The fatty acid composition of enriched artemia and larvae rearing: An overview of common snook (Centropomus undecimalis) culture

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ABSTRACT

A holistic understanding of the supply line of nutrients is important for developing diets in marine fish larval culture. This includes adaptation of rearing conditions that meet the larval requirements for the optimal presentation of food organisms and micro diets. This paper combines published literature and unpublished data on common snook reproduction, early life history and on growing in the wild and in captivity. The main focus is to review reports and articles in order to give the best recommendation for commercial scale production in common snook. A general summary of snook culture is presented. The importance of hatchery fry production as an essential culture pre-requisite is emphasized. In addition, an analysis of the main difficulties and constraints for future development is explored. This review pinpoints the gaps in knowledge regarding larval nutritional requirements, the nutritional value of live feeds, challenges and opportunities in the development of the enrichment process. Fatty acid composition in Artemia was analysed after 12 hours enrichment period using three different types of enrichment diets; easy DHA, Algamac 3050 and Algamac 3050+Algamac Enhance. The SFA varies from 16.6-17.1% respectively. MUFA varies from 13.8-30.6% between diets. This percentage shows some difference in comparison with diet 2 and 3, (15.0, 13,8%). PUFA percentages ranges between 47.5-65.8%. The results demonstrate that different methods of enrichment affect the composition of fatty acid and may reach the requirements for marine fish larvae. Information about larvae rearing for common snook has been investigated. It is vital to understand how a better survival rate can be obtained. Some of the most important factors involved in reaching this goal are described.

This paper should be cited as:
Bennett Wilson, K. 2018. *The fatty acid composition of enriched artemia and larvae rearing: An overview of common snook (Centropomus undecimalis) culture*. United Nations University Fisheries Training Programme, Iceland. Final project. http://www.unuftp.is/static/fellows/document/Keith17prf.pdf

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1 INTRODUCTION

The Government of Nicaragua has recognized aquaculture as an important economic activity for the generation of employment, food and nutrition security, and as a source of foreign exchange. It should be compatible with national policies for the environmental conservation and protection of natural resources. During the past 20 years, substantial changes in the world fish market have been observed, with a large increase in the production of farmed fish. This is due to new technology, the decline in wild fish catches, over exploitation, human population growth, and increasing demand for fish and sea food. As intensive aquaculture industries grow, new species are likely to be farmed**.** Common Snook (*Centropomus undecimalis*) is one of the largest fish in the *Centropomidae* family. It has fast growth, high market value, excellent flesh quality and is important to sport fishing in the American continent. It is also considered an emerging species for intensive fish culture in the Americas. The species is a protandrous hermaphrodite and great efforts have been made to understand and improve its reproductive process in captivity (Aliaume, Zerbi, Joyeux, & Miller, 2000).

INPESCA (Nicaraguan Institute of Fisheries and Aquaculture) in coordination with FAO (Food and Agriculture Organization of the United Nations), GRCCS (Regional Government of the South Caribbean Coast), BCIE (Central Bank for Economic Integrations), FIDA (Fund for International Development of Archives) and other actors are implementing projects on the Atlantic coast of Nicaragua to produce juveniles of marine fish, including common snook. The objectives are for job creation, enhancement of wild stocks, increased aquaculture production, improved food and nutrition security and reduced fishing pressure on this species (figure 1) (INPESCA, 2016).

Marine fish larvae are very vulnerable during the first stages of development and have strict requirements for biotic and abiotic conditions to survive, develop and grow properly. The production of fish larvae and viable fry has been a major obstacle in the development of marine aquaculture industry. The challenges of larvae first feeding are compelx, and the physical-chemical, nutritional and microbial condition of the larvae must all meet their requirements. Live feeds are used as an essential component during the larval stages of most marine finfish species in aquaculture (Reitan, Rainnuzzo, Oie, & Olsen , 1997). The schematic processing lines from the production of live feed to larval feeding are almost identical for all cultured marine fish species worldwide. The main modifications needed to adapt the technology are related to the method of n-3 HUFA (highly unsaturated fatty acids) enrichment of rotifer and artemia (Castell, et al., 2002). The nutritional composition provided in the diet of common snook larva is crucial for their survival, development and growth potential. Good knowledge of the larval nutritional requirements throughout development would contribute to optimize diets and feeding protocols, and thereby improve larval and juvenile quality and survival rate.

Nutritional composition provided in the diet of common snook larva is crucial for their survival, development and growth potential. The importance of this project is to provide scientific data of how to meet the nutrition requirement for marine larvae, especially for common snook, considering the species as a new candidate for intensive culture in Central America, and Nicaragua specifically. The aim is to recognize their general requirement in culture and understand some of the main issues related to these requirements. The objective also is to evaluate the factors in the weaning protocol, the importance of the weaning process, and the

role of environmental factors in this process. The main task is to evaluate which procedure may fit for development of common snook fingerling production.

1.1 Aquaculture in Nicaragua

Nicaragua began to develop aquaculture in the 1950s, with actions taken by the government to integrate fish farming into traditional agriculture. In 1980, the Nicaraguan Institute of Fisheries and Aquaculture (INPESCA) was established as an autonomous entity of the State. It is the governing body of the fishing industry and authorized to organize companies, associations and production cooperatives. Many technological and political challenges in recent years have been experienced in Nicaragua during the development of tilapia culture. The inaccurate use of water and escapes of tilapia into the wild led in 2006 to an alarm call for users of the lakes and lagoons. Various organizations of civil society, mayors and environment protection officers placed demands on public institutions and private companies. Raising tilapia using cages in lakes and lagoons, for alleged non-environmentally responsible practices was the main issue. The government decided to terminate such activities in 2007 (Martínez, 2016).

Majority of aquaculture in the Northern Pacific coast region (figure 2), occurs in semi-intensive industrial farms which have an important economic impact, however local employment is insufficient (Martínez, 2016). Small scale aquaculture producers are the major contributors towards food security for more than 100,000 families in the region. The development of this subsector is heavily contingent on the creation of niche markets, particularly local markets. In addition, there is a low consumption of fish which hinders the progressive growth of the market.

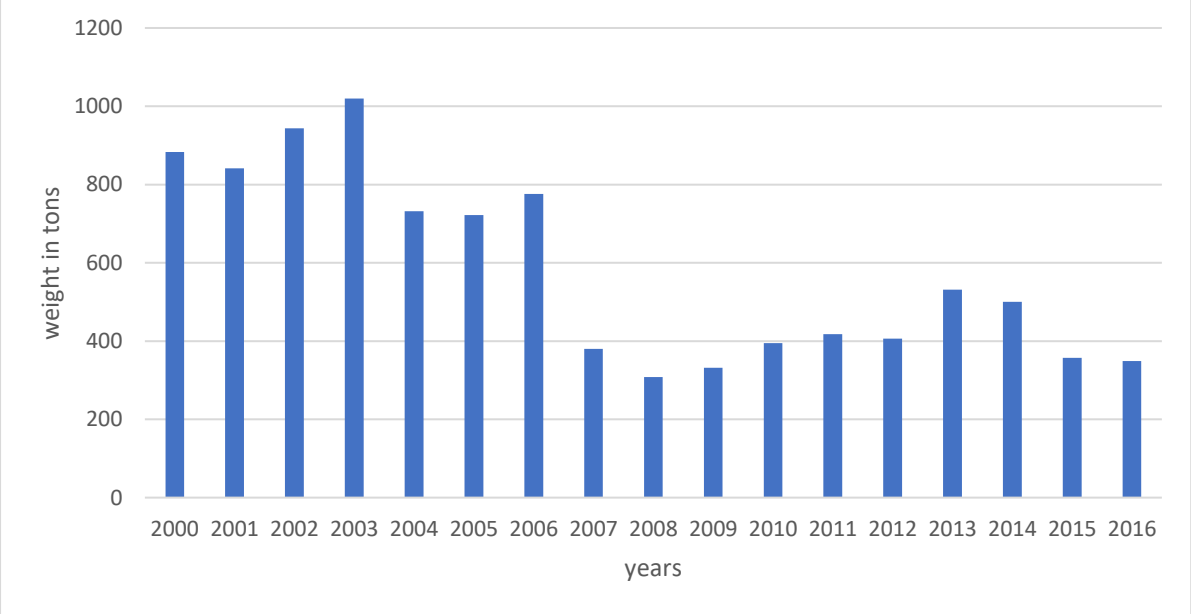

Figure 1. Artisanal fishery of common snook in Nicaragua (Data from INPESCA, annual report 2016, 2017).

Figure 2. The northern pacific coast (Chinandega) region where most semi-intensive farms are located (INPESCA, 2015)

1.2 General objective

- Investigate feeding techniques and management used for improving marine fish larvae survival rate. To evaluate which procedure may fit for development of common snook fingerling production.

