Food intake and absorption are affected by dietary lipid level and lipid source in seabream (*Sparus aurata* L.) larvae

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Abstract

Marine larval nutrition studies have classically focused on essential fatty acid (EFA) requirements and very little is known regarding the effect of total lipid level or lipid source on food ingestion and absorption, which are important factors determining growth. In the present work two experiments analysed food intake and nutrient absorption in seabream larvae in response to two dietary lipid levels (17-18% and 25-28%). The first experiment tested *Artemia* enriched on two levels of a fish oil emulsion (higher and lower – HF and LF,

respectively), while in the second experiment larvae were co-fed *Artemia* enriched on one of two levels of a soybean oil emulsion and a microdiet (MD) containing one of two levels of soybean oil as the main lipid source (higher and lower – HS and LS, respectively). Food intake and nutrient absorption were determined by performing radioactive trials using *Artemia* radiolabelled with [1-^{14}C] oleic acid in the first experiment (at 26 and 33 days after hatching - DAH) and MD labelled with [1-^{14}C] oleic acid or glycerol tri[1-^{14}C]oleate (31 and 32 DAH) in the second experiment. The dietary treatments did not induce significant differences in larval dry weight in the first experiment, while food intake was significantly higher and nutrient absorption significantly lower in larvae fed the HF diet, compared to the LF treatment. In the second experiment, a significantly higher dry weight was achieved by larvae fed on the LS diet, which was also significantly more ingested and absorbed. The fish oil experiment supports the hypothesis that a higher food intake may cause a decrease in nutrient absorption efficiency, possibly through a faster gut transit, but in the soybean oil experiment total absorption appears to have simply reflected food intake. The results show that dietary lipid level significantly affects larval food intake and absorption efficiency but the effect was dependent on lipid source, suggesting that dietary fatty acid (FA) composition might be a more determinant factor than total lipid level. Food intake was apparently not regulated to meet a requirement for EFA. Lipid source or FA composition may regulate food intake through pre- or post-absorptive mechanisms, such as through effects on palatability, digestibility and stimulation of neuroendocrine pathways.

Keywords: assimilation; fatty acid composition; fish larvae; ingestion; lipid nutrition

1. Introduction

The larval rearing of most marine fish species is still based on live prey, mainly rotifers and *Artemia*. Although these organisms are not optimal nutritionally, particularly in their essential fatty acid (EFA) content, they are widespread since they are easy to culture in large quantities and are readily ingested by the larvae. To overcome their nutritional deficiency, enrichment products and protocols have been developed over the last decades to enrich these zooplankton with sufficient levels of EFA (e.g., Rodríguez et al., 1996; Rainuzzo et al., 1997; Han et al., 2000). In order to meet the EFA requirements of the larvae and thus achieve good growth, survival and larval quality, the general approach is to simulate the biochemical composition of the larva's natural diet (marine plankton), which is particularly rich in (n-3) polyunsaturated fatty acids (PUFA). However, the natural marine phytoplankton and zooplankton is composed mostly of (n-3) PUFA-rich phospholipids, while many of the enrichment methodologies use lipid sources rich in triacylglycerols, which have a lower (n-3) PUFA content compared to polar lipids (Sargent et al., 1989). Therefore, in order to supply high levels of EFA, rotifers and *Artemia* fed to larvae may have an excessive total lipid content having a high neutral lipid composition. In fact, quantitative imbalances in the macronutrient composition of the diet, particularly neutral lipid excess, has been hypothesized to lead to lower larval digestion and absorption ability (Kjørvik et al., 1991; Hoehne, 1999; Olsen et al., 2000; Morais et al., in press) resulting in poor growth and larval performance. In a previous study conducted with Senegalese sole (*Solea senegalensis*) larvae it has been shown that a diet with a higher neutral lipid content may lead to a reduced fatty acid (FA) absorption efficiency, possibly related to the accumulation of lipid droplets within the gut enterocytes (Morais et al., in press). However, other factors may also account for a lower growth, which may be obtained when rearing larvae on high lipid diets. A lower ingestion of high lipid diets has been shown to occur in several juvenile and adult fish species, thus

resulting in a lower intake of protein. Nevertheless, in most cases, growth is similar to that obtained with a lower lipid diet, as a result of a protein-sparing effect (Lee and Putnam, 1973; Marais and Kissil, 1979; Boujard and Médale, 1994; Santinha et al., 1999; Ogata and Shearer, 2000; Yamamoto et al., 2000; Sæther and Jobling, 2001; Gélineau et al., 2001, 2002; Boujard et al., 2004; Skalli et al., 2004). The results obtained in another study conducted with Senegalese sole larvae (Morais et al., 2005) indicated that the protein and amino acid absorption may be slower in larvae fed a higher neutral lipid diet, which may potentially result in an overall lower food (and protein) intake. In addition, Gawlicka et al. (2002), working with white sturgeon (*Acipenser transmontanus*) larvae, found a lower growth in larvae fed high lipid diets and suggested that this might have been caused by a reduced food intake. The regulation of food intake according to the lipid level and FA composition of the diet has not yet been examined in fish larvae and will be the focus of the experiments reported here.

Two experiments were conducted in order to study food intake and nutrient absorption in seabream (*Sparus aurata* L.) larvae in response to feeding on diets quantitatively different in neutral lipid level but also qualitatively different in their FA composition. In the first experiment larval performance was tested when the fish were fed *Artemia* enriched on one of two levels of a fish oil emulsion, while in the second experiment larvae were co-fed *Artemia* enriched on one of two levels of soybean oil emulsion together with a microdiet (MD) containing soybean oil, at a corresponding lipid level. The oils used in the experiments are a rich source of neutral lipid (triacylglycerol) and not of phospholipids. After a period of feeding on the experimental diets, trials were conducted using ^{14}C-markers in order to quantify food ingestion and absorption.

2. Materials and methods

2.1. Larval rearing

All the experiments were conducted at the National Center for Mariculture, Eilat, using seabream (*Sparus aurata* L.) larvae that originated from spawns of captive broodstock maintained at the ARDAG Red Sea Mariculture Ltd. (Eilat, Israel). Larvae were reared according to Tandler et al. (1987), in 400 L conical tanks supplied with 10 μm filtered and UV-treated seawater (25 g L^{-1}), with a flow rate of one tank exchange per day and a temperature that was raised gradually from 19 to 24 ºC. At 20-21 days after hatching (DAH) larvae were transferred to 27 L aquaria, supplied with 10 μm filtered and UV-treated seawater (25 g L^{-1}), and stocked with 500 larvae each. During the acclimation period to the aquaria system (2-3 days), all larvae were fed on *Artemia* (A&P Holdings Inc., Salt Lake City, Utah, USA) enriched with AquaGrow Advantage (Advanced Bio Nutrition Corp., Maryland, USA), according to manufacturer's instructions. Larvae were fed twice daily, with 1 metanauplii mL^{-1} in the morning and in the afternoon. Green water was maintained during the acclimation period through the addition of 1 L of concentrated fresh *Nannochloropsis* spp culture, in the morning.

