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Impacts of three different microdiets on Florida Pompano, *Trachinotus carolinus*, weaning success, growth, fatty acid incorporation and enzyme activity

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Abstract:

In this study, three microdiets were tested on weaning of Florida pompano larvae: Otohime, Gemma and a reference diet LR803. The experimental system was stocked with 11-day-old larvae, which were co-fed micro-diets and live food from 11 dph to 17 dph then micro-diets only until 28 dph. Survival from 11 dph to 28 dph was similar for all treatments, with an average of 33%. At the end of the trial, the Gemma larvae were significantly longer and heavier than those larvae fed the other diets. Significant differences were observed in fatty acid composition of the diets and larvae between treatments. The Gemma larvae incorporated the lowest amount of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA). However, they had the highest DHA/EPA and ARA/EPA ratios, which is in agreement with the concept that the proportions of polyunsaturated fatty acids could be of greater importance than their absolute amount. Results from the enzyme analysis suggest that fishmeal is suitable as the main protein source for Florida pompano larvae. This study gives new insights on Florida pompano early nutritional requirements and demonstrated the full functionality of the pancreas at 16 days post hatch, opening possibilities of an earlier weaning time.

Highlights

► Survival was similar for Florida pompano larvae weaned on three different diets. ► Growth was enhanced by the Gemma diet compared to the two other diets. ► Essential fatty acids ratios appeared more important than absolute inclusion levels. ► Enzyme analyses showed the full functionality of the pancreas by 16 days post hatch.

Keywords: Enzyme ; Fatty acids ; Fish larvae ; Microdiets ; *Trachinotus carolinus* ; Weaning

1. INTRODUCTION

The Florida pompano, *Trachinotus carolinus*, is found along the coasts of the eastern Atlantic ocean and Gulf of Mexico, from Massachusetts down to Brazil, with the highest abundance along the coast of Florida (Gilbert, 1986; Smith-Vaniz, 2002). Belonging to the Carangidae family, it is a popular food and game fish (Iversen and Berry, 1969; Weirich et al., 2006). The commercial fishery in the United States has remained small and the demand is continuously increasing and higher than the supply (Weirich et al., 2006). In 2011, with only 102.4 metric tons of commercial landing, the whole-fish dockside price reached US$ 8.92/kg (NOAA/NMFS, 2013). In addition to a high retail price and consumer demand, the Florida pompano has a fast growth rate and can withstand high densities, making it a prime candidate for aquaculture production (Iversen and Berry, 1969; Moe et al., 1968; Weirich et al., 2006). However, difficulties in producing large quantities of juveniles have hindered the development of Florida pompano farming and the development of reliable hatchery protocols is essential (Cavalin and Weirich, 2009; Riley et al., 2009). One of the main challenges of larval rearing is to provide adequate nutrition to support the fast growth and development of larvae. Traditionally, most marine finfish larvae are first fed rotifers then *Artemia* until the end of metamorphosis when they are slowly weaned on to a commercial dry diet (Rosenlund et al., 1997). However, live food production is costly, time consuming and *Artemia* nutritional value varies tremendously depending on strain, origin and even batches from the same location (Conceição et al., 2010; Lavens and Sorgeloos, 2000). Rotifers and *Artemia* are both deficient in essential fatty acids, which are a fundamental source of energy and structural components for larval development (Sargent et al., 1997). Indeed, marine fish larvae are unable to elongate and desaturate 18:3n-3 and 18:2n-6 to polyunsaturated fatty acids (PUFAs) and these PUFAs must therefore be supplied by the diet (Bell, 2003; Sargent et al., 1999b, 1989). In addition to a poor nutritional profile live food also transfers harmful

bacteria to the larvae and the microbial control of live food cultures is a difficult procedure (Olafsen, 2001).

To achieve an economical and reliable production of juveniles in marine finfish aquaculture it is critical to develop micro-particulate diets that provide the adequate nutrition to the larvae. Research from the past decade showed that unlike previously suggested (Dabrowski, 1984; Kolkovski et al., 1993; Lauff and Hofer, 1984), fish larvae do possess the necessary enzymes to digest an inert diet at the onset of exogenous feeding (Cahu and Zambonino-Infante, 2001). However, fish larvae have very different nutritional requirements compared to juveniles. For example, it was found that dietary lipids (Coutteau et al., 1997) and protein hydrolysates (Zambonino-Infante et al., 1997) are two crucial components for the growth and development of larvae but are not essential for juveniles. Therefore, larval diets must be specifically designed to meet these particular needs. In addition, the design of the diet must take into consideration the reduced attraction of larvae for inert particles (Cox and Pankhurst, 2000; Fernandez-Diaz et al., 1994), the risk of leaching of nutrients due to the small surface to volume ratio (Kvåle et al., 2006; Langdon, 2003) and the effects on water quality (Bonaldo et al., 2011; Fletcher et al., 2007). While Cahu et al. (2003) successfully reared seabass *Dicentrarchus labrax* on an experimental diet with total elimination of live food, a live food period is still required for most marine fish larvae.

Co-feeding microdiets and live food has been shown to improve larval performances compared to feeding microdiets or live food alone in many species such as red sea bream *Pagrus major* and Japanese flounder *Paralichthys olivaceus* (Kanazawa et al., 1989), yellow-finned black porgy *Acanthopagrus latus* (Leu et al., 1991), red drum *Sciaenops ocellatus* (Holt, 1993), Atlantic cod *Gadus morhua* (Callan et al., 2003), barramundi *Lates calcarifer* (Curnow et al., 2006) or Senegalese sole *Solea senegalensis* (Engrola et al., 2009). Two main factors are contributing to the success of co-feeding protocols. First, live food visual and

chemical stimulation facilitates the ingestion of the microdiet, conditioning the larvae to prey on inert particles and allowing for an earlier weaning to dry feed (Canavate and Fernandez-Diaz, 1999; Rosenlund et al., 1997). Second, live prey carry numerous nutritional factors stimulating pancreatic enzyme secretions and endocrine responses which contributes to the maturation of the digestive system (Kolkovski et al., 1997; Koven et al., 2001). The maturation of the digestive system is characterized by the progressive decline of the early mode of protein digestion and absorption (through pinocytosis and intracellular digestion) and the simultaneous increase of extracellular digestion and membrane transport as the enterocytes brush border membrane develops (Govoni et al., 1986). The study of the pancreatic, cytosolic and brush border membrane enzymes give essential information on this maturation process (Zambonino-Infante and Cahu, 2001).

The goal of this research was to gain knowledge on Florida pompano larvae nutritional requirements and digestive abilities by studying the impact of co-feeding three different microdiets on larval performances, lipid incorporation and digestive enzyme activity.

2. MATERIALS AND METHODS:

2.1 Experimental set up

Eggs were obtained from a captive spawn at the Mote Marine Laboratory Aquaculture Research Park in Sarasota, Florida. The broodstock population was collected in 2008 off the southwest coast of Florida and conditioned using temperature and photoperiod control in a 25m^3 tank equipped with a recirculating filtration system. After 6 weeks under simulated natural conditions, fish were sampled to check their reproductive status and females with oocytes in late secondary growth (410 ± 21 µm, n=14 females, 50 oocytes per females) were implanted with Ovaplant® at 50µg/kg (Western Chemical Inc.). Eggs were incubated in a 100L conical hatching tank with an upwelling water flow recirculating with a 3.3m^3 tank equipped with a filtration system. After hatching, the water flow was reduced and the screen of the overflow pipe removed to allow for a gentle release of the larvae in the tank and the collection of dead embryos and egg casings at the bottom of the hatching tank.

Approximately 200,000 larvae were reared in the 3.3 m^3 tank (temperature 26 ± 1 °C, salinity 35 ± 1 g L^{-1}, dissolved oxygen of 6 ± 1 mg L^{-1} and pH of 8 ± 0.5) up to 11 days post hatch (dph). Rotifers enriched with Algamac 3050 (Aquafauna Bio-Marine Inc., Hawthorne, CA, USA) were fed to the larvae at 5 ml^{-1} from 2 to 11 dph and *Artemia* were introduced from 9 dph at 3 ml^{-1}. Up to 11 dph, the tank water was shaded with RotiGrow *plus* (Reed Mariculture Inc, CA, USA) at 500,000 cells L^{-1}. At dph 11, the water level in the production tank was lowered and the larvae were collected and transferred to twelve 130 L experimental tanks (870 larvae $tank^{-1}$, counted manually, equivalent to 6-7 larvae L^{-1}) corresponding to three microdiets tested in quadruplicate: 1) Otohime (Commercial diet, Marubeni Nisshin Feed Co. Japan), 2) Gemma (Commercial diet, Skretting, France) and 3) Larval Reference Diet 803 – LR803 (Experimental diet, Agricultural Research Service, USA). The diet ingredients are listed in Table 1.

Larvae were co-fed one of three microdiets, rotifers at 3 ml^{-1} and *Artemia* at 2 ml^{-1} from 11 to 13 dph, followed by microdiet and *Artemia* at 1 ml^{-1} until 16 dph. From 17 to 28 dph ~~(end of the experiment),~~ larvae were only fed microdiets through automatic feeders. Microdiets were overfed to ensure larvae satiation and tanks were siphoned on a daily basis to maintain tank hygiene. The experiment was ended at dph 28 when fish had completed metamorphosis, had been fully weaned for over 10 days and required transfer to a larger system.