1.3 Specific objectives

- To identify key parameters for successful marine larvae rearing.
- To evaluate factors affecting mortalities and quality in larvae rearing.
- To evaluate enrichment techniques in live feed to meet nutritional requirement of marine fish larvae.
- To evaluate the factors incorporated into the weaning protocol and the role of environmental factors involved in this process.

Information is gathered through review of relevant published and unpublished literature from journal articles and reports. The aim is to get an overview of common snook larvae rearing, developing a better understanding of the different methods and techniques that have been useful in hatchery settings, resulting in improved larvae survival.

2 LITERATURE REVIEW

2.1 Common snook biology

Common snook is widely distributed in the Western Atlantic: southern Florida (USA), south-eastern coast of the Gulf of Mexico, most of the Antilles and Caribbean coast of Central and South America. Its range extends southward to Rio de Janeiro, Brazil, up to the coast of North Carolina and the coast of Texas USA (figure 3). Adults inhabit coastal waters usually at depths less than 20 meters, estuaries and lagoons, penetrating into freshwater. As a marine carnivorous species, it feeds on smaller fishes and crustaceans. Mature individuals congregate at mouths of passes and rivers during the spawning season which usually take place from May through September. The maximum length registered is 140 centimeters, and the maximum published weight 24.3 kilograms (figure 4). Common length is approximately 50 centimeters. The habitats are mostly marine, freshwater, brackish, reef-associated ranging tropical climate of 25°C (Luna, 2017).

Figure 3. Distribution of common snook over the Americas continent. The red areas indicate distribution pattern (Luna 2017).

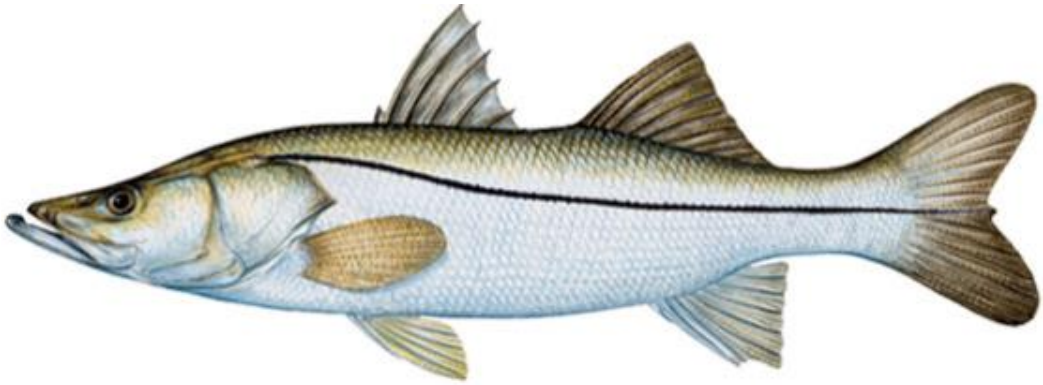

Figure 4. Adult common snook (Centropomus undecimalis) (Fishbase, 2017)

2.2 Common snook natural habitat

Common snook larvae can be found in polyhaline and euryhaline waters near estuarine passes or adjacent river mouths (Tucker, Kennedy, & David, 2009). According to the distribution of larvae, the eggs hatch near the mouths of estuaries and the larvae remain near the bottom where flood tides transport them into the bay (Robert, Muller, Ronald, & Taylor, 2012). Developing larvae prefer low water currents, shallow waters, often near overhanging shoreline vegetation or seagrass fields. Primary nursery habitat has been described as brackish, shallow, warm-water (between 25 °C and 32 °C) with streams or dredged canals slow currents, un-vegetated bottoms and overhanging or submerged mangrove prop roots (Stevens, D, Blewett, & Poulakis, 2007).

Juvenile common snook demonstrate an ontogenetic shift in habitat. This is related to mouth gape morphology and diet requirements. These small common snook (10 cm), actively move further up the estuary and may migrate as far as small streams that are completely fresh water. This remote habitat provides three critical requirements for these diminutive common snook: 1) a low-water current environment, 2) abundant quantities of the proper-sized prey to accommodate the developing gape, and 3) a safe respite from larger predators (Blewett, Steven, Champeau, & Taylor, 2009).

At a larval stage the requirement of protein and highly unsaturated fatty acids for this carnivorous species is very important (Soligo, Garcia, & Cerqueira, 2011). Marine copepods and phytoplankton are their main sources of protein and lipids, especially for highly unsaturated fatty acids, but also to carbohydrates and enzymes. This important fatty acid in the diet contributes to the development of central nervous system and maintaining the structure and function of the cell membrane. It also helps in developing the eyes vision and stress tolerance (Yanes, Roca, & Rhody, 2009).

2.3 Reproduction

Common snook is protandrous hermaphrodite. Differences in growth and maturity between sexes is significantly connected. Majority of small common snook are male and most large common snook are female (Lowerre, Barbieri, Vose, & Whittington, 2003). Though most adults may utilize freshwater habitats, they are unable to spawn in freshwater. The sperm become activated only in saline waters. They congregate for spawning at the mouths of rivers, inlets and canals. This activity is positively correlated with monthly rainfall patterns, tide, moon phase, amplitude temperature (28 °C), salinity (35 ppt) and pH of 7.8–8 (Muller, Murphy, & Kennedy, 2001). Spawning takes place mostly in the evening, happening between 14:00 and 20:00 hours, over the course of several days. Two spawning peaks are observed: the first in June/July, the second in August/September (Taylor & Grier, 1998). Common Snook are multiple spawners. Females probably spawn throughout the summer and ovulate as frequently as every two weeks. Histological evidence indicates that the ovary may return to spawning condition in less than two weeks. Ovaries contain three or more batches, or groups, of oocytes at different developmental stages, at each time.

2.3.1 Egg nutritional content

Most marine fish spawn pelagic eggs that are fertilized externally and float individually near surface of the sea (Fabra, Raldua, Power, Deen, & Cerda, 2005). Pelagic eggs are generally small (0.4-0.8 mm). Little variation is in egg characteristics such as size, quantity, oil globules, pigmentation and the morphology of the embryo within species. The development time is highly temperature dependent, and species specific. Large eggs generally have a longer incubation time (Small & Bates, 2001).

About 90-95% of the yolk mass in pelagic eggs is water, which makes them buoyant. However, the yolk also should contain all the necessary nutrients and energy for normal embryonic development. These nutrients contain components such as proteins, free amino acids, lipids, vitamins and minerals (Samaee, Este, Gime, & Lahnsteiner, 2009) (table 1).

Table 1. Essential biochemical components in fish eggs (Samaee, Este, Gime, & Lahnsteiner, 2009).

Essential biochemical components in fish eggs			
Pigment	Vitamins	Organic Components Vitellogenin	Minerals ions
Astaxanthin	Vitamin A		Calcium
	Vitamin B 6-12	Free amino acids	
Other carotenoids	Vitamin C	Essential fatty acids	Magnesium,

A comparative study was made by Hauville et al., (2015) on eggs from captive and wild common snook broodfish, with the objective to compare the fatty acid composition (table 2). The fatty acid composition in wild eggs is a good indicator on the fatty acid requirement of the hatched larvae during initial feeding. They concluded that captive eggs contained significantly lower SFA (saturated fatty acid) levels compared to wild eggs (25.4±0.3 and 30.6±0.6% of TFA (total fatty acid) respectively). The MUFA (monounsaturated fatty acids) is significantly lower in captive eggs compared to wild eggs (23.3 ± 0.3and 29.5 ± 0.2% of TFA respectively). The ARA (arachidonic acid) contents are significantly higher in wild fish eggs compared to captive fish eggs (5.4±0.3 and 3.8±0.2% of TFA respectively).

The EPA (eicosapentaenoic acid) contents are significantly lower in wild eggs than in captive eggs (2.4 ± 0.4 and 4.2 ± 0.2% of TFA respectively). Consequently, ARA/EPA ratio in wild eggs is significantly higher than that in captive eggs (2.3±0.6 and 0.9±0.1 respectively). The ratio of DHA/EPA is higher in wild eggs with 6.5± 0.7% and 6.6 ± 0.3% respectively. However, DHA (docosahexaenoic acid) contents are significantly lower in wild eggs compared to captive eggs (14.5 ± 0.2 and 27.3 ± 0.4% of TFA respectively. The total PUFA (polyunsaturated fatty acid) in wild eggs is significantly lower than in captive eggs (33.6 ± 0.5 and 47.0 ± 0.3% of TFA respectively) (Hauville, et al., 2015).

