Co-feeding of live feed and inert diet from first-feeding affects *Artemia* lipid digestibility and retention in Senegalese sole (*Solea senegalensis*) larvae

Mônica Giacometti Mai[a,b], Sofia Engrola[a], Sofia Morais[c], Maria Célia Portella[d], José Roberto Verani[b], Maria Teresa Dinis[a], Luís E.C. Conceição[a]

[a] CCMAR, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

[b] UFSCar - Federal University of São Carlos, 13565-905 São Carlos, Brazil

[c] Institute of Aquaculture - University of Stirling. Stirling FK9 4LA, Scotland, UK

[d] São Paulo State University - Aquaculture Center, 14.884-900 Jaboticabal, Brazil

Corresponding author: M. G. Mai, UFSCar - Federal University of São Carlos, 13565-905 São Carlos, Brazil. E-mail address: monica_mai@hotmail.com . Phone and fax number 55 16 3351 8310

Abstract

The present study intended to evaluate the effects of early introduction of inert diet in lipid digestibility and metabolism of sole, while larval feed intake, growth and survival were also monitored. *Solea senegalensis* larvae were reared on a standard live feed regime (ST) and co-feeding regime with inert diet (Art R). Trials using sole larvae fed with *Artemia* enriched with two different lipid emulsions, containing glycerol tri [1-^{14}C] oleate (TAG) and L-3-phosphatidylcholine-1,2-di-[1-^{14}C] oleoyl (PL), were performed at 9 and 17 days after hatching (DAH) to study lipid utilization. Co-feeding did not affect sole survival rates (ST 59.1 ± 15.9 %; Art R 69.56 ± 9.3 %), but was reflected in significantly smaller final weight at 16 DAH (ST 0.71 ± 0.20; Art R 0.48 ± 0.14 mg). Higher feed intake was observed in sole larvae fed on *Artemia* enriched with labeled PL at 9 DAH but not at 17 DAH. At 17 DAH, the smaller larvae (Art R treatment) ingested

proportionally more *Artemia* in weight percentage, independently of enrichment. At 9 DAH lipid digestibility was equal among treatments and higher than 90%, while at 17 DAH it was higher in ST treatment (around 73 %) compared to the Art R group (around 66 %). Lipid retention efficiency at 9 DAH was higher in the Art R treatment, reaching values of 50 %, while these values almost duplicated at 17 DAH, ranging up to 80 % in both treatments without significant differences. These results show that co-feeding of live feed and inert diet from first-feeding in Senegalese sole has a toll in terms of growth and lipid digestibility but does not seem to compromise lipid metabolic utilization.

Keywords: *Solea senegalensis;* Weaning; Lipid metabolism; Digestibility; Feed Intake; Metamorphosis.

1. Introduction

In order to successfully achieve the objective of a significant partial replacement of live feed by inert diets from first feeding, a detailed understanding of the larval digestive physiology and how it may be influenced by the dietary components is indispensable (e.g., Cahu and Zambonino Infante, 2001; Morais, 2005; Engrola, 2008).

The Senegalese sole (*Solea senegalensis*, Kaup 1858) is a flatfish found along the Mediterranean and Atlantic coasts, and is a promising candidate for aquaculture in Europe since the nineties due to good market prices (Howell, 1997; Dinis et al., 1999; Imsland et al., 2003). Despite its high potential as an aquaculture species, only a few studies looking at sole larvae rearing conditions (Esteban et al., 1995; Dinis et al., 1999) and weaning strategies (Marin-Magan et al., 1995; Cañavate and Fernández-Díaz, 1999; Ribeiro et al., 2002; Engrola et al., 2005, 2007, 2009a) are available. Moreover,

weaning success of Senegalese sole is still a critical step, with two strategies being possible: sudden weaning and weaning in co-feeding with *Artemia* metanauplii (Engrola et al., 2007). In spite of recent progress in sole larvae nutritional requirements and understanding of larval digestive physiology, weaning results obtained so far are variable and difficult to reproduce (Conceição et al., 2007b). Therefore, hatchery protocols for Senegalese sole still rely on live preys during the period before the metamorphosis, which occurs between 12 and 20 DAH, when they can be gradually substituted by frozen *Artemia* metanauplii. Recently it has been demonstrated that protein digestibility and retention are depressed by co-feeding with high levels of *Artemia* replacement by inert diet, and thereby lead to lower growth (Engrola, 2008). However, when a moderate level of *Artemia* replacement is used, sole are able to adapt their protein metabolism and enhance protein utilization in the long term, with a growth promoting effect at complete weaning (Engrola et al., 2009b).

Fish larvae diets, and particularly enriched *Artemia*, tend to be rich in triacylglycerols (TAG) as lipid source (Morais et al., 2006), in an attempt to meet essential fatty acids (EFA) requirements, namely in terms of n-3 polyunsaturated fatty acids (PUFA) (Sargent et al., 1989). This can be a problem since the high levels of lipids as well as the unbalances in lipid class composition found occasionally in enriched live preys have been suggested to affect fatty acid (FA) digestion and absorption (Salhi et al., 1995, 1997, 1999; Díaz et al., 1997; Morais et al., 2007). In the marine environment, high levels of phospholipids (PL) are normally found in the total lipid fraction of phytoplankton and zooplankton ingested by fish larvae (Sargent et al., 1989). A beneficial effect of dietary PL supplementation in purified diets in terms of survival, growth, resistance to stress, and lower occurrence of deformities has been demonstrated in larval and juvenile stages of various species of fish and crustaceans

(Geurden et. al., 1995; Coutteau et al., 1997; Koven et al., 1998; Cahu and Zambonino Infante, 2001).

The present work intended to evaluate the effects of co-feeding live feed with inert diet from mouth opening on lipid digestive capacity and metabolism of sole larvae, compared to a standard feeding regime using only live feed. To achieve this, two metabolic trials using radiolabeled *Artemia* enriched with lipid emulsions containing either glycerol tri [1-^{14}C] oleate (TAG) or L-3-phosphatidylcholine-1,2-di-[1-^{14}C] oleoyl (PL) were performed with sole larvae before (9 days after hatching, DAH) and during the metamorphosis climax (17 DAH). The digestibility, retention and catabolism of the radiolabel incorporated in *Artemia*, as well as larval *Artemia* intake, were measured. In addition, the use of different sources of radiolabeled lipid (TAG or PL) enabled to verify whether these effects depend also on the molecular moiety in which the FA were supplied to *Artemia*.

2. Materials and Methods

2.1 Larval rearing

Senegalese sole eggs used in the experiment were obtained from natural spawning of captive breeders maintained in IPIMAR-CRIPSul, Olhão, Portugal. The larvae were stocked in 100 L cylindro-conical tanks at a density of 100 larvae L^{-1}. The green water technique was used in the rearing tanks with a 1:1 mixture of *Tetraselmis chuii* and *Isochrysis galbana* in a recirculation system, at a temperature of 19.8 ± 0.4 ºC and a salinity of 37.8 ± 1.5 ‰. Oxygen saturation was 96.4 ± 9.6% and a 12/12-h light/dark cycle was adopted. Water renewal was increased from 4 times/day from 0 DAH to 8 times/day from 13 DAH until the end of the experiment, which lasted 19 days.

