



## Effects of fatty acid composition and spawning season patterns on egg quality and larval survival in common snook (*Centropomus undecimalis*)

Carlos Yanes-Roca<sup>b,\*</sup>, Nicole Rhody<sup>a,1</sup>, Michael Nystrom<sup>a,1</sup>, Kevan L. Main<sup>a,1</sup>

<sup>a</sup> Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, Florida 34236, USA

<sup>b</sup> Institute of Aquaculture, Stirling University, Stirling, Scotland, FK9 4LN, UK

### ARTICLE INFO

#### Article history:

Received 19 February 2008

Received in revised form 17 October 2008

Accepted 22 October 2008

#### Keywords:

Common snook

Egg quality

Fatty acids

Larval survival

### ABSTRACT

Common snook (*Centropomus undecimalis*) is a new candidate species for aquaculture. Its reproductive cycle has not been completed in farmed fish since knowledge of their behaviour in the wild and its reproductive physiology remains incomplete, and the only source of seeds comes from wild broodstock. This study was undertaken to examine the fatty acid profile of common snook eggs throughout the spawning season (May to September) in relation to egg quality and larval survival. The fatty acid (FA) composition of eggs collected from wild broodstock stripped on the field (2002–2005), was determined over the spawning season. In general the FA profile observed was consistent with that observed in marine fish apart from a high level of arachidonic acid (ARA) (3.68% of Total FA). The profile of polyunsaturated fatty acids (PUFA) changed over the spawning season (37.5%–29.4% Total FA) and egg quality was best in May, June and July. Eggs with higher concentration (13% of Total FA) of docosahexaenoic acid (DHA) were found to have higher fertilization, hatching and larval survival rate.

© 2008 Elsevier B.V. All rights reserved.

### 1. Introduction

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). Snook are protandric hermaphrodites: some males develop into females between 1 and 7 years of age, having a maximum 20-year lifespan. 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).

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 (Kjorsvik 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 (Kjorsvik et al., 1990).

Snook is a seasonal spawner, with spawning activity determined by the water temperature. Salinity, tidal current and moon phase are other physical parameters that determine the snook-spawning period, which runs from May till September (Yanes Roca, 2006). Prior work done on snook egg quality (Neidig et al., 2000) resulted in poor egg quality from hormone induced captive broodstock.

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 research 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 in certain amount to satisfy biological demands (Kjorsvik et al., 1990).

Apart of their role as being a major source of metabolic energy (Sargent et al., 1989; Froyland et al., 2000; Sargent et al., 2002; Tocher, 2003), Fatty acids (FA), and particularly Polyunsaturated fatty acids (PUFA), are functionally essential for normal growth, development and reproduction in fish (Sargent et al., 1989, 2002).

Lipids and their constituent FA 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. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the major FA in the total lipid of eggs of most fish and these fatty acids markedly influence the reproductive parameters. DHA is especially abundant in the retina and brain and has a

\* Corresponding author. Tel.: +34 6668 62806; fax: +34963543576.

E-mail addresses: [carlos.yanes@uv.es](mailto:carlos.yanes@uv.es), [mechacyr@hotmail.com](mailto:mechacyr@hotmail.com) (C. Yanes-Roca).

<sup>1</sup> Tel.: +1 941 388 4441.

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., 1999). In addition, 20:4n-6 as a major fatty acid in phosphatidylinositol (PI) and precursor of prostaglandin E<sub>2</sub>, 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).

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. 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 those 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 its development and functionality.

The main aims of this study were to describe the fatty acid composition of wild common snook eggs and assess the direct influence that fatty acids composition have on egg and larval quality.

## 2. Materials and methods

### 2.1. Sampling sites

The capture of wild stock for eggs and milt collection took place from April to September over the course of 4 years (2002–2005). A total of 96 field trips were made to 7 locations spread along the coast of Sarasota County, Manatee County and Port Charlotte (Florida, USA) (Fig. 1). The sites were located at passes and estuaries, characterized by seagrass and sandy bottoms. Broodstock sampling took place through all the lunar phases (i.e. full moon, new moon, etc.) and was collected during the outgoing tides between 15:00 and 20:00 o'clock.