Table 2. Fatty acid composition in common snook eggs from wild and captive broodstock (Hauville, et al., 2015)

Fatty acid composition in common snook eggs			
	in the wild		in captivity
Fatty acids	Eggs		Eggs
SFA	30.6±0.6%		25.4±0.3%
ARA	5.4±0.3%		3.8±0.2%
EPA	2.4±0.4%		4.2±0.2%
DPA	2.7±0.1%		3.2±0.1%
DHA	14.5±0.2%		27.3±0.4%
PUFA	33.6±0.5%		47.0±0.3%
MUFA	29.5±0.2%		23.3±0.3%
EPA+DHA	16.9±0.2		31.5±0.3
DHA/EPA	6.5±0.7		6.6±0.3%
ARA/EPA	2.3±0.6%		0.9±0,1%

2.4 Physical tolerances

Common snook is a eurythermal species, but sensitive to cold water, with lethal minimum temperatures between 6-13°C. Laboratory experiments demonstrated that they stop feeding at 14.2°C, lose equilibrium at 12.7°C, and die at 12.5°C (Shafland & Foote, 1983). Below 25 °C and above 33 °C larvae did not survive long after hatching (Robert, Muller, Ronald, & Taylor, 2012). Lethal maximum temperatures were shown to be influenced by acclimation temperature. In laboratory experiments, lethal maximum temperature was between 38.7°C and 40.7°C (Hill, 2005).

Salinity is one of the most important factors determining common snook distribution. Broodstock reproduce in oceanic waters and the juveniles migrate to brackish and low salinity waters to continue their growth (0-10ppt). When juveniles reach maturity, they move to the open ocean where they join the breeding population (Gracia, Rosas, & Brito, 2006). Common snook can survive high turbidity and dissolved oxygen (DO) as low as 0.9– 1.0 mg/L (even 0.3–0.4 mg/L) for a short time but with stress.

3 AQUACULTURE PROSPECTS ON COMMON SNOOK

Common snook has been identified as a promising candidate for aquaculture and stock enhancement (Brennan, Walter, & Leber, 2008). In 2006, after more than 20 years of research trials at laboratories in Florida, Texas, Mexico and Brazil, wild-caught common snook were successfully matured in captivity. In years past, eggs were obtained from wild mature fish, caught near the time of ovulation. Spawning fish were stripped, and eggs fertilized in the field during the natural spawning season but with low larvae survival. With the use of photothermal conditioning and hormonal induction (GnRHa) voluntary spawning was achieved (Rhody, 2014).

3.1 Broodstock management

Broodstock management involves all the appropriate measures taken by an aquaculturist to enable a captive group of fish to undergo reproductive maturation, spawning, and produce fertilized eggs (Mylonas, Fostier, & Zanuy, 2010). A period of three years should be considered a generation interval for common snook. The required number of effective breeders is 25 males and 25 females. A sex ratio of 1:1 is recommended and adopted by most marine fish hatcheries to minimize genetic risk. However, only half of breeders in hatcheries are known to be effective, the required number of breeders is 50 males and 50 females (Alvarez & Ronald, 2008).

For common snook it is very important to maintain broodstock in optimal conditions to obtain efficient spawning outcome. Larvae survival in common snook has usually been very low (2%), with an exceptional 7% obtained since 1998. High mortality is reported to occur during the first 7-10 days post-hatch (dph) (Kennedy, et al., 1998). A balanced diet for broodstock can increase egg quality which has a positive impact on larvae survival (42% 3 dph) to first feed (Neidig, Skapura, Grier, & Dennis, 2000).

3.2 Larvae rearing

Common snook eggs stocking density in an incubator show a relation between density and larvae survival. The survival increases at a low stocking egg density (figure 5). However, temperature also plays an important role in the hatching success. In comparison of different temperature experiments, the highest hatching percentage was observed at 28^{o}C (figure 6).

In captivity, larvae first feed 45-50 hours after hatching, at a size of about 2.5-2.6 mm TL. Their yolk is significantly consumed by the end of their second dph, while the lipid globule lasts until their fifth or sixth dph respectively (Tucker, 2005). Adding microalgae in the rearing tank (green water system) from two days after hatching until day 14, has a positive impact on snook larvae survival (figure 7). This was done in comparison with larvae diet that has no phytoplankton (clear water) just with SS type rotifer (super small) (Yenes-Roca, 2006).

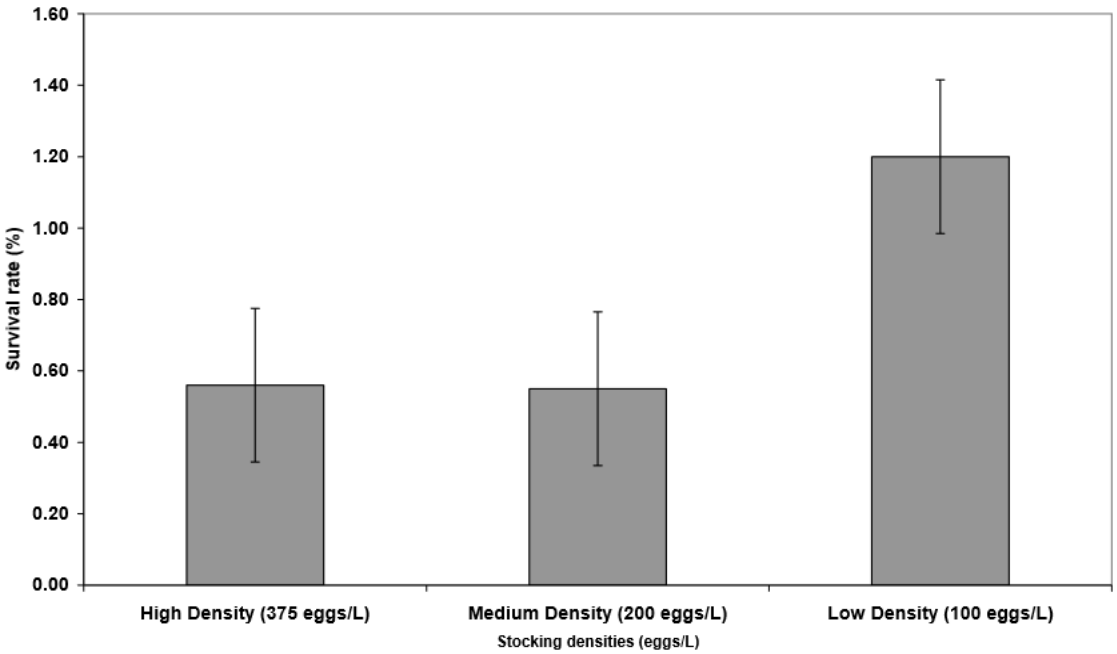

Figure 5. Larvae survival rate from three stocking densities 14 days after hatch (Yenes-Roca, 2006)

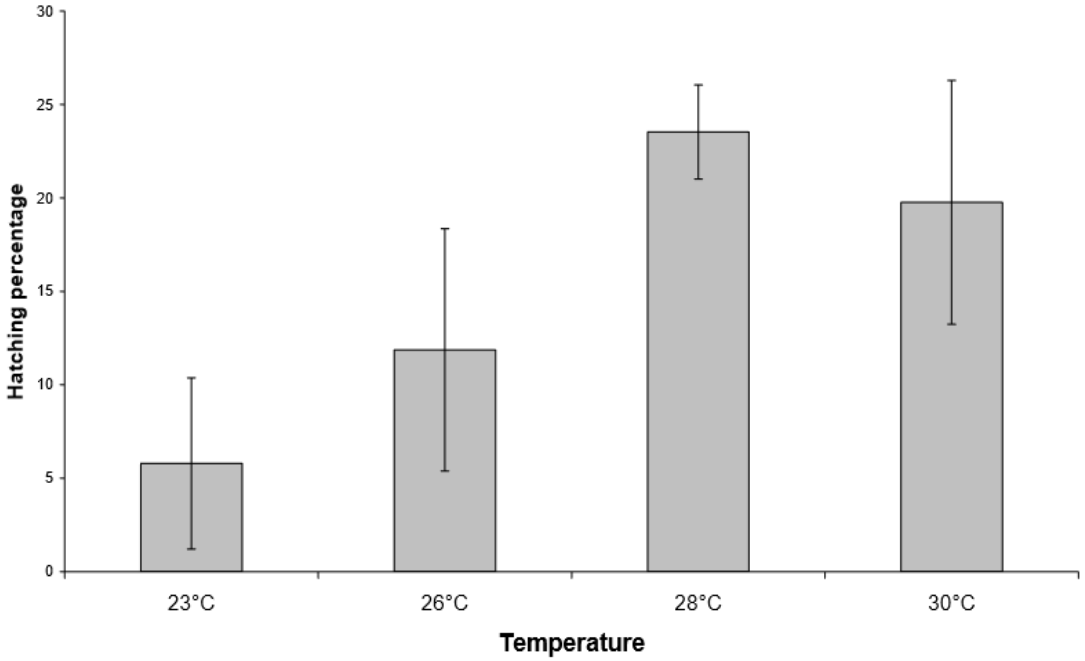

Figure 6. Influence of four temperatures on snook hatching percentage (Yenes-Roca, 2006).