2.2 Feeding regimes

Two different feeding regimes were randomly assigned in triplicate during the pelagic phase: standard live feed (Standard, ST) and live feed co-fed with inert diet from mouth opening (*Artemia* Replacement, Art R). The feeding was based on rotifers (*Brachionus rotundiformis*) enriched with Red Pepper (BernAqua, Olen, Belgium) from 2 to 4 DAH for both treatments; *Artemia* nauplii (INVE Aquaculture NV) from 4 to 9 DAH for both treatments; and *Artemia* metanauplii enriched for 12 hours, at 250 nauplii mL^{-1}, with 0.4 g L^{-1} in two doses (at 3 and 6h, following the manufacturer's instructions) of a 1:1 mixture (weight basis) of Easy DHA Selco® (INVE Aquaculture NV) and Micronised Fishmeal® (Ewos, Scotland) for both treatments until the end of experiment. The amount of *Artemia* supplied to the Art R treatments was gradually reduced during the experiment (see Table 1). At the end of the experiment Art R sole were being offered 45 % frozen *Artemia* metanauplii and 55 % inert diet (*Proton* 100-200 µm; INVE Aquaculture NV, Dendermonde, Belgium) in proportion (weight basis) to total daily ration. Between days 13 and 16, the *Artemia* metanauplii supply to both treatments was gradually changed from live to frozen *Artemia*. *Artemia* metanauplii were harvested, washed in seawater, counted, and frozen at -20 ºC. Fifteen minutes before feeding, *Artemia* was thawed in seawater. Table 1 shows the feeding regimes in detail.

Table 1

The larvae were fed daily at 11:00 am, 14:00 pm and 17:00 pm. The first meal was composed by 50 % of the daily feeding dose, and the remaining 50 % was shared between the two following meals.

2.3 Sampling

Samples were taken for the determination of individual dry weight (DW) at: 2 DAH, at mouth opening (*n*=30 per treatment), 8 DAH (*n*=30 for each replicate) and 16 DAH (*n*=15 for each replicate). The larvae were stored at - 20 ºC and afterwards freeze-dried for 48 h in a Savant SS31 (Savant Instruments Inc., Hokbrook, NY, USA). The DW of the larvae was determined in a Sartorius type M5P scale (precision of 0.001mg; Sartorius micro, Göttingen, Germany). Survival was determined at the end of the experiment, by counting the larvae remaining in the rearing tanks.

2.4 Lipid metabolism trials

Two trials were performed using *Artemia* labeled with different ^{14}C-lipid sources to analyze the effects of the feeding regimes on the digestive capacity and metabolism of sole larvae: the first at 9 DAH, in the pelagic phase, and the second at 17 DAH, during the metamorphosis climax.

2.4.1 Artemia [1-^{14}C] labeling

Two lipid emulsions were prepared using 0.09 g of Easy DHA Selco plus either 50 µL (50 µCi) of glycerol tri [1-^{14}C] oleate (TAG; 3·7MBq/mL) or 100 µL (10 µCi) of L-3-phosphatidylcholine-1,2-di-[1-^{14}C] oleoyl (PL; 0·925MBq/mL) (Amersham Pharmacia Biotech Ltd., UK). After mixing the radiolabeled lipids, the solvent in which the radiolabel came dissolved was evaporated by flushing N_2. The eppendorfs with the emulsions were covered with parafilm and submitted to 5 min of ultra-sound bath at 30 ºC, vigorously shaken for 2 minutes and then stored at - 20 ºC. The enrichment was made by adding and mixing the TAG or PL emulsions to 150 mL of seawater before introducing *Artemia* (200 metanauplii/mL). The incubation lasted 14 h to allow for

complete lipid incorporation (Morais et al., 2004b), being also a common enrichment period used with several commercial products. After incubation, *Artemia* metanauplii was washed thoroughly, counted and samples were taken to measure the incorporated radiolabel.

2.4.2 Cold chase - set up

In both trials, approximately 14 h prior to the start of radiolabeled *Artemia* feeding and 30 min following their last meal, 10 larvae from each triplicate larval rearing tank were transferred to smaller trays in the experimental radioisotope room and acclimatized. Each of the four trays (two trays per treatment; one for each type of radiolabel tested) contained thus 30 larvae pooled from the triplicate larval rearing tanks. An excess of larvae was sampled from the rearing tanks, in relation to the requirements of the cold chase trial, in case there were any mortalities during overnight acclimation or in case some of the larvae would not ingest the radiolabeled Artemia Each tray received approximately 10,000 *Artemia* metanauplii incubated with one of the lipid radiolabels, resulting in the following treatments: ST – TAG (Standard + *Artemia* incubated with ^{14}C-glycerol trioleate); ST – PL (Standard + *Artemia* incubated with ^{14}C-phosphatidylcholine); Art R – TAG (*Artemia* Replacement + *Artemia* incubated with ^{14}C-glycerol trioleate) and Art R - PL (*Artemia* Replacement + *Artemia* incubated with ^{14}C-phosphatidylcholine). Sole larvae were allowed to feed on the radiolabeled *Artemia* during 30 min. This period is a trade-off between the time necessary for a complete meal size and to avoid significant losses by larvae catabolism. After that time, 15 larvae from each treatment presenting food in their stomachs (assessed visually) were carefully transferred, one by one with an inverted Pasteur pipette, through two tanks with clean seawater (to eliminate any ^{14}C-lipid that could be present in the surface of the fish), and

subsequently transferred to individual incubation vials. After 2 h, each larva in the incubation vial was fed non labeled *Artemia* in the same concentration normally offered in the rearing tanks. This feeding of radiolabeled *Artemia* followed by feeding with non labeled *Artemia* characterizes a cold chase-type trial (Conceição et al., 2007a). During the course of the trials, mortality was negligible - 2 dead larvae in ST PL; 1 dead larvae in ArtR TG and 1 dead in ArtR PL treatments, in the first trial (9 DAH), and no mortality was recorded during the second trial (16 DAH).

2.4.3 Determination of radiolabeling

The method employed allows following the metabolic fate of a tracer nutrient into different compartments of individual larvae: retention in body (larvae), catabolism (CO_2 trap) and evacuation (incubation water) (Rønnestad et al., 2001). The metabolic chambers (7.5 mL of seawater) were connected to a metabolic trap (5.0 mL of KOH 0.5 M), to capture the $^{14}CO_2$ eliminated by larvae. After 24 h, each larva was sampled and placed in a 6 mL scintillation vial (Sarstedt, Rio de Mouro, Portugal). Hydrochloric acid (HCl 0.1 M) was gradually added to the remaining water, resulting in a progressive decrease of pH causing the diffusion of any remaining CO_2 in the incubation water (Rønnestad et al., 2001). As a result, the radioactivity found in the water of the incubation chambers corresponds to the evacuated, i.e., non absorbed, labeled *Artemia*. Tissue solubilizer (Solvable, Packard Bioscience, Groningen, Netherlands) was added to the vials containing the sampled larvae and the labeled *Artemia*. The vials were then placed overnight in an oven to allow tissue solubilisation. After cooling, 5 mL of Ultima Gold XR (Packard Bioscience) was added. To the vials containing the larva's incubation water and the KOH (metabolic trap), 5 mL of Ultima Gold XR was also added. The samples were counted on a Beckman LS 6000IC liquid scintillation counter

(Beckman Instruments Inc., Fullerton, CA, USA) and the results are presented as a percentage of disintegrations per minute (DPM) in each compartment in relation to total counts. Feed intake (FI) and feed utilization of larvae on each dietary treatment fed *Artemia* enriched with either one of the emulsions (4 combinations; *n*=15 per combination) were determined at 9 DAH and 17 DAH. Feed intake (% BDW) during the cold chase was determined as:

$$FI = [(R_{total}/SR_{Artemia})/DW_{fish}] \times 100$$

as described by Conceição et al. (1998), where R_{total} is the sum of the radioactivity in the incubation seawater, in the CO_2 trap and in fish (DPM), $SR_{Artemia}$ is the specific radioactivity in *Artemia* samples (DPM/mg *Artemia* DW), and DW_{fish} is the fish dry weight (mg).