### 2.2. Capture of wild stocks, eggs and milt extraction and fertilization

When spawning activity (agglomeration of fish in one spot at the water surface) was identified, a 91 m by 2.1 m seine net, equipped with a purse at the centre, was deployed from a boat, enclosing the wild broodstock within the net and the embankment. The net was then pulled onto the shore and trapped fish were collected. The captured snook were then sexed (Male snooks were identified by their pin-like genital pore, while female snooks were identified by their larger, swollen, genital pore) and placed in designated male and female nets (1.2 m by 1.2 m) until broodstock were manually stripped. In order to avoid any gamete pollution with sea water or urine all stripped fish pelvic and abdominal area was dried and cleaned. A minimum of 5 and a maximum of 20 females fish were stripped during each spawning trip. Their eggs were stripped and collected in a dry, clear 1000 ml plastic container with a lid. Two replicate samples of 1 ml of eggs were taken and fixed in 3 ml of chloroform/methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene (BHT) and stored at -30 °C for later lipid analysis. Milt was stripped from male snooks and collected in graduated, dry plastic syringes and chilled with ice packs until needed for fertilization. The ratio of males contributing

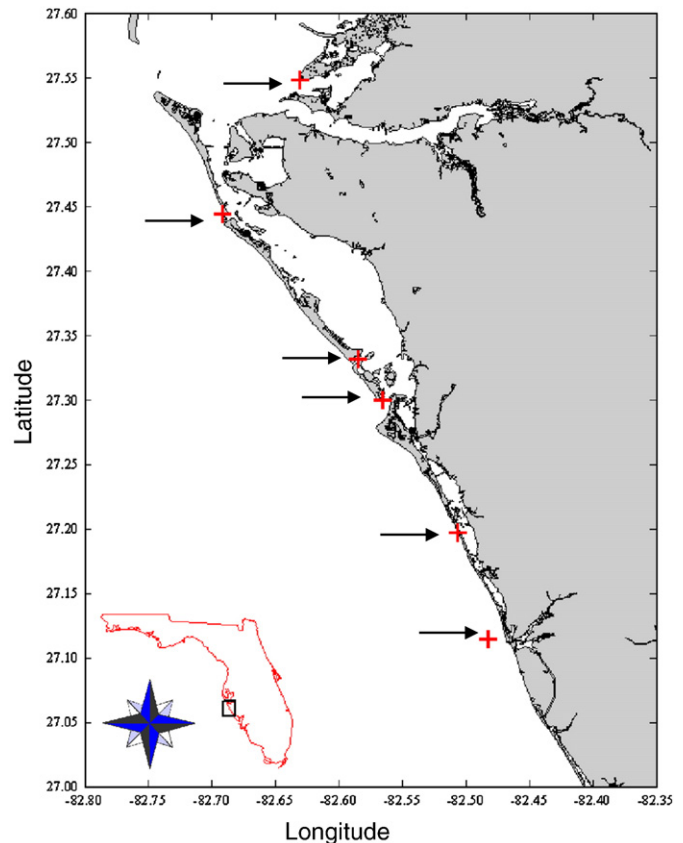


Fig. 1. Map of sampling sites in Central West Florida. Arrows marked sampling sites.

milt to females contributing eggs was 5:1 (5 males to 1 female). For fertilization, 1 ml of milt was added to the container holding 300 ml of eggs (1 ml=approximately 2800 eggs (Neidig et al., 2000)). After mixing the eggs and milt thoroughly, approximately 200 ml of filtered seawater at 28 °C were added to activate the sperm. The activated mixture was held for 2 min and then the eggs were rinsed in a 100 µm sieve with filtered sea water.

### 2.3. Egg transportation and stocking

The eggs are then rinsed into a plastic bag with 6 l of filtered seawater. The bag was topped up with pure oxygen (14 mg/l of dissolved oxygen), sealed with rubber bands and placed in a cooler at 28 °C for transport.

After transportation (average transportation time was 2 h) from the sampling site to the laboratory, 3 samples containing 1 ml of fertilized eggs were examined under a light microscope to assess their fertilization rate, egg size and morphology. Other samples of fertilized eggs were collected and placed in 3 floating hatching cells that were, at the same time, placed in the production tanks and collected after eggs hatched the following day. These hatching cells consisted of a 5 cm PVC piece of tubing open on the top end, and 100 µm mesh on the bottom, 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 (2 ml/l) 1 min prior to stocking in tanks. Water in all tanks was between 28 and 29 °C, at 35 ppt and 6–8 mg/l of dissolved oxygen. Prior to stocking, production tanks were set up with very light aeration and no water circulation in order to reduce water movement to the minimum. All the larvae used for this experiment were taken from the 2 l experimental tanks, part of a re-circulating system, where the water