2.2. Enrichment of the experimental Artemia

The emulsions for lipid enrichment were prepared with either fish oil (Matmor Feeds, Israel) or commercial soybean oil. In each 100 g of emulsion, 8 g of Phospholipon 80H (Phospholipid GmbH, Köin, Germany) were mixed in 88 g of oil at *ca* 50 ºC (5 ºC above the phase transition temperature), using a magnetic stirrer. After cooling, 2 g of Alginic acid, sodium salt (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 1 g of vitamin C (Stay C, Hoffman LaRoche, Switzerland) and 1 g of vitamin E (BASF, Germany) were added and mixed. *Artemia* was hatched according to Van Stappen (1996) and, at instar II, was enriched

in 20 L cylindrical-conical tanks (150 nauplii mL^{-1}, 25 g L^{-1}), at 28 ºC (±1, S.D.) and with strong aeration. The fish and soybean oil emulsions were blended with water (1:200 w/w) in a high speed blender and enrichment was conducted over a period of 16 h. In the high lipid treatments (HF and HS), the first dose (0.4 g L^{-1}) was added at the start of enrichment and a second dose (0.2 g L^{-1}) was added 8 h later, while in the low lipid treatments (LF and LS), a single dose of 0.04 g L^{-1} was added at the start of enrichment. In the first (fish oil) experiment, 50 mg liposomes L^{-1} were added to the enrichment tanks of both HF and LF treatments, at the start of the enrichment period (same conditions as when radiolabeling the *Artemia* for the radioactive trials – see below).

2.3. Liposome preparation

Liposomes were prepared by evaporating a mixture containing hydrogenated soy lecithin (Epikuron 200 SH, Lucas Meyer GmbH, Hamburg, Germany; 100 mg mL^{-1} chloroform), cholesterol (Riedel-deHaën, Seelze, Germany; 40 mg mL^{-1} chloroform) and chloroform (1:1:50 v/v) in a rotary evaporator Rotavapor R110 (Büchi Laboratoriums-Technik AG, Schweiz, Switzerland), at 60 ºC, until a film was formed on the wall of the round flask. The flask was then kept in a dessicator, under vacuum, for *ca* 30 min. After adding distilled water (1:5 v/v, lecithin:water) and glass beads, the flask was slowly rotated in the water bath (60 ºC) of the rotary evaporator during *ca* 1 h, to form multi-lamellar vesicles (MLV) or liposomes.

2.4. Experimental system for the radioactive trials

The radioactive trials were conducted in an experimental system consisting of thirty 1 L glass beakers, located in the radioisotope room. Each beaker was supplied with 900 mL of filtered seawater (25 g L^{-1}) at 22 ºC, which entered at the bottom and exited near the surface of the beaker through a 350 μm mesh. The beakers were stocked with 25-30 larvae in the evening before the study and the larvae were acclimated overnight and unfed until the food

intake experiment the following morning. In the morning of the trials of the first experiment, 2700 metanauplii (3 *Artemia* mL^{-1}) were fed to each beaker, after thoroughly washing and counting the radiolabelled *Artemia*. In the second (MD co-feeding) experiment the radioactive trials were conducted using ^{14}C-labelled MD fed in an amount (67.5 mg per beaker) equal to the weight equivalent (2.5 µg per metanauplii) of the 2700 *Artemia* fed to each beaker.

2.5. First experiment: Effect of Artemia enriched with high and low levels of fish oil emulsion

Seabream larvae were fed from 23 DAH on *Artemia* enriched, as described above, on a higher or lower dose of fish oil emulsion (HF and LF, respectively). Each treatment was tested in five 27 L aquaria and the water temperature varied from 20 to 23 ºC during the experimental period. Larvae were fed 2-4 *Artemia* mL^{-1} day^{-1} in two daily rations. At 26 DAH (short-term effect; 3 days on the experimental diets) and 33 DAH (long-term effect; 10 days on the experimental diets), radioactive trials were conducted in order to analyse the intake of radiolabelled *Artemia* and the absorption efficiency of the labeled nutrients. At 26 and 34 DAH, samples of pooled larvae were taken from each aquaria, washed in distilled water and dried at *ca* 60 ºC for 24 h for dry weight (DW) determination. At 34 DAH, 20 larvae per aquaria were also sampled, washed in distilled water and frozen at –30 ºC, for FA analysis.

Food intake and label absorption were studied using a cold-chase approach where larvae were allowed to feed on radiolabelled *Artemia* having different total lipid levels during a period of 2 h (established previously as time to gut fullness), after which a group of larvae were sampled. After sampling, the remaining radioactive *Artemia* and much of the water was siphoned and the larvae resuspended in fresh seawater. Approximately 30 min later (2:30 h after start feeding) non-labelled treatment *Artemia* was added. The larvae were then sampled at 3, 3:30, 4 and 5 h after start feeding on the radiolabelled *Artemia*. This approach assumes that the subsequent rate of clearance of radioactivity in the gut is influenced by food intake rate as well as absorption from the lumen. The sampled larvae were later dissected under a

binocular microscope and the digestive tract was separated from the body, in order to quantify separately the radioactivity in each of these compartments. Pooled guts from each time point replicate were transferred to 5 mL plastic scintillation vials (Packard Bioscience, Gronungen, The Netherlands) while the pooled bodies were placed in 20 mL glass scintillation vials (Packard Bioscience). The guts and bodies were separately solubilized with 300 and 700 µL of Soluene (Packard Bioscience), respectively, at 60 ºC during 2 h and, after cooling, 4 mL (guts) and 10 mL (bodies) of scintillation liquid (Ultima Gold, Packard Bioscience) were added. Radioactivity (disintegrations per minute – dpm) was counted in a TRI-CARB 2100 TR (Packard Bioscience) liquid scintillation analyzer.

Radiolabelled liposomes were prepared as described above, except that [1-^{14}C] oleic acid (Amersham Pharmacia Biotech Ltd, Little Chalfont, Bucks, UK) (1µCi mg^{-1} liposomes) was added to the chloroform mixture, before evaporation. *Artemia* was labeled by introducing ^{14}C-radiolabelled liposomes into the enrichment media (50 mg liposomes L^{-1}), at the start of the 16 h enrichment period. *Artemia* radiolabeling was conducted under a fume hood, in 400 mL beakers, and under the same conditions as described above for non-radioactive enrichment. After 16 h, the radiolabelled *Artemia* was filtered and washed on a 150 µm mesh filter and ressuspended in 400 mL of fresh seawater. Four counted samples of radiolabelled *Artemia* were transferred to filter paper, washed with distilled water and the filter was placed on the bottom of a 5 mL plastic scintillation vial. The *Artemia* were solubilized with 200 µL of Soluene and 4 mL of scintillation cocktail was added. Total radioactivity per sample was counted and the amount of radioactivity per *Artemia* metanauplii was then calculated.

2.6. Second experiment: Effect of co-feeding microdiet (MD) and Artemia enriched with high and low levels of soybean oil emulsion

In this experiment larvae were co-fed enriched *Artemia* and MD (50:50), from 23 to 33 DAH, in five replicate 27 L aquaria. Two gelatine-encapsulated MD (closed formula) were

formulated to contain two total lipid levels (15% and 25%) but having the same absolute levels of n-3 HUFA. This was carried out by using different amounts of soybean oil but including a constant level (mg g^{-1} DW diet) of EPAX 1050 TG (Pronova Biocare AS, Ălesund, Norway), the sole lipid source of EFA. The lower lipid MD was co-fed with *Artemia* enriched with a low dose of soybean oil emulsion (LS), while the higher lipid MD was co-fed with *Artemia* enriched with a high dose of soybean oil emulsion (HS). At 23 DAH, when the larvae started feeding on the experimental treatments, the daily ration was calculated on the basis of 3 metanauplii mL^{-1} day^{-1}. Larvae began feeding on 80% of *Artemia* ration, which was reduced to 70% on 24 DAH and finally to 50% from 25 DAH. The amount of food given to the larvae was increased 10% daily and feeding frequency was twice for *Artemia* and four times daily for the MD. Water temperature varied between 21 and 24 ºC.