Larvae were sampled at 11, 16, 22, 26 and 28 dph to record standard length and body depth (10 larvae $tank^{-1}$, 40 $treatment^{-1}$). Body depth was measured from the insertion of the first dorsal spine to the to the most ventral point on the base of the body, as pictured in Cavalin and Weirich, 2009. Wet weight was recorded on 22, 26 and 28 dph only due to the very low weight of the larvae prior to 22 dph and the lack of analytical precision (10 larvae $tank^{-1}$). At 16, 22 and 28 dph, 10 larvae from each tank were preserved for enzyme analysis. At the end of the trial (28 dph), all fish were counted to determine survival and 10 larvae from each tank were preserved for proximate and fatty acid analysis.

2.2 Enzyme analysis

Enzyme analyses were performed at the Functional Physiology of Marine Organisms Unit at Ifremer, Brest, France. Larvae were dissected under a microscope on a glass plate at 0 °C to separate the pancreatic segment from the intestinal segment as described in Cahu and Zambonino-Infante (1994). Trypsin and amylase activities were assayed according to Holm et al. (1988) and Métais and Bieth (1968) respectively. Enzymes of the brush border membrane, alkaline phosphatase (AP) and leucine aminopeptidase (AN), were assayed according to Bessey et al. (1946) and Maroux et al. (1973) respectively. Leucine–alanine peptidase (leu-ala) assays were performed using the method of Nicholson and Kim (1975).

Enzyme activities are expressed as specific activities (i.e. U/mg protein). Protein was determined by the Bradford procedure (Bradford, 1976).

2.4 Proximate and lipid analyses

Proximate analyses were performed at the University of Stirling Institute of Aquaculture Nutrition Group, in Stirling, Scotland. Proximate composition of microdiets and larvae at the end of the trial were determined according to standard procedures (AOAC, 2000). Prior to analysis, the larvae from each tank were pooled and minced. Moisture content was determined by drying the samples at 105 °C for 24 h. Ash content was determined after 24 h in crucibles at 600 °C. Crude protein content (Nx6.25) was determined using the automated Kjeldahl method (Tecator Kjeltec Auto 1030 analyzer, Foss, Warrington, U.K). Crude lipid content was determined after extraction according to Folch et al. (1957).

Lipid analyses were performed by the Fisheries and Mariculture Laboratory at the University of Texas Marine Science Institute (UTMSI) in Port Aransas, Texas, USA. Lipids were extracted according to Folch et al. (1957) and the fatty acid composition was determined by gas-liquid chromatography after preparation of fatty acid methyl esters (FAMEs) according to Morrison and Smith (1964). FAMEs were separated and quantified on a gas chromatograph (Shimadzu GC-2014, Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a Phenomenex ZB-WAX plus capillary column (30 m long, 0.53 mm internal diameter, 1.0 μm thickness; Phenomenex, Torrance, CA, USA) with on-column injection and flame ionization detection, using helium as carrier gas (4 mL min^{-1}) and injector and detector temperatures of 250 and 260 °C respectively. Temperature was held at 160°C for 5 min then increased up to 220°C at 3°C per minute and maintained at this temperature for 30 minutes. FAMEs peaks were identified by comparison with known standards (Supelco, Inc., Bellefonte, Pennsylvania, USA).

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178 **2.5 Statistical analysis**

179 Statistical analysis was performed with MINITAB® version 16.0 (Minitab Ltd., Coventry,
180 UK). Normality and homogeneity of variance were confirmed using Kolmogorov−Smirnov
181 test. Growth, body depth, wet weight and enzyme activities were compared using a General
182 Linear Model (GLM) with all time and treatment interactions being analyzed and significant
183 differences grouped by a Tukey post hoc test with 95 % confidence. Survival, proximate
184 analysis and fatty acid data were arcsine square root transformed before a one-way ANOVA
185 followed by a Tukey post hoc test with 95 % confidence. Linear regression was performed to
186 evaluate the incorporation of selected fatty acids from the diet into larval tissues. All data are
187 presented as mean ± standard error of the mean (SEM) and level of statistical significance
188 was set at $P<0.05$.

3. RESULTS

3.1 Survival

Survival during the experiment (from 11 to 28 dph) was similar for all treatments with 32.1 ± 1.2 %, 32.6 ± 0.8 % and 33.3 ± 0.9 % for the fish fed the Gemma, LR803 and Otohime diets, respectively ~~(data not shown)~~.

3.2 Growth

From 11 to 22 dph, larvae standard length was similar for all treatments (Fig. 1A). By 26 dph, the Gemma larvae were significantly longer than the larvae fed the other diets. This trend continued at 28 dph with Gemma > LR803 > Otohime larvae. In addition, from 26 to 28 dph, the Otohime larvae exhibited a slower growth compared to the other treatments with an average length at 28 dph not statistically different from that of 26 dph (Fig. 1A).

The first significant difference in body depth was observed at 16 dph with the Gemma larvae being larger than the LR803 and Otohime larvae (Fig. 1B). The same trend was observed at 28 dph.

From 26 dph onwards, the Gemma larvae were significantly heavier than the LR803 and Otohime larvae (Fig. 1C). No difference was observed between the other two diets.

3.3 Proximate analysis

Lipid content was significantly different between microdiets (Table 2). Highest lipid content was found in the LR803 diet (26.8 ± 0.1 %), compared to the Otohime diet (21.2 ± 1.6 %) and the Gemma diet (18.9 ± 0.5 %). At the end of the trial, no difference was observed in the lipid content of the larvae.

Protein content was significantly different between diets, with the Gemma diet containing 57.6 ± 0.1 % of protein, a significantly higher content than that of the Otohime diet (52.3 ± 0.1 %) and the LR803 diet (50.9 ± 0.1 %). The LR803 diet had a significantly lower protein content compared to the two other diets. At the end of the trial, the Gemma and Otohime larvae had similar protein contents (16.3 ± 0.3 % and 16.1 ± 0.3 % respectively), significantly higher than that of the LR803 larvae (14.5 ± 0.8 %).

Moisture and ash content were significantly lower for the LR803 diet (respectively 5.6 ± 0.1 and 3.7 ± 0.1%) compared to the other diets. Moisture content was similar for the Gemma and Otohime diets (6.4 ± 0.1 %) while ash content was significantly higher for the Otohime diet compared to the Gemma diet (respectively 12.8 ± 0.1 % and 10.8 ± 0.1 %). However, at the end of the trial no difference was observed in the moisture and ash content of the larvae fed the different treatments with an average moisture content of 61.2 ± 0.3 % and an average ash content of 10.6 ± 0.4 % (Table 2).

3.4 Fatty acid analysis

Fatty acid profile of the larvae at the end of the trial was strongly influenced by the fatty acid profile of the microdiets (Table 3). The main differences were observed in linoleic acid (LA), ARA, EPA and DHA content.

LA content contrasted greatly between the diets, with LR803 and Gemma containing 19.84 ± 0.04 and 30.40 ± 0.05 % of LA respectively, as opposed to only 4.54 ± 0.02 % for Otohime diet. LA content in the larvae was correlated (R^2=0.96) with the diet content. The LR803 and Gemma larvae incorporated respectively 20.06 ± 0.06 % and 24.40 ± 0.32 % of LA, against 4.51 ± 0.13 % for the Otohime larvae.

There was also a strong correlation (R^2=0.98) between the ARA content of the microdiets and the ARA content of the larvae at the end of the trial. Highest ARA content was observed in

the LR803 diet and larvae with respectively 0.55 ± 0.00 % and 0.59 ± 0.01 %. The Otohime diet had a similar ARA content (0.54 ± 0.01 %), however the Otohime larvae incorporated only 0.52 ± 0.01 % of ARA, significantly less than the LR803 larvae. A significantly lower ARA content was observed in the Gemma diet and larvae, with respectively 0.34 ± 0.00 % and 0.30 ± 0.00 %.

The Otohime and LR803 diet and larvae contained the highest proportion of EPA with respectively 10.79 ± 0.05 % and 9.01 ± 0.08 % for the diet and 7.28 ± 0.08 % and 6.10 ± 0.13 % for the larvae. The Gemma diet and larvae contained the lowest proportion of EPA with respectively 4.12 ± 0.03 % and 2.21 ± 0.04 %.

The Otohime diet contained the highest DHA content (9.57 ± 0.01 %), significantly greater than the Gemma diet DHA content (7.32 ± 0.05 %) and the LR803 DHA content (5.36 ± 0.01 %). The Otohime larvae incorporated the highest DHA content with 11.22 ± 0.29 %. However, even though the Gemma diet contained significantly higher DHA content than the LR803 diet, the larvae fed these diets incorporated similar DHA contents (7.37 ± 0.11 and 7.12 ± 0.13 %) for the Gemma and LR803 larvae respectively.

3.5 Enzyme analyses

Trypsin activity in the pancreatic segment did not vary for the Otohime larvae (Fig. 2A). However, for the Gemma and LR803 larvae, the activity increased two-fold from 16 to 22 dph, and then decreased from 22 to 28 dph to levels similar to 16 dph. In the intestinal segment, trypsin activity remained steady in the LR803 larvae throughout the trial period, while a two-fold increase was observed for the Gemma larvae (Fig. 2B). A strong increase (4 fold) was observed in the Otohime larvae during the first half of the trial followed by a decrease in activity from 22 to 28 dph.

Amylase activity in the pancreatic segment exhibited a similar pattern for the larvae from all treatments with a sharp decrease between 16 and 22 dph and stabilization from 22 and 28 dph (Fig. 2C). In the intestinal segment, amylase specific activity did not vary significantly in the Gemma and LR803 larvae (Fig. 2D). However, a two-fold increase was observed between 16 and 22 dph in the Otohime larvae, that decrease between 22 and 28 dph, and reach levels similar to 16 dph.