**Table 1**  
Common snook egg fatty acid composition, average values from 48 samples

Fatty Acid	Eggs total FA % area
14:0	1.36±0.1
15:0	0.81±0.2
16:0	20.70±0.5
18:0	5.26±0.2
20:0	0.17±0.1
22:0	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

(Total fatty acid percentage area) means given with ±standard deviation.

temperature was at 28 °C, with a salinity of 35 ppt and with constant dissolved oxygen concentration of 10 mg/l. Larvae were fed S Type rotifers at a density of 30 rotifers/ml. Five larvae were randomly collected daily from day 0 until day 30.

#### 2.4. Lipid and data analysis

All fixed samples were analysed at the Institute of Aquaculture, University of Stirling. Lipid extraction was carried out following the protocol of Folch et al. (1957). To determine the fatty acid composition

of the total lipid extracts, aliquots were subjected to acid-catalysed transesterification (Christie, 2003) and the resulting fatty acid methyl esters purified by thin layer chromatography on silica-coated glass plates using iso-hexane:diethyl ether (90:1 v/v) as developing solvent. After recovery from the adsorbent by elution with iso-hexane: diethyl ether (1:1 v/v) containing 0.01% BHT, the purified fatty acid methyl esters were analysed by capillary gas chromatography.

Excel 2000 and SPSS 12th edition were used to analyse the data collected. 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.

### 3. Results

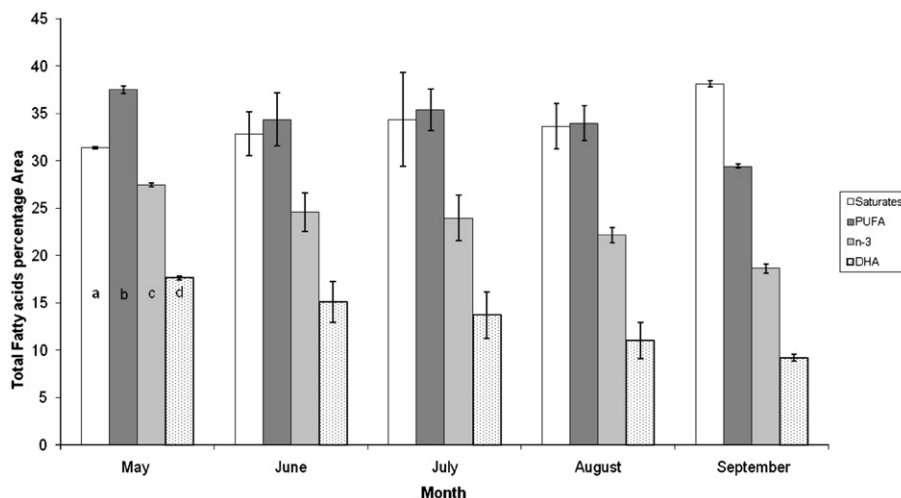
Table 1 shows the overall average fatty acid composition in the 48 samples of unfertilised snook eggs collected between 2002 and 2005 together with the range of individual fatty acids observed.

The most abundant saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) observed in common snook eggs were palmitic acid (16:0), oleic acid (18:1n-9) and docosahexaenoic acid (22:6 n-3), respectively. SFA accounted for an average of 34.5% of the total fatty acids in snook eggs. Similar proportions of MUFA (31.5%) and PUFA (34.9%) were observed. Within the PUFA, the n-3 were more abundant than those of the n-6 series with an overall ratio (n-3:n-6) of 2.5% TFA area. The mean EPA to DHA ratio was 0.18 and the average ARA, (20:4n-6) to EPA was 1.55 (Table 1).

#### 3.1. Changes in common snook egg fatty acid composition during spawning season

A significant difference ( $p=0.041, 0.021, 0.006, 0.016$ , respectively) was found between the spawning months and the SFA, showing an increasing trend from May to September (Fig. 2), at the same time the PUFA (Fig. 2), the omega 3 FA (Fig. 2) and the DHA (Fig. 2) showed a significant decreasing trend. May was the month with the highest value in all the above FA except for the SFA, where September had the highest average.

On the other hand, no significant difference was found between the spawning months and the MUFA, the omega 6 FA, the EPA and the ARA ( $p=0.756, 0.224, 0.493, 0.739$ , respectively) (Table 2). Overall, no significant difference in MUFA values was found between the different months.