At 31 and 32 DAH, two cold-chase trials were conducted separately in which the larvae were fed either high or low lipid MD (corresponding to the dietary treatment previously fed) that had been radiolabelled with triolein, glycerol tri[1-^{14}C]oleate or [1-^{14}C] oleic acid (Amersham Pharmacia Biotech Ltd, Little Chalfont, Bucks, UK), respectively. This allowed the examination of ingestion and absorption efficiency of different lipid class labels as a function of dietary total lipid. The radioactive concentrations (dpm μg^{-1} MD) of the diets were determined by liquid scintillation counting, after solubilizing 10-20 mg MD in 100 µL of Soluene (60 ºC during 2 h) and adding 4 mL of scintillation cocktail. At 34 DAH the DW of the larvae was determined and 10 larvae per aquaria were sampled for FA analysis, as described for the first experiment.

2.7. Artemia proximate composition and FA analysis

Samples of the enriched *Artemia* treatments were collected and dry matter was calculated from weight loss after drying at 100 ºC for 24 h. Crude protein content of the dried and ground samples was determined by Kjeldahl (AOAC, 1980) and the total lipid extracted

(Folch et al., 1957) was measured gravimetrically. Ash content was calculated from weight loss after incineration of the samples in a muffle furnace for 24 h at 550 ºC (AOAC, 1980).

For FA analysis of the enriched *Artemia*, MD and larvae, samples were lyophilized and total lipid was extracted according to Folch et al. (1957). The total lipid in the larvae was quantified gravimetrically (Sartorius BP 210 S; ± 0.1 mg) and then transmethylated to their corresponding fatty acid methyl esters (FAME) by acidified methylation overnight at 50 ºC in 1% H_2SO_4 in methanol (v/v). After purification on 20 x 20-cm TLC plates pre-coated with silica gel (G60, Merck) using the solvent system hexane/diethyl ether (1:1, v/v), the resulting FAME were concentrated in hexane (2 mg FAME mL^{-1} hexane). The samples were injected onto a TraceGC ultra (Thermo Finnigan, Italy) gas chromatograph equipped with a 30 m x .32 mm Stabilwax capillary column (Restek, USA) that used hydrogen as a carrier gas. The temperature of the column was raised from 50-180 at 40 ºC min^{-1}, from 180-225 at 3 ºC min^{-1}, with a final hold at 225 ºC for 13 min. FAME were identified by known purified standards and quantified using a response factor to an internal nonadecanoic acid standard (19:0; Sigma-Aldrich, St. Louis, MO, USA).

2.8. Statistical analysis

The results obtained from the proximate composition analysis, main FA classes and EFA of the treatment *Artemia* and MD, as well as larval growth, food intake and label absorption in larvae submitted to the different dietary treatments were compared by one-way analysis of variance (ANOVA). All percent data was arcsine-transformed and, after performing the Bartlett's test to check the assumption of homogeneity of variance, data was analysed at a significance level of 0.05 (Zar, 1996). When significant differences were found, the Tukey HSD multiple range test was performed, using the software Statistica 6 (StatSoft Inc., Tulsa, USA). All data is given as mean values and standard deviations (S.D.).

3. Results

3.1. Proximate and FA composition of the dietary treatments and reared larvae

The proximate composition analysis of the *Artemia* used in the two experiments (Table 1) revealed a significantly higher total lipid level and a corresponding significantly lower total protein content in the high lipid treatments (HF and HS). In terms of ash and dry matter content, significant differences were found only in the first experiment, where a lower ash content and a higher dry matter content were measured in the HF treatment.

The FA analysis of the experimental *Artemia* used in the first experiment (Table 2) revealed, as expected, a significantly higher total FAME content (mg g^{-1} DW) of the HF diet, compared to LF *Artemia*. This resulted from the higher levels achieved in most analysed FA, with significantly higher absolute amounts being measured for total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA. The HF *Artemia* also contained significantly larger amounts of the EFA arachidonic acid (ARA; 20:4n6), eicosapentaenoic acid (EPA; 20:5n3) and docosahexaenoic acid (DHA; 22:6n3). In the second experiment (Table 2), the *Artemia* enrichment with soybean oil emulsion produced similar results in terms of total FAME and FA classes but in this experiment the variability of the analysed *Artemia* samples was too high to achieve statistic power to detect eventual significant differences. As for the formulated MD, the diet containing a higher lipid level tended to have, as expected, higher amounts of total FAME and significantly higher levels of SFA, MUFA and n-6 PUFA (including ARA), but not of n-3 PUFA. The MD were formulated to contain similar absolute amounts of the EFA DHA and EPA, which was accomplished. In addition, as soybean oil does not contain EFA, the amounts of ARA and EPA (DHA was not detected) did not differ significantly between the HS and LS *Artemia*. However, if the data is expressed on a relative basis (% total FAME; not shown), the higher lipid *Artemia* and MD presented a lower percentage of EFA.

At the end of the first experiment, larvae fed on HF *Artemia* presented a slightly higher, but non significant, total lipid content (17.2 ± 0.8; n=5) than larvae fed on the LF diet (15.4 ± 2.1; n=5). In terms of FA composition (Table 3), larvae submitted to the HF treatment showed a significantly higher total FAME content, as a result of the significant larger amount of n-3 PUFA, particularly ARA, EPA and DHA. No significant differences were found in total SFA, MUFA and n-6 PUFA between larvae submitted to the two fish oil treatments. In the second experiment, when larvae were co-fed either HS or LS *Artemia* and MD, the larvae on the high lipid treatment presented a significantly higher total lipid level (20.7 ± 1.1 vs. 16.0 ± 1.1; n=5) and accumulated a significantly larger amount of most of the analysed FA, particularly of 18:2n-6 and 18:1n-9 (reflecting dietary composition). No significant differences were found in n-3 PUFA, ARA and EPA but the larvae on the lower lipid diet retained a significantly larger amount of DHA.

3.2. Growth, food intake and nutrient absorption in larvae fed Artemia enriched with high and low levels of fish oil emulsion

Feeding seabream larvae on either a HF or LF diet did not result in significant differences in larval dry weight, either at 26 DAH (3 days of feeding on the experimental diets) or at 34 DAH (11 days of feeding on the experimental diets) (Figs. 1A and 1B).

Cold-chase radioactive trials were conducted at 26 and 33 DAH (Fig. 2), in order to quantify the intake and absorption of radiolabelled *Artemia*, enriched in the same manner as the treatment *Artemia*. In both trials a significantly higher amount of radiolabelled *Artemia* was ingested by larvae feeding on the HF diet, as can be seen by the significantly larger amount of *Artemia* in the larval gut at 2 h (before starting the cold-chase) and by the more rapid decline in the gut of these larvae during cold-chase. At 5 h after start feeding on the labeled *Artemia* (or 2:30 h after starting the cold-chase) all label is expected to have been absorbed into the gut tissues (i.e., is no longer in the lumen). Therefore, the results obtained at

5 h in the gut compartment indicate a higher nutrient absorption in the LF treatment (although only significant at 33 DAH). Similarly, a higher absorption into the body was observed in the LF larvae that was significant at both ages and in all time points analyzed.

