning and settlement of intertidal (Taylor, 1984) and pelagic-spawning fish and invertebrates (Crabtree 1995, Robertson et al., 1999). Common snook spawning has been documented to follow a lunar cycle (Roberts *et al*., 1999). Lunar cycles in fish behaviour have long been recognized and exploited by artisanal and commercial fisheries (Johannes 1981, Parrish 1999). The implications of lunar cycles for the design of sampling programs are discussed by Gaudreau & Boisclair (2000).

3.1.4 Seasonal influence on spawning activities

Common snook is a seasonal spawner (Taylor *et al.* 2000). The spawning season for snook in the study area occurs from May until the end of October. This seasonal pattern is directly related to Florida's west coast water temperature variation (Figure 3.1). The common snook is very sensitive to temperature with detrimental effects (cessation of feeding, malformations, high mortalities) at approximately 15°C or lower (Marshall 1958, Gilmore *et al.* 1983). In early life, snook eggs and larvae have been proven to successfully

develop at 28°C ± 1 (Shafland and Koehl 1980, Lau and Shaftland 1982, Tucker 1987). At the same time larvae propagated in the laboratory have been successfully reared at 24.6 to 32.5°C (Shafland and Koehl 1980, Lau and Shafland 1982, Tucker 1987). Snook larvae have also been collected from the Naples Bay, Florida, at temperatures ranging from 28.7 to 31.4°C (Tolley *et al*. 1987). In a hatchery study, snook reared at 24°C did not survive, and development rates increased with incubation temperature (Lau & Shafland, 1982). All the above suggested that temperatures over 27°C are necessary for snook spawning as well as snook larval development. Therefore this study was based on fieldwork between April to October.

Overall a better understanding of key environmental factors on the spawning behaviour of wild common snook will improve field work efficiency, increase the rearing success through collection of good quality gametes and reduce the costs for their procurement.
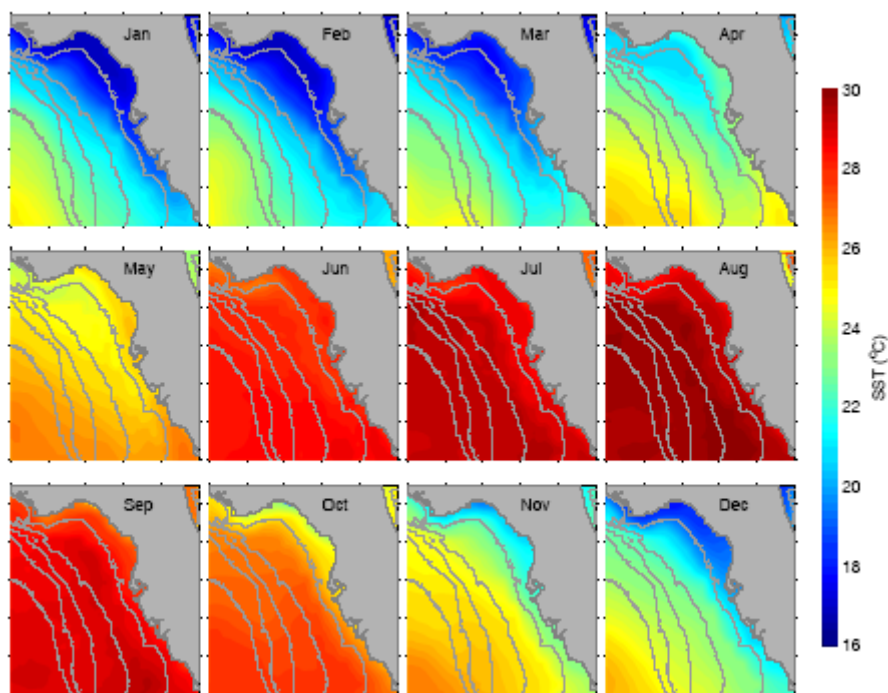
Figure 3.1. Average water temperatures of Florida's West coast (1998-2004), (NOAA, 2005)

3.1.5 Aims of this study

The objective of this study was to establish a reliable source of common snook gametes for egg and larval development studies under captive conditions.

Three main aspects were investigated:

i. The influence of location on successful capture of wild stocks, to identify the best locations to capture wild common snook.

ii. The influence of lunar phase on the spawning of wild common snook.

iii. To establish monthly spawning activity of wild common snook to identify the periods when common snook spawning activity is optimal.

3.2 Materials and Methods

3.2.1 Spawning Sites

Seven sites along the Florida West coast between Terra Ceia, the most northern site (Manatee county) and Cayo Costa (Port Charlotte county) the most southern site were used for this study (Figure 3.2.)
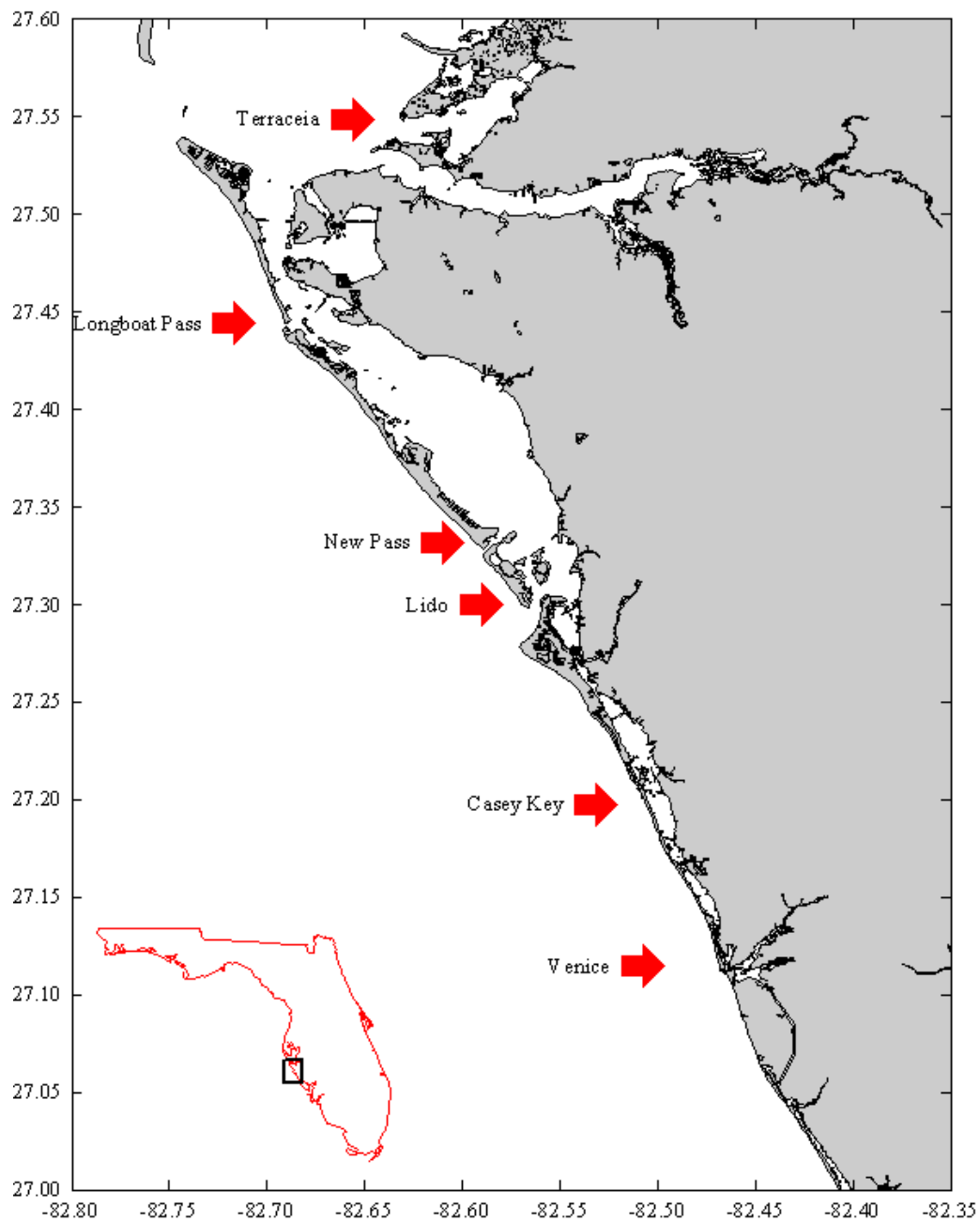
Figure 3.2. Manatee and Sarasota county sampling sites, names and location

3.2.1.1 McGill island, Terraceia Bay.

Terraceia is the most northern site of this study (27°32'55"N, 82°37'53W) located at the mouth of the Manatee river, an 80 Km river that flows SW and W, located in Manatee county.

Only accessible by boat, the site is an inhabited mangrove island located at Terraceia Bay (Figure 3.3). The shore profile is shallow (Figure 3.4) surrounded by sand banks and grass flats. Due to its shallow profile, water tidal speed is moderate.

The site is frequently used by recreational fishermen searching mainly for snook, but due to its remote location from boat ramps and inaccessibility by land the site remains fairly free of fishing and boating pressure compared with other sites. Snook congregations at the McGill island site were observed from April through October
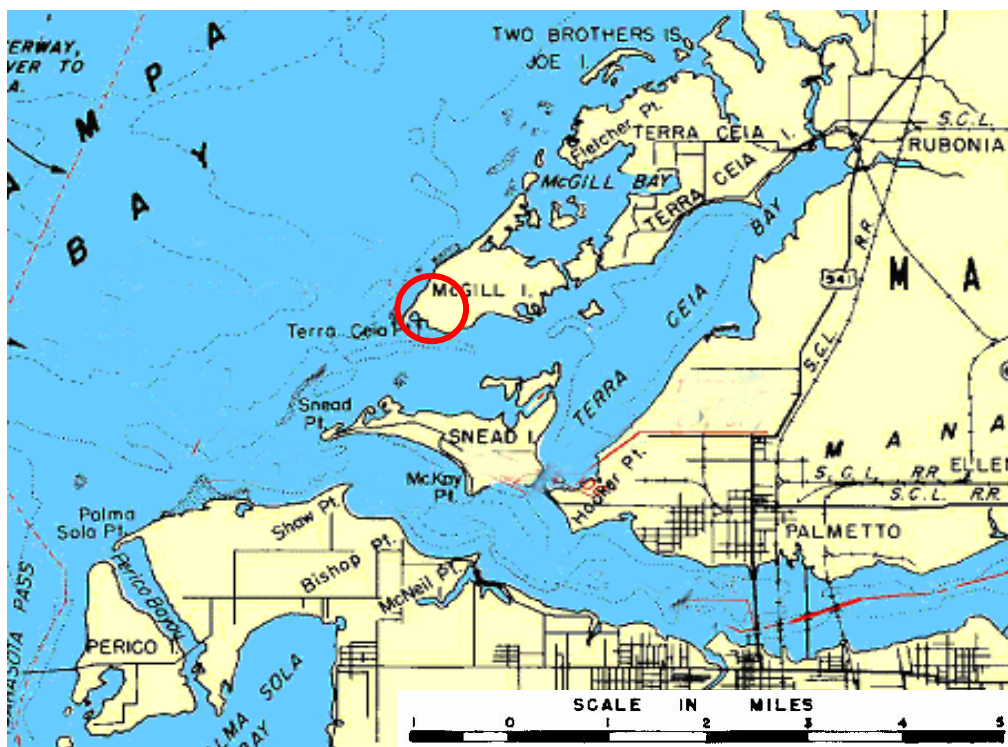
Figure 3.3. McGill island Location, FL (US. Corps of engineers, 1999)
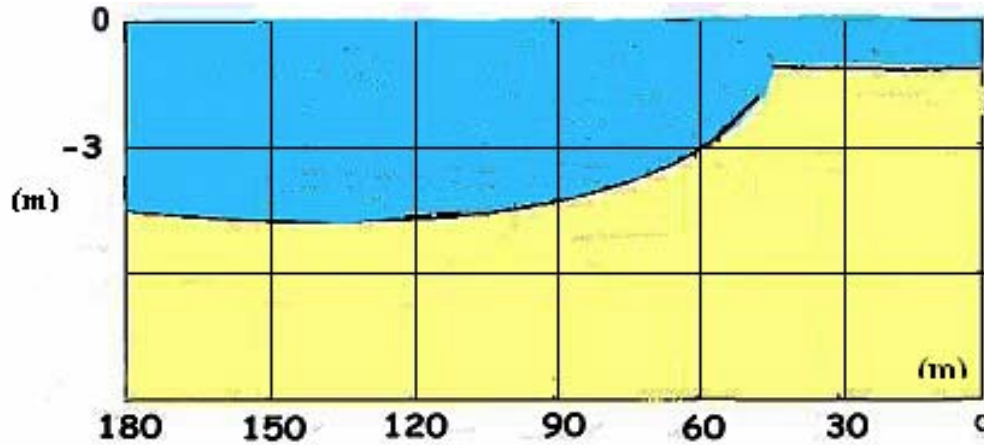
Figure 3.4. McGill Island beach profile (US. Corps of engineers, 1999)

3.2.1.2 Long boat, Beer can island

This site is located in Manatee county at Beer Can island (27°26'39"N, 82°41'31"W) and next to Long Boat Pass (Figure 3.5), in this case the site is not at a river mouth but in a barrier island pass.

This site is located next to a highly built up area. Well known in the area for its scenic beauty, and fishing tradition, the site is easily accessible by land as well as by boat, making the site a popular choice for boaters and recreational fishermen. Snook congregations are observed from April through October. This sandy site has a steep and deep profile (Figure 3.6) due to the strong tidal current that moves through the pass.

Figure 3.5. Long Boat Site location, FL (US. Corps of engineers, 1999)
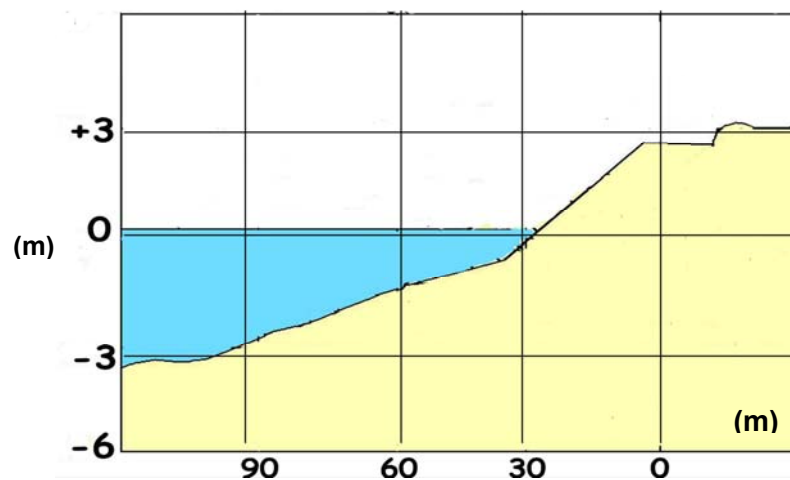
Figure 3.6. Beer can island, beach profile (US. Corps of engineers, 1999)

3.2.1.3 New Pass

This site is located (27° 19'55''N, 82°35'07''W) in Longboat Key southern tip right at a pass called New Pass (Figure 3.7). This site is one of the main accesses by boat to the Sarasota Bay, making it a busy location in terms of boating traffic. Characterized by its sandy bottom and strong currents due to the tidal water currents, this is not usually frequent by recreational fishermen but during the weekends a small shallow sheltered cove next to the site gets crowded with boaters.

Fish congregations occur from May to October, especially during the different moon phases, large females are observed on a regular basis. This site is located 5 minutes away (by boat) form the main Mote Marine Laboratory, which makes it the site closest to the laboratory (Figure 3.7).

The beach profile is directly determined by the proximity to the pass canal which is regularly dredged making it unusually deep (10-15 m), this increases the amount of water washing in and out the bay, sharpening the pass edges were the site is located. The site is characterized by a sharp drop 5-10 m from

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the shore (Figure 3.8). Two private docks and 2 jetties are present along the beach, breaking the beach continuity.

Figure 3.7. New Pass and Lido beach location (US. Corps of engineers, 1999)
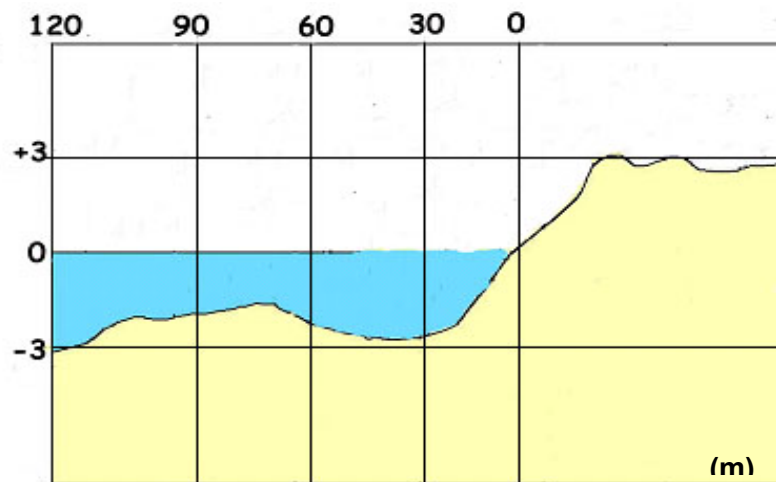
Figure 3.8. New Pass beach profile (US. Corps of engineers, 1999)

3.2.1.4 Lido beach

This site is located at the southern tip of Lido key (27°17'59"N, 82°33'56"W), right at the biggest pass in the Sarasota county, the Big Pass (Figure 3.7). Like 'New Pass' this site has a sandy bottomed beach, which is located in a county park, therefore is accessible by either land or boat. Recreational boat fishing is not usual at this site due to proximity to the recreational park, which is usually used by bathers, jet skies, wind surfers and kite surfers. This beach is characterized for its strong currents especially during outgoing tides. Although this pass has the biggest boating traffic in the county the site is not affected by it, due to the pass width.

The beach profile (Figure 3.9) is similar to the New Pass, with a sudden drop 5-10 m from the shore, although there are no docks or jetties along the beach. Some bottom sea grass can be found at the northern part of the beach in shallow waters (< 0.5 m).
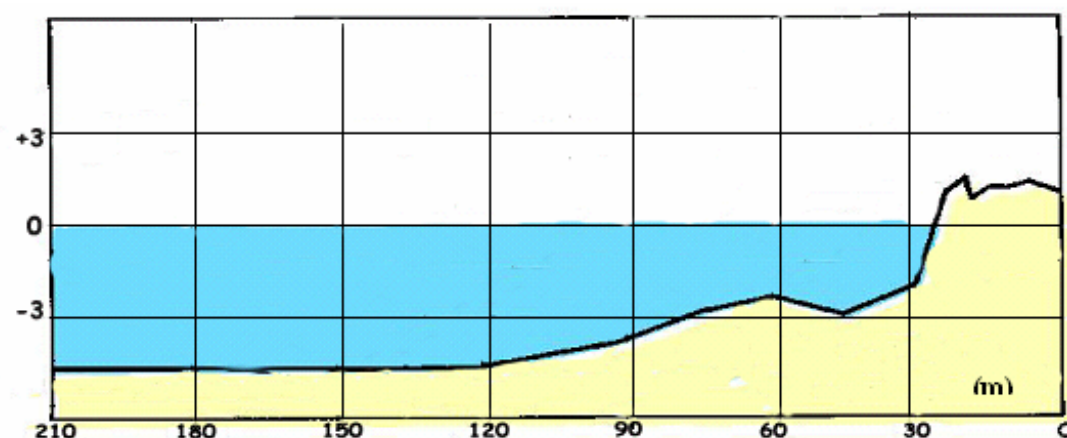
Figure 3.9. Lido beach profile (US. Corps of engineers, 1999)

3.2.1.5 Venice

Venice is the most Southern site in the Sarasota county (27°06'53"N, 82°28'57"W), is located in Casey's Key southern tip, next to the Venice inlet (Figure 3.10), This site is located in a narrow natural canal, characterized for the abundant presence of mangroves and sea grass bottom with patchy sandy parts.

Boating traffic is high due to the proximity to the Venice inlet, but recreational boat fishing is unusual as are most types of water sports. The site is located in a recreational park often used by bathers.
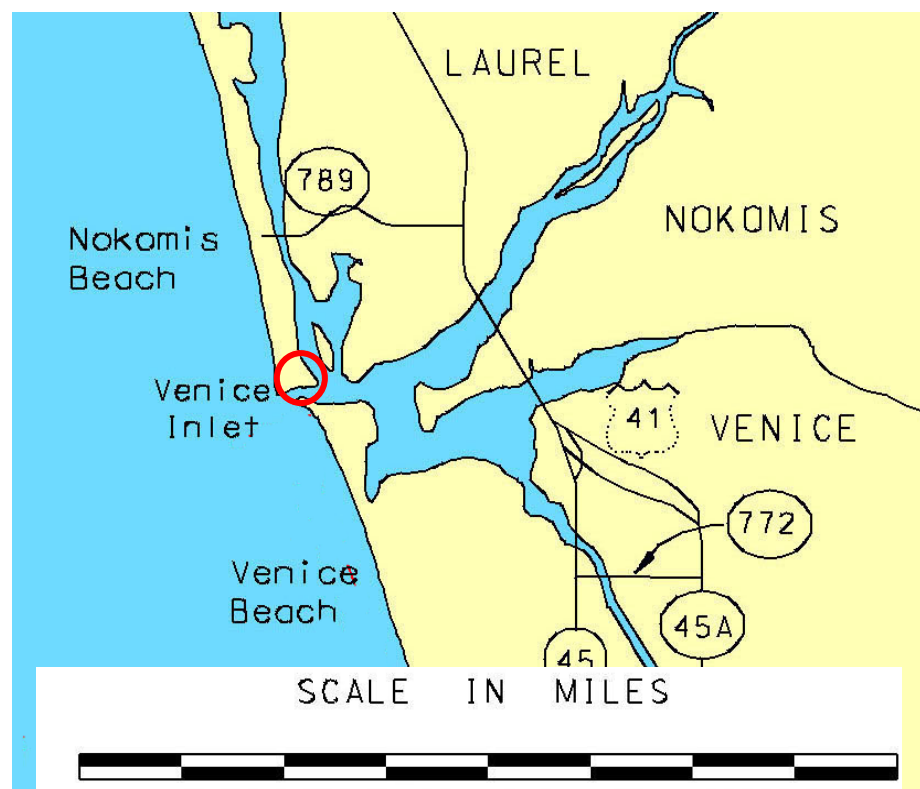
Figure 3.10. Venice sampling site (US. Corps of engineers, 1999)

The site profile is typical of a canal, although the influence from the water currents due to the proximity to the Venice inlet has produced a sand flat in between the canal and the mangrove shore. Although there is a sharp drop 10 m from the shore, the depth it does not go below the 2 m (Figure 3.11).
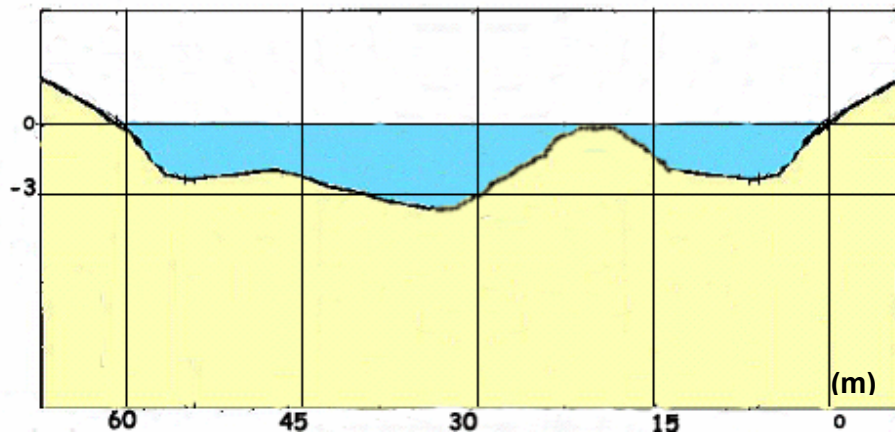
Figure 3.11. Venice site profile. (US. Corps of engineers, 1999)

3.2.1.6 Cayo Costa Island, Charlotte Harbour

Cayo Costa Island is the study's southern sampling site (26°69" N, 82°24" W), located on the county border between Lee and Charlotte counties (Figure 3.12). Although not directly in the pass, the sampling site is next to the Captiva Pass on the eastern part of the island. This island is only accessible by boat, recreational fishing boats are common in the area, mainly targeting red fish (*Sciaenops ocellatus*) which are common in these waters. Charlotte harbour is characterized for its remote location away from built-up areas and related pollution sources. Water sports and bathing are rare. Sea grass bottom alternates with sandy bottoms and no mangroves are found in this area.

The beach profile (Figure 3.13) follows a very smooth increase in depth with sand flats in between. Currents, although fairly strong, are moderate in relation to other sites in this study such as Lido Beach or Beer Can Island, due to its relative distance from the Captiva Pass.

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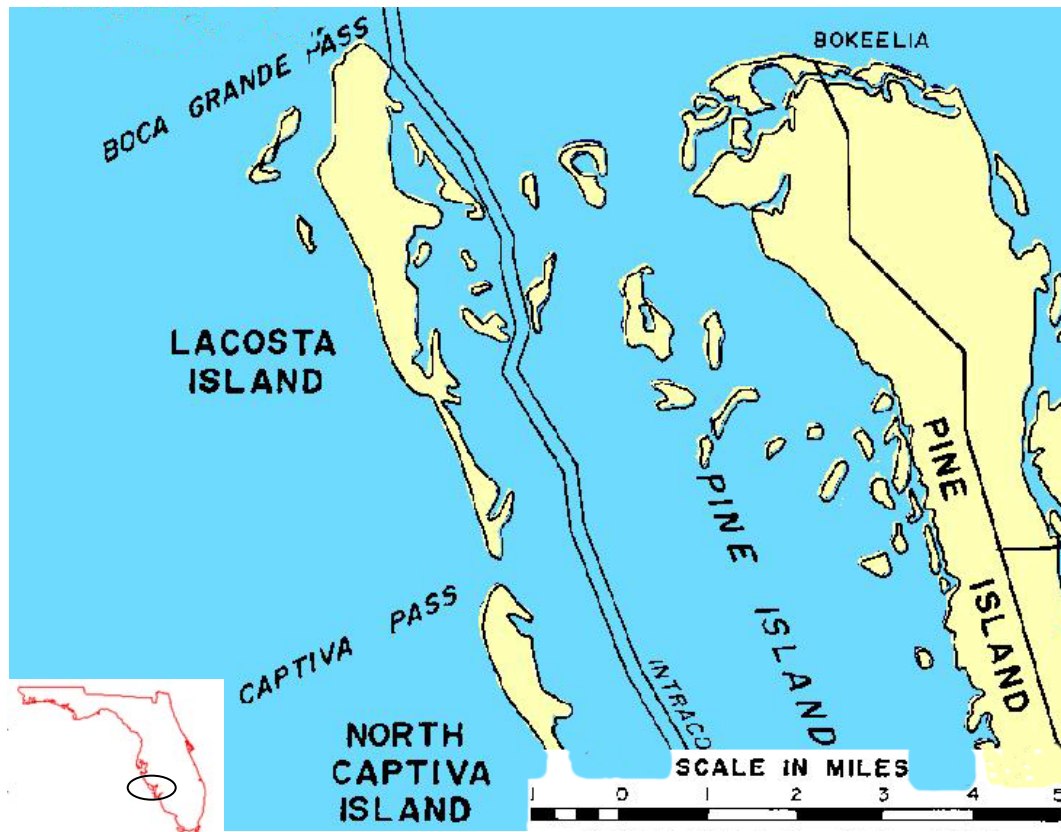
Figure 3.12. . Cayo Costa Island site location (US. Corps of Engineers, 1999).
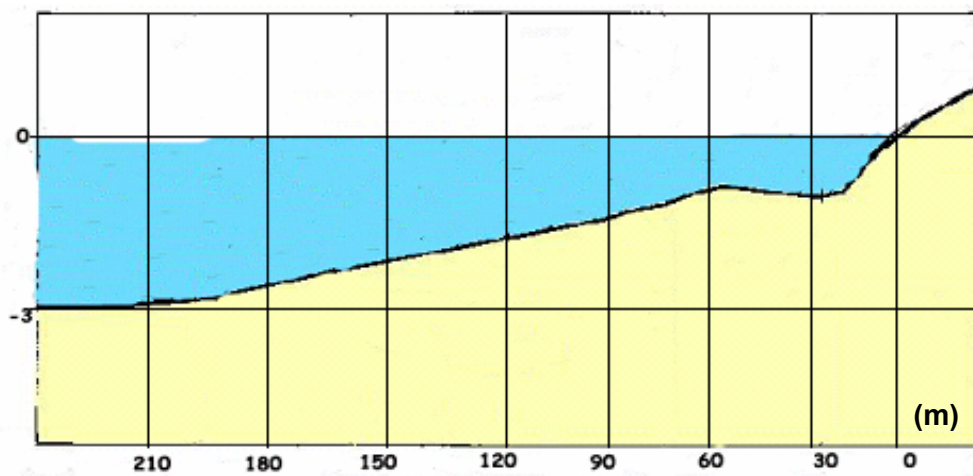
Figure 3.13. Cayo Costa Island beach site profile (US. Corps of Engineers, 1999)

3.2.2 Sampling regime

Fieldwork started in May 2002 and finished in August 2005 (Table 3.1), principally in the summer months, including April and September. However, no fieldwork was carried out between September and the following April, due low water temperatures (Figure 3.1) for spawning.

The 2002 fieldwork was used to assess the sampling area in order to develop a sampling regime for the following year. Sampling locations were chosen following three key factors; (i) snook congregations, (ii) accessibility, and (iii) proximity to the hatchery. Also local fishermen and scientists working in the area were consulted.

A total of 22 field trips were conducted between 29th May and 9th September 2002. Five sampling locations were identified during 10 different moon phases. The 2003 fieldwork was based on a sampling schedule using results from the 2002 fieldwork. Forty-six field trips were carried out between 18th April and 29th August 2003.

The 2004 season fieldwork was narrowed down to those dates that 2002 and 2003 fieldwork results showed to be optimal for sampling, in terms of seed quality and cost-efficiency. During this season 11 field trips were conducted between 21st June and 19th August 2004.

During the final season in 2005, the fieldwork schedule followed the same pattern as the 2004 season, although due to a strong red tide presence along

three counties some adjustments were made to avoid sites affected by such algal blooms. One more site was added (Cayo Costa, Lee County), that was free of a red tide. Eighteen field trips were carried out between 25th May and 8th August 2005.

Table 3.1. Study sampling trips by month*, moon phase and location (● New moon, ○ Full Moon, + days past, - days before)

3.3 Results

3.3.1 Wild common snook captured numbers from 2002-2005

Over the four spawning seasons (2002-05) a total of 5077 fish were captured of which 4502 were males (88%) and 575 were females (12%). Seventy one percent of the total numbers of fish captured were caught in Terra Ceia, followed by Longboat (16%), Cayo Costa (10%), New Pass (3%), Lido (0.5%) and Casey Key (0.5%).

A total of 244 females and 760 males were stripped from 2002-2005. The season in 2005 had the most fish stripped, with 302 males and 66 females. This was followed by 2003 season with 179 males and 70 females (Figure 3.14)
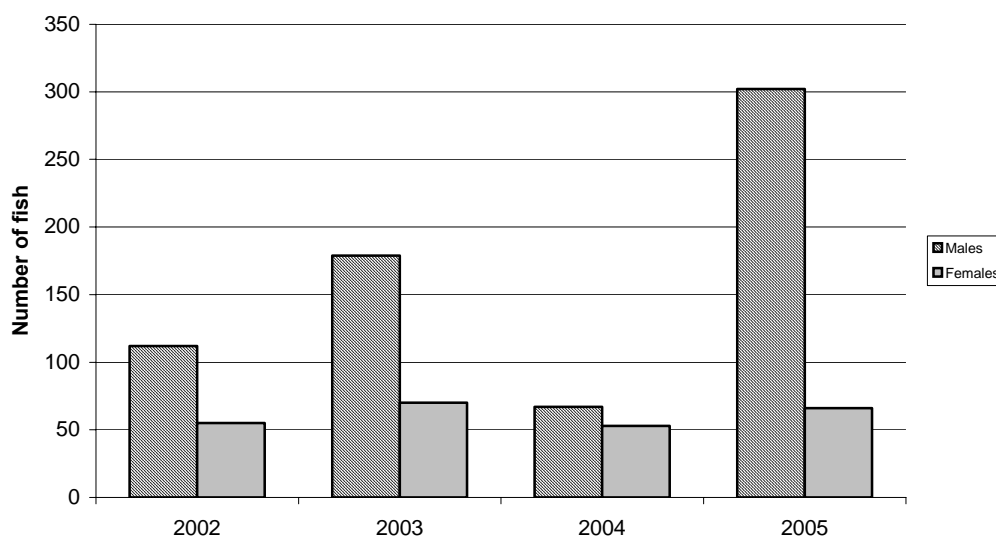
Figure 3.14. Total number of stripped **Year** **fish by year**

3.3.2 Wild common snook spawning results by location

3.3.2.1 Results from the 2002-2003 season

Out of the 6 locations sampled during the 2002/2003 season, a clear pattern can be observed (Figure 3.15). Two locations appear more productive in terms of wild snook caught, Terra Ceia (McGill Island) with 1309 animals, and Longboat (Beer Can island) with 478 animals. Venice and New Pass landed considerably lower numbers (123,113, respectively). The percentage of males within the total catch was the same in all locations.
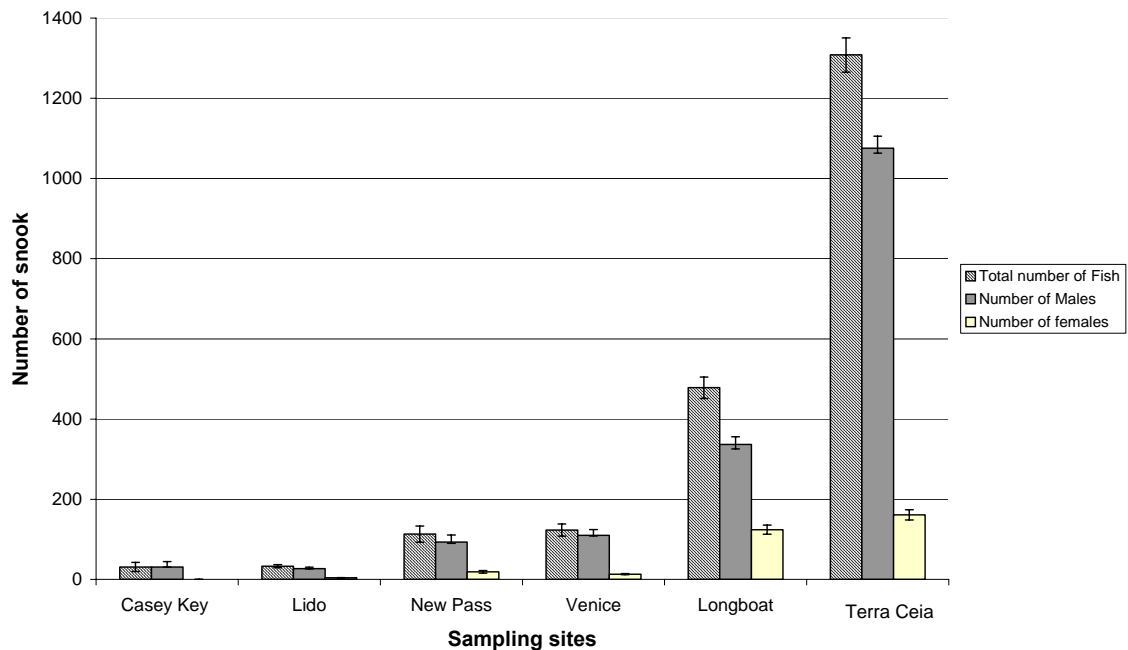
Figure 3.15. Total catch at the different sampling sites during 2002-2003

Similar patterns can be observed when average catch per trip are considered by location (Figure 3.16). Sampling sites followed the same classification with Terra Ceia as the most productive (62 fish, 54 males and 8 females) followed by Longboat with 30 fish 21 males and 9 females per trip. In the case of females, Terra Ceia and Longboat have a similar amount of females caught in average. Venice and New Pass had an average of 14 and 13 fish per trip.
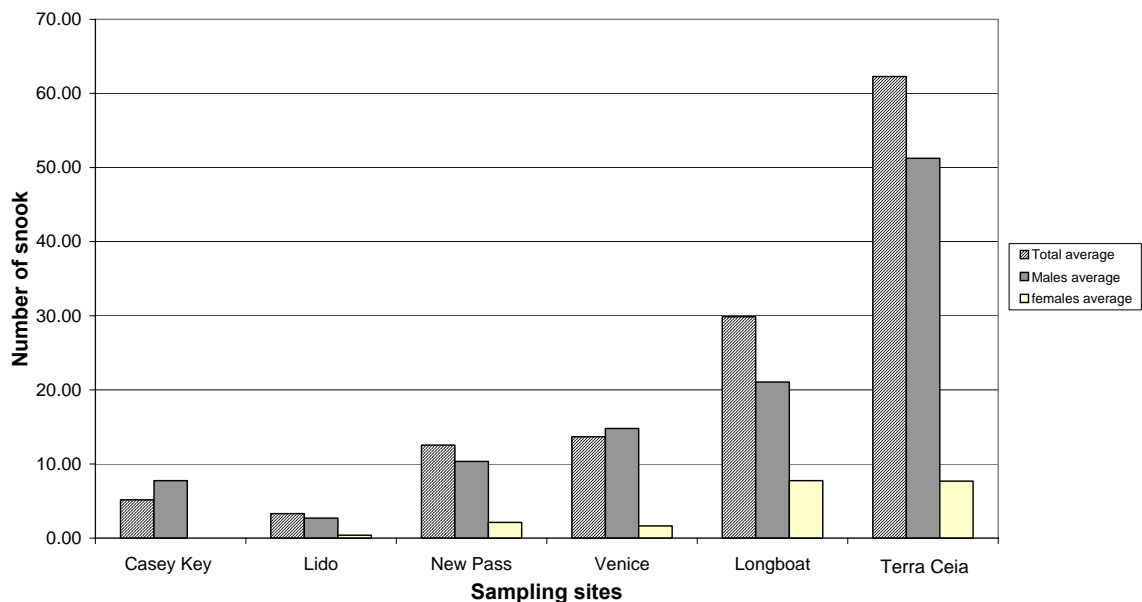
Figure 3.16. Average number of fish caught per trip by location during the 2002-2003 seasons

The frequency with which a site was visited for collection, as well as the success in terms of spawning (days where fish were able to be stripped), is shown (Figure 3.17). Three parameters were examined; spawning success, overall success and sampling frequency. Spawning success shows the number of times that spawning has been successful compared to the total number of field trips carried out at the sampling site. Overall success

compares individual site success with total number of successful spawnings at all sites. Sampling frequency is the number of field trips that took place at each site compared to the total number of field trips in the first two seasons ('02 and '03).

The highest spawning success took place at Terra Ceia (53%), followed by Longboat (44%) and Venice (30%). New Pass, Lido and Case Key appeared to have lower spawning success, each site was less than 5% (Figure 3.17).
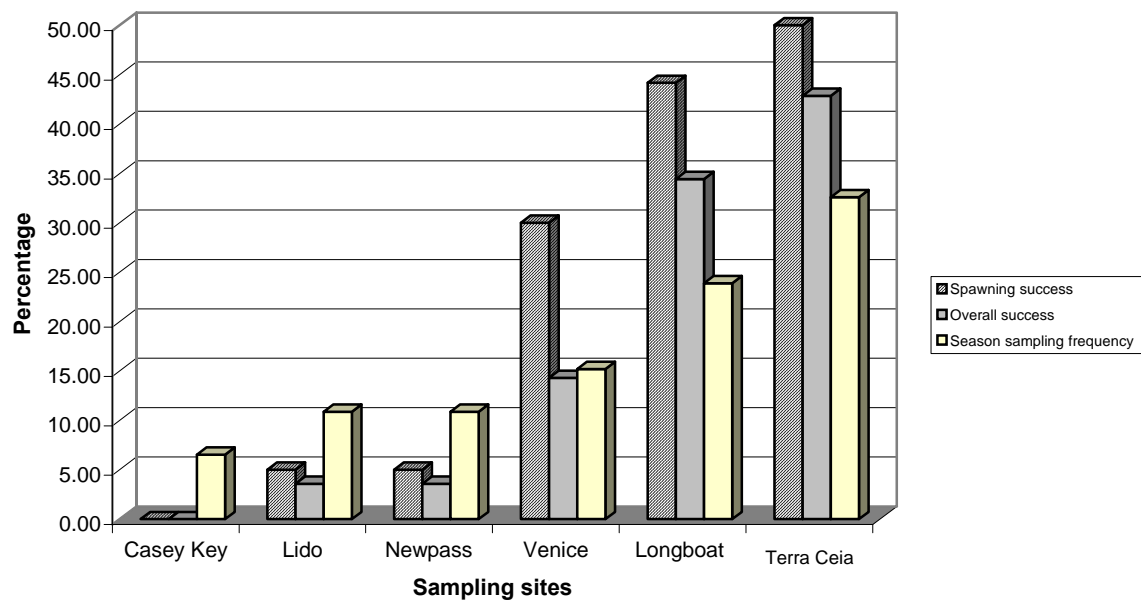
Figure 3.17. Spawning success, overall success (site individual success against the total number of successful spawnings between all the sites) and frequency by location during the 2002-2003 season

3.3.2.2. Results from the 2004-2005 season

In the 2004 and 2005 season, results from the 2002-2003 seasons were used to focus sampling effort at those locations where the best results were obtained. Those locations were Terra Ceia, Longboat, New Pass and Cayo

Costa (Figure 3.18). Cayo Costa was a new site that was introduced due to the effects of Hurricane Charlie (August '04) on the Longboat site topography that made the site unsatisfactory for sampling. Additionally, there was a heavy red tide presence in the Sarasota Bay area.

Results from the 2004-2005 season confirmed Terra Ceia to be the most productive (2150 fish, 146 females) of the sites, although in 2005 Cayo Costa (466 fish, 62 females), and Longboat in 2004 (344 fish and 41 females), had high numbers. New Pass was used as a back up site in case of unexpected problems arose at the other sites and this is reflected in the productivity (Figure 3.18).
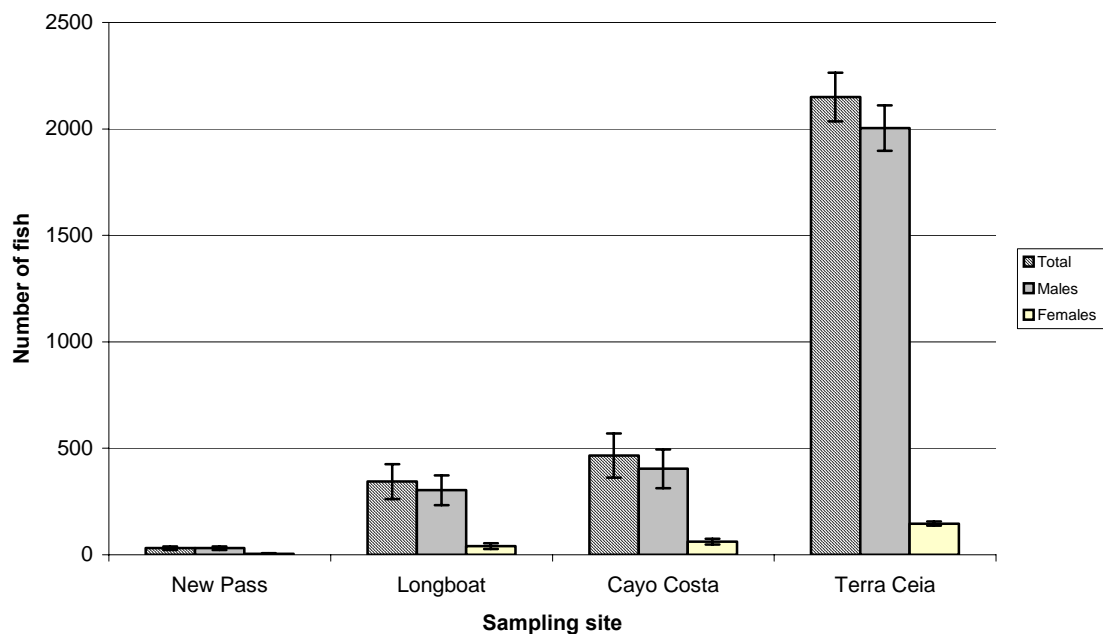
Figure 3.18. Total fish caught by location during the 2004-2005 season

In comparing the average catch per trip during the 2004 and 2005 season Terra Ceia was, once again, the highest (249 fish and 12 females). During the 2005 season Cayo Costa averaged 93 fish and 12 females, making this site

the second most productive. Longboat was third with 57 fish and 7 females (Figure 3.19).
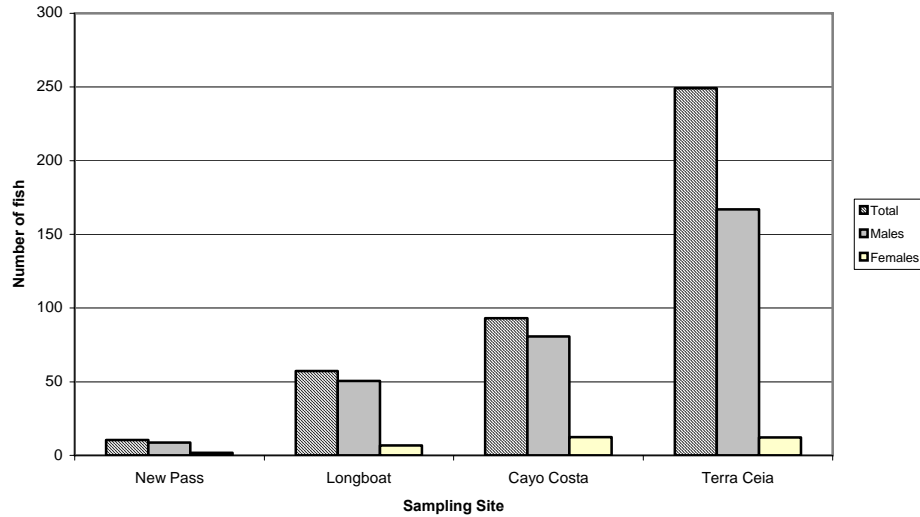
Figure 3.19. 2004-05 average total number of fish caught per trip by location.