**Fig. 2.** Temporal changes in mean monthly values (% area) from 2002–2005 in snook eggs: a) saturated fatty acids; b) polyunsaturated; c) omega-3; and d) DHA.

**Table 2**  
Fatty acids composition variation by month from the different years (2002–2005)

Month	Fatty acid	2002	2003	2004	2005
May	MUFA	0	30.92±0.1	0	0
	n-6	0	7.80±0.1	0	0
	ARA	0	3.40±0.0	0	0
	EPA	0	1.79±0.0	0	0
	EPA/DHA	0	0.10±0.0	0	0
	ARA/EPA	0	1.90±0.0	0	0
June	MUFA	33.76±0.6	31.96±1.3	32.00±2.6	32.47±1.6
	n-6	5.40±0.5	8.35±0.1	9.90±0.8	7.58±1.3
	ARA	1.55±0.2	3.47±0.4	4.38±0.4	3.29±0.8
	EPA	1.28±0.2	2.39±0.5	2.39±0.3	2.23±0.2
	EPA/DHA	0.09±0.0	0.19±0.0	0.17±0.0	0.12±0.0
	ARA/EPA	1.21±0.1	1.50±0.3	1.87±0.4	1.48±0.4
July	MUFA	25.65±4.7	30.64±0.5	32.34±2.8	31.12±1.3
	n-6	9.44±2.0	9.23±0.0	10.49±2.2	8.81±3.0
	ARA	3.82±0.2	3.82±0.5	4.65±0.7	3.89±1
	EPA	2.41±0.1	2.25±0.6	3.36±0.9	3.03±0.4
	EPA/DHA	0.23±0.0	0.17±0.1	0.24±0.0	0.19±0.1
	ARA/EPA	1.59±0.1	1.79±0.5	1.41±0.2	1.29±0.4
August	MUFA	28.58±0.4	31.02±2.5	30.70±1.4	34.08±0.4
	n-6	11.10±0.6	7.20±0.6	10.79±0.6	11.86±0.0
	ARA	3.55±0.3	3.08±1.3	4.66±1.3	5.07±0.2
	EPA	2.53±0.0	1.78±0.4	3.34±0.4	2.74±0.0
	EPA/DHA	0.29±0.0	0.12±0.0	0.28±0.1	0.28±0.0
	ARA/EPA	1.40±0.0	1.38±0.7	1.40±0.7	1.85±0.1
September	MUFA	32.30±0.2	0	0	0
	n-6	10.06±0.3	0	0	0
	ARA	3.56±0.2	0	0	0
	EPA	1.26±0.0	0	0	0
	EPA/DHA	0.14±0.0	0	0	0
	ARA/EPA	2.84±0.0	0	0	0

Means given with ± standard deviation.

At the same time no significant difference was found between the spawning months and both EPA/DHA and ARA/EPA ratios ( $p=0.360$ ,  $0.062$ , respectively) (Table 2).

**3.2. Influence of eggs and larvae DHA levels on fertilization rates, hatching percentage and larval survival**

DHA levels were found significantly correlated with fertilization rate ( $p=0.002$ ), hatching percentage ( $p=0.009$ ) and larval survival ( $p=0.001$ ). It can be observed that DHA levels in snook eggs having a 10% TFA had a fertilization percentage under 50% (Fig. 3). At the same time, snook eggs with DHA values over 12% total lipid area had a fertilization percentage over 60 (Fig. 3).

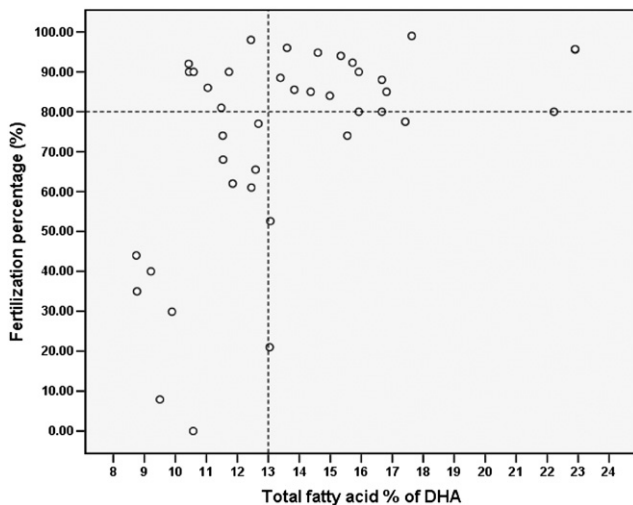


Fig. 3. Snook eggs DHA values against fertilization percentage (2002–2005).