Different *Artemia* utilization parameters were determined: digestibility (D, %), retention (R, %) and catabolism (C, %). These were calculated as:

$$D = [(R_{body} + R_{CO_2\ trap})/(R_{body} + R_{CO_2\ trap} + R_{water})] \times 100;$$

$$R = [R_{body}/(R_{body} + R_{CO_2\ trap})] \times 100;$$

$$C = [R_{CO_2\ trap}/(R_{body} + R_{CO2\ trap})] \times 100;$$

where R_{body} is the total radioactivity in fish body (DPM), $R_{CO_2\ trap}$ is the total radioactivity per CO_2 trap (DPM), and R_{water} is the total radioactivity in the incubation seawater (DPM).

2.5 Statistical analysis

The data obtained for each treatment were compared through one-way (growth and survival) or two-way (distribution of label in each compartment in metabolic trials) analysis of variance (ANOVA), using the software Statistica 6 (StatSoft Inc., Tulsa, USA). For two-way ANOVA, the combined effects of the factors “feeding regime” (ST

or Art R) and "lipid source" (TAG or PL) were tested. The assumption of homogeneity of variance was checked using the Bartlett's test and a significance level of 0.05 was employed (Zar, 1996). Data from the labeled *Artemia* feeding trial (percentage of counts found in each compartment) and all other percentage data were arcsin($x^{1/2}$) transformed. When significant differences were found in one way and two way ANOVA, the Tukey's test and Newman Keuls test was performed, respectively. All data are given as mean values with standard deviations (SD).

3. Results

3.1 Larval growth and survival

The initial DW of sole larvae was 42.68 ± 13.91 µg for all treatments. No significant differences were observed in sole survival between the two feeding regimes (ST 59.1 ± 15.9 %; Art R 69.6 ± 9.3 %) at 16 DAH, but the final mean DW was significantly higher in larvae fed live feed alone (ST treatment) at this time (Fig. 1b). However, this difference in DW was still not significant at 8 DAH (Fig. 1a).

Figure 1

3.2 Feed Intake

The handling to transfer the larvae with the Pasteur pipette was a methodology that has been previously used in other study (Engrola et al., 2009b), in which it was observed that the larvae were actively feeding on Artemia in the incubation vials and, therefore, it is believed that this factor did not affect the results, regarding the stress caused to larvae.

Artemia labeling with emulsions containing either ^{14}C-TAG or ^{14}C-PL resulted in average DPM values of 78.6 ± 7.5 DPM/*Artemia* and 37.9 ± 2.9 DPM/*Artemia* for TAG

and PL labeling, respectively, at the 9 DAH metabolic trial; and 65.8 ± 1.8 and 54.8 ± 4.9 DPM/*Artemia* for TAG and PL labeling, respectively, at 17 DAH.

At 9 DAH, sole fed *Artemia* enriched with PL (standard - ST - and co-feeding -Art R) presented a significantly higher feed intake compared to those fed TAG-enriched *Artemia* ($P < 0.001$), while no significant differences were observed between the two feeding regimes. The number of *Artemia* consumed per sole larvae at 9 DAH was ST - TAG 9.1 ± 2.3; ST – PL 23.8 ± 6.7; Art R – TAG 11.6 ± 4.1; Art R - PL 19.2 ± 6.9. Considering that there were no differences in the DW of the larva at this age, the percentage of ingested *Artemia* weight in relation to the sole larva weight was significantly higher in the treatments fed ^{14}C - PL labeled *Artemia* (Fig. 2a).

Towards the end of the experiment (17 DAH), larvae from the ST and Art R treatments consumed the same amount of labeled *Artemia*, independently of the type of lipid emulsion used to label them: ST - TAG 40.9 ± 16.2; ST - PL 41.8 ± 10.5; Art R - TAG 43.7 ± 10.8; Art R - PL 42.1 ± 7.5 *Artemia*/sole larvae. Nevertheless, at 17 DAH the DW of larvae reared on the standard live feed regime (ST) was higher than that on Art R (Fig.1b), which means that the smallest larva (Art R) ingested proportionally more *Artemia* in weight percentage ($P < 0.001$) than the sole fed live feed alone (ST group) (Fig. 2b). At this time, no significant different were observed between treatments fed *Artemia* enriched with either PL or TAG.

Figure 2

3.3 Digestibility and larval metabolism

There were no significant differences in *Artemia* digestibility among treatments at 9 DAH (Fig. 3a). At 17 DAH, however, sole from ST treatment fed with *Artemia* enriched with TAG had significantly higher lipid digestibility (88.7%) ($P < 0.001$

276 "feeding regime" and $P < 0.05$ for "lipid source"). Sole from Art R treatment fed PL
277 Artemia had the lowest lipid digestibility, 66.0% (Fig. 3b).
278 Figure 3
279 Sole larvae at 9 DAH presented significantly higher label retention (% of label
280 absorbed) in the co-feeding treatments (Art R) ($P < 0.05$) and no statistical differences
281 were found for "lipid source" ($P > 0.07$; Fig. 4a). Lipid retention values were 48.8 ±
282 11.2 % in Art R – TAG; 40.4 ± 17.2 % in Art R – PL; 32.3 ± 10.6 % in ST – TAG and
283 40.0 ± 11.0 % in ST - PL. Concomitantly, the larvae submitted to the ST feeding regime
284 presented a significantly higher catabolism, when analyzed by two-way ANOVA, i.e,
285 disregarding the lipid source utilized for *Artemia* enrichment (67.6 ± 10.6% in ST –
286 TAG and 59.9 ± 12.7% in ST – PL; 51.1 ± 11.2% in Art R – TAG; 59.5 ± 17.2% in Art
287 R – PL). Similar results were observed when analyzing retention, catabolism and
288 evacuation expressed as DPM/ mg of sole larva DW at 9 DAH (results not shown).
289 Figure 4
290 At 17 DAH, there were no longer statistical differences in label retention or
291 catabolism (Fig. 4b) between dietary treatments and also between sole fed *Artemia*
292 enriched with either PL or TAG. The same was observed when data were expressed in
293 DPM/mg of DW (results not shown) instead of been expressed in % of DPM, even
294 when corrected for the different *Artemia* labeling (DPM/*Artemia*) in *Artemia* enriched
295 with TAG or PL.
296
297 **4. Discussion**
298 *4.1 Co-feeding inert diet from the first feeding affects larval growth but not survival*
299 The survival rates observed in the present study are in the upper range of values
300 observed in previous studies with Senegalese sole larvae (Dinis et al., 1999; Cañavate

and Fernández-Díaz, 1999). Furthermore, early introduction of an inert diet since mouth opening (treatment Art R), did not affect the survival rate of Senegalese sole, compared to the standard feeding regime until 19 DAH. Similar results were found for other species (Kolkovski et al., 1997; Roselund et al., 1997), when an early co-feeding was attempted. Curnow et al. (2006) co-fed Asian sea bass *Lates calcarifer* on two different inert diets, and verified lower or higher survivals depending on fish size and diet type. It has also been suggested that co-feeding larval sole from 1 mg of larval weight may even improve survival rates at weaning, since it might enhance digestive maturation and/or stimulate digestive secretion (Engrola et al., 2007), even though a higher size dispersion could be observed.