Spawning success and sampling frequency were highest at Terra Ceia at 75% and 35%, respectively. This was also the most sampled site (38%). The second most successful site was Cayo Costa with 60% success and an 11% overall success rate, but was the third most sampled site with a 19% total frequency, behind Longboat with 23% (Figure 3.20)
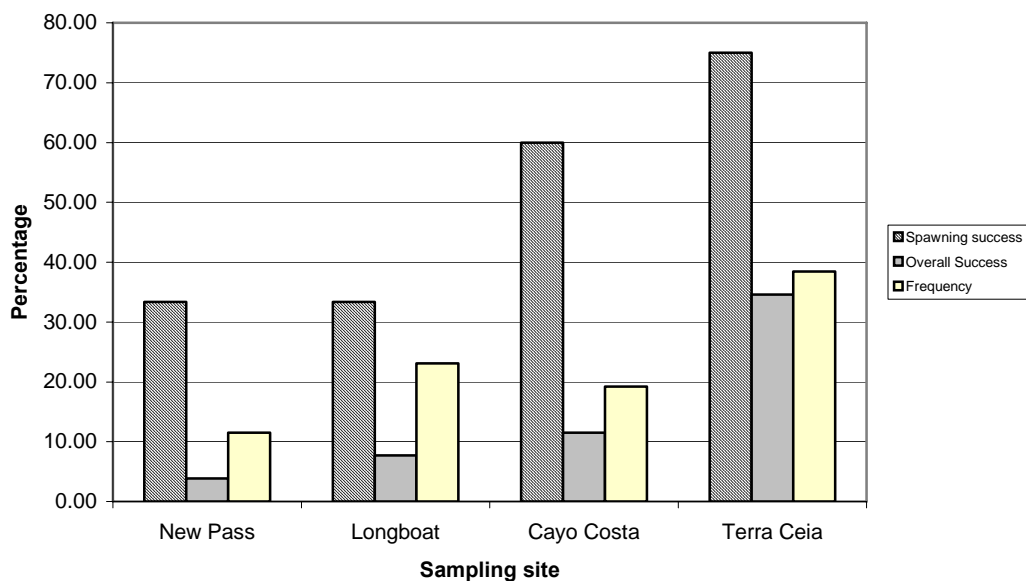
Figure 3.20. Spawning success and sampling frequency by location in 2004-2005

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3.3.3 Influence of lunar cycle on common snook spawning success and egg quality

Over the course of 4 seasons, sampling was carried out during fourteen moon phases. Overall, more fish were caught during the later stages of the moon phases. Fewer females were caught on the second day after a new or full moon compared to other days (Figure 3.21). When sampling took place two and three days after a new moon, total fish capture success was at its highest (1093 and 858), both in total number and in terms of males captured (990 and 789). On the other hand, the number of females caught on the third day after a full moon (189) was greater than any time in the lunar cycle. This compares to the number of females caught on the second day after a new moon (69) and on the third day after a new moon (104). Results from sampling on days before new and full moons showed lower catch numbers (Figure 3.21)
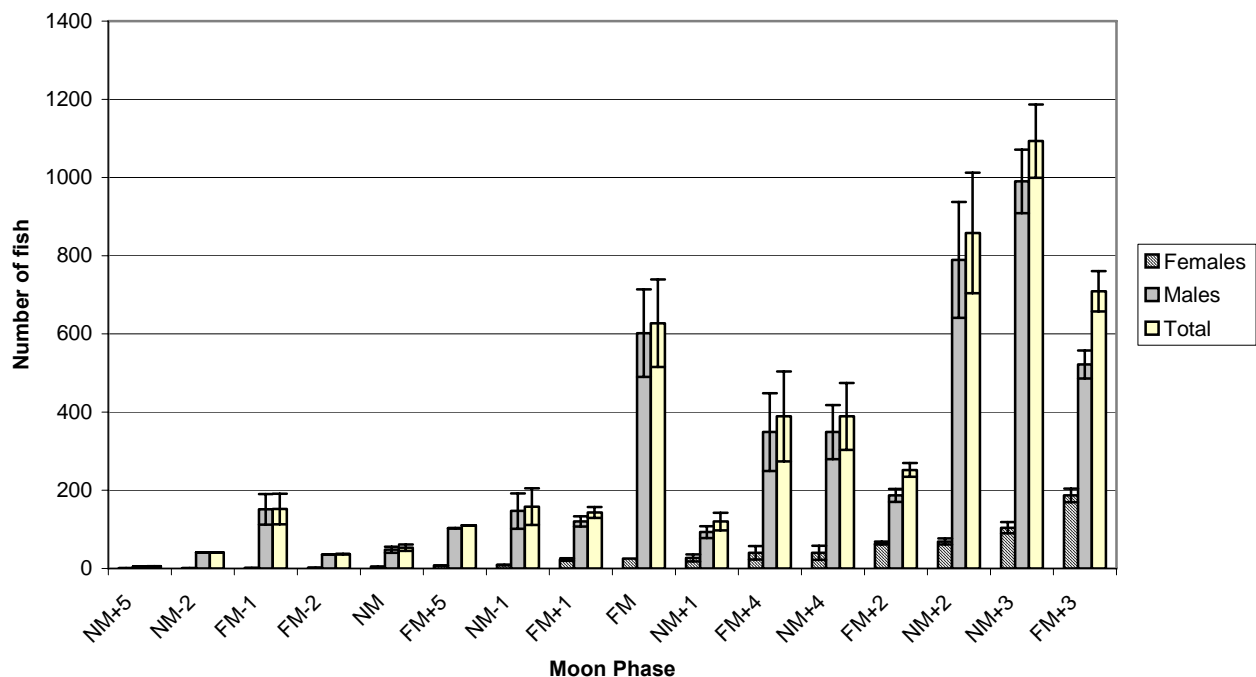
Figure 3.21. Total catch by moon phase during 2002-2005 (NM=New moon, FM=Full moon, numbers after i.e. –1, determine the days before (-) or after (+)the moon phase)

In comparing spawning success with the total number of fish captured, a similar pattern can be observed (Figure 3.22). The spawning success was greater on days following a full and new moon, especially on the second day following a new moon (62%) and on the third day following a full moon (75%). On the other hand, in the early stages of either a new or full moon, spawning success was below 40% (Figure 3.22).
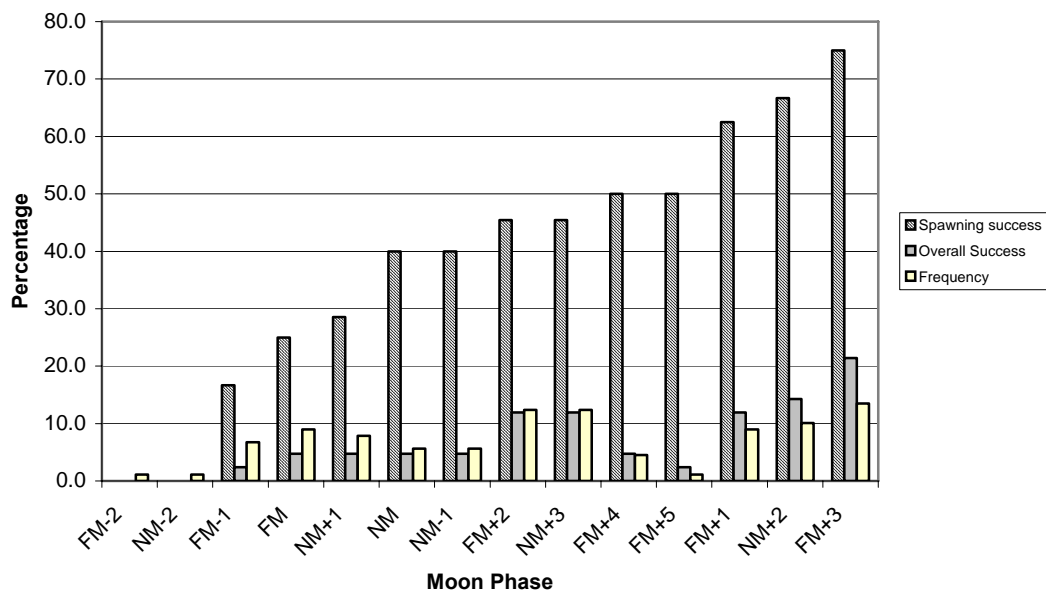
Figure 3.22. Spawning Success by moon phase during 2002-2005 (NM=New moon, FM=Full moon, numbers after i.e. –1, determine the days before (-) or after (+)the moon phase

Overall spawning success suggests that the first, second or third day after a new or full moon are best for procuring spawning broodstock. Results for the overall spawning success obtained from sampling on these days were always greater than 10%, especially on the second after a new moon with a 14% overall success and on the third day after a full moon with a 21% spawning success rate. For the days running up to a new or full moon, and the fourth or

greater days following a new or full moon, the overall success rate was lower than 5%. The third day after a full moon had the highest sampling frequency result for all four sampling seasons (13.5%). The sampling on the second or third day after a new or full moon always had a sampling frequency greater than 10%, with second day after a full moon and third day after a new moon having the greatest sampling frequency of 12.4% (Figure 3.22).

Moon phases had no significant influence on the fertilization rate (p= 0.477) and hatching rates of eggs (p=0.216) collected during successful spawning trips (Figure 3.23). Sixty percent of all moon phases had a fertilization rate over the 80%, Samples collected during the full moon, one day after the new moon, and four days after a new moon, had an average of greater than 94% fertilization. The fertilization rate of eggs collected one day before a full moon showed the lowest average rate of 7.9%.
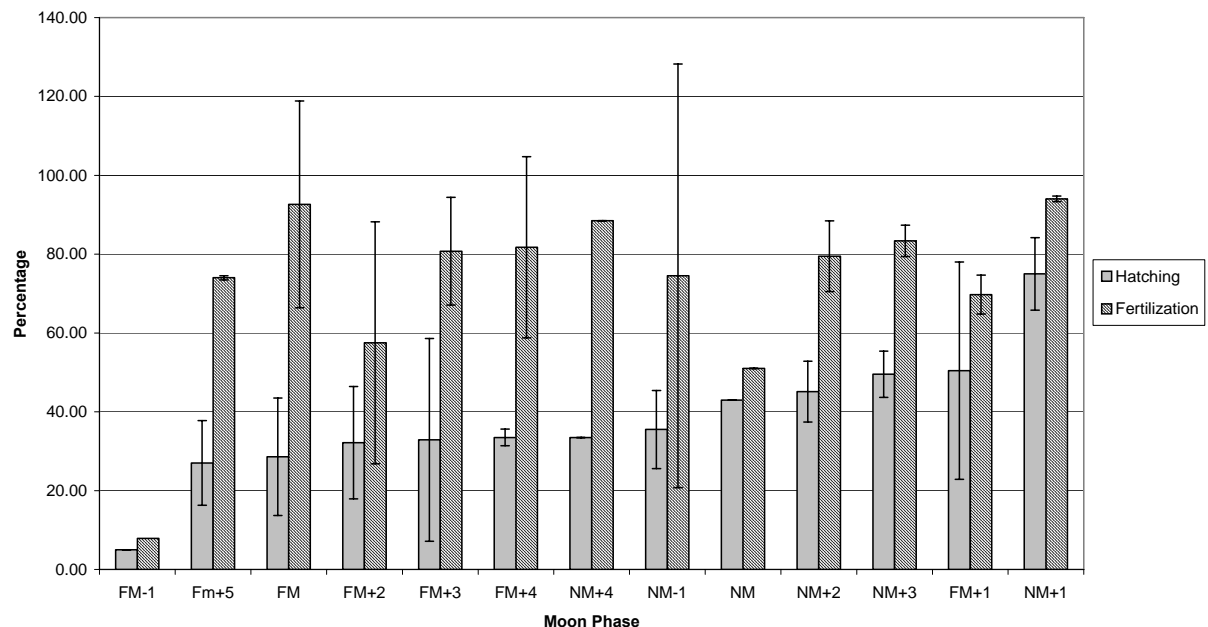
Figure 3.23. Egg quality by moon phase Spawning Success by moon phase during 2002-2005 (NM=New moon, FM=Full moon, numbers after i.e. –1, determine the days before (-) or after (+)the moon phase

The second day after a new or full moon produced lower values of 54 and 51% respectively. In comparing hatching rates for specific days over new and full moons, the samples collected on most days had rates of less than 50%. Only on the first day after a new moon was the success rate noticeably higher at 75% (Figure 3.23).

3.3.4 The influence of month on the common snook spawning and egg quality

The number of fish caught in each month during the 4 sample seasons is given in Figure 3.24. The highest number of fish (1599 males and 395 females) were caught in June, followed by July (1455 males and 177 females), and then May and August. No fish were captured in April and only 104 fish were caught in September.
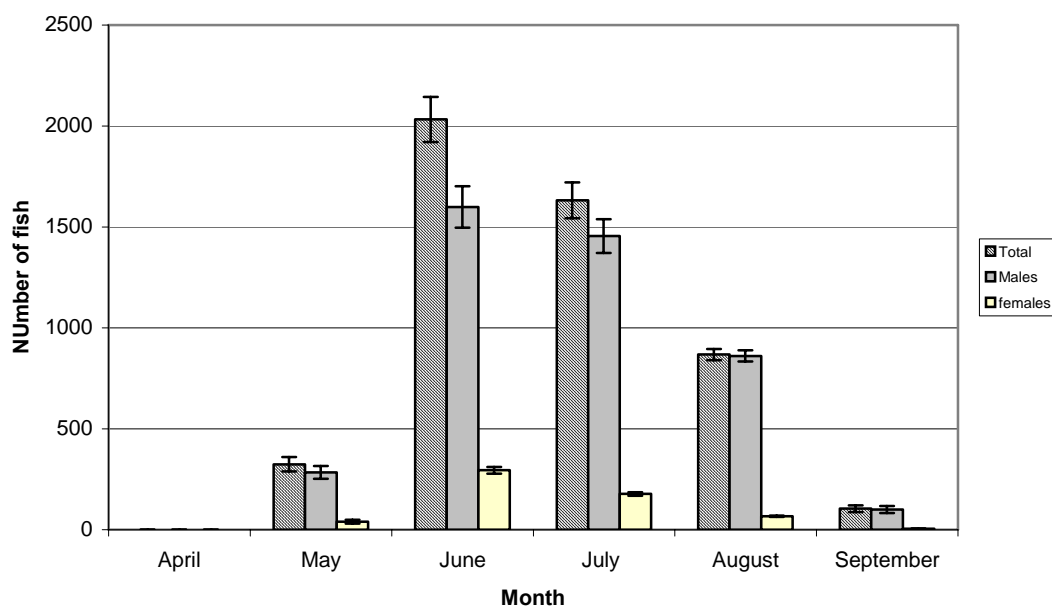
Figure 3.24. Total catch of common snook between 2002-2005

The average number of fish caught per trip in each month showed a similar pattern to the total number of fish captured. June had the highest value (76 males and 14 females) on average per trip, followed by July (66 males and 8 females) and August (33 males and 4 females) (Figure 3.25).
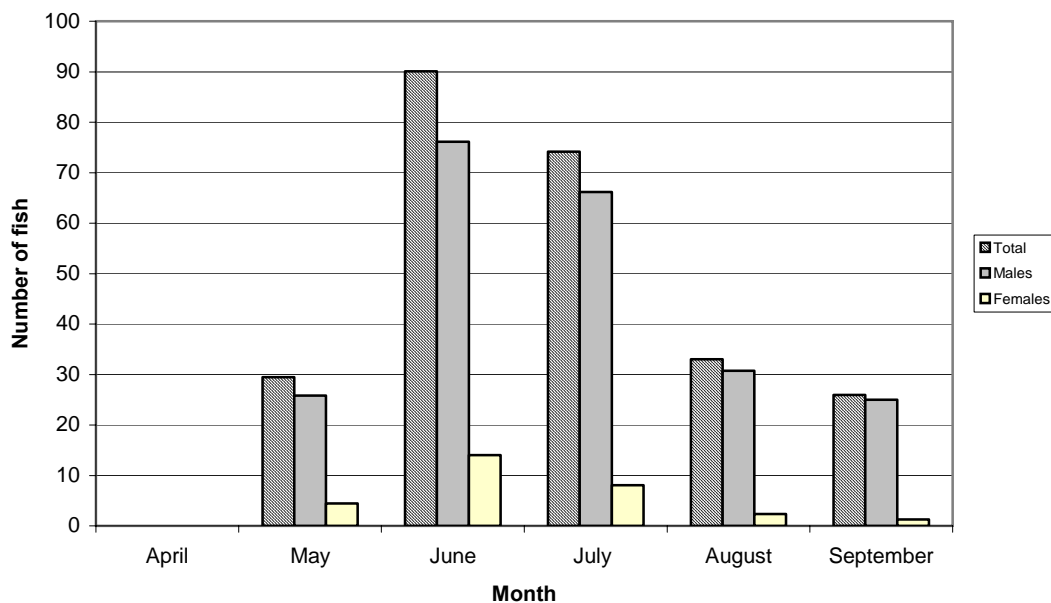
Figure 3.25. 2002-2005 Average catch per trip by month

During trips that took place over the month of June, spawning success results were shown to be more productive (53%) in catching females ready to spawn. The overall success of June accounted for 35% of the total successful spawning trips (Figure 3.26). July and August had lower percentages of both spawning success and overall success (39 and 28% and 34 and 28% respectively). April showed zero percentage success since no females ready to spawn were found.

Sampling frequency in August accounted for 30% of the total sampling effort, 50% of the total sampling effort was carried out between June and July (24 and 26% respectively). The months where less sampling took place were April and September with 2 and 4% of the total sampling effort (Figure 3.26)
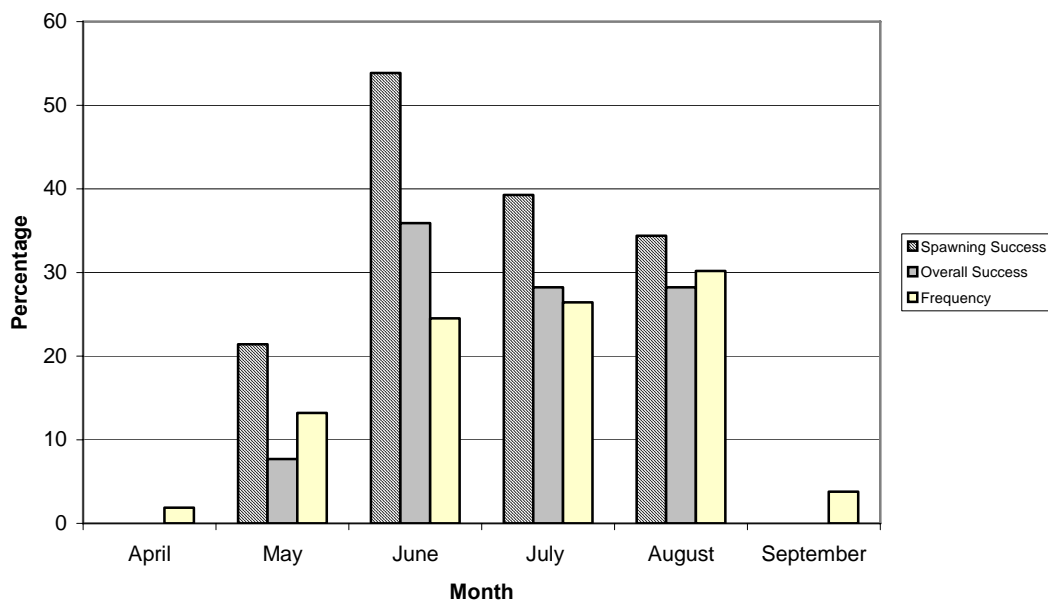
Figure 3.26. 2002-2005 Spawning success and sampling frequency by month

The two main criteria used for egg quality were the fertilization rate and the hatching rate of the collected eggs. Results from the total sampling period (2002-2005) showed a significant difference (p= 0.021) between months with a decreasing pattern from May to September. Average results for fertilization rates were highest in May (93.5%), percentages fell in June (87%) and rose in July (81%), August and September values fell again to 62 and 46% respectively.

Hatching percentage rates (Figure 3.28) showed a similar pattern to fertilization rates; but differences were not significant (p= 0.345). May had the highest mean hatching percentage value (37.75%), June and July values

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were lower (36.83 and 37%), and September values were the lowest for both

parameters (Figure 3.27).
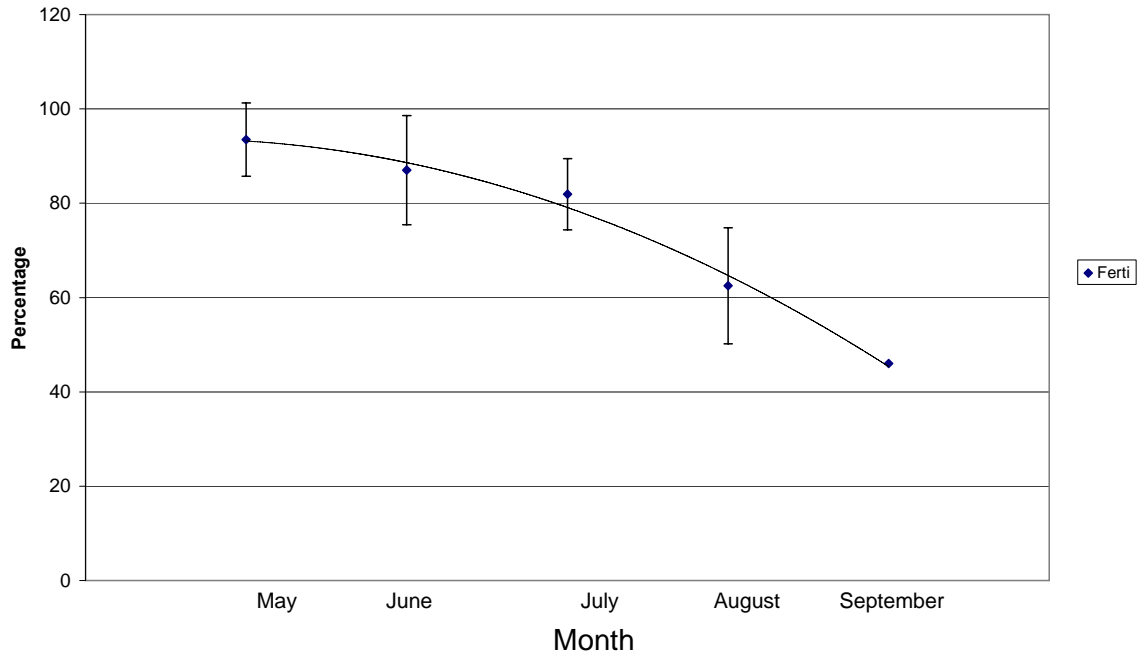
Figure 3.27. 2002-2005 average fertilization by month
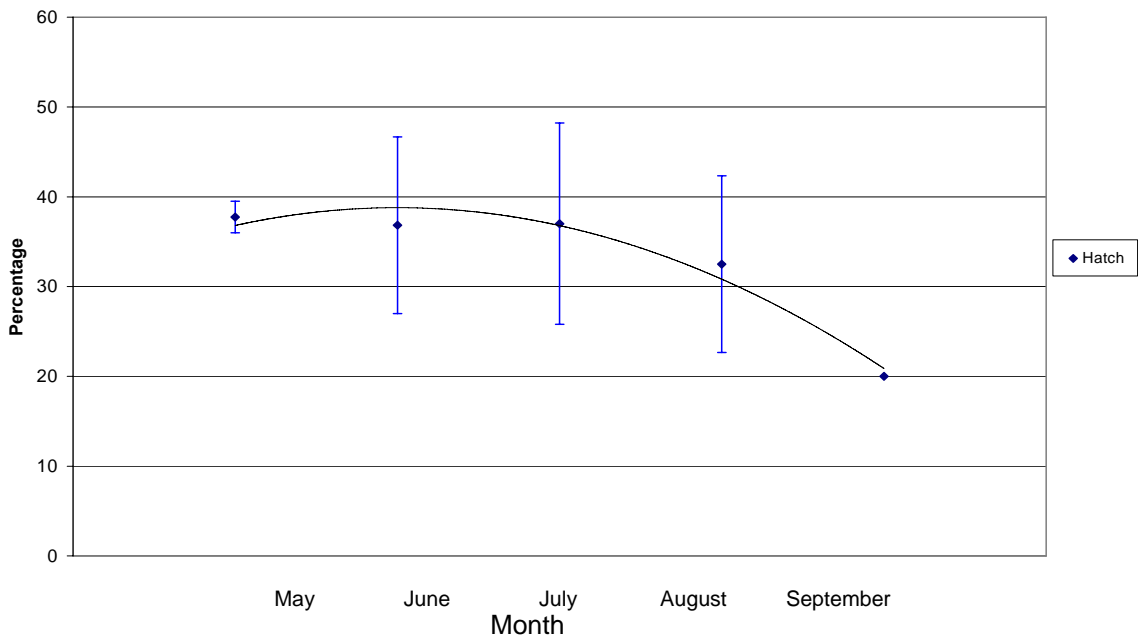
Figure 3.28. 2002-2005 average hatching rate by month

3.4 Discussion

Many tropical fish species aggregate during specific times and at specific locations for spawning. Spawning aggregations of fish can be influenced by season, lunar phase, and temperature and commonly form at traditional spawning sites (Taylor 1984). The types of fishes that aggregate to spawn range from predatory serranids (Smith 1972; Samoilys & Squire 1994), trevallies (Thresher 1984; Johannes 1981) and snappers (Carter & Perrine 1994) to herbivorous parrot fishes and surgeonfishes (Colin & Clavijo 1988; Myrberg et al. 1988).

3.4.1 Spawning sampling site selection

The first aim in this study was to establish the best sites for catching spawning common snook and to establish which environmental factors and activity influenced spawning. Information from snook related literature (Taylor, 1998) and local knowledge were used to select the sampling sites. Originally, a few factors were chosen to determine the most productive locations to maximize gamete quality, improve sampling efficiency, increase larval production and minimize cost and time. The geography and the topography of a site were found to be important to fish in relation to spawning success (Squire & Samoilys 2000). Similarly, geography and topography were found to be critical to the practical use of particular sites. The sampling gear was only suitable for certain topographic profiles. Practical considerations also impacted on geographic location. The distance from the hatchery was limited to less than three hours travel. This was a reflection of the ability of the egg to cope with

transport stress up to the blastula stage, which usually occurred about three hours after fertilization. Also it was important not to use east coast sample sites in an effort to restrict contamination of the west coast gene pool.

There are many possible reasons why fish aggregate to spawn at specific locations. Spawning aggregations typically form at sites with several key characteristics. It has been suggested that water movements which transport pelagic eggs and larvae into the water column or offshore facilitate the pelagic phase of development (Thresher 1984). This is also the case for snook where spawning aggregations take place during the outgoing tides, (Peters, *et al* 1998) making the sampling task more complicated. There is an opinion that suggests that large numbers of pelagic eggs released simultaneously may overwhelm the ability of egg predators to feed (Johannes 1978). Also, spawning aggregations might facilitate the ability of individuals to find a mate and to synchronize physiological readiness to spawn (Colin & Clavijo 1988).

There was a clear tidal influence on male/female presence at the spawning sites. Nets collected during incoming tides or during slack water always contained only males. It was only during outgoing tides that females were present. Despite this pattern sampling success was unpredictable.

Site topography was found to be one the of key factors for sampling success. Sites where the topography was both steep and deep (i.e. Lido Beach, New Pass) resulted in situations where use of gear and man power were inefficient. In these cases the bottom would be deeper than the seine net. Also water current would be too strong for the net to be pulled by a 10 man crew, or to keep the net in the correct position.

Terra Ceia was the most suitable site, its smooth topography (Figure 3.4), relatively slow tidal water current, abundant snook aggregations, and isolation from boat and fishing crowds, made this site the most successful in terms of spawning (Figure 3.17) and total number of fish caught (Figure 3.15).

Another successful site was Cayo Costa (Figure 3.12). However, this site was added to the sampling locations only after the Beer Can Island site suffered a total change of topography due to the passage of Hurricane Charlie (August 2004), and became unsuitable for sampling. Cayo Costa had identical characteristics to Terra Ceia but the distance from the hatchery (3 hours), made Terra Ceia a more suitable site.

The sampling regime could not be followed as initially proposed. The original regime attempted to sample all the sites equally (Gaudreau & Boisclair, 2000) during the first two years, in order to get the most accurate results. However, problems involving location related issues resulted in a redesign of the sampling regime. Florida has the most boat licenses in the United States. This fact plus the favorable weather conditions for boating makes some locations very popular for either fishing, bathing or water sports. This created a problem when using the original sampling regime. Changing the sampling regime reduced the competition for sampling sites. This competition made sampling during the weekend difficult, and so, additionally, sampling occurred mainly on weekdays. Another problem linked to location was the influence of the severe summer weather that occurs over the Florida coast every year, such as evening's summer storms and hurricanes. Hurricanes and tropical storms

accelerate shore erosion and can significantly change the topography of some sites, making them unsuitable for sampling. This happened at Longboat and New Pass, which made sampling more difficult than the previous seasons, other sites suffering less significant erosion.

Other problems encountered included; broken boats, nets trapped in rocks, old piers, and manatees. These problems occasionally made it impossible to capture fish.

In summary Terra Ceia, Longboat, New Pass, Lido Beach, Casey Key, Venice and Cayo Costa are, by the published definition (Samoilys 1997; Zeller 1997), primary spawning aggregation sites for snook, which are highly predictable from year to year. Only the site topography made Terra Ceia and Cayo Costa more suitable.

3.4.2 Influence of lunar cycle over common snook spawning and egg quality

Spawning aggregations of fishes can vary considerably both within and between fish species. Spawning aggregations can form on a daily basis with associated movements over short distances (Colin and Clavijo 1988; Myrberg et al. 1988), or on a seasonal basis as a result of large-scale migrations (Colin 1992; Shapiro *et al.*, 1993). In general terms, a spawning aggregation is defined as 'a group of con-specific fish gathered for the purpose of spawning with fish densities or numbers significantly higher (3 fold increase) than those found in the area of aggregation during the non-reproductive periods' (Domeier & Colin, 1997).

Snook spawning season is known to occur from April until October (Taylor, *et al.,* 1998). However, previous studies have not identified the optimal days for egg collection or days where more spawning activity occurs.

Taylor, *et al.,* (1998) reported that spawning occurs independent of either tidal condition (in relation to spring and neap tides) or lunar phase. However, Roberts, *et al.,* (1999) found the gonadosomatic index of adult snook to be at its highest during the new moon.

The present study has shown spawning activity to be higher around the lunar phases, not only in terms of fish caught but also in terms of spawning success. Conversely, days running up to new or full moons, and the days following the fourth day after a new or full moon, showed a decrease in the values for spawning success. Additionally, no significant difference was found with respect to egg quality. This all indicates that snook spawning aggregations are influenced by the lunar cycle.

The original sampling regime could not be followed in some lunar phases due mainly to the fact that no sampling was carried out during the weekend. However, sampling was still well spread through the different lunar phases and the four years of data collection have given sufficient data to produce reliable results. Also, despite the fact that many other factors could have had an influence on the results, such as red tide, and monthly variance, lunar influence over the snook aggregations still showed itself to be particularly significant.

3.4.3 . Period of selection for spawning sampling and influence on egg quality

The snook spawning season on the West coast of Florida is reported to take place from April to December (Marshall, 1958, Fore and Schmidt, 1973, Matlock & Osburn 1987, McMichael et al, 1989, Peters et al, 1998, Taylor, 1998). The present study has shown that the snook spawning season starts in the last quarter of May and finishes early in September. In considering the parameters; total number of fish caught, number of females caught, spawning success, and egg quality parameters, all parameters showed a similar trend, values peaking in June and July and slowly decrease through August until the end of the spawning period in September. Marshall, (1958) and Roberts *et al.,* (1999) found June and July also were the peak months of the spawning season.

The results obtained in this study differed with most previously published studies when referring to the start and end of the spawning season. Such contradictory results can be attributed to the fact that temperature has been shown to be the primary environmental cue stimulating maturation at different stages of reproduction in some teleosts (review by Lam & Munro, 1987; Andersson & Förlin., 1992). During this study no spawning success, nor spawning aggregation occurred when water temperatures were below 27°C. Peters, *et al* (1998) reported 22°C as the lower limit for spawning in the wild. In April 2003 when water temperature was below the 25 °C, no snook were caught, but aggregation of males were seen and low number of males were

caught early in May (average water temperature 26°C) at the regular spawning grounds although no females were captured.

During the 4 sampling seasons, water temperatures did not exceed 28°C before the third quarter of May and water temperatures started decreasing early in September except in the 2005 season. Therefore, the snook spawning season was determined by the West Coast water temperature (Figure 3.1). In May 2005 the water temperatures in the Bay Area were unusually cold (26°C) compared to the previous years. In May 2005 no females snook were found during sampling. Additionally, lower numbers of males were seen. This compared to previous years when the first spawning aggregations occurred at the end of May, high numbers of snook were caught, and specimens were mature and flowing. A similar case happened to Roberts et al. 1999, where endocrine levels on fish were lower in samples taken in April 1988 than in April 1989, where water temperatures were higher in April 1989.

The sampling area was regularly affected by red tide blooms (*Karenia brevis*). *Karenia brevis* is a dinoflagelatte that produces a nerve toxin that paralyzes the fish's gills and causes death by lack of oxygen (Kirkpatrick *et al* 2004). Red tide toxins can affect invertebrate animals, such as crabs, shrimp, and lobsters, as well as marine teleosts like menhaden, mullet, pinfish, anchovies, pigfish, etc., which are part of the snook diet (Fore and Schmidt 1973). In 2002, and 2005, the red tide concentrations reached values that were fatal for many marine species. Fish kills appeared on a daily basis especially in 2005. Snook mortalities are not easily identified, only when levels reach record high

can snook kills be seen. In 2002 over three hundred mature snook were found dead at Lido beach.

The ability of this species to move into inland waters when the red tide is present might be a reason why snook kills are not as common as other marine fish species kills, whose movements are restricted to the seawater environment.

The sampling regime tried to avoid locations which had high red tide concentrations, but the long terms effects on the snook aggregations and most importantly on the females are unknown. Poor egg quality during 2002 and 2005 could be related to the presence of red tide, and it is likely that, to some degree, the snook population in the area did suffer from it.

Overall in this study spawning snook aggregations followed a yearly pattern that is triggered by the water temperature in the West Coast of Florida. During the spawning season peaks occurred regularly in June and July. Although temperatures in August remained, on average, the same, the spawning success and egg quality decreased. This fact could be explained by the snook's reproductive nature, which is sequential and batch spawning (Taylor et al., 1998). This means that as the season goes by, and females spawn daily, synchronous with the lunar cycle, the energy exhaustion which this behavior involves will have an effect on the females lipid threshold.

Chapter 4:

The influence of lipids and fatty acids on snook eggs and larval quality

4 The influence of lipids and fatty acids on snook eggs and larval quality

4.1 Introduction

4.1.1 Lipids and fatty acids in fish

Lipids are a large group of heterogeneous compounds, having in common the property of insolubility in water, but solubility in organic solvents such as chloroform, hydrocarbons or alcohols (Gurr and James, 1975). Animal lipids can be divided into two broad classes, neutral lipids that are completely soluble in non-polar solvents and polar lipids.

The main neutral lipids of fish are triacylglycerols (often termed triglycerides), wax esters and sterols (Table 4.1). Triacylglycerols and wax esters are very important energy storage substances, while sterols have a structural function in cell membranes. Triacylglycerols are by far the most abundant single lipid class in fish lipids and consist of three molecules of fatty acids esterified to the three alcohol groups of glycerol (a trihydric alcohol). Wax esters are very abundant in marine zooplankton and consist of a single molecule of a fatty acid esterified to a single molecule of a fatty alcohol. Sterols are tetracyclic hydrocarbon compounds, the most common of which is cholesterol.

Unesterified (or free) fatty acids are also classed as neutral lipids. Free fatty acids are present in very low amounts in living animals and fresh tissues but may be liberated from any of the more complex lipids by post-mortem enzymatic hydrolysis.

Phospholipids, sulpholipids and glycolipids, and sphingolipids are the polar lipids, which share with sterols a structural function in animal cell membranes

and influence various membrane functions including ion channelling and transport, endocytocis and exocytosis, activities of membrane-associated receptors and enzymes, amongst others.

With the exception of cholesterol, the basic unit of all the above mentioned lipid classes is the fatty acid, of which around 40 commonly occur in nature (Christie, 2003). They may be classified as saturated fatty acids (often abbreviated as SFA), which are those that do not possess any double bonds in their carbon chain, monounsaturated (MUFA) if they contain one double bond and polyunsaturated fatty acids (PUFA) having more than one double bond.

Ackman, 1989; Henderson and Tocher, 1987; Yurkowski, 1989; and Steffens, 1997 all reviewed the fatty acid (FA) composition of fish. The predominant SFA that occur naturally in fish lipids, as in all animal fats, are palmitic acid (16:0) and stearic acid (18:0), but a range of chain lengths from C_{12} to C_{24} can be found in minor amounts.

The most abundant MUFA in fish tissues, Oleic acid (18:1n-9) is found in virtually all lipids of animal and plant origin. Palmitoleic acid (16:1n-7) is a component of most animal fats and may be present in somewhat greater amounts in fish. Vaccenic acid (18:1n-7) is also found in small amounts in fish oils.

In fish, the major PUFA are arachidonic acid (20:4n-6) and its metabolic precursor, linoleic acid (18:2n-6), together with eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) and their metabolic precursor, linolenic acid (18:3n-3). Several other PUFA of C_{16}, C_{18}, C_{20} and C_{22} can be also found in fish tissues but usually in minor amounts. In contrast to other animal and plant fats and oils, fish in general have a greater amount of the n-3 HUFA, and particularly 20:5n-3 and 22:6n-3. PUFA are very susceptible to oxidative deterioration, the more double bonds they possess, the greater their susceptibility.

In fish, the fatty acid content and lipids composition are generally reported to vary considerably, both within and between species (Puustinen *et al.*, 1985; Henderson and Tocher, 1987; Ackman, 1989; Ahlgren *et al.*, 1994; Andrade *et al.*, 1995; Sargent *et al.*, 1995; Zenebe *et al.*, 1998). The fatty acid composition of fish is influenced by the dietary lipid (Henderson and Tocher, 1987; Sargent *et al.*, 1989). The extent to which FA composition varies between different fish species depends also on whether the species is freshwater or marine, wild or reared in captivity, and on environmental influences, such as water temperature and seasonal fluctuations (Henderson and Tocher, 1987)

In general, the lipids of marine fish species are formed by lower proportions of SFA and C_{18} PUFA and higher levels of C_{20} and C_{22} PUFA than the lipids of freshwater fish species (Hilditch and Williams, 1964). In addition, the FA composition of marine fish is characterized by lower levels of n-6 PUFA, especially 18:2n-6 and 20:4n-6, and their ratio of n-3/n-6 PUFA is higher than that of freshwater fish (Henderson and Tocher, 1987; Steffens, 1997)

The n-3/n-6 PUFA ratio of marine fish is typically in the range of 4.7 to 14.4 (Henderson and Tocher, 1987), which reflects the higher amounts of n-3 HUFA, specifically 20:5n-3 and 22:6n-3, in their lipids.

Sargent *et al.* (1989) have described the influence of the food chains in marine and freshwater aquatic environments on the biochemical characteristics of fish lipids. Marine microalgae that constitute the primary producers in marine ecosystems contain up to 50% of their FA as n-3 PUFA. Diatoms tend to be rich in 20:5n-3 and dinoflagellates tend to be rich in 22:6n-3. As the n-3 PUFA in the marine phytoplankton are retained in zooplankton lipids, such as that of copepods, so they are retained in the depot neutral lipids of marine zooplanktonivorous fish, such as capelin, mackerel and herring amongst others.

Zooplanktonivorous fish in turn are major prey of various other fish that constitute major marine fisheries and thus n-3 PUFA are transmitted to them. In summary fish are rich in n-3 PUFA, including the former two HUFA (Ahlgren *et al.*, 1994; Steffens, 1997).

The FA composition of fish can also be influenced by numerous other factors. It is well established that it is influenced by the temperature of the water (Henderson and Tocher, 1987), with the degree of unsaturation of FA in fish tissue lipids generally increasing at low temperatures (Hazel 1979; Farkas *et al.*, 1980, Farkas, 1984). Food deprivation is also known to affect tissue FA composition. De Silva *et al.* (1997) showed that in starved red hybrid tilapia (*O. mossambicus* × *O. niloticus*) the relative percentage weights of MUFA decreased and those of PUFA, and in particular 22:6n-3, increased.

Table 4.1. List of fatty acids, classification and nomenclature

Systematic name	Trivial name	Shorthand designation
Saturated fatty acids		
Dodecanoic	Lauric	12:00
Tetradecanoic	Myristic	14:00
Pentadecanoic	-------	15:00
Hexadecanoic	Palmitic	16:00
Octadecanoic	Stearic	18:00
Eicosanoic	Arachidic	20:00
Docosanoic	Behenic	22:00
Tetracosanoic	Lignoceric	24:00:00
Monounsaturated fatty acids		
7-hexadecenoic	Palmitoleic	16:1 n-7
9-octadecenoic	Oleic	18:1 n-9
11-octadecenoic	Vaccenic	18:1 n-7
9-eicosenoic	Gondoic	20:1 n-11
11-eicosenoic	Gadoleic	20:1 n-9
11-docosenoic	Cetoleic	22:1 n-11
13-docosenoic	Erucic	22:1 n-9
15-tetracosenoic	Nervonic	24:1 n-9
Polyunsaturated fatty acids		
9,12-octadecadienoic	Linoleic	18:2n-6
6,9,12-octadecatrienoic	γ-linolenic	18:3n-6
8,11,14-eicostrienoic	Dihomo-g-linoleic acid	20:3n-6
5,8,11,14-eicosatetraenoic	Arachidonic	20:4n-6
7,10,13,16-docosatetraenoic	Adrenic	22:4n-6
4,7,10,13,16-docosapentaenoic	Docosapentaenoic	22:5n-6
9,12,15-octadecatrienoic	α-linonelic	18:3n-3
6,9,12,15-octadecatetraenoic	Stearidonic	18:4n-3
5,8,11,14,17-eicosapentaenoic	Timnodonic	20:5n-3
7,10,13,16,19-docosapentaenoic	Clupanodonic	22:5n-3
4,7,10,13,16,19-docosahexaenoic	Cervonic	22:6n-3

Changes associated with adaptation from freshwater to sea-water have also been demonstrated in the FA patterns of tissue lipids in several fish species (Henderson and Tocher, 1987). Factors affecting tissue FA composition also include seasonality (Bergstrom, 1989) and size (Tidwell and Robinette, 1990) amongst others.

4.1.2 Fatty acid biosynthesis

Fatty acids are incorporated in the body lipids of fish from their diet but they can also be formed endogenously (lipogenesis). In fish, the major site of lipogenesis is the liver but adipose tissue and the intestinal epithelium also have a lipogenic capacity (Sargent *et al.*, 1989). Although the enzymes have not been fully characterised, it is generally assumed that the pathways of fatty acid biosynthesis (lipogenesis) in fish are qualitatively similar to those in mammals (Gurr and Harwood, 1991). Thus, carbon derived from carbohydrate and amino acid catabolism, can be used for the formulation of citrate, which is transferred from the mitochondrion to the cytosol and converted to acetyl-CoA and oxaloacetate. The first step in fatty acid synthesis is the carboxylation of the two-carbon acetyl-CoA unit to malonyl-CoA, which is subsequently converted to fatty acids by the fatty acid synthetase complex via a series of condensation and reductions involving the utilisation of NADPH.

Palmitic acid and stearic acid are the major products of the fatty acid synthetase system in fish (Sargent *et al.*, 1989), as in all known organisms (Cook, 1996), while other saturated fatty acids can also be formed to a lesser extent (Henderson, 1996). Likewise, all organisms including fish are capable

of desaturating 16:0 and 18:0 by a microsomal $\Delta 9$ fatty acid desaturase to yield respectively 16:1n-7 and 18:1n-9. The $\Delta 9$ desaturation system has been particularly well characterized in fish, but less well studied is the extent to which 16:1n-7 and 18:1n-9 are chain elongated to higher homologues, including 18:1n-7, 20:1n-9, 22:1n-9 and 24:1n-9, by the conventional microsomal elongation pathway that occurs in higher terrestrial mammals (Tocher, 2003).

4.1.3 Importance of fatty acids in fish

A major role of fatty acids in fish, as in all organisms, is to generate metabolic energy in the form of ATP via principally mitochondrial β-oxidation (Sargent *et al.*, 1989; Froyland *et al.*, 2000). Fatty acids are not only the major source of metabolic energy in fish for growth (Sargent *et al.*, 2002; Tocher, 2003), they also are the major source of metabolic energy for reproduction (Sargent *et al.*, 1989, 2002), when the production of large gametes, particularly eggs is very energy intensive. Fatty acid oxidation is also an important source of energy for sustained swimming and migration, where energy requirements of the red muscle are high (Henderson, 1996).

 Another major role for the C_{20} PUFA, specifically 20:4n-6 and 20:5n-3, is as precursors for the group of highly biologically active compounds called eicosanoids (Bell *et al.*, 1986). Eicosanoids are produced virtually in every body tissue and generally in response to stressful conditions. Eicosanoid production is a normal physiological process, with excess eicosanoid production derived from 20:4n-6 often occurring in pathological conditions. The metabolic precursors of eicosanoids, 20:4n-6 and 20:5n-3, compete for

the same enzyme systems and thus eicosanoids actions are determined by the ratio 20:4n-6/20:5n-3 in cellular membranes, which in turn is determined by the dietary intake of n-6 and n-3 PUFA.

4.1.4 Fatty acid role in embryonic and larval development

Apart from their role as being a major source of metabolic energy, FA, and particularly PUFA, are functionally essential for normal growth, development and reproduction in all vertebrates.

DHA is especially abundant in the retina and brain and has a particularly important role in maintaining the structure and function of the cell membranes of these tissues (Bell *et al.*, 1995). This in turn has particular implications for fish larval nutrition as an insufficiency of 22:6n-3 in fish larval diets is likely to impair neural and visual development with negative consequences for a range of physiological and behavioural processes (Sargent *et al.*, 1999b)

Lipids and fatty acids have a particularly important role in the reproductive parameters of fish such as, egg quality, spawning, hatching rate and survival of larvae (Sargent *et al.*, 1989, 2002; Rainuzzo *et al.*, 1997). Lipids are utilized as energy sources throughout embryogenesis, and particularly in the later stages of development prior to hatching. EPA and DHA are the major FA in the total lipid of eggs of most fish and these fatty acids markedly influence the reproductive parameters. In addition, 20:4n-6 as a major fatty acid in PI and precursor of prostaglandin E_2, stimulate ovarian and testicular steroidogenesis and is assumed to be involved in embryonic development of the immune system, hatching and early larval performance (Mustafa and Srivastava, 1989; Wade and Van Der Kraak, 1993; Sorbera *et al.*, 1998).

4.1.5 Requirements of fatty acids in fish

PUFA are essential fatty acids (EFA), which cannot be synthesised *de novo* by fish, nor in general by all animals, and thus must be supplied pre-formed through the diet. Dietary requirements of essential fatty acids in fish tissues depends on a complex interplay between the fish's genes and their diet (Sargent *et al.* 1999a, b, 2002). There are species-specific differences, principally between freshwater and marine fish, in their ability to synthesise C_{20} and C_{22} PUFA, which are the biologically active forms of the n-3 and n-6 PUFA. Therefore, in species that cannot substantially perform these conversions the C_{20} and C_{22} PUFA, particularly 20:5n-3, 22:6n-3 and 20:4n-6, are dietary EFA and their C_{18} homologues, viz. 18:3n-3 and 18:2n-6, do not satisfy EFA requirements (Sargent *et al.*, 1995, 2002).

The exact dietary requirements of EFA in fish requires consideration not only of the relative and absolute amounts of individual fatty acids in the fish diets, but also the fish's innate abilities to metabolise these fatty acids, whether anabolically or catabolically (Sargent *et al.*, 2002).

Current estimates of the EFA requirements of marine fish indicate that these can be met by 20:5n-3, 22:6n-3 and 20:4n-6 and that requirements for n-3 are higher than n-6 PUFA.

Little is known about the biological requirements of the *Centropomus* species, no work on the lipid requirements has been reported to date. Seiffert *et al.* (2001), experimented with feeding n-3 enriched rotifers to *Centropomus parallelus* larvae and found no difference between the various enrichments. Previous research conducted on marine species, as mentioned before, lead to

the conclusion that common snook also depend on fatty acids for their development and functionality

4.1.6 Deficiency of fatty acids in fish

EFA are required for normal growth, development and reproduction of all known organisms. In fish, a number of deficiency symptoms and pathologies have been reported. Poor growth and low feed conversion are the main symptoms of EFA deficiency which have been shown in various species, including tilapia (Castell *et al.*, 1972; Kanazawa *et al.*, 1980). Juvenile herring (*Clupea harengus*) deprived of 22:6n-3 had impaired vision and impaired ability to capture prey at low light intensities (Bell *et al.*, 1995). In rainbow trout, an EFA insufficiency has been linked with fin erosion (Castell *et al.*, 1972), while in turbot with fin rot of both the tail and dorsal fin (Bell *et al.*, 1985). In the latter species, malpigmenation has also been linked to a sub-optimal EFA supply (Sargent *et al.*, 1999a). Other common symptoms include pale, swollen liver in salmonids, enlarged hearts, lower content of haemoglobin in blood, increased water content in muscle and viscera (Castell *et al.*, 1972). Reproductive performance was poor in fish fed diets containing no PUFA. They produced lower number of eggs, lower hatching rates and production of larvae with deformities (Watanabe *et al.*, 1982, 1984).

4.1.7 Egg quality variation during spawning season

The production of a large number of high quality eggs is important for natural populations in the wild. Parental genes, fish size and nutritional status are known to have major effects on egg production (Lambert & Thorsen, 2003). Whilst broodstocks are carefully selected and benefit from a controlled environment resulting in high reproductive output, fish in the wild are exposed to a host of environmental factors that fluctuate in an uncontrolled manner. Species respond to this situation by using a number of different reproductive strategies. American eel and pink salmon, which have high-energy expenditures during the spawning period, do not survive to second reproduction (Moriarty, 2003; Bazarkin, 1990). Lake whitefish and arctic char, in contrast, may skip reproduction to enhance survival, and nutritional condition is thought to determine the length of the spawning interval (Klemetsen *et al.*, 2003). In Atlantic cod that usually reproduce annually, when mature, nutritional condition may bear upon their reproductive success. In slow growing cod, their condition during spawning declines to levels close to the range where energy reserves are completely exhausted (Lambert *et al.*, 2000)

Environmental factors also have a direct effect on the food availability, producing a food restriction which can seriously affect spawning success. A reduction in feeding rate has been reported to cause an inhibition of gonadal maturation in several fish. Species, including goldfish *Carassius auratus*, (Sasayama and Takahashi, 1972) , European seabass *Dicentrachus labrax*, (Cerda et al., 1994) and male Atlantic salmon ´*Salmo salar*, (Berglund, 1995) . In seabass, after 6 months of feeding broodstock with a half food ration,

growth rates decreased and spawning time was delayed and eggs as well as newly hatched larvae were smaller than those obtained from fish fed full rations (Cerda et al., 1994).

4.1.8 Objectives

Lipid and fatty acid composition has been used to assess the direct influence that these fatty acids have on egg and larval quality and survival.
The main aims are to:

I. Describe the fatty acid composition of wild common snook egg

II. Locate the period of time during the spawning season when egg quality is at its best, to increase to a maximum the larval survival.

III. Assess the larval feeding efficiency in the rearing tanks, with special attention to the first feeding period.

IV. Assess the influence of DHA composition of eggs on egg fertilization, hatching percentage and overall larval survival rate.

V. Evaluate the feasibility of using DHA levels as a predictive tool of egg quality, prior to stocking wild eggs in rearing tanks.

4.2 Materials and Methods

4.2.1 Lipid Analysis

All fixed samples were analyzed in the Lipid group laboratory, part of the Institute of Aquaculture, University of Stirling. Lipid extraction was carried out following the protocol of Folch *et al.,* (1957). Fatty acids of the total lipid samples were converted to methyl esters by acid-catalysed trans-estherification (Christie 2003) for later use in the preparation and purification of the fatty acids methyl esters.

The fatty acid composition was determined by gas-liquid chromatography .

All the detailed information relating to the above protocols is described in chapter two.

4.3 Results

4.3.1 Fatty acid composition of unfertilised eggs

Forty-eight samples of 1 ml of unfertilised snook eggs were collected during 2002-2005, this was the material used to obtained the fatty acid profile of common snook eggs (Table 4.2).

The most abundant fatty acid in terms of total lipid percentage area in the common snook eggs were: palmitic acid (16:0), oleic acid (18:1n-9), docosahexaenoic acid (22:6 n-3), Palmitoleic acid (16:1(n-7), stearic acid (18:0), vaccenic acid (18:1(n-7), arachidonic acid 20:4(n-6), and docosapentaenoic acid (22:5(n-3)), eicosapentaenoic acid (20:5(n-3)), linoleic acid 18:2(n-6) and myristic acid (14:0), (Table 4.2).

Total saturated fatty acids (SFA) in snook eggs had a 34.5% total lipid area, which is higher than the total monosaturated (MUFA) fatty acids (31.5%). Polyunsaturated fatty acids (PUFA) represent 34.9% of the total lipid area (Table 4.2).