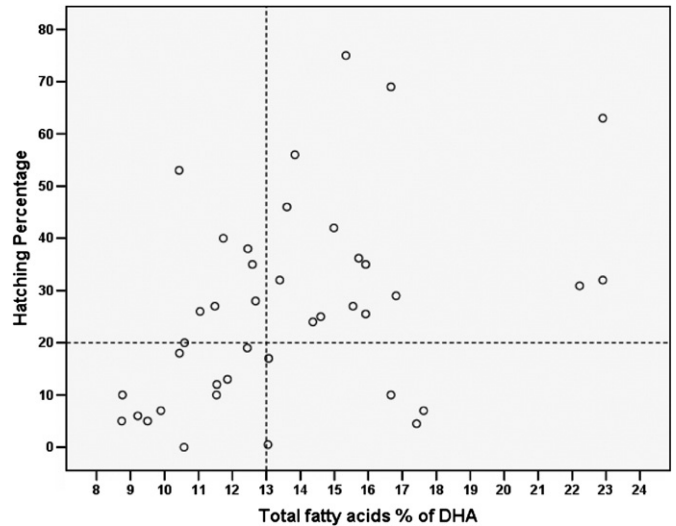


Fig. 4. Influence of snook eggs DHA levels on hatching percentage.

The DHA/fertilization correlation showed that 81% of egg samples, with DHA values higher than a 13% of TFA, had a fertilization rate of 80% or higher. On the other hand, 61% of eggs samples with DHA values lower than 13% TFA had a fertilization rate below 80%. Overall 70% of all the snook eggs samples analysed with a 13% TFA or higher had a fertilization percentage of 80% or higher.

In terms of hatching percentage, 80% of the snook larvae with an egg DHA content of 13% or higher of TFA, had at least a hatching percentage of 20%; on the other hand, only 39% of snook eggs with an egg DHA content lower than 13% of TFA had a hatching percentage higher than 20% (Fig. 4). More in depth, 55% of snook larvae with an egg DHA content higher than 13% had a hatching percentage of 30% or higher. At the same time, only 22% of larvae with an egg DHA content lower than 13% of TFA had a hatching percentage of 30% or higher.

Larvae survival increased with the increased of DHA levels in eggs (Fig. 5). Seventy six percent of snook larvae with DHA egg contents higher than 12% of TFA survived past day 6 (Fig. 5), on the other hand, 94% of snook larvae with DHA eggs content lower than 13%TFA dropped out by day 6 (Fig. 5).

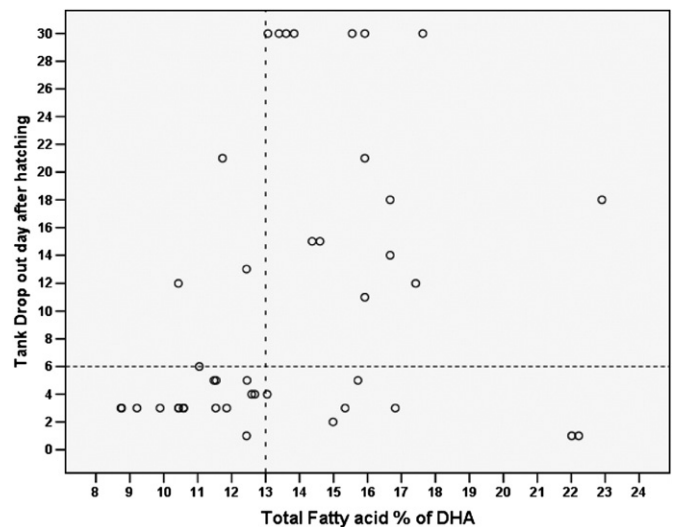


Fig. 5. Influence of snook egg DHA levels on larvae survival. Each spot in the graph represents the day in which a tank stocked with larvae die out and the DHA % area TFA when it happened.

#### 4. Discussion

Snook egg fatty acid composition fits the general marine fish fatty acid profile (Kaitaranta and Linko 1984; Tocher and Sargent 1984; Wiegand, 1996; Sargent et al., 2002).