In the brush border membrane, AP specific activity decreased by half between 22 and 28 dph in the Otohime larvae (Fig. 3A). In contrast, AP activity in the Gemma larvae appeared to increase between 22 and 28 dph though it was not statistically significant (40%, $p=0.087$). Activity levels did not vary significantly for the LR803 larvae.

Activity levels of AN increased more than two-fold between 22 and 28 dph in the Gemma larvae (Fig. 3B). A 50 % increase was also observed for the Otohime larvae, while no significant difference was found in the LR803 larvae. During the trial period, an increase in leu-ala activity was observed in larvae from all treatments although only significantly for the Otohime and Gemma larvae (Fig. 3C). Between 16 and 22 dph, a two-fold increase was observed in the Gemma larvae and a three-fold increase in the Otohime larvae. Between 22 and 28 dph, no significant variations were observed in the Gemma larvae while a 20 % decrease was observed in the Otohime larvae.

4. DISCUSSION

This study showed that microdiet composition has a major impact on Florida pompano larvae, influencing growth, fatty acid incorporation and activity of digestive enzymes. Standard length, body depth and wet weight were all enhanced by the Gemma diet compared to the Otohime and LR803 diet. Survival was similar for all treatments hence all diets were able to support the growth of larvae after the end of the live food period.

No difference was observed in the lipid, moisture and ash content of the larvae despite significant differences in the proximate composition of the microdiets. This shows that the minimal lipid requirement was met and demonstrates the ability of Florida pompano larvae to tolerate some variations in the lipid dietary intake. However, significant differences were observed in the fatty acid profiles of the diets and larvae.

As previously mentioned, marine fish are unable to synthetize PUFAs *de novo*. The dietary importance of PUFAs has been widely studied and reviewed (Sargent et al. 1989; Watanabe and Kiron 1994; Izquierdo 1996; Sargent et al. 1999b; Sargent et al. 2002). However, major uncertainties remain in PUFA dietary requirements as they vary between species and are determined not only by the absolute amount of PUFAs, but also by their relative ratios (Hamre et al., 2013; Izquierdo et al., 2000; Sargent et al., 1997).

DHA plays a critical role in the structure of cell membrane, especially for the correct development of the visual and immune systems (Bell et al., 1995; Rodríguez et al., 1997; Sargent, Bell, et al., 1999; Koven, 2003; Benítez-Santana et al., 2007). DHA and EPA compete for the same enzyme to esterify fatty acid into phospholipid structures (Sargent, Mcevoy, et al., 1999). Several studies have demonstrated that DHA has a more dominant role in growth and membrane structure than EPA, suggesting that the relative proportions of DHA and EPA may have a greater impact than the absolute amount, with the necessity of a greater proportion of DHA than EPA (Glencross and Rutherford, 2011; Glencross, 2009; Reitan et

al., 1994; Rodríguez et al., 1998; Watanabe, 1993). Our results agree with that hypothesis, where the Otohime diet and larvae displayed the highest DHA and EPA contents but also displayed the lowest growth. In contrast, the Gemma larvae, with the lowest DHA and EPA contents but with a DHA:EPA ratio more than double of that of the Otohime or LR803 treatments, exhibited the fastest growth. At the end of the experiment, DHA:EPA ratios in the larvae were significantly different from the ratios in the diet. The amount of EPA in the larvae was significantly lower than that of the diet for all treatments. In contrast, the amount of DHA was significantly higher in the Otohime and LR803 larvae than in the diets while it did not vary in the Gemma treatment. This resulted in significantly higher DHA:EPA ratios in the larvae than in the diet for all treatments. This suggests the ability of the larvae to modulate, to some degree, the incorporation of EPA and some activity of the Δ6 desaturase, even though the activity of this enzyme seems too low to convert enough EPA to DHA to compensate the dietary deficiency (Vagner and Santigosa, 2011).

Among the HUFAs, DHA and EPA are the most abundant in marine fish eggs and tissues and the dietary requirements for these two fatty acids have been widely studied in comparison to ARA, though the latter plays a critical role in the development of the larvae (Sargent, Bell, et al., 1999; Bell, 2003). ARA is specifically concentrated in fish eggs, attesting to its high biological importance during the early phases of larval development (Bell et al., 1997).

EPA and ARA compete for the enzymes involved in the production of eicosanoids, with eicosanoids produced from ARA being more biologically active, implying that, like DHA and EPA, the ratio of ARA to EPA might be of greater importance than the absolute quantity (Sargent, Mcevoy, et al., 1999; Tocher, 2003). Results from this experiment are consistent with these earlier observations. Both the Gemma diet and larvae presented a lower ARA and EPA content than for the other treatments. However, they presented the highest ARA:EPA ratio and displayed the fastest growth. These results reinforce the fact that HUFA dietary

requirements need to be considered as a whole, where the amount of each HUFA influences the final ratios and the required metabolic pathways. An important difference in the fatty acid profile of the different diets tested in this trial was the amount of LA. Despite the higher levels of LA in the Gemma and LR803 diet, no increase in the content of the desaturation/elongation pathway products was observed and it is likely that this fatty acid was primarily used as a source of energy.

In addition to their impact on larvae development and fatty acid incorporation, microdiets can strongly influence the development of the digestive system (Cahu and Zambonino-Infante, 2001). Marine fish larvae have to switch from a primary mode of digestion to an adult mode of digestion; the maturational process is characterized by the acquisition of a progressive efficient secretion of pancreatic enzymes, and the transition from a cytosolic to a brush border membrane digestion at the intestinal level (Dabrowski, 1984; Ronnestad et al., 2013; Zambonino-Infante et al., 1997).

An increase in growth and survival has been reported in correlation with an increase of both pancreatic and intestinal enzyme activity in sea bass (Cahu and Zambonino-Infante, 1995), sole (Ribeiro et al., 1999) and cod (Wold et al., 2007). Pancreatic enzymes specific activity (activity per gram of protein) follow the same pattern in temperate marine fish, with an increase during the first days/weeks of the life cycle depending on the species, followed by a decrease to a constant level, not due to a decline in the amount of digestive enzymes, but as a result of the increase of tissue proteins in the growing larvae (Zambonino-Infante and Cahu, 2001). Changes in the enzyme activity during ontogeny are genetically programmed, however the diet influences the plateau level of enzymes and can delay or stop the digestive system maturation process if inadequate (Cahu and Zambonino-Infante, 2001; Krogdahl and Sundby, 1999). In this trial, the composition of the different diets impacted the development of digestive functions of the larvae. Results from the amylase and trypsin analyses indicated

355 that the pancreas is fully functional at 16 dph, which does not exclude that the pancreas may
356 be functional at an earlier date. Amylase specific activity is high in young fish larvae then
357 declines to a constant low level (Cahu and Zambonino-Infante, 1994; Péres et al., 1998;
358 Ribeiro et al., 1999), a pattern comparable to the decline of lactase expression during the
359 development of mammals (Freund et al., 1990). In the present study, the strong decline in
360 amylase activity was observed in the pancreatic segment from 16 dph for all treatments. In
361 the intestinal segment, amylase specific activity was significantly higher for larvae fed the
362 Otohime diet indicating a stimulation in amylase secretion likely due to the potato starch
363 contained in this diet. Indeed, starch content in the diet can influence amylase expression as
364 demonstrated in sea bass (Péres et al., 1998, 1996), red drum (Buchet et al., 2000) and yellow
365 croaker *Pseudosciaena crocea* (Ma et al., 2005).
366 Trypsin is responsible for the digestion of proteins during the early development of the larvae
367 and its activity is influenced by both the source and quantity of protein in the diet (Guerreiro
368 et al., 2010; Péres et al., 1996; Zambonino-Infante et al., 1996). In the present study, the
369 higher specific activity of trypsin observed in the intestinal segment of the larvae fed
370 Otohime suggests that the peak of specific activity in the pancreatic segment occurred before
371 16 dph and was likely a sign of a higher pancreatic secretory activity. The Gemma diet
372 includes the highest amount of protein followed by the Otohime diet and then the LR803 diet.
373 Therefore the increase of trypsin activity was not a result of the protein content of the diet but
374 probably the result of differences in the source and molecular form of the protein in the diet.
375 Indeed, each diet had a different main source of protein with the predominent ingredient
376 being krill meal in Otohime, fish meal (native protein and protein hydrolysate) in Gemma and
377 squid meal in LR803. Protein sources in microdiets have to provide an appropriate amino
378 acid profile and also have to be highly digestible given the larvae's poor digestive capacities;
379 in addition, they should have low water solubility due to the small surface to volume ratio of

the diet particles (Nankervis and Southgate, 2006).

To facilitate proteins digestion and assimilation by the larvae, pre-digested proteins (protein hydrolysates) are frequently incorporated into microdiets. Low to moderate levels of protein hydrolysates have proven beneficial in several species including gilthead sea bream *Sparus aurata* (Zambonino-Infante et al., 1997) and European sea bass (Cahu et al., 1999) while high levels were detrimental in these same two species (Cahu et al., 1999; Kolkovski and Tandler, 2000). In the present study, the exact ingredient quantities and the presence or proportions of protein hydrolysates in the diets are not known. Therefore, it is difficult to characterize the effect of each source of protein on larvae development. However, the satisfactory results obtained with the Gemma larvae suggest that fish meal including a mix of native proteins and protein hydrolysates is an appropriate source of protein for pompano larvae.