On the other hand, looking at fatty acid ratios, mean n-3/n-6 ratio is 2.5; EPA/DHA ratio gives a mean value of 0.18 and the ARA/EPA ratio is average 1.55 (Table 4.2)

Table 4.2. Common snook egg Fatty acid composition, average values from 48 samples.
(Total lipid percentage area)

Fatty Acid	Eggs total lipid % area
14	1.36 ±0.1
15	0.81 ±0.2
16	20.70 ±0.5
18	5.26 ±0.2
20	0.17 ±0.1
22	0.01 ±0.1
∑ Saturated	34.46 ±0.6
16:1(n-9)	0.73 ±1
16:1(n-7)	5.70 ±0.7
18:1(n-9)	14.82 ±1
18:1(n-7)	4.24 ±0.1
20:1(n-11)	0.25 ±0.1
20:1(n-9)	0.75 ±0.3
20:1(n-7)	0.22 ±0.0
∑ Monounsaturated	31.48 ±0.3
18:2(n-6)	1.37 ±0.1
18:3(n-6)	0.28 ±0.1
20:2(n-6)	0.18 ±0.0
20:3(n-6)	0.38 ±0.1
20:4(n-6) AA	3.68 ±0.3
22:4(n-6)	0.74 ±0.1
22:5(n-6)	1.26 ±0.0
∑ n-6 PUFA	9.45 ±0.7
18:3(n-3)	0.67 ±0.1
18:4(n-3)	0.50 ±0.1
20:3(n-3)	0.13 ±0.0
20:4(n-3)	0.34 ±0.1
20:5(n-3) EPA	2.38 ±0.1
22:5(n-3)	2.75 ±0.1
22:6(n-3) DHA	13.73 ±1.6
∑ n-3 PUFA	23.81 ±0.8
∑ PUFA	34.86 ±0.6
n-3:n-6	2.52 ±0.8
EPA/DHA	0.18 ±0.0
ARA/EPA	1.55 ±0.1

Means given with ±Standard deviation. PUFA: polyunsaturated fatty acids; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; AA arachidonic acid;

4.3.2 Common snook egg fatty acid level changes during the spawning season

4.3.2.1 Common snook egg saturated fatty acid (SFA) level variation during the spawning season

The mean monthly SFA values in common snook from 2002-2005 ranged from 29 (total lipid percentage) in June 2005 to 41 in June 2002 (Figure 4.1). In 2002 the mean monthly SFA values were significantly higher (p>0.05) than the following years averaging a 37.8 total lipid percentage. Also, no significant difference (p<0.05) was observed between the monthly values within any year of the study.
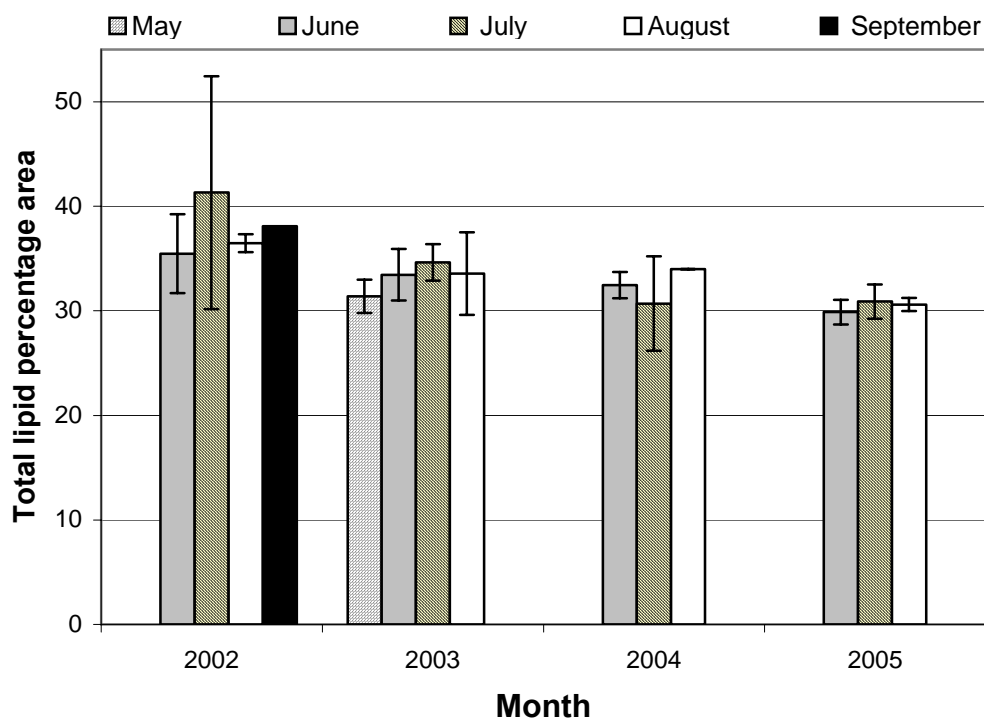
Figure 4.1. Changes in average saturated fatty acid values in common snook eggs by month per year (2002-2005)

The 2005, SFA values in snook eggs were the lowest ones on average with 30% of total lipid. Months were constant without a significant difference (p<0.05), (Figure 4.2).

A significant (p= 0.041) correlation between month and SFA % area was observed, where SFA values increased through the summer (Figure 4.2), indicating a significant month by month increase in SFA values over the summer.
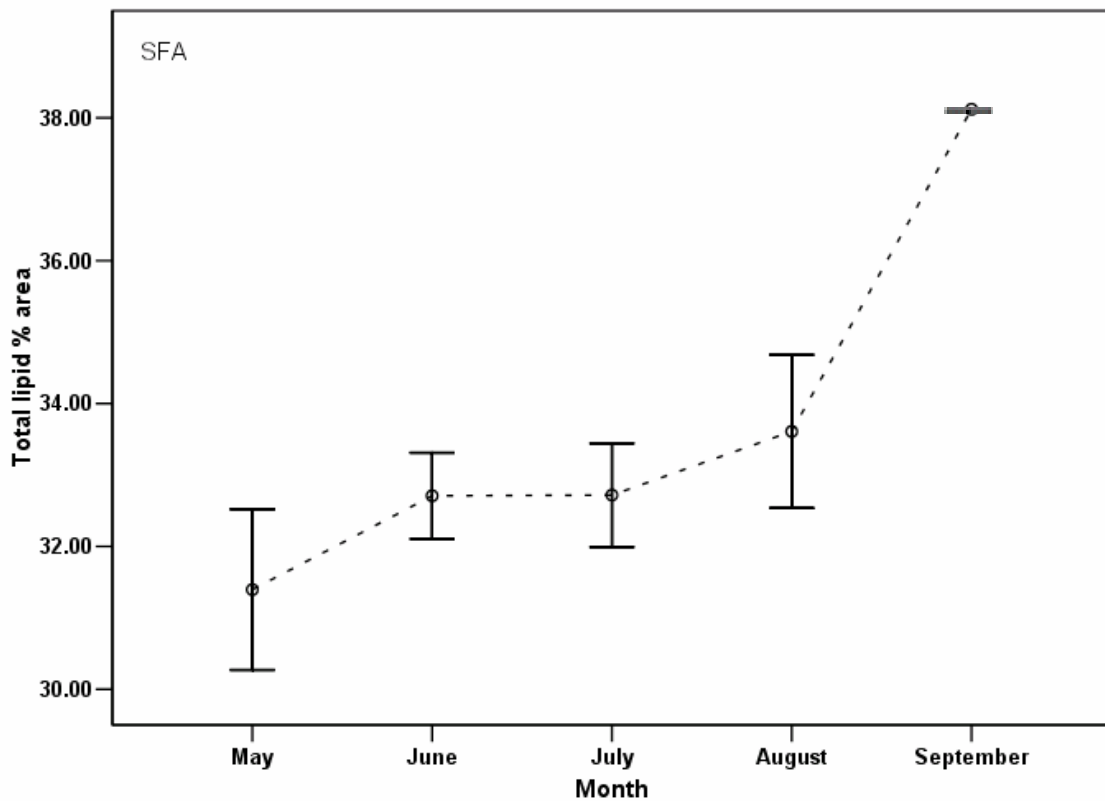
Figure 4.2. Temporal changes in mean monthly saturated fatty acid (% area) values from 2002-2005 in snook eggs

4.3.2.2 The variation of monounsaturated fatty acid (MUFA) levels in common snook eggs during the spawning season.

The mean 2002 MUFA values in common snook eggs were lower compared with the following years with an average value of 29.5% of total lipid (Figure 4.3). Monthly mean values in 2002 are significantly different ($p < 0.05$) especially comparing June, August and September. The wide variation within July made further comparison difficult. There was also a significant difference ($p < 0.05$) between the values for July and August 2005. No significant difference was observed ($p < 0.05$) between any other mean monthly values.
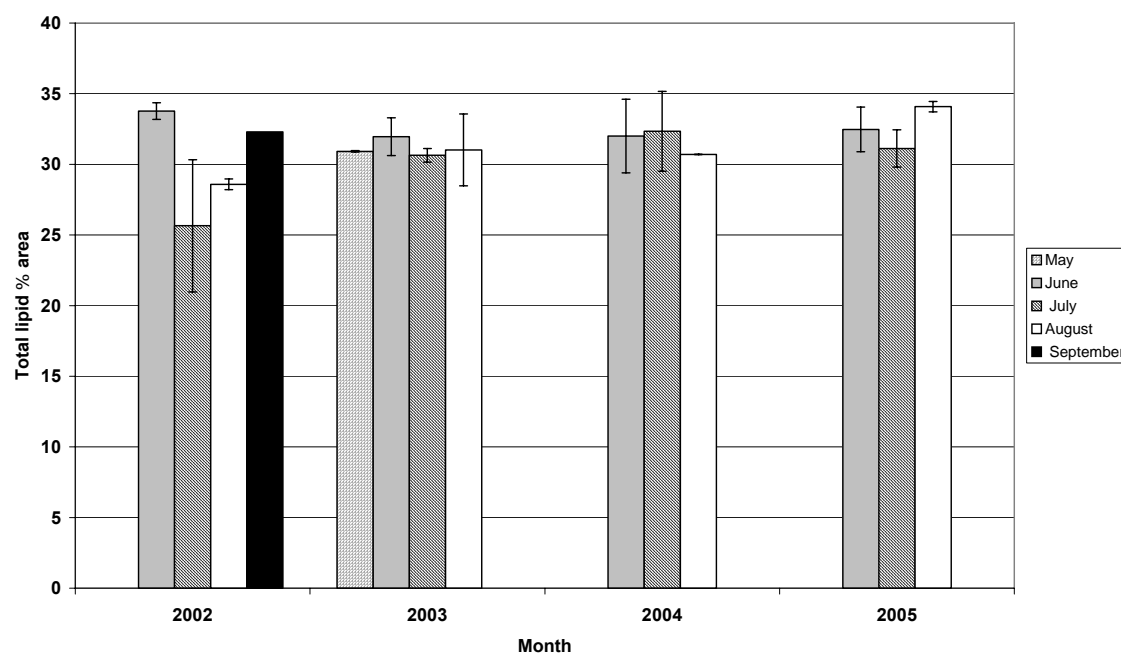
Figure 4.3. Changes in average monounsaturated fatty acid values in eggs by month per year (2002-2005)

Overall, the snook eggs MUFA values stayed constant throughout the spawning season. No significant difference ($p = 0.756$) was found between the different months. June values were the highest (32.5% total lipid) and July the lowest with a 29.9 % total lipid (Figure 4.4)
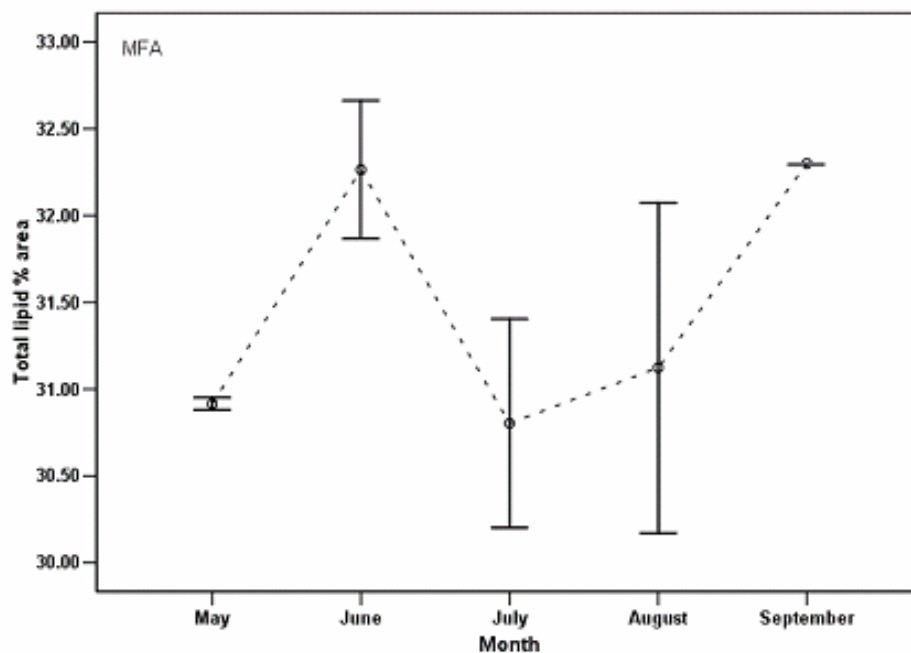
Figure 4.4 Temporal changes in mean monthly monounsaturate fatty acid % values from 2002-2005 in snook eggs

4.3.2.3 The variation of polyunsaturated fatty acid (PUFA) levels in common snook eggs during the spawning season.

The mean PUFA values in common snook eggs for 2002 were the lowest compared with the following years, averaging 31.9% of total lipids. No significant difference ($p<0.05$) was observed between the 2002 mean month values except the August and September values were significantly different (Figure 4.5). In 2003, there was a significant difference between values for May, July and August ($p<0.05$). The mean month values in 2004 were constant, although July showed a wide range in values. The 2005 mean PUFA values were the highest in this study with a 36.5% of total lipid, monthly values were constant (Figure 4.5).
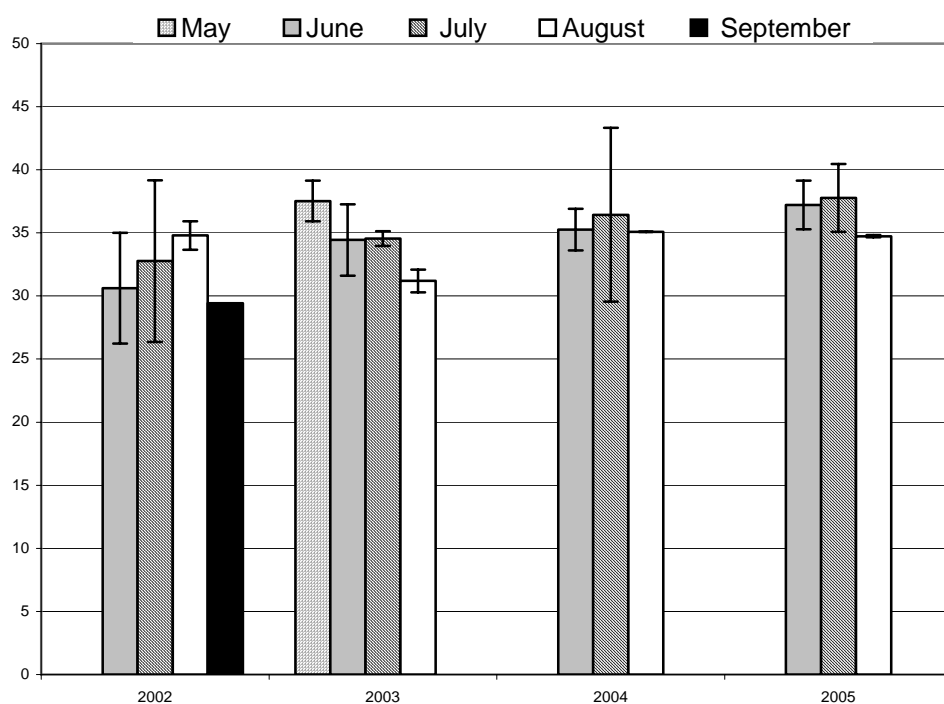
Figure 4.5. Mean polyunsaturated fatty acid values by month per year (2002-2005)

The comparison of mean PUFA values through the spawning season for specific months from 2002-2005 (Figure 4.6) showed a peak in May (37.5%). June and July values remained constant (p> 0.05 between June and July). August and September values decreased significantly (p>0.05). Overall, PUFA values from May to September shows a significant (p= 0.021) decreasing trend (Figure 4.6).
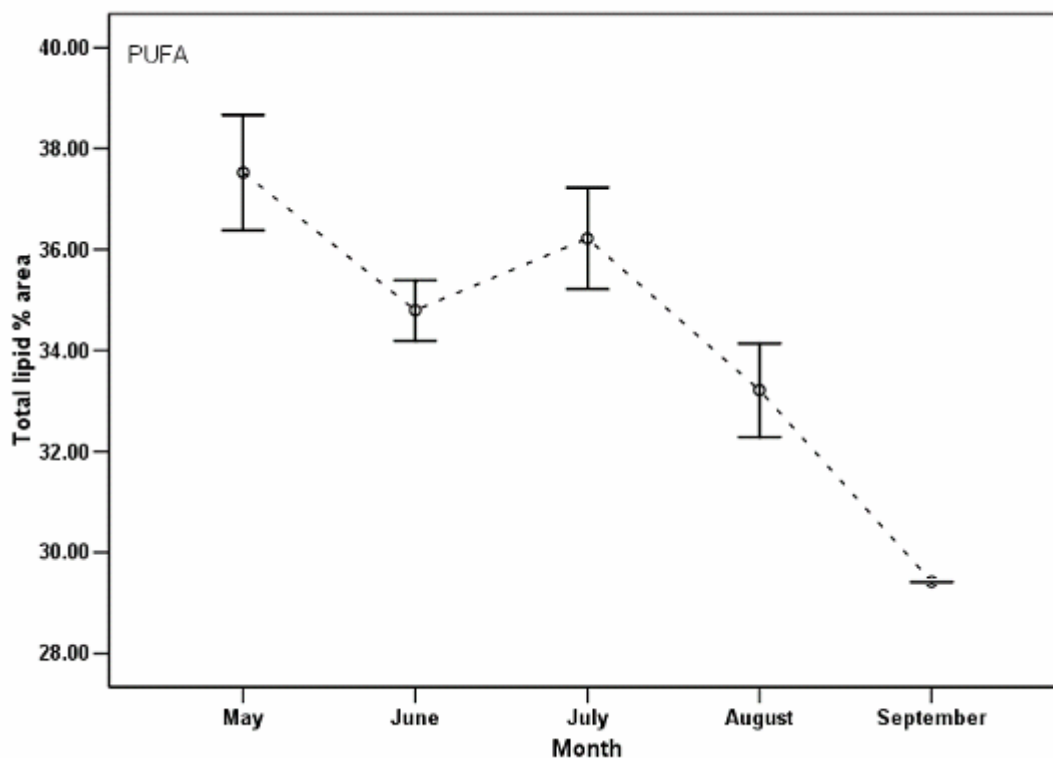
Figure 4.6. Temporal changes in mean monthly polyunsaturated (% area) values from 2002-2005 in snook eggs

4.3.2.4 The variation of omega-3 (n-3) levels in common snook eggs during the spawning season.

In 2002, the n-3 values only showed a significant difference ($p<0.05$) between June and August, and June and September (Figure 4.8). In 2003, the only significant difference ($p>0.05$) was observed between May and July, and May and August (Figure 4.7).

The 2004, n-3 monthly values did not have significant correlation ($p<0.05$). In July it was observed a significant range of values were observed (Figure 4.8). In 2005, the mean omega 3 values decreased from June through August, showing a significant difference ($p<0.05$) between those two months. The

omega 3 values in June and July of 2005 were the highest recorded in this study (2002-2005) with 27.6 and 27% respectively (Figure 4.7).
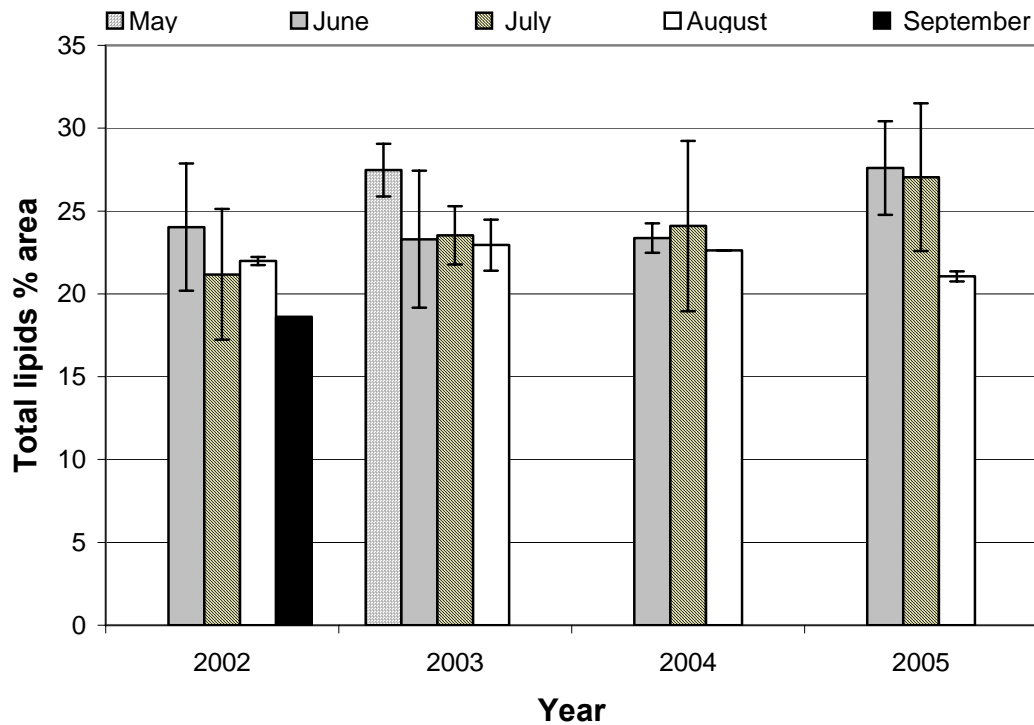
Figure 4.7. Average monthly omega-3 values during 2002-2005

The 2002-2005 overall mean values obtained during this study are presented in Figure 4.8. The omega 3 values of common snook eggs peaked in May, the values from the following months (June, July and August) showed a significant decreasing pattern (p= 0.006) with the lowest mean value in September (18.6%)
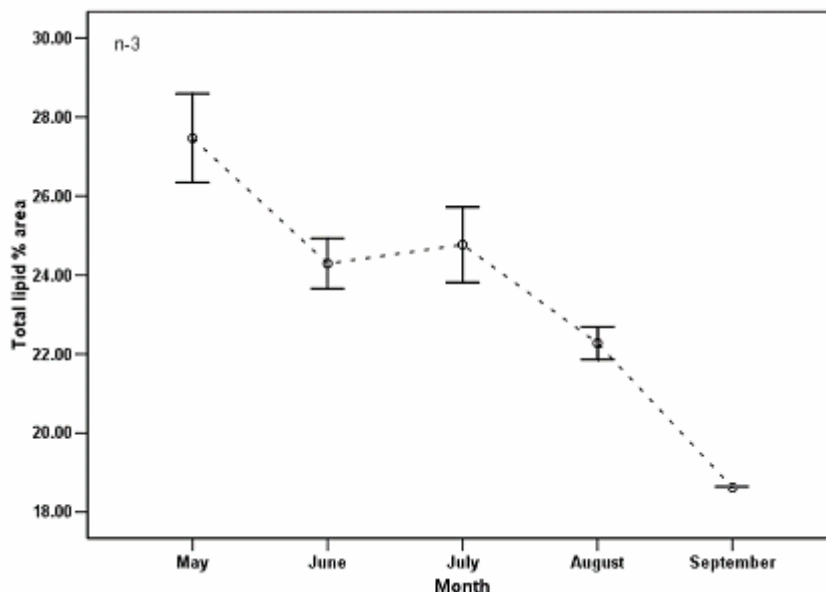
Figure 4.8. Temporal changes in mean monthly omega-3 (% area) values from 2002-2005 in snook eggs

4.3.2.5 The variation of omega-6 (n-6) levels in common snook eggs during the spawning season.

The n-6 values during this study showed an increasing trend through the spawning season (Figure 4.9). In 2002, a significant ($p < 0.05$) increasing trend was observed between June and September. The 2003 values showed a similar trend to 2002 with the lowest value in May, increasing significantly ($p < 0.05$) in the following months (Figure 4.9). In 2004 n-6 values were the highest of all the other years, but no significant difference was found between the different months (Figure 4.9). Finally, in 2005 a significant difference ($p < 0.05$) was observed between June and August.
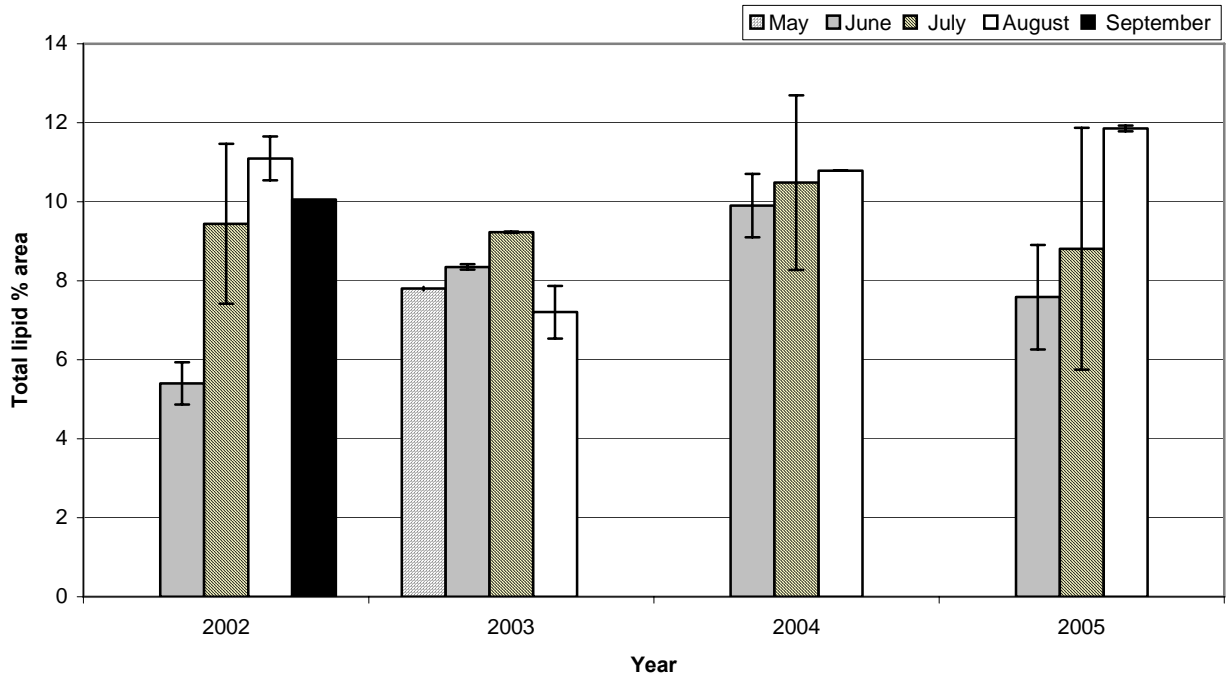
Figure 4.9. Average omega-6 values per month during 2002-2005

Although no significant difference ($p= 0.224$) was found, it was observed that the average n-6 values through the spawning season during 2002 to 2005 increased (Figure 4.10)
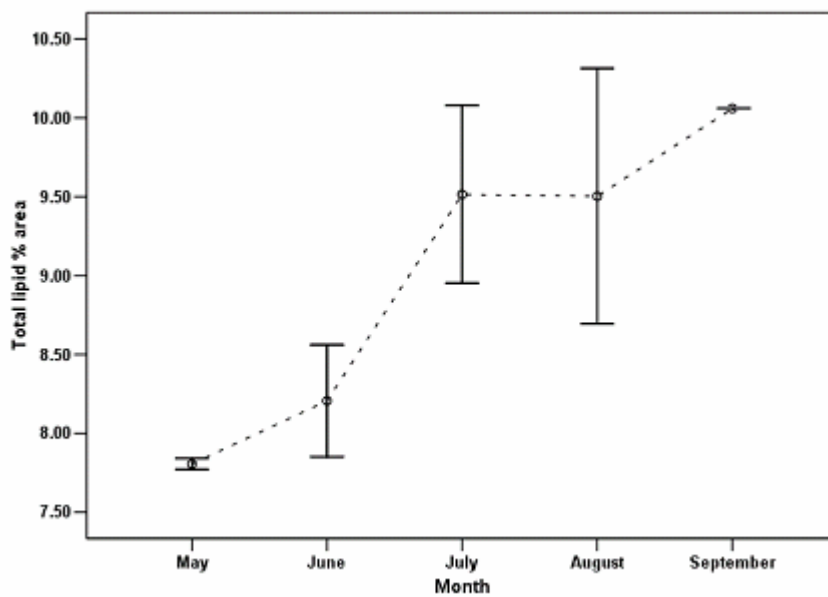
Figure 4.10. Temporal changes in mean monthly omega-6 (% area) values from 2002-2005 in snook eggs

4.3.2.6 The variation of DHA levels in common snook eggs during the spawning season.

In 2002, June had the highest DHA value (14.7%), and a significant difference between months was observed ($p<0.05$) (Figure 4.11). During 2003, May had significantly the highest DHA value, but no significant difference ($p>0.05$) was observed between the other months. In 2004, no significant difference ($p>0.05$) was observed between June and July values but a significant difference was observed when compared to August values (Figure 4.11). The 2005 DHA values showed similar decreasing patterns as 2004, no significant difference was observed ($p>0.05$) between June and July but August was a significantly different to June and July ($p<0.05$). Total values were higher than any other year (Figure 4.11).
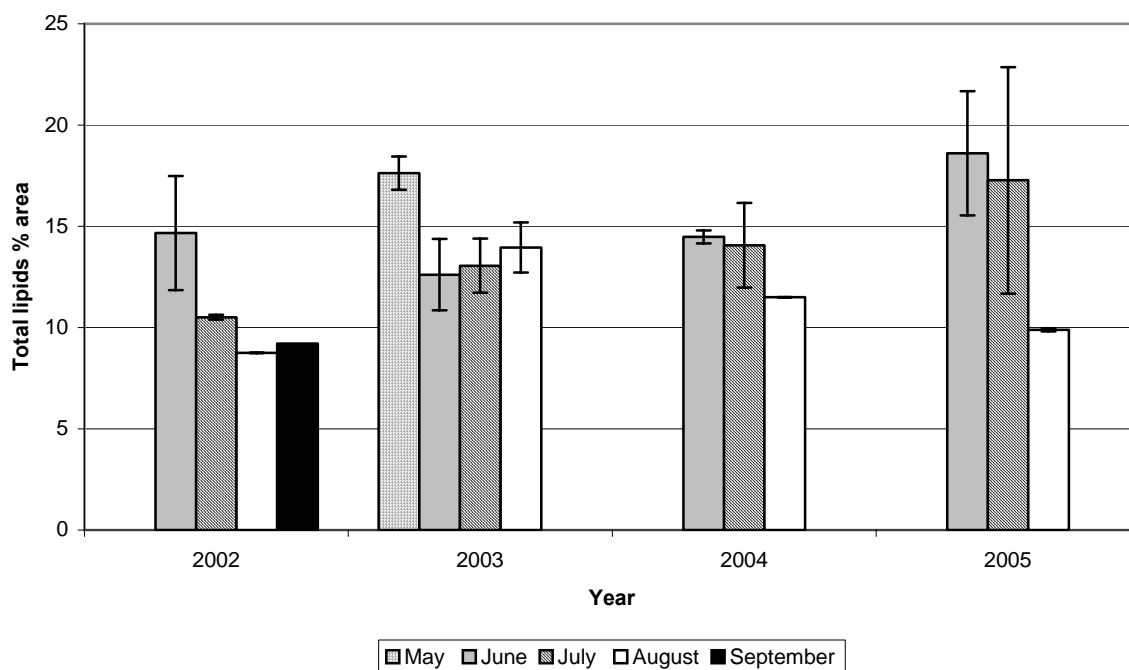
Figure 4.11. Average DHA values per month during 2002-2005

On average, the snook eggs DHA values through the spawning season showed a significant (p= 0.016) decreasing pattern from May till September (Figure 4.12). May had the highest mean value and September the lowest.
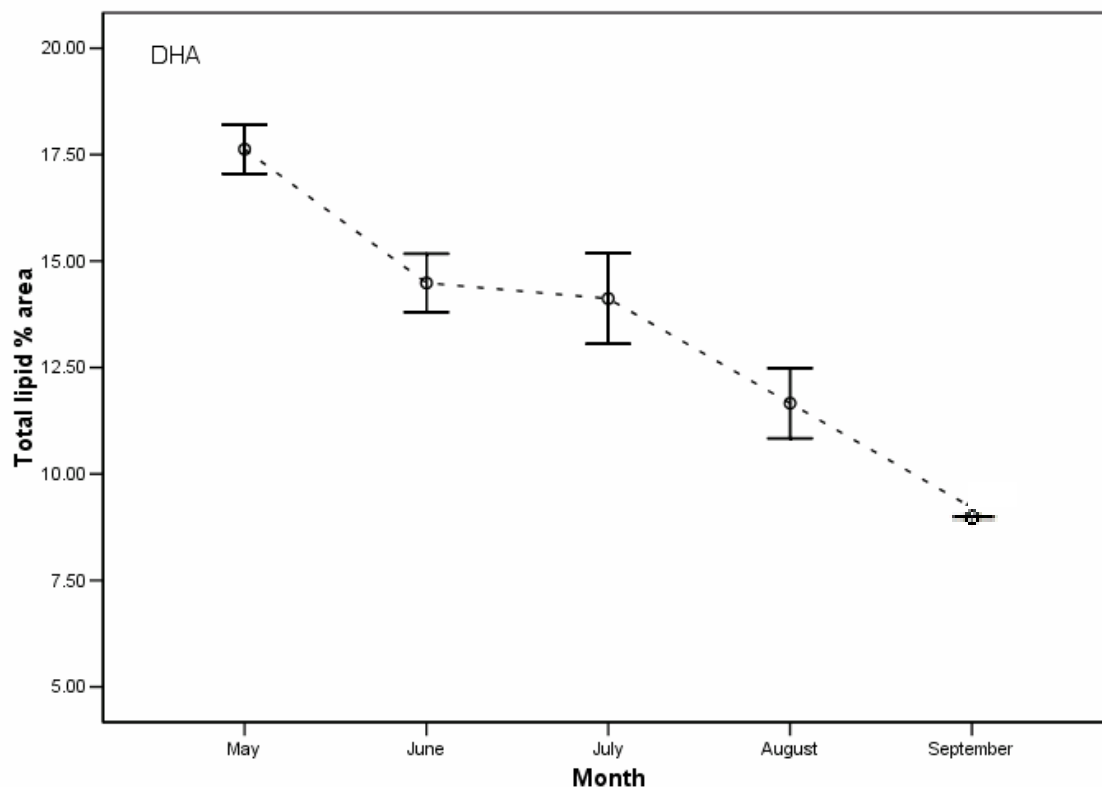
Figure 4.12. Temporal changes in mean monthly DHA (% area) values from 2002-2005 in snook eggs

4.3.2.7 The variation of ARA levels in common snook eggs during the spawning season.

The common snook eggs arachidonic acid values in 2002 (Figure 4.13) remained constant (p<0.05) except for the June month value, which was significantly different (p<0.05) to the other months. During the 2003 spawning season no significant difference was observed between months (Figure 4.13)

Overall, ARA mean values in 2004 were the study's highest, but month values remained constant showing no significant difference ($p<0.05$). In 2005, a significant ($p>0.05$) increasing pattern was observed from June to August.
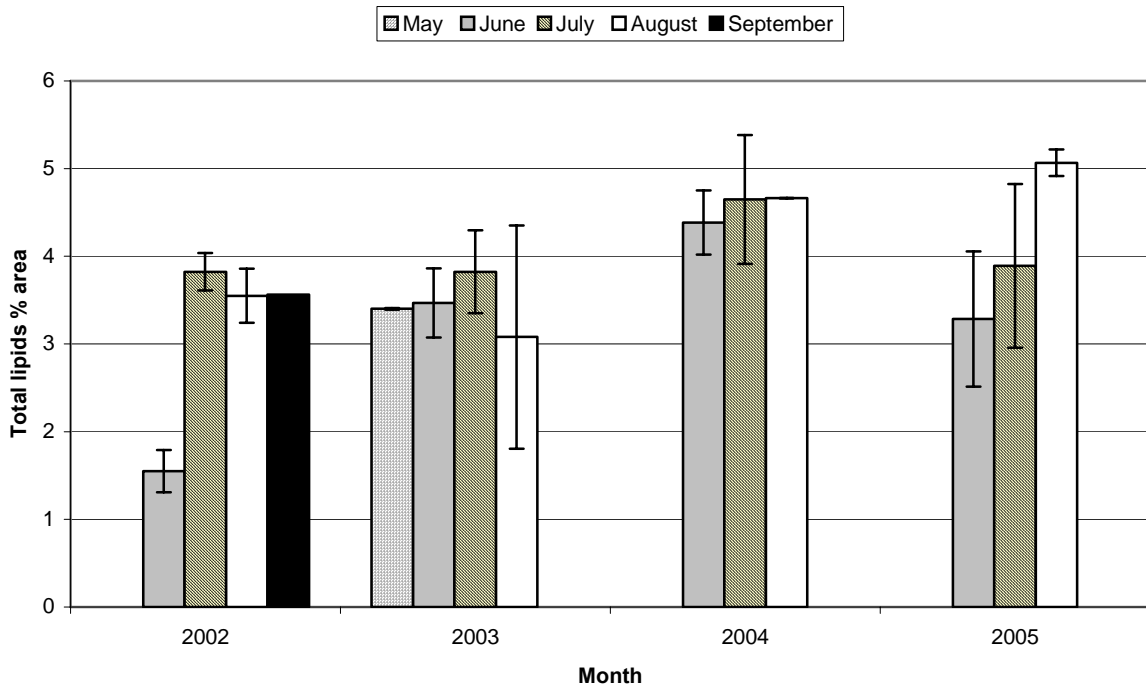
Figure 4.13. Average ARA values per month during 2002-2005

Overall, during the snook spawning season no significant differences were observed ($p=0.739$) on ARA monthly mean values, although May and June values were significantly different to the other months (Figure 4.14).
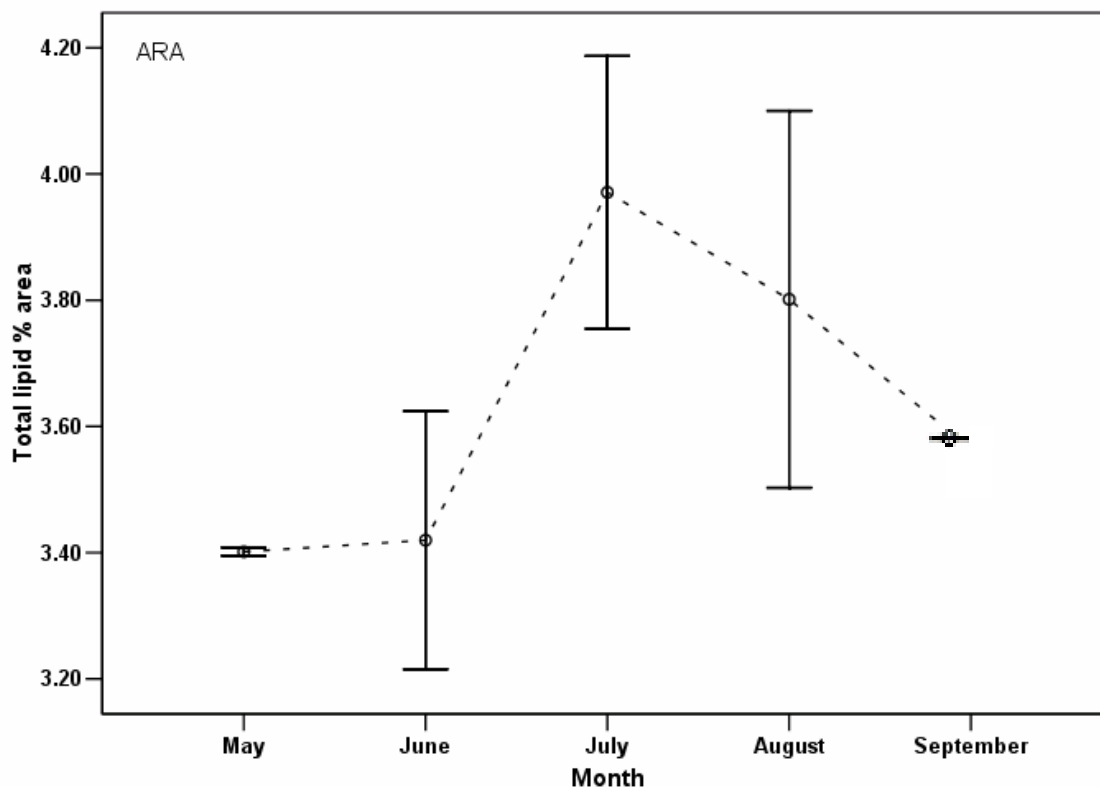
Figure 4.14. Temporal changes in mean monthly ARA (% area) values from 2002-2005 in snook eggs

4.3.2.8 The variation of EPA levels in common snook eggs during the spawning season.

In 2002, the mean EPA value was the lowest out of the 4 years. The June 2002 value was significantly different ($p > 0.05$) to the July and August values, although no significant difference was found overall ($p < 0.05$), (Figure 4.15). The following year (2003), EPA values remained constant, observing no significant difference between months. The 2004 and 2005 overall EPA mean values were higher than in the previous two years (Figure 4.15). In 2004, no significant difference was found between months ($p < 0.05$), although June had the lowest value. In 2005, no significant difference was observed between months ($p < 0.05$), but June values were significantly different to the July and August values ($p > 0.05$).
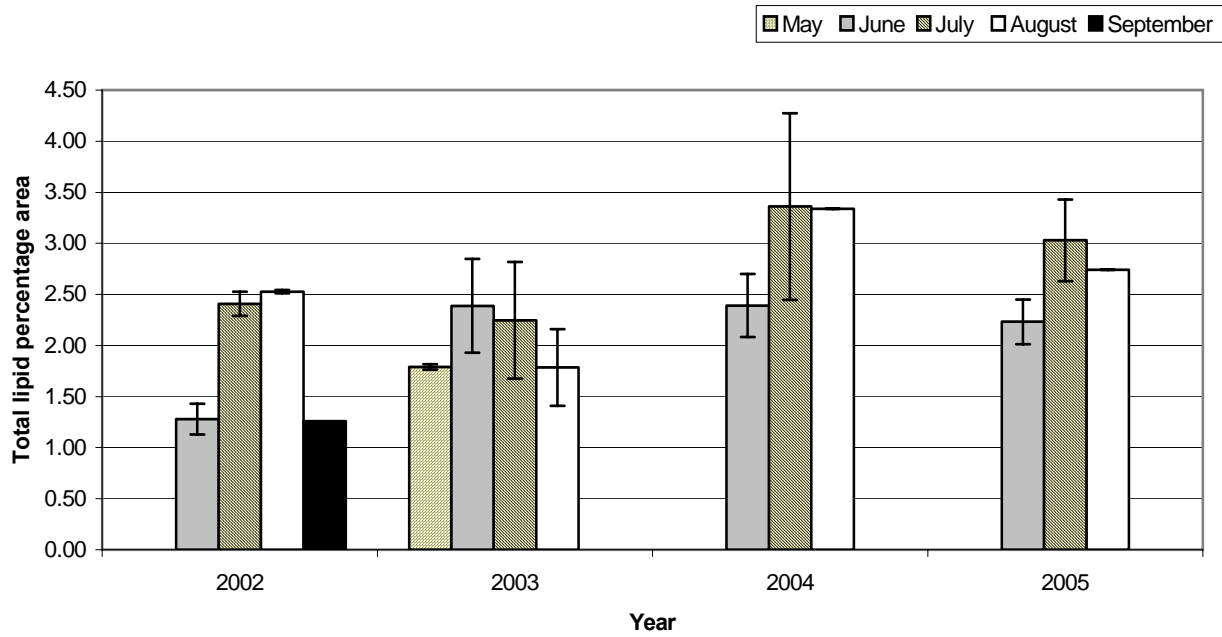
Figure 4.15. Average EPA values per month during 2002-2005

Overall the EPA values during the 2002 to 2005 spawning season (Figure 4.16), from May to September, showed no significant difference (p= 0.493) May and September values were significantly different to June, July and August (p<0.05).
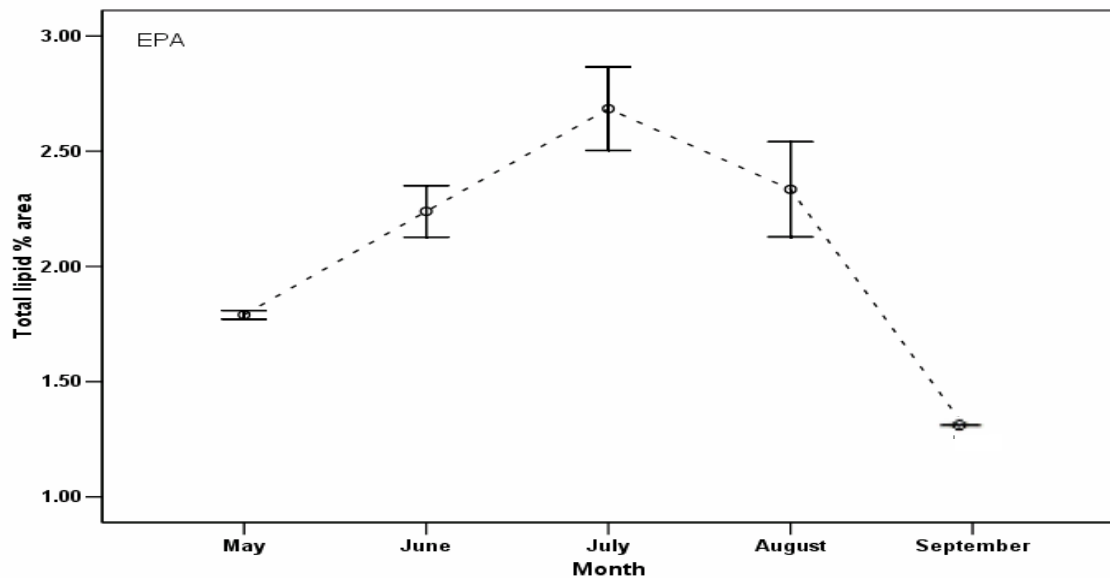
Figure 4.16. Temporal changes in mean monthly EPA (% area) values from 2002-2005 in snook eggs

4.3.2.9 The variation in EPA/DHA levels in common snook eggs during the spawning season.

The EPA/DHA ratio values obtained in this study showed a similarity in pattern between 2002, 2004 and 2005, all of them showing an increasing pattern from the begining of the season (Figure 4.17), on the other hand 2003 had a decreasing pattern. The difference between the month's values was significant in 2002, 2004 (p<0.05). The August values in 2002, 2004 and 2005 were the highest of the year (Figure 4.17).
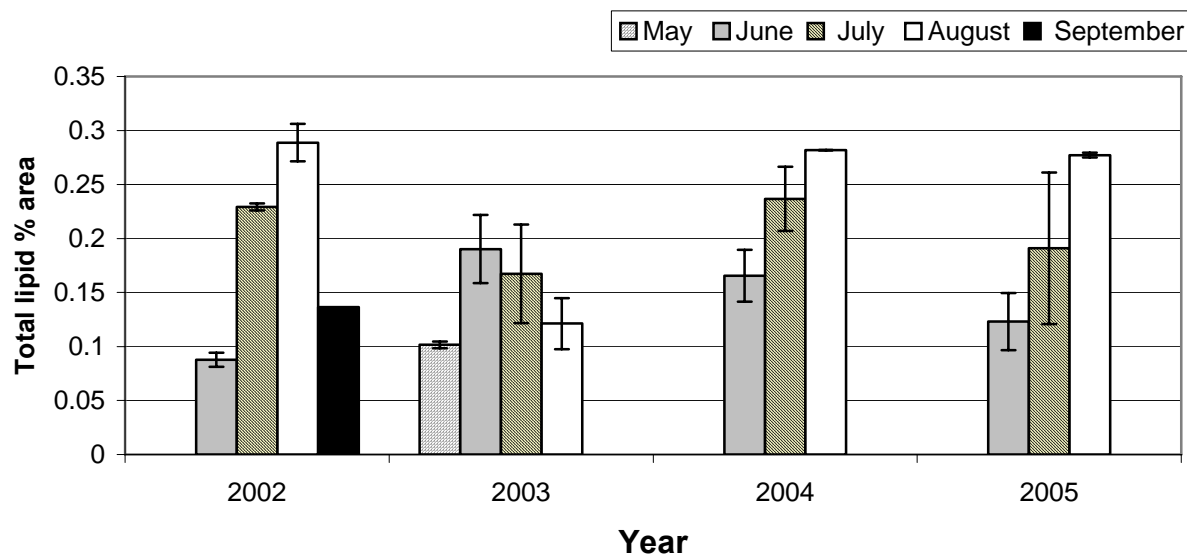
Figure 4.17. Average EPA/DHA values per month during 2002-2005

The EPA/DHA ratio through the spawning season (Figure 4.18), results showed a steady increase in the values from May till August, but no significant difference was found between months (p=0.360) to define a decreasing or increasing pattern. May and September were significantly different (p>0.05) to June, July and August.
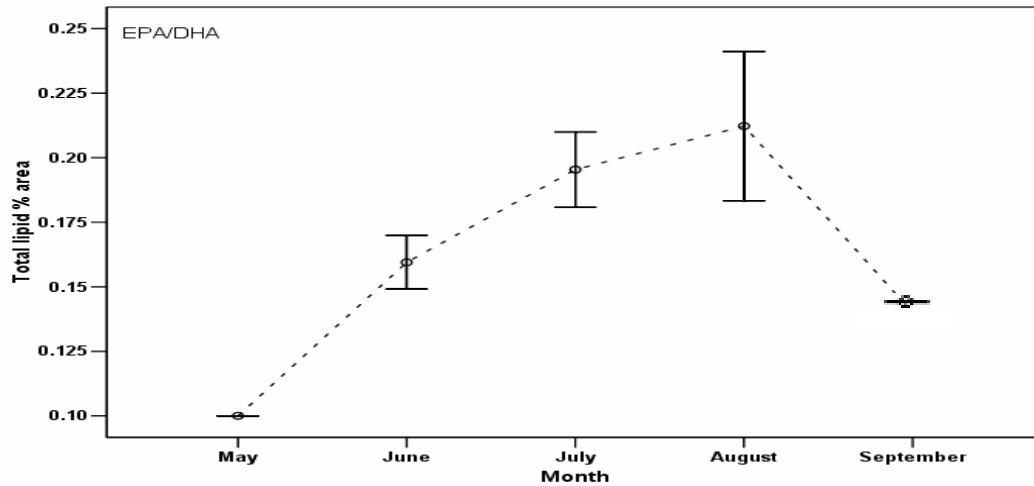
Figure 4.18. Temporal changes in mean monthly EPA/DHA (% area) values from 2002-2005 in snook eggs