Growth was significantly lower in the *Artemia* replacement treatment, compared to sole larvae fed only on live feed until the end of the pelagic phase. However, long-term effects of early co-feeding strategies in different species have been shown: enhanced growth and survival after weaning in Senegalese sole (Cañavate and Fernández-Díaz, 1999; Engrola et al., 2009a); increased survival rates and equivalent growth in length and weight in dourado *Salminus brasilienses* (Vega-Orellana et al., 2005); and improved growth and survival of tongue sole *Cynoglossus semilaevis* (Chang et al., 2006). Additionally, Yúfera et al. (2003) have demonstrated that Senegalese sole grew at a lower rate when fed exclusively with inert diet from 13 DAH onwards, than when fed on live feed.

Still, even if the growth rates observed in the *Artemia* replacement treatment were lower compared to the control group, they are within normal values for the Senegalese sole larval rearing (Cañavate and Fernández-Diáz, 1999; Engrola et al., 2007).

4.2 Lipid source and Artemia labeling

In this study *Artemia* was labeled by including a ^{14}C-oleic acid tracer in the enrichment emulsion, supplied esterified either to TAG or to PL. However, *Artemia* lipid digestibility and metabolism results cannot be completely and directly related to lipid class effect since it is well known that *Artemia* metanauplii cannot be considered a passive carrier of FA, and both labeled TAG and PL may have been transformed into other lipids by *Artemia*. In fact, an important fraction of the filtered lipids is digested, assimilated into the *Artemia* body and metabolised, and not just simply accumulated in the gut (Ando et al., 2004).

In addition to the differential metabolism of certain fatty acids, incorporated fatty acids redistribute themselves among lipid classes with high unpredictability, both during enrichment and particularly in starving conditions, after being added to the larval rearing tanks (Watanabe et al., 1982; Léger et al., 1986, 1987; Takeuchi et al., 1992; McEvoy et al., 1995, 1996; Navarro et al., 1999). In the present study, the lipid composition of the radiolabeled *Artemia* at the end of the enrichment period was not analyzed. Still, it is believed that the methodology used is valid to study the effect of enriching Artemia with PL or TAG, on lipid utilization by fish larvae.

This is the first time that *Artemia* enriched with ^{14}C-labeled lipids is used to study diet utilization in fish larvae. Previous metabolic studies have been carried out using a ^{14}C–protein hydrolysate where the label was incorporated in *Artemia* protein, in order to study the ontogeny of protein digestive capacity (Morais et al., 2004a) and the effect of feeding regime on protein utilization (Engrola et al., 2009b).

4.3 Artemia enrichment with phospholipids stimulates feed intake in young sole larvae

At 9 DAH sole larvae fed *Artemia* enriched with radiolabeled PL presented a 2.6-fold (ST) and 1.6-fold (Art R) higher feed intake when compared to the treatments fed

Artemia enriched with radiolabeled TAG. Furthermore, when the additional quantity of PL supplied by the radiolabeled lipid emulsion was calculated, a very low value (0.111 x 10^{-9} µg of L-3-phosphatidylcholine-1,2-di-oleoyl per *Artemia*) was found. This value is further reduced if it is considered that only a proportion would be found intact (i.e., non assimilated and metabolized) in the *Artemia* digestive tract at the time of larval ingestion. *Artemia* enriched with labeled L-3-phosphatidylcholine-1,2-di-[1-^{14}C] oleoyl (PL) seem to have a feeding stimulation effect in sole larvae at 9 DAH. However, the content of PL in the *Artemia* was not verified in the present study. Nevertheless, in postlarval penaeid shrimp it was demonstrated that dietary enrichment of *Artemia* with phosphatidylcholine did not enhance the *Artemia's* PC content (Tackaert et al., 1991). In gilthead seabream larvae an increased ingestion of microdiets with a higher level of PC was observed (Koven et al., 1994, 1998; Izquierdo et al., 2001). The dietary PC was found to be a feeding stimulant/attractant and significantly increased ingestion rates in 15-26 DAH gilthead seabream larvae; however it was no longer effective in 28 DAH and older larvae (Koven et al., 1998). The efficacy of dietary PL thus appears to reduce with age and may reflect the immature nature of the digestive system in marine fish larvae which lack a fully functional digestive tract until completion of metamorphosis (Munilla-Moran and Stark, 1989; Bisbal and Bengtson, 1995; Ribeiro et al., 1999; Bell et al., 2003). Iritani et al. (1984) have also shown that fish larvae have limited capacity for endogenous "de novo" PL biosynthesis, which may be insufficient to maintain an optimal rate of lipoprotein synthesis. This is in line with Morais (2005), who suggested that higher dietary requirement for PL is also probable in the earlier larval stages to sustain the fast growth and organogenesis, which likely require a high rate of membrane synthesis and turnover.

In Senegalese sole, at 17 DAH the level of ingestion of *Artemia* per sole larvae was independent of the type of *Artemia* enrichment. On the other hand, the smaller larvae from the Art R treatment ingested significantly more *Artemia* as percentage of DW compared to sole larvae fed live feed alone. This may eventually be explained by an attempt to compensate for the energy expended in metamorphosis in the *Artemia* replacement larvae, together with its delay in weight gain at this stage. Similar results were obtained by Engrola et al. (2009b) in similar ages.

4.4 Early co-feeding strategy affects lipid digestive capacity of larval sole

The higher *Artemia* intake when the enrichment with PL was used at 9 DAH in both ST and Art R treatments was not translated into statistical differences in digestibility. At this age, sole larvae seem to have a high digestive capacity to deal with lipid in live preys (up to 90% digestibility), independently of their feeding regime and of the source of lipid used to enrich *Artemia*. Therefore, sole early larvae seem to have better digestive capacity for lipids compared to protein since protein digestibility in 8 DAH sole was found to be 72.4% on a standard feeding regime and 70.4% on a co-feeding regime (Engrola et al., 2009b), and 83% at 12 DAH (Morais et al., 2004a).

At 17 DAH a significantly lower lipid digestibility was measured in the Art R treatment compared to the ST treatment and also in relation to 9 DAH. Sole larvae co-fed with inert diet from mouth opening have also a reduced protein digestibility during metamorphosis climax (16 DAH), compared to younger and older ages (Engrola et al. 2008). In fact, the metamorphosis climax is a critical developmental stage for sole larvae, and early adaptation to inert diets, in particular, seems to have a toll in terms of digestive efficiency. At this stage, growth, ingestion rates and oxygen consumption have been shown to decrease (Parra, 1998). The higher *Artemia* lipid digestibility in

larvae fed the ST dietary treatment when compared with the larvae co-fed with inert diet at 17 DAH, independently of the label used, may be explained by a faster development of the digestive system of these larvae, related to the higher larval DW in these treatments (ST). Cahu and Zambonino Infante (2001) observed that intestinal maturation might be stimulated but also irreversible impaired, depending on how co-feeding of live prey and inert diets is performed. In addition, Fernández-Díaz et al. (2006) observed that sole exclusively fed with microencapsulated diets had altered hepatic and gastrointestinal structures when compared to live feed-fed sole.