The  $n-3/n-6$  PUFA ratios in snook eggs is lower (2.52) than the typical marine fish (2.9) (Henderson and Tocher 1987), although still reflecting the higher amounts of  $n-3$  HUFA, specifically 20:5 $n-3$  and 22:6 $n-3$ , in their lipids. The EPA value is in the mid low range of those reported fish eggs (e.g. Eldridge et al., 1983; Kaitaranta and Linko 1984; Tocher and Sargent 1984), 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 (mainly MUFA) found in the common snook 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 (~2.5% area TFA) (e.g. Tocher et al., 1985; Fraser et al., 1988; Ulvund and Grahl-Nielsen 1988).

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., 1999). Consequently the unusually high ARA value observed 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). The broodstock remain exposed to pure marine conditions during the spawning season, which affects their feeding habits. It has been shown for other species that, not only the quality of eggs, but also their chemical components, are influenced by their nutritional composition of diets eaten by the broodstock before, and during, spawning (Leray et al., 1985; Watanabe et al., 1991; Harel et al., 1994; Watanabe and 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 are investigated, the extent to which diets can influence egg quality may be seen. So far there are contradicting 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, while PUFA, and omega 3 fatty acids 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. (1985) have suggested that DHA has a more important biochemical function in 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. 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 and 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.

A positive correlation between total egg lipid content and hatching has been well established Zhukinsky and Kim (1981) and hatching percentage and fertilization are useful parameters to assess egg quality (e.g. Springate et al., 1984; Springate and Bromage 1985; Nomura et al., 1974). This study used both parameters together with the fatty acid composition to assess good egg viability. The above parameters (fertilization and hatching) by themselves are not definite criteria of egg quality (Kjorsvik and Lonning, 1983; Blaxter 1955; Dushkina, 1975), therefore in order to get reliable results to determine wild snook eggs 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 fertilization percentage, hatching percentage and larval survival where the higher the DHA snook eggs concentration, the higher fertilization percentage, hatching percentage and larval survival. 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. All the above findings confirmed the importance of DHA for embryonic development and larval survival as well as giving us a prediction tool for egg viability and larval rearing success.

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 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.

#### Acknowledgements

This work was supported by grants from the Institute of Aquaculture at Stirling University, the Florida Fish and Wildlife Conservation Commission, the National Oceanic and Atmospheric Administration funded research consortium, the Science Consortium for Ocean Replenishment (SCORE), and the Mote Scientific Foundation.

Special thanks to the Center for Aquaculture Research and Development at Mote Marine Laboratory, FL, especially to Dr. Kevan Main for her unconditional support through the four years. Thanks also to the Institute of Aquaculture at the University of Stirling and the Lipids group for the help given.

#### References

- Almansa, E., Martin, M.V., Cejas, J.R., Badia, P., Jerez, S., Lorenzo, A., 2001. Lipid and fatty acid composition of female gilthead seabream during their reproductive cycle: effects of a diet lacking  $n-3$  HUFA. *Journal of Fish Biology* 59, 267–286.
- Bell, J.G., Sargent, J.R., 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture* 218, 491–499.
- Bell, M.V., Henderson, R.J., Sargent, J.R., 1985. Changes in the fatty acid composition of phospholipids from turbot (*Scophthalmus maximus* L.) in relation to dietary polyunsaturated fatty acid. *Comparative Biochemistry and Physiology* 81, 193–198.