AP and AN are intestinal enzymes mainly located in the brush border membrane of enterocytes while leu-ala peptidase is an intestinal enzyme mainly located in the cytosol of enterocytes (Cahu et al., 1998). As the enterocytes mature and proliferate, the brush border membrane develops and the associated enzyme activities increase while cytosolic enzyme activities decline, indicating the establishment of the adult digestive system (Boglino et al., 2012; Zambonino-Infante and Cahu, 2001). In this trial, no decline in leu-ala activity was observed in Gemma and LR803 larvae, while a peak was observed at 22 dph for Otohime larvae but with an activity level at 28 dph superior to 16 dph. Cytosolic digestion in Florida pompano seems to remain elevated after the onset of BBM enzymes; in consequence, the maturation of the enterocytes should be evaluated primarily through the activity of the BBM enzymes (i.e. AP and AN). Very minor changes were observed in the specific activity of AP and AN in the LR803 larvae, suggesting a delay in the maturation of the enterocytes in this group. At 22 dph, highest AP and AN specific activities were observed in the Otohime larvae. Contrariwise, this positive sign of enterocyte maturation was not confirmed at 28dph, particularly for AP. In contrast, larvae fed Gemma exhibited an appropriate maturation of the BBM enzymes. This suggests that the Otohime diet was probably adequate for young larvae before 22dph, but failed to sustain an appropriate development of Florida pompano larvae after this developmental date. Contrastingly, the larval development seemed to be more continuous and balanced with the Gemma diet, as demonstrated by the growth and enzymatic results.

In conclusion, this study brings new knowledge on Florida pompano dietary requirements and digestive development. Results first suggest that a diet including 20 % lipids, 55 % proteins, a DHA:EPA ratio greater than 1 and a ARA/EPA ratio of at least 0.08 is an adequate weaning diet for Florida pompano since the Gemma diet, with similar characteristics, promoted the best larvae performance in this experiment. Second, results

from the enzyme analysis showed that the pancreas is fully functional by 16 dph suggesting that weaning onto a dry diet could occur earlier than in the present study. Additional research is needed to determine more precisely the Florida pompano larval nutritional requirements . Indeed, even though lipids are one of the most important nutritional factors known to affect larvae growth and survival, numerous other macro and micro-nutrients can either enhanced or inhibit larvae development. This includes ingredients and supplementations present in low quantities in the diets tested in this study such as taurine (Pinto et al., 2013, 2010; Schreck et al., 2012), yeast (Tovar-Ramírez et al., 2010, 2004) or vitamins and minerals (Hamre et al., 2013; Moren et al., 2011) which were beyond the scope of this study. However, the present results give a baseline of a suitable weaning diet, which can be used in future trials to determine the optimal weaning time for Florida pompano.

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458 **REFERENCES**

459 AOAC, 2000. Official methods of analysis. Association of Official Analytical Chemists,
460 Gaithersburg, Maryland, USA.

461 Bell, G., 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities.
462 Aquaculture 218, 491–499.

463 Bell, G., Farndale, B.M., Bruce, M., Navas, J.M., Carillo, M., 1997. Effects of broodstock
464 dietary lipid on fatty acid compositions of eggs from sea bass (*Dicentrarchus labrax*).
465 Aquaculture 149, 107–119.

466 Bell, M. V, Batty, R.S., Dick, J.R., Fretwell, K., Navarro, J.C., Sargent, J.R., 1995. Dietary
467 deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile
468 herring (*Clupea harengus* L.). Lipids 30, 443–449.

469 Benítez-Santana, T., Masuda, R., Juárez Carrillo, E., Ganuza, E., Valencia, Hernández-Cruz,
470 C.M., Izquierdo, M.S., 2007. Dietary n-3 HUFA deficiency induces a reduced visual
471 response in gilthead seabream *Sparus aurata* larvae. Aquaculture 264, 408–417.

472 Bessey, O.A., Lowry, O.H., Brock, M.J., 1946. A method for the rapid determination of
473 alkaline phosphates with five cubic millimeters of serum. J. Biol. Chem. 164, 321–9.

474 Boglino, A., Darias, M.J., Ortiz-Delgado, J.B., Özcan, F., Estévez, A., Andree, K.B.,
475 Hontoria, F., Sarasquete, C., Gisbert, E., 2012. Commercial products for Artemia
476 enrichment affect growth performance, digestive system maturation, ossification and
477 incidence of skeletal deformities in Senegalese sole (*Solea senegalensis*) larvae.
478 Aquaculture 324-325, 290–302.

Bonaldo, A., Parma, L., Badiani, A., Serratore, P., Gatta, P.P., 2011. Very early weaning of common sole (*Solea solea* L.) larvae by means of different feeding regimes and three commercial microdiets: Influence on performances, metamorphosis development and tank hygiene. Aquaculture 321, 237–244.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–54.

Buchet, V., Zambonino-Infante, J.L., Cahu, C.L., 2000. Effect of lipid level in a compound diet on the development of red drum (*Sciaenops ocellatus*) larvae. Aquaculture 184, 339–347.

Cahu, C.., Zambonino Infante, J.., Quazuguel, P., Le Gall, M.., 1999. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass *Dicentrarchus labrax* larvae. Aquaculture 171, 109–119.

Cahu, C.L., Zambonino-Infante, J.L., 1994. Early weaning of sea bass (*Dicentrarchus labrax*) larvae with a compound diet: Effect on digestive enzymes. Comp. Biochem. Physiol. Part A Physiol. 109, 213–222.

Cahu, C.L., Zambonino-Infante, J.L., 1995. Effect of the molecular form of dietary nitrogen supply in sea bass larvae: Response of pancreatic enzymes and intestinal peptidases. Fish Physiol. Biochem. 14, 209–214.

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

Cahu, C.L., Zambonino-Infante, J.L., Barbosa, V., 2003. Effect of dietary phospholipid level and phospholipid:neutral lipid value on the development of sea bass (*Dicentrarchus labrax*) larvae fed a compound diet. Br. J. Nutr. 90, 21–28.

Cahu, C.L., Zambonino-Infante, J.L., Peres, A., Quazuguel, P., Gall, M.M. Le, 1998. Algal addition in sea bass (*Dicentrarchus labrax*) larvae rearing□: effect on digestive enzymes. Aquaculture 161, 479–489.

Callan, C., Jordaan, A., Kling, L.., 2003. Reducing Artemia use in the culture of Atlantic cod (*Gadus morhua*). Aquaculture 219, 585–595.

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

Cavalin, F.G., Weirich, C.R., 2009. Larval performance of aquacultured Florida pompano (*Trachinotus carolinus*) fed rotifers (*Brachionus plicatilis*) enriched with selected commercial diets. Aquaculture 292, 67–73.

Conceição, L.E.C., Yúfera, M., Makridis, P., Morais, S., Dinis, M.T., 2010. Live feeds for early stages of fish rearing. Aquac. Res. 41, 613–640.

Coutteau, P., Geurden, I., Camara, M.R., Bergot, P., Sorgeloos, P., 1997. Review on the dietary effects of phospholipids in fish and crustacean larviculture. Aquaculture 155, 149–164.

Cox, E.S., Pankhurst, P.M., 2000. Feeding behaviour of greenback flounder larvae, *Rhombosolea tapirina* (Günther) with differing exposure histories to live prey. Aquaculture 183, 285–297.

522 Curnow, J., King, J., Bosmans, J., Kolkovski, S., 2006. The effect of reduced Artemia and
523 rotifer use facilitated by a new microdiet in the rearing of barramundi *Lates calcarifer*
524 (BLOCH) larvae. Aquaculture 257, 204–213.

525 Dabrowski, K., 1984. The feeding of fish larvae□: present «□state of the art□» and
526 perspectives. Reprod. Nutr. Dev. 24, 807–833.

527 Engrola, S., Figueira, L., Conceição, L.E.C., Gavaia, P.J., Ribeiro, L., Dinis, M.T., 2009. Co-
528 feeding in Senegalese sole larvae with inert diet from mouth opening promotes growth
529 at weaning. Aquaculture 288, 264–272.

530 Fernandez-Diaz, C., Pascual, E., Yúfera, M., 1994. Feeding behaviour and prey size selection
531 of gilthead seabream, *Sparus aurata*, larvae fed on inert and live food. Mar. Biol. 118,
532 323–328.

533 Fletcher, R.C., Roy, W., Davie, A., Taylor, J., Robertson, D., Migaud, H., 2007. Evaluation
534 of new microparticulate diets for early weaning of Atlantic cod (*Gadus morhua*):
535 Implications on larval performances and tank hygiene. Aquaculture 263, 35–51.

536 Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and
537 purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509.

538 Freund, J.N., Torp, N., Duluc, I., Foltzer-Jourdainne, C., Danielsen, M., Raul, F., 1990.
539 Comparative expression of the mRNA for three intestinal hydrolases during postnatal
540 development in the rat. Cell. Mol. Biol. 36, 729–36.

541 Gilbert, C., 1986. Species profiles: life histories and environmental requirements of coastal
542 fishes and invertebrates (south Florida): Florida pompano. Biol. Rep. 82, 24.

543 Glencross, B., Rutherford, N., 2011. A determination of the quantitative requirements for
544 docosahexaenoic acid for juvenile barramundi (*Lates calcarifer*). Aquac. Nutr. 17,
545 e536–e548.

546 Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by
547 aquaculture species. Rev. Aquac. 1, 71–124.

548 Govoni, J.J., Boehlert, G.W., Watanabej, Y., 1986. The physiology of digestion in fish
549 larvae. Environ. Biol. Fishes 16, 59–77.

550 Guerreiro, I., de Vareilles, M., Pousão-Ferreira, P., Rodrigues, V., Dinis, M.T., Ribeiro, L.,
551 2010. Effect of age-at-weaning on digestive capacity of white seabream (*Diplodus*
552 *sargus*). Aquaculture 300, 194–205.