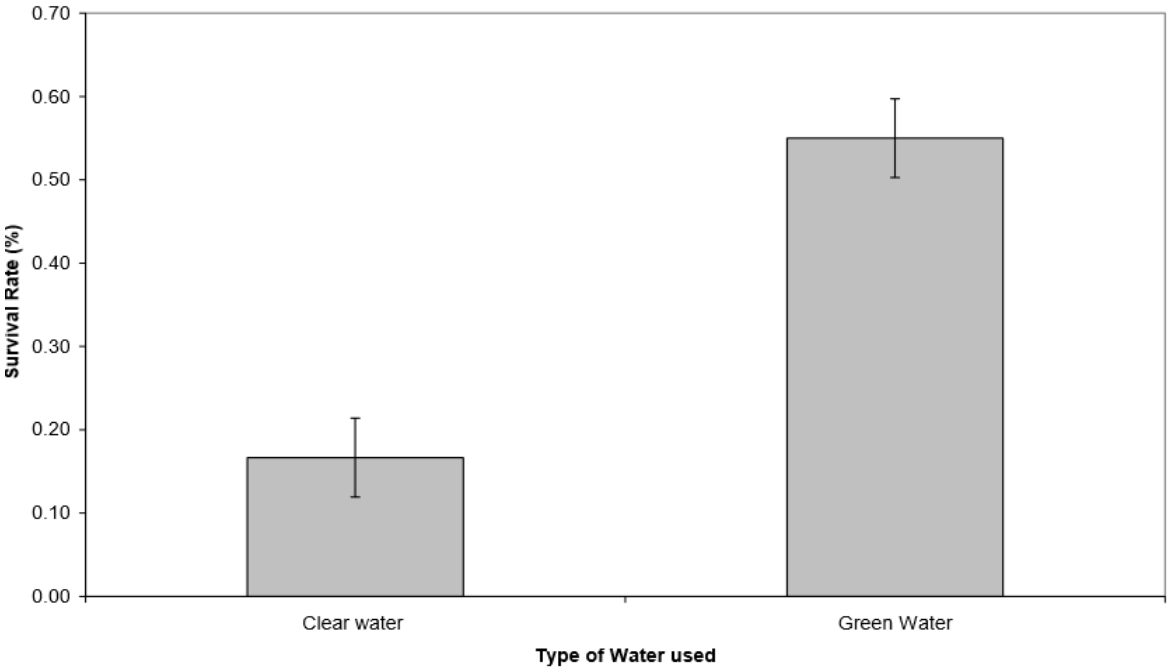

Figure 7. Survival of snook larvae 14dph from tank with microalgae *Nannochlropsis oculata* (green water) and without any phytoplankton (clear water) (Yenes-Roca, 2006).

To date, final fry densities in production trials, have ranged from 2-5 fries per litre. Larval survival has been low in most cases with the highest reported at 7% at 55 dph (Tucker & Kennedy, 2001). Metamorphosis to juveniles occurs at 35 dph (35-40 mm TL) in common snook. Snook larvae can be adapted to fresh water at about 15 dph (Tucker, 2005). In Florida, nursery trials produced fingerlings ready to stock in grow-out systems with 10 cm TL (total length), and about 10 grams in weight at three months of age. Snook can grow up to 20 grams in 3-4 months when fed on experimental salmon starter diets (Tucker, 1987).

Sefling (1998) recommended density of 2-5 fry/L during the first feeding and weaning period for common snook (Sefling, 1998). In experiments reported by Edward, a mean survival of 22.6% (2.2-52.6%) at 30 dph with a mean harvest density of 2.9 fry/L was obtained. Larvae can be weaned to artificial feeds at an earlier age (35-38dph) reaching at least 50mmTL (2g) at 50dph (Tucker, 2005).

3.3 Nutritional requirements for marine fish larvae

The production of fish larvae and viable fry has been a major obstacle in the development of marine aquaculture industry. The challenges of larvae culture during first feeding are highly multidisciplinary, and the physical-chemical, nutritional and microbial conditions for the larvae must all meet their requirements. Consequently, many specific processes cannot directly be generalised from findings obtained in typical species and require specific studies for each species (Weirich & Reigh, 2001). These culture adjustments must consider the biological, chemical and physical factors affecting the fish larvae in its natural environment.

Nutritional requirements are frequently defined as the 'requirement for maximal growth and/or survival' where the relation fish-diet-feeding has an important effect in the determination of the quantitative and quality needs (Izquierdo & Lall, 2004). Obtaining knowledge of the larval nutritional requirements throughout development contribute to optimize diets and feeding protocols, and thereby improve larval and juvenile quality. As fish larvae are vulnerable, it is always difficult to identify and meet nutritional requirements when several physiological and metabolic constraints are linked, which may prevent growth or an appropriate development (Hamre, et al., 2012).

The design and formulation of diets requires conversion of the nutritional requirements into the nutrient content in the diet. Micronutrient supplies of proteins, amino acids, fatty acids and so forth, are often given as nutritional absorption elements. Fish larvae grow extremely fast, feed continuously and, therefore, the total breakdown of nutrients must be high.

3.3.1 Lipids

Lipids can be more formally defined as substances such as a fat, oil or wax that dissolve in alcohol but not in water. It includes carbon, hydrogen and oxygen but have far less oxygen proportionally than carbohydrates. Together with carbohydrates and proteins, lipids are the main elements of plant and animal cells. Lipids are grouped as neutral or polar lipids, depending on their polarity. The lipid content and composition of marine fish eggs is essential for survival, and as such should be as similar as possible to that of the resulting larvae and of the larvae's natural copepod diet (McEvoy, Navarro, Hontoria, Amat, & Sargent, 1996).

Although in some cases it is considered that by giving 10% of a marine phospholipid in the diet for larvae, there is no need for the fish to biosynthesise glycerophosphoinositol or glycerophosphocholine. Supposition concern is that fish larvae have a limited ability to biosynthesise phospholipids de novo. Neither is there any requirement to add free inositol or free choline to the diet. The levels of 22:6n-3 and 20:5n-3 provided in the diet by phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine sufficiently meet the published n-3 HUFA requirements for marine fish larvae and also generate the required ratio of DHA/EPA (22:6 n-3:20:5 n-3) of 2:1 (Reitan, Rainuzzo, & Olsen, 1994).

3.3.2 Fatty acids

Fatty acids are characterised by the number of their carbon atoms and double bonds. Some of these are essential (EFA) for growth and development of the vertebrates. EFA must be supplied in the food and are grouped into two families: the linoleic acid (n-6) and the linolenic acid (n-3) families. HUFA are the ones with 20 or more carbons atoms (Bell, McEvoy, Estevez, Shields, & Sarjent, 2002).

Many marine species have little metabolic capacity to elongate and desaturate the shorter C18-precursors of the n-6 and n-3 families, but they can modify HUFA through catabolic chain shortening reactions (Sargent, Bell, Bell, Henderson, & Tocher, 1991). High contents of n-3 HUFA are found in aquatic plants and animals. These fatty acids are essential components for neural tissue, cell membrane functionality and many regulatory functions. The n-3 fatty acids dominate the n-6 in marine food webs, but the n-6 HUFA arachidonic acid (ARA) is also essential for marine fish.

Larvae requirements of n-3 HUFA (highly unsaturated fatty acid) docosahexaenoic acid (DHA (22:6 (n-3)), eicosapentaenoic acid (EPA, 20: 5(n-3)), and arachidonic acid (ARA, 20: 4 (n-6)) are strongly related to growth, survival rate and tissue differentiation. This includes eye, brain and neural development. Neural tissues contain very high amounts of DHA (Mourente, 1991). In most cases for larvae, these elements can only be delivered by including live feed as first diet due to small size and often poorly developed digestive system in the fish.

In most cases the EPA and ARA of the phospholipids are antecedents in prostaglandin synthesis. The prostaglandin are precursors for several regulating compounds known as tissue hormones. Prostaglandin G_3 is synthesised from EPA and acts as a regulatory to prostaglandin $G_{2,}$ which is synthesised from ARA. Both ARA and EPA are derived from the membrane phospholipids (PL) through the action of phospholipases. The G_3 and G_2 ratio, which is believed to modify cellular processes, will depend on the ratio of EPA to ARA in the membrane, and ultimately in the diet. In general, DHA and EPA ratio of 2:1 is found in marine species larvae, which has been suggested as adequate for larval nutrition (Sargent, McEvoy, & Bell, 1997).