At 17 DAH a significantly higher digestibility was also observed in TAG-labeled compared to PL-labeled *Artemia*. As already mentioned, the fatty acid and lipid class composition of the radiolabeled *Artemia* was not determined and therefore the higher digestibility of TAG-labeled *Artemia* has to be discussed with caution. Earlier studies have showed that phospholipids have higher digestibility compared to TAG (Morais et al., 2007). Still, the present study suggests that enrichment of *Artemia* with PL may be less efficient to deliver oleic acid, and eventually other fatty acids, compared to TAG during metamorphosis climax of sole. These findings clearly deserve further study.

4.5 Co-feeding affects lipid retention and catabolism in sole larva

At 9 DAH lipid catabolism was significantly reduced and, concomitantly, body retention was significantly increased, when larvae were co-fed an inert diet since first feeding (Art R treatment). It has been suggested that young larvae may have the ability to compensate for an eventual lower digestibility with a higher retention of absorbed amino acids (Morais et al., 2004a). The same idea could be used to explain the present results in relation to lipids, if it is assumed that the inert diet is less digestible than live

prey during the initial stages of development. In that case, larvae co-fed simultaneously with live prey and inert diet might have a lower overall digestive efficiency.

At 17 DAH both lipid catabolism and retention presented no statistical differences between treatments which, comparing with data at 9 DAH, suggests metabolic changes as metamorphosis proceeds. In fact, lipid catabolism was 2-fold higher (ca. 50% compared to ca. 20% of total absorbed lipid) at 9 DAH compared to 17 DAH. This suggests that lipid may be less important as an energy fuel during metamorphosis climax. Alternatively, this may be a result of a selective pressure towards an increase in body lipid as energy reserves in the stages prior to metamorphosis (hence higher lipid retention), to compensate for the reduction in prey consumption during this period (Youson, 1988; Parra, 1998). Then, an eventual high mobilization of endogenous lipid reserves during metamorphosis climax may explain the present results. Still, the present study demonstrates that lipid retention efficiency remains constant independently of feeding regime and lipid source used in *Artemia* enrichment during metamorphosis climax. Therefore, it seems that partial replacement of *Artemia* by an inert diet does not seem to compromise larval lipid metabolism.

5. Conclusion

Co-feeding sole larvae with inert diet since mouth opening did not affect survival rate but resulted in smaller size of sole larvae. Although the lipid tracer used to radiolabel the *Artemia* was most likely at least partly assimilated and metabolized by the *Artemia* in an unpredictable way, a fraction of the PL label remaining in the *Artemia* digestive tract might have had a stimulant effect in feed intake at 9 DAH, but no longer at 17 DAH. In addition, while no difference was noticed at 9 DAH in lipid digestibility, the ST-TAG treatment showed the highest lipid digestibility at 17 DAH, most probably as a

result of a more advanced maturation of the digestive system. Furthermore, at 9 DAH the co-feeding regime reduced lipid catabolism and concomitantly increased lipid retention in larval sole, which may be an adaptation to a feeding regime with lower digestibility. However, lipid retention was high in all treatments at 17 DAH, most likely as a response to morphological and physiological changes that takes place in the larval body during the metamorphosis climax.

In short, co-feeding of live fed and inert diet from first-feeding in Senegalese sole (*Solea senegalensis*) has a toll in terms of growth and lipid digestibility but does not seem to compromise lipid metabolic utilization.

6. Acknowledgements

The authors wish to thank Helena Teixeira for practical assistance. Grant to Mônica Mai (SWE 201887/2007-0) from "Conselho Nacional de Desenvolvimento Científico e Tecnológico" - CNPq (Brasil) supported this work. This study benefited from funding by Project PROMAR/SP5.P117/03 (program INTERREG III A, co-funded by FEDER, European Commission).

7. References

Ando, Y., Samoto, H., Murayama, Y. 2004. Positional distribution of DHA and EPA in triacyl-*sn*-glycerols (TAG) of *Artemia franciscana* nauplii enriched with fish oils ethyl esters and TAG. Aquaculture 233, 321-335.

Bell, J.G., McEvoy, L.A., Estevez, A., Shields, R.J., Sargent, J.R. 2003. Optimising lipid nutrition in first-feeding flatfish larvae. Aquaculture 227, 211-220.

474 Bisbal, G.A., Bengtson, D.A. 1995. Development of digestive tract in larval summer
475 flounder. J. Fish Biol. 47, 277–291.
476 Cahu, C., Zambonino Infante, J. 2001. Substitution of live food by formulated diets in
477 marine fish larvae. Aquaculture 200, 161-180.
478 Cañavate, J.P., Fernández-Díaz, C. 1999. Influence of co-feeding larvae with live and
479 inert diets on weaning the sole *Solea senegalensis* onto commercial dry feeds.
480 Aquaculture 174**,** 255-263.
481 Chang, Q., Liang, M.Q., Wang, J.L., Chen, S.Q., Zhang, X.M., Liu, X.D. 2006.
482 Influence of larval co-feeding with live and inert diets on weaning the tongue
483 sole *Cynoglossus semilaevis*. Aquacult. Nutr. 12 (2), 135-139.
484 Conceição, L.E.C., Verreth, J.A.J., Verstegen, M.W.A., Huisman, E.A. 1998. A
485 preliminary model for dynamic simulation of growth in fish larvae: application
486 to the African catfish (*Clarias gariepinus*) and turbot (*Scophthalmus maximus*).
487 Aquaculture 163, 215-235.
488 Conceição, L.E.C., Morais, S., Rønnestad, I. 2007a. Tracers in fish larvae nutrition: A
489 review of methods and applications. Aquaculture 267, 62-75.
490 Conceição, L.E.C., Ribeiro, L., Engrola, S., Aragão, C., Morais, S., Lacuisse, M.,
491 Soares, F., Dinis, M.T. 2007b. Nutritional physiology during development of
492 Senegalese sole (*Solea senegalensis*). Aquaculture 268, 64-81.
493 Coutteau, P., Geurden, I., Camara, M.R., Bergot, P., Sorgeloos, P. 1997. Review on the
494 dietary effects of phospholipids in fish and crustacean larviculture. Aquaculture
495 155, 149-164.
496 Curnow, J., King, J., Partridge, G., Kolkovski, S. 2006. Effects of two commercial
497 microdiets on growth and survival of barramundi (*Lates calcarifer* Bloch) larvae
498 within various early weaning protocols. Aquacult. Nut. 12, 247–255.

