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**Weaning strategies affect larval performance in yellowfin seabream (*Acanthopagrus latus*)**

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**Running head:** Effect of weaning strategies on yellowfin seabream larvae

**Keywords:** Alkaline phosphatase, larviculture, leucine-alanine peptidase, live food, Sparidae

**Abstract**

A 30-day study was carried out to determine the influence of different weaning strategies (WS) on growth and survival rates, stress resistance, digestive enzyme activities and whole body fatty acid profiles in yellowfin seabream (*Acanthopagrus latus*) larvae. Thus, eight WS were designed by combining live foods (LF) (rotifers and *Artemia*) and a microdiet (MD) (Gemma Micro$^{TM}$, Skretting). In the control group (100 LF), larvae were fed only LF (100%), including enriched rotifers (from 2 to 20 days post hatching, dph) and *Artemia* (nauplii + enriched metanauplii, from 18 to 30 dph). The other treatments were based on different proportions of LF with the MD: 75LF-25MD, 50LF-50MD, 25LF-75MD and 100 MD in which 25, 50, 75 and 100% of LF that applied in the control group were replaced with a fixed ration of MD, respectively. In addition, for determining the appropriate time of weaning, three WS were applied including: sudden weaning (SW): larvae were weaned on to MD with only rotifer from 2 to 5 days post hatch (dph); early weaning (EW): larvae were weaned on to MD with only rotifer from 2 to 14 dah and late weaning (LW): larvae were weaned on to MD with rotifer (from 2 to 14 dph) and *Artemia* nauplii (from 12 to 20 dph). Larvae were hand-fed with the MD hourly from 08:00 h to 18:00 h (11 times a day). Results showed that the lowest survival rates were found among larvae from 100MD (1.5%) and SW (1.9%) groups, whereas the highest survival rates were found in larvae from 100LF (31.0 ± 1.7%), 75LF-25MD (34.7 ± 4.5%) and 50LF-50MD (36.2 ± 4.0%). Larvae from the 50LF-50MD group showed the highest resistance against air exposure (70.0%) and osmotic stress (100.0%) challenges. At the end of the trial, larvae from 100LF, 75LF-25MD, 50LF-50MD and LW groups showed higher growth performance than the other groups. At 30 dph, larvae from the LW group had the highest level of alkaline phosphatase to leucine-alanine peptidase ratio, while those from

the 100MD, SW and EW treatments showed the lowest ones. The fatty acid profile of larvae reflected that of the diet. In particular, larvae from 100MD and SW groups had generally lower levels of n-3 long chain polyunsaturated fatty acids compared to the other treatments. In conclusion, results of the present study indicated the time of weaning and proportion of LF and MD remarkably affect larvae performance in terms of growth, survival, digestive function and stress tolerance. In addition, the replacement of LF with MD up to 50% after mouth opening of larvae neither have detrimental effects on growth, survival and digestive enzymes activities, meanwhile enhanced stress resistance of *A. latus* larvae.

## 1. Introduction

The availability of high-quality fingerlings for on-growing is one of the most critical factors affecting commercial success in fish farming. Successful larval production depends on a wide range of biotic and abiotic factors, as well as on the optimization of the zootechnical conditions for rearing (*e.g.* larval density, feeding protocol, health management, among others). In this sense, the main challenge in the mass propagation of marine fish is the high mortality during larval stages that it is mostly related to lack a fully functional immune system, sensory organs, undeveloped digestive system and, inappropriate nutrition and culture conditions during this critical developmental period (Hamre et al., 2013). The limited digestive capacities in marine finfish larvae, especially at the onset of exogenous feeding, restrict them to feed on specific food types, mainly live food (LF) organisms, which are characterized by their high digestibility (Zambonino-Infante and Cahu, 2001). However, the nutritional quality of conventional LF, namely rotifers (*Brachionus* spp.) and *Artemia* nauplii and metanauplii, is inferior (*e.g.* nutritional deficiencies on long chain polyunsaturated fatty acids (LC-PUFA), phospholipids, water-soluble nutrients, vitamins and trace elements) compared to natural preys such as copepods (Kandathil