3.4 Enrichment in live feed

Only two zooplankton families have so far shown that they can be produced regularly and efficiently at an acceptable cost: rotifer Brachionus species and brine shrimp Artemia species. The most commonly used species within these groups are *Brachionus plicatilis* and *Artemia franciscana*. In most cases they are used alone or in appropriate combination as live feed for most marine species in culture. Despite the convenience of the production of rotifers and Artemia there is a main downside which resides in their poor nutritional profile. Both prey types are deficient in essential HUFA which has been identified as critical nutritional factors in marine fish larval development and survival (Hamre, et al., 2012).

Microalgae are important components of fish larvae diets, either directly or indirectly and as food for rotifer and Artemia. It is important to know whether short term n-3 HUFA enrichment is needed and when both lipid and fatty acid composition can be controlled during cultivation of life feed. Lipid content becomes low in rotifer and Artemia 13- 15% of dry matter with extreme value of 10-25%. On the other hand, short-term enrichment using emulsified oil will normally bring the lipid level to above 25% of dry matter (Hamre, et al., 2012).

Artemia production is different from that of rotifer because they are hatched from resting cysts that are commercially available. There is no need for biomass production and the maintenance of culture as for rotifers. The biochemical structure and nutritional value are far more stable and reproducible in hatched nauplii than in rotifer. However, there is a strong need to increase the content of essential n-3 HUFA in Artemia to obtain a live-feed quality that can meet the requirement of the fish larvae. Short-term enrichment of Artemia is the important enrichment method used. Effective enrichment of live feed can only be successful with enrichment mixtures containing high proportion of lipids. Table 3 demonstrates the different composition of fatty acids between copepods and Artemia nauplii. Copepods are considered one of the main live prey for marine larvae due to their small size and high fatty acid content.

Table 3. Comparison of the fatty acid composition of total lipids of copepods and live feeds. (Artemia) (Tocher & Sargent, 1984)

	22:6*n*−3	20:5*n*−3	20:4*n*−6	18:3*n*−3	18:2*n*−6	18:1*n*−9	16:0
Calanoid copepods[c]	32.2	12.1	1.0	1.7	2.0	7.0	18.1
Artemia nauplii[d]	0.0	3.9	1.1	22.1	5.9	17.4	11.6

4 WEANING PROCESS

The weaning of pelagic marine fish larvae is the transition from a live feed diet, such as rotifer, Artemia, or any other cultured or harvested marine zooplankton to a formulated diet. Traditionally this transition started once the fish larvae has completed metamorphosis. At this stage, the fish accept and utilize formulated diet without any problem. Feeding with living organisms can conclude slowly. The bigger the fish at weaning, the easier the weaning process and the lower the losses. Often the fish have reached sizes of 50-250 milligrams of wet weight when weaning take place using particulate feed of around 0.3 millimetre in diameter (Naess, Hamre, & Holm, 2001).

The natural diet of carnivorous fish is low in carbohydrates. Fish have a limited capacity for handling dietary carbohydrates due to low activities of both digestive and metabolic enzymes. Nutritional studies have been hindered by the lack of efficient formulated diets for larvae. However, diets for juveniles are available. The nutrient requirement of juveniles may with caution, be induced into the larvae stage. Success during weaning stage depends on a set of husbandry techniques which are adjusted to specific species. This also includes specific conditions at the rearing site. Although good diets for early weaning may be on the market, in some cases it may be less demanding to continue growing larvae on live feed (Baskerville & Kling, 2004).

4.1 Growth potential and grow out method

Common snook have shown notable tolerance to the main environmental conditions in tropical areas. They show considerable versatility in adapting to culture systems (cages, ponds and tanks), and culture intensity (extensive, semi-intensive, intensive and super intensive). Good outcomes were obtained especially in estuarine and coastal sites and ponds (Alvarez-Lajonchère & Tsuzuki, 2008). Common snook is not an active fish. This means that the feed is used very efficiently, with FCRs (food conventional ratio) ranging from 0.73 to 1.10 (Tucker, 1987). Fish in the range 504-726g mean BW (body weight) gained 4.1g day^{-1} (0.67% day^{-1}) with an FCR of 1.1 (Tucker, 1987). Monitoring growth indicated that they can reach 2kg in 24 months (Tucker, 2005). Snook juveniles (from10cm TL) show an excellent survival outcome (80-100%) in cages. It may be worthwhile to cultivate these juveniles on a long-term scale, to obtain a better profit (Tucker, Russell, & Rimmer, 2005).

In Mexico, wild common snook juveniles of 20-25g body weight, grown in cylindrical 20 m^3 tanks with seawater and fed dry feed reached about 300g in 6 months, a growth rate of up to (2.7 g/day^{-1})-or 1,4%. In small non-drainable earthen ponds 800g fish harvested after 1 year, yielded 4-5 tonnes per hectare (Sanchez, Rosas , Duruty, & Suarez, 2002). Growth in floating cages can be a better alternative for snook rearing. The use of stocking densities up to 200 fishes per m^3 did not affect the survival, growth performance and FCR of fat snook juveniles of 156dph (5.7g). Stocked in floating cages for 60 days (Tsuzuki, Cardoso, & Cerqueira, 2008), which probably also can be applied for common snook.

4.2 Current situation challenges

Progress continues to be made to improve larval rearing in common snook. Although there exist many theories regarding the diet of larvae, there are no reported studies on this topic. Common snook larval diets are one of the major bottlenecks in common snook aquaculture. In addition, issues such as prey type and size during the first 5–7 days post hatch are still poorly understood (Carlos, Yanes, Roca, & Kevan, 2012).

5 MATERIALS AND METHODS

A trial was set up to test and practice enrichment process of Artemia and the effect of different enrichment material on fatty acid composition in the live feed. Enrichment of Artemia was carried out at the Marine Research Institute (MRI) aquaculture facility at Staður, in Grindavík, Iceland in January 2018. Samples were collected and analysed at the Icelandic Food and Biotech R&D Company (Matis) in Reykjavik. Fatty acids composition was analysed in the three experimental samples.

5.1 Experimental setup

This experiment was carried out with three different enrichment diets using the same duration period. The duration period of the enrichment was 12 hours. All experimental treatments were conducted in duplicate. Enrichment diets for each tank are described in Table 4. Experimental design is described in Figure 8.

5.1.1 Enrichment diets used

- Tank # 1: Easy DHA Selco (INVE aquaculture 2014).
- Tank # 2: Algamac 3050 flake (Bio-Marine).
- Tank # 3: Algamac 3050 flake with Algamac Enhance (Bio-Marine).

Table 4. Enrichment diets prepared for each tank grams per tank.

Enrichment diets for each tank. Grams per tank.			
Diets	Water/L	g/L	Total added/g
Easy DHA	50L	0.6g/L	30 grams
Algamac 3030	50/L	0.6g/L	30 grams
Algamac 3050 + Algamac Enhance	50/L	0.6g/L	30 grams (15g+15g)

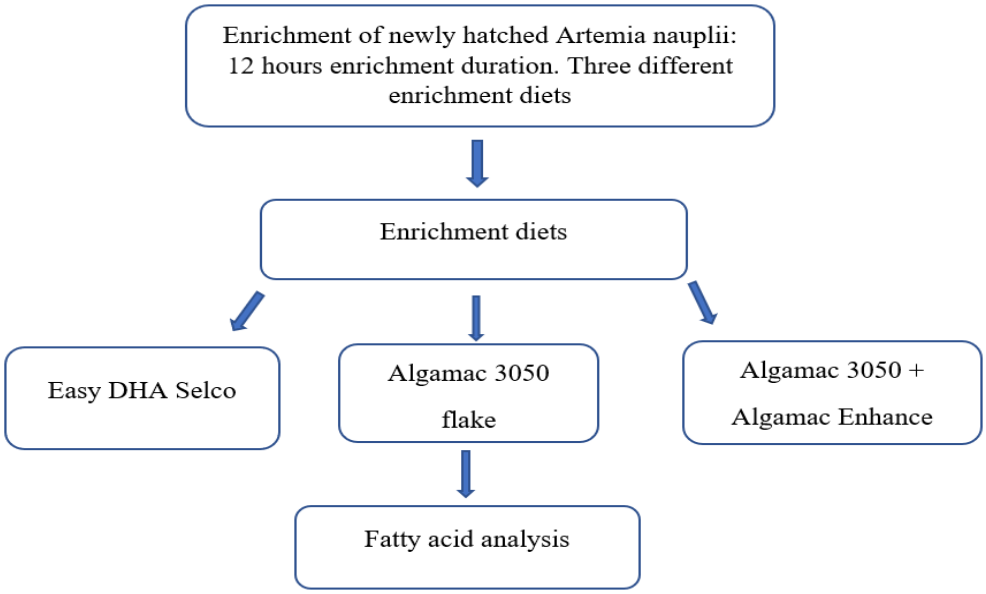

Figure 8. Experimental setup with the three-different diets, 12-hour enrichment period.