4.3.2.10 The variation of ARA/EPA levels in common snook eggs during the spawning season.

The ARA/EPA ratio in 2002 showed a significant difference between months (p<0.05). Results from 2003, 2004 and 2005 showed no significant difference between months remaining constant month by month (Figure 4.19).
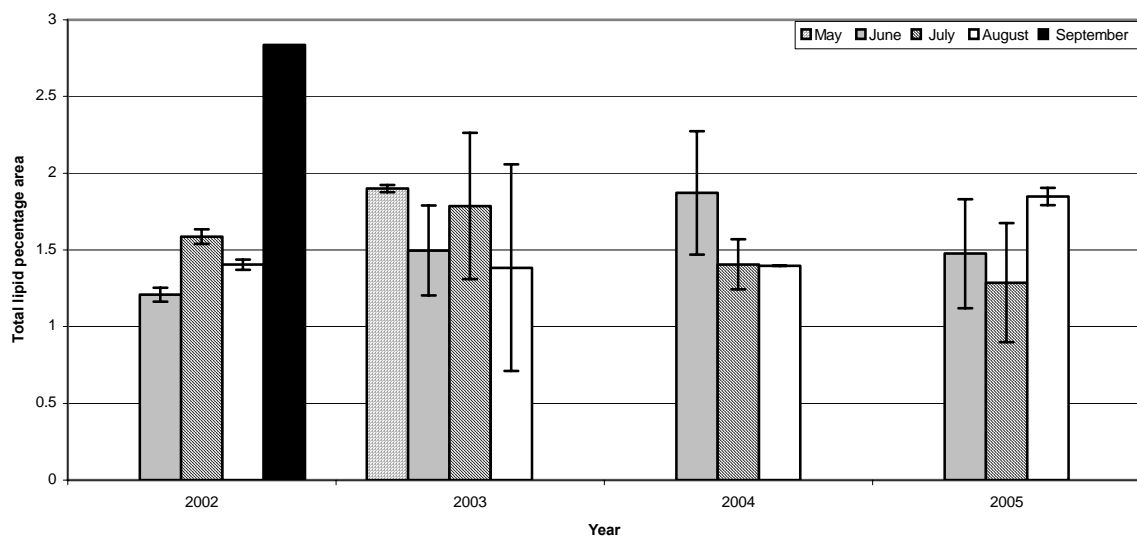
Figure 4.19. Average ARA/EPA values per month during 2002-2005

Overall, mean ARA/EPA ratio values showed no significant difference in the ARA/EPA monthly values (p=0.062), (Figure 4.20), only September is significantly different to the other months (P<0.005).
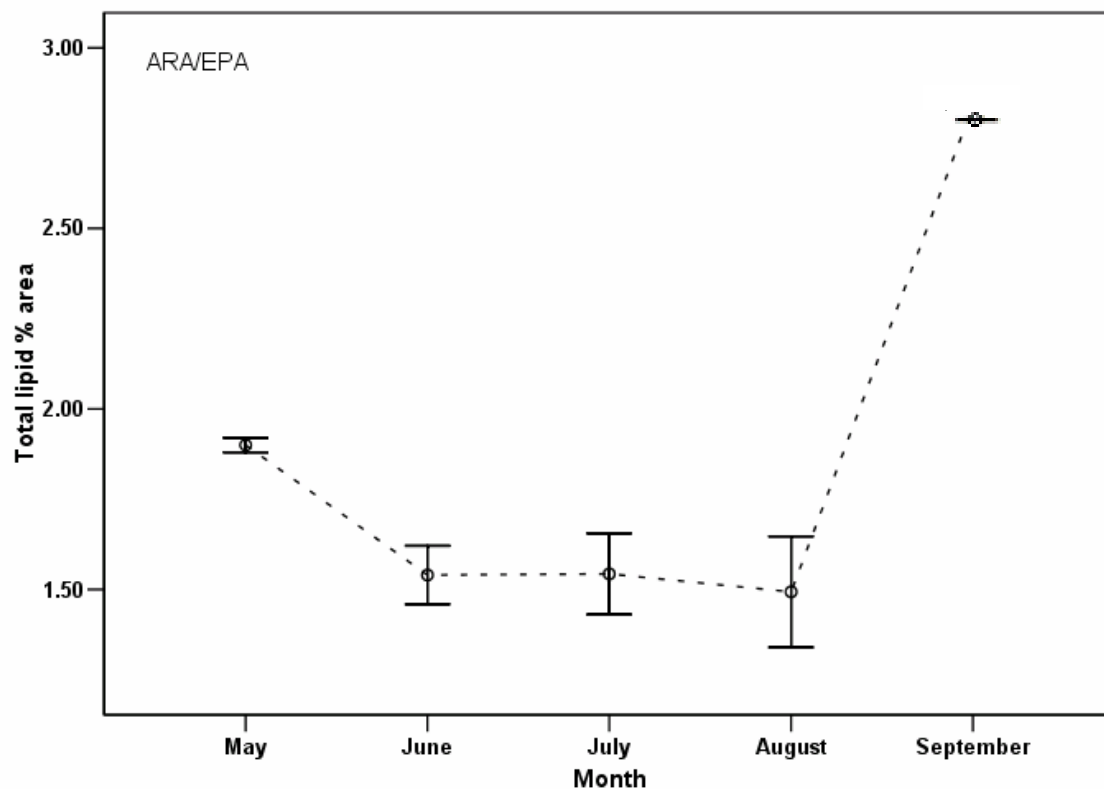
Figure 4.20 . Temporal changes in mean monthly ARA/EPA (% area) values from 2002-2005 in snook eggs

4.3.2.11   Influence of egg DHA levels on fertilization rates

The DHA levels in snook eggs having a 10% total lipid area had a fertilization percentage under 50% (Figure 4.21). Snook eggs with DHA values over 12 % total lipid area had a fertilization percentage over 60 (Figure 4.21). The fertilization rate of snook eggs was significantly correlated (p= 0.002) to DHA levels.
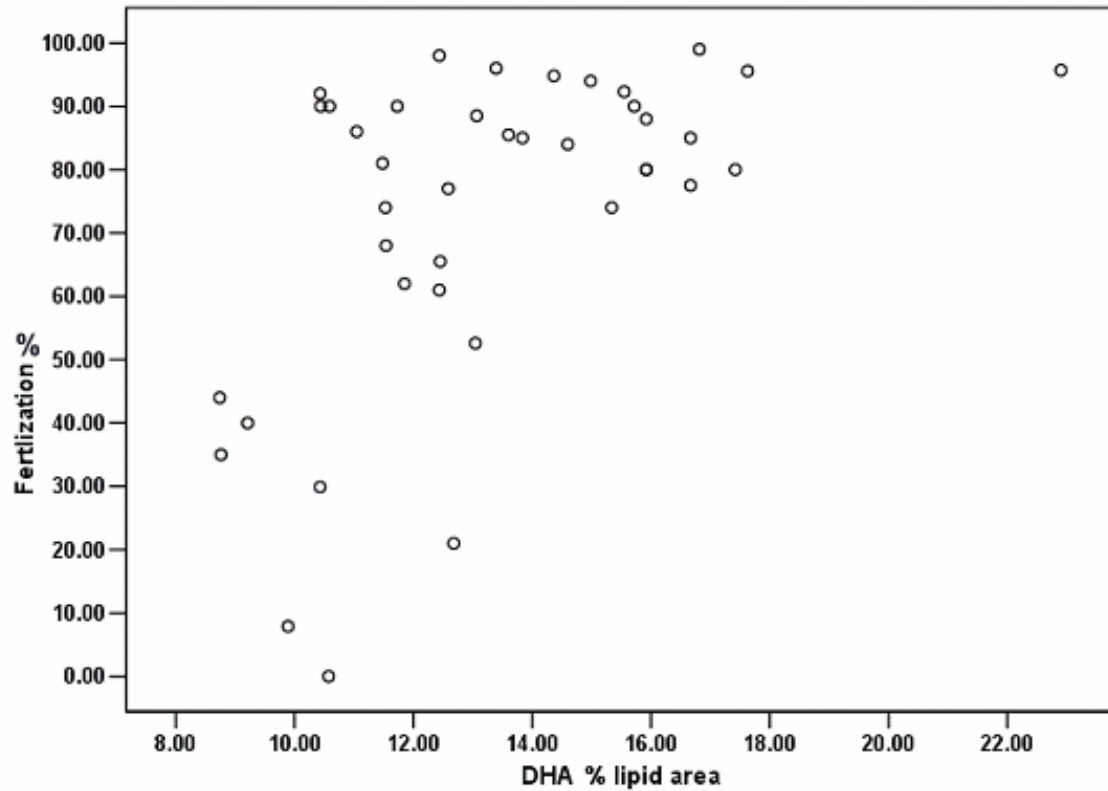
Figure 4.21. Snook egg DHA values against fertilization percentage (2002-2005)

The DHA/fertilization correlation (Figure 4.22) showed that 81% of egg samples, with DHA values higher than a 13% of TLA, had a fertilization rate of 80% or higher. On the other hand, 61% of eggs samples with DHA values lower than 13% TLA had a fertilization rate below 80% (Figure 4.22). Overall 70% of all the snook eggs samples analysed with a 13% TLA or higher had a fertilization percentage of 80% or higher (Figure 4.22)
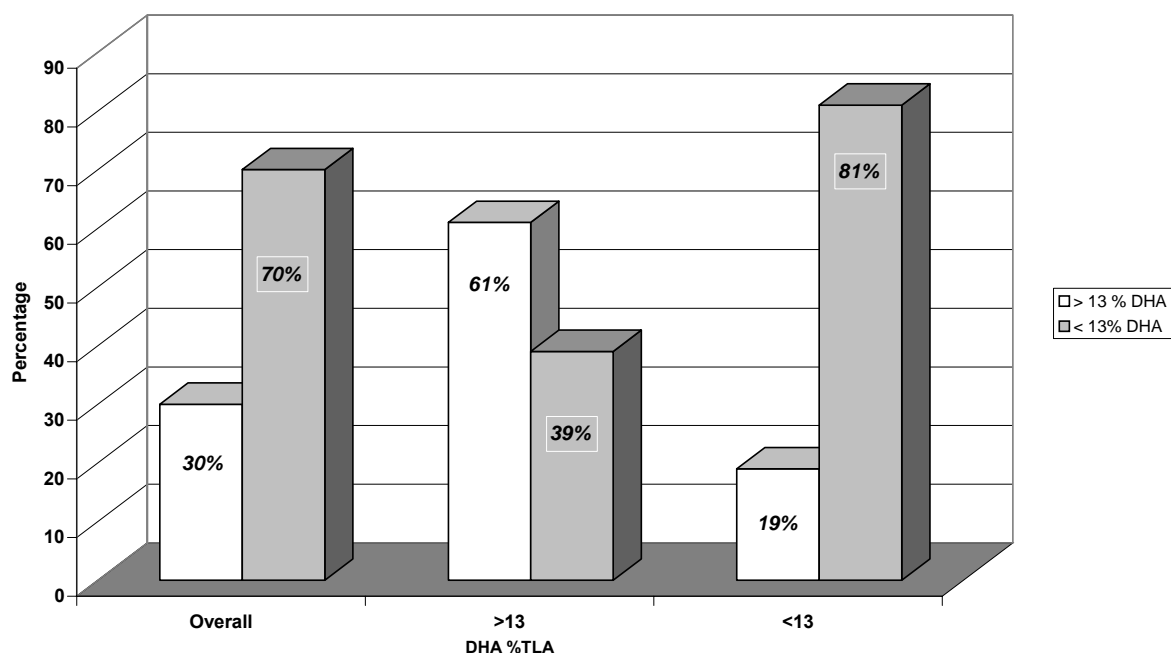
Figure 4.22. Percentage of eggs samples with an 80% fertilization rate or higher against eggs samples with DHA levels below or above 13 total lipid area %

4.3.2.12   Influence of egg DHA levels on hatching percentage

There was a significant correlation (p= 0.009) between DHA and hatching rate (Figure 4.23). Eighty percent of the snook larvae with an egg DHA content of 13% of TLA and higher had a hatching percentage of 20% or higher, on the other hand, only 39% of snook eggs with an egg DHA content lower than 13% of TLA had a hatching percentage higher than 20% (Figure 4.23). More in depth, fifty five percent of snook larvae with an egg DHA content higher than 13% had a hatching percentage of 30% or higher. On the other hand, only 22% of larvae with an egg DHA content lower than 13% of TLA had a hatching percentage of 30% or higher.
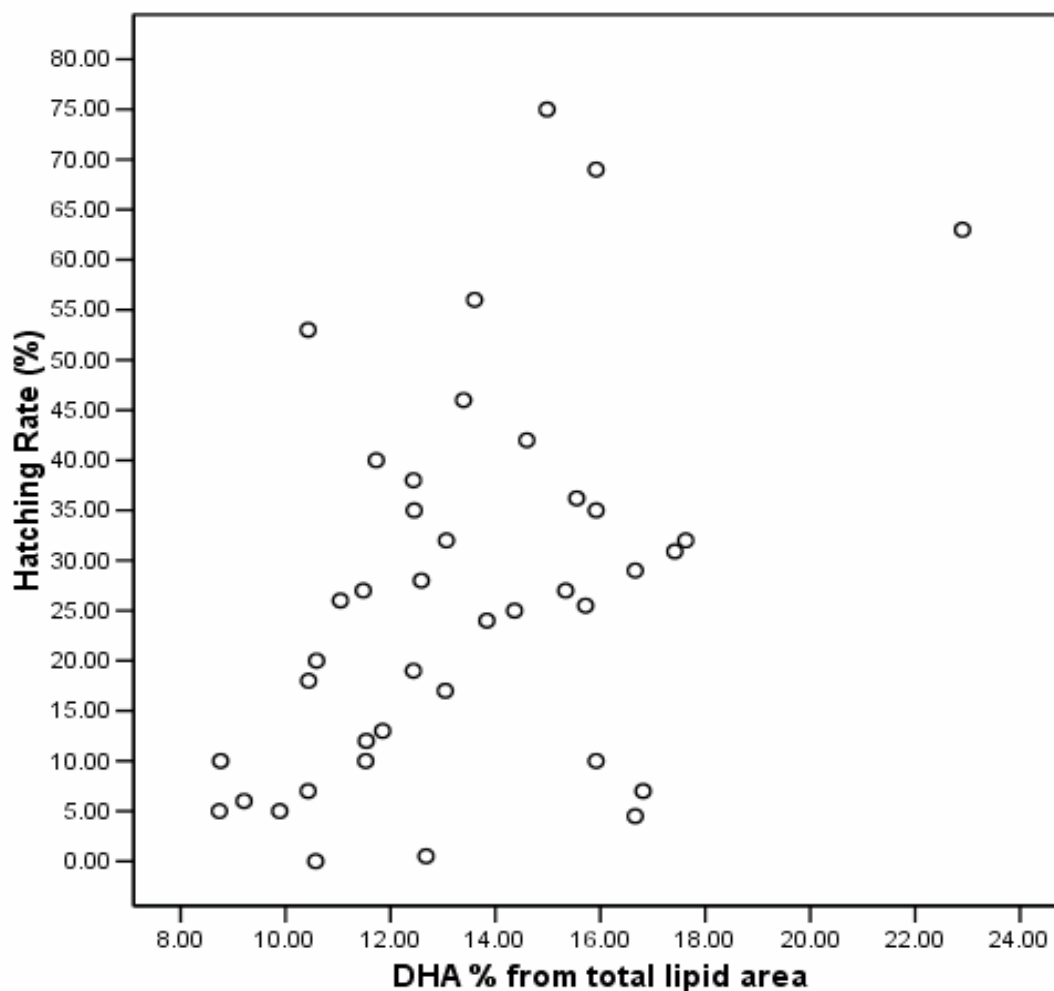
Figure 4.23. Influence of snook egg DHA levels on hatching percentage

4.3.2.13   Influence of egg DHA level on larval survival

A significant correlation (p= 0.001) between egg DHA contents and larval survival was found, where larval survival increased with the increase of DHA levels in eggs (Figure 4.24). Seventy six percent of snook larvae with DHA egg content higher than 12% of TLA survived past day 6 (Figure 4.24), on the other hand, 94% of snook larvae with DHA eggs content lower than 12 % TLA dropped out by day 6 (Figure 4.24).

4.3.2.14   DHA level fluctuations through larval development from common snook larvae reared in captivity.

The DHA levels from common snook larval development, was investigated from the embryonic stage to 80 days post hatching (Figure 4.25). Using the average embryonic DHA level (14%) as a starting reference, the fluctuations in DHA levels during larval development showed a significant decrease (p=0.02) during the first six days (Figure 4.25). After day 8 post hatching, DHA levels started to significantly increase (p=0.02), reaching values over 10% by day 60 post hatching.
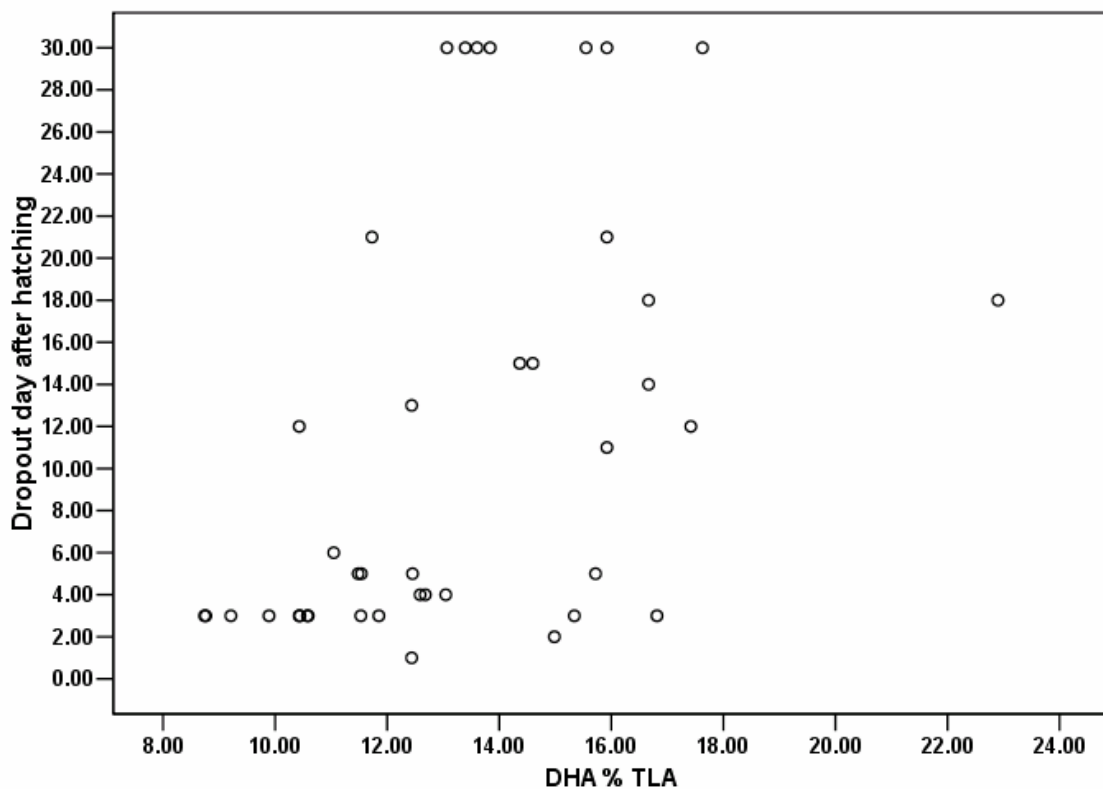
Figure 4.24. Influence of snook egg DHA levels on larval survival
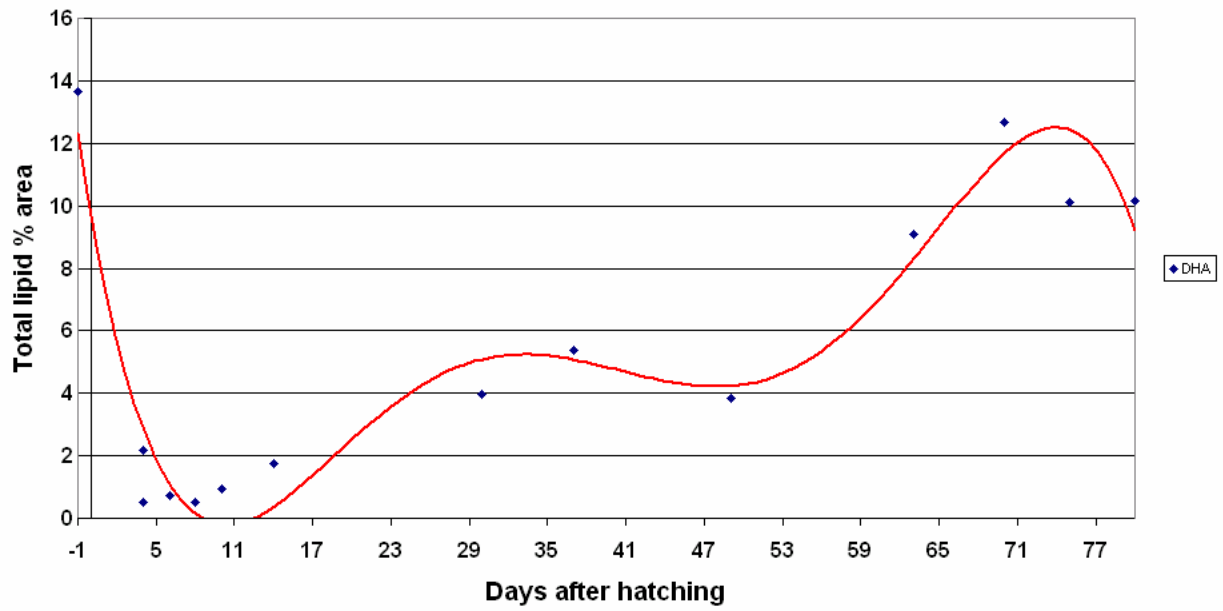
Figure 4.25. Common snook larval DHA levels changes with age from egg to 80 days post hatching (-1 value on the graph refers to the average egg DHA value)

4.4 Discussion

One of the limiting factors for successful mass production of fish fry and recruitment of wild stocks is the variability of egg quality, since poor egg quality may decrease the survival potential of the hatched larvae; increasing the knowledge on this topic could result in a better larval survival (Kjørsvik *et al.,*1990).

The potential to produce viable fry is determined by several physical, genetic and chemical parameters, as well as the initial physiological processes occurring in the eggs, therefore if one of these essential factors is lacking, or is incomplete, egg development may fail (Kjørsvik et al., 1990).

Snook is a seasonal spawner, with spawning activity determined by the water temperature, as discussed in chapter 3. Salinity, tidal current and moon phase are other physical parameters that determine the snook-spawning period, which runs from May till September. Using this spawning season as a reference, and using the influence of lipids on egg quality, the identification of the most productive months in terms of egg viability was investigated.

This chapter has focused on the chemical content of wild snook eggs. The biochemical composition of a healthy egg reflects the embryonic demands both for nutrition and growth. Some components are considered essential for an organism and have to be present at a certain level to satisfy biological demands (Kjørsvik et al., 1990). This study focussed on the organic composition of snook eggs, looking at the fatty acid content as a main parameter, to identify egg viability and influence over other parameters such as; fertilization rate, hatching rate, and larval survival.

Snook egg fatty acid composition fits the general marine fish fatty acid profile with predominant SFA, mainly palmitic acid (16:0), important as an energy source. It is also structurally important in the sn-1 position of membrane phospholipids, (Sargent, 1995) and stearic acid (18:0) and, with a range of chain lengths from C_{12} to C_{24}, can be found in minor amounts. Oleic acid (18:1n-9) is the main MUFA and it confers fluidity on membranes when inserted in the sn-1 position of phosphatidyl ethanolamine. It also supplies energy for embryos (Dey *et al.*, 1993) and is the most abundant, followed by Palmitoleic acid (16:1n-7) and Vaccenic acid (18:1n-7). This is the general pattern reported for the fatty acid composition of the eggs of many fish species (Kaitaranta & Linko 1984; Tocher & Sargent, 1984; Wiegand, 1996a; Sargent *et al.*,. 2002).

The n-3/n-6 PUFA ratios in snook eggs is lower (2.52) than the typical marine fish (Henderson and Tocher, 1987), although still reflecting the higher amounts of n-3 HUFA, specifically 20:5n-3 and 22:6n-3, in their lipids. The EPA value is in the mid low range of those reported fish eggs (Eldridge *et al.*, 1983; Kaitaranta & Linko, 1984; Tocher & Sargent, 1984; Cowey *et al.*, 1985; Ulvund & Grahl- Nielsen, 1988; Falk-Petersen *et al.*, 1989; Ashton *et al.*, 1993; McEvoy *et al.* 1993; Vazquez *et al.* 1994; Jobling *et al.*, 1995; Silversand *et al.,* 1996; Wiegand, 1996a; Bell *et al.*, 1997; Almansa *et al.*, 1999; Bruce *et al.*, 1999; Morehead *et al.* 2001; Furuita *et al.*,2002), but fatty acid composition values vary with species (Sargent *et al.* 1995)*.* A more detailed study on the wild snook diet should be carried out to find out the cause of this lower value.

The high levels of the major fatty acids found in the common snook eggs shows their importance as energy store for embryonic development (Almansa *et al.*, 2001). The most unusual value obtained for snook eggs in this study was the ARA value, which was significantly higher than for other marine species (Eldridge *et al.*, 1983; Kaitaranta & Linko, 1984;Tocher & Sargent, 1984; Cowey *et al.*, 1985; Tocher *et al.*, 1985; Fraser *et al.*, 1988; Ulvund & Grahl-Nielsen, 1988; Falk- Petersen *et al.*, 1989; Ashton *et al.*, 1993; McEvoy *et al.*, 1993; Vazquez *et al.*, 1994; Silversand *et al.*, 1996; Wiegand, 1996b; Pickova *et al.*, 1997; Almansa *et al.*, 1999; Bruce *et al.*,1999; Morehead *et al.*, 2001; Furuita *et al.*, 2002; Furuita, *et al.*, 2003).

The egg lipid composition is directly affected by the parental diet as well as the physical conditions to which they are exposed (Sargent *et al*, 1999b). This unusual ARA value may be due to the temporally alteration of habitat that wild snook adults are exposed to during the spawning season (from upstream in brackish canals and creeks to the mouth of estuaries and beaches). Wild common snook broodstock remain exposed to pure marine conditions during the spawning season which affects their feeding habits. It has been shown that, not only the quality of eggs, but also their chemical components, are influenced by the nutritional composition of diets eaten by broodstock before, and during, spawning (Leray *et al.*, 1985; Watanabe *et al.*, 1991; Harel *et al.*, 1994; Watanabe & Kiron, 1994; Fernandez-Palacios *et al.*, 1995). However, the duration of the period that broodstock should feed on a diet, in order to affect the chemical composition of the eggs and the spawning quality, is not clear. Arachidonic acid (ARA) values tend to increase when fish are exposed to unusual environments, or situations increasing their stress levels. That may

be one of the reasons why ARA values are high in snook eggs. However, in order to get more conclusive, and reliable, results, more work should be done on the wild snook diet and habits in the wild. Once the common snook feeding habits during the spawning period is investigated, the extent to which diets can influence egg quality may be seen. So far there are contradictory results from species such as rainbow trout, where only a long period of omega 3 fatty acid deficiency in the broodstock diet affected the egg lipid composition (Leray *et al.*, 1985). In contrast, in sparids (Zohar, *et al.*, 1984), egg quality seems to be affected by dietary lipid just prior to spawning and even during spawning. Evans *et al.*1998, suggest that the decline in the unsaturated to saturated fatty acids ratio, and total lipid indicates a reduction in egg membrane fluidity. This decline can be observed on the snook eggs during the spawning season, as the season progresses. During this period SFA values significantly increased from May to September, whilst PUFA, and omega 3 fatty acid values, significantly decreased during the same period.

Watanabe (1993) suggest that DHA, as an EFA, plays a more important role in the enzyme activity of the cell membrane and in physiological balance than EPA does. Deficiencies in DHA could lead to behavioural impairment in larvae (Sargent, 1995). Bell *et al.*, (1985b) have suggested that DHA has a more important biochemical function as the lipid than EPA. This was later confirmed by Koven *et al.*, (1993), who observed gilthead sea bream larvae had conserved DHA over EPA during deprivation.

During the present study, no significant differences were found between EPA levels throughout the season. In contrast, DHA values declined significantly from May to September.

The EPA:DHA ratio from May to August showed a significant increase during the spawning season and AA:EPA ratio showed a decline (although not significant) during the same period. Ibrahim (2004), obtained the same patterns when looking at wild goldlined seabream. Pickova *et al.*, (1997) positively correlated DHA:EPA ratio with egg symmetry and viability. On the other hand, Bell & Sargent, (2003) suggested that some ratios, such as AA:EPA, are species specific, dependent to the environment the species inhabits. Tveiten *et al.*, (2004) and Ibrahim, (2004) also suggested that there is no general requirement for high AA:EPA in order to increase egg survival. Zhukinsky and Kim, (1981), concluded a positive correlation between total egg lipid content and hatching. Based on this conclusion, and to the fact that hatching percentage and fertilization are useful parameters to assess egg quality (Springate et al., 1984, Springate and Bromage, 1985, Nomura et al., 1974, Escaffre and Billard, 1979, Sakai et al., 1975, Thorpe et al., 1984, Dinis, 1982, Hirose et al., 1979, Kjørsvik and Lønning, 1983, Kjørsvik et al. 1984, Dushkina, 1975). This chapter used both parameters together with the fatty acid composition to assess good egg viability. The above parameters (fertilization and hatching) are not a reliable criterion of egg quality (Kjørsvik and Lønning, 1983, Blaxter, 1955; Dushkina, 1975), therefore in order to get reliable results to determine wild snook egg viability, fatty acid composition has been used as the main parameter compared to time, fertilization, hatching rate, and larval survival. As a result, a significant correlation was found between the DHA concentrations of snook eggs and their fertilization percentage, where the higher the DHA snook egg concentration, the higher the fertilization percentage. This finding can be used as an important, and

quick, diagnostic tool to predict the egg viability of wild snook in order to save time and money. Another significant correlation was found between the snook egg DHA concentration and the hatching percentage. The higher the snook egg DHA concentration, the better the hatching percentage. This correlation can also be used as another diagnostic tool to predict egg viability and larval survival.

Part of this study investigated the correlation between snook egg DHA concentration and the larval survival, as a result, a significant correlation was found between these two parameters, where the higher the snook egg concentration, the better the larval survival.

All the above findings confirmed the importance of DHA for embryonic development and larval survival as well as giving us a predictive tool for egg viability and larval rearing success.

The present study was carried out over four years, and in each year environmental and logistical factors differed. Although sampling was kept as continuous and regular as possible there were disruptions, such as: sampling site competition with recreational boaters and anglers; crew restrictions; weather limitations, such as storms, tropical storms, hurricanes; and red tide. Although no study has been carried out on the effects of red tide over the wild snook spawning, some preliminary work has been done using this study data. This showed significant effects on the fatty acid concentrations on those eggs that were exposed to heavy red tide condition. This can be observed, when looked at the 2002 fatty acid results. This year was characterized by the heavy presence of red tide along the Bay area, producing mass fish kills on a daily basis. The results for this year have, on average, the lowest

concentrations of MUFA, PUFA, n-3, DHA and EPA, and the highest concentrations of SFA.

More in depth studies are needed to be done in this topic. Since no work with lipids has been done with wild common snook, a better understanding of its biochemical needs, and behaviour, will help to; culture captive broodstock, close their reproduction cycle, and free the wild stocks from the sampling stress that they are subjected to every summer.

Overall most of the results obtained in the chapter demonstrates that snook egg quality decreases as the summer season advances. The end of May, June and July are the best months to obtain viable eggs. At the same time, the use of fatty acid analysis has shown significant correlations of fatty acid composition with fertilization percentage, hatching percentage and larval survival, creating an important tool for the prediction of egg viability and larval culture success.

Chapter 5:

Improving larval culture and rearing techniques

5 Improving larval culture and rearing techniques

5.1 Introduction

The ultimate objective of hatchery-based snook juvenile production is the supply of high quality animals for stock enhancement in the Sarasota Bay area.

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 healthy seed. 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 (Chapter 6) is necessary. Additionally, an understanding of both larval behaviour and the response of larvae to feeds and environmental conditions, is fundamental (Chiu Liao et al., 2001).

5.1.1 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 (Chiu Liao *et al*., 2001)

Common snook culture is at present limited to Mote Marine Laboratory and Florida State Hatchery, and they rely on wild seed collection. Additionally, the larviculture practices are under-developed compared with those for many other marine fish species.

The present chapter focuses on larval rearing during the first 14 days after hatching. The techniques that need to be investigated relate to the design of a larviculture system, larval feed requirements, and system management.

5.1.1.1 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 small yolk sac (Calzada *et al.,* 1998). This first feeding phase is critical for larval survival; and therefore successful synchronization between exhaustion of indigenous 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, which 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 (ie. 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 (ie. 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)

5.1.1.2 Importance of temperature on embryonic and larval development

Nearly every aspect of early fish development is affected by temperature, (such as fertilization, hatching, and 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 also affected by temperature are the yolk conversion efficiency as demonstrated in salmonid embryos (Heming, 1982; Heming and Buddington, 1988; Marr, 1996; and Peterson & Martin-Robichaud, 1995), as well as in striped bass by 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).

5.1.1.3 Larval stocking densities

One of the key aspects of successful large-scale culture is determining the optimum culture densities. For several species of fish, such as sea bass (*Dicentrarchus labrax*), or sea-bream (*Sparus aurata*), optimum culture

densities are well studied. 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). No density studies for common snook have been conducted, although some work has been done on fat snook in relation to growth and effects of larvae, and juvenile density (*Centropomus parallelus*) (Cerqueira *et al.*, 1995).

5.1.1.4 Prey density

It has been shown that prey density (Werner and Blaxter, 1981) is one of the factors affecting feeding efficiency and consequently larval growth and survival under culture conditions. Enhancement of the 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 of the larvae (Duray *et al*, 1996).

5.1.1.5 Background phytoplankton ('Green water')

Most marine fish larvae are visual feeders and the feeding success of the larvae at various developmental stages depends on the provision of suitable

food and 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 micro-algae 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 may be used as a food source. In recent years, the use of `green water' in larval tanks is considered to be of optical rather than nutritional value to fish larvae (Marliave 1994).

5.1.1.6 Artificial micro-diets

A number of studies were carried out to find satisfactory, formulated diets that would substitute for natural 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 show an optimum 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).

5.1.2 Rotifers and their nutritional value

Live food will remain an important food source for the first feeding of early larval stages. One of the important starter feeds used 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 of this live food (Candreva *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 metazoa 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 size 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 (Figure 5.1).

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 from which originates the name Rotatoria (bearing wheels). The retractable corona assures locomotion and a whirling water movement,
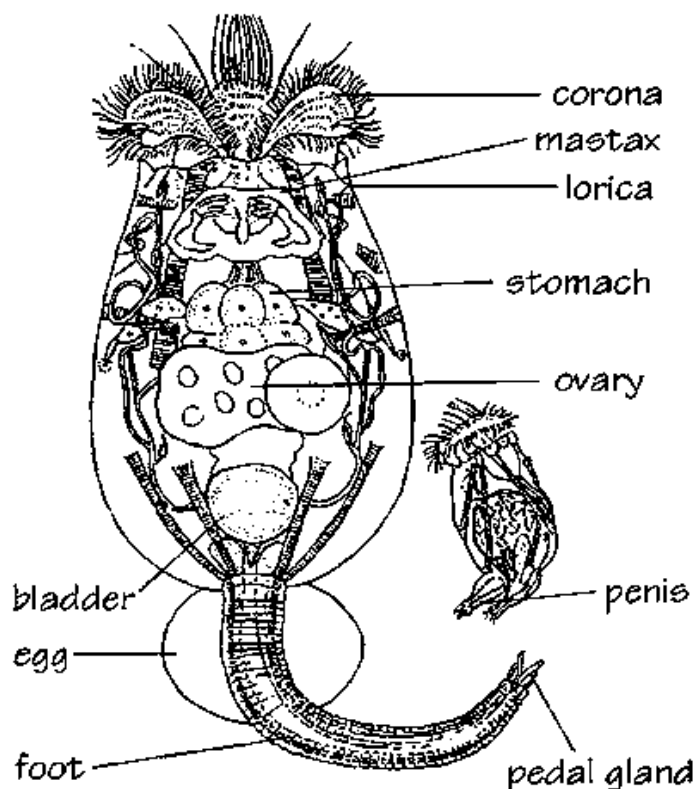
Figure 5.1. Brachionus plicatilis, female and male (Koste, 1980)

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 (Figure 5.1).

Only a few rotifer species belonging to the genus *Brachionus* are used in aquaculture. As outlined in the introduction the most widely used species is *Brachionus 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, and this can influence its suitability as a starter feed for marine larvae, like 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.

5.1.3 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 (Brachionus spp.) 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 may be obvious, for example deformities or malpigmentation, but in many cases may be obscure, as in 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), these deficiencies 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)

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5.1.4 Snook Aquaculture

Collection of data on the conditions required for spawning, larval rearing, and release into 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 was also obtained on the lower lethal temperature (15°C) for juveniles (Howells *et al.*, 1990) and on the development of laboratory reared larvae and juveniles (Lau and Shafland, 1982).

All the above studies have 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 1970s, 1980s and 1990s, research efforts have left gaps in understanding of larval rearing and broodstock management.

5.1.5 Objectives

The objective of this chapter was to improve the larval survival during the first 14 days after hatching, a time when most of the mortality occurs (85%).

The main aims are to:

    I. Investigate the influence of temperature over hatching rate.

   II. Establish the effect of egg stocking densities over larval survival.

  III. Establish the optimal flow rate during incubation and larval rearing.

  IV. Investigate the effect of the green water technique on larval survival and growth.

   V. Determine the optimal prey density to increase larval survival and growth.

  VI. Investigate alternative preys more suitable for the larval mouth gape.

 VII. Determine the optimal daily feeding regime

VIII. Evaluate the use of artificial diets during the pre-weaning period.

  IX. Determine the influence of enriched rotifers on larval survival and growth.

   X. Investigate the effect of different rearing tanks over larval survival

5.2 Materials and Methods

All the larval rearing experiments carried out during this study took place at the Mote Marine Laboratory facilities, located in Sarasota, Florida. During the first two years experiments were carried out at the main aquaculture facility located at Lido Key barrier island (Figure 5.2). The following two years, due to the expansion of the Aquaculture facilities, the experiments were carried out 20 miles inland at the new Mote Aquaculture Park (Figure 5.2), part of the center for aquaculture, research and development (C.A.R.D)

Figure 5.2. Aerial photo from Lido Key (left) and the Mote Aquaculture Park (right)

Although artificial sea water (Instant Ocean ®) was used at both locations, conditions at the first location (Mote Marine Laboratory at Lido Key) were not as hygienic or controlled as in the second location (Aquaculture park), due to the construction work that was taken place on the surroundings.

The main difference between the two locations was the possibility to have the rearing systems in a temperature-controlled environment. That was the case of the Aquaculture Park, characterised by their state of art technology, which allowed the control of air and water temperature, light intensity, as well as having the system in an isolated room without human traffic. Also, the

absence of dust and insects, such as ants, was an important factor compared to the first site.

5.2.1 Live culture

The suitability of three types of live feeds (Microalgae, Rotifers and copepods) were investigated in parallel. Each culture was independent from the other, in separate rooms, using water from a different water reservoirs, in order to avoid any possibility of cross-contamination.

5.2.1.1 Microalgae

The main microalgae used in this study was *Nannochloropsis occulata* (Figure 5.3)*;* this is a 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 and chlorella because of its lack of chlorophyll b. These algae are a popular food source for rotifers and filter feeders in general.
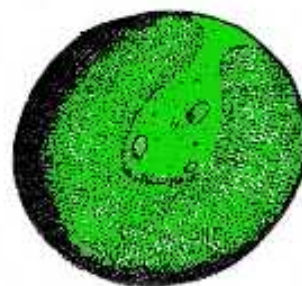
Figure 5.3. Nannochloropsis oculata

The *Nannochloropsis occulata* was used to feed the rotifer cultures and to create a green water environment in the larval system.

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 are transferred into 250 ml flasks and later (1 week) into larger 19L 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 ensured a reliable *Nannochloropsis* production (Figure 5.4). All the cultures were exposed to 24 hours white light condition (1000 lux), water temperature was kept at 29 ºC with light, constant aeration.
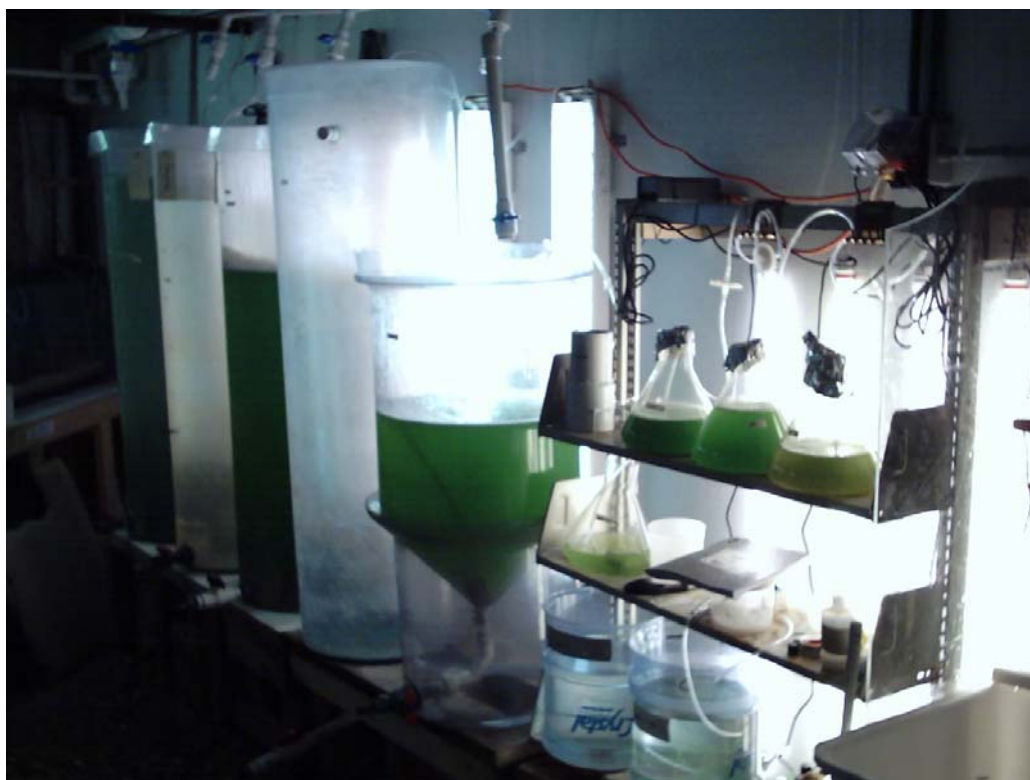
Figure 5.4. *Nannochloropsis oculata* **culture tanks**

Nannochloropsis oculata was also obtained from Reed Mariculture, as a paste. This paste consists in a highly concentrated media (68 billion/ml) of *Nannochloropsis oculata* that was kept frozen till the day prior to use. This alternative was used in order to reduce the time involved in the batch culture and to test the difference between fresh and frozen paste algae.

5.2.1.2 Rotifers

During the course of this study four different types of rotifers were used.

In aquaculture, a simple classification is used, which is based on two different morphotypes, namely *Brachionus rotundiformis* or small (S-type) rotifers and *Brachionus plicatilis* or large (L-type) rotifers. The two types can be clearly distinguished by their morphological characteristics: the lorica length of the L-type ranging from 130 to 340 µm (average 239 µm), and of the S-type ranging from 100 to 210 µm (average 160 µm). Moreover, the lorica of the S-type shows pointed spines, while of the L-type has obtuse angled spines (Figure 5.5).
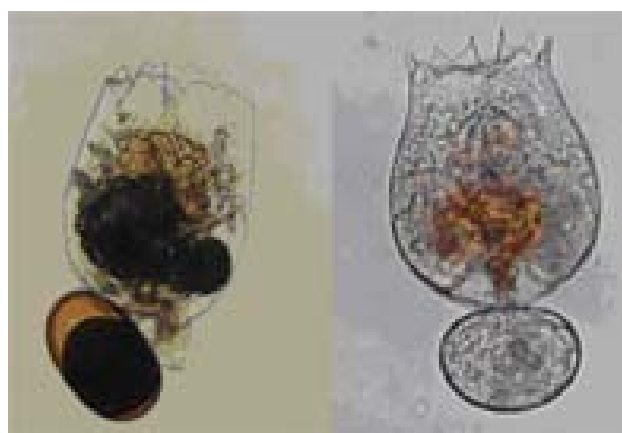
Figure 5.5. Brachionus plicatilis (left) rotundiformis (right)

Apart from the L and S type rotifers described above, two other types of rotifers were used:

1) The SS type rotifer (Super small rotifers) ranging 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 at start feeding of less than 100 µm). Those rotifers, however, are not genetically isolated from S-strains, but are smaller than common S-strains (Person Le Ruyet *et al.* 1993)

2) Another strain of the SS rotifers from the University of Ghent, Belgium, were genetically modified to resist warmer temperatures (above 30 ºC)

All the above mentioned rotifer strains were cultured to feed the experimental common snook larvae. The chosen method of cultivation was batch culture. 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 the batch culture, a total harvest of the rotifers is applied with part of the rotifers used as food for fish larvae and part used as inoculum for the next culture (Hirata, 1980; Lubzens, 1987). All the rotifers were fed with *Nannochloropsis oculata*.

5.2.1.3 Copepods culture

The calanoid copepod *Acartia tonsa* (*Acanthacartia*) (Figure 5.6) was cultured for some of the feeding experiments. This species was chosen due its small size (80-100µm), nutritional value and availability.

These 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 kept in the fridge at 4 °C till need it.

Prior to feeding, copepods eggs were taken out of the 100 ml flask and placed in a 500 ml transparent flask with seawater at 35 ppt and 28 ºC under a 12 hours light period (12-12). No aeration was need it during the 48 hours

hatching period. After this period *Acartia tonsa* were fed to the snook larvae. Feeding densities varied depending on the experiment.

Figure 5.6. Calenoid copepod *Acartia tonsa* eggs and naupulii

5.2.2 Larval rearing systems

All the experiments run during this study were conducted using two independent experimental systems.

5.2.2.1 System A "Microcosms"

System A (Microcosms), was a self-contained re-circulating system (Rana, 1986). The system was made of transparent plexiglass, contained 48x2L tanks (Figure 5.7), which allowed several different experiments to be run at the same time, with appropriate replication. The system dimensions were 1.25 m in length by 70 cm in width by 20 cm in height. The single unit tank dimensions (Figure 5.7) were 12x10x20 cm, the screen (75 µm mesh size), the drain hole was 17.5 cm from the bottom. Apart from the larval containing tanks, the system was formed by two sumps (Figure 5.8), a 140 liter sump A

filled with bio-filtration beads and a 120 liter sump B, with a fluidised bed and a carbon filter. Air stones, were placed in Sump A as well as in sump B, a pure oxygen ceramic stone was also present at sump B, in order to keep the dissolved oxygen levels between 8 to 10 mg/l
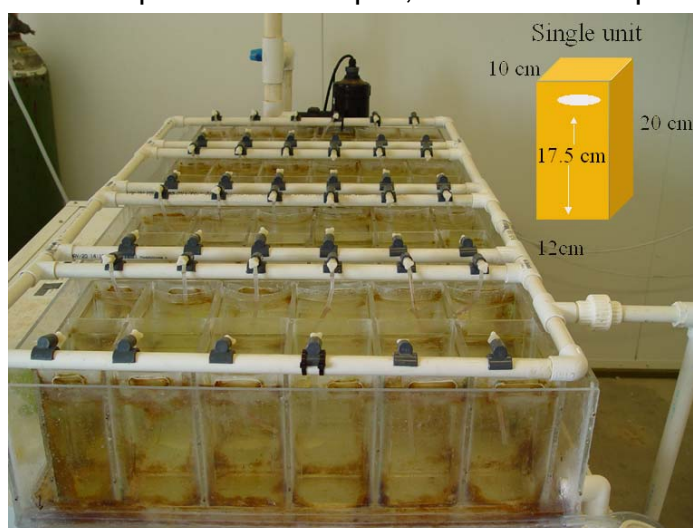
Figure 5.7.System A (Microcosms) and individual tank dimensions

Each compartment flow rate was individually regulated, through a drip valve. The flow rates varied between tanks depending on the experimental requirements. Detailed information on flow rates is given in section 5.2.3.3. The tank re-circulation system was based on an overflowing system, with a drip valve on the inflow and a 75µm meshed rectangular hole as the tank's out flow. The first 3 days after hatching a transparent plastic separator was placed close to the outflow to avoid egg and larvae agglomeration against the mesh to reduce possible mortalities. All the tank outflows ran to a common drain canal through the UV light filter (Figure 5.8) and into the sumps.

Figure 5.8. Microcosm filtration system, A) UV light, B) bio-filtration beads C) Fluidised bed and Carbon filter (right)

Daily 10% water exchange was conducted. Water quality was checked three times a day (every five hours), the parameters checked daily were, water temperature, salinity, dissolved oxygen and pH. Nitrites, ammonia and nitrates 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, as given in the feeding experiment protocols.

5.2.2.2 The B system

The B system was a re-circulating system, built in 2005 as an alternative experimental system for the microcosms; although it was run at the same time, in order to compare the influence over the larval survival. The main difference lay in the individual tank water volume, which was higher (6 l).

This system was based on a green bottom fiberglass raceway, where individuals tanks were dipped in (Figure 5.9). Twelve (6 l) tanks shared the same filtration system, which was formed by a UV light, a protein skimmer, bio-filtration beads and a fluidised bed. The system had two 300-litre sumps underneath the raceway, where all the filtration system was set up; also air stones were placed to keep the dissolved oxygen at the desired levels. Water

heaters were also placed on the bottom of the raceways to keep the temperature constant.

Figure 5.9. Larval rearing System B. Full system and individual rearing tanks (left to right)

Inflow water flow was regulated, individually per tank, and outflow water was passed through a 75µm mesh standpipe, draining into a common draining canal (Figure 5.9, middle picture) leading to the first sump. Slight aeration was also supplied to the individual tanks. A 25% water change was carried out every week. As in the microcosms, water quality (water temperature, salinity, dissolved oxygen and pH) was checked three times a day (every five hours). Nitrites, ammonia and nitrates were checked on a weekly basis.

5.2.3 Experimental methodology

Snook eggs were stocked in the experimental systems 2/3 hours after fertilization. Eggs were stocked in all the experiments at a salinity of 35ppt, temperature of 28°C (except for the temperature experiments), 9-10 mg/l of dissolved oxygen and at pH 7.9.

During the first two years larvae were fed 3 days after hatching and 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.

5.2.3.1 Influence of temperature on hatching percentage

After collecting common snook eggs in the field, eggs were fertilized and transported to the laboratory, where they were stocked in 2 l PVC floating containers (Figure 2.3), similar containers were at the same time floating in a 300 l raceway tank. Four raceway tanks were used, each one of them had a different temperature (23, 26, 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 between the acceptable values. Twenty hours after fertilization, tanks were removed from the raceways and hatching percentage calculations were made (see the formula below). This experiment was repeated four times to obtain more reliable results.