- Bell, M.V., Batty, R.S., Dick, J.R., Fretwell, K., Navarro, J.C., Sargent, J.R., 1995. Dietary deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.). *Lipids* 30, 443–449.
- Blaxter, J.H.S., 1955. Herring rearing. The storage of herring gametes. *Marine Research* 3, 1–12.
- Christie, W.W., 2003. *Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids*, Third edition. Oil Press, Bridgwater, England.
- Dushkina, L.A., 1975. Viability of herring (*Clupea*) eggs and fertilization capacity of herring sperm stored under various conditions. *Journal of Ichthyology* 15, 423–429.
- Eldridge, M.B., Joseph, J.D., Taberski, K.M., Seaborn, G.T., 1983. Lipid and fatty acid composition of the endogenous energy sources of striped bass (*Morone saxatilis*) eggs. *Lipids* 18, 510–513.
- Evans, R.P., Parrish, C.C., Zhu, P., Brown, J.A., Davis, P.J., 1998. *Marine Biology* 130, 367–376.
- Fernandez-Palacios, H., Izquierdo, M., Robaina, L., Valencia, A., Salhi, M., Vergara, J.M., 1995. Effect of *n*-3 HUFA level in broodstock diets on egg quality of gilthead seabream *Sparus aurata* L. *Aquaculture* 132, 325–337.
- Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipid from animal tissues. *Journal of Biological Chemistry* 226, 497–509.
- Fraser, A.J., Gamble, J.C., Sargent, J.R., 1988. Changes in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (*Gadus morhua*). *Marine Biology* 99, 307–313.
- Froyland, L., Lie, O., Berge, R.K., 2000. Mitochondrial and peroxisomal beta-oxidation capacities in various tissues from Atlantic salmon, *Salmo salar*. *Aquaculture Nutrition* 6, 85–89.
- Harel, M., Tandler, A., Kissil, G.W., 1994. The kinetics of nutrient incorporation into body tissues of gilthead seabream *Sparus aurata* females and the subsequent effects on egg composition and egg quality. *British Journal of Nutrition* 72, 45–48.
- Henderson, R.J., Tocher, D.R., 1987. The lipid composition and biochemistry of freshwater fish. *Progress in Lipid Research* 26, 281–347.
- Howells, R.G., Sonski, A.J., Shafland, P.L., Hilton, B.D., 1990. Lower temperature tolerance of snook, *Centropomus undecimalis*. *Northeast Gulf Science* 11, 155–158.
- Ibrahim, F.S., 2004. *Reproductive Biology of Wild Goldlined Seabream, Rhabdosargus sarba* Captive Breeding and Larval Development in the Sultanate of Oman. Doctoral Thesis. Stirling University.
- Kaitaranta, J.K., Linko, R.R., 1984. Fatty acids in the roe lipids of common food fishes. *Comparative Biochemistry and Physiology* 79, 331–334.
- Kjorsvik, E., Lonning, S., 1983. Effects of egg quality on normal fertilization and early development of the cod, *Gadus morhua* L. *Journal of fish biology* 23, 1–12.
- Kjorsvik, E., Mangor-Jensen, A., Holmejoerd, I., 1990. Egg quality in fishes. *Advances in Marine Biology* 26, 71–113.
- Koven, W.M., Tandler, A., Sklan, D., Kissil, G.W., 1993. The association of eicosapentaenoic and docosahexaenoic acids in the main phospholipids of different age *Sparus aurata* larvae with growth. *Aquaculture* 116, 71–82.
- Leray, C., Nonnotte, G., Rouband, P., Leger, C., 1985. Incidence of (*n*-3) essential fatty acid deficiency on trout reproductive processes. *Reproduction, Nutrition and Development* 25, 567–581.
- Mustafa, T., Srivastava, K.C., 1989. Prostaglandins (eicosanoids) and their role in ectothermic organisms. *Advances in Comparative and Environmental Physiology* 5, 157–207.
- Neidig, C., Skapura, D.P., Grier, H.J., Dennis, C.W., 2000. Techniques for spawning common snook: broodstock handling, oocyte staging, and egg quality. *North American Journal of Aquaculture* 62, 103–113.
- Nomura, M., Sakai, K., Takashima, F., 1974. The overripening phenomenon of trout, temporal morphological changes of eggs retained in the body cavity after ovulation. *Bulletin of Japanese Society of Scientific Fisheries* 40, 977–984.
- Pickova, J., Dutta, P.C., Larsson, P.O., Kiessling, A., 1997. Early embryonic cleavage pattern, hatching success and egg lipid fatty acid composition: comparison between two cold stocks. *Canadian Journal of Fisheries and Aquatic Sciences* 54, 2410–2416.
- Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. *Aquaculture* 155, 103–115.
- Sargent, J.R., 1995. Origins and functions of lipids in fish eggs: nutritional implications. In: Bromage, N.R., Roberts, R.J. (Eds.), *Broodstock Management and Egg and Larval Quality*. Blackwell Science, Oxford, pp. 353–372.