3.3. Growth, food intake and FA absorption in larvae co-fed MD and Artemia enriched with high and low levels of soybean oil emulsion

When larvae were co-fed either a high lipid (soybean-based) MD + HS *Artemia* or a lower lipid MD + LS *Artemia* during a period of 11 days, significant differences were found in larval dry weight at 34 DAH, with a lower lipid diet (LS) resulting in significantly larger larvae (Fig. 1C).

The results from the cold-chase trial performed at 31 DAH with triolein-labeled MDs (Fig. 3) showed that the larvae had a significantly higher food intake of the lower lipid diet (LS), as can be seen by comparing the amount of labeled MD in the gut at 2 h. In terms of absorption into the body, a significantly larger amount of label was incorporated into the body tissues of larvae fed the LS diet. At 32 DAH a similar radioactive trial was performed, this time using oleic acid-labeled MDs (Fig. 3). Similarly to the results obtained with the triolein-labeled MDs, a larger food intake and higher label absorption were measured in larvae feeding on the LS diet. A significantly higher amount of label was measured in the gut of LS-fed larvae at all sampling points and, in this case, a decrease in label after the cold-chase was noted (particularly in the LS treatment). At 5 h after start feeding on the labeled MD, a significantly larger amount of label was found in the LS treatment, indicating a larger absorption into the gut enterocytes. As for the absorption into the body, it was also significantly larger at all times (except at 2 h) in the LS treatment. In general, larger amounts of label were absorbed into the gut and body tissues by the larvae fed on oleic acid-labeled MDs, compared to larvae fed on triolein-labeled MDs.

4. Discussion

The voluntary regulation of food intake based on dietary digestible energy content has been demonstrated in many studies performed with several juvenile and adult fish species (Lee and Putnam, 1973; Marais and Kissil, 1979; Boujard and Médale, 1994; Santinha et al., 1999; Ogata and Shearer, 2000; Yamamoto et al., 2000; Sæther and Jobling, 2001; Gélineau et al., 2001, 2002; Boujard et al., 2004; Skalli et al., 2004), even though not all studies are consensual in this respect (Alanärä, 1994). However, to the best of our knowledge, this is the first reported study investigating the effect of dietary lipid level on food intake in marine fish larvae. The results obtained in the present work indicate that in fish larvae food intake does not appear to be regulated by total lipid level in the diet and that dietary FA composition may have a more important role in controlling ingestion. When seabream larvae were fed diets containing higher and lower amounts of soybean oil (HS and LS), the total lipid level of the diet appeared to negatively affect food intake, as observed with older fish, given that larvae fed significantly more on the LS diet. However, when higher and lower lipid-enriched *Artemia* were prepared using fish oil instead (HF and LF), the opposite was noted, i.e., larvae on the HF diet had a significantly higher food intake.

In the case of fish larvae, a stringent control of food intake according to dietary energy level or a lipostatic regulation of food intake (Yamamoto et al., 2002), as described for older fish, may not be so likely, due to several reasons. Fish larvae have a much higher demand for growth, with growth rates that may vary between 10 and 100% per day, which implies very high requirements for energy and structural components (Conceição, 1997). On the other hand, lipid is important for early organ development and for physiologically demanding processes such as metamorphosis and smoltification in salmonids (Berril et al., 2004). Therefore, it is more likely that lipid accumulates in depots which may have a negative feedback on appetite (lipostatic control) (Yamamoto et al., 2002) in juvenile and adult fish

than in larvae. Finally, due to the patchy nature of their natural habitat, i.e., as a consequence of temporal and spatial food availability in the aquatic environment, planktonic fish larvae probably maximize the numbers of preys fed, that is, fish larvae in the sea are believed to maximize their ingestion rate, even if that results in a reduced efficiency (Boehlert and Yoklavich, 1984; Checkley, 1984).

The type of oil used in the work reported here affected differently larval food intake. If fish regulate their food intake to not only balance their energy but also their macronutrient intake, as has been shown through self-selection experiments using encapsulated pure macronutrients (Rubio et al., 2003), it is not surprising that the FA composition of the lipid fraction may also exert some effect. These self-selection studies have shown that in adult seabass the regulation of food intake was independent of orosensory properties and suggested the existence of postingestive (chemosensory detection at the gastrointestinal level) and/or postabsorptive mechanisms (Rubio et al., 2003), which may also be affected by dietary FA composition. In the present study, food intake does not appear to have been regulated to meet a requirement for EFA as both diets which were more ingested (HF and LS) presented higher or similar levels of EFA than their same oil counterpart treatment. The absolute level of EFA was similar in the HS and LS diets (both in *Artemia* and MD) but in relative terms the percentage of EFA was higher in the LS diet, as a result of dilution of the naturally occurring EFA in *Artemia* and in the MD by increasing the neutral lipid level using an EFA-deficient oil.

Different factors may possibly explain the differences observed in food intake in relation to the dietary lipid source. The biochemical composition of the food may have an indirect regulatory effect on feeding by influencing the digestive and absorptive processes and in this way affect the rate of gut clearance. If the digestion and/or absorption of a meal occur faster, then the gut may be cleared sooner, thus leading to sustained feeding and appetite and indirectly leading to a higher food intake. The results of Hadas et al. (2003) showed that when seabream larvae were fed a phosphatidylcholine-supplemented MD, which was responsible

for an enhanced ^{14}C-oleic acid transport from the gut enterocytes into the larval body, an increased ingestion rate of this diet over the control diet was measured. It was hypothesized that an increase in lipid absorption may have increased the clearance rate of the chyme, thus leading to an increased ingestion rate, after a few hours of feeding. Therefore, nutrient digestibility may have an indirect effect on food intake. FA digestibility in fish decreases with increasing chain length and increases with unsaturation (Austreng et al., 1979; Sigurgisladottir et al., 1992; Olsen et al., 1998; Johnsen et al., 2000; Caballero et al., 2002; Morais et al., in press). SFA are less digestible and high levels of SFA in the diet might reduce the apparent digestibility of other FA (Austreng et al., 1979; Caballero et al., 2002). Consequently, dietary FA composition may affect the total digestible energy content of the diet. Given the high digestibility of n-3 PUFA, in particular, the long chain EPA and DHA, *Artemia* enriched with fish oil may be more digestible for marine fish larvae than with soybean oil. In fact, *Artemia* enriched on higher levels of soybean oil (HS) may have detrimental effects on lipid digestion and absorption. Differences in dietary oil digestibility could therefore be a factor explaining the experimental observations. The HF diet had higher absolute and relative amounts of n-3 PUFA and EFA than LF *Artemia*, while the LS diets had similar absolute levels (but a higher relative amount) of EFA and lower absolute and relative amounts of less digestible FA, such as SFA and of 18:1n-9 and 18:2n-6 (present in high amounts in soybean oil) than in the HS diets. However, as will be discussed below, the absorption results do not support this hypothesis, as they indicate that the absorption efficiency of the LF diet was higher than that of the HF *Artemia*, while the soybean oil diets appear to have been both equally efficiently absorbed.