Hatching rate= Hatched larvae *100/ Total number of eggs stocked

5.2.3.2 Influence of egg stocking densities on larval survival and growth.

Nine tanks were used for this experiment; all were stocked at the same time. Tanks were stocked with three different egg densities; in triplicate. The densities were: high density (375 egg/l), medium density (200 eggs/l) and low density (100 eggs/l). All tanks were fed S type rotifers three times a day at a concentration of 30 rot/ml. After 14 days from hatching all tanks were harvested, and larvae counted and measured (total length and myomere

height). This experiment was run in both system A (microcosmos) and B (raceway).

5.2.3.3 Influence of tank flow rate on larval survival

Common snook eggs were stocked at a density of 200 eggs per L. All tanks were fed three times a day with SS type rotifer at a concentration of 30 rotifers/ml. Tanks stocked with snook embryos were exposed to three different flow rates (No Flow, slow flow (10 ml/min) and high flow (30 ml/min)).

Twenty seven tanks were used in this experiment, nine per flow treatment. Larvae were harvested on day 3, 6 and 10 after hatching in order to check for survival. Each flow treatment had 3 replicates per day. A slow flow rate was set at 10 ml/min and high flow rate was set at 30 ml/min. The experiment was terminated at day 10 after hatching, all remaining tanks were harvested.

5.2.3.4 Effect of background phytoplankton in the water (Green water technique) on larval survival.

Ten tanks were used in this experiment. All were stocked at the same time and at the same standard density (200 egg/l). Flow rate was set at 15ml/min. All tanks were fed SS type rotifers three times a day with a concentration of 30 rot/ml. Five tanks were stocked with *Nannochloropsis oculata* at 1000cells/ml (green water) and five tanks without (clear water). The experiment was terminated at 14 days post hatching, all larvae were harvested to establish larval survival.

5.2.3.5 Influence of rotifer density on larval survival

This experiment used 15 tanks, five tanks per density. 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 days after hatching and counted to calculate survival. This experiment was run in systems A (microcosmos) and B (raceway).

5.2.3.6 Comparative influence between copepods and rotifer feeding on larval survival and growth.

Twelve tanks were used in each system for this experiment, three tanks per experiment diet. Four diets were given to the larvae: i) 100% rotifers, ii) 75% rotifers and 25% copepods, iii) 50% rotifers and 50% copepods and iv) 75% copepods and 25 % rotifers. All the tanks were stocked at the same time and fed 30 prey/ml; the amount of each prey (rotifer or copepod) was determined by the above-mentioned percentages. Flow rate was maintained at 10ml/min during the whole experiment, which lasted 14 DAH. Total length and myomere height were taken on day 14 DAH, also stomach contents were looked at. This experiment was run in both systems.

5.2.3.7 Acceptance of micro-diet feeding by larvae

A total of 21 (2L PVC) floating tanks were used during this experiment placed on a green fiberglass race way. Each tank was stocked at the same time with

300 egg/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 with a specific diet. The three diets were: i) a SS type strained (150 µm) rotifers diet at a density of 30 rot/ml fed three times a day, ii) A 100 µm artificial micro-diet fed twice a day, iii) A 150 µm artificial micro-diet fed twice a day. Every day three tanks were taken out and larval stomach contents were examined under a microscope.

5.2.3.8 Influence of four feeding regimes on larval survival

Twelve tanks from system A were stocked with 200 eggs/L for 14 days. All the tanks were fed enriched SS type rotifers at a density of 30 rot/ml. The flow rate was set at 10 ml/min. Four feeding regimes were experimented with: 1) Feeding once a day (8 am), 2) Feeding twice a day (morning and afternoon), 3) Feeding three times a day (8 am, 12 pm and 4 pm), 4) Feeding 4 times a day (8 am, 12 pm and 4 pm and 8 pm). Fourteen days after hatching all the tanks were harvested and total survival was calculated.

5.2.3.9 Effect of enriched rotifer feeding on larval survival and growth

Ten tanks were stocked with 200 eggs/l at the same time. Five tanks were fed S type rotifers at a density of 30 rot/ml and the other five tanks were fed DHA/Selco enriched S type rotifers at a 30 rot/ml density. Larvae were fed three times a day (8 am, 12 pm, 4 pm). Flow rate was set at 10 ml/min. 14 days after hatching all the larvae were collected, counted and measured (Total length and myomere height)

5.3 Results

5.3.1 Influence of temperature on hatching rate

The influence of incubation temperature on hatching rate was investigated to establish the optimal temperature for incubating snook eggs. Embryos exposed to 23ºC water had the lowest mean hatching rate of 5.9%, Hatching percentage ranged from 0.8 to 11.4% (Figure 5.10). Embryos stocked at 26ºC had a higher mean hatching percentage (11.9%) and values ranged from 4.9 to 17.5%. The second highest hatching percentage was found with embryos that were stocked at 30ºC water with a mean hatching percentage of 19.8, values ranged from 10.5% to 25.3% (Figure 5.10). The temperature to which the mean highest hatching percentage happened was at 28ºC, with a value of 23.5%, ranging from 21.7% to 27%.
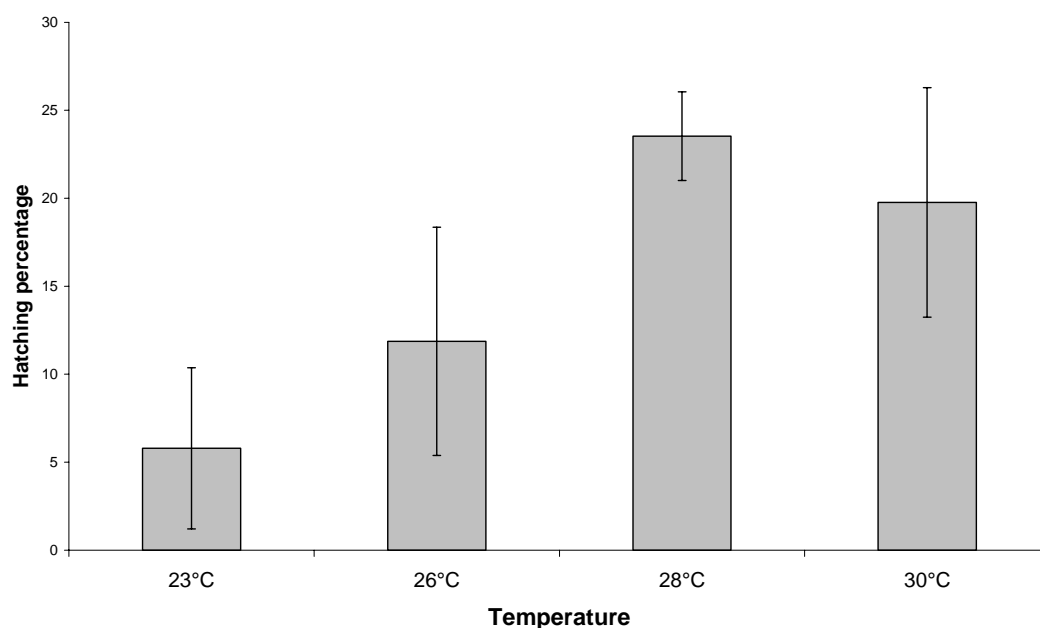
Figure 5.10. Influence of four temperatures on snook larval hatching percentage

No significant difference in hatching rate (p> 0.05) was found between the 28 and 30°C treatment, but a significant difference (p<0.05) with respect to the 26 and 23°C was found.

5.3.2 Influence of egg stocking densities on larval survival and growth

The effect of egg stocking density on larval survival at 14 days post hatching is given in Figure 5.11. 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 percentage was obtained at the low density stocking (100 egg/l) with a mean overall 1.2 % survival by day 14 after hatching (Figure 5.11). 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.
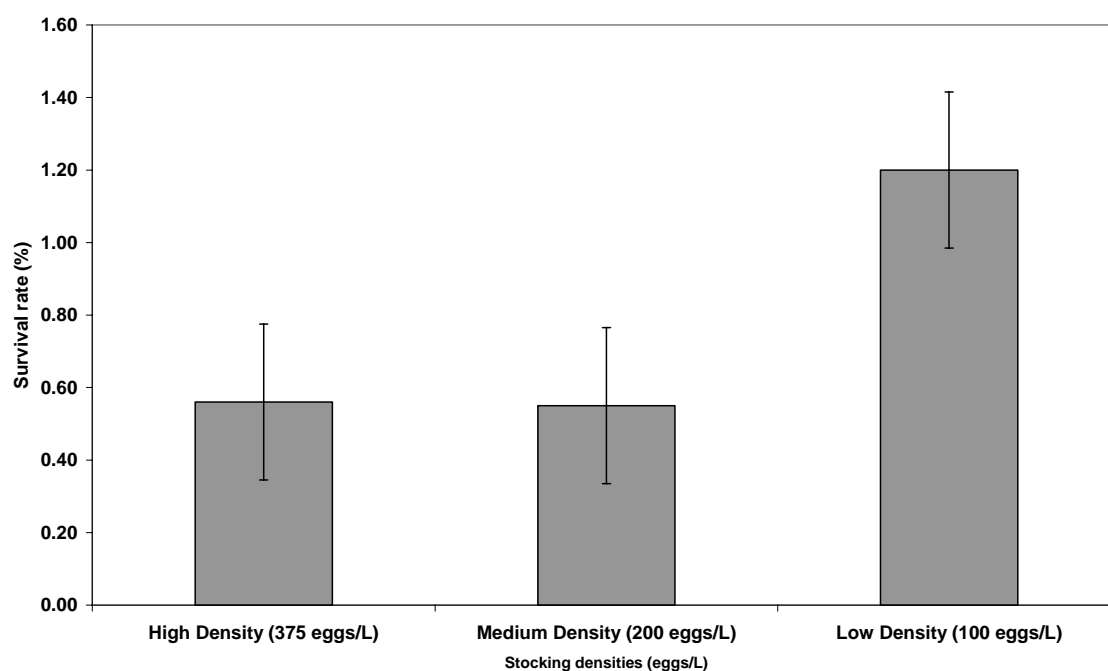
Figure 5.11. Larval survival from three stocking densities after 14 DAH

5.3.3 Influence of tank flow rate over larval survival

Snook larvae were exposed to three different flow rates. Those fish exposed to no flow during the first 10 days (Figure 5.12) had a mean 45 % survival rate by day 3 after hatching, survival then decreased to 19%by day 6. At 10 DAH, larval mean survival was only 5% from the initial stocking density. The slow flow (10ml/min) treatment had a mean survival of 40% at 3 DAH, decreasing 3 days later to a 32% survival. Finally a mean 21% survival was observed 10 DAH (Figure 5.12). The third experiment had the lowest values in terms of survival values. At high flow (30ml/min), survival from 3 DAH was 16%, decreasing on Day 6 to a mean 10% survival and by day 10 after hatching larval mean survival was 9% (Figure 5.12). 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.
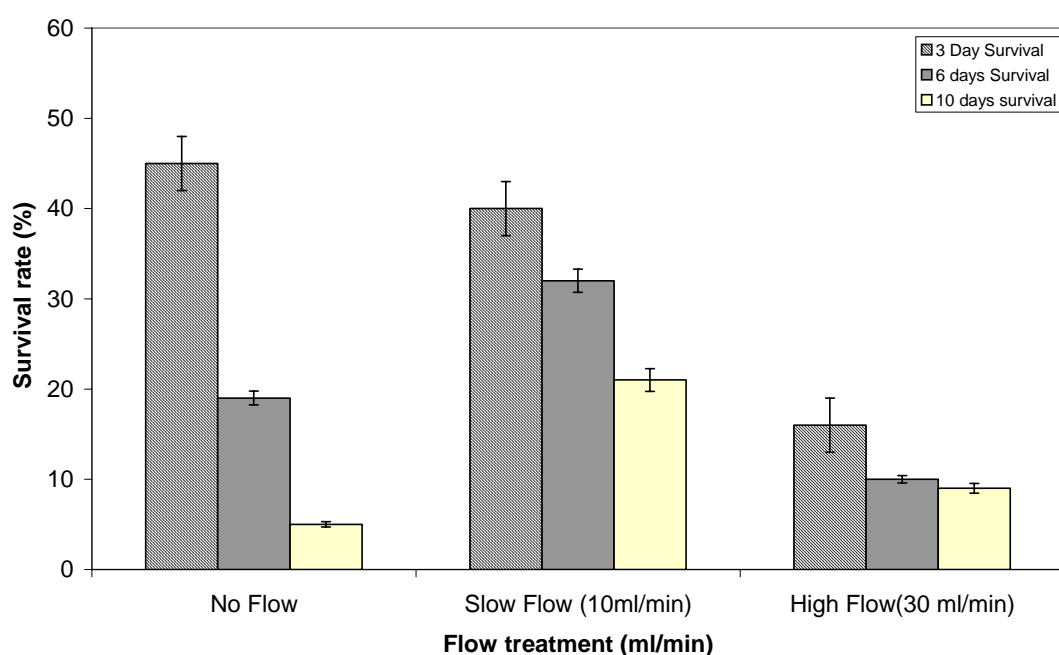
Figure 5.12. Snook larval survival results on three types of flow during 10 DAH

5.3.4 Effect of background phytoplankton (green water technique) on larval survival

Larvae were stocked in water without any algae and in water with algae (*Nannochloropsis occulata*) at a concentration of 1000 cells/ml. Larvae tanks without any algae are referred to as 'clear water' in this study (Figure 5.13). The survival of larvae was significantly ($p < 0.05$) influenced by the presence of *Nannochloropsis 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 5.13).
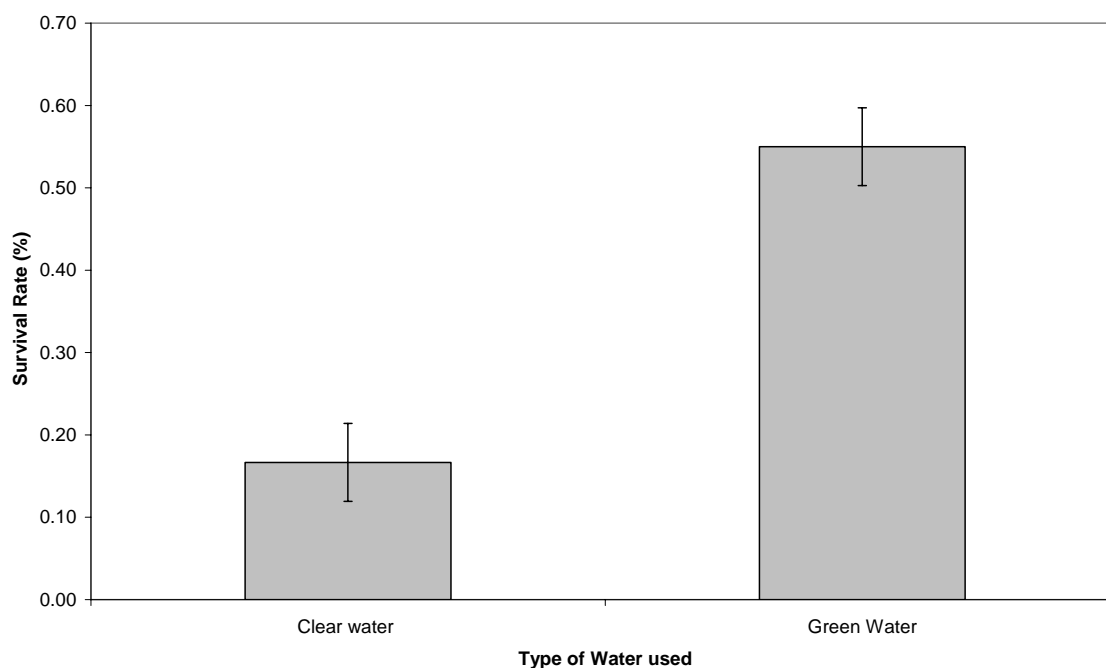
Figure 5.13. 14 DAH snook larval survival from tanks with Nannochloropsis oculata (Green water) and without any phytoplankton (clear water)

Length and myomere height from the clear water tanks larvae had an average standard length (SL) of 3.20 mm and average myomere height of 0.70 mm (Figure 5.14);) 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 values from the green water tanks are higher, no significant difference was found (p= 0.053).
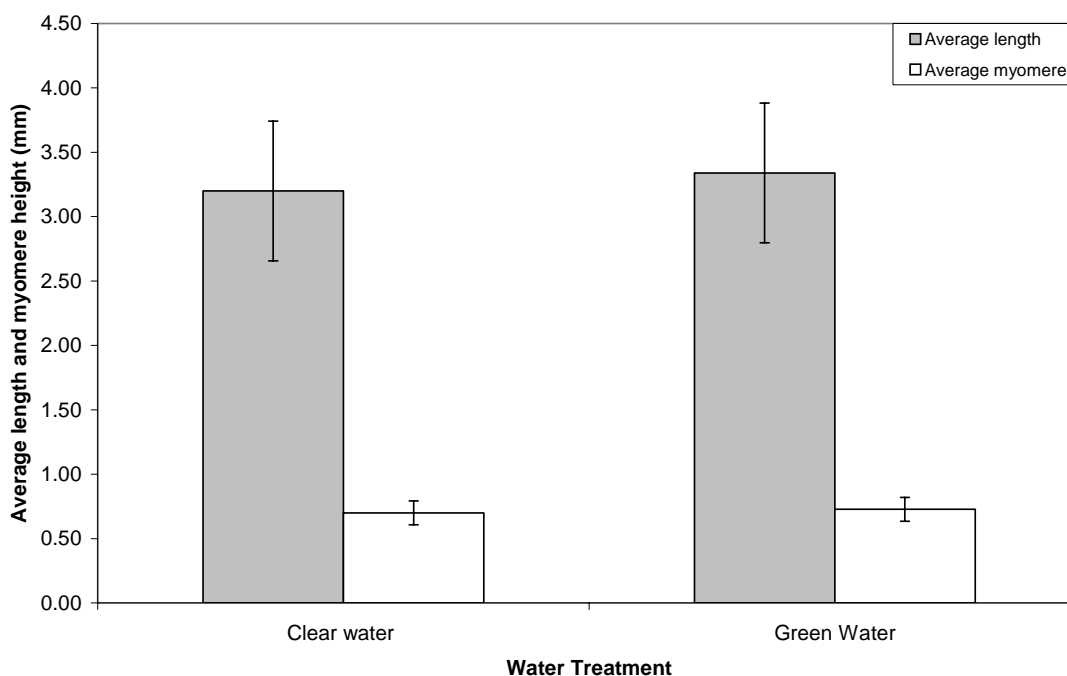
Figure 5.14. Average snook larval length (SL) and myomere height from green water and clear water tanks

5.3.5 Rotifer density feeding experiment

Three different rotifer densities were tested on growth and survival of common snook larvae (Figure 5.15). After 14 days, larvae fed a 5 rotifer/ml diet had an average survival rate of 0.86% and only 13% tank 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 (Figure 5.15). The third experiment using a 30 rotifers/ml diet, 20% of all tanks had live larval and a total average of 3.67% larval survival. No significant differences were found in larval survival between tanks fed 15 and 30 rotifer/ml (p=0.053).
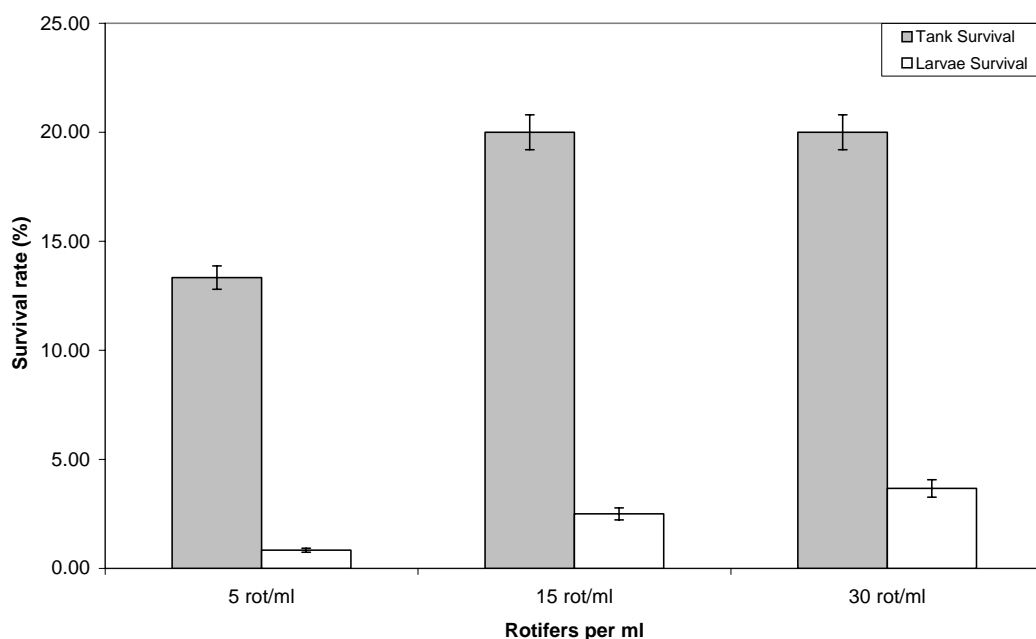
Figure 5.15. Rotifer density experiment using three densities 5, 15 and 30 rot/ml

Increasing rotifer concentrations from 5 to 30 rotifers/ml did not significantly (p>0.05) affect the larval length or myomere height (Figure 5.16). Larvae fed with 15 rotifers per ml had the highest mean length (SL) with 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 (Figure 5.16)

5.3.6 Comparative influence between copepod and rotifer feeding on larval survival and growth

Four different diets were used in this experiment; 3 using *Acartia tonsa* and SS type rotifers, one with 100% rotifers. The diet with 75% copepod and 25% rotifers (Figure 5.17) had the highest mean survival with a 1.44%; the second highest average larvae survival was the diet formed by 100% rotifers with a

survival of 0.83%. The other two diets (50% rotifers 50% copepods and 25% copepods 75% rotifers) had a similar result with a 0.61% mean survival (Figure 5.17). A significant difference was found between the 75/25 copepod/rotifers diet and the other three diets ($p > 0.05$), on the other hand no significant difference was found between the 50/50 and the 25/75 diets ($p < 0.05$)
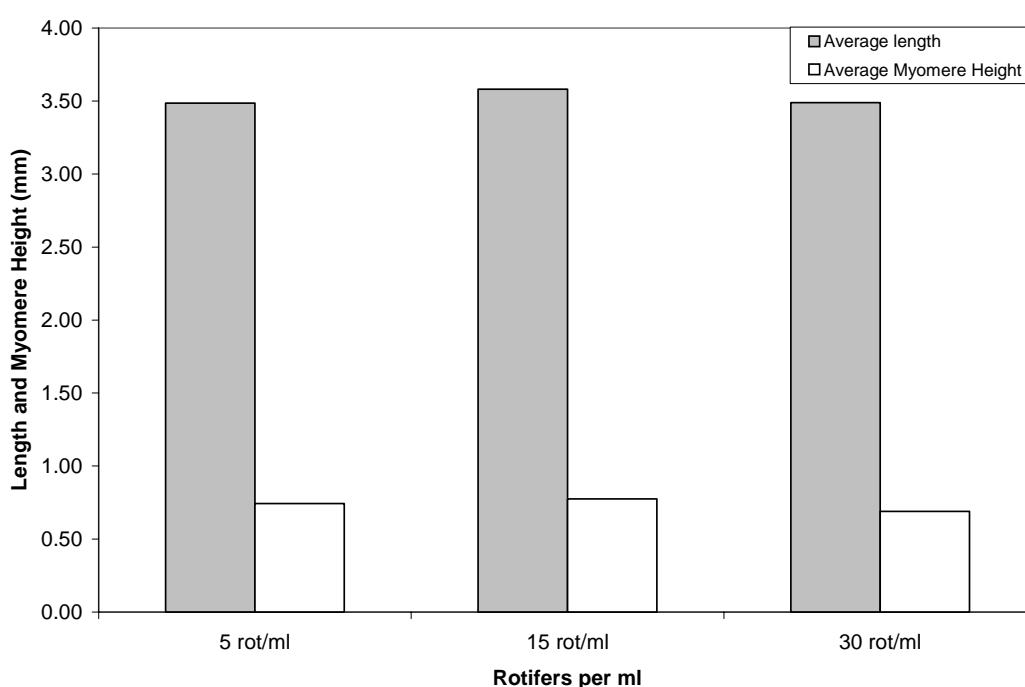
Figure 5.16. Snook larval length (SL) and myomere height from three rotifer density diets

The influence of live feed combinations on larval growth is presented in figure 5.18. Live feed combinations on larval growth had no significant ($p < 0.05$) effect. The 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, very similar to the 100% rotifers diet which had an 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 (Figure 5.18).
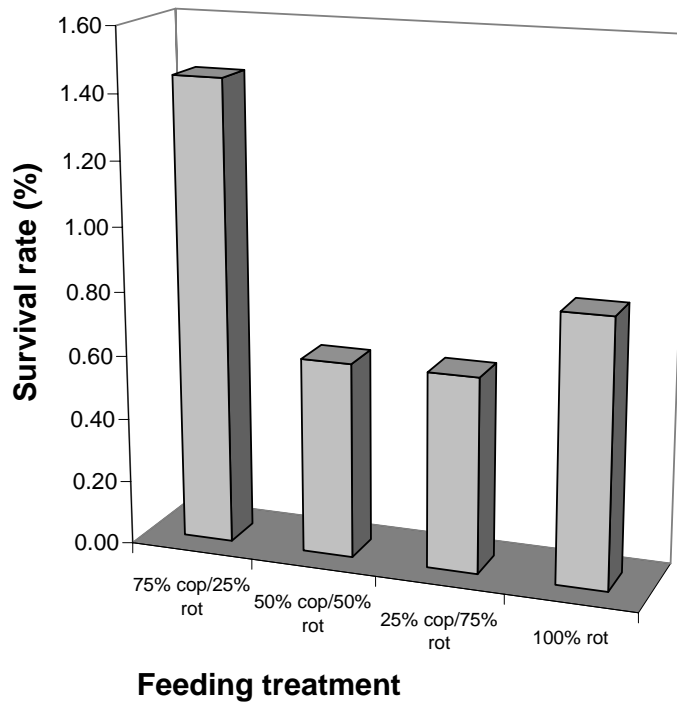
Figure 5.17.Snook larval survival percentage after 14 days from four diets that combined copepods and rotifers at different percentages. Feeding treatment 75/25 consisted on 75% copepods and 25% rotifers, 50/50 consisted on 50% copepods and 50% rotifers and the 25/75 consisted of a diet formed by 25% copepods and a 75% rotifer
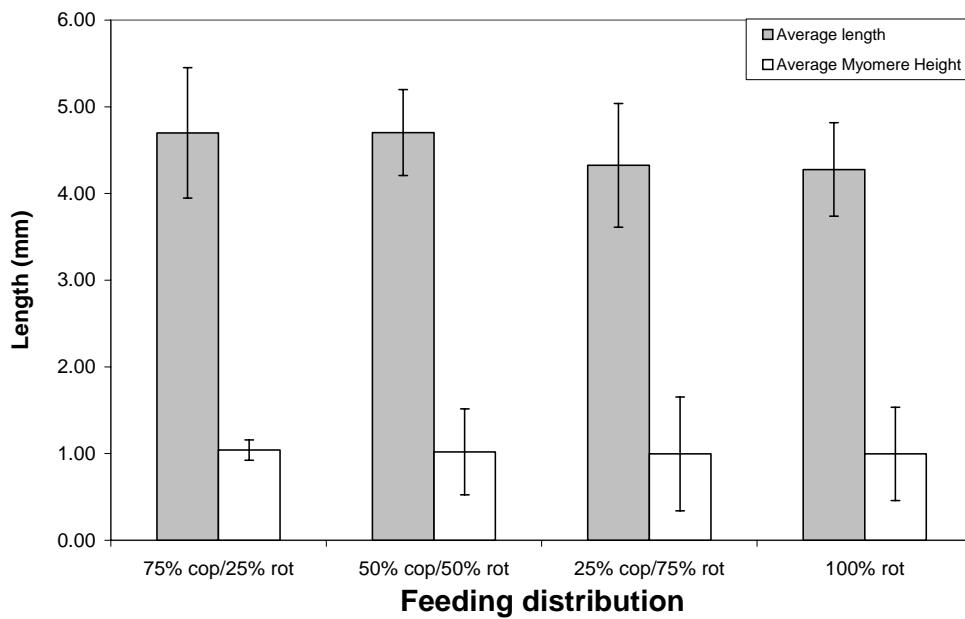
Figure 5.18. Average length (SL) and myomere height from larvae exposed to four diets
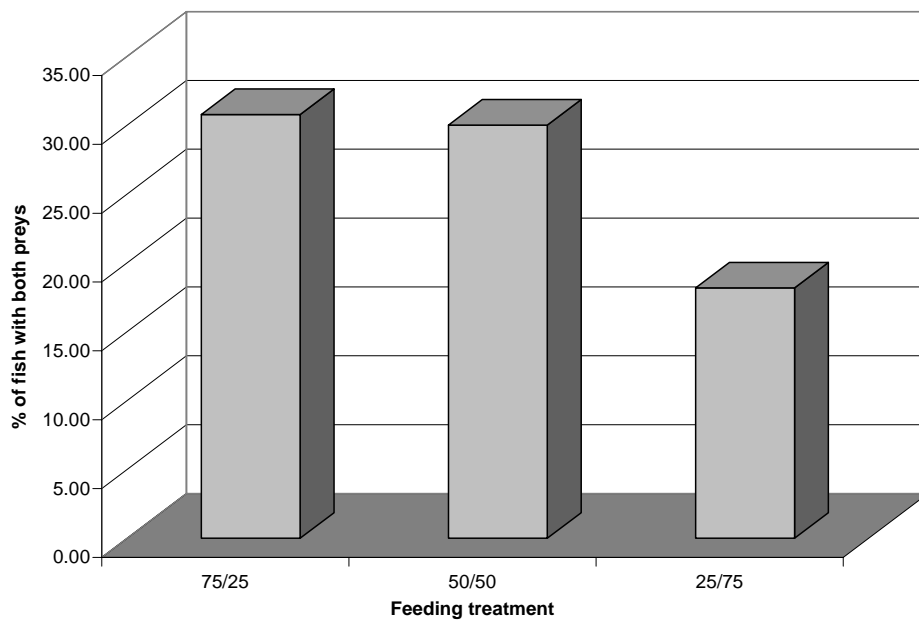
Figure 5.19. Percentage of rotifers and copepods found in the snook larvae stomach contents from four diets

The stomach contents of 14 day old larvae were examined for food items (Figure 5.19), 30.8 % of larvae from the 75/25 treatment had both rotifers

and copepods in their guts, from the 50/50 diet, 30% of the larvae had also both rotifers and copepods and the 25/75 diet had 18 % of the larvae with both rotifers and copepods in their guts. The rest of the larvae only had rotifers.

5.3.7 Acceptance of micro-diet feeding by larvae

The stomach contents from larvae fed micro-diets were examined in this experiment (Figure 5.20). The experiment duration was 7 days, starting from day 2 after hatching.

In tanks fed with rotifers, the percentage of larvae with rotifers in the stomach, after 7 days were: 10, 15, 24, 24, 35, 37, and 19%, respectively (Figure 5.20). Tanks where 100µm micro-diet food was offered, percentage of larvae with food was: 8, 10, 6, 20, 15, 25, and 28%, respectively. Tanks where the 150µm micro-diet was offered the percentage of larvae with food in their stomach were: 12, 7, 18, 15, 27, 20, and 27% respectively (Figure 5.20)
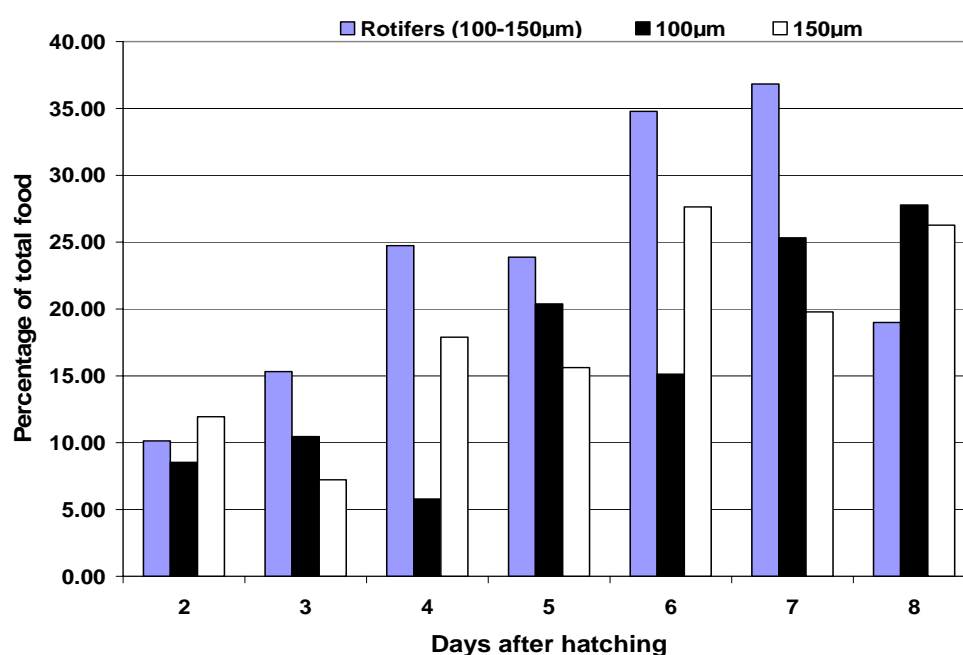
Figure 5.20. Percentage of larvae with food in the digestive system from three diets, SS type rotifers, 100 and 150µm micro-diets

5.4 Discussion

5.4.1 Importance of temperature during incubation

Fish are affected by many intrinsic and extrinsic factors. These can affect developmental controls resulting in phenotype alterations (Johnston *et al.*,1996; Adriaens & 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). As well as having a direct effect on their physiology through its effects on enzyme reaction rates (Hochachka and Somero 2001).

In the present study, hatching rates varied with temperature. Eggs incubated at 28°C showed the best hatching rates, although no larval growth study was carried out. These results confirms findings of Rideout *et al.*, (2004) who pointed out the direct influence of temperature during the incubation period over larval growth, which also has a direct effect over hatching percentage (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).

5.4.2 Influence of 'green water' on larvae

Food availability is a key factor during first feeding and the consumption rate is dependent on it. Additionally, the developmental stage of the individual affects the consumption rate (Houde and Schekter, 1980; Kentouri, 1985). Based on the previous findings a simple experiment was run, where food was offered to the larvae from day one after hatching to be compared against larvae that were not offered anything till the first feeding day. Final results showed a significant difference in survival between the two treatments, where larvae offered *Nannochloropsis 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 supports the idea that microalgae should be 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 hypothesis that has been put forward to explain the role of phytoplankton in the 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). Recently, phytoplankton has been reported as being a protecting agent, antagonistic towards pathogenic bacteria (Kennedy et al., 1998; Støttrup *et al.*, 1995). A further hypothesis is the effect that a concentration of phytoplankton has on the water, producing a background effect; which allows the fish to better locate its prey (Marliave, 1994). This effect has been documented to improve larval rearing in many fish species, such as 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* (Dendrinos *et al.* 1984), barramundi *Lates calcarifer* (Pearce 1991); and red porgy *Pagrus pagrus* (Rotllant *et al.* 2003). Also, Skjermo and 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.

5.4.3 Effect of enriched rotifers on common snook larviculture

The transition from endogenous to exogenous feeding is a vulnerable period for many species, when endogenous factors may acquire some importance (Kamler, 1992a). In fat snook larvae, Seiffert. *et al.,* (2001) observed during day 6-9 high mortality rates at yolk sac depletion, attributing this fact to possible larvae weakness or malformations. Brügger, (1995), found similar problems with the same species during his experiments, also Rodriguez *et al.*, (1998), observed high mortality of gilthead sea bream larvae during the first 2-3 days of external feeding**.**

Seiffert *et al.*, (2001) concluded that the improvement of dietary values of rotifers was not sufficient to solve low survival during larvae rearing. Tamaru *et al.,* 1993, Koven *et al.*, (1990), Mourente *et al.*,(1993), Rodriguez *et al.*, (1998), and Dhert *et al.*, (1990), all found no improvement in larval survival

when fed enriched rotifers. Taking the above conclusions into consideration, the larval rearing experiments in this study focused on basic larval rearing techniques in order to develop a reliable common snook rearing protocol; establish stocking densities, background phytoplankton, optimal flow rate, prey selection, prey densities. Since rotifer enrichment tend to foul the experimental systems, and no improvement in larval survival may occur (Tamaru *et al.,* 1993, Koven *et at.,* 1990, Mourente *et al.,*1993, and Rodriguez *et al.,* 1998, Dhert *et al.,* 1990), no rotifer enrichment was carried out during any of the experiments in which rotifers were used. A trial using enriched rotifers versus non-enriched rotifers was attempted but not enough data was obtained due to the very low survival on both treatments.

5.4.4 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 egg/l) to be the one with the higher survival, this finding matches Hernandez-Cruz *et al.,* (1999) who obtained low survival in red porgy when eggs were stocked in high density results. On the other hand, Tagawa *et al.,* (1997) found higher mortality following lower rearing densities in Japanese flounder and also in three other marine teleosts (Kaji *et al.,* 1999). Tagawa *et al.,* (2004) found higher survival in larvae reared at higher densities, attributing the fact 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 of this study showed clearly the low survival at high densities, which

might be due to the high level of prey competition or stocking over-crowding preventing the environment to meet basic conditions.

5.4.5 Importance of prey density on common snook larviculture

Although not at the same level of importance, prey density is an important factor when larvae and juveniles are reared (Fushimi, 1983). Low density can cause larval starvation or nutritional deficiencies leading to high mortalities. High densities can produce water quality deterioration, system fouling, decreasing Oxygen levels, increasing ammonia levels, etc... finding the right prey density is crucial to avoid the already mentioned problems and also to reduce labor and costs.

During the rotifer density experiments, the 15 rotifers/ml treatment gave the best results in terms of tank survival although 30 rotifers/ml treatments had the highest larval survival, but the difference between the two treatment was not significant, therefore and since the 30 rot/ml treatment has a higher production cost, the 15 rot/ml treatment seems to be the optimal density from the three treatments run. However, recent attempts to calculate the optimal prey density for cod larvae (*Gadus morhua*) have found that survival increases to a maximum 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 rotifer densities differ between species, for instance the black sea bream needs 1-3 rotifers/ml (Kafutu & 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, and further experiments need to be carried out to find out the optimal density, this time focusing between 10 to 20 rotifers per ml.

5.4.6 Optimal 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 of 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, cited by Hagiwara *et al.*, 2001). During the course of this study four strains of rotifers and one copepod were tested in order to find the optimal prey type for the common snook larvae; prey that will suit the physical needs as the larvae develop and grow in size.

The previous work done in snook larval culture used L type rotifers and had little success (mainly for stock enhancement). The experiments run with the L type rotifers on the experimental system showed an extremely low survival, also DHA analysis of larvae showed a steep decrease in DHA from 1 day post hatching to 6 days post hatching, DHA values in 6 days post hatching larvae were below 1% of total lipid area (Chapter 4). In both experimental and production systems mass mortality regularly occurred between day 5-6 (Chapter 4), 75-85% of all stocked tanks did not have live larvae after Day 6 (Chapter 4). Those results plus the fact that only 5% of the larvae fed L type rotifers had food in their stomachs lead to the conclusion that snook larvae

were dying of starvation, mainly due to the prey size which was bigger than the snook larvae mouth gape. Common snook larvae rearing has produced satisfactory results only when Lau & Shafland (1982), fed common snook larvae with copepods grown naturally in outdoor ponds, supporting the prey size hypothesis.

In order to solve this problem, new prey other than L type rotifers were used to feed the snook larvae. 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* (Hamilton). In common with the snook larvae this fish species are known to have a small mouth size.

Results from *Acartia tonsa* feeding experiments showed an increase in larval survival, matching the results from StØttrup & Norsker (1997) also found an increase in survival when rotifer feeding was supplemented with *Tisbe* sp copepods.

5.4.7 The use of artificial diets during the pre-weaning period

Production of marine fish juveniles in commercial hatcheries still depends on the supply of live prey, such as rotifers and *Artemia*. Compound diet substitution for live prey is crucial for lowering production costs and for sustaining production of high and constant quality juveniles (Cahu & Zambonino-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* Linnaeus, 1758) larvae to metamorphosis using an artificial diet (Adron *et al.,* 1974), many trials have been conducted, with different degrees of success, to

utilize artificial diets in larval rearing of other 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 due to conclusive results obtained in the laboratory. In European sea bass *Dicentrarchus labrax,* Person Le Ruyet *et al.* (1993) sustain good growth and survival*,* also Zambonino-Infante *et al.*, (1997), found significant growth and good survival when only fed compound diet from day 20. In the same species Cahu *et al.*, (1998) reported that 35% of larvae were fed exclusively on 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).

In this study common snook larvae were offered two sizes of micro-diets (100µm, 150µm) and rotifers for 7 days from 2 days after hatching till 9 days after hatching, although there was higher percentage of larvae with food in their stomachs on those tanks fed with SS type rotifer, a significant number of larvae offered the micro-diet had food in their stomach, similar results were observed in pike-perch (Ostaszewska *et al.*, 2005), where feeds 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 have suggested that co-feeding with live feed 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 & Zambonino-Infante, 1997; Kolkovski *et al.*, 1993). Future research on this topic should be carried out, although neither survival or growth parameters were examined in this study, snook larvae seemed to accept the artificial diet and based on the already mentioned literature findings in several marine larval species' survival and growth, the common snook larval pre-weaning period could be reduced.

5.4.8 Conclusions

Larval rearing has been the most difficult and challenging part of this thesis, it has been a learning curve from the first year, when larval survival results were zero. During the first two years, the rearing protocols were based on the previous work done with common snook, aimed at mass production of larvae for stock enhancement, at Mote Marine Laboratory. In the following 2 years, the larval rearing protocols were modified based on earlier findings and published literature. The fourth year was the most productive year when common snook larvae managed to survive past the 14 days in the experimental systems. The most important changes made were: a) the earlier first feeding day. Initial feeding commenced at 2 days after hatching rather than day 3; and b) smaller size prey were used. Smaller prey were offered as food source (SS type rotifer and Acartia sp.). These two changes improved the larval survival significantly.

The experimental period was limited to three months in any year due to the fact that seed were obtained from wild broodstock during the natural spawning season (May-August), this slowed the progress and unfortunately did not allow all the experiments to be completed.

Common snook larval survival has been improved but mortality is still high, more improvements in rearing techniques are needed to increase survival. Many factors can be dictating the low survival rate, a possible one could be the bacterial effect on the snook larvae, which was not looked at during this study, mainly coming from the food source, the rotifers. Rotifers are major carriers of bacteria (Muroga and Yasunobu, 1987; Munro *et al.*, 1993, 1994). Although in most hatcheries, special efforts are made to keep the rotifer cultures as clean as possible (Minkoff, 1994; Tanasomwang and Muroga, 1989), the billions of rotifers and their accompanying food inevitably create a high load of organic material, which is rapidly colonized by bacteria. These rearing conditions allow the bacteria to grow fast (Skjermo *et al.*, 1997; Skjermo and Vadstein, 1999; Verschuere *et al.*, 1997, Olafsen, 2001) and can also change the normal interaction between bacteria and rotifers to one that is detrimental. Vadstein *et al.*,(1993) and Gatesoupe, (1991) showed that an antibiotic treatment of rotifers prior to the administration to the larvae resulted in an increased survival of the larvae. Additionally, the egg microflora may be another source for bacterial infection; published data reported the exponential growth of opportunistic bacteria when eggs are sterilized, disturbing the balance of microbial communities (Prieur, 1982; Li and Dickie, 1985; Jeanthon *et al.,.*1988; Baticados and Pitogo, 1990; Salvensen *et al.* 2000a,b). The low competition for nutrients allows opportunistic bacteria with high growth rates

to proliferate, such bacteria may be favorable for larval development or pathogenic, the process is unpredictable (Andrew & Harris, 1986). In summary, bacterial studies on snook larval culture may help the overall survival.

During the first three years, experiments were run only in the 2 litre rearing tanks but in the fourth year different experimental tanks were used simultaneously (6L), survival results seem to be better from the bigger tanks but more research is needed to obtain more quality data. Therefore tank type could be another important factor to look into.

Overall, after four years of research on the snook larval rearing techniques, positive improvements have been made and the improvements on the existing protocol have identified the most critical bottle neck and its solutions, but more research is needed to develop such protocols.

Chapter 6:

Embryonic and larval development

6 Embryonic and larval development

6.1 Introduction

The identification of the critical embryonic and larval stages, such as eye and gut formation, first feeding, and swim bladder inflation, is essential for a better understanding of any fish species. This understanding may help to improve the common snook larval rearing techniques and therefore increase larval survival rates.

6.1.1 The digestive system and its development

Although less developed than adults, the larval digestive tract is functional when feeding begins (Govoni *et al.*, 1986). Additionally, the digestive tract evolves as the larvae grow. This facilitates changes in the rates of ingestion and digestion, and the assimilation efficiency resulting in improved larval growth (Sarasquete *et al.*, 1995).

The development of the digestive system tract, as well as the possible abnormalities and deficiencies which result from the absence or the inadequacy of food, have been studied in several teleosts (Cousin & Baudin-Laurencin, 1985; Avila and Juairo, 1987; Eckman, 1987; Ferrais *et al.*, 1987, Deplano *et al.*, 1991; Boulhic and Gabaudan, 1992). Such development is determined by the development of some key digestive system structures, which are described below.

6.1.1.1 Digestive system physiology

The gut is a tubular structure beginning at the mouth and ending at the anus. It is commonly divided into four parts. The most anterior part, the head gut, is most often considered in terms of its two components, the oral (buccal) and gill (branchial, pharyngeal) cavities. Often the mouth is not completely formed at hatching, but rapid development in many marine fish larvae leads to the possibility of taking external food before the yolk is finally absorbed. Without success in feeding there is eventually self-metabolism and loss of weight. Yolk may be transported and stored within the body, especially in the base of the primordial fin and other subdermal spaces (Shelbourne, 1956) and fat may be stored in the mesenteries of the larval gut. After the final absorption of the yolk, the larvae retain their potential to feed for some days depending on species, egg size and temperature.

Most fish larvae are predatory with a large mouth and well-developed eyes. In herring, the gape of the jaw increases by 50% during the yolk sac stage, the gape of 0.3-0.4 mm at first feeding depends on the length of larvae and, therefore to some extent, on the original egg size (Blaxter 1965). An elastic ligament at the articulation makes it possible for larger organisms to be taken (Flüchter, 1962).

The foregut begins at the posterior edge of the gills and includes the oesophagus, the stomach, and the pylorus. The foregut consists of the oesophagus and the intestine anterior to the opening of the bile duct. This posterior demarcation is arbitrary, and primarily for convenience during gross dissection, and may have little relation to the functional aspects. The midgut

includes the intestine posterior to the pylorus, although pyloric caecae are always absent in fishes which lack stomachs. The midgut is always the longest portion of the gut and may be coiled into complicated loops (often characteristic for each species) when longer than the visceral cavity. In some fish, the beginning of the hidgut is the anus. Only rarely is there a hindgut caecum in fish comparable to that found in mammals. A cloaca (a chamber common to anal and urogenital openings and formed from the infolded body wall) never occurs in teleost fish, except the Dipnoi, although it is universal in sharks and rays.

The onset of digestive functions, associated with morphological transformations, follows a sequential chronology in developing fish like that in developing mammals. The digestive tract of fish larvae is not functional at hatching, but undergoes major developmental changes over several weeks (Vu, 1983; Cousin and Baudin Laurencin, 1985; Boulhic and Gabaudan, 1992). Notably, the stomach is not differentiated at hatching. The development of this organ occurs several days or weeks later in marine fish depending on the species.

Movement along the gut is mainly by peristalsis, although Iwai (1964) found cilia in the gut of ayu (*Plecoglossus altivelis*) especially in positions posterior to the liver. Backwardly directed ciliary currents and forward peristalsis seemed to cause a circulation of the gut contents, perhaps to aid digestion. Reduction of cilia in later stages suggested a transition from cilia to microvilli.

In sea bass, for example, the stomach develops from days 25 to 30 (Vu, 1983). Pepsin activity is detected from this date, but the lack of a stomach does not hinder protein digestion, since protein hydrolysis is ensured by several pancreatic and intestinal enzymes (Zambonino-Infante and Cahu, 1994). Recent studies have concentrated on the functional changes in the digestive tract during larval development by studying the onset and the variation of pancreatic and intestinal digestive enzymes and the response of these enzymes to diet concentration and composition (Ma *et al.*, 2005).

Veggetti *et al.* (1999) stated the importance of the digestive system ultrastructure development for determining the dietary requirements of the larvae at the specific development stage of its digestive system.

6.1.2 Vision system and development

The eye development for most fish species is critical for their survival, especially once the exogenous feeding starts (Mani-Ponset et al., 1996), mainly due to their visual feeding nature (Blaxter, 1986, Batty, 1987). Prey capture, orientation, schooling and eluding predators are other basic activities that rely on vision (Porter & Theilacker, 1999, Blaxter 1986, Paul, 1983).

The role of vision in feeding has been tested experimentally and thresholds of light intensity measured. The fall off in rate of feeding corresponds with dusk and dawn periods. Some species can take food in the dark, e.g., Cisco (*Leucichthys artedi*) (Jonh and Hasler, 1956), especially when it is present in high concentrations. Some, like sole (*Solea solea*), take food in the dark for most of the larval life and others, like plaice (*Pleuronectes platessa*), only at later stages around metamorphosis (Blaxter, 1968).

Kawamura (1984), Pankhurst (1996) and Roo *et al.* (1999) reported major changes in the vision system in the lecitotrophic phase as preparation for onset of feeding in sparids. At the same time Roo *et al.* (1999) studied in *Pagrus pagrus* the relation between gut and eye development, demonstrating that vision was developed before the onset of exogenous feeding.

6.1.2.1 The vision system: the retina

The initial processing of the image that occurs in the layers of the retina is some of the most complex that makes up the sense of vision. The direct transmission path is photoreceptor to bipolar cells to ganglion. Rods and cones (Figure 6.1) are photoreceptors cells found in the outer-limiting membrane (OLM) that forms a dense outer layer of the retina. This layer is where transduction of light actually occurs.

Rods are more sensitive to light than are cones, and rods are particularly important for night vision. On the other hand cones, operate at higher light intensities and are the main receptor of daylight vision, since rods saturate at very low light level and essentially cease to function. All colour distinction is due to cone function, based on the existence of three subtypes of cones sensitive to three distinct wavelengths.