553 Hamre, K., Yúfera, M., Rønnestad, I., Boglione, C., Conceição, L.E.C., Izquierdo, M., 2013.
554 Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances
555 in larval rearing. Rev. Aquac. 5, S26–S58.

556 Holm, H., Hanssen, L.E., Krogdahl, A., Florholmen, J., 1988. High and low inhibitor soybean
557 meals affect human duodenal proteinase activity differently: in vivo comparison with
558 bovine serum albumin. J. Nutr. 118, 515–520.

559 Holt, G.J., 1993. Feeding Larval Red Drum on Microparticulate Diets in a Closed
560 Recirculating Water System. J. World Aquac. Soc. 24, 225–230.

561 Iversen, E.S., Berry, F.H., 1969. Fish Mariculture: Progress and Potential. Proceeding Gulf
562 Caribb. Fish. Inst. 21, 163–176.

563 Izquierdo, M.S., 1996. Review Article Essential fatty acid requirements of cultured marine
564 fish larvae. Aquac. Nutr. 2, 183–191.

565 Izquierdo, M.S., Socorro, J., Arantzamendi, L., Hernández-Cruz, C.M., 2000. Recent
566 advances in lipid nutrition in fish larvae. Fish Physiol. Biochem. 22, 97–107.

567 Kanazawa, A., Koshio, S., Teshima, S.-I., 1989. Growth and survival of larval Red Sea
568 Bream *Pagrus major* and Japanese Flounder *Paralichthys olivaceus* fed microbound
569 diets. J. World Aquac. Soc. 20, 31–37.

570 Kolkovski, S., Koven, W., Tandler, A., 1997. The mode of action of Artemia in enhancing
571 utilization of microdiet by gilthead seabream *Sparus aurata* larvae. Aquaculture 155,
572 193–205.

573 Kolkovski, S., Tandler, A., 2000. The use of squid protein hydrolysate as a protein source in
574 microdiets for gilthead seabream *Sparus aurata* larvae. Aquac. Nutr. 6, 11–15.

575 Kolkovski, S., Tandler, A., Kissil, G.W., Gertler, A., 1993. The effect of dietary exogenous
576 digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream
577 (*Sparus aurata*, Sparidae, Linnaeus) larvae. Fish Physiol. Biochem. 12, 203–209.

578 Koven, W., 2003. Key factors influencing juvenile quality in mariculture: a review. Isr. J.
579 Aquac. 55, 283–297.

580 Koven, W., Kolkovski, S., Hadas, E., Gamsiz, K., Tandler, a, 2001. Advances in the
581 development of microdiets for gilthead seabream, *Sparus aurata*: a review. Aquaculture
582 194, 107–121.

583 Krogdahl, A., Sundby, A., 1999. Characteristics of pancreatic function in fish, in:
584 Pierzynowski, S.G., Zabielski, R. (Eds.), Biology of the Pancreas in Growing Animals.
585 Elsevier Science, Amsterdam, pp. 437–458.

586 Kvåle, A., Yúfera, M., Nygård, E., Aursland, K., Harboe, T., Hamre, K., 2006. Leaching
587 properties of three different micropaticulate diets and preference of the diets in cod
588 (*Gadus morhua* L.) larvae. Aquaculture 251, 402–415.

589 Langdon, C., 2003. Microparticle types for delivering nutrients to marine fish larvae.
590 Aquaculture 227, 259–275.

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

593 Lavens, P., Sorgeloos, P., 2000. The history, present status and prospects of the availability
594 of Artemia cysts for aquaculture. Aquaculture 181, 397–403.

595 Leu, M.-Y., Liou, C.-H., Wu, C.-H., 1991. Feasibility of using micro-coated diet fed to larval
596 yellow-finned black porgy, *Acanthopagrus Latus* (Houttuyn). J. Fish. Soc. Taiwan 18,
597 287–294.

598 Ma, H., Cahu, C.L., Zambonino-Infante, J.L., Yu, H., Duan, Q., Le Gall, M.-M., Mai, K.,
599 2005. Activities of selected digestive enzymes during larval development of large
600 yellow croaker (*Pseudosciaena crocea*). Aquaculture 245, 239–248.

601 Maroux, S., Louvard, D., Barath, J., 1973. The aminopeptidase from hog intestinal brush
602 border. Biochim. Biophys. Acta - Enzymol. 321, 282–295.

603 Métais, P., Bieth, J., 1968. Determination of alpha-amylase by a microtechnic. Ann. Biol.
604 Clin. (Paris). 26, 133–42.

605 Moe, M.A.J., Lewis, R.H., Ingle, R.M., 1968. Pompano mariculture: preliminary data and
606 basic considerations. Florida State Board Conserv. Mar. Res. Lab. - Tech. Ser. 55, 65.

607 Moren, M., Waagbo, R., Hamre, K., 2011. Micronutrients, in: Holt, G.J. (Ed.), Larval Fish
608 Nutrition. Wiley-Blackwell, Oxford, UK.

609 Morrison, W.R., Smith, L.M., 1964. Preparation of fatty acid methyl esters and
610 dimethylacetals from lipids with boron fluoride--methanol. J. Lipid Res. 5, 600–608.

611 Nankervis, L., Southgate, P.C., 2006. An integrated assessment of gross marine protein
612 sources used in formulated microbound diets for barramundi (*Lates calcarifer*) larvae.
613 Aquaculture 257, 453–464.

614 Nicholson, J.A., Kim, Y.S., 1975. A one-step l-amino acid oxidase assay for intestinal
615 peptide hydrolase activity. Anal. Biochem. 63, 110–117.

616 NOAA/NMFS, 2013. Annual Commercial Landing Statistics [WWW Document]. URL
617 http://www.st.nmfs.noaa.gov/commercial-fisheries/commercial-landings/annual-
618 landings/index

619 Olafsen, J.A., 2001. Interactions between fish larvae and bacteria in marine aquaculture.
620 Aquaculture 200, 223–247.

621 Péres, A., Cahu, C.L., Zambonino-Infante, J.L., Gall, M.M., Quazuguel, P., 1996. Amylase
622 and trypsin responses to intake of dietary carbohydrate and protein depend on the

developmental stage in sea bass (*Dicentrarchus labrax*) larvae. Fish Physiol. Biochem. 15, 237–242.

Péres, A., Zambonino-Infante, J.L., Cahu, C.L., 1998. Dietary regulation of activities and mRNA levels of trypsin and amylase in sea bass (*Dicentrarchus labrax*) larvae. Fish Physiol. Biochem. 19, 145–152.

Pinto, W., Figueira, L., Ribeiro, L., Yúfera, M., Dinis, M.T., Aragão, C., 2010. Dietary taurine supplementation enhances metamorphosis and growth potential of *Solea senegalensis* larvae. Aquaculture 309, 159–164.

Pinto, W., Rønnestad, I., Dinis, M.T., Aragão, C., 2013. Taurine and fish development: insights for the aquaculture industry, in: Idrissi, A. El, L'Amoreaux, W.J. (Eds.), Taurine 8. Springer New York, pp. 329–34.

Reitan, K., Rainuzzo, J., Olsen, Y., 1994. Influence of lipid composition of live feed on growth, survival and pigmentation of turbot larvae. Aquac. Int. 2, 33–48.

Ribeiro, L., Zambonino-Infante, J.L., Cahu, C.L., Dinis, M.T., 1999. Development of digestive enzymes in larvae of *Solea senegalensis*, Kaup 1858. Aquaculture 179, 465–473.

Riley, K., Charles, W., Cerino, D., 2009. Development and growth of hatchery-reared larval Florida pompano (*Trachinotus carolinus*). Fish. Bull. 107, 318–328.

Rodríguez, C., Pérez, J.A., Badía, P., Izquierdo, M.S., Fernández-Palacios, H., Lorenzo Hernandez, A., 1998. The n-3 highly unsaturated fatty acids requirements of gilthead seabream (*Sparus aurata* L .) larvae when using an appropriate DHA EPA ratio in the diet. Aquaculture 169, 9–23.

Rodríguez, C., Pérez, J.A., Diaz, M., Izquierdo, M.S., Fernández-Palacios, H., Lorenzo Hernandez, A., 1997. Influence of the EPA / DHA ratio in rotifers on gilthead seabream (*Sparus aurata*) larval development. Aquaculture 150, 77–89.

Ronnestad, I., Manuel, Y., Ueberschar, B., Ribeiro, L., Sæle, Ø., Boglione, C., 2013. Feeding behaviour and digestive physiology in larval fish□: current knowledge , and gaps and bottlenecks in research. Rev. Aquac. 5, S59–S98.

Rosenlund, G., Stoss, J., Talhot, C., 1997. Co-feeding marine fish larvae with inert and live diets. Aquaculture 155, 183–191.

Sargent, J.R., Bell, G., McEvoy, L.A., Tocher, D.R., Estévez, A., 1999a. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191–199.

Sargent, J.R., Henderson, R.J., Tocher, D.R., 1989. The lipids, in: Halver, J.E. (Ed.), Fish Nutrition. Interscience Publishers, New York, pp. 153–218.

Sargent, J.R., Mcevoy, L., Estévez, A., Bell, G., Bell, M. V, Henderson, R.J., Tocher, D.R., 1999b. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179, 217–229.

Sargent, J.R., McEvoy, L.A., Bell, G., 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture 155, 117–127.