In addition, the palatability of the oils used in the present study may have also affected the results. When feeding *Artemia*, even if a direct orosensory or taste effect of the oils is unlikely, as they are absorbed and converted into the *Artemia*'s body nutrients, the possibility exists that washing the *Artemia* after enrichment is insufficient to remove all residues of the

lipid emulsions from the exosqueleton. In the case of the formulated MD used in the second experiment, taste may have had a more important effect. In a study conducted by Marais and Kissil (1979) with older seabream, a low soybean oil diet was more highly ingested by the fish and the authors speculated whether a high level of soybean oil in the diets could have made them less attractive.

Food intake is an important parameter affecting growth but other factors, including digestive capacity and absorption efficiency, may have a considerable influence. Some of these factors may actually be interrelated. In fact, a high food intake has been shown to negatively affect nutrient assimilation efficiency, as a result of more rapid passage through the gut, while the assimilation efficiency increases at a lower ration, through an increase of the residence time of food particles (Werner and Blaxter, 1980; Ryer and Boehlert, 1983; Boehlert and Yoklavich, 1984). Therefore, in the present study, the absorption of different labels was also analyzed. In the first experiment, HF and LF *Artemia* were radiolabelled using liposomes containing [1-^{14}C] oleic acid. In order to have an idea of the fate of the label, once incorporated by the *Artemia*, its distribution was determined at the end of the enrichment period, by extracting the *Artemia* total lipid and collecting separately the remaining non-lipid fraction, followed by separation of polar and neutral lipid classes through TLC (data not shown). An important percentage of the label (about 30-40%) was found to be incorporated in the non-lipid fraction of the *Artemia*, being this more elevated in *Artemia* enriched on a low lipid dose. The label incorporated in the lipid fraction was found mostly as part of polar lipid (around 98-99% of label in lipid), with only a minor percentage being in the form of neutral lipid. The second experiment, using radiolabelled MDs instead of *Artemia*, had the advantage of allowing determination of intake and absorption of a free FA (oleic acid) or a triacylglycerol (triolein) tracer which does not undergo modification during diet radiolabeling.

In both experiments, a higher absolute nutrient absorption was measured in larvae fed lower lipid diets (LF and LS). Despite the fact that the LF diet was significantly less ingested

than the high lipid diet, it was markedly more absorbed, indicating much higher absorption efficiency in larvae fed this diet. In the second experiment, however, even though there was a higher label absorption in the LS treatment, larvae on this diet also ingested a larger amount of food. Therefore, if the radioactivity in the body is expressed as percentage of total radioactivity, no significant differences in absorption efficiency were noted between the LS and HS treatments (data not shown). Therefore, the results in the fish oil treatments support the hypothesis that a higher food intake may cause a decrease in nutrient absorption efficiency, while in the soybean oil experiment total absorption appears to be correlated with food intake.

A higher absorption efficiency of a diet containing a lower neutral lipid level has been previously described in Senegalese sole larvae (Morais et al., in press). However, in Senegalese sole, this occurrence was coincident with a lower accumulation of lipid droplets within the enterocytes, which were suggested to potentially slow down lipid absorption. In the present study, histological analysis of the gastrointestinal tract was performed at the end of both experiments and no obvious difference in lipid accumulation was found between larvae fed the higher and lower lipid diets, independently of the lipid source (data not shown). This may be due to species-specific differences or to the fact that in the present experiments there was a higher dietary supply of phospholipids, in the form of liposomes (added to the *Artemia* enrichment) in the first experiment or included in the MD in the second experiment. It has been frequently suggested that the endogenous synthesis of phospholipids may be insufficient to maintain an optimal rate of lipoprotein synthesis in fish larvae and, therefore, that dietary phospholipid has to be supplied in order to avoid problems in lipid transport which may lead to the accumulation of large lipid vacuoles in the enterocytes (Fontagné et al., 1998; Olsen et al., 1999; Salhi et al., 1999; Izquierdo et al., 2000). The lipid class analysis conducted on the enriched *Artemia* (data not shown) revealed that the lower lipid-enriched *Artemia* had, as expected, a higher relative level of phospholipids, as a result of presenting the same absolute

amount of phospholipids but a lower amount of neutral lipids, compared to the *Artemia* enriched with a higher dose of lipid emulsion. Therefore, a higher relative polar lipid content of LF *Artemia* may have been an additional factor explaining the significantly better absorption of this diet, compared to HF. However, other factors than phospholipid content and lipid droplet accumulation in the enterocytes may be involved and explain differences in absorption efficiency in larvae fed different levels of dietary neutral lipid.

A long history of feeding on the dietary regimes does not seem necessary for the dietary effects on food intake and nutrient absorption efficiency to manifest themselves. In the first experiment, when larvae were submitted to the radioactive trial after only 3 days of feeding on the experimental diets, similar results were obtained as those measured after 10 days of feeding on the diets. In the second experiment, a short-term effect was not analyzed but preliminary trials that were performed using HS and LS radiolabelled *Artemia* (data not shown) indicated that LS *Artemia* is more ingested and more absorbed, just as observed in the present work, after only 2 days of feeding on the experimental diets, i.e., before any significant difference is induced in growth by feeding the diets over a long period of time.

In the second experiment, much higher label absorption was measured in the trial using oleic acid as a marker, compared to the triolein trial. A better assimilation of oleic acid in a free form, rather than bound to a triacylglycerol (triolein), would be expected and has been reported before (Morais et al., in press). However, it should also be noted that there was a low intake of the triolein-labeled MDs as, after introducing new non-labeled MD, there was no decrease in the amount of label in the gut, as would have been expected. It thus appears that at 2 h most of the label was no longer in the gut lumen but was already incorporated into the gut tissues. One of the problems encountered when using the MD was the difficulty in controlling and standardizing particle size and the size of the triolein-labeled MD might have not been the most adequate for the larval age being studied. Nevertheless, the same trend was noted in terms of food intake and absorption irrespective of the form of the label (FA in the free form

vs. bound), which indicates that the differences observed between the HS and LS diets were not due to differences in the capacity to digest the diets and may be more a question of FA absorption.

Larvae fed the HF and LF *Artemia* diets tested in the first experiment did not differ in terms of growth, which may indicate that the LF diet met the minimum EFA requirement for growth in seabream larvae. The FA composition of the larvae at the end of the experimental period appears to confirm the higher FA absorption efficiency in larvae fed LF *Artemia*, as no significant differences were found in the larval content of SFA, MUFA and n-6 PUFA in larvae fed the fish oil treatments. The exception was in the level of the EFA which are absorbed more efficiently than other FA (Sigurgisladottir et al., 1992; Olsen et al., 1998; Caballero et al., 2002; Morais et al., in press). At the end of the second experiment, a significantly higher dry weight was achieved when larvae were fed the LS diet, compared with the HS treatment. This result was not surprising given the significantly higher food intake, as well as more efficient FA absorption, that were measured in larvae fed the LS diet. In addition, in spite of no significant differences being found in the absolute amounts of EFA in the diets, a higher relative level (% total FAME) of EFA was present in the LS diet, where less dilution by the soybean oil occurred. In this experiment, the larval FA composition provided indirect confirmation of the food intake results, as larvae fed on the LS diet, in spite of presenting lower levels of total FAME, had a larger amount of DHA in their tissues. DHA, being an EFA with an important structural role, tends to be selectively absorbed and conserved in marine fish larvae (Sargent et al., 1989). As the soybean oil enriched *Artemia* did not present DHA in its composition and both high lipid and low lipid MDs supplied an equivalent absolute amount of this EFA, it can be assumed that larvae on the low lipid treatment had a higher MD intake over the course of the experiment.