Rods and cones synapse into a column of interneurons (bipolar and horizontal cells), which process the light signal into a sensible image (Figure 6.1), and are located at the Inner Nuclear Layer (INL). The output of their interaction with the receptor cells, and with each other, converges onto the ganglion cell layer (GCL), which are the output layers from the retina to the brain. The ganglion layer (Figure 6.1) is the innermost layer of the retina. It has large

cells bodies and from these arise long myelinated axons that will exit the eye and make up the optic nerve.
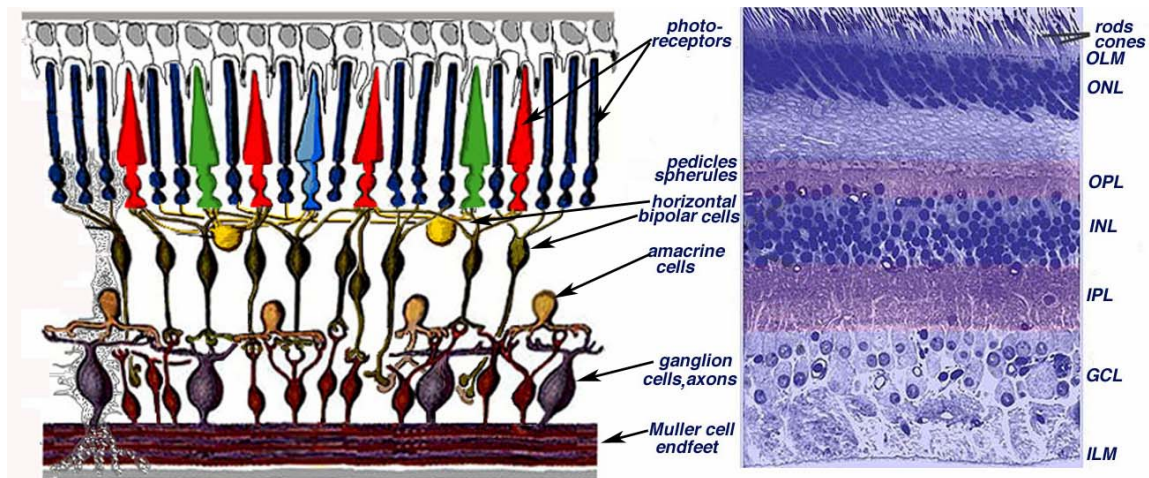
Figure 6.1. Retina ultrastructure diagram and TEM picture (OLM: outer limiting membrane, ONL: outer nuclear layer, OPL: outer plexiform layer, INL: inner nuclear layer, IPL: inner plexiform layer, GCL: ganglion cell layer, ILM: inner limited membrane) (**Kolb** *et al*, **2005**)

The Centropomus family larvae was first described by Lau & Shafland in 1982, they described the *Centropomus undecimalis* larval gross morphology for the first 47 days, focusing on their pigmentation and osteological development. Later on Alvarez-Lajonchere *et al.* (2002) described the fat snook (*Centropomus parallelus)* embryonic and larval development. Common snook embryonic development has not been described also no ultrastructure work on the larval development has been done.

6.1.3 Objectives

The main objective of this chapter is to:

I. Identify the time when the *Centropomus undecimalis* larva is able to start exogenous feeding.

II. Describe the main physiological embryonic and larval features.

III. Understand larval ultrastructure development to determine eye and gut development and timing.

6.2 Materials and Methods

6.2.1 Gamete sampling

One hour after fertilization snook embryos were stocked in a 50 litre tank at a constant temperature of 28°C and at a salinity of 35ppt, no aeration was used, dissolved oxygen was constant at 8mg/l. Eggs were kept in the dark, simulating night time light characteristics. A sample of ten embryos was collected every hour till hatching. Once collected, samples were placed under a light microscope (Olympus 3500x) with a dark field Egg diameter was measured and embryonic development was observed and documented using an attached 35 mm camera (Nikon 500). This sampling was repeated three times during three nights at different spawning events.

6.2.2 Larval sampling

All the larvae used for this experiment were taken from a 3300L production tank described in Chapter two, the water temperature was at 28°C, with a salinity of 35ppt and a with constant dissolved oxygen of 10mg/l.

Five larvae were randomly collected daily from day 0 till day 3 and every two days from day 4 till day 14; after day 14, five larvae were collected every 15 days till day 80 after hatching.

Once collected they were placed under a light microscope (Olympus 4000), which had a digital camera mounted (Sony 600), larval pictures were taken

under dark field conditions. Standard length (SL) and myomere height from the specimens were also recorded using a calibrated microscope reticule.

This sampling regime was repeated on seven different spawning events, collecting a total of 280 larvae, between day 0 till 14 and 60 samples from day 15 till day 80.

Another set of ten larvae was collected from day 1 after hatching till day 3 after hatching (included), once collected larvae were fixed for TEM and SEM work.

6.2.3 Electron microscopy specimen fixation and preparation

Larvae collected for TEM were fixed in Karnovsky's fixative at 4°C for two hours and placed in cacodylate buffer (pH 7.5) at 4°C until further processing at the Stirling University Histology department. Once at the processing unit specimens were processed as detailed in table 6.1.

Once processed, block sectioning was carried out using a glass knife for semi thin sections (0.5µm) to be observed under the light microscope. For TEM, block sectioning was carried out with a diamond knife to take ultra thin sections (50nm). Ultra thin sections were then mounted on a coper grid and stained with a metal stain, this step was carried out following a metal stain protocol described below. A drop of saturated uranyl acetate was placed on a piece of dental wax and the grid was floated over the drop for 30 minutes in the dark. The grid was then rinsed firstly with 50% alcohol and then with distilled water and floated on a drop of lead citrate for 25 minutes. Once again the grid was thoroughly rinsed, dried on filter paper and immediately removed

to avoid dust accumulation on the grid. The section was then ready to screen.

Sections were viewed and photographed at the Institute of Aquaculture

Transmission Electron Microscope (FEI Tecnai E2 Biotwin).

Table 6.1. Processing protocol for TEM specimens

CHEMICAL/MATERIAL		TIME (hr:min)
primary fixation in Karnovsky's at 4°C , pH 7.2		02:00
rinse in cacodylate Buffer at 4°C twice		01:00 each
post-fix in 1% osmium in distilled water		01:00
rinse in distilled water twice		01:00 each
en-bloc stain:2% uranyl acetate in 30% Acetone		01:00
dehydrate through acetone series at room temperature		
	25%	00:10
	75%	00:10
	95%	00:10
	100% (4 changes)	00:10 each
intermidiate rinse with propylene oxide (2 changes)		00:05 each
Infiltrate with aradite epoxy resin		
	Propylene oxide: Araldite 1:1	01:00
	propylene oxide: Araldite 1:3	00:30
	araldite 100% at 37°C	01:00
	araldite 100% at 60°C	00:30
	araldite 100% at 60°C	00:30
Place both the embedding resin and the tissue in vaccumed oven at 60°C to eleiminate bubbles		00:05
embed tissues in green block moulds		
polymerise in oven at 60°C		16:00

Specimens for Scanning Electron Microscopy (SEM) were fixed using a

different method to those for TEM. Fixing and processing protocol for SEM

can be found in table 6.2.

Table 6.2. SEM larvae fixative and processing protocol

CHEMICAL/MATERIAL		TIME (hr:min)
Fix larvae in 1% Gluteraldehyde in 0.1M Sodium Cacodylate at 4°C		02:00
Transfer to 3% Gluteraldehyde in 0.1M Sodium Cacodylate at 4°C		48:00
wash well in Buffer and carry out remainder of procedure in fume cupboard		
Post-fix in 1% Osmium Tetroxide in buffer		02:00
Dehydrate through ethanol series		
	30% Ethanol	00:30
	60% Ethanol	00:30
	90% Ethanol	00:30
	100% Ethanol	00:30
50:50 100% Ethanol/HMDS		00:30
Trasfer directly to Hexamethyldisilazane (HMDS), 2 changes		00:30 each
Air dry at room temperature in the fume cupboard		00:30
Mount on stubs and sputter coat		

6.3 Results

6.3.1 Embryonic development

Development at 28°C took 15 hours from fertilization to hatching. The fertilized eggs were spherical, with a homogenous yolk, a smooth chorion, and a single oil droplet. Egg diameters ranged from 0.65mm to 0.72mm (mean 0.69, n=300) and the oil droplet diameter ranged from 0.15 to 0.30 (mean=0.26mm n=300)

Fertilization was carried out in the field therefore no embryonic development was documented up to the blastodisc stage (first hour after fertilization). Although not observed it is estimated that the blastoderm separated from yolk approximately 30 minutes after fertilization, shortly after the blastoderm was then divided in two blastomeres.

The following is a description of development at approximate one-hour intervals:

One hour and thirteen minutes after fertilization the second segmentation or 4-cell stage occurred. The second cleavage furrow develops on two blastomeres at a right angle to the first cleavage plane. It deepens until each blastomere is divided into two cells of the same size. The oil droplet is larger and gathers toward the vegetal pole. (Figure 6.2). One hour and thirty-two minutes after fertilization another division occurs this time the blastoderm is divided in 16 blastomeres (Figure 6.3), also called the 16 cell stage, where the

fourth cleavage plane, which is parallel to the second, divides the two rows of 4 blastomeres into 4 rows of 4 blastomeres (Figure 6.3).

Figure 6.2. Four cell stage 1h AF
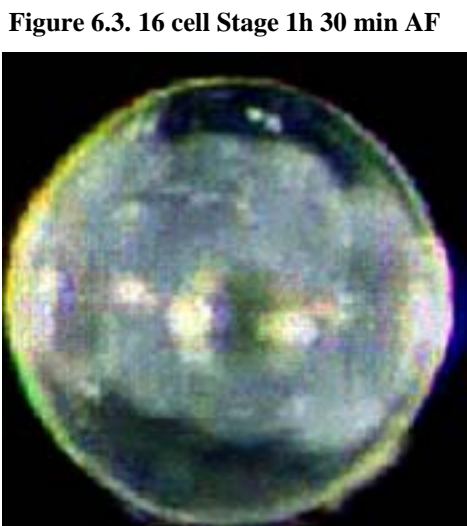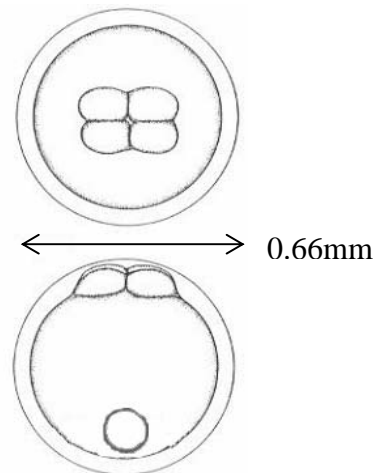
Figure 6.3. 16 cell Stage 1h 30 min AF
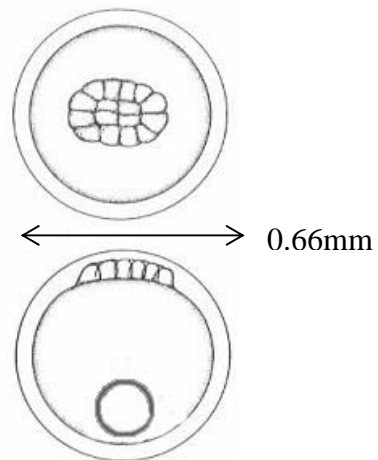
At two hours after fertilization another division occurs. This time the blastoderm is divided in 32 blastomeres (Figure 6.4), the 32-cell stage, where the fifth cleavage plane divides the marginal 12 blastomeres meridionally into 24, and the central 4 blastomeres horizontally into 8 thereby forming 2 layers, an outer and an inner layer, in the central region. The number of marginal

cells is 14 (Figure 6.4). Three hours after fertilization the blastomeres were still distinct but the rapid division is too advanced to know the number of blastomeres, this is called the late morula stage (Figure 6.5) this time the planes of the sixth and the later cleavages were difficult to precisely trace. The blastomeres (256-512) have different cleavage planes depending on their positions within the dome-shape blastoderm and were arranged in 3 layers. The peripherial blastoderms (21-24) were flattened in shape. The cells were arranged in 3-4 layers but still easily dissociated from each other (Figure 6.5).
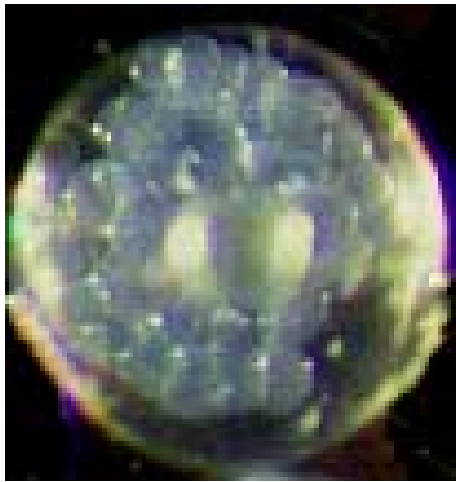
Figure 6.4. 32 cell stage (2 h AF)

0.66mm
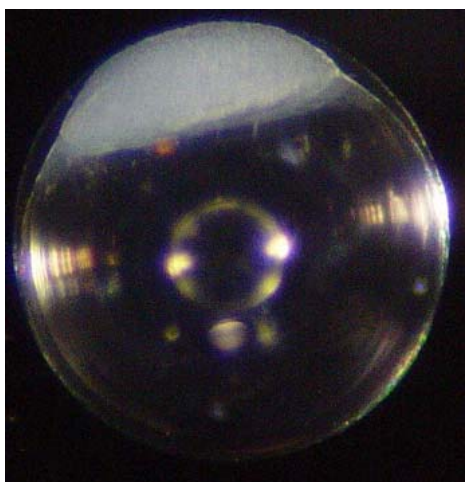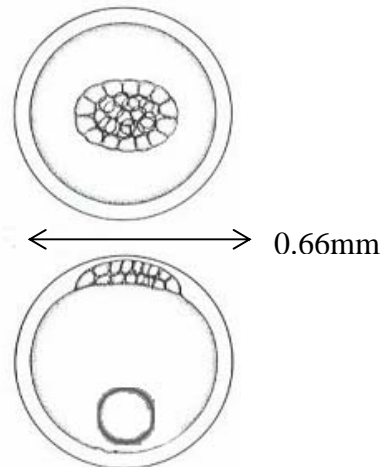
Figure 6.5. Late morula stage (3 h AF)

0.66mm
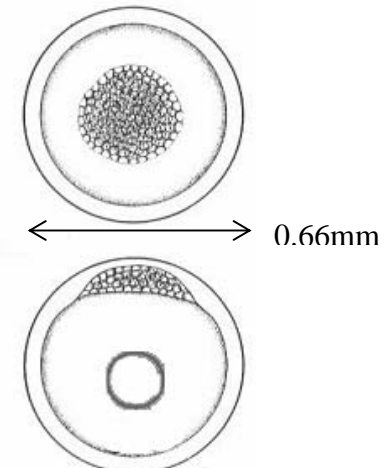
Four hours and ten minutes after fertilization, the blastomeres were no longer distinguishable and the blastocoel, or segmentation cavity, is beginning to form, this is the blastula stage (Figure 6.6). Projection of the underside of the blastoderm (central cells) into the yolk sphere is observed. In this stage, some blastomeres begin to cleave asynchronously and to migrate. Several rows of periblast nuclei were visible around the blastoderm (Figure 6.6)

Five hours and 12 minutes after fertilization the blastoderm now covers more than half the yolk (Figure 6.7) and the blastocoel is completely formed (Figure 6.7). This is the mid gastrula stage, where a streak is visible in the midline of the embryonic shield projecting into the germ ring area (Figure 6.8)

Six hours and twelve minutes later, the blastoderm covers ¾ of the yolk sphere (Figure 6.9), and the embryonic shield becomes more clearly visible as a narrow streak. The enveloping layer expends uniformly over the yolk sphere until this stage (Figure 6.9).

0.66mm
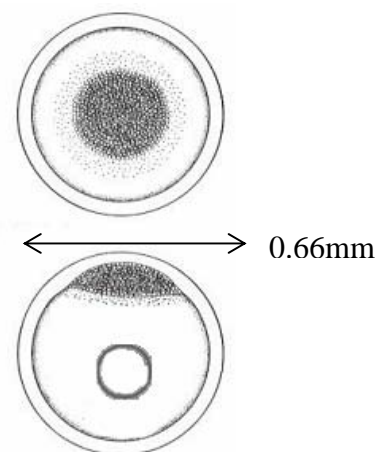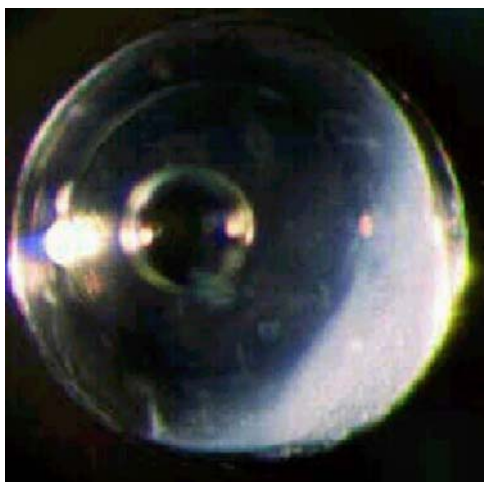
Figure 6.6. Blastula stage (4h AF)
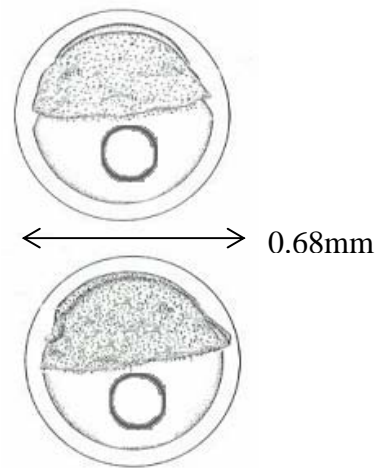
Figure 6.7. Mid Gastrula stage (5h AF)

Seven hours and 15 minutes after fertilization, the blastoderm now covers more than four fifths of the surface of the yolk, leaving a small area around the vegetal pole exposed (Figure 6.10), this stage is known as the neurula stage; the head is recognized anteriorly in the distinct embryonic body. A beak-like mass of cells is seen in front of the head, the embryo completes an arc of 150°.

Figure 6.8. Mid gastrula stage from opposite angle

The brain and nerve cord in the arrow-shaped embryonic body develop as a solid rod of cells. A solid optic bud appears on each side of the cephalic end. The beak-like cell mass is still visible and the blastopore is closed (Figure 6.10).

0.68mm
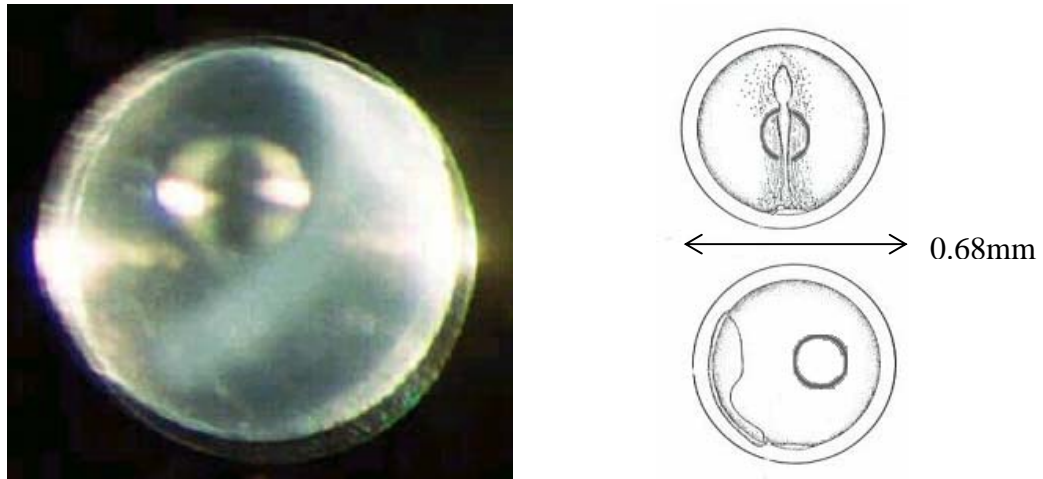
Figure 6.9. Late gastrula stage (6h AF)

Eight hours and 10 minutes after fertilization, melanophores were visible on the embryo for the first time (Figure 6.11), and the optic vesicles were clearly outlined. This is the somite stage, where a pair of otic (auditory) vesicles appears at the posterior region of the head. Depressions begin to form at the dorsal surface of the eye vesicles (Figure 6.11)

Nine hours and 21 minutes after fertilization, the embryo is strongly pigmented (Figure 6.12), especially dorso-laterally. Melanophores on the yolk concentrate on the ventral surface on each side of the embryo. Some melanophores were also apparent on the oil droplet (Figure 6.12).

Ten hours and 12 minutes after fertilization (Figure 6.13) the optic vesicles differentiate to form the optic cup and the lenses begin to form. The small optic vesicles appear, but they lack otoliths. The regions of the brain were well defined, and the neural fold is seen as a median line along the body (Figure 6.13).
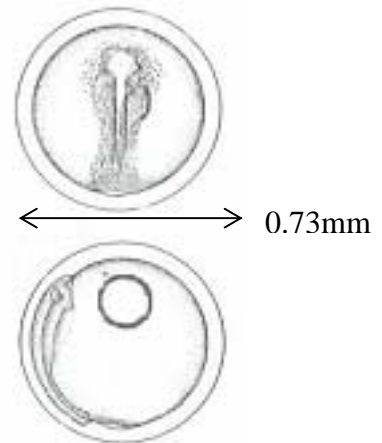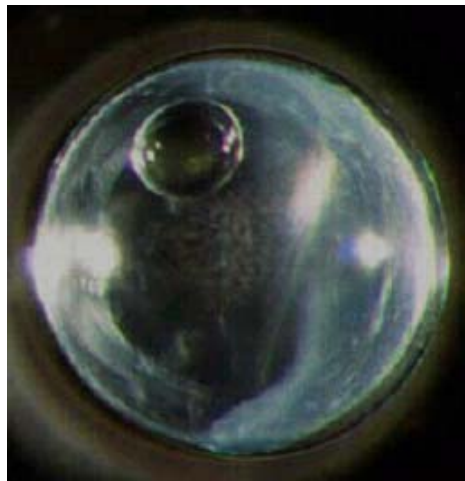
Figure 6.10. Neurula stage (7h AF)
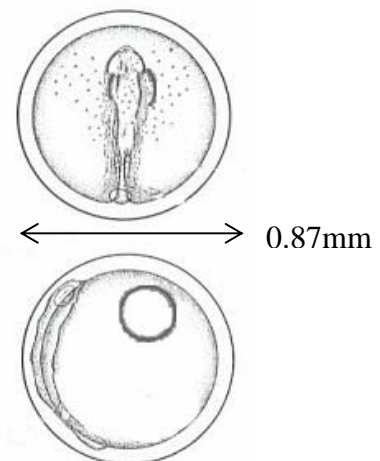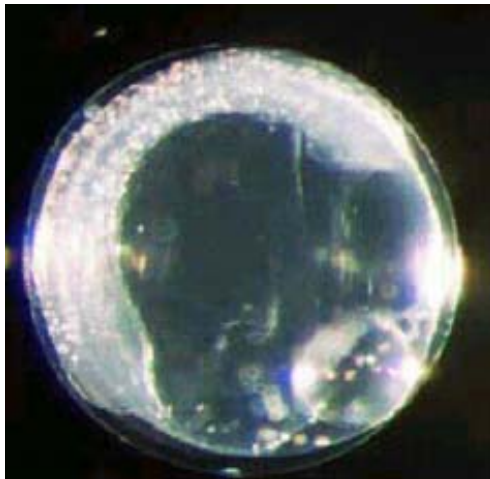
Figure 6.11. Early somite stage (8h AF)

Eleven hours and twelve minutes after fertilization (Figure 6.14) the tubular heart appears underneath the head from the posterior end of the mid brain to the anterior end to the hind-brain. The body cavity extends further toward the posterior end of the eye vesicles. The melanophores on the oil droplet become larger and more distinct; those on the head expanded to outline the olfactory lobes and optic vesicles. The tail has separated from the yolk (Figure 6.14)

0.86mm
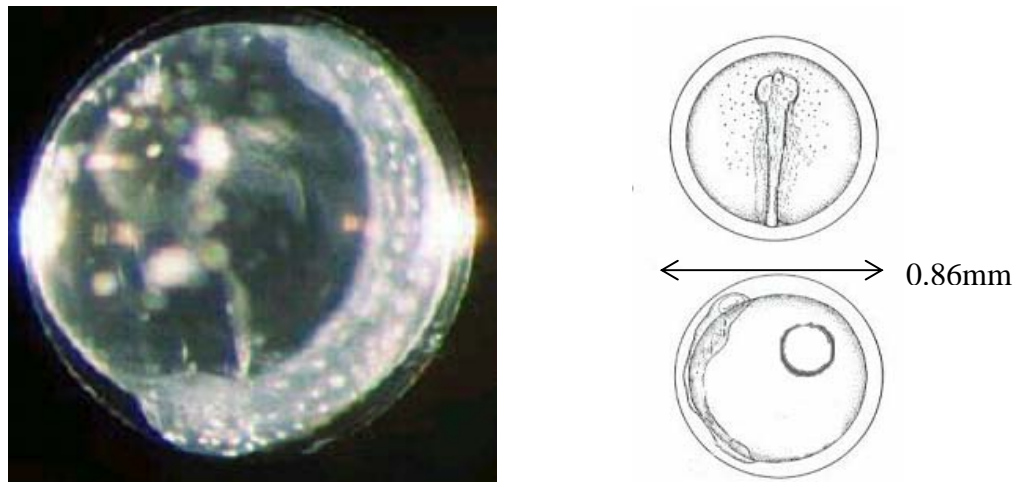
Figure 6.12. Somite stage (9h AF)

Twelve hours and thirty minutes after fertilization (Figure 6.15), the melanophores were much larger and fewer, forming aggregations. The anterior portion of the heart, which exhibits a slow pulsation, extends up to the anterior end of the fore-brain. Cuverian ducts and the vitello-caudal vein were still incomplete. The embryonic body encircles nearly ¾ of the yolk sphere (Figure 6.15).

0.89mm
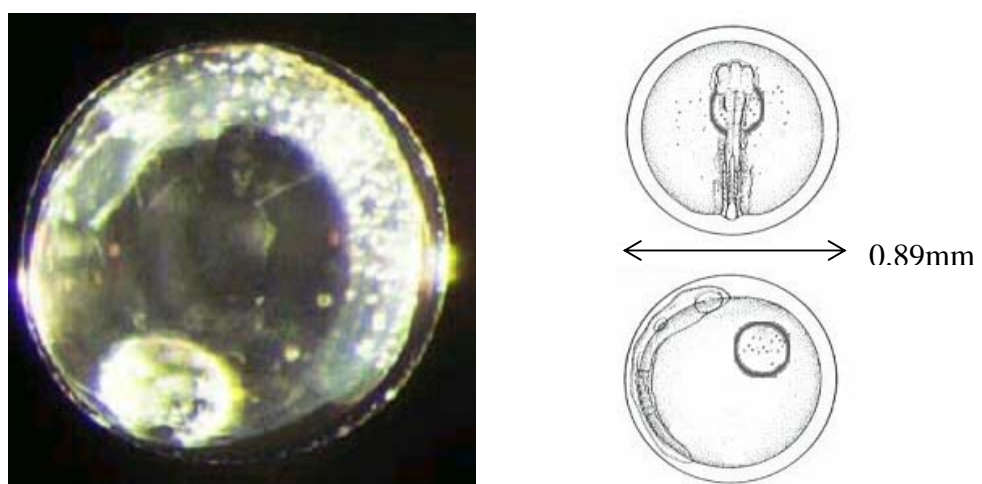
Figure 6.13. Somite stage (10h AF)

Thirteen hours and 5 minutes after fertilization (Figure 6.16), the blood circulation begins and the heart beat is faster and more constant. At the same time the embryo starts moving with quick but short movements mainly produced by the tail. Melanophores were more compact and they accumulated in four places along the embryo's body (Figure 6.16)

Figure 6.14. Tubular heart formation (11 AF)
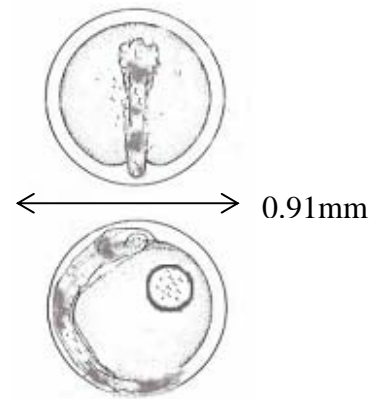
Figure 6.15. Heart beating start (12h AF)
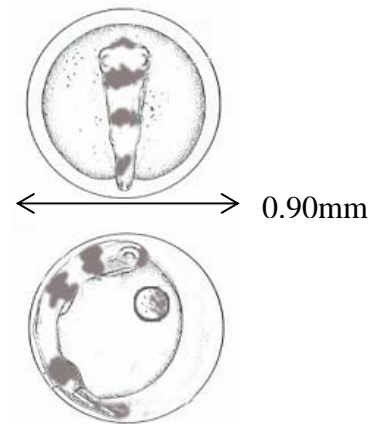
Fourteen hours and twenty two minutes after fertilization the first larvae hatched (Figure 6.17). At this time the melanophore accumulations can be

observed clearly. Five groups spread along the newly hatched larvae. There were two on the head region one over the olfactory region and another around the eyes. The other two were, one situated in the mid-laterally and dorso-laterally area and another one on the post anal area (Figure 6.17).

Fifteen hours after fertilization massive hatching took place, 90 % of the eggs hatched at that time.
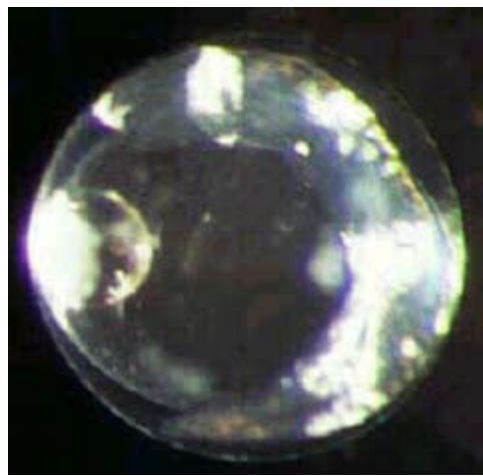
Figure 6.16. Pre hatching (13h AF)
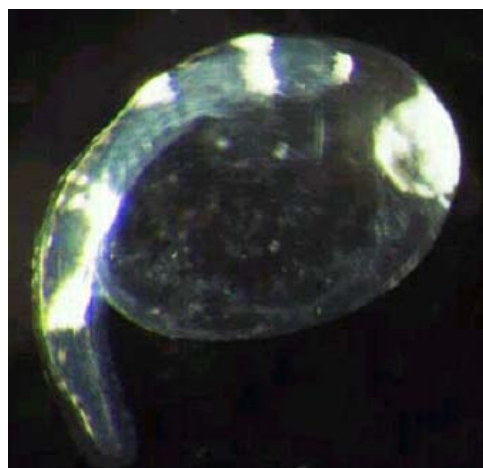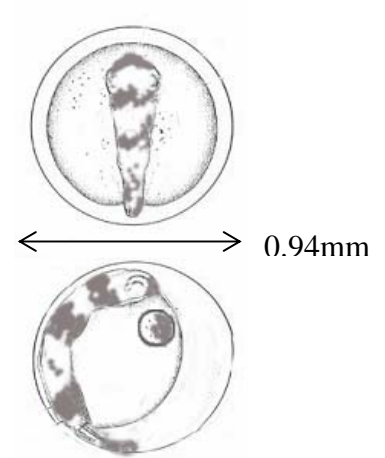
Figure 6.17. Newly hatched larvae (14 h and 22 min AF)

6.3.2 Larval development

6.3.2.1 Hatching stage

Centropomus undecimalis larvae hatched 15 hours after fertilization at 28°C (Figure 6.17), measuring a mean SL of 1.71 ranging from 1.38 to 1.84mm (n= 35). The body of newly hatched yolk sac larvae is very elongate with an oval shape and with a mean size of 0.91mm in length ranging from 0.85- 1.02mm (n=300) and with a mean width of 0.58 ranging between 0.4-0.7mm. A single oil droplet was present, located on the yolk sac's frontside underneath the head. A transparent voluminous finfold covers most of the body (Figure 6.17). Eyes and mouth were not formed, no eye pigmentation was present. Larvae were concentrated on the surface, floating and moving mainly in circles due to their limited fin development.

6.3.2.2 Yolk sac stage

On Day 0 (24 hours after fertilization) common snook larvae (Figure 6.19 and 6.20a) measured a mean standard length (SL) of 1.78mm ranging from 1.49- 2.075mm (n=35) and a mean myomere height (MH) of 0.164mm ranging from 0.152-0.171mm. The yolk sac had reduced in size with a mean length of 0.51mm ranging from 0.31-0.63mm and mean width of 0.35mm ranging from 0.29-0.45mm. Eyes were starting to form and some pigmentation was observed. The mouth was not formed although some definition could be observed. The pectoral fins were starting to develop.

In terms of larval behaviour, larvae were mainly at the surface, although some larvae were distributed below in the water column but close to the surface. Swimming movements were more directional.

Day 1 larvae (Figure 6.19) had a mean SL of 2.07 mm ranging from 1.92-2.17mm and with a an average MH of 0.202mm ranging from 0.180-2.07mm (n=35). The yolk sac although reduced to half the size from the previous day was still present with a mean length of 0.16mm ranging from 0.12-0.20mm. The eyes were starting to gain definition and the retina could now be differentiated with eye pigmentation observed but not fully completed. The optic tectum or primary optic center of the brain is large (Plate 6.1) as in most fish which rely on their sense of vision. Lenses were fully complete and visible (Plate 6.1) and the internal layer organization was well established, although layer thickness is still low. The cornea and the cartilaginous ring were present as well as the optic chiasma right below the infundibulum. The retina layer organization could be observed (Plate 6.2) and the main layers were visible such as the outer limiting layer (OLM) the outer nuclear layer (ONL) where the columnar nuclear bodies (CNB) were already present although undeveloped. Also, the outer plexiform layer (OPL) and the inner nuclear layer (INL) could be observed, yet most of the layers were not fully developed with some main organelle missing, as can be observed in plate 6.3, where the OLM and the ONL were still lacking organelles definition. Some pigments in the epithelium were present, although still low level (Plate 6.4).

The alimentary canal of a one day old larva showed some differentiation along its length. Cilia, which help to circulate the contents could be observed in the lumen (Plate 6.14, 6.15), at the same time some irregular small microvilli

appeared along the digestive wall. Other organelles observed include the mitochondrion and the nucleus of the epithelial cells, although these organelles were present in low numbers. Other structures, such as the non-villous region (NVR) and the terminal web (TW), were clearly defined although they were lacking in thickness (Plate 6.15)

On Day 2 (Figure 6.19 and 6.20b), SL has increased to a mean 2.26mm ranging from 2.12-2.71mm and with an average MH of 0.211mm ranging from 0.185-0.282mm (n=35). At this time the yolk sac has been nearly absorbed reducing its size to a mean length of 0.15mm ranging from 0.11-0.20mm. The oil droplet was still present although has reduced it size (Figure 6.19 and 6.20b). Eyes were formed and pigmented (Plate 6.5-6.6), the cornea has gained in thickness and it now tight against the lens. The retina layer was now more defined and each layer was now thicker. The optical nerve was fully formed and connected to the main nervous system (Plates 6.5-6.6-6.10). For the first time the clear layer of pigment epithelium cells was present and the other layers such as the ONL were gaining in complexity (Plate 6.6-6.7) with the development of organelles such as the cones and rods (Plate 6.8) nearly completed (Plate 6.9).

The mouth was formed and open (Figure 6.19, 6.20b) with the main cartilages, such as; Meckel's cartilage, the hyposynplectic cartilage, and the basihyal cartilage, already present (Plate 6.5). Also the tongue can be observed (Figure 6.20b, Plate 6.5). The digestive system was straight and long, extending past the posterior margin of the yolk sac, and into the ventral finfold, and although undeveloped had some food inside. The digestive system walls were defined, no cilia were observed in the lumen and the

microvilli layer was now well established along the walls (Plate 6.16) also the microvili were long and compact (Plates 6.17, 6.18). The epithelium cell structure was forming and the main organelles were observed, such as; the mitochondrion, the clear and dark apical cells, and the epithelium nucleus (Plates 6.17, 6.18). However, numbers were low.

Pectoral fins were well developed and functional. Slight caudal fin definition can be observed. Larvae showed a photopositive reaction gathering at those areas with more light. Now the distribution was more diverse, having larvae in the water column as well as in the surface. Burst movements towards prey were commonly seen.

On day 3 (Figure 6.19 and 6.20c), snook larvae had a mean SL of 2.31 ranging from 2.15 to 3.1mm and a mean MH of 0.214mm ranging from 0.20 to 0.35mm (n=35). The yolk sac had been nearly completely absorbed and the oil droplet was still present, although severely reduced.

The eyes had gained in pigmentation and were developed. The retina layer was well structured due the development of most of the layer organelles with the cornea fully formed (Plates 6.11, 6.12). All the different layers were clearly differentiated such as the outer ganglion layer (OGL), the inner nuclear layer (INL) and the inner ganglion layer (IGL) (Plates 6.12, 613). The pigment epithelium cell layer was fully formed with photoreceptor inclusions (cones) (Plate 6.13), the bodies of the photoreceptors (B) were also now well defined (Plates 6.12, 6.13).

The maxilliary jaws were developing and mouth gap was increasing. The alimentary canal was no longer a long straight tube, some structure could be

observed especially in the anal region, which, by this stage, was well developed. Structural epithelium organelles, such as; the nucleus, the mitochondrion, and the dark vesicles, were all present and in high numbers (Plate 6.20 and 6.21). The microvilli layer had gained in length and was more compact (Plates 6.20). Other important organelles were also present, such as the Golgi apparatus (GA), the rough endoplasmatic reticulum (RER), the desmosome of apical juction (D) and the pinocytotic vesicle (P) (Plate 6.21). Food was observed in the gut, mainly rotifers and algae.

The swim bladder was formed and showed signs of inflation. Swimming speed had increased as well as the larvae motility.

6.3.2.3 Pre flexion Stage

Day 4 larvae (Figure 6.19 and 6.20d) have a mean SL of 2.35mm ranging from 2.22-3.23mm; the mean MH was 0.211 ranging from 0.20 to 0.28mm (n=35). By this day the yolk sac had been totally absorbed and the oil droplet was not present anymore (Figure 6.19 and 6.20d). The medulla oblongata could be observed as well as the cerebellum, which was fully formed. The eyes were fully pigmented and the mouth was fully functional with more develop jaws, teeth formation could also be observed. The swim bladder was positioned posterior to the pectoral fin base and above the stomach, and was developed and inflated. The gut gained in thickness and become partitioned. Rotifers were observed in the gut.

At this stage larvae were scattered throughout the water column, although no larvae could be seen on the tank bottom. There were many active swimmers which spent most of the time seeking prey.

On Day 6 (Figure 6.19 and 6.20e), snook larvae mean SL was 2.51 mm ranging from 2.1 to 3.41mm and with a mean MH of 0.245mm ranging from 0.214 to 0.351mm (n=35). Rows of teeth were present at the same time that the mouth gap had increased. The gas bladder was fully inflated. The gut was now well partitioned and food was commonly observed inside with 90 % of larvae observed having full stomachs. The finfold around the larvae was no longer present and the fins were starting to take shape, especially the caudal and dorsal fins, the pectoral fins were fully functional but still developing.

Snook larvae 8 days old (Figure 6.19 and 6.20f) had a mean SL of 2.55mm ranging from 2.41 to 3.7mm and with a mean MH of 0.276mm ranging from 0.21 to 0.34mm (n=35). Snook larvae at this stage gained in body depth and an increase in the head size in relation to eye size occurred. The notochord was fully formed and ended at the caudal fin. There was an increase in volume of the digestive system, and definition of the different organs was more apparent. The caudal fin started to develop the shape of an adult caudal fin going from a more rounded initial shape towards a forked fin shape.

6.3.2.4 Flexion Stage

Ten days after hatching (Figure 6.19 and 6.20g) common snook larvae have a mean SL of 3.136mm ranging from 3 to 4.2 mm (n=35), on the other hand, the mean MH was 0.381mm with values ranging from 0.291 to 0.41mm. Notochord flexion started. At this stage the larvae had increased their head size and both the maxilliary and premaxilliary bones were gaining in thickness and strength. Teeth were present now in both jaws bones. The digestive system was now well differentiated, with all the organs gaining in volume and

structure. Also, 90 % of the observed stomachs were full, rotifers were observed all along the digestive system. Fins formation continued, no spine formation was apparent but fin shape definition kept developing, especially the dorsal and caudal fins (Figure 6.19 and 6.20g).

Common snook larvae 12 days old (Figure 6.19 and 6.20h) had a mean SL of 3.57 mm with values ranging from 3.41 to 3.84 mm and with average MH of 0.401 mm, ranging from 0.266 to 0.467 mm (n=35). The development of the dorsal and anal fin bases could be observed. At the same time notochord flexion was nearly completed. Larval body depth continued to increase and the head size was still proportionally bigger in width than the rest of the body. By day 14 after hatching common snook larvae (Figure 6.19 and 6.20i) had a mean SL of 4.43 mm with values ranging from 3.57 to 5.71 mm and the mean MH was 0.41 mm ranging from 0.36 to 0.46 mm. By this stage the lower dorsal fin was developing faster than the upper one and 9 rays could be observed. Similar development happens to the anal fin although rays were not as developed. Notochord flexion was completed.

Common snook development during the first 14 days after hatching followed a rapid development (Figure 6.18). In terms of standard length; three stages were observed: the first stage was during the yolk sac stage where the SL increased from 1.78 mm to 2.26 mm in a period of 2 days. The next stage was during the pre-flexion stage where the SL was of 2.35 mm to 2.56 mm in 4 days (Figure 6.18). The last stage during the flexion stage, the average

common snook SL went from 2.56 mm to 4.43 mm in 6 days increasing exponentially every day. All the average standard length and myomere height measurements can be seen in table 6.3.

Table 6.3. Common snook mean Standard lenght and Myomere height during the first 14 days after hatching (***Standard deviation, n= number of speciemens) (mm)**