499 Díaz, J.P., Guyot, E., Vigier, S., Connes, R. 1997. First events in lipid absorption during
500 post-embryonic development of the anterior intestine in gilt-head sea bream. J.
501 Fish Biol. 51, 180-192.
502 Dinis, M.T., Ribeiro, L., Soares, F., Sarasquete, C. 1999. A review on the cultivation
503 potential of *Solea senegalensis* in Spain and in Portugal. Aquaculture 176**,** 27-
504 38.
505 Engrola, S. 2008. Improving growth performance of Senegalese sole postlarvae. PhD
506 Thesis. Universidade do Algarve, Faro, Portugal, 167 pp.
507 Engrola, S., Conceição, L.E.C, Gavaia, P.J., Cancela, M.L., Dinis, M.T. 2005. Effects of
508 pre-weaning feeding frequency on growth, survival, and deformation of
509 Senegalese sole, *Solea senegalensis* (Kaup, 1858). Isr. J. Aquac.-Bamidgeh 57,
510 10-18.
511 Engrola, S., Conceição, L.E.C., Dias, L., Pereira, R., Ribeiro, L., Dinis, M.T. 2007.
512 Improving weaning strategies for Senegalese sole: effects of body weight and
513 digestive capacity. Aquacult. Res. 38, 696-707.
514 Engrola, S., Figueira, L., Conceição, L.E.C., Gavaia, P., Ribeiro L., Dinis M.T. (2009a).
515 Co-feeding in Senegalese sole larvae with inert diet from mouth opening
516 promotes growth at weaning. Aquaculture (in press).
517 doi:10.1016/j.aquaculture.2008.12.010
518 Engrola, S., Mai, M.G., Dinis, M.T., Conceição, L.E.C. (2009b). Co-feeding of inert
519 diet from mouth opening does not impair protein utilization by Senegalese sole
520 (Solea senegalensis) larvae, Aquaculture 287, 185–190.
521 Esteban, J.C., Calderon J.A., Carrascosa M., Pecci, F., 1995. Cria larvaria, destete y
522 preengorde del lenguado (*Solea senegalensis*) realizado en el departamento de
523 cultivos marinos del IES Sancti-Petri. In: F. Castelló i Orvay, A. Calderer i Reig

(Eds.), V Congresso Nacional de Acuicultura. Universitat de Barcelona, May 10-13, Sant Carles de la Ràpita, Spain, pp. 426-431.

Fernández-Díaz, C., Kopecka, J., Cañavate, J.P., Sarasquete, C., Sole, M. 2006. Variations on development and stress defences in Solea senegalensis larvae fed on live and microencapsulated diets. Aquaculture 251, 573-584.

Geurden, I., Radünz-Neto, J., Gergot, P. 1995. Essentiality of dietary phospholipids for carp (*Cyprinus carpio* L.) larvae. Aquaculture 131, 303-314.

Howell, B.R. 1997. A re-appraisal of the potential of the sole, *Solea solea* (L.), for commercial cultivation. Aquaculture 155, 359-369.

Imsland, A.K., Foss, A., Conceição, L.E.C., Dinis, M.T., Delbare, D., Schram, E., Kamstra, A., Rema, P., White, P. 2003. A review of the culture potential of *Solea solea* and *S. senegalensis*. Rev. Fish Biol. Fish. 13**,** 379-407.

Iritani, N., Ikeda, Y., Fukuda, H., Katsurada, A., 1984. Comparative study of lipogenic enzymes in several vertebrates. Lipids 19, 825-835.

Izquierdo, M.S., Tandler, A., Salhi, M., Kolkovski, S. 2001. Influence of dietary polar lipids' quantity and quality on ingestion and assimilation of labelled fatty acids by larval gilthead seabream. Aquacult. Nutr. 7, 153-160.

Kolkovski, S., Tandler, A., Izquierdo, M. S. 1997. Effects of live food and dietary digestive enzymes on the efficiency of microdiets for seabass (*Dicentrarchus labrax*) larvae. Aquaculture 148, 313-322.

Koven, W.M., Henderson, R.J., Sargent, J.R. 1994. Lipid digestion in turbot (*Scophthalmus maximus*). I: Lipid class and fatty acid composition of digesta from different segments of the digestive tract. Fish Physiol. Biochem. 13, 69-79.

Koven, W.M., Parra, G., Kolkovski, S., Tandler, A. 1998. The effect of dietary phosphatidylcholine and its constituent fatty acids on microdiet ingestion and

549 fatty acid absorption rate in gilthead seabream, Sparus auratus, larvae. Aquacult.
550 Nutr. 4, 39-45.
551 Léger, P., Bengtson, D.A., Simpson, K.L., Sorgeloos, P., 1986. The use and nutritional
552 value of *Artemia* as a food source. Oceanogr. Mar. Biol. Annu. Rev. 24, 521-
553 623.
554 Léger, P., Bengtson, D.A., Sorgeloos, P., Simpson, K.L., Beck, A.D., 1987. The
555 nutritional value of *Artemia*: a review. In: Sorgeloos, P., Bengtson, D.A.,
556 Decleir, W., Jaspers, E. (Eds.), *Artemia* Research and its Applications. Ecology,
557 Culturing, Use in Aquaculture. Universa Press, Wetteren, pp. 357-372.
558 Marin-Magan V., Anguis V., Cañavate, J.P., 1995. Uso de alimento inerte en larvas y
559 alevines del lenguado Solea senegalensis. In: F. Castelló i Orvay, A. Calderer i
560 Reig (Eds.), V Congresso Nacional de Acuicultura. Universitat de Barcelona,
561 May 10-13, Sant Carles de la Ràpita, Spain, pp. 432-436.
562 McEvoy, L.A., Navarro, J.C., Bell, J.G., Sargent, J.R. 1995. Autoxidation of oil
563 emulsions during the *Artemia* enrichment process. Aquaculture, 134, 101-112.
564 McEvoy, L.A., Navarro, J.C., Hontoria, F., Amat, F., Sargent, J.R. 1996. Two novel
565 *Artemia* enrichment diets containing polar lipid. Aquaculture, 144, 339-352.
566 Morais, S., Lacuisse, M., Conceição, L.E.C., Dinis, M.T., Rønnestad, I. 2004a.
567 Ontogeny of the digestive capacity of Senegalese sole (*Solea senegalensis*), with
568 respect to digestion, absorption and metabolism of amino acids from *Artemia*.
569 Mar. Biol., 145, 243-250.
570 Morais, S., Conceição, L.E.C., Dinis, M.T., Rønnestad, I. 2004b. A method for
571 radiolabeling *Artemia* with applications in studies of food intake, digestibility,
572 protein and amino acid metabolism in larval fish. Aquaculture, 231, 469-487.

573 Morais, S. 2005. Digestive physiology and food intake in marine fish larvae with
574 respect to dietary neutral lipids . PhD Thesis. Universidade do Algarve, Faro,
575 219 pp.
576 Morais, S., Torten, M., Nixon, O., Lutzky, S., Conceição, L.E.C., Dinis, M.T., Tandler,
577 A., Koven, W. 2006. Food intake and absorption are affected by dietary lipid
578 level and lipid source in seabream (*Sparus aurata* L.) larvae. J. Exp. Mar. Biol.
579 Ecol. 331, 51-63.
580 Morais, S., Conceição, L.E.C., Rønnestad, I., Koven, W., Cahu, C. 2007. Dietary
581 neutral lipid level and source in marine fish larvae: Effects on digestive
582 physiology and food intake. Aquaculture 268, 106-122.
583 Munilla-Moran, R., Stark, J.R. 1989. Protein digestion in early turbot larvae,
584 *Scophthalmus maximus* (L.). Aquaculture 81, 315-327.
585 Navarro, J.C., Henderson, R.J., McEvoy, L.A., Bell, M.V., Amat, F. 1999. Lipid
586 conversions during enrichment of *Artemia*. Aquaculture 174, 155-166.
587 Parra, G. 1998. Fisiología y balance energético durante el desarrollo larvario de peces
588 marinos (*Solea senegalensis* Kaup y *Sparus aurata* Linneo) en cultivo. Tesis
589 doctoral, Universidad de Cádiz, 176 pp.
590 Ribeiro, L., Zambonino-Infante, J.L., Cahu, C., Dinis, M.T. 1999. Development of
591 digestive enzymes in larvae of *Solea senegalensis*, Kaup 1858. Aquaculture 179,
592 465-473.
593 Ribeiro, L., Zambonino-Infante, J.L., Cahu, C., Dinis, M.T. 2002. Digestive enzymes
594 profile of *Solea senegalensis* post larvae fed *Artemia* and a compound diet. Fish
595 Physiol. Biochem. 27**,** 61-69.