Sargent, J.R., Tocher, D.R., Bell, G., 2002. The Lipids, in: Halver, J., Hardy, R. (Eds.), Fish Nutrition. Elsevier, San Diego, pp. 181–257.

664 Schreck, C.B., Björnsson, B.T., McCormick, S.D., McGinnity, P., Stefansson, S.O., Ueda,
665 H., Donaldson, E.M., Salze, G., McLean, E., Craig, S.R., 2012. Dietary taurine enhances
666 growth and digestive enzyme activities in larval cobia. Aquaculture 362, 44–49.

667 Smith-Vaniz, W.F., 2002. Carangidae, in: Carpenter, K.E. (Ed.), The Living Marine
668 Resources of the Western Central Atlantic, Volume 3: Bony Fishes Part 2
669 (Opistognathidae to Molidae). FAO Species Identification Guide for Fishery Purposes
670 and American Society of Ichthyologists and Herpetologists Special Publication No. 5,
671 Rome, pp. 1426–1440.

672 Tocher, D.R., 2003. Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish.
673 Rev. Fish. Sci. 11, 107–184.

674 Tovar-Ramírez, D., Mazurais, D., Gatesoupe, F.-J., Quazuguel, P., Cahu, C.L., Zambonino-
675 Infante, J.L., 2010. Dietary probiotic live yeast modulates antioxidant enzyme activities
676 and gene expression of sea bass (*Dicentrarchus labrax*) larvae. Aquaculture 300, 142–
677 147.

678 Tovar-Ramírez, D., Zambonino Infante, J., Cahu, C., Gatesoupe, F.., Vázquez-Juárez, R.,
679 2004. Influence of dietary live yeast on European sea bass (*Dicentrarchus labrax*) larval
680 development. Aquaculture 234, 415–427.

681 Vagner, M., Santigosa, E., 2011. Characterization and modulation of gene expression and
682 enzymatic activity of delta-6 desaturase in teleosts: A review. Aquaculture 315, 131–
683 143.

684 Watanabe, T., 1993. Importance of Docosahexaenoic Acid in Marine Larval Fish. J. World
685 Aquac. Soc. 24, 152–161.

686 Watanabe, T., Kiron, V., 1994. Prospects in larval fish dietetics. Aquaculture 124, 223–251.

687 Weirich, C.R., Groat, D.R., Reigh, R.C., Chesney, J., Malone, R.F., 2006. Effect of Feeding
688 Strategies on Production Characteristics and Body Composition of Florida Pompano
689 Reared in Marine Recirculating Systems. N. Am. J. Aquac. 68, 330–338.

690 Wold, P.-A., Hoehne-Reitan, K., Cahu, C.L., Zambonino-Infante, J.L., Rainuzzo, J.,
691 Kjørsvik, E., 2007. Phospholipids vs. neutral lipids: Effects on digestive enzymes in
692 Atlantic cod (*Gadus morhua*) larvae. Aquaculture 272, 502–513.

693 Zambonino-Infante, J.L., Cahu, C.L., 2001. Ontogeny of the gastrointestinal tract of marine
694 fish larvae. Comp. Biochem. physiolgy Part C 130, 477–87.

695 Zambonino-Infante, J.L., Cahu, C.L., Peres, A., 1997. Partial substitution of di- and
696 tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval
697 development. J. Nutr. 127, 608–14.

698 Zambonino-Infante, J.L., Cahu, C.L., Peres, A., Quazuguel, P., Gall, M.M. Le, 1996. Sea
699 bass (*Dicentrarchus labrax*) larvae fed different Artemia rations□: growth , pancreas
700 enzymatic response and development of digestive functions. Aquaculture 139, 129–138.

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Figure headlines

Figure 1. Standard length (A) Body depth (B) and wet weight (C) of Florida pompano larvae fed three different diets. Values are mean ± SEM (n = 4 tanks, 10 larvae per tank and time point). Letters indicate significant differences between treatments and time points (Tukey test, $p<0.05$).

Figure 2: Specific activity (mU/mg protein) of trypsin and amylase in the pancreatic segment and intestinal segment of Florida pompano larvae fed different microdiets. Values are mean ± SEM (n = 4 tanks, 10 pooled larvae per tank and time point). Letters indicate significant differences between treatments and time points (Tukey test, $p<0.05$).

Figure 3. Specific activity (mU/mg protein) of alkaline phosphatase and leucine aminopeptidase in the brush border membrane (A and B), and of leucine alanine peptidase in the intestinal segment (C) of Florida pompano larvae fed different microdiets. Values are mean ± SEM (n = 4 tanks, 10 pooled larvae per tank and time point). Letters indicate significant differences between treatments and time points (Tukey test, $p<0.05$).

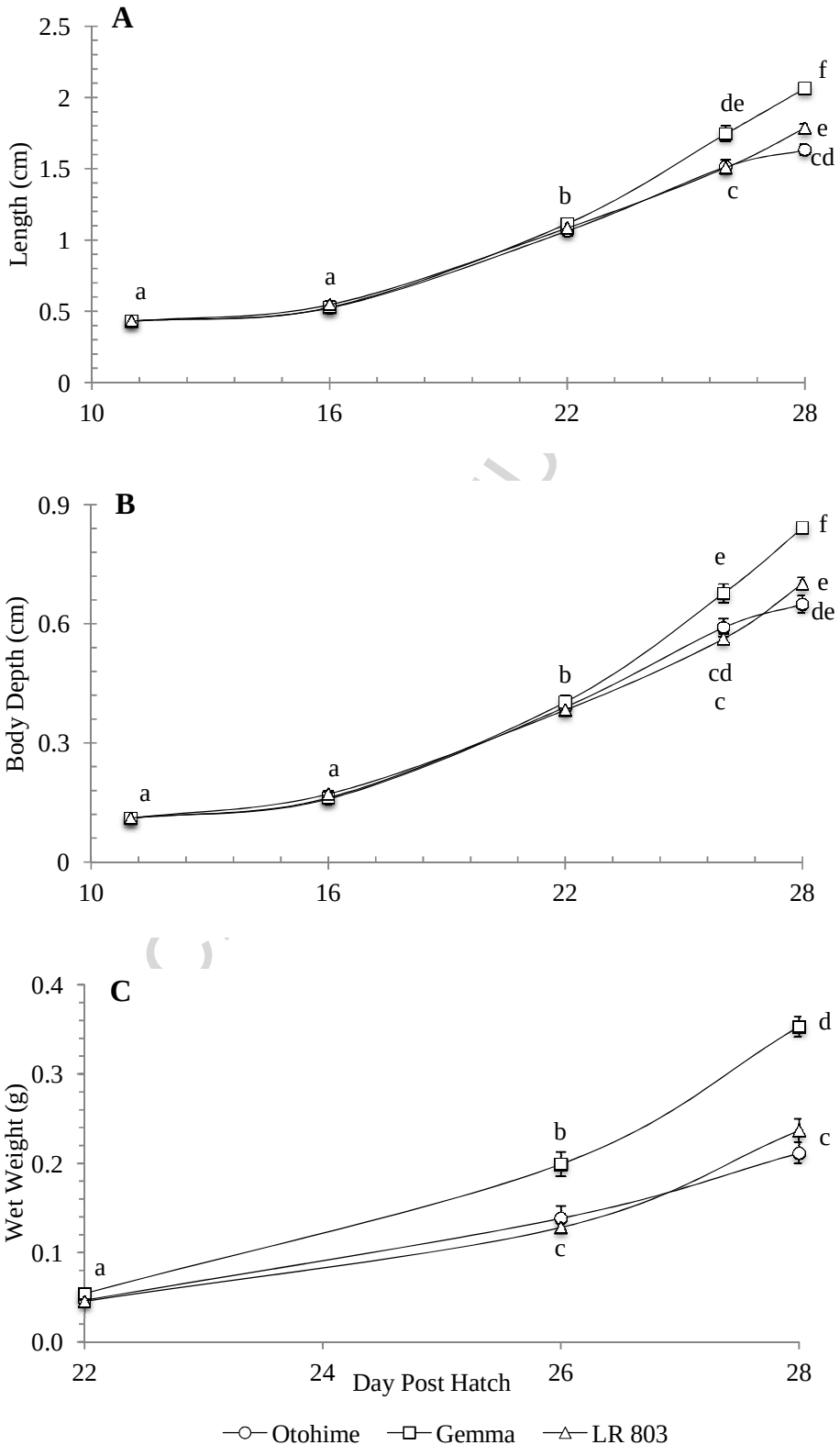
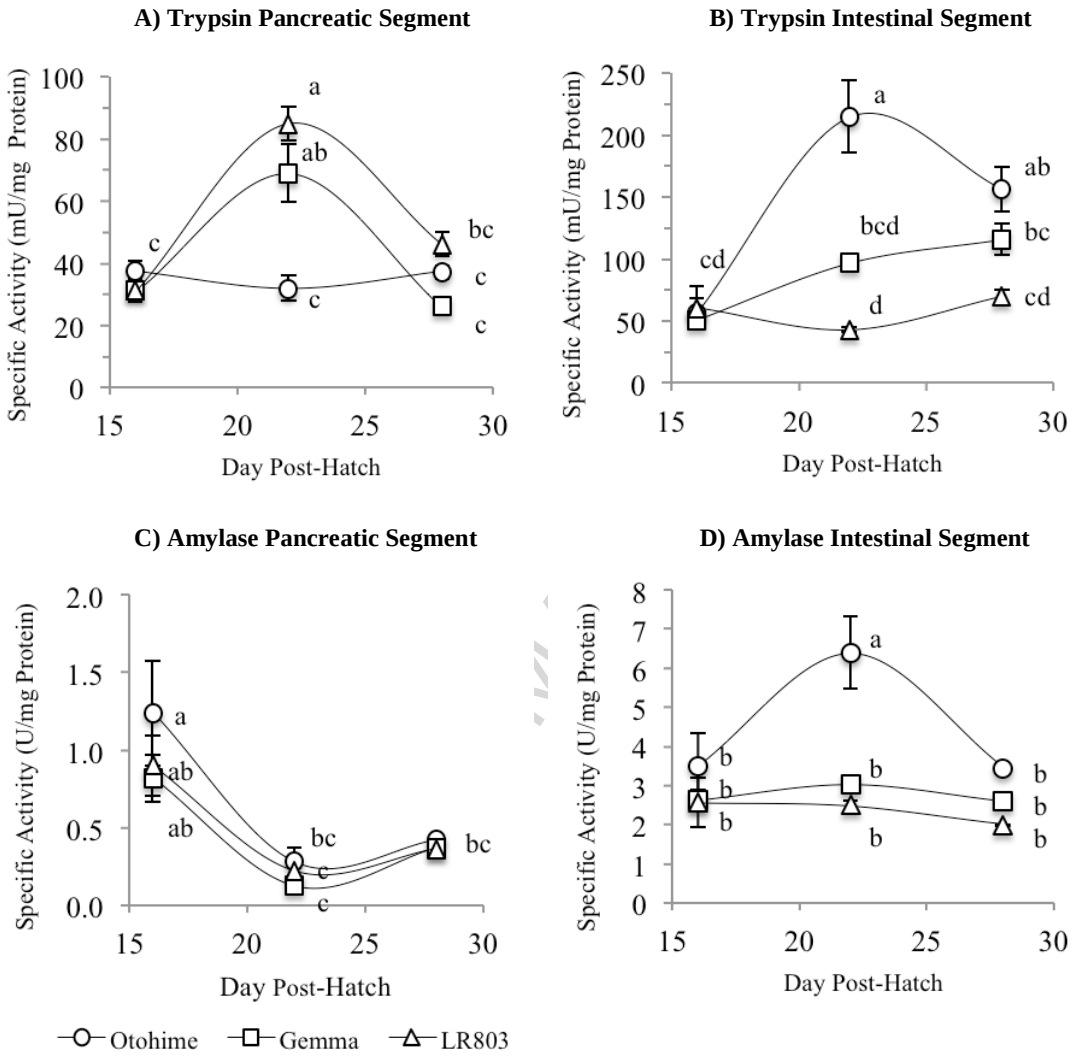