Day	Mean Standard Length (SL)	Mean Myomere Height (MH)	
0	1.785 ±0.289*	0.165 ±0.015*	n=35
2	2.311 ±0.434*	0.211 ±0.024*	n=35
4	2.266 ±0.360*	0.211 ±0.059*	n=35
6	2.520 ±0.515*	0.246 ±0.036*	n=35
8	2.556 ±0.435*	0.277 ±0.040*	n=35
10	3.137 ±0.495*	0.368 ±0.047*	n=35
12	3.580 ±0.454*	0.401 ±0.055*	n=35
14	4.433 ±0.780*	0.549 ±0.025*	n=35
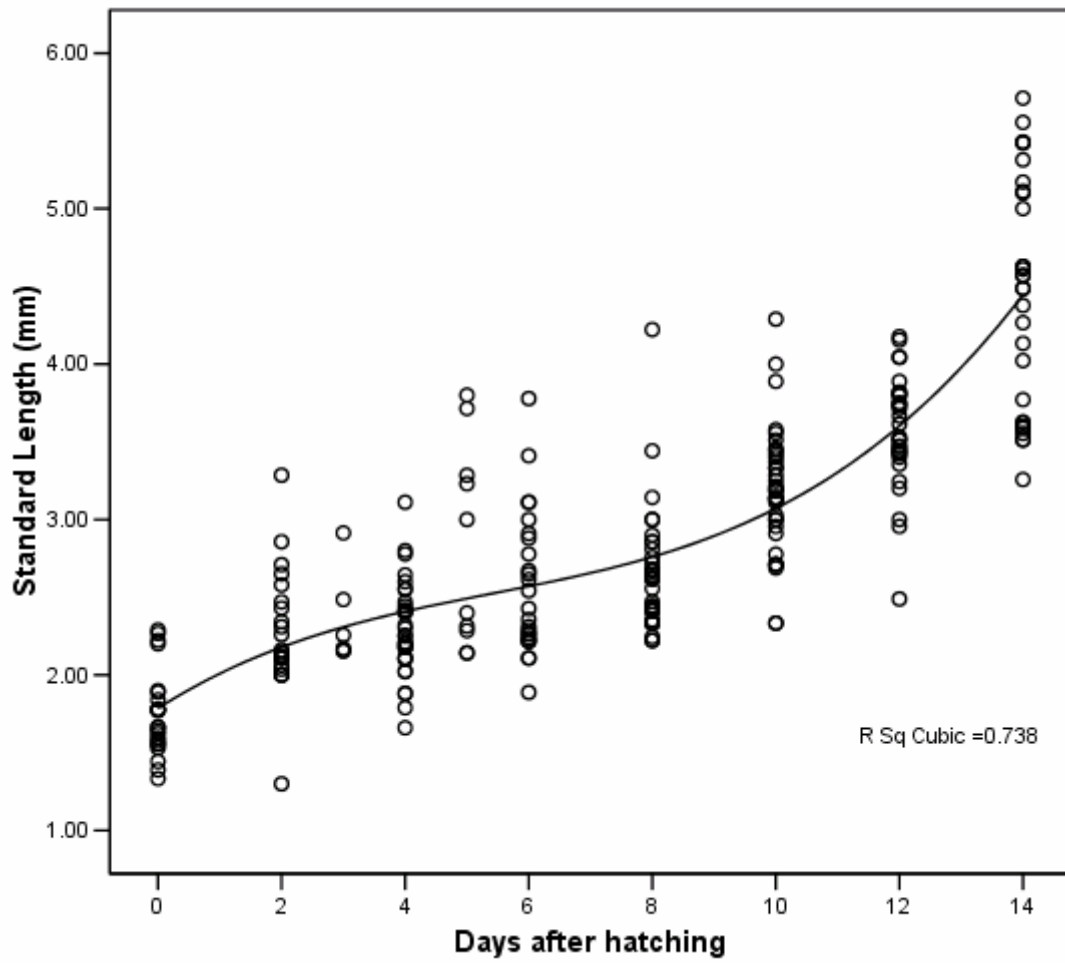
Figure 6.18. Common snook larval growth during the first 14 days after hatching
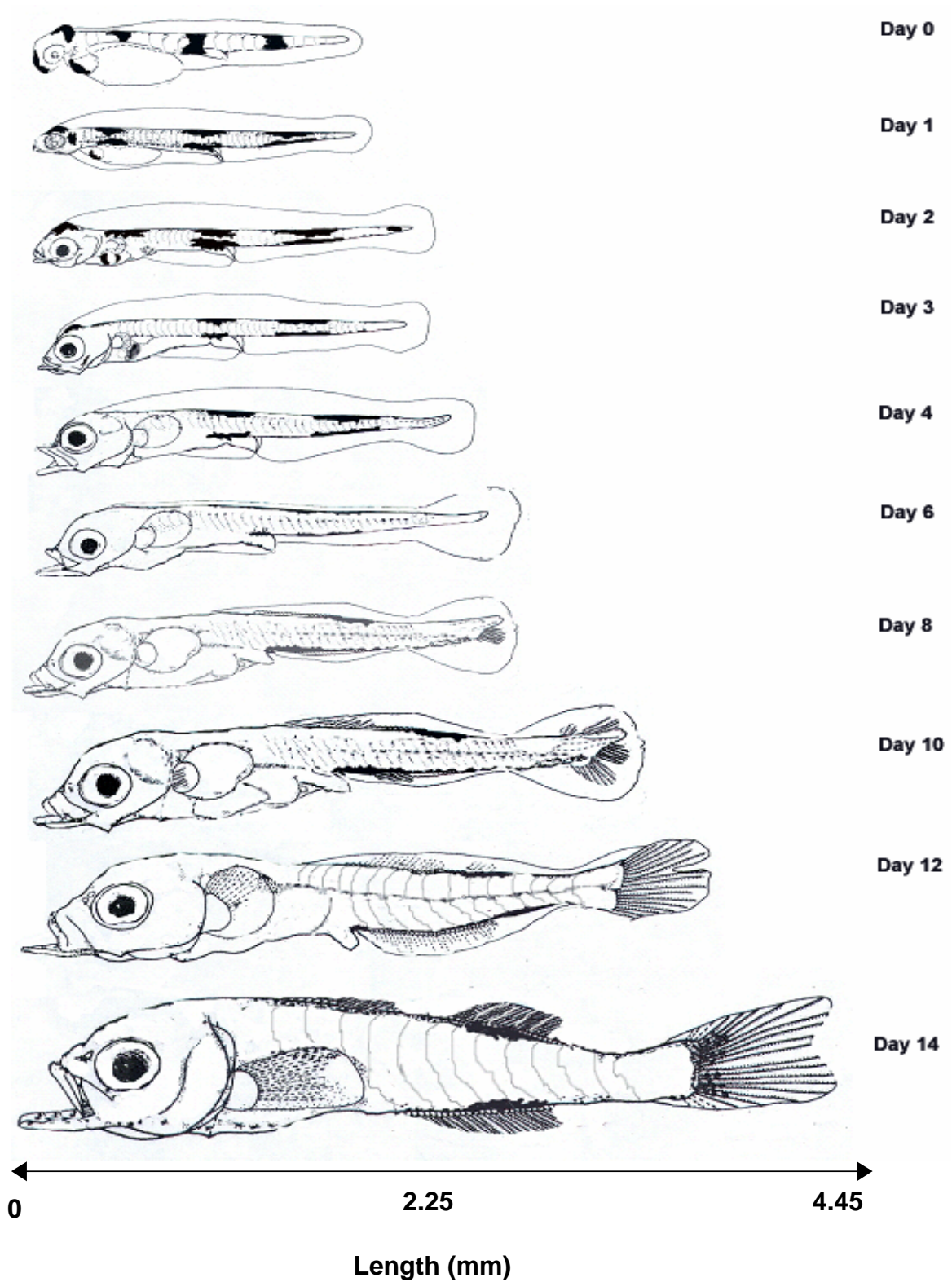
Figure 6.19. Common snook larval development from Day 0 to Day 14 After Hatching
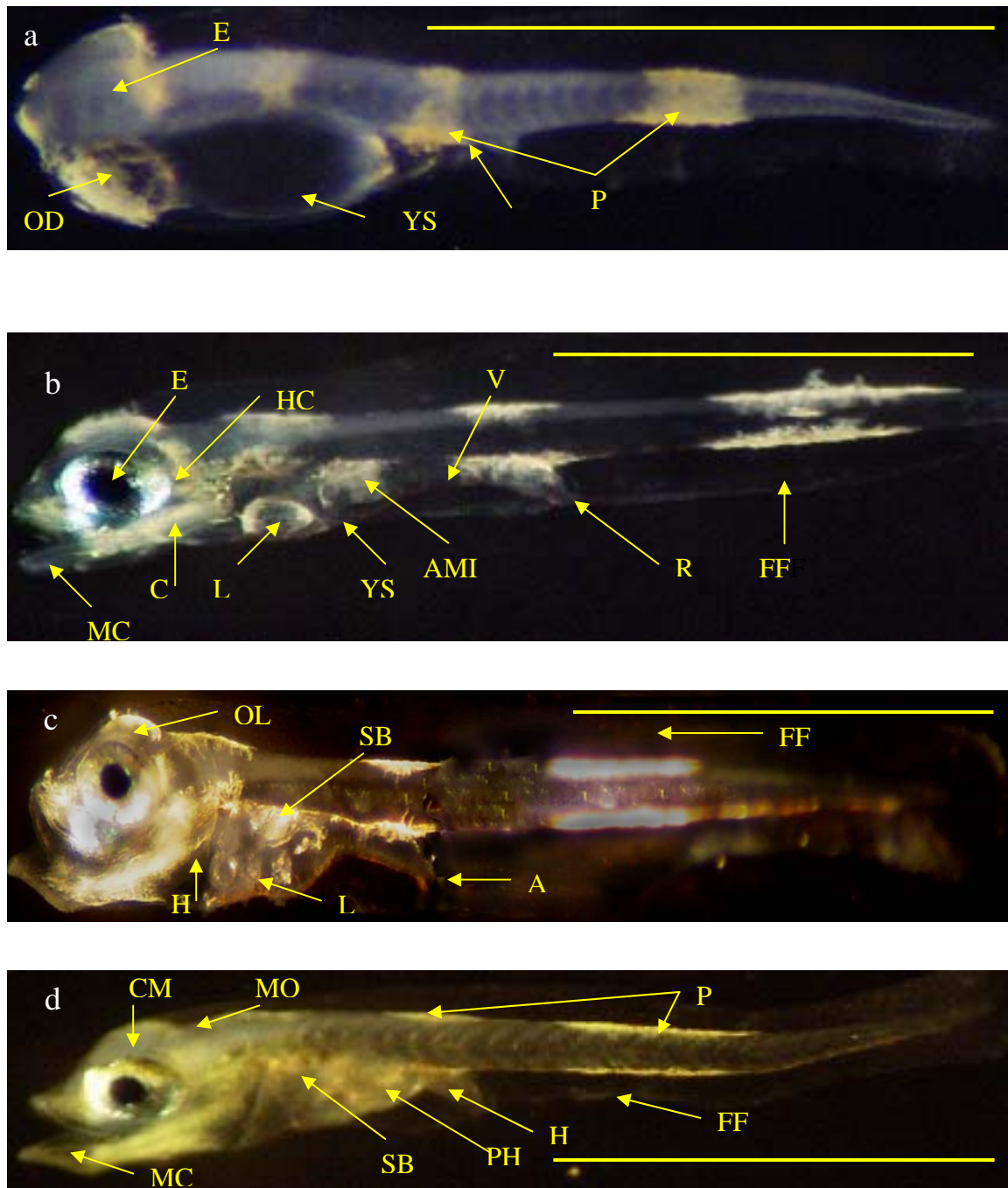
Figure 6.20. Common snook larval development from hatching until Day 14 after hatching (scale bar 1 mm)
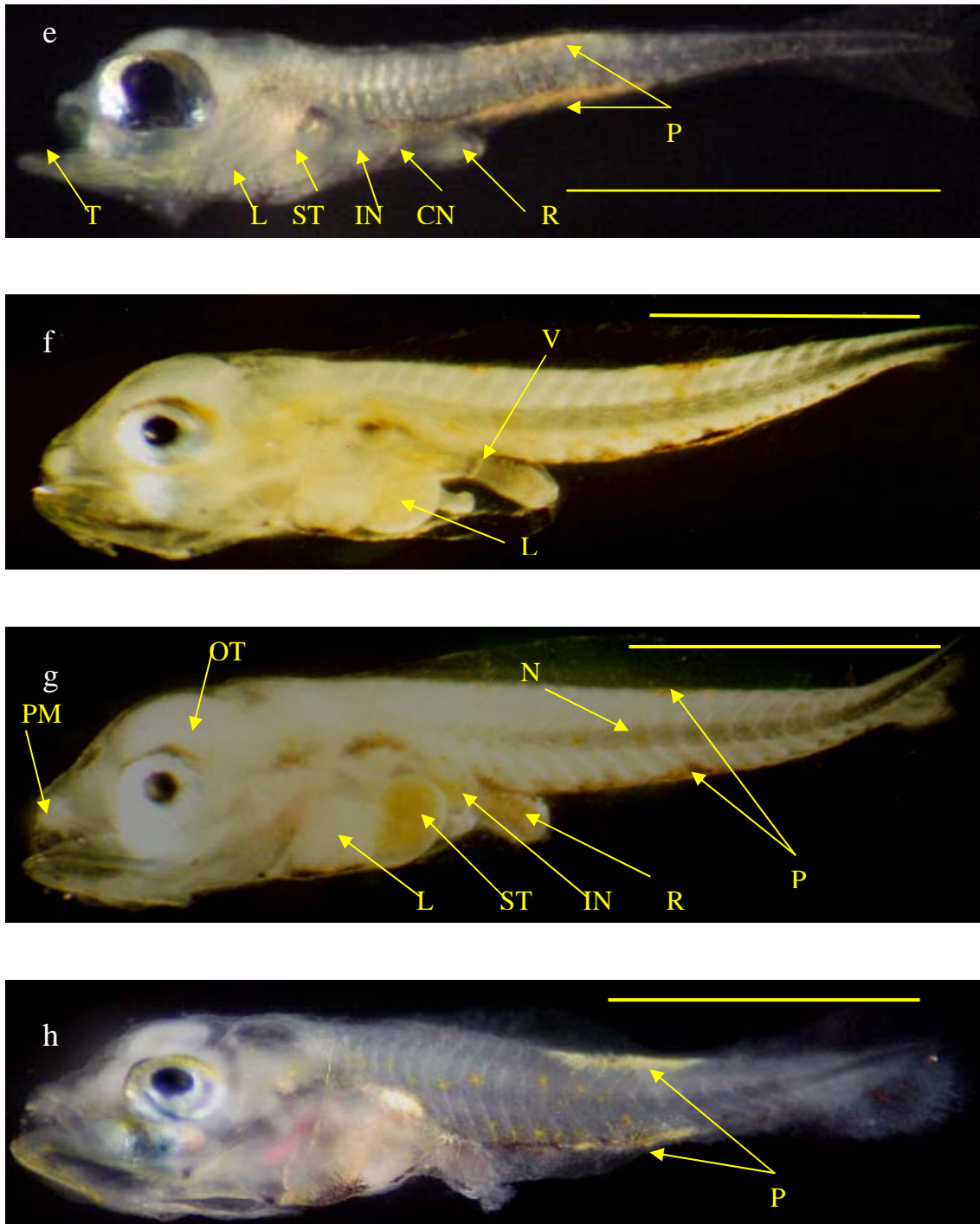
Figure 6.20 (continued). Common snook larval development from hatching until Day 14 after hatching (scale bar 1mm)
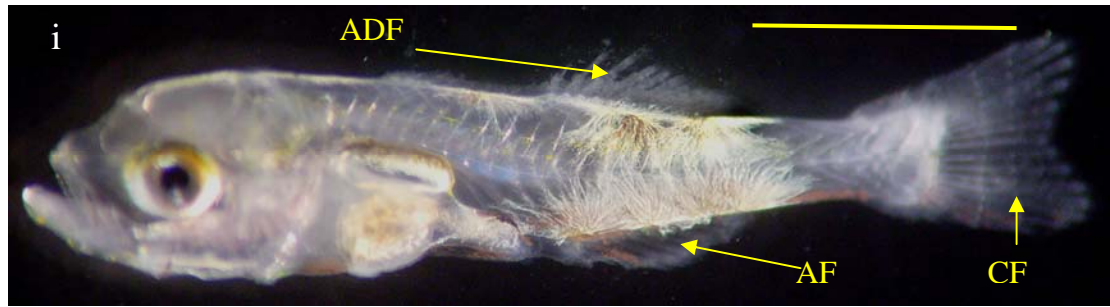
Figure 6.20 (continued). Common snook larval development from hatching until Day 14 after hatching. Scale bar 1mm

Photograph a.   Day 0 common snook larvae

Photograph b.   Day 2 common snook larvae

Photograph c   Day 3 common snook larvae

Photograph d   Day 4 common snook larvae

Photograph e   Day 6 common snook larvae

Photograph f   Day 8 common snook larvae

Photograph g   Day 10 common snook larvae

Photograph h   Day 12 common snook larvae

Photograph i   Day 14 common snook larvae

A	anus opening	MC	Meckel's cartilage
ADF	anterior dorsal fin	MO	medulla oblongata
AF	anal fin	N	Notochord
AMI	antero-media instestine	OD	Oil droplet
C	ceratohyal	OL	optic lobe of brain
CF	caudal fin	OT	optic tectum
CM	cerebellum	P	Pigments
CN	constriction	PH	posterior paret of hindg
E	eye	PM	premaxilia
FF	finfold	R	rectal area
H	Heart	SB	swim bladder
HC	hyposynplectic cartilage	T	teeth
IN	instestine	V	intestino-rectal valve
L	Liver	YS	yolk sac
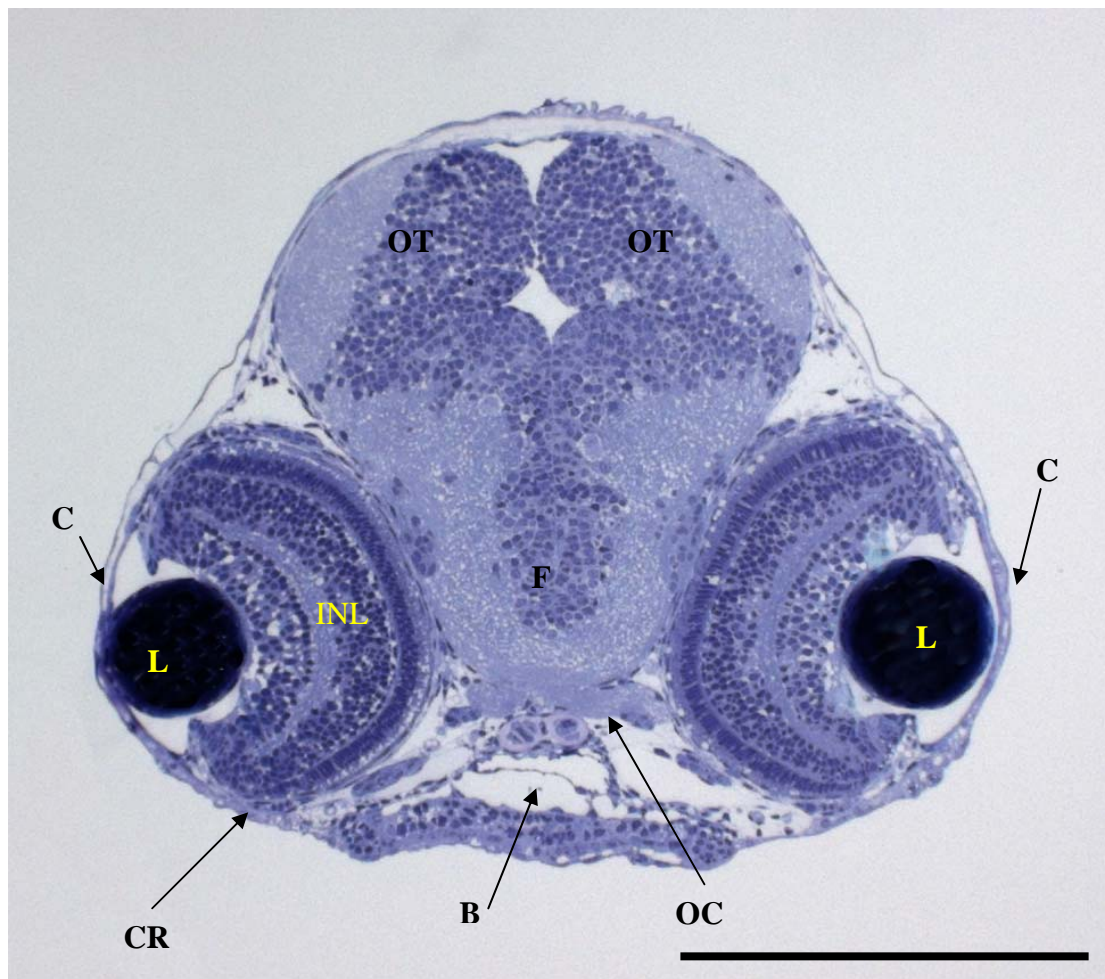
Plate 6-1. Head semi-thin cross section from a 1DAH common snook larva

Scale bar 65μm

B   buccal cavity
C   cornea
CR   cartilaginous ring
F   Infundibulum
INL   inner nuclear layer
L   lens
OLM   outer limiting membrane
ONL   outer nuclear layer
OC   optic chiasma
OPL   outer plexiform layer
OT   optic tectum
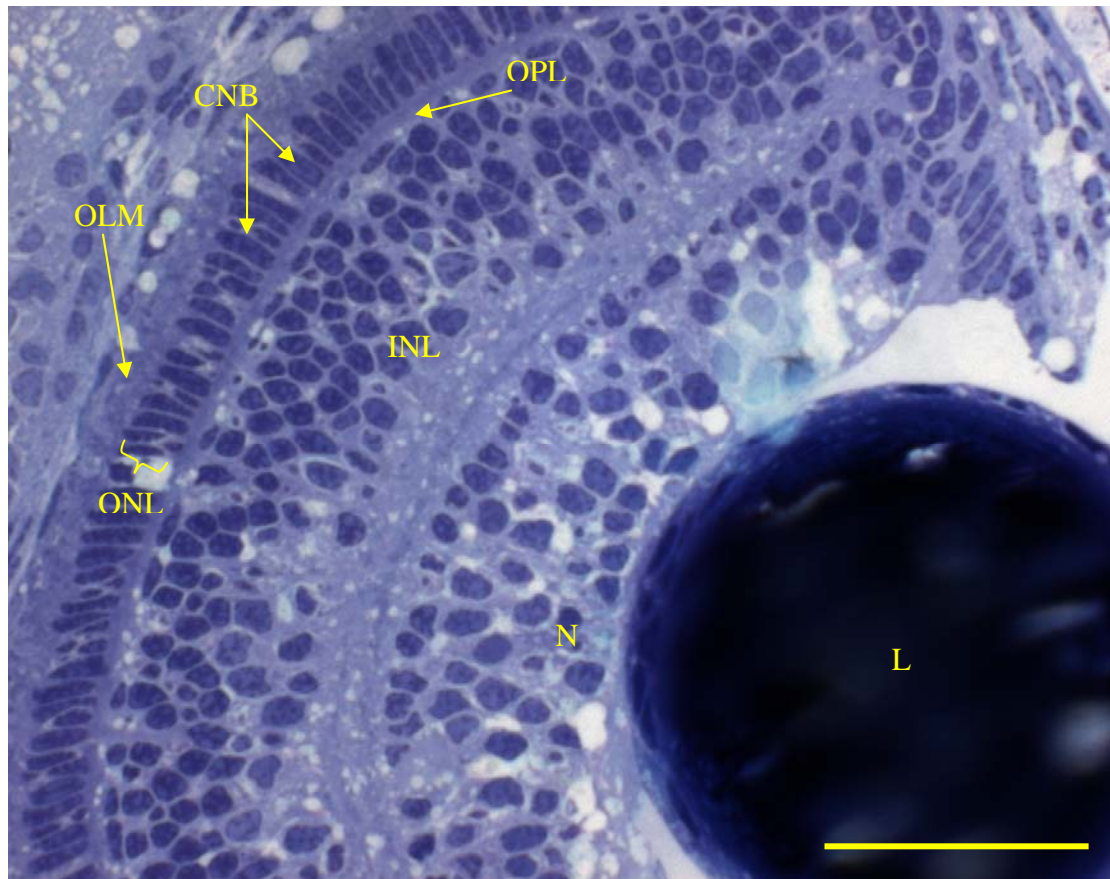
Plate 6-2. Electron micrograph of cross section through eye of common snook larva at one DAH

Scale bar 15 μm

CNB   columnar nuclear bodies (nuclei of photoreceptors)
INL   inner nuclear layer
L   Lens
N   neurons
OLM   outer limiting layer
ONL   outer nuclear layer
OPL   outer plexiform layer
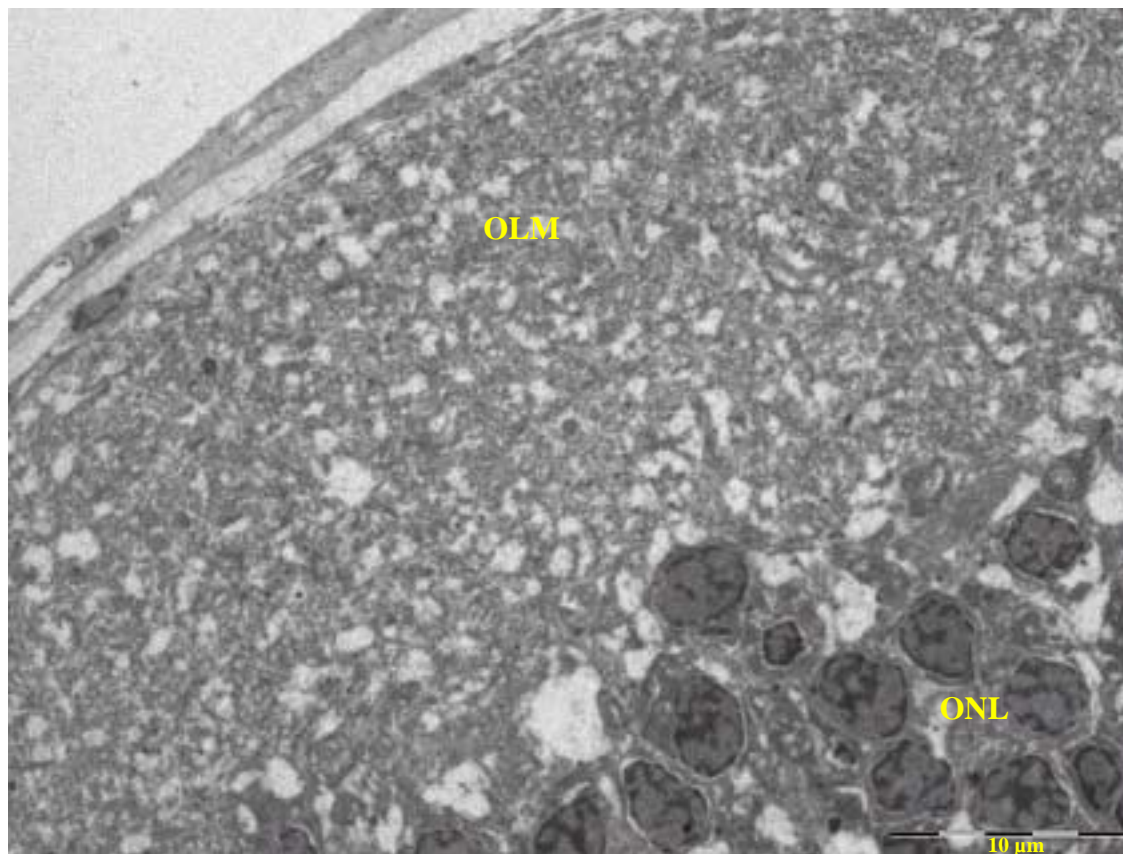
Plate 6-3. Electron micrograph of cross section through eye of common snook larva at one DAH

Scale bar 10µm

OLM   outer limiting layer
ONL   outer nuclear layer
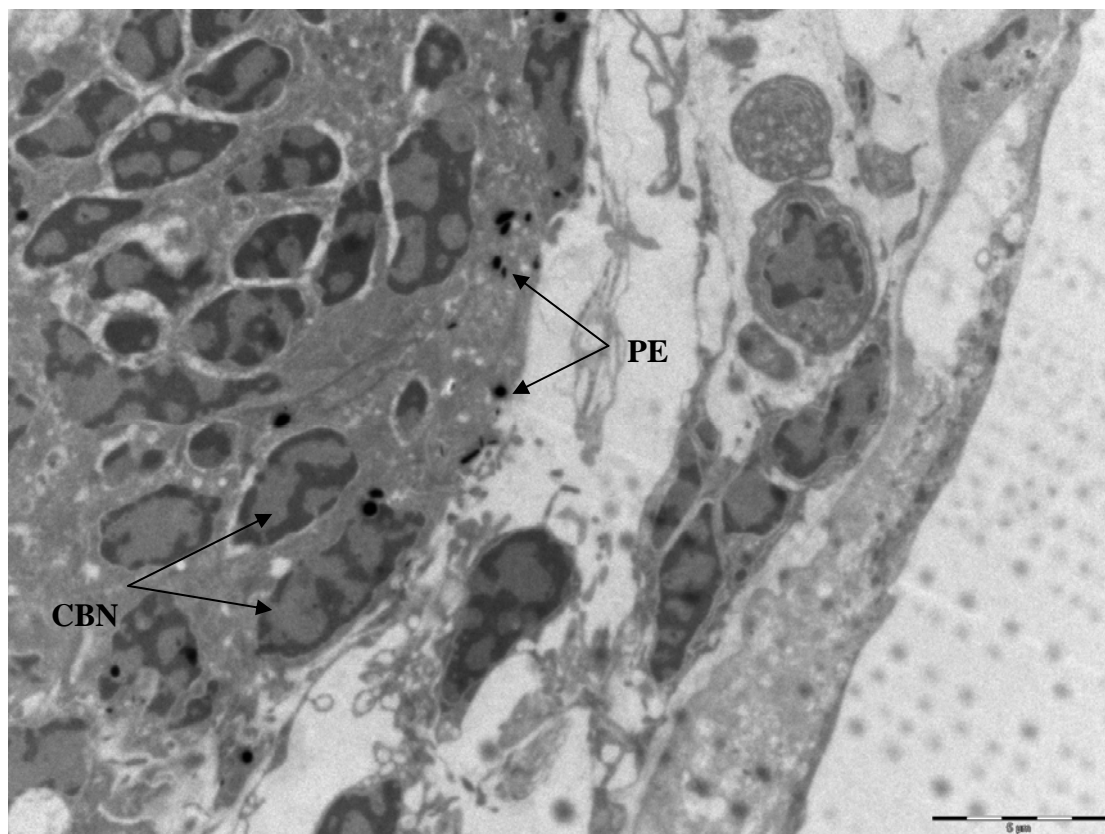
Plate 6-4. Electron micrograph of cross section through eye of common snook larva at one DAH

Scale bar 5µm

CNB   columnar nuclear bodies (nuclei of photoreceptors)
PE   Pigment epithileium
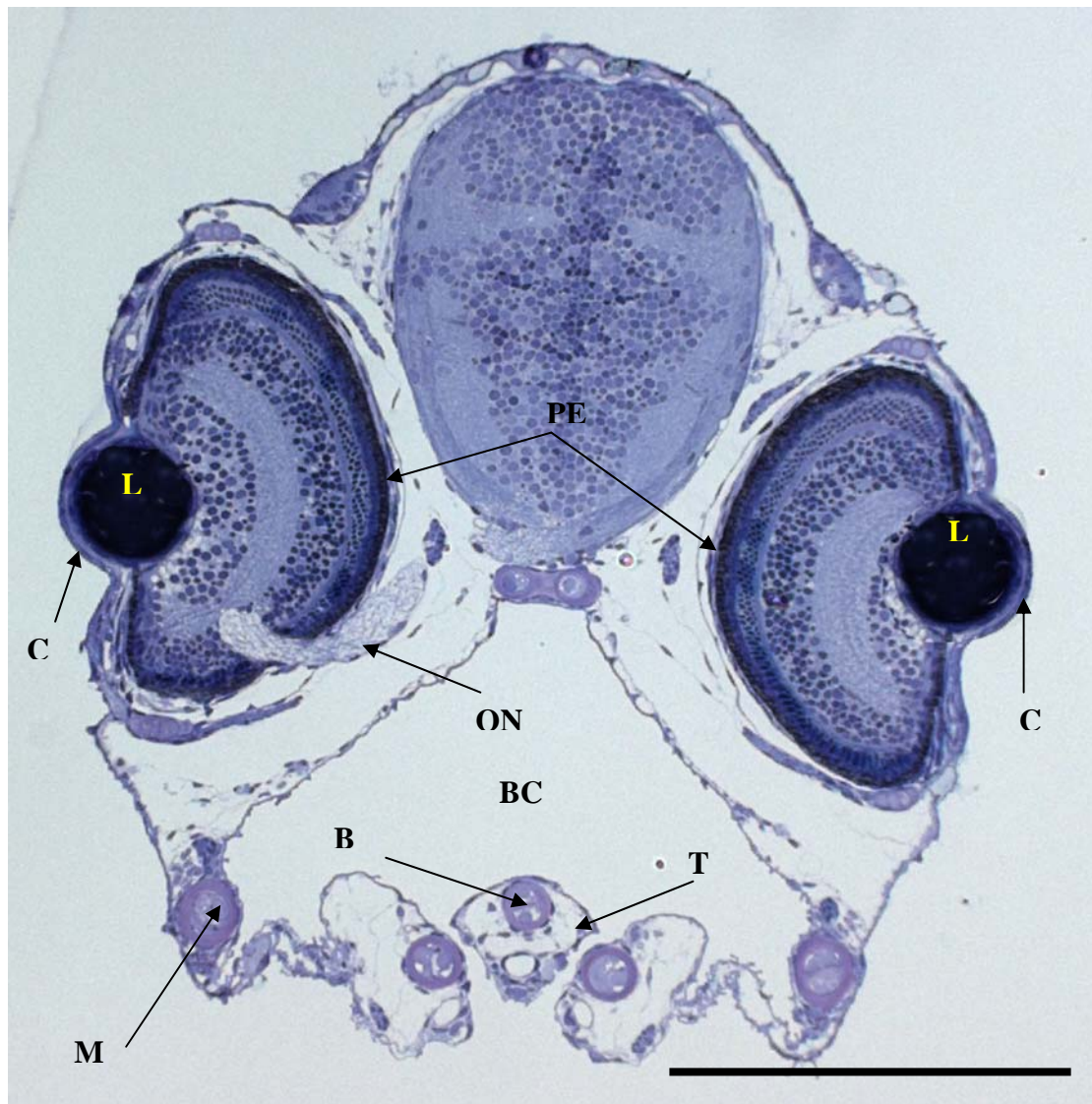
Plate 6-5. Head semi-thin cross section from a 2DAH common snook larva

Scale bar 90µm

B   basihyal cartilage
BC   buccal cavity
C   cornea
L   lens
M   Meckel's cartilage
ON   optical nerve
PE   pigment epithelium
T   tongue
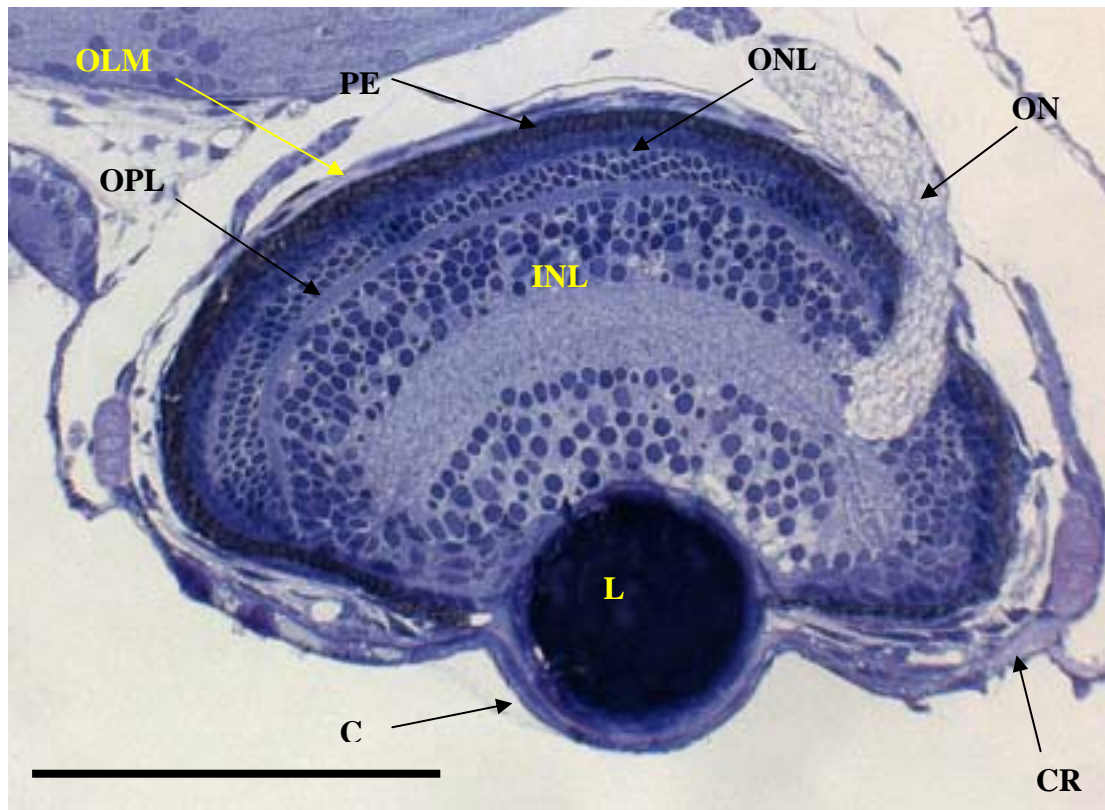
Plate 6-6. Head semi-thin cross section from a 2DAH common snook larva

Scale bar 40µm

C   cornea
CR   cartilaginous ring
INL   inner nuclear layer
L   lens
OLM   outer limiting membrane
ONL   outer nuclear layer
OPL   outer plexiform layer
ON   optical nerve
PE   pigment epithelium
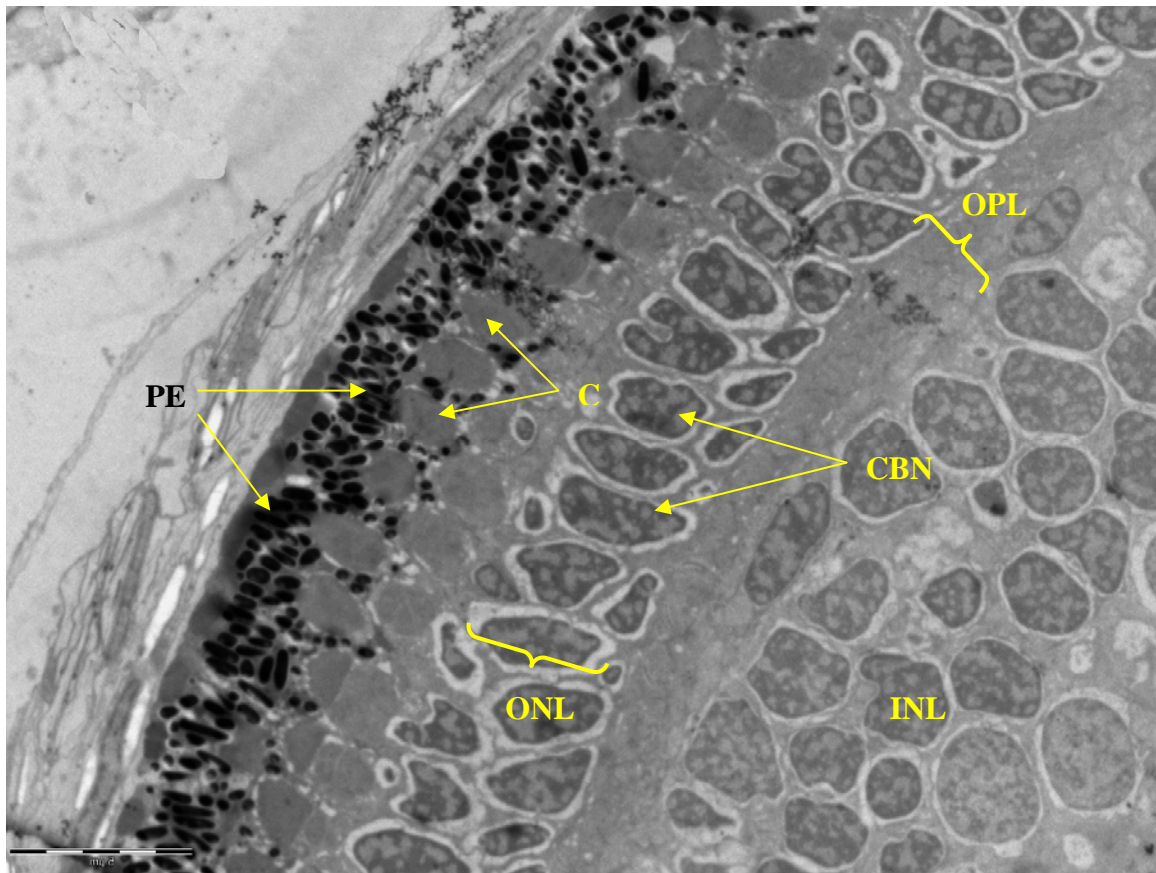
Plate 6-7. Electron micrograph of cross section through eye of common snook larva at two DAH

Scale bar 5µm

C   cones (outer segment)
CNB   columnar nuclear bodies (nuclei of photoreceptors)
INL   inner nuclear layer
L   Lens
N   neurons
OLM   outer limiting layer
ONL   outer nuclear layer
OPL   outer plexiform layer
PE   pigment epithelium
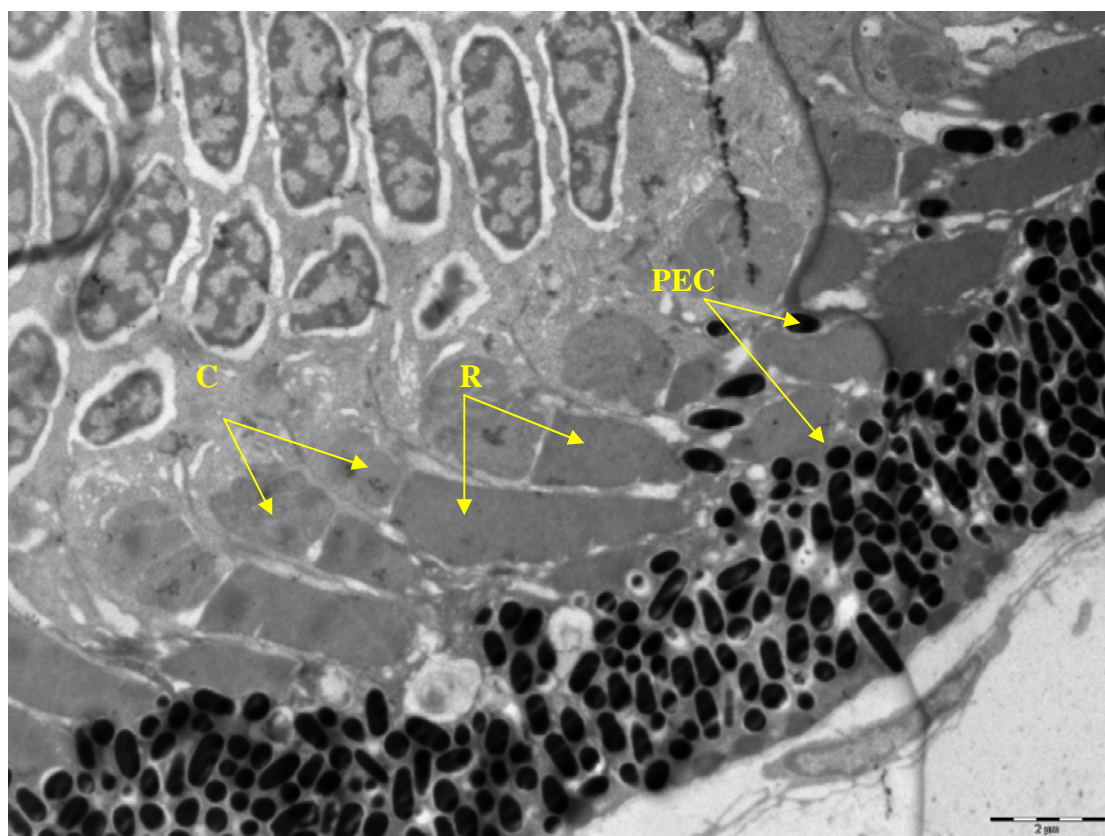
Plate 6-8. Electron micrograph of cross section through eye of common snook larva at two DAH

Scale bar 2µm

C   cones (outer segment)
PEC   pigment epithelium cell granule
R   rods
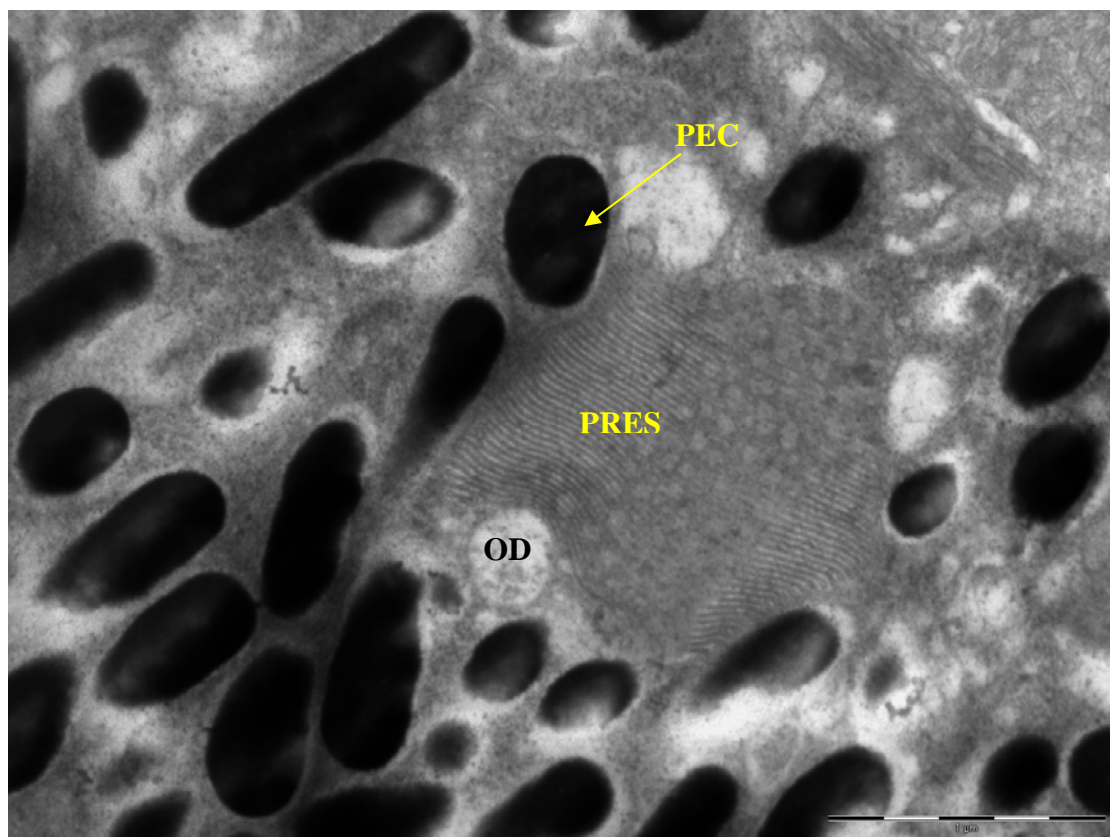
Plate 6-9. Electron micrograph of cross section through eye of common snook larva at two DAH

Scale bar 1μm

OD   oil droplet
PE   pigment epithelium cell granule
PRES   photoreceptor outer segment
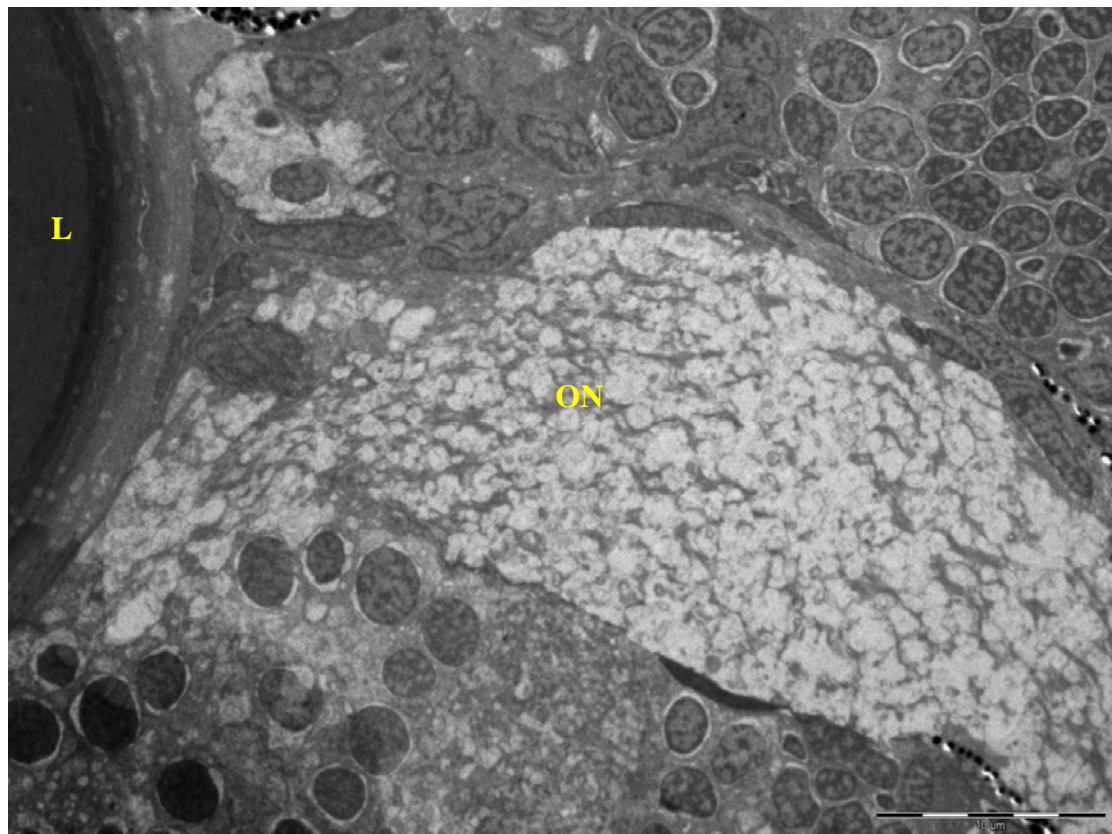
Plate 6-10. Electron micrograph of cross section through eye of common snook larva at two DAH

Scale bar 10µm

L   lens
ON   optical nerve
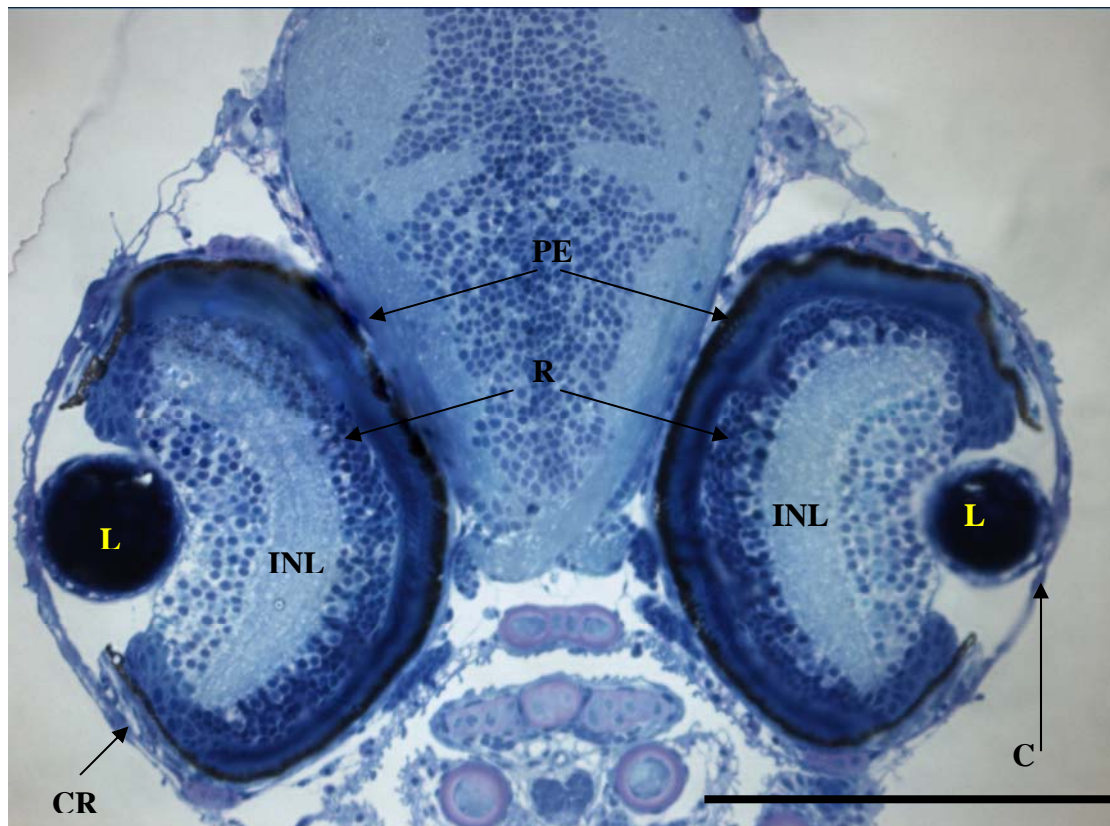
Plate 6-11. Head semi thing cross section from a 3 DAH common snook larva

Scale bar 100µm

CR   cartilaginous ring
INL   inner nuclear layer
L   lens
R   retina
PE   pigment epithelium layer
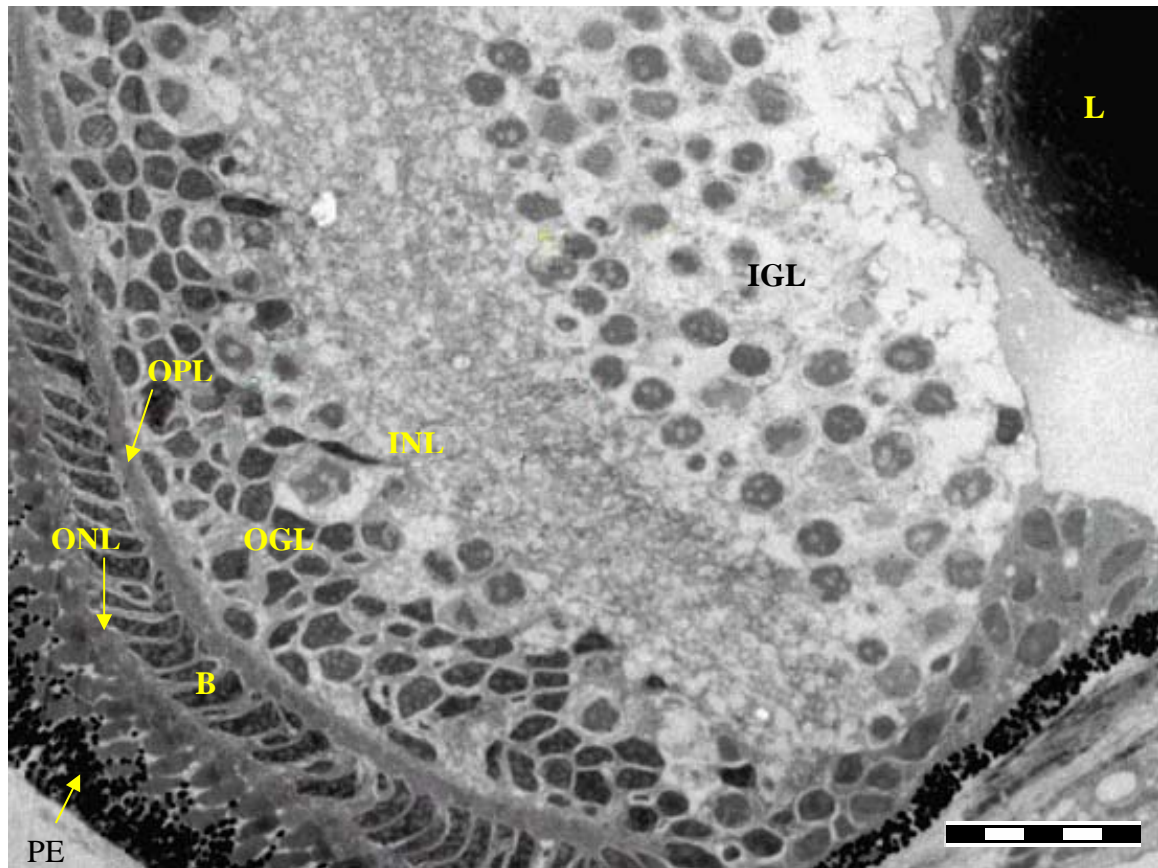
Plate 6-12.Electron micrograph of cross section through eye of common snook larva at three DAH