5. Conclusions

The results obtained in the present study revealed that food intake in seabream larvae does not appear to be regulated by total lipid (thus total energy) level in the diet, as observed in most juvenile and adult fish. On the other hand, dietary FA composition had a significant effect on ingestion. In addition, the observed effects were noticeable after only a short period of feeding on the dietary regimes. Food intake does not seem to have been regulated to meet a requirement for EFA and it is suggested that differences in diet digestibility, by potentially affecting the rate of gut clearance, may explain, at least partially, the results obtained in the present experiments. However, the absorption results did not support this hypothesis. Palatability of the diets may have also exerted some influence. The results obtained in the experiment using fish oil support the idea of a higher food intake causing a decrease in nutrient absorption efficiency (possibly by decreasing the gut retention time) but in the soybean oil experiment total absorption appears to have simply reflected food intake.

Acknowledgements

This work and Sofia Morais were supported by the "Fundação para a Ciência e a Tecnologia", Portugal (PhD grant SFRH/BD/4902/2001).

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Table 1. Proximate composition of the enriched non-labelled *Artemia* used in the two experiments. Values are means ± S.D. (1st experiment, n=7; 2nd experiment, n=5). Different superscript letters in the same column, within each experiment, indicate significant differences (P<0.05). NS = non significant, at P<0.05.

	Total lipid (%DW)	Total protein (%DW)	Ash (%DW)	Dry matter (%WW)
1st Experiment				
HF	28.1 ± 2.7 [a]	50.7 ± 2.7 [a]	8.6 ± 1.0 [a]	9.2 ± 0.5 [a]
LF	17.0 ± 1.3 [b]	60.4 ± 0.8 [b]	10.5 ± 1.1 [b]	8.3 ± 0.6 [b]
2nd Experiment				
HS	25.3 ± 3.5[a]	54.8 ± 1.9[a]	9.4 ± 0.7 [NS]	10.0 ± 1.3 [NS]
LS	18.0 ± 1.1[b]	61.5 ± 2.4[b]	9.2 ± 0.6 [NS]	9.0 ± 1.5 [NS]

Table 2. Fatty acid analysis of the experimental diets used in the two experiments. Values are means ± S.D. (1st experiment, n=5; 2nd experiment, n=2, except for LS *Artemia*, where n=3). Different superscript letters indicate significant differences (P<0.05) between treatments, for the analysed FA or FA classes. NS = non significant, at P<0.05.

FAME	1st Experiment		2nd experiment			
(mg/g DW)	HF	LF	Art-HS	Art-LS	MD-HS	MD-LS
14:0	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.14	0.14 ± 0.07	0.18 ± 0.02	0.13 ± 0.01
14:1	0.61 ± 0.26	0.50 ± 0.18	0.04 ± 0.03	0.11± 0.04	0.28 ± 0.29	0.02 ± 0.03
15:0	0.45± 0.18	0.27 ± 0.07	0.06 ± 0.03	0.18 ± 0.12	0.11 ± 0.11	0.18 ± 0.10
16:0	18.51 ± 1.72	8.10 ± 0.96	7.35 ± 7.01	6.70 ± 2.48	13.04 ± 0.41	3.12 ± 0.75
16:1n-7	6.63 ± 1.20	2.63 ± 0.38	0.56 ± 0.72	0.72 ± 0.26	0.21 ± 0.01	0.18 ± 0.05
16:2	1.09 ± 0.12	1.01 ± 0.10	0.06 ± 0.05	0.09 ± 0.04	0.03 ± 0.01	0.00 ± 0.00
16:3	0.82 ± 0.13	0.56 ± 0.06	0.07 ± 0.06	0.21 ± 0.24	0.01 ± 0.02	0.00± 0.00
18:0	17.32 ± 5.32	9.21 ± 0.50	11.20 ± 7.61	3.01 ± 1.18	3.14 ± 0.05	1.03 ± 0.42
18:1n-9	25.04 ± 1.86	11.81 ± 1.47	20.04 ± 19.00	10.67 ± 8.47	30.20 ± 0.63	5.63 ± 1.87
18:1n-7	9.85 ± 0.76	7.88 ± 0.75	3.72 ± 3.48	7.34 ± 3.62	0.61 ± 0.13	0.15 ± 0.09
18:2n-6	7.82 ± 0.59	3.71 ± 0.51	18.88 ± 24.93	6.08 ± 2.88	49.84 ± 0.53	3.97 ± 1.33
18:3n-3	20.02 ± 1.78	19.38 ± 3.87	11.62 ± 14.02	12.73 ± 6.72	2.37 ± 0.35	0.23 ± 0.08
18:4n-3	3.23 ± 0.21	2.46 ± 0.32	1.79 ± 2.32	4.48 ± 5.91	0.46 ± 0.09	0.31 ± 0.34
20:0	0.37 ± 0.04	0.20 ± 0.01	0.05 ± 0.05	0.03 ± 0.02	0.65 ± 0.79	0.97 ± 0.40
20:1n-9	0.77 ± 0.39	0.36 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.02
20:2n-6	0.38 ± 0.06	0.18 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.06	0.04 ± 0.06
20:3n-6	0.14 ± 0.04	0.12 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.21	0.04 ± 0.05
20:4n-6	1.05 ± 0.14 ^{a}	0.46 ± 0.07 ^{b}	0.06 ± 0.06 ^{NS}	0.12 ± 0.02 ^{NS}	0.03 ± 0.02 ^{a}	0.33 ± 0.02 ^{b}
20:4n-3	0.75 ± 0.11	0.46 ± 0.11	0.04 ± 0.05	0.04 ± 0.04	0.38 ± 0.06	0.46 ± 0.09
20:5n-3	14.96 ± 3.18 ^{a}	5.44 ± 2.02 ^{b}	0.23 ± 0.19 ^{NS}	0.46 ± 0.20 ^{NS}	19.11 ± 0.18 ^{NS}	23.20 ± 8.01 ^{NS}
22:1n-9	0.28 ± 0.05	0.22 ± 0.13	0.00 ± 0.00	0.10 ± 0.18	0.12 ± 0.09	0.07 ± 0.05
21:5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.14	0.23 ± 0.30
22:5n-6	0.37 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.14 ± 0.14	0.17 ± 0.02
22:5n-3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.56 ± 0.58	1.55 ± 0.16
22:6n-3	18.70 ± 2.84 ^{a}	1.07 ± 0.60 ^{b}	0.00 ± 0.00 ^{NS}	0.00 ± 0.00 ^{NS}	42.57 ± 0.01 ^{NS}	51.25 ± 18.81 ^{NS}
Totals						
FAME	149.14 ± 15.79 ^{a}	76.11 ± 5.95 ^{b}	75.89 ± 75.10 ^{NS}	53.19 ± 15.24 ^{NS}	165.54 ± 2.32 ^{NS}	93.26 ± 31.41 ^{NS}
SFA	36.65 ± 6.85 ^{a}	17.77 ± 1.41 ^{b}	18.79 ± 14.83 ^{NS}	10.04 ± 3.64 ^{NS}	17.12 ± 1.06 ^{a}	5.42 ± 0.64 ^{b}
MUFA	43.18 ± 3.84 ^{a}	23.47 ± 2.55 ^{b}	24.36 ± 23.23 ^{NS}	18.93 ± 5.71 ^{NS}	31.46 ± 0.94 ^{a}	6.06 ± 1.84 ^{b}
PUFA	69.32 ± 7.90	34.87 ± 3.60	32.74 ± 37.04	24.21 ± 6.42	116.96 ± 0.33	81.79 ± 28.92
n-3 PUFA	57.65 ± 7.32 ^{a}	28.81 ± 3.42 ^{b}	13.67 ± 11.94 ^{NS}	17.72 ± 3.89 ^{NS}	66.45 ± 1.13 ^{NS}	77.00 ± 27.32 ^{NS}
n-6 PUFA	9.76 ± 0.86 ^{a}	4.48 ± 0.60 ^{b}	18.95 ± 24.99 ^{NS}	6.19 ± 2.90 ^{NS}	50.23 ± 0.67 ^{a}	4.55 ± 1.31 ^{b}
n-3/n-6	5.91 ± 0.61	6.53 ± 1.23	2.35 ± 2.47	3.23 ± 1.47	1.32 ± 0.04	16.74 ± 1.17
DHA/EPA	1.27 ± 0.15	0.19 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	2.23 ± 0.02	2.20 ± 0.05