Figure 1



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728 Figure 2

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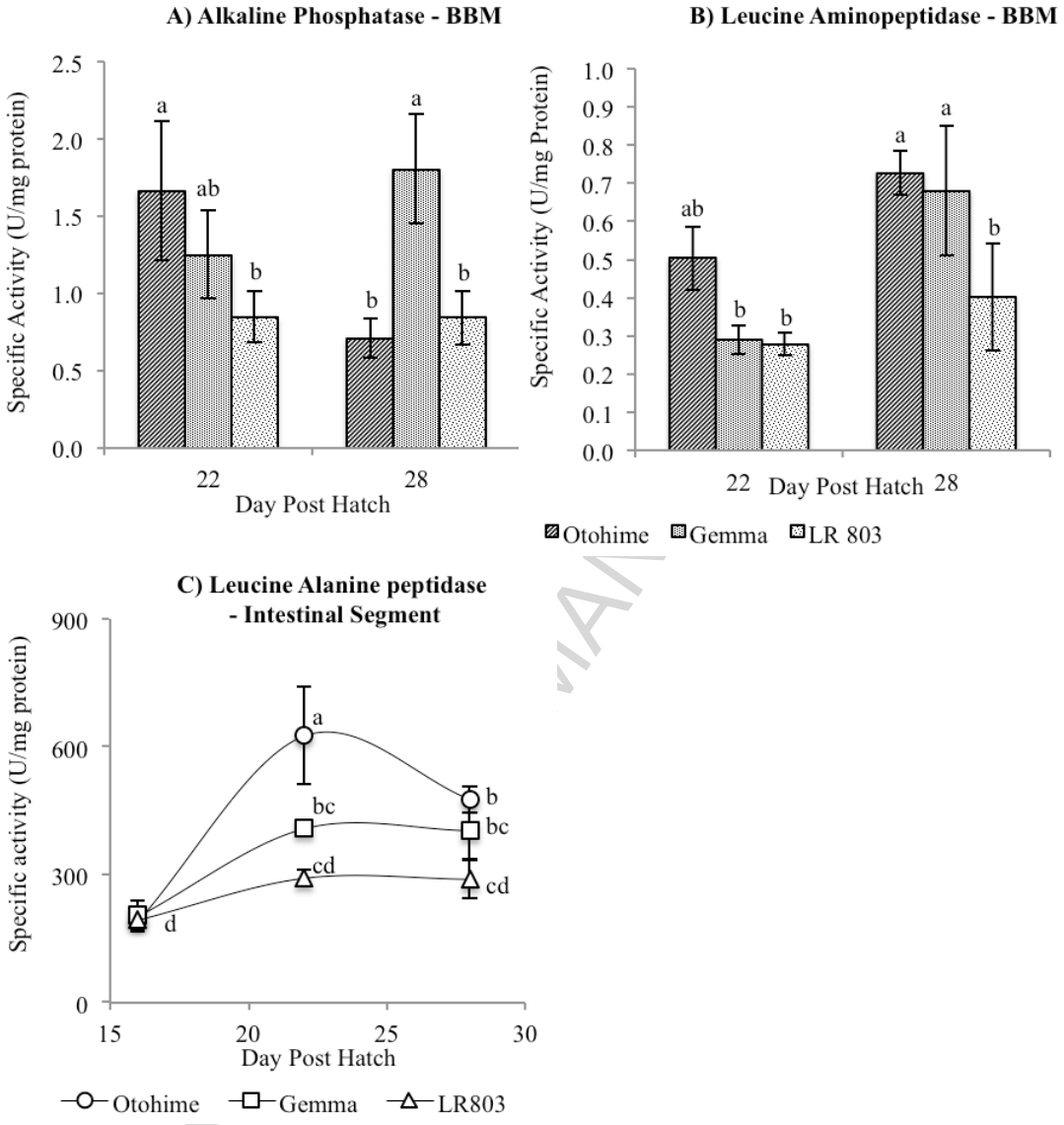

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731 Figure 3

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Highlights:

- Survival was similar for Florida pompano larvae weaned on three different diets
- Growth was enhanced by the Gemma diet compared to the two other diets
- Essential fatty acids ratios appeared more important than absolute inclusion levels
- Enzyme analyses showed the full functionality of the pancreas by 16 days post hatch

745 Table 1**.** Ingredients in the microdiets tested, as communicated by the manufacturer.

Microdiet	Ingredients
Otohime	Krill meal, fish meal, squid meal, potato starch, wheat flour, fish oil, brewers yeast, calcium phosphate, guar gum, soy lecithin, betaine, licorice plant, apple extract, wheat germ.
Gemma	Fish meal, algae, fish oils, lecithin, betaine, wheat gluten, vitamins, minerals.
LR803	Squid meal, krill meal, anchovy oil, lecithin, wheat gluten meal, vitamins, di-calcium phosphate, taurine, vitamin C, astazanthin.

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Table 2**.** Proximate analysis (% of wet weight) of the different microdiets and larvae (values ± SEM, n = 4 tanks, 10 pooled larvae per tank) at the end of the trial. Letters indicate significant differences within the same column (Tukey test; $p<0.05$).

Sample	% Lipid	% Protein	% Moisture	% Ash
Gemma diet	18.9^{b} ± 0.5	57.6^{d} ± 0.1	6.4^{b} ± 0.1	10.8^{b} ± 0.1
Otohime diet	21.2^{b} ± 1.6	52.3^{c} ± 0.1	6.4^{b} ± 0.1	12.8^{c} ± 0.1
LR803 diet	26.8^{c} ± 0.1	50.9^{c} ± 0.1	5.6^{a} ± 0.1	3.7^{a} ± 0.1
Larvae fed Gemma diet	9.9^{a} ± 0.8	16.3^{b} ± 0.3	61.4^{c} ± 0.3	10.2^{b} ± 0.2
Larvae fed Otohime diet	10.5^{a} ± 0.9	16.1^{b} ± 0.3	60.6^{c} ± 0.3	10.8^{b} ± 0.6
Larvae fed LR803 diet	10.7^{a} ± 0.6	14.5^{a} ± 0.8	61.6^{c} ± 0.2	10.8^{b} ± 0.3

Table 3. Fatty acid profile of microdiets tested and the larvae sampled at the end of the trial (values are ± SEM, n = 4 tanks, 10 pooled larvae per tank). Letters indicate significant differences within a same row (Tukey test, $p<0.05$).