596 Rønnestad, I., Rojas-García, C.R., Tonheim, S.K., Conceição, L.E.C. 2001. In vivo
597 studies of digestion and nutrient assimilation in marine fish larvae. Aquaculture
598 201, 161-175.
599 Rosenlund, G., Stoss, J., Talbot, C. 1997. Co-feeding marine fish larvae with inert and
600 live diets. Aquaculture 155, 183-191.
601 Salhi, M., Kolkovski, S., Izquierdo, M.S., Tandler, A. 1995. Inclusion of lecithin and
602 polar or neutral lipids high in n-3 HUFA in microdiets for gilthead seabream
603 *Sparus aurata* larvae. In: Lavens, P., Jaspers, E., Roelants, I. (Eds.), Proceedings
604 of the Fish and Shellfish Larviculture Symposium - Larvi'95 (European
605 Aquaculture Society, Special Publication, vol. 24. European Aquaculture
606 Society, Gent, Belgium, pp. 184-187.
607 Salhi, M., Izquierdo, M.S., Hernandez-Cruz, C.M., Socorro, J., Fernández-Palacios, H.
608 1997. The improved incorporation of polyunsaturated fatty acids and changes in
609 liver structure in larval gilthead seabream fed on microdiets. J. Fish Biol. 51,
610 869-879.
611 Salhi, M., Hernández-Cruz, C.M., Bessonart, M., Izquierdo, M.S., Fernández-Palacios,
612 H. 1999. Effect of different dietary polar lipid levels and different n-3 HUFA
613 content in polar lipids on gut and liver histological structure of gilthead
614 seabream (*Sparus aurata*) larvae. Aquaculture 179, 253- 263.
615 Sargent, J., Henderson, R.J., Tocher, D.R. 1989. The lipids. In: Halver, J.E. (Ed.), Fish
616 Nutrition. Academic Press, London, pp. 154-218.
617 Tackaert, W., Camara, M.R., Sorgeloos, P. 1991. The effect of dietary
618 phosphatidylcholine in postlarval penaeid shrimp: I. Diet preparation. In:
619 Lavens, P., Sorgeloos, P., Jaspers, E., Ollevier, F. (Eds.), Larvi'91: Fish and

620 Crustacean Larviculture Symposium. European Aquaculture Society, Special
621 Publication No.15, Gent, Belgium, pp. 76-79.
622 Takeuchi, T., Arakawa, T., Satoh, S., Watanabe, T., 1992. Supplemental effect of
623 phospholipids and requirement of eicosapentaenoic and docosahexaenoic acid of
624 juvenile striped jack. Nippon Suisan Gakk. 58, 707-713.
625 Vega-Orellana, O.M., Fracalossi, D.M., Sugai, J.K., 2006. Dourado (*Salminus*
626 *brasiliensis*) larviculture: Weaning and ontogenetic development of digestive
627 proteinases. Aquaculture 252, 484-493.
628 Youson, J.H. 1988. Fish metamorphosis. In: Hoar, W.S., Randall, D.J. (Eds.), Fish
629 Physiology, vol. XI, Part B. Academic Press, New York, pp. 135-196.
630 Yúfera M., Kolkovski S., Fernández-Díaz C., Rinchard J., Lee K.J., Dabrowski K..
631 2003. Delivering bioactive compounds to fish larvae using microencapsulated
632 diets. Aquaculture 227, 277-291.
633 Watanabe, T., Ohta, M., Kitajima, C., Fujita, S. 1982. Improvement of dietary value of
634 brine shrimp *Artemia* salina for fish larvae feeding them on w-3 highly
635 unsaturated fatty acids. B. Jpn. Soc. Sci. Fish. 48, 1775-1782.
636 Zar, J.H. 1996. Biostatistical Analysis. Prentice Hall International, NJ, USA. 662 pp.

637
638

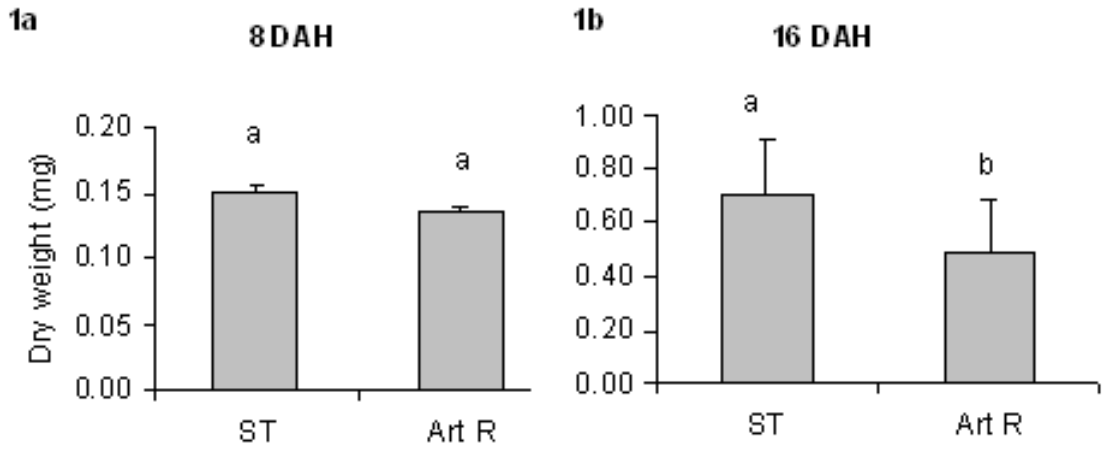
Figure 1 – Senegalese sole dry weight (mg) at 9 days after hatching (DAH) (1a, n = 30 pooled larvae) and 16 DAH (1b, n = 15). ST: Standard feeding regime; Art R: Partial *Artemia* replacement by inert diet from mouth opening. Values are means ± SD. Different letters for the same age indicate statistical differences between treatments ($P < 0.05$, ANOVA).

Figure 2 – *Artemia* intake of sole larvae at 9 (2a) and 17 days after hatching (DAH) (2b). Values are means ± SD (2a: n=13 to ST PL; n=15 to ST TAG; n=14 to ArtR TAG; n=14 to ArtR PL. 2b: n=15 for all treatments). ST – TAG (Standard feeding regime + *Artemia* enriched with glycerol tri [1-^{14}C] oleate - TAG); ST – PL (Standard feeding regime + *Artemia* enriched with L-3-phosphatidylcholine-1,2-di-[1-^{14}C] oleoyl - PL); Art R – TAG (*Artemia* replacement feeding regime + *Artemia* enriched with TAG) and Art R - PL (*Artemia* replacement feeding regime + *Artemia* enriched with PL). Different letters at the same age indicates statistical differences ($P < 0.05$, two way ANOVA) between feeding regimes (a, b) or lipid sources (x, y).