Table 3. Fatty acid analysis of the larvae, at the end of the experimental period (34 DAH). Values are means ± S.D. (n=5). Different superscript letters indicate significant differences (P<0.05) between treatments, for the analysed FA or FA classes. NS = non significant, at P<0.05.

FAME	1st experiment		2nd experiment	
(mg/g DW)	HF	LF	HS	LS
14:0	0.00 ± 0.00	0.00 ± 0.00	0.16 ± 0.04	0.10 ± 0.06
14:1	0.19 ± 0.01	0.24 ± 0.02	0.07± 0.03	0.04± 0.03
15:0	0.59 ± 0.71	0.24 ± 0.06	0.33 ± 0.29	0.26± 0.30
16:0	9.92 ± 1.23	8.53 ± 0.84	7.64 ± 1.76	6.10 ± 0.46
16:1n-7	1.58 ± 0.47	1.44 ± 0.40	0.14 ± 0.04	0.14 ± 0.09
16:2	0.59 ± 0.07	0.76 ± 0.10	0.19 ± 0.08	0.13 ± 0.02
16:3	0.48 ± 0.07	0.48 ± 0.07	0.19 ± 0.07	0.13 ± 0.06
18:0	6.27 ± 0.34	6.55 ± 0.45	5.53 ± 0.42	3.22 ± 1.09
18:1n-9	11.40 ± 0.72	10.58 ± 0.76	15.10± 3.33	8.54 ± 1.07
18:1n-7	5.61 ± 0.20	6.42 ± 0.56	3.24 ± 0.67	2.79 ± 0.48
18:2n-6	3.67 ± 0.16	3.49 ± 0.33	12.68 ± 3.65	4.32 ± 0.70
18:3n-3	6.27 ± 0.37	9.26 ± 0.91	4.13 ± 2.41	4.48 ± 0.74
18:4n-3	0.89 ± 0.06	1.08 ± 0.10	0.15 ± 0.11	0.17 ± 0.09
20:0	0.13 ± 0.01	0.15 ± 0.01	0.07 ± 0.02	0.03 ± 0.02
20:1n-9	0.38 ± 0.02	0.37 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
20:1n-7	0.09 ± 0.04	0.12 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
20:2n-6	0.27 ± 0.02	0.23 ± 0.01	0.07 ± 0.06	0.03 ± 0.02
20:3n-6	0.23± 0.01	0.30 ± 0.02	0.12 ± 0.17	0.13 ± 0.20
20:4n-6	1.27 ± 0.06 [a]	1.03 ± 0.07 [b]	0.28 ± 0.22 [NS]	0.31 ± 0.21 [NS]
20:4n-3	0.58 ± 0.03	0.65 ± 0.33	0.09 ± 0.02	0.11 ± 0.05
20:5n-3	8.27 ± 0.48 [a]	5.40 ± 0.96 [b]	1.21 ± 0.21 [NS]	1.53 ± 0.68 [NS]
22:1n-9	0.20 ± 0.02	0.18 ± 0.06	0.04 ± 0.03	0.02 ± 0.03
21:5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.04
22:5n-3	0.96 ± 0.07	0.49 ± 0.25	0.07 ± 0.04	0.20 ± 0.12
22:6n-3	11.27 ± 1.02 [a]	4.23 ± 1.17 [b]	1.66 ± 0.12 [a]	3.73 ± 1.04 [b]
Totals				
FAME	71.12 ± 4.00 [a]	62.21 ± 5.14 [b]	53.16 ± 7.08 [a]	36.58 ± 4.96 [b]
SFA	16.91 ± 1.66 [NS]	15.47 ± 1.10 [NS]	13.73 ± 2.27 [a]	9.71 ± 1.60 [b]
MUFA	19.45 ± 1.13 [NS]	19.35 ± 1.50 [NS]	18.58 ± 4.04 [a]	11.56 ± 1.45 [b]
PUFA	34.75 ± 1.72	27.39 ± 3.10	20.85 ± 5.53	15.31 ± 2.62
n-3 PUFA	28.24 ± 1.58 [a]	21.10 ± 2.71 [b]	7.31 ± 2.50 [NS]	10.22 ± 2.03 [NS]
n-6 PUFA	5.45 ± 0.21 [NS]	5.05 ± 0.39 [NS]	13.16 ± 3.72 [a]	4.80 ± 0.77 [b]
n-3/n-6	5.18 ± 0.13	4.17 ± 0.36	0.56 ± 0.19	2.14 ± 0.28
DHA/EPA	1.36 ± 0.08	0.78 ± 0.12	1.40 ± 0.18	2.84 ± 1.37

Figure captions

Fig. 1. Larval dry weight (mg), at 26 DAH (A) and 34 DAH (B) in the first experiment and at the end (34 DAH) of the second experiment (C). Values are means (n=5, variable number of pooled larvae) ± S.D. Asterisk represents significant differences between the treatments. NS = non significant, at $P<0.05$.

Fig. 2. Food (equivalent to number of *Artemia*) present in the gut (A and C) and absorbed into the body (B and D) of seabream larvae fed *Artemia* enriched on a high (HF) or low (LF) level of fish oil emulsion, at 26 DAH (A and B) and 33 DAH (C and D). Values for each treatment and time point are means (n=3, 30 pooled larvae) ± S.D. Asterisks represent significant differences between the treatments at the analyzed time point.

Fig. 3. Microdiet (μg MD/larva) present in the gut (A and C) and absorbed into the body (B and D) of seabream larvae fed triolein-labeled MD, at 31 DAH (A and B) or oleic acid-labeled MD, at 32 DAH (C and D). Values for each treatment and time point are means (n=3, 25 pooled larvae) ± S.D. Asterisks represent significant differences between the treatments at the analyzed time point.