Scale bar 5µm

B   bodies of photoreceptors
IGL   inner ganglion layer
INL   inner nuclear layer
L   Lens
OGL   Outer ganglion layer
ONL   outer nuclear layer
OPL   outer plexiform layer
PE   pigment epithelium
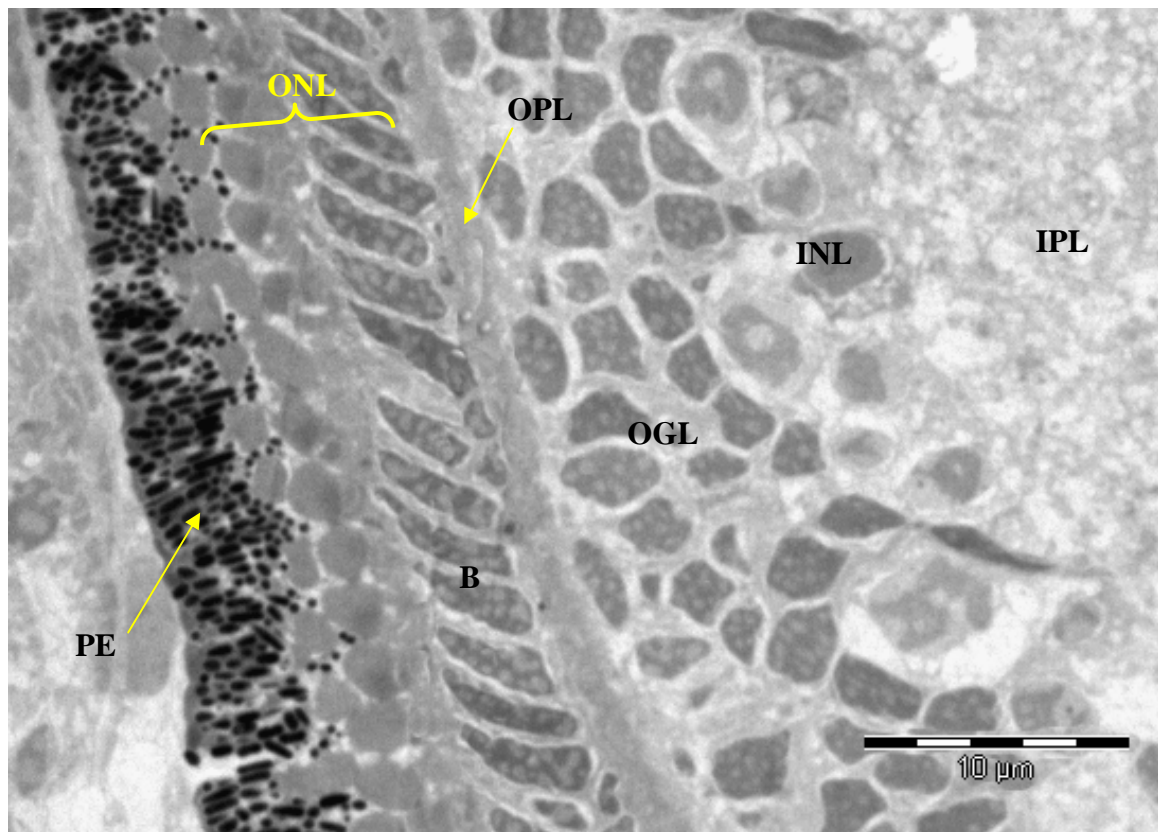
Plate 6-13. .Electron micrograph of cross section through eye of common snook larva at three DAH

Scale bar 10μm

B   Bodies of photoreceptors
INL   inner nuclear layer
IPL   inner plexiform layer
OGL   Outer ganglion layer
ONL   outer nuclear layer
OPL   outer plexiform layer
PE   pigment epithelium

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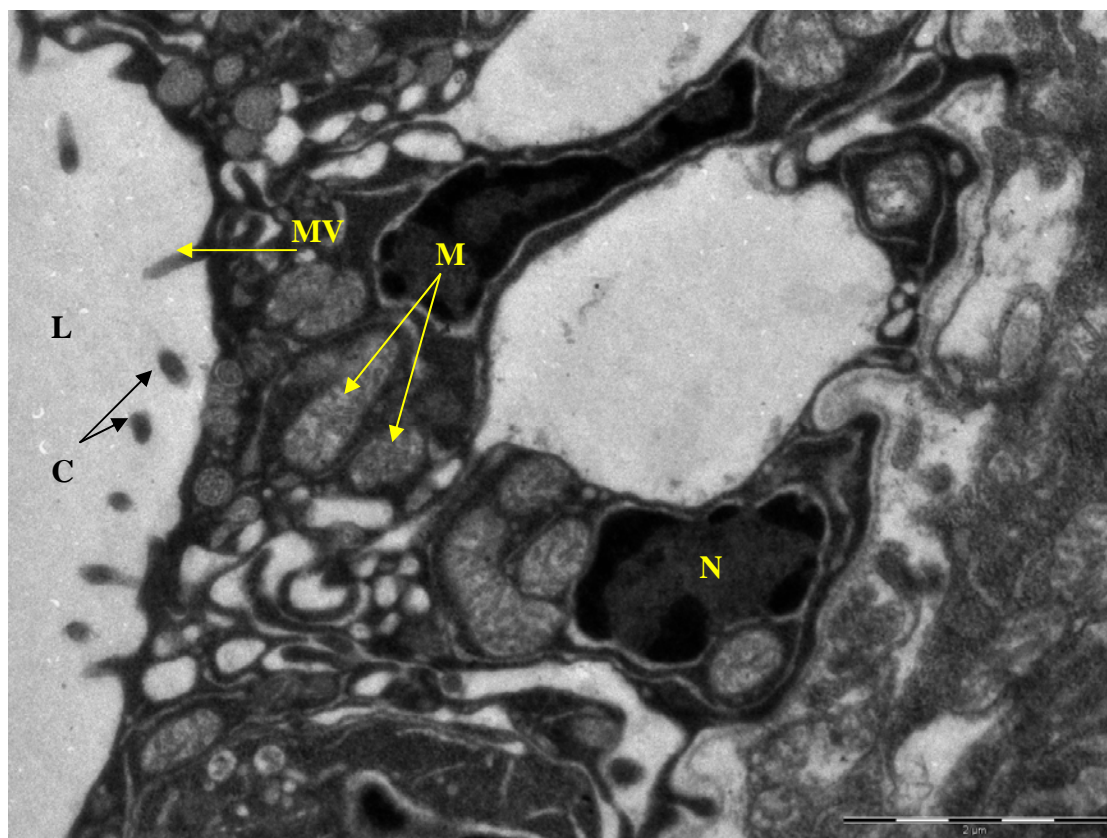
Plate 6-14. . Electron micrograph of cross section through antero-median intestine of common snook larva at one DAH

Scale bar 2µm

C   cilia
L   lumen
M   mitochondrion
MV   microvilli
N   nucleus
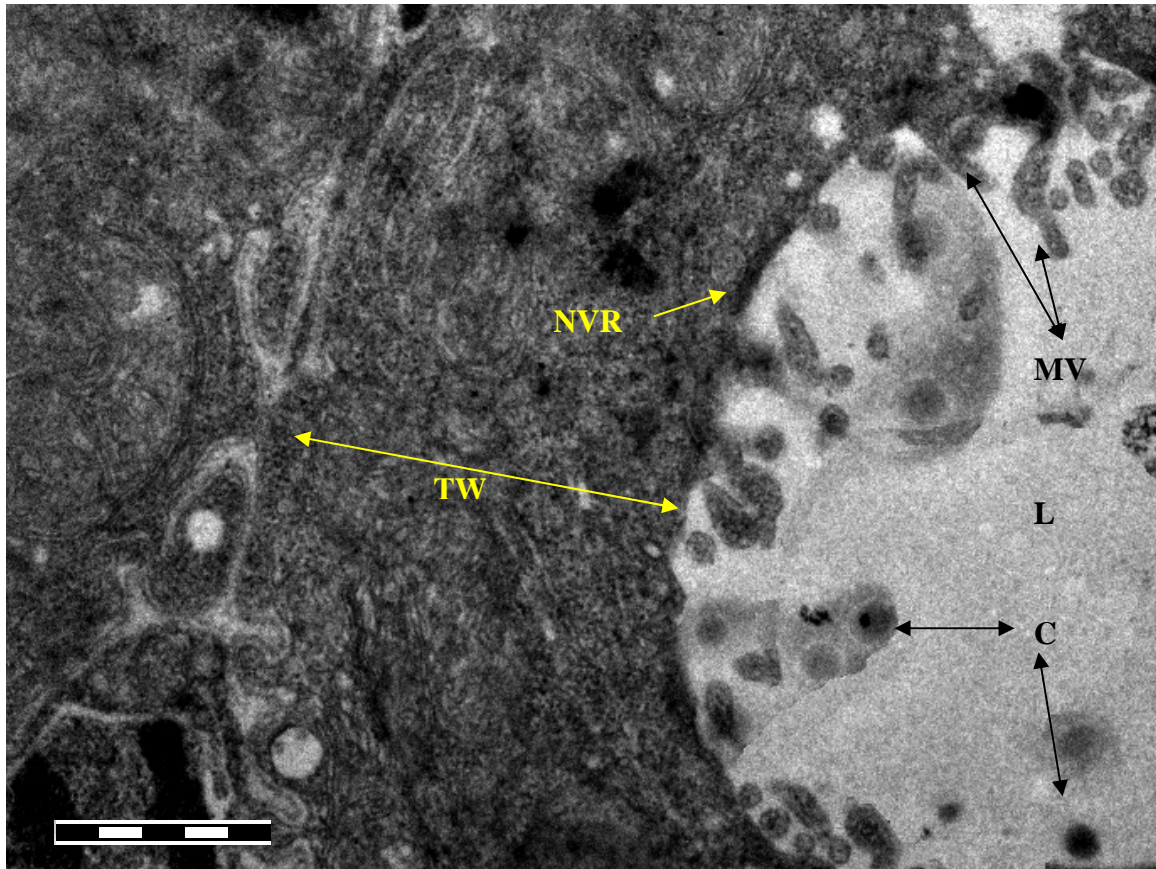
Plate 6-15. Electron micrograph of cross section through rectal area of common snook larva at one DAH

Scale bar 2µm

C   cilia
L   lumen
MV   microvilli
NVR non-villous region
TW   terminal web

Plate 6-16. Antero-media intestine semi-thin cross section from a 2 DAH common snook larva

Scale bar 70µm

CA   clear apical cell in epithelial
DA   dark apical cell in epithelial
L   lumen
N   nucleus of columnar cell
DSW   digestive system walls
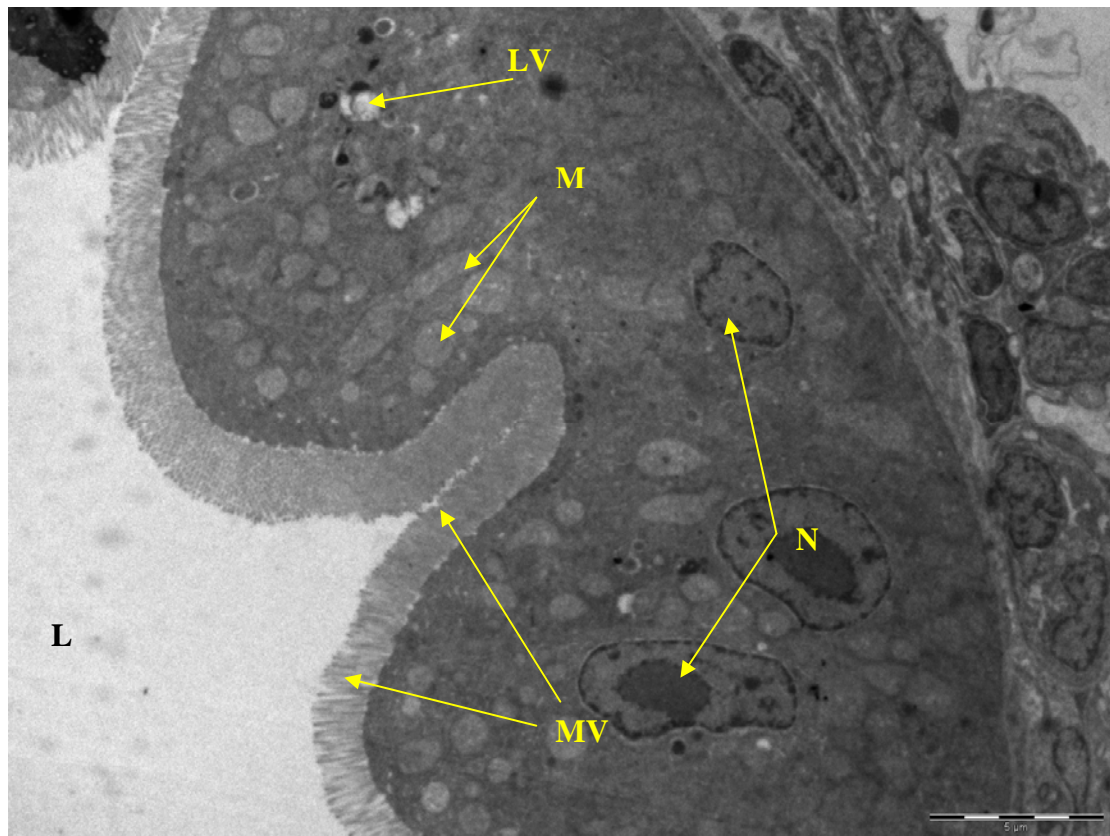
Plate 6-17. . Electron micrograph of cross section through antero-median intestine of common snook larva at two DAH

Scale bar 5µm

L   lumen
LV   Light vesicles
M   mitochondrion
MV   microvilli
N   nucleus
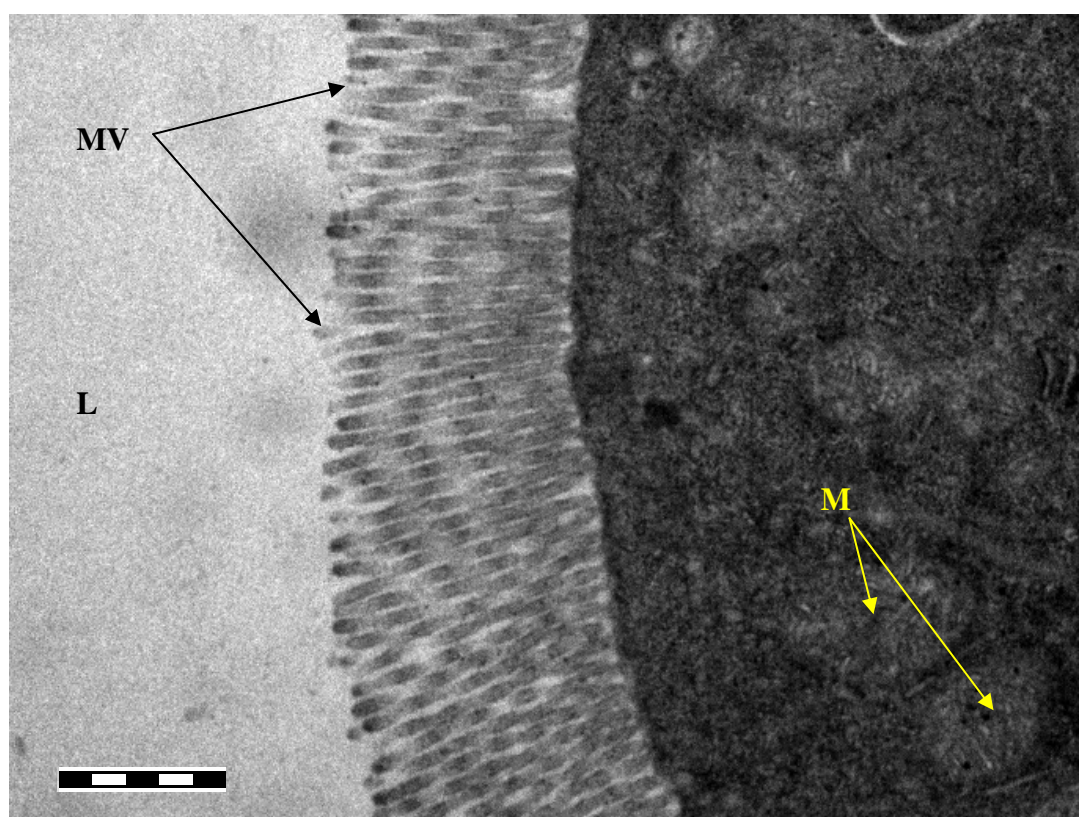
Plate 6-18. Electron micrograph of cross section through rectal area of common snook larva at two DAH

Scale bar 1µm

L   lumen
M   mitochondrion
MV   microvilli
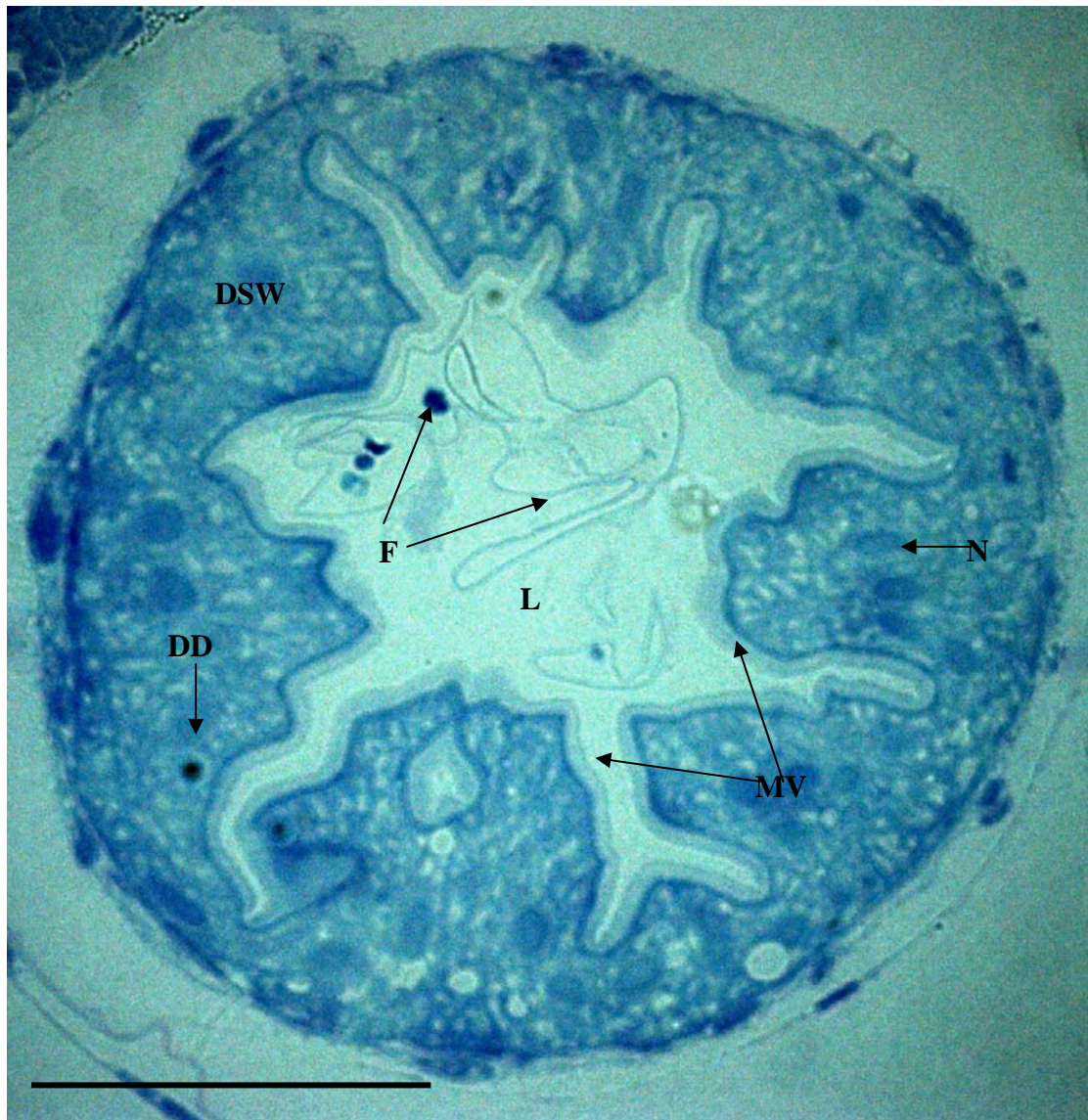
Plate 6-19. Antero-media intestine semi-thin cross section from a 3 DAH common snook larva

Scale bar 90µm

DD   dark droplet
DSW   digestive system wall
F   food particles
L   lumen
MV   microvilli layer
N   nucleus of enterocyte
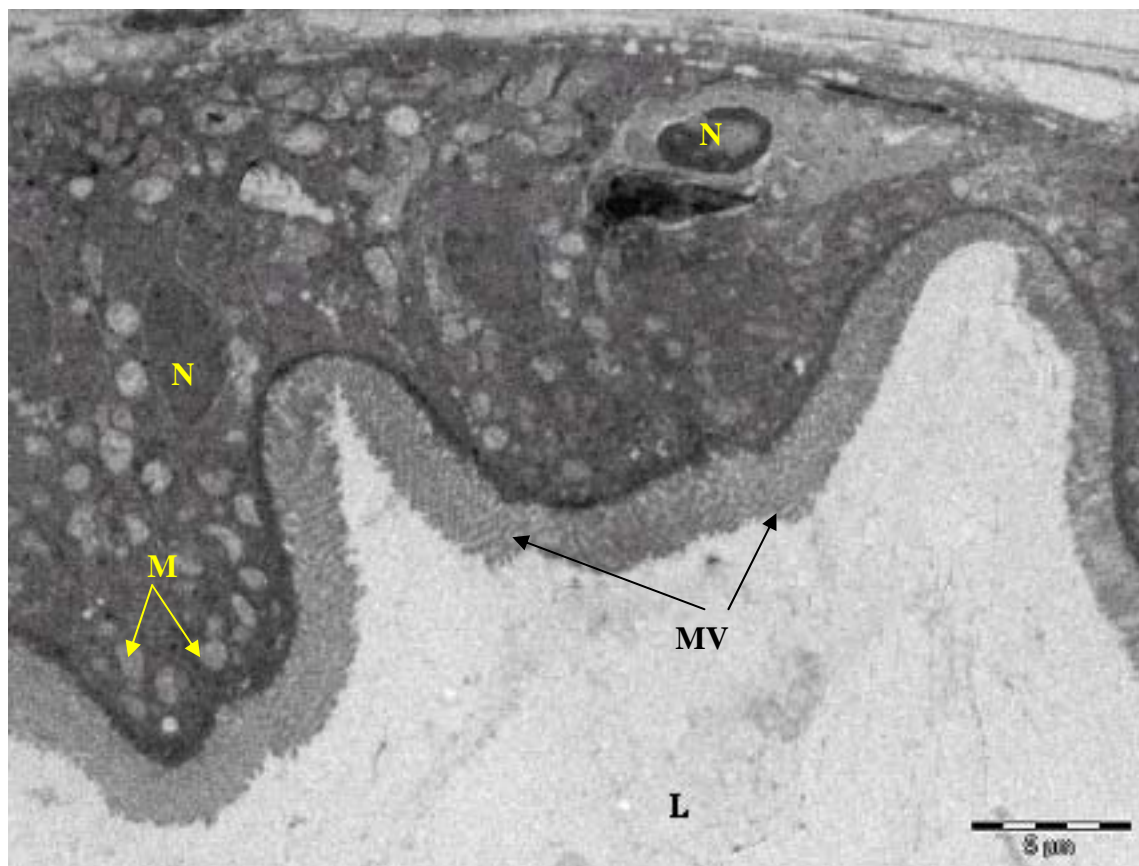
Plate 6-20. Electron micrograph of cross section through antero-median intestine of common snook larva at three DAH

Scale bar 5µm

L   lumen
M   mitochondrion
MV   microvilli
N   nucleus
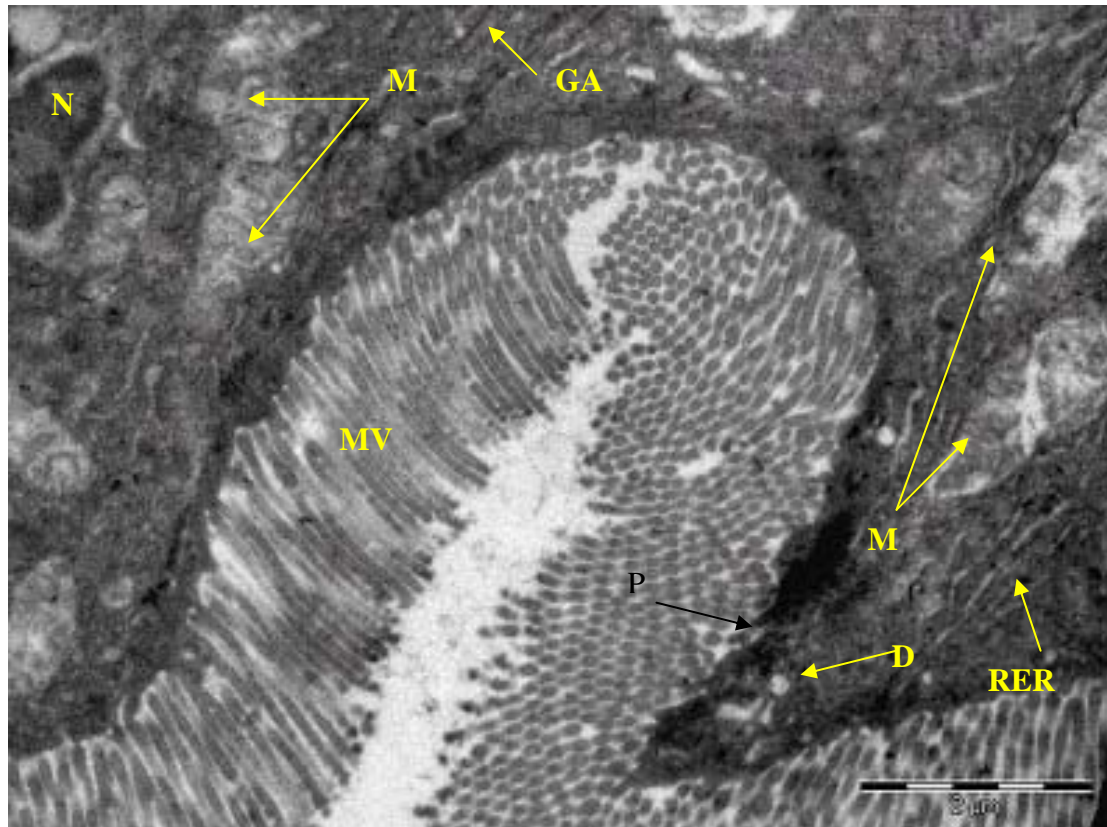
Plate 6-21. Electron micrograph of cross section through rectal area of common snook larva at three DAH

Scale bar 2µm

D   desmosome of apical juction
GA   golghi apparatus
M   mitochondrion
MV   microvilli
N   nucleus
P   pinocytotic vesicle
RER   rough endoplasmic reticulum
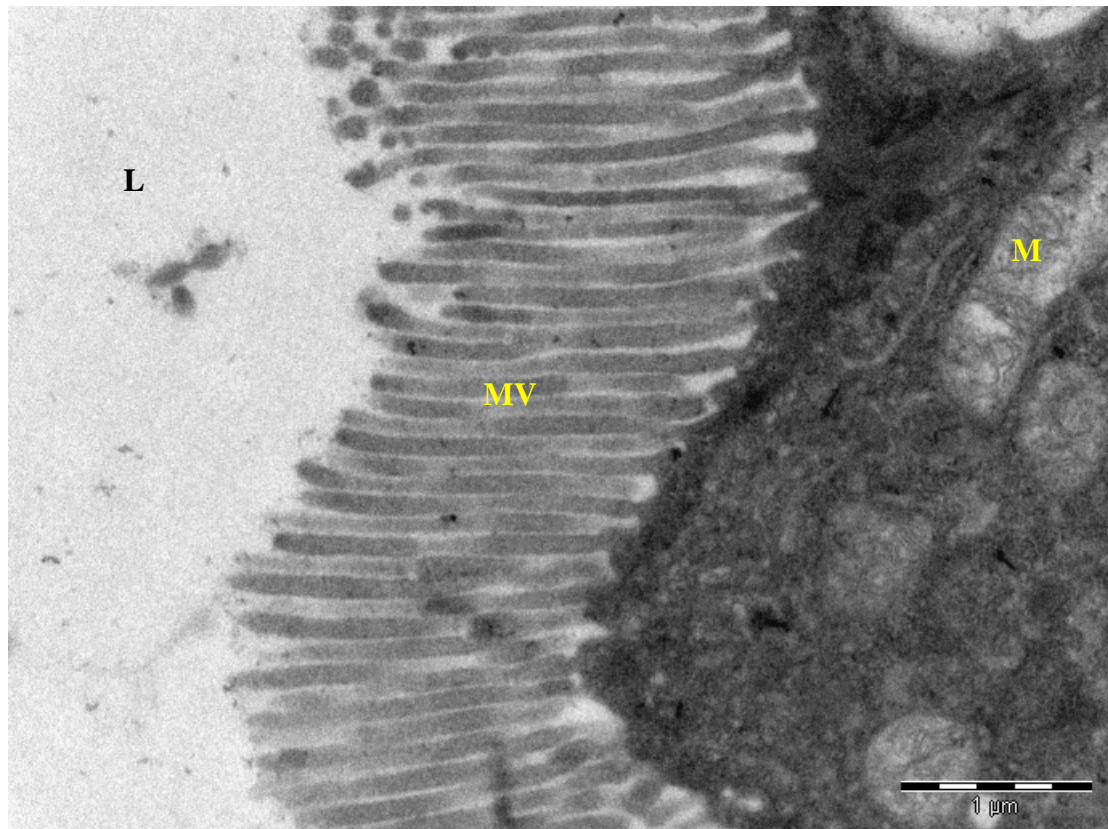
Plate 6-22. Electron micrograph of cross section through antero-median intestine of common snook larva at three DAH

Scale bar 1µm

L   lumen
M   mitochondrion
MV   microvilli

6.4 Discussion

6.4.1 *Centropomus undecimalis* **embryonic development**

The developmental features of *Centropomus undecimalis* described in this chapter are typical of most teleost species with planktonic eggs. No common snook eggs have been reported in the wild, although it is known that, immediately after spawning, eggs are taken towards the open sea by the outgoing tide and it is assumed that the following inward tide brings them back into the estuarine and mangrove environment. However, the youngest reported common snook found in the wild was 12 days old (Lau & Shafland, 1982). Sampling efforts to find the wild common snook eggs have been carried out, but as mentioned before, the quest has been so far unsuccessful. Many factors may have resulted in this unsuccessful outcome, such as sampling gear, location, time and the inability to recognize the common snook eggs once collected due to the fact that no embryonic development description had been done previously. The embryonic development carried out during this study was aimed to help to identify the common snook eggs in the wild, as well as to describe their development.

The search for buoyant planktonic embryos in the wild is a key issue so that it will be possible to then mimic the incubation conditions in the laboratory. This is important since the influence of the environmental conditions influences the early development and physiology of the offspring (Blaxter, 1992).

During incubation the process of cleavage, formation of layers, and morphogenesis of teleost eggs have been described in a number of standard

textbooks such as Rudnick (1955), Waddington (1956) and Smith (1957), with Oppenheimer (1947) and Devillers (1961) stressing structural changes from the viewpoint of experimental embryology. Most of those descriptions were done in freshwater species, although extensive work have been done in some marine species including *Seriolella punctata* (Grimes & Robertson, 1981), Sea Bream (*Sparus aurata)*, Flounder (*Platichthys flesus*), Dab (*Limanda limanda*), Herring *(Clupea harengus*), Cod (*Gadus morhua*), Plaice (*Pleuronectes platessa*), and salmon (*Salmo salar*).

Common snook are similar to many other fish species in that their eggs are round, although in the anchovy (*Engraulis)* and bitterling (*Rhodeos)* they are ovoid, and in certain gobies they are pear-shaped. At the same time the common snook embryo has only one oil globule whereas more than one can be found in other marine species (Simpson, 1956), and in most fish species that have telolecithal eggs with yolk more concentrated at the vegetative pole.

As with hagfish and elasmobranchs, common snook has a meroblastic cleavage even though snook are teleosts. They do not have the holoblastic cleavage that characterizes lampreys. Other groups such as bowfin (*Amia),* gar (*Lepidosteous)*, and sturgeon (*Acipenser*) have intermediate features.

All the above embryo characteristics plus the melanophore patterns, egg size and oil droplet size all described in the results section will make the recognition of common snook embryos more accurate, as well as providing a tool for egg quality evaluation in common snook culture.

As mentioned previously, the common snook larvae location in the planktonic column is unknown as well as their feeding habits and behaviour. This chapter has described the common snook larval development from the first 14 days of

larval culture in captivity, in order to facilitate the identification process for future studies on wild common snook larvae as well as for aquaculture purposes. Although there are many theories regarding the diet of common snook larvae during first feeding, there are no reported studies on this topic and so common snook first feeding is still a major problem for snook culture. In addition issues such as prey and prey size during the first 5 to 7 days are still underdeveloped. Therefore finding wild larvae of such an age will help to find the optimal prey, improve the rearing protocol and increase the knowledge on the wild common snook larvae ecology

6.4.2 *Centropomus undecimalis* morphological development

The common snook larval development was described in 1982 by Lau & Shafland, although they focused on larvae 14 days old and beyond, mainly looking at the osteo and fin formation; other than this no reported common snook development studies have been carried out.

Common snook larvae during the first 12 days of life have a characteristic pigmentation pattern localized in four main patches on the ventral and dorsal area. Pigmentation starts spreading along the larvae as it get older, and then begins to fade away with formation of the main fins. Although the finfold is present till day 12, the pigmented larval surface area c is significantly reduced from day 8. Caudal fin and pectoral fins are the first ones to develop from around day 12, the dorsal and the ventral fins start to gain definition by day 12. Head formation during the first 10 days occurs fast, and basic brain structure is well develop by day 3. Notochord flexion occurs around day ten.

From this day onwards, common snook larvae gain definition in fins, head features and organs.

6.4.3 Eye development

At hatching common snook larvae have unpigmented and non-functional visual system like many other species (e.g., Sole *Solea solea,* mackerel *Scomber scombrus,* whiting *Merlangius merlangus,* pilchard *Sardina pilchardus,* and sardine *Sardinops caerulea*); others have pigmented eyes (e.g. plaice *Pleuronectes platessa*, cod *Gadus morhua*, herring *Clupea harengus*, and salmonids), (Blaxter & Staines, 1970).

Blaxter, (1986) described the important role that vision has on fish larvae orientation, as a consequence of them being visual feeders. Fish species with a relatively small yolk sac need to develop fast in order to survive. In common snook larvae just like in sparids such as *Pagrus major (*Kawamura, 1984) and *Pagrus auratus (*Pankhurst, 1996) the most important changes in the eye structure occur in the lecitotrophic larvae as a preparation for prey capture.

Common snook one-day-old larvae had all the basic structural elements necessary for visual function, but most of them were not complete. This was an indication that the eye was about to become functional. Kawamura (1984) found that the visual system of *P. major* is functional at 36 h post-hatch when visual cells and pigments are present and nerve optic fibres connect with the optic tectum. In common snook, the visual system could not be functional at Day 1 post-hatching, principally because the pigmentation pattern, responsible for photon absorption was very sparse at this stage.

Retinas of most fish larvae mainly have green-sensitive single cones (Evans & Browman, 2004). This is the case of 2 day old common snook larvae: although the retina is not as fully developed as in the adult stage, all the retinal structural layers were complete but not fully functional. Histological observations support the partial functionality of the eye; by this time the pigment cell layer was present and the optic nerves are connected to the optic tectum. Thus the visual system was completely ready for prey capture. The pure cone retina has been found in the earlier stages of many teleost larvae (*Oncorhynchus* spp. (Ali, 1959), *Clupea harengus* (Blaxter & Jones, 1967), and *Pleuronectes platessa* (Blaxter, 1968). However, at first feeding Snook are only equipped with simple cones, as with *Pagrus major* (Kawamura, 1984) and *Pagrus auratas* (Pankhurst, 1996), and rods and twin cones appear at metamorphosis (Blaxter & Staines, 1970).

At day 3 the common snook larval retina has well developed presumptive cone receptors, the pigmentation layer is fully complete, structural layers are fully functional and differentiated, and the formation of the rod precursors can be spotted although they are scarce.

Overall, common snook larvae by day 2 post hatching have their visual system developed sufficiently to locate and capture prey, although due to the underdeveloped stage of the rods (which provide better vision under low light intensity levels, (O'Connell, 1981; Kawamura, 1984; Pankhurst, 1996)), adequate light conditions are necessary to optimize their ability to capture prey (Huse, 1993). Taking the rod development into consideration, light intensity during larval development should be altered accordingly, and to

investigate this matter more samples of older larvae should be colleted for histology.

6.4.4 Digestive system development

At hatching common snook larvae had a simple undifferentiated straight gut linked to an unstructured mouth and anus, as described in other teleost species (Roo *et al.*, 1999; Govoni, 1980; Govoni *et al.*, 1986; Stroband & Dabrowski, 1979; Bisbal & Bengston, 1995; Cousin & Baudin-Laurencin, 1985; Boulic & Gabaudan, 1992; Peña *et al.*, 2003). It is generally assumed that lipid absorption takes place in the anterior intestine, and based on this assumption and the importance that lipids have over the larval development, all the histological work done in this study was based on the antero-intestine development.

During the first 2 days the common snook larval digestive system undergo major changes. The anus and the mouth open; gut cells undergo significant growth; development of organelles is increased; and the intestinal valve is formed.

On day one, the alimentary canal differentiation is starting to appear, the mouth is starting to be formed but jaw cartilages are still developing. At the cell level, organelles can be observed but their underdeveloped stage and low numbers, rendering the digestive system unable to function. The alimentary canal epithelium at some parts of the luminal surface showed the presence of microvilli, although in low number and underdeveloped, at the same time ciliated cells were present in the lumen, such ciliated cells were not found after day 1, similar findings were observed in other species (Calzada *et al.*,

1998; Loewe & Eckman, 1988; Govoni *et al.*, 1986). The presence of these ciliated cells in larvae (which did not have any peristaltic movements) were reported by Iwai (1964) and contribute to the circulation of the intestinal contents (mainly yolk).

Common snook larvae by day 2 post-hatch had absorbed the yolk sac; similar results were observed in goldlined sea-bream (Ibrahim, 2004), and in seabass (Walford & Lam, 1993). Other species such as cod and sheephead sea-bream (Kjørsvik *et al.*, 1991; John & Tucker, 1987) exhausted their yolk sac at four days after hatching. The mouth was open and the main jaw cartilages such as the Meckel's cartilage or the hyomandibulare cartilage were present, these results matched other species such as *Etheostoma caeruleum*, *Catostomus commersoni*, *Persina caprodes* and *Gadus morhua* (McElman & Balon, 1981; Paine & Balon, 1985a,b; Kjørsvik *et al.*,1991), the dermal bones, such as the maxilliary and the pre-maxilliary are formed later on. At the same time food was observed in the gut.

Morphological and histological observations suggest that 2 days after hatching common snook larvae possess digestive organs enabling digestion, absorption and metabolization of endogenous food. At the onset of day 2 post hatch, enterocytes are morphologically developed, yet as with cod larvae (Kjørsvik *et al.*, 1991), the digestive mechanisms are immature and their functionality relies on lipid absorption and possibly temporary lipid storage in the anterior part of the gut (Tanaka, 1972a; Stroband & Dabrowski, 1979). Although no histology of the rectal area was done, the epithelial cells are probably responsible for food protein ingestion and intracellular digestion (Iwai & Tanaka, 1968; Tanaka, 1972b; Watanabe, 1982a,b).

Day 3 old common snook larvae had continued to develop. Mouth jaw cartilages were gaining in definition and speeding their functionality. The alimentary canal is now more structured with a clear differentiation between the different gut parts. Organelles had increased in numbers, such as mitochondrian, rough endoplasmatic reticule and the golghi apparatus. The microvilli layer is increased in length and consistency,

In conclusion, the alimentary canal of the common snook larva develops from an undifferentiated tube at hatching to a complex tract before the onset of day 2 after hatching (when the yolk sac is exhausted), this fast development of the digestive system is parallel to the visual system formation, showing the formation timing between these two systems; timing that is highly important for the beginning of prey capture and predatory avoidance. Together with the digestive and visual system development, common snook larvae have a partially developed fin structure that allows them to move on the water column and approach prey.

Chapter 7:

General Discussion

7. General Discussion

This study investigated the life cycle of the common snook (*Centropomus undecimalis*) and its larval culture, focusing on the early life stages from fertilization to the larval stage. Due to the current incomplete broodstock-breeding program at Mote Marine Laboratory (the reproductive cycle is not closed), the only supply of seed comes from wild broodstock. This reliance on the wild broodstock, and the lack of knowledge regarding some areas of the life cycle, allowed the present study to expand the research into areas beyond the aquaculture related issues. The results from the present study have increased the knowledge of embryonic and larval development. At the same time, a useful tool for predicting egg quality has been developed, and also current larval rearing protocols have been improved increasing the larval survival and quality.

The first step of this study was to secure a supply of healthy seed for rearing. This focused on the wild spawning cycle that occurs in the study area from May to September with a peak in June and July. The timing of this spawning cycle was directly related to the water temperature, which seems to be the key factor for the initiation of the spawning cycle. Therefore, the spawning cycle period will vary dependent on the water temperature. For instance, during the 2004 spawning season, the water temperatures in May and early June were lower than usual delaying the spawning season by one month. In terms of identifying the months where spawning activity was the highest, the various factors investigated such as; number of broodstock captured, amount of eggs obtained, successful spawning, and egg quality, indicated the peak

spawning months. These were from the last quarter of May to the first quarter of July, contradicting McMichael *et al.*, (1989) conclusions, but such contrary results could be due to water temperature differences at the time the studies were carried out. Independent of when the spawning period starts or ends, results in the present study indicate the peak spawning activity occurred at the beginning of the spawning period and lasted two months. Following this, egg quality, spawning success, egg quantity and broodstock capture decreased progressively. As discussed in chapter four, this decline is probably due to the physical exhaustion that wild broodstock are exposed to during the spawning period. Species such as artic char lose up to 80% of their lipid body contents during their spawning periods (Jørgensen *et al.*, 1997; Jobling *et al.*, 1998). Also, common snook broostock are in a different environment during the spawning season (upstream brackish water). These unusual physical and nutritional factors at the spawning site can directly affect common snook egg quality, as reported in other species (Sargent *et al*, 1999b; Zohar, *et al.*, 1984). It can be argued that even though the environment of the common snook breeding ground is different to the upstream brackish mangrove waters, this species has the ability to cope with the change (Volpe, 1959; Ager *et al.*, 1978; Shafland & Koehl, 1980) and the influence over the egg quality is minimal or nonexistent since the mouths of estuaries and coastal beaches are their natural spawning sites. On the other hand, the marine environment conditions by themselves are not the cause for the decrease in egg quality, but rather the continuous long exposure to such conditions. In summary, the exhausting physical demands that broodstock suffer during the spawning season, as well as the long exposure to an unusual environment, and the

change of diet, may be the reasons for the decline in egg quality as the spawning season progresses.

Within any month in the spawning season, snook in common with other species (Korringa 1947, Taylor 1984), also followed a spawning pattern, congregating for spawning at certain days. The results of the present study identified this pattern and related it to the moon cycle, matching the observations of Roberts *et al.*, (1999). Common snook spawning activity increased during different phases of the moon. Using the results obtained from egg quality, number of broodstock captures, and spawning success, high spawning activities were observed around the full moon and the new moon phases. Early stages of the moon cycle had a low spawning activity, but it increased slowly towards the full and new moon days and then increased exponentially during the first three days after both moon phases (full and new) before declining after the 4th day. In summary, chapter three has answered in detail the questions of 'where', 'when' and 'how' to get the best quality eggs for captive breeding.

The biggest remaining issue that requires additional extensive research is the effect of red tide over wild broodstock and their reproductive performance. This plankton bloom has occurred almost every year over the past five years and affects most of the aquatic fauna present in the bay area, resulting in mass fish kills. Concentrations of red tide tend to increase during the summer months, which coincide with the common snook spawning period. The effect of this is unknown. Adverse effects have been reported in other species such as red drum (*Scianops ocellatus*) (Riley *et al.*, 1989) and medaka (*Oryzias latipes*) (Kimm-Brinson & Ramsdell, 2001), showing that the red tide affects

their embryonic development, hatching rate and larval survival. Although common snook kills have been reported, especially in 2002, when more than 30 large mature females where found dead, common snook kills are not usual. What is clear, is that the environment around the spawning period is greatly affected by the bloom and, directly or indirectly, common snook could be affected in some way.

Transportation times from the spawning ground to the hatchery ranged from 20 minutes to 2 and a half hours. Although conditions inside the bag were controlled within the standard range, the influence of the transportation over development of the eggs has not been studied. Cleary et al., (2002) reported adverse effects in snapper egg survival due to handling and transportation. Other studies with species such as catfish, reported adverse effects on hatching success and larval survival when transportation time was extended (Small, 2005). Future work on this issue is recommended in order to limit disruption of egg development. Finding sites closer to the hatchery will reduce the transport time and, thus, the possible effects on egg development. A comparative study between the different type of transport containers may improve hatching success.

Based on the positive correlations between firstly, fertilization rate and secondly, hatching percentage, with the total egg lipid contents (Springate et al., 1984, Springate and Bromage, 1985, Nomura et al., 1974, Escaffre and Billard, 1979, Sakai et al., 1975, Thorpe et al., 1984, Dinis, 1982, Hirose et al., 1979, Kjørsvik and Lønning, 1983, Kjørsvik et al. 1984, Dushkina, 1975), DHA levels were chosen to investigate the influence that lipid content has over egg fertilization and hatching. Once the positive correlation was established, a fast

tool for predicting the development of healthy larvae was produced. This tool uses fertilization and hatching rate values as references for egg quality. Low values have been found to have reduced DHA levels, decreasing the chances for larval survival. At the same time, it was observed that larval survival from eggs with low DHA levels was reduced, matching the fertilization and hatching rates, and increasing the value of the egg quality prediction tool, as well as the direct effect that DHA levels have over egg and larval development. In summary, lipid analysis is a time consuming process that does not permit the prediction of egg quality in-situ (in 2 days from fertilization). But the positive correlation obtained from DHA levels with fertilization rate, hatching rate and survival, will predict the degree of larval success from just fertilization and hatching rates. Such methods of assessment give an idea of the egg quality without assuring larval survival, since other important factors, like first feeding, directly affect larval survival after hatching.

First feeding has been another important topic investigated during this study, due to its impact on larval survival (Person Le Ruyet *et al*. 1993). Lipids analysis assisted in identifying the first feeding period, and the point of no return, where starvation was the main cause of death. Therefore, the analysis of larval samples collected through development from day 2 post hatching to day 80 post hatching, showed that DHA levels fluctuate throughout development. The results showed a decrease in DHA levels during the first 6 days, nearly reaching the 0%. Watanabe, (1993) reported a decrease in DHA levels during marine embryonic development and during the time prior to first feeding, due to the high metabolic activity, However, the low levels reached by the common snook larvae indicated a very low lipid reserve, suggesting

that after the yolk sac exhaustion (Day 3), larvae were not ingesting any food. Following 6 days post hatch, the larvae collected began to show a slow increase in DHA levels. The tank mortality results obtained from both production and experimental systems reflect patterns seen in the lipid analysis results. In both systems over 70% of all tanks had no live larvae by day 6 ph. This reaffirms the fact that if by day six a common snook hasn't been able to feed it will not survive. Such results lead to the conclusion that feeding conditions for common snook at first feeding are not viable. In order to solve the problem, two main questions were asked: when are common snook larvae able to start feeding, and what prey and prey size can be ingested? The first question was answered by investigating both the gross structure, and the ultra structure of larvae during development, especially during the first three days. Gross morphology development showed that 2 day post hatched larvae had a partially-formed but functional open mouth, together with well differentiated eyes and, although still underdeveloped, ventral fins. At the same time unidirectional, predatory 'snap' movements were observed.

When the ultra structural development was investigated, a rapid development of structures in the alimentary canal was observed from day 1 to day 2 post-hatch. By then the gut, although not fully functional due to the lack of some organelles, was able to take food in and digest it mainly by lipid absorption. Food was also observed in the gut of those larvae fed from day two. At the same time, by day 2 post hatching the eye development was also partially completed and the eyes were partially functional since most of the organelles required for it were present, the brain was formed, and the connection between the eye and the optic nerve was in place. The partial eye

functionality is due mainly to lack of rods and the predominance of one type of cone opsin, suggesting limited visual acuity and limited wave-length sensitivity, which would reduce target contrast (Evans & Browman 2004). This should be taken into account when dealing with artificial light conditions during culture. Finding the optimal background conditions to increase the prey contrast, modifying the light conditions, and the tank colour, should be tried. Positive results were found during this study when modifying the tank's background using the green water technique. In summary, digestive tract and eye development were coordinated, reaching functionality at the same time allowing the larvae to feed. Protocols prior to this study started feeding at day three, a day later than they were able to feed. This could be one of reasons for the mass mortality at day 6.

The other significant problem observed was the size of prey. This issue restricted larval survival to larger than the average animals. The previous protocol used L type rotifers as first feeding prey. Larger larvae could feed on the rotifers, smaller larvae could feed on rotifer eggs. However, average sized larvae were unable to feed on either. A smaller prey was needed to increase the first feeding larval survival, three prey sizes were tested, S type rotifer, SS type rotifer, and copepods (*Acartia tonsa*). Larval survival and food found in the gut increased as the size was reduced, but larval survival was still low. Many factors affect larval survival (Chiu Liao *et al.*, 2001), this study analysed main factors affecting larval survival determining their influences over egg quality and larval survival. During first feeding a mix of SS type rotifers and copepods should be given from day two, with a high percentage of copepods (*Acartia tonsa*) and a low SS type rotifer percentage. After this there should

be a slow shift towards a diet based on SS type rotifers once prey size is not restricting. Another option will be the use of Bay water for the larval system for the first 6 to 8 days, as this water is rich in copepods and other species. This is a more natural food supply for common snook larvae. Constraints to this option are the potential presence of red tide and other harmful organism such as jellyfish larvae.

The first 10 days of the life cycle remain unknown, and this gap needs to be closed with studies of the habitat conditions and feeding habits, so this information can be applied to snook cultivation. Also, once the eggs are released in the water column, their movements with the tide and later settlement can be investigated. Acceptance of micro-diets by common snook larvae is an important topic. The positive preliminary results obtained during this study could lead to a substantial reduction in production costs and also a reduction in nutritional deficiencies such as lordosis, characterized by the poor nutritional quality of rotifers. The search for a more suitable micro diet for snook larvae in terms of nutritional value and acceptability may help to obtain acceptable survival results.

Overall, the present study of common snook culture has been demanding. The novelty of this species to aquaculture and to the lack of captive broodstock, limited the experimental period to four months of the year. In order to make faster progress on their culture it is critical to produce F1 in captivity, so problems associated with sampling could be removed, allowing studies to focus on larval rearing protocols. At Mote Marine laboratory, mature wild broodstock were collected over two years and kept in tanks under controlled lunar cycle light and temperature conditions that mimicked those in

the wild. Initially, no hormone injections were used in an attempt to allow the brooders to settle and adapt to the captive conditions and maybe spawn naturally. Prior to this study, induced spawning of common snook had produced poor results (Neidig *et al.* 2000). After two years, no signs of gonadal maturity were observed. Latterly, some gonad development has been observed. Such preliminary results may lead to captive spawning.

The egg quality analysis carried out during this study will be very helpful in the future to compare egg quality of captive broodstock with wild broodstock in order to assess egg viability.

Common snook culture will in the future be viable if more intensive research is carried out. Their small size at hatching, the difficulty to breed them in captivity, and the protected species status in Florida have been the main reasons for the discontinuous research. The market value that common snook has in South and Central America makes that region the ideal place to develop the cultivation due to the economic incentive that common snook culture farming will have.

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