5.2 Water parameters

Artemia was cultivated in 80L of sea water at a salinity of 35 ppt and at temperature of 30°C. pH level was maintained at 8-8.5. Aeration was constant during the hold process at the bottom on each tank. Oxygen level was maintained above 75%. Light intensity at the surface of each tank was 1000 lux.

5.3 De-capsulation of Artemia

The Artemia magnetic cysts (Sep-Art Technology) used in this experiment originated from the Great Salt Lake (GSL) in Utah (INVE Aquaculture 2014). Non-decapsulated cysts were inoculated at 2g/L according to the instructions provided by the industry. Sanocare ACE (INVE), (0.6 g/L) and Pyceze (0.1 g/L) were added to the inoculation to enhance water quality and reduce microbial levels.

Table 5. Decapsulation of Artemia setup, grams per litre of water

start-up decapsulation set up for Artemia			
Decapsulation	Water /L	g/L	Total grams added
Artemia	80 L	2g/L	160g
ACE		0.6g/L	48g
Pyceze		0.1g/L	8g

After hatching, the nauplii were separated from the cysts using the SEP-Art magnetic separator. For harvest, the hatching water (containing Artemia nauplii and shells) were run through the separator at a flow rate of 8-10 L/min. Shells were attracted to the magnets inside the tube and thus separated from the Artemia nauplii which pass freely through the tube. Nauplii was then collected at the end of the tube (figure 9).

Figure 9. Separation method of Artemia newly hatched nauplii from the cysts using the magnetic separator.

The cleaning process was done by washing with clean seawater before being stocked into the enrichment tanks. Each enrichment was done using 50 L tanks (figure 10). The stocking density were 185 nauplii/ml for each experiment. The anti-microbial agents (Sanocare ACE 0.6g/L and Pyceze, 0.1g/L) were added immediately to all the tanks. Samples were collected after 12 hours and stored in a freezer (-15 °C).

Figure 10. Enrichment tank with 50L of water per tank

5.4 Laboratory Analysis

The enriched Artemia samples were taken to fatty acid analysis. The procedure can be divided into three steps.

5.4.1 Fat extraction

The method that was used for the fat extraction was based on Bligh & Dyer. 25g of enriched Artemia for each sample was weighed out. The use of 250 ml centrifuge bottles intended for organic solvents. Proceeded by adding 25 ml chloroform and 50 ml methanol. This was mixed for two minutes and then another 25 ml chloroform was added and mixed for one additional minute. 25 ml 0,88% KCl-solution was added and continue mixing for one minute. The sample was centrifuged for 20 minutes, at 2500 rpm (rotations per minute) at 0-5°C. A 25 ml pipette was used to remove the upper phase of the solution. The lower chloroform phase is then filtrated via disodium sulphate (Na_2SO_4) through a glass microfibre filter (Whatman GF/C). The suction flask content is then poured into a 50 ml volumetric flask (which is made up to mark). Fat determination is then made like in Folch description (evaporation of 3 ml chloroform phase at 50-60°C under nitrogen stream).

5.4.2 Methylation

Methylation was done based on AOCS Official Method Ce 1b-89 with minor adjustments.

- ✓ 90 mg from the extracted fat in a test tube with stopper was weighed out.
- ✓ 1,5 mL 0,5N NaOH, was mixed and heated for seven minutes at 100°C.
- ✓ When cooled down, 2 mL BCl3 12% in methanol was added, then heated for 30 minutes at 100°C.
- ✓ Sample was cooled down again and added one ml of standard solution (for instance C11:0 methyl ester at around 1-2 mg/ml) and five ml concentrated NaCl solution. Mixed on vortex for 30 seconds.
- ✓ When the isooctane layer was separated from the aqueous layer, then is was transferred to a clean test tube with a small amount of natriumsulfate.

5.4.3 Gas chromatography

Fatty acid methyl esters (FAME) were separated on a Varian 3900 Gas Chromatograph equipped with a fused silica capillary column (Omegawax^{TM}250 30m x 0,25mm x 0,25µm film), split injector and flame ionisation detector fitted with Galaxie Chromatography Data System, Version 1.9.3.2 software. The oven was programmed as follows: 100°C for 4 min, then raised to 240°C at 3°C/min and held at this temperature for 15 min. Injector and detector temperature are 225°C and 285°C, respectively. Helium was used as a carrier gas at the column flow 0.8 mL/min; split ratio, 200:1. The programme was based on AOAC 996.06.

6 RESULTS

6.1 Fatty acid composition

The composition of saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acids (PUFAs) and essential fatty acid plush docosahexaenoic acid (EPA+DHA) percentage is shown in figure 11. This was analysed after the 12 hours of Artemia enrichment. Overall, the PUFA contents of Artemia varied from 47.5% to 65.8% of total fatty acid between the three results. The highest percentage of PUFA was obtained in diet 3 (Algamac 3050+Algamac Enhence), with 65.8%. The lowest percentage of PUFA was obtained in diet one (Easy DHA) with 47.5%.

Comparing the percentage of MUFA obtained in the experiment, Artemia from diet one shows the highest percentage with 30.6%. On the other hand, diet two and three were just 15.0 and 13.8% respectively. The SFA percentage was most likely with small variations in all three diets. For diet one, SFA was 17.1%, diet two, SFA was 16.6% and diet 3, 16.7%.

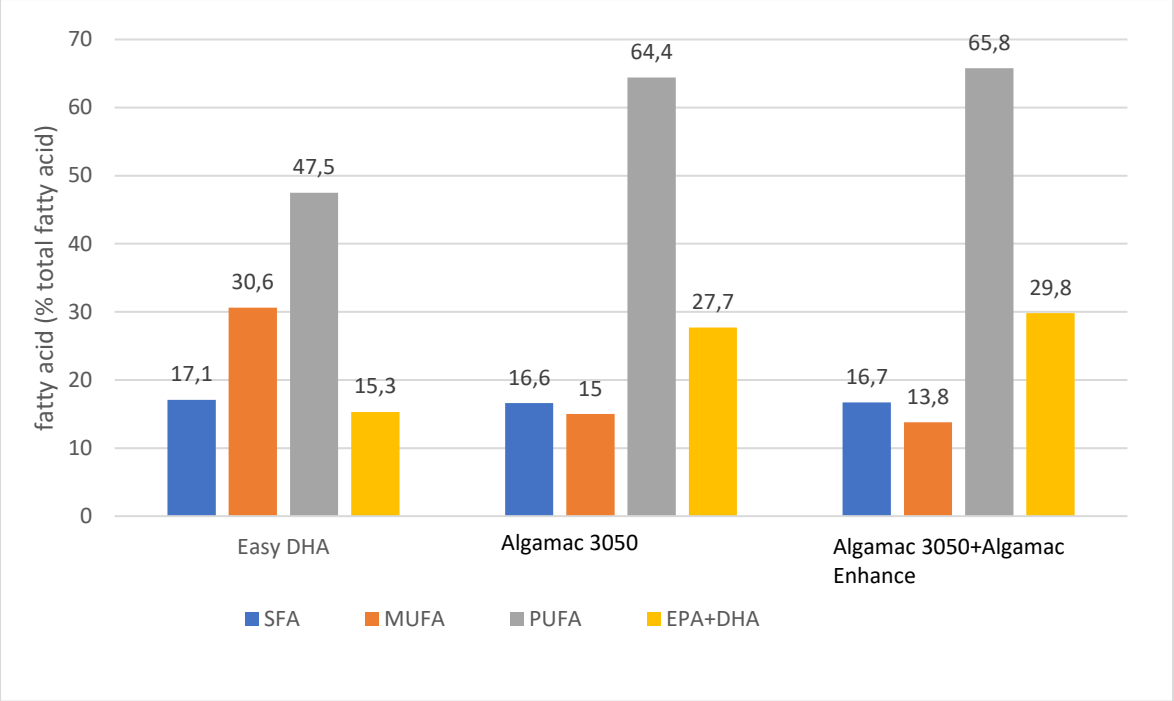

Figure 11. Percentage of fatty acid composition for SFA, MUFA, PUFA and EPA+DHA (% of total fatty acid) in enriched Artemia using the three diets. Easy DHA, Algamac 3050 and Algamac 3050+Algamac Enhanced

There was some difference in the result shown within the essential fatty acids such as EPA and DHA in the all three diets, as shown in Table 6. The EPA composition ranges from 4.1 to 6.5 ratio between the diets. In the first diet EPA was 6.5%. In comparison with the second and third diets the EPA was 4.8 and 5.1% respectively. The DHA percentage was highest in diets three with 24.7% followed by diet two with 22.9%. The lowest DHA ratio was obtained in the diet one with 8.8. A considerable difference is clear in fatty acid content between diet one compared to diet two and three. Figure 12 below illustrates the ratio of fatty acid composition in the diets.