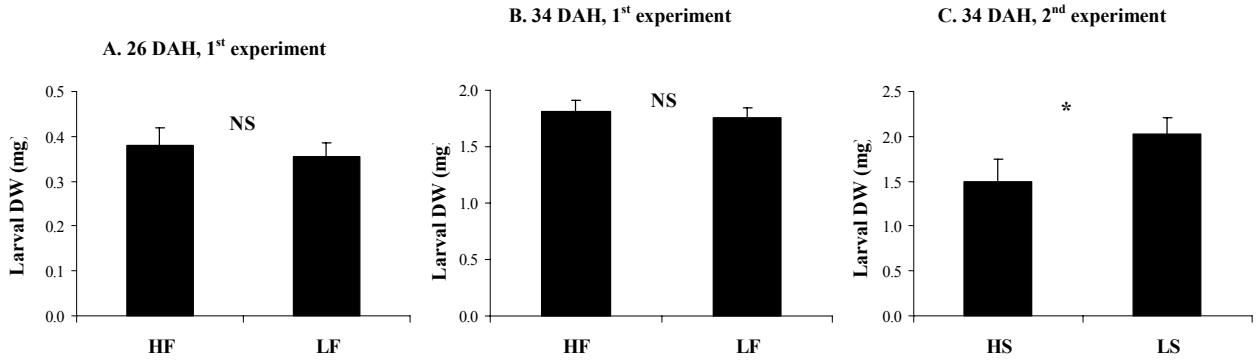
A. 26 DAH, 1st experiment
B. 34 DAH, 1st experiment
C. 34 DAH, 2nd experiment
Larval DW (mg)
NS
NS
*
HF
LF
HF
LF
HS
LS

Fig. 1.

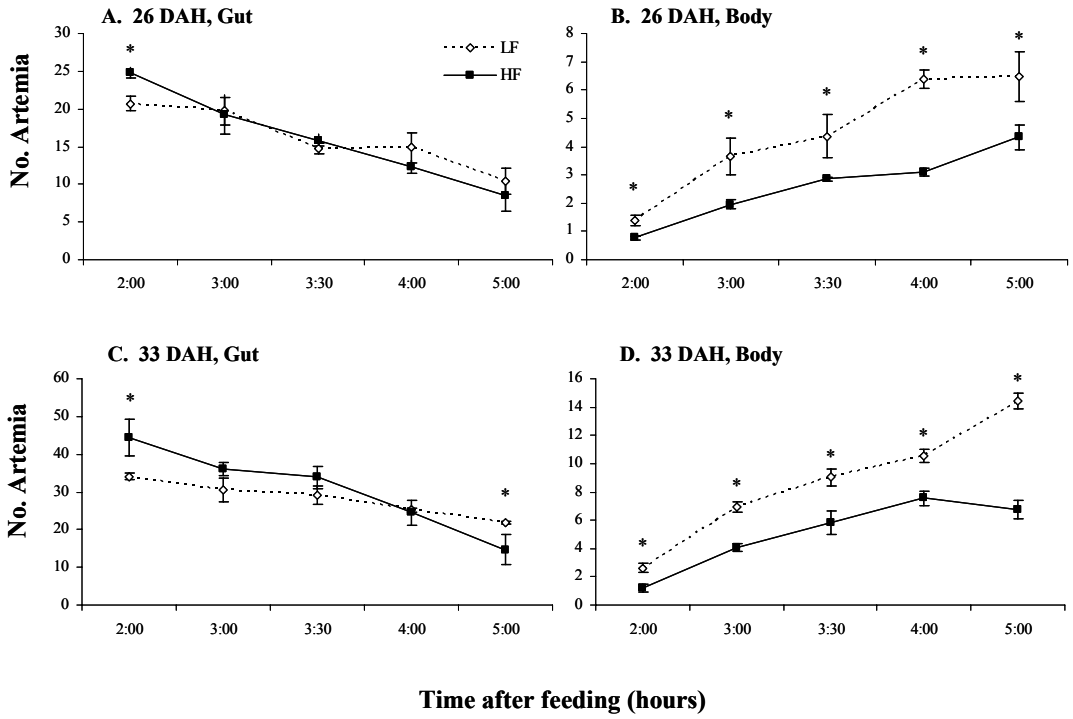
A. 26 DAH, Gut
B. 26 DAH, Body
C. 33 DAH, Gut
D. 33 DAH, Body
LF
HF
No. Artemia
No. Artemia
2:00
3:00
3:30
4:00
5:00
Time after feeding (hours)

Fig. 2

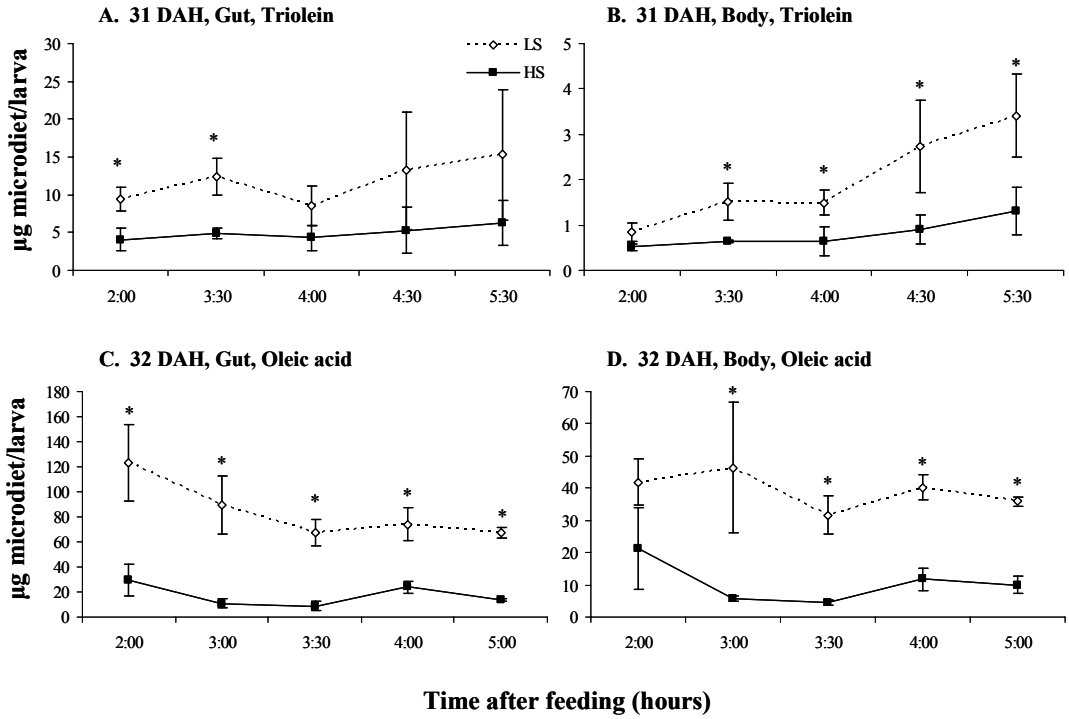
A. 31 DAH, Gut, Triolein
B. 31 DAH, Body, Triolein
C. 32 DAH, Gut, Oleic acid
D. 32 DAH, Body, Oleic acid
LS
HS
µg microdiet/larva
Time after feeding (hours)

Fig. 3