Figure 3 – *Artemia* lipid digestibility in sole at 9 (3a) and 17 days after hatching DAH (3b). Values are means ± SD (3a: n=13 to ST PL; n=15 to ST TAG; n=14 to ArtR TAG; n=14 to ArtR PL. 3b: n=15 for all treatments). ST – TAG (Standard feeding regime + *Artemia* enriched with glycerol tri [1-^{14}C] oleate - TAG); ST – PL (Standard feeding regime + *Artemia* enriched with L-3-phosphatidylcholine-1,2-di-[1-^{14}C] oleoyl - PL); Art R – TAG (*Artemia* replacement feeding regime + *Artemia* enriched with TAG) and Art R - PL (*Artemia* replacement feeding regime + *Artemia* enriched with PL). Different letters at the same age indicates statistical differences ($P < 0.05$, two way ANOVA) between feeding regimes (a, b) or lipid sources (x, y).

Figure 4 – Lipid retention efficiency and catabolism determined in sole at 9 (4a) and 17 days after hatching DAH (4b). Retention (R, %) and Catabolism (C, %) were calculated as: $R = [R_{body}/(R_{body} + R_{CO_2\ trap})] \times 100$; $C = [R_{CO_2\ trap}/(R_{body} + R_{CO_2\ trap})] \times 100$; respectively, where R_{body} is the total radioactivity in fish body (DPM) and $R_{CO_2\ trap}$ is the total radioactivity per CO_2 trap (DPM.

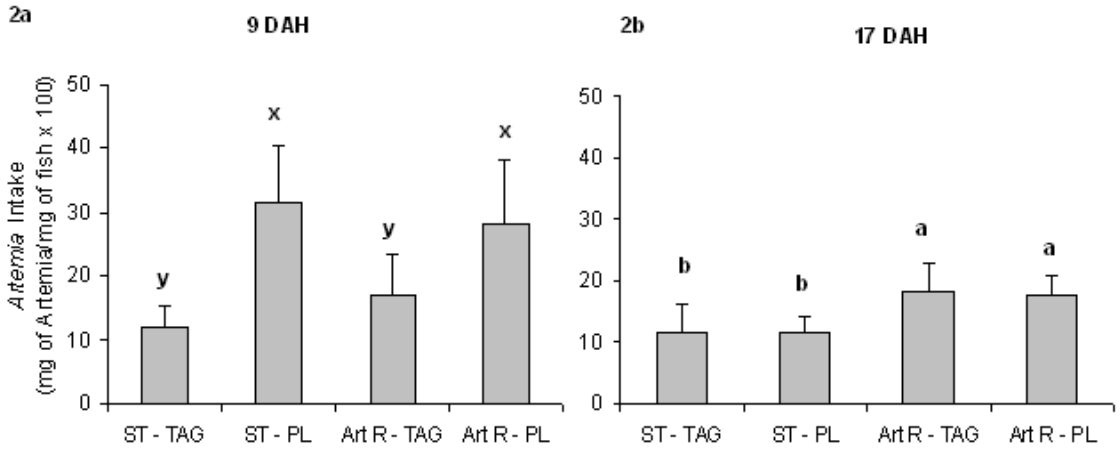
Values are means ± SD (4a: n=13 to ST PL; n=15 to ST TAG; n=14 to ArtR TAG; n=14 to ArtR PL. 4b: n=15 for all treatments). ST – TAG (Standard feeding regime + *Artemia* enriched with glycerol tri [1-^{14}C] oleate - TAG); ST – PL (Standard feeding regime + *Artemia* enriched with L-3-phosphatidylcholine-1,2-di-[1-^{14}C] oleoyl - PL); Art R – TAG (*Artemia* replacement feeding regime + *Artemia* enriched with TAG) and Art R - PL (*Artemia* replacement feeding regime + *Artemia* enriched with PL). Different letters at the same age indicate statistical differences ($P < 0.05$, two way ANOVA) between feeding regimes (a, b).



679

680 Fig 1

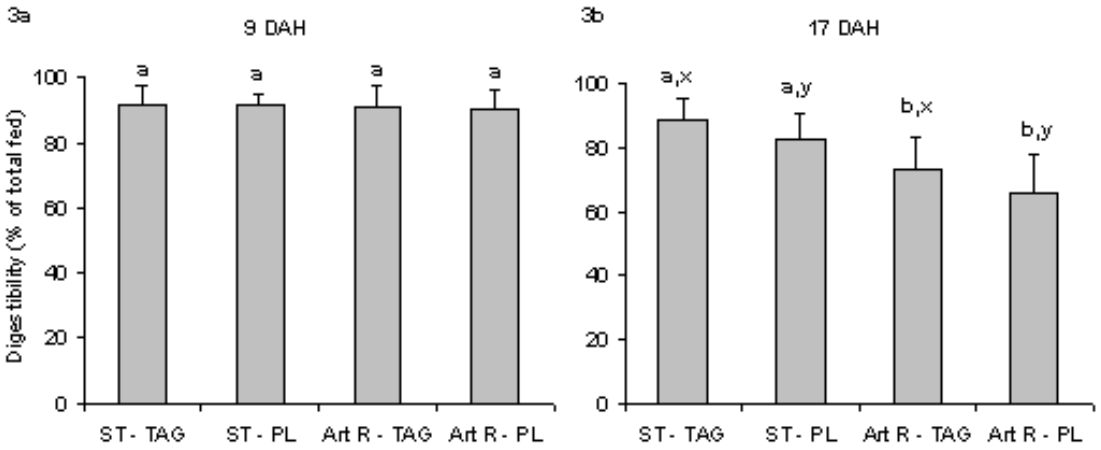
681



682

683 Fig 2

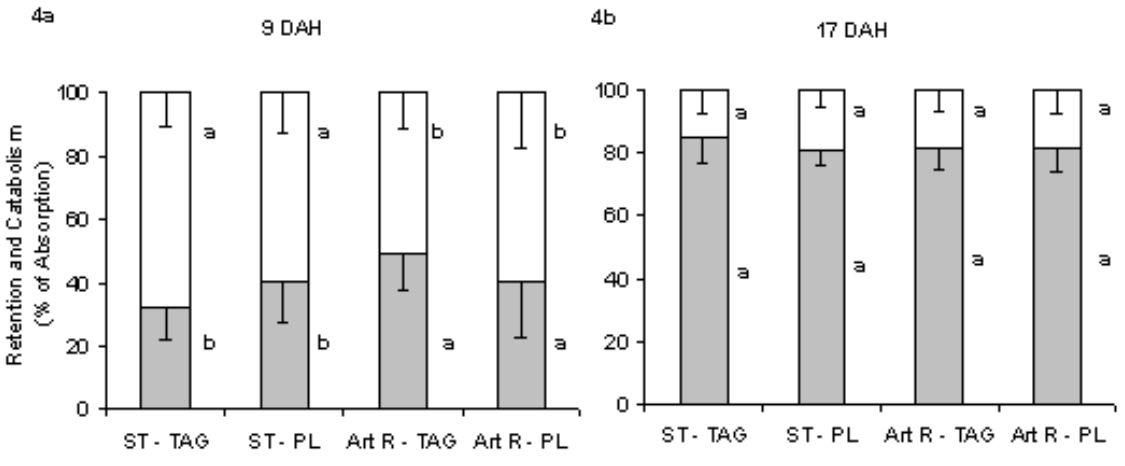
684



685

686 Fig 3

687


4a
9 DAH
4b
17 DAH
Retention and Catabolism (% of Absorption)
100
80
60
40
20
0
a
b
a
b
b
a
b
a
a
a
a
a
a
a
a
a
ST - TAG
ST - PL
Art R - TAG
Art R - PL
ST - TAG
ST - PL
Art R - TAG
Art R - PL

688

689 Fig 4