Fatty acids as % of total fatty acids		Gemma diet	Otohime diet	LR803 diet	Larvae fed the Gemma diet	Larvae fed the Otohime diet	Larvae fed the LR803 diet
Saturated fatty acids							
12:0	Lauric	$0.06^{c} \pm 0.00$	$0.14^{e} \pm 0.00$	$0.11^{d} \pm 0.00$	$0.03^{a} \pm 0.00$	$0.06^{c} \pm 0.00$	$0.05^{d} \pm 0.00$
14:0	Myristic	$2.97^{ab} \pm 0.02$	$7.40^{b} \pm 0.05$	$6.83^{ab} \pm 0.04$	$2.36^{a} \pm 0.02$	$4.64^{ab} \pm 0.07$	$3.72^{ab} \pm 1.23$
15:0	Pentadecanoic	$0.28^{a} \pm 0.00$	$0.46^{d} \pm 0.00$	$0.43^{cd} \pm 0.00$	$0.35^{b} \pm 0.01$	$0.33^{b} \pm 0.01$	$0.42^{c} \pm 0.00$
16:0	Palmitic	$17.93^{a} \pm 0.06$	$18.34^{a} \pm 0.05$	$19.94^{b} \pm 0.07$	$21.25^{c} \pm 0.17$	$20.77^{bc} \pm 0.28$	$18.87^{a} \pm 0.28$
17:0	Heptadecanoic	$0.23^{a} \pm 0.00$	$0.23^{a} \pm 0.00$	$0.33^{c} \pm 0.00$	$0.28^{b} \pm 0.01$	$0.25^{ab} \pm 0.01$	$0.36^{d} \pm 0.01$
18:0	Stearic	$3.16^{a} \pm 0.00$	$3.09^{a} \pm 0.01$	$3.54^{a} \pm 0.01$	$5.55^{bc} \pm 0.20$	$5.90^{c} \pm 0.31$	$4.80^{b} \pm 0.11$
Total		$24.62^{a} \pm 0.08$	$29.67^{bc} \pm 0.1$	$31.17^{bc} \pm 0.1$	$29.81^{bc} \pm 0.4$	$31.95^{c} \pm 0.6$	$28.22^{b} \pm 1.3$
Mono-unsaturated fatty acids							
15:1	cis-10 pentadecanoic	$0.04^{a} \pm 0.00$	$0.09^{c} \pm 0.00$	$0.07^{b} \pm 0.00$	$0.04^{a} \pm 0.00$	$0.07^{b} \pm 0.00$	$0.07^{b} \pm 0.01$
16:1n7	Palmitoleic	$2.19^{a} \pm 0.01$	$5.26^{d} \pm 0.02$	$6.28^{e} \pm 0.02$	$2.40^{b} \pm 0.02$	$4.84^{c} \pm 0.06$	$6.38^{e} \pm 0.06$
18:1n9	Oleic	$11.72^{b} \pm 0.02$	$12.25^{b} \pm 0.03$	$9.92^{a} \pm 0.02$	$13.92^{c} \pm 0.27$	$15.59^{d} \pm 0.51$	$11.24^{b} \pm 0.07$
18:1n7	Vaccenic	$1.47^{a} \pm 0.01$	$4.21^{c} \pm 0.02$	$3.11^{b} \pm 0.02$	$1.42^{a} \pm 0.06$	$4.01^{c} \pm 0.17$	$3.46^{b} \pm 0.03$
20:1n9	Eicosenoic acid	$3.23^{b} \pm 0.00$	$5.37^{c} \pm 0.05$	$0.98^{a} \pm 0.01$	$3.53^{b} \pm 0.04$	$3.75^{b} \pm 0.26$	$0.68^{a} \pm 0.18$
Total		$18.66^{a} \pm 0.02$	$27.18^{d} \pm 0.06$	$20.36^{b} \pm 0.06$	$21.30^{c} \pm 0.2$	$28.26^{e} \pm 0.1$	$21.84^{c} \pm 0.15$
Poly-unsaturated fatty acids							
16:2n4	Hexadecadienoic	$0.45^{b} \pm 0.00$	$0.75^{d} \pm 0.01$	$1.42^{f} \pm 0.00$	$0.38^{a} \pm 0.01$	$0.64^{c} \pm 0.01$	$1.03^{e} \pm 0.01$
16:3n4	Hexadecatrienoic	$0.29^{a} \pm 0.00$	$0.5^{c} \pm 0.00$	$0.99^{d} \pm 0.00$	$0.27^{b} \pm 0.01$	$0.46^{c} \pm 0.02$	$0.74^{e} \pm 0.01$
18:2n6	Linoleic (LA)	$30.40^{d} \pm 0.05$	$4.54^{a} \pm 0.02$	$19.84^{b} \pm 0.04$	$24.40^{c} \pm 0.32$	$4.51^{a} \pm 0.13$	$20.06^{b} \pm 0.06$
18:3n6	γ-linolenic	$0.03^{a} \pm 0.01$	$0.14^{b} \pm 0.00$	$0.21^{b} \pm 0.00$	$0.06^{a} \pm 0.00$	$0.17^{b} \pm 0.00$	$0.15^{b} \pm 0.02$
18:3n4	Octadecatrienoic	$0.07^{a} \pm 0.00$	$0.16^{b} \pm 0.00$	$0.27^{c} \pm 0.00$	$0.12^{a} \pm 0.01$	$0.17^{b} \pm 0.01$	$0.34^{c} \pm 0.02$
18:3n3	α -linolenic	$3.59^{e} \pm 0.01$	$1.18^{b} \pm 0.01$	$3.08^{d} \pm 0.01$	$2.81^{c} \pm 0.05$	$1.07^{a} \pm 0.01$	$2.77^{c} \pm 0.02$
18:4n3	Stearidonic	$1.63^{b} \pm 0.01$	$2.53^{d} \pm 0.01$	$1.48^{b} \pm 0.01$	$0.92^{a} \pm 0.03$	$1.73^{bc} \pm 0.02$	$0.83^{a} \pm 0.21$
20:2n6	Eicosadienoic	$0.15^{a} \pm 0.00$	$0.15^{a} \pm 0.00$	$0.09^{a} \pm 0.00$	$0.78^{b} \pm 0.04$	$0.22^{a} \pm 0.01$	$0.30^{a} \pm 0.10$
20:3n6	Eicosatrienoic	$0.05^{a} \pm 0.00$	$0.09^{b} \pm 0.00$	$0.20^{d} \pm 0.02$	$0.12^{bc} \pm 0.01$	$0.14^{c} \pm 0.01$	$0.21^{d} \pm 0.01$
20:4n6	Arachidonic (ARA)	$0.34^{a} \pm 0.00$	$0.54^{bc} \pm 0.01$	$0.55^{bc} \pm 0.00$	$0.30^{a} \pm 0.01$	$0.52^{b} \pm 0.01$	$0.59^{c} \pm 0.01$
20:3n3	Eicosatrienoic	$0.10^{a} \pm 0.00$	$0.16^{b} \pm 0.00$	$0.16^{b} \pm 0.00$	$0.26^{d} \pm 0.01$	$0.18^{b} \pm 0.01$	$0.21^{c} \pm 0.01$
20:4n3	Eicosatetraenoic	$0.30^{a} \pm 0.00$	$0.56^{b} \pm 0.01$	$0.56^{b} \pm 0.03$	$0.56^{b} \pm 0.02$	$1.03^{d} \pm 0.03$	$0.87^{c} \pm 0.03$
20:5n3	Eicosapentaenoic (EPA)	$4.12^{b} \pm 0.03$	$10.79^{f} \pm 0.05$	$9.01^{e} \pm 0.08$	$2.21^{a} \pm 0.04$	$7.28^{d} \pm 0.08$	$6.10^{c} \pm 0.13$
22:5n6	ω6 Docosapentaenoic	$0.17^{c} \pm 0.00$	$0.10^{a} \pm 0.01$	$0.16^{bc} \pm 0.00$	$0.18^{c} \pm 0.01$	$0.14^{b} \pm 0.00$	$0.20^{d} \pm 0.00$
22:5n3	ω3 Docosapentanoic	$0.59^{a} \pm 0.01$	$0.95^{b} \pm 0.00$	$1.18^{c} \pm 0.00$	$1.01^{b} \pm 0.04$	$2.58^{d} \pm 0.06$	$2.55^{d} \pm 0.05$
22:6n3	Docosahexaenoic (DHA)	$7.32^{b} \pm 0.05$	$9.57^{c} \pm 0.01$	$5.36^{a} \pm 0.01$	$7.37^{b} \pm 0.11$	$11.22^{d} \pm 0.29$	$7.12^{b} \pm 0.13$
Total		$49.60^{d} \pm 0.09$	$32.69^{a} \pm 0.04$	$44.54^{c} \pm 0.1$	$41.75^{b} \pm 0.5$	$32.06^{a} \pm 0.34$	$44.06^{c} \pm 0.37$
Total ω3		$17.66^{b} \pm 0.09$	$25.73^{d} \pm 0.06$	$20.82^{c} \pm 0.09$	$15.14^{a} \pm 0.2$	$25.09^{d} \pm 0.35$	$20.44^{c} \pm 0.23$
Total ω6		$31.13^{d} \pm 0.04$	$5.55^{a} \pm 0.01$	$21.04^{b} \pm 0.05$	$25.84^{c} \pm 0.36$	$5.70^{a} \pm 0.12$	$21.51^{b} \pm 0.15$
ω3/ω6 ratio		$0.57^{a} \pm 0.00$	$4.63^{c} \pm 0.02$	$0.99^{b} \pm 0.00$	$0.59^{a} \pm 0.01$	$4.40^{c} \pm 0.13$	$0.95^{b} \pm 0.01$
ARA/EPA ratio		$0.08^{d} \pm 0.00$	$0.05^{a} \pm 0.00$	$0.06^{b} \pm 0.00$	$0.14^{f} \pm 0.00$	$0.07^{c} \pm 0.00$	$0.1^{e} \pm 0.00$
DHA/EPA ratio		$1.78^{e} \pm 0.01$	$0.89^{b} \pm 0.00$	$0.59^{a} \pm 0.01$	$3.33^{f} \pm 0.08$	$1.54^{d} \pm 0.05$	$1.17^{c} \pm 0.03$