Table 6. Result of fatty acid composition (% of total fatty acid) of Artemia after 12 hours of enrichment using three different diets. Easy DHA, Algamac 3050, Algamac 3050+Algamac Enhance

Fatty acids composition %	Easy DHA Selco (diet 1)	Algamac 3050 (diet 2)	Algamac 3050 + Algamac Enhance (diet 3)
EPA C20:5n3	6.5	4.8	5.1
ARA C20:4n6	1.2	2.7	2.9
DPA C22:5n3	0.8	0.4	0.5
DHA C22:6n3	8.8	22.9	24.7
SFA	17.1	16.6	16.7
MUFA	30.6	15.0	13.8
PUFA	47.5	64.4	65.8

DHA/EPA	1.33	4.77	4.84
ARA/EPA	0.18	0.56	0.57
EPA+DHA	15.3	27.7	29.8
Total omega 3	36,5	48,9	49,8

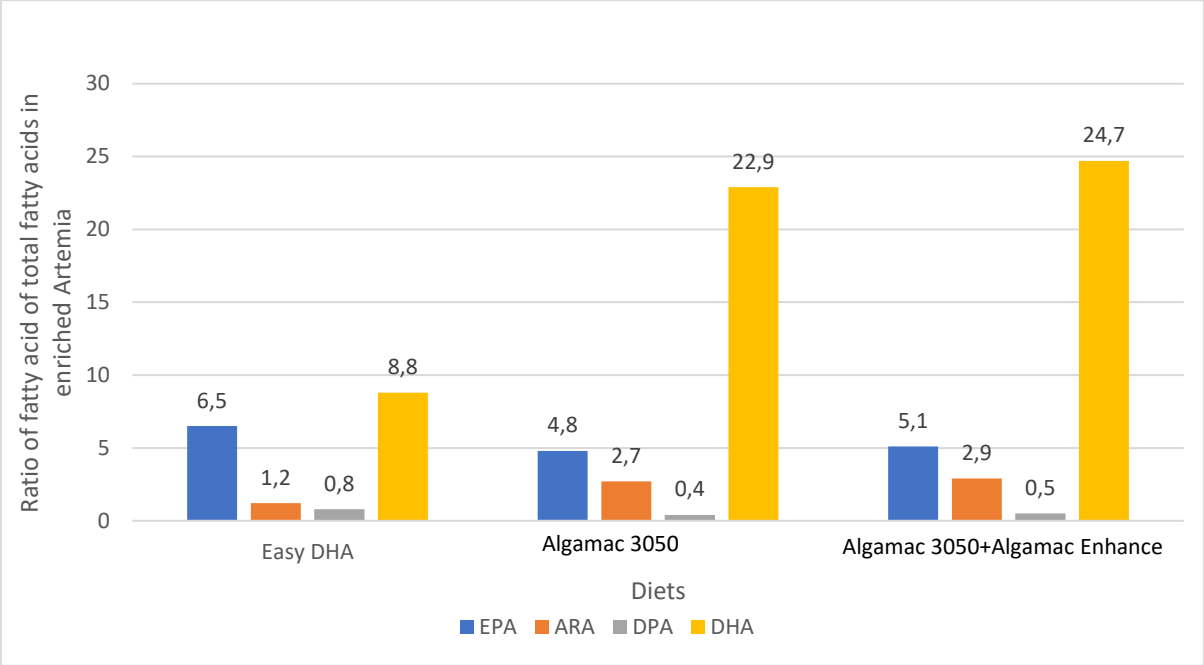

Figure 12. Ratio of fatty acids composition percentage in diets (EPA, ARA, DPA, DHA).

The ratio of fatty acids of the different diets is shown in figure 13. The DHA/EPA ratio range between 1.35 and 4.84. The diet 3 demonstrated the highest ratio with 4.84 followed by diet 2 with 4.77. Diet 1 had the lowest ratio of all three with 1.35. On the other hand, the ARA/EPA ratio rages from 0.18 to 0.57. Diet 3 represents the highest ratio of 0.57 in comparison with diets 1 and 2 which was 0.18 and 0.56 respectively.

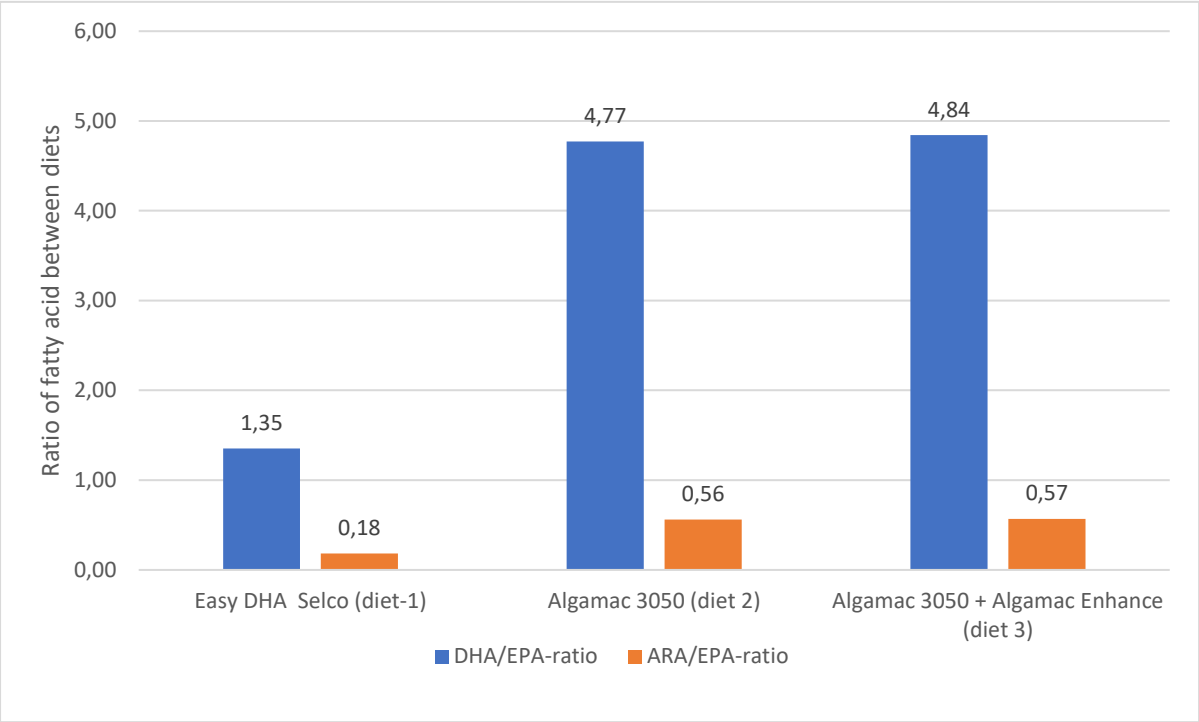

Figure 13. DHA/EPA ratio and ARA/EPA ratio in the three diets.

7 DISCUSSION

One of the limiting factors for successful mass production of most fish juveniles is the variability of egg quality and first feed management. Poor egg quality may decrease the survival potential of a hatched larvae. The potential to produce viable fingerling is determined by several biological, physical, and chemical parameters, in addition to some genetic factors. This includes the physiological processes that occur during the larvae development.

In this study the enrichment of Artemia was carried out to meet the requirements of essential fatty acids for marine fish larvae. The proportion of these acids are generally low in live feed such as rotifer and Artemia.

Fatty acid content, predominantly essential fatty acid, of live feeds such as Artemia is critical for larval farming. Consequently, enrichment is essential to improve essential fatty acid levels. The composition of fatty acid in enriched Artemia varies as a function of the enrichment protocol. Nevertheless, in this study, the fatty acid composition of enriched Artemia demonstrates some differences between diets.

The EPA ratio in wild snook eggs and captive ranges from 2.4 ± 0.4 and 4.2 ± 0.2 (Hauville, et al., 2015). This result shows that all three enrichment diets reached the ratio that are found in wild and captive eggs 4.8-6.5 respectively. This means that all three diets can be used for enriching Artemia as live feed for snook larvae.