- Sargent, J.R., Henderson, J.R., Tocher, D.R., 1989. The lipids. In: Halver, J.E. (Ed.), *Fish Nutrition*, 2nd edition. Academic Press, New York, pp. 153–218.
- Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1995. Requirement criteria for essential fatty acids. *Journal of Applied Ichthyology* 11, 183–198.
- Sargent, J.R., McEvoy, L., Estevez, A., Bell, J.G., Bell, M.V., Henderson, J.R., Tocher, D.R., 1999. Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture* 179, 217–229.
- Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds.), *Fish Nutrition*, 3rd edition. Academic Press, San Diego, pp. 181–257.
- Seiffert, M.E.B., Cerqueira, V.R., Madureira, L.A.S., 2001. Effect of dietary (*n*-3) highly unsaturated fatty acids on growth and survival of fat snook (*Centropomus parallelus*, Pisces: *Centropomidae*) larvae during first feeding. *Brazilian Journal of Medical and Biological Research* 34, 645–651.
- Sorbera, L.A., Zanuy, S., Carrielo, M., 1998. A role for polyunsaturated fatty acids and prostaglandins in oocyte maturation in the sea bass (*Dicentrarchus labrax*). In: Vandry, H., Tonon, M.C., Roubos, E.W., Loof, A. (Eds.), *Trends in Comparative Endocrinology and Neurology: From Molecular to Integrative Biology*. New York Academy of Sciences, New York, pp. 535–537.
- Springate, J.R.C., Bromage, N.R., 1985. Effects of egg size on early growth and survival in rainbow trout (*Salmo gairdneri*, Richardson). *Aquaculture* 47, 163–172.
- Springate, J.R.C., Bromage, N.R., Elliot, J.A.K., Hudson, D.L., 1984. The timing of ovulation and stripping and the effects on the rates of fertilization and survival to eying, hatch and swim up in the rainbow trout (*Salmo gairdneri*). *Aquaculture* 43, 313–322.
- Taylor, R.G., Grier, H.J., Whittington, J.A., 1998. Spawning rhythms of common snook in Florida. *Journal of Fish Biology* 53, 502–520 (Abstract).
- Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science* 11, 107–184.
- Tocher, D.R., Sargent, J.R., 1984. Analyses of lipid and fatty acids in ripe roes of some northwest European marine fish. *Lipids* 19, 492–499.
- Tocher, D.R., Fraser, A.J., Sargent, J.R., Gamble, J.C., 1985. Fatty acid composition of phospholipids and neutral lipids during embryonic and early larval development in Atlantic herring (*Clupea harengus* L.). *Lipids* 20, 69–74.
- Tveiten, H., Jobling, M., Anderssen, I., 2004. Influence of egg lipids and fatty acids on egg viability, and their utilization during embryonic development of spotted wolf-fish (*Anarchichas minor* Olafsen). *Aquaculture Research* 35, 152–161.
- Ulvund, K.A., Grahl-Nielsen, O., 1988. Fatty acid composition in eggs of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* 45, 898–901.
- Wade, M.G., Van Der Kraak, G., 1993. Regulation of prostaglandins E and F production in the goldfish testes. *Journal of Experimental Zoology* 266, 108–115.
- Watanabe, T., 1993. Importance of docosahexaenoic acid in marine larval fish. *Journal of World Aquaculture Society* 24, 495–501.
- Watanabe, T., Kiron, V., 1994. Broodstock management and nutritional approaches for quality offsprings in the red seabream. *Proceedings, Broodstock Management and Egg and Larvae Quality*.
- Watanabe, T., Lee, M., Mizutani, J., Yamada, T., Satoh, S., Takeuchi, T., Yoshida, N., Kitada, T., Arakawa, T., 1991. Effective components in cuttlefish meal and raw krill for improvement of quality of red seabream *Pagrus major* eggs. *Nippon Suisan Gakkaishi* 57, 681–694.
- Wiegand, M.D., 1996. Composition, accumulation and utilization of yolk lipids in teleost fish. *Fish Biology and Fisheries* 6, 259–286.
- Yanes Roca, C., 2006. *Husbandry and Larval Rearing of Common Snook (Centropomus undecimalis)*. University of Stirling. PhD thesis.
- Zhukinsky, V.N., Kim, D., 1981. Characteristics of age related variability in the composition of amino acids and lipids in mature and overripe eggs of the Azov roach *Rutilus rutilus* and the bream *Abramis brama*. *Journal of Ichthyology* 20, 121–132.
- Zohar, Y., Billard, R., Weil, C., 1984. La reproduction de la dourade (*Sparus aurata*) et du bar (*Dicentrarchus labrax*): connaissance du cycle sexual et controle de la gametogenese et de la ponte. In: Barnabe, G., Billard, R. (Eds.), *L'Aquaculture du bar et des sparides*. INRA, Paris.