The ARA ratio in wild and captive snook eggs varies between 5.4±0.3 and 3.8±0.2%. On the other hand, the ratio of ARA obtained from all three diets ranges from 1.2-2.9% respectively. This result shows that the all three diets did not reach the ratio ranges in wild and captive eggs. On the other hand, all three enrichments reached more than 1.0% ration that was obtained in copepods (table 3) which is considered one of the main prey for snook at the larvae stage.

The DHA composition in wild snook eggs compared to captive eggs is 14.5% ± 0.2 and 27.3% ± 0.4 (Hauville, et al., 2015). In comparison with the results obtained for all three diets, which ranges from 8.8-24.7%. The diets 2 and 3 show the highest results with 22.9% and 24.9% respectively.

The requirement of marine fish larvae for (n-3) PUFA level of DHA and EPA is high. For finfish species, they incline to a requirement of high DHA and ARA levels and a lower EPA level. This translates into high DHA: EPA and ARA: EPA ratios (Yanes, Roca, & Rhody, 2009). These requirements were obtained in this study by the enrichment of Artemia. On the other hand, common snook larvae may require a high level of ARA in diets. This conclusion was made due to the high percentage of ARA found in wild snook eggs. (Yenes-Roca, 2006). Consequently, most diets which have high levels of (n-3) PUFA, especially DHA and ARA levels, could be suitable for common snook.

This result shows the percentage of PUFA in all three sample ranges between 47.5-65.8%. Observing the ratio of PUFA in wild and captive snook eggs (table 2), shows that it varies from 33.6-47.0%. Comparing both results, all three-enrichment diets give a higher PUFA ratio than wild and captive snook eggs. The percentage of MUFA varies from 13.8-30.6% between the diets. Diet one shows the highest ratio with 30.6% of MUFA, followed by diet 2 and 3 with 15.0 and 13.8% respectively. In comparison with the percentage of MUFA obtained in wild and captive snook eggs which were 29.5 and 23.3% respectively. Showing that diet 1 had a higher percentage of MUFA in comparison with wild and captive snook eggs. On the other hand, diets 2 and 3 demonstrate a lower percentage. The SFA percentage obtained from the three diets varies from 16.6-17.1%. The result was obtained from wild and captive snook eggs which is 30.6-25.4%. In this case snook eggs show a higher percentage of SFA than all three experimental diets. According to the nutrition content obtained in diet 2 and 3, they can be supplied as feed for snook larvae. Due to the size of the larvae during the first days, Artemia prey should only be applied from day 7 dph of the larvae.

The incorporation of fatty acids in the diet of marine fish larvae has a positive effect on the survival rate. A good relation between DHA/EPA and ARA/EPA ratio is important. This relation is considered critical for larval viability, reducing the percentage of larvae abnormalities and a better pigmentation (Bell, McEvoy, Estevez, Shields, & Sarjent, 2002). The relation between DHA/EPA ratio diet 2 and diet 3 is satisfactory for snook larvae. The ratio varies from 4.77 and 4.84 for diet 2 and 3 respectively. The ratio of DHA/EPA in diet 1 is 1.35 which is considered less effective in comparison with the other two.

The ARA/EPA ratio obtained in diet 3 had the highest percentage in comparison with diet 1 and was 0.57. In diet two the ratio was 0.56 and diet 3 was 0.18. The ratio of ARA/EPA obtained in copepods in 0.08. Considering copepods as snook larvae main prey, all three diets reach an acceptable ratio for the larvae. The DHA/EPA ratio is 6.5 ±0.7, which was obtained also in wild snook eggs (Hauville, et al., 2015). The results obtained after the enrichment demonstrate that the diets 2 and 3 reach the basic requirement for snook larvae.

Snook egg fatty acid composition is found strongly related to the general marine fish fatty acid profile. In most cases high mortality occurs during the development of larvae in captivity because of the lack of acceptable feed (prey size, density, quality). However, the use of fatty acid analysis in snook eggs and live feed, has shown significant correlation of fatty acid composition with eggs fertility and larvae survival. Fatty acids play an important role in snook diets. The composition of fatty acids in snook eggs give a better understanding about what could be their requirement at the larvae stage.

There are some other factors that could also be of major importance and which could also influence the survival rate of the larvae. Keeping the optimal temperature (28 ^{0}C) can improve hatching percentage and larvae survival for common snook. The stocking density for snook eggs should always be low (100 eggs/L). Low density stocking gives higher percentage of larvae survival (figure 5). The availability of live prey is important during the feeding period. The density of live prey should always be adequate (30 rotifer/ml). This also can prevent starvation and cannibalism within the larvae. Flow rate is another factor that should be controlled during snook larvae rearing. A slow flow rate (10ml/min), in suitable for snook larvae.

8 CONCLUSION AND RECOMMENDATIONS

Some factors are considered essential for an organism and must exist at a certain level to fulfil their biological requirements. The enrichment diets which were supplied to newly hatched Artemia in this experiment were acceptable. This can be used successfully in the rearing of Artemia for short term enrichment to increase the fatty acid content. The percentage of fatty acids obtained can meet the requirements of marine larvae.

The results obtained in the enrichment diets demonstrate that diets 2 and 3 are highly recommended for common snook larvae. This is because both diets demonstrate high fatty acid composition. The ratio of fatty acid composition obtained in the wild eggs is similar to the ratio obtained in the enrichment diets 2 and 3. Also the enrichment period of 12 hours had been effective in this experiment. More experiments are recommended using a longer-term enrichment period (24 hours), to be able to evaluate the percentage of fatty acid between time periods.

To be able to improve the survival of common snook larvae, it is important to consider:

- The initial feeding day – onset of feeding. Larvae start feeding from day two after hatching. It is highly recommended to start feeding at this time.
- Use small size prey. Small prey size (SS type rotifer) improved the larvae survival significantly.
- Antibiotic treatment applied to live feed (rotifers- artemia-zooplankton) before given as feed is important. This may reduce the possibility of adding negative bacteria into the larval tank or getting bacterial infection into the larvae.
- Adding (Green water system) microalgae to the rearing tank as supplement also has a positive impact on the larvae survival.
- Optimal rearing temperature is 28 ^{O}C.

Not at the same level of importance, prey density is an important factor during egg, larvae and juvenile rearing process. Prey density (rotifer) of 12-15 rotifer/ml is recommended for common snook. On table 7, some basic guidelines are presented for larvae rearing in common snook.

Although common snook larvae survival has improved in recent years, still more improvement needs to be achieved. Commercial scale production of juveniles is still struggling in the hatcheries. Getting a better understanding of larvae biology in the wild can lead to significant improvement for their culture. Also, broodstock diet should be closely monitored, which has a huge impact on egg quality and hatching outcome.

Table 7. Larvae rearing protocol for common snook.

Common snook larvae rearing recommended protocol									
DAYS after hatch	Larvae Size, SL.	Temp. ^{O}C	Microalgae	Prey type & density	pH	salinity	Oxygen	photoperiod	Stock/density
0	1.71mm	28 ^{0}C	------	-----	7.8	35 ppt	10mg/L	14 hours light, 10 hours dark.	20lav/L
1	2.07mm		-------	-----		35 ppt			20lav/L
2	2.17mm		1000/ml	15s.rot/ml		35ppt			20lav/L
3	2.26mm		1000/ml	30s.rot/ml		35ppt			15lav/L
4	2.35mm		1000/ml	30s.rot/ml		33ppt			15lav/L
6	2.51mm		------	s.rotifer + enr.Artemia —30/ml		33ppt			15lav/L
8	2,55mm		------			30ppt			15lav/L
10	3.14mm		------			30ppt			15lav/L
12	3.37mm		-------	Enr.Art+Macro diet		28ppt			15lav/L
14	4.43mm		-------			28ppt			15lav/L

9 ACKNOWLEDGEMENTS

I give God thanks for the life, health and strength that he gives me during all these days here in Iceland. My sincere gratitude goes to my supervisor, Associate Prof. Ólafur Sigurgeirsson, for his support, advice and guidance during my project. A special thanks to Agnar Steinarsson for his assistance during the experiment at the Marine Research Institute (MRI) at Grindavík, Iceland. To Ingibjorg Rosa Þorvaldsdottir at Matis for her great laboratory work and patience during the analysis of the samples.

I would like to give thanks to the UNU-FTP staff here in Iceland. Tumi Tomasson, Thor Asgeirsson, Mary Frances Davidson, Julie Ingham and Stefan Ulfarsson, for support and advice while conducting this training program.

Thanks to the INPESCA staff in Nicaragua for support and feedback during the development of my project. Special thanks to the executive president Dr. Edwardo Jackson Abella to Ms. Ana Rosa Mayorga and Mr. Eustace Wiltshire Hamilton. Also, I would like to give thanks to my family especially my wife, Kathy Howard for her moral support and constant encouraging words.

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