Radhakrishnan et al., 2020). Furthermore, the manipulation of their nutritional value is difficult especially regarding their protein content and water-soluble nutrients (*e.g.* taurine, selenium) (Hamre et al., 2008; Kandathil Radhakrishnan et al., 2020). In addition, the production of LF is a labor intensive activity that requires a large amount of hatchery spaces and specialized infrastructures, which involves a non-negligible production cost. Moreover, it has been suggested that LF can carry a high load of bacteria, including opportunistic bacteria namely *Vibrio* spp. that may cause larval diseases and mass mortality episodes (Vadstein et al., 2013). Thus, the substitution of LF by artificial microdiets (MD) through an optimal weaning procedure would reduce larval production costs, promote the standardization of larval rearing procedures, and control the accurate delivery of nutrients to fish larvae according to their nutritional requirements (Hamre et al., 2013; Wang et al., 2020). In this regard, the determination of the appropriate time for the onset of larval weaning, optimal feeding frequency and ration should be considered for establishing an optimal weaning strategy during fish larval stages (Rosenlund et al., 1997; Hamre et al., 2013). Other parameters that may also affect the success of larval weaning are the developmental stage (*e.g.* size, digestive system and sensory organs), behavior of larvae, the physico-chemical characteristics of MD (*e.g.* nutritional composition, attractability, palatability, digestibility, color, shape, sedimentation rate and size), weaning strategy (WS) and culture environment (Faulk et al., 2007; Conceição et al., 2010; Sáenz de Rodrigáñez et al., 2011; Hamre et al., 2013; Kandathil Radhakrishnan et al., 2020 among others). An improper WS could result in a progressive starvation, chronic stress, retardation of larval development, compromising larval performance that finally may led to high mortalities or improper larval quality (Cahu and Zambonino-Infante, 2001; Engrola et al., 2007; Faulk and Holt, 2009; Pradhan et al., 2014; Mesa-Rodriguez et al., 2018 among others). Different WS have been widely used in larviculture studies.

For instance, weaning can be done gradually by co-feeding larvae with LF and MD, and progressively replacing one type of one prey type by another, or the transition from LF to MD may be conducted abruptly. However, several studies reported that first-feeding of marine fish larvae with MD may result in low survival rates and growth reduction, which has been mainly attributed to several factors, such as i) low palatability, inferior nutritional quality and digestibility of MD; ii) difficulties in MD digestion due to lower activity of digestive enzymes and lack of acid digestion in newly hatch larvae; and iii) nutrients leakage from MD that reduce the nutritional quality of MD and result in poor water quality (Hamre et al., 2013). Thus, the application of a co-feeding strategy based on the progressive transition from LF to MD can alleviate the disadvantageous characteristics of MD (Holt et al., 2011), although this approach needs to be done at a species-specific level. Furthermore, LF have various nutritional factors that not only compensate nutritional values of MD, but also promote gut maturation by inducing pancreatic enzymes secretion and hormones/neurotransmitters and allow conditioning of larvae to early weaning (Kolkovski et al., 1997; Kolkovski, 2001; Fletcher et al., 2007; Conceicao et al., 2010; Hamre et al., 2013). On the other hand, it has been suggested that early weaning facilitate the efficient conditioning of larvae to accept MD easier, stabilize the larval nutritional condition and result in shorter weaning period (Hamre et al., 2013). In addition, several studies reported the pronounced effects of different WS on the whole body fatty acid profile of marine fish larvae such as common sole (*Solea solea* L., Bonaldo et al., 2011), red porgy (*Pagrus pagrus*, Andrade et al., 2012), gilthead sea bream (*Sparus aurata* L., Pantazis et al., 2014) and Florida Pompano (*Trachinotus carolinus*, Hauville et al., 2014). In marine fish larvae, LC-PUFA have vital role in organogenesis and formation of cellular membranes and remarkably affect physiological processes such as growth, survival and stress resistance (Tocher, 2010; Hamre et al., 2013). Marine fish larvae have

no or negligible capacity in biosynthesis of LC-PUFA; thus, the schedule of WS should be designed to ensure the provision of extra level of LC-PUFA for larvae. This is of special relevance when using enriched live preys during early life stages, since enrichment procedures may substantially affect the fatty acid profiles of rotifers and *Artemia* metanauplii, whereas that in terms of protein and amino acid content are considered to be more stable (Øie et al., 2011; Maehre et al., 2013)

The yellowfin seabream (*Acanthopagrus latus*) has the desirable characteristics for aquaculture such as a great resistance to environmental changes (*e.g.* temperature and salinity), high fecundity, adaptation to reproduce in captivity, tolerance to the high stocking densities, suitable growth rate and feed conversion ratio (Vahabnezhad et al., 2016). Previous study by Leu et al. (1991) demonstrated that replacement of 67% LF (rotifer and *Artemia*) with MD remarkably improved growth performance and survival rates in *A. latus* larvae from 10 to 30 dph; but total replacement of LF with MD drastically reduced survival and growth in this species. In the previous study, authors used handmade zein micro-coated diet which have inferior quality compared to novel high-tech commercial MD. In addition, Sarvi et al. (2010) reported that up to 50% of rotifer (*B. rotundifourmis*) can be replaced by MD (Caviar, Bernaqua, Olen, Belgium) from 2 to 15 dph; however, over 50% replacement pronouncedly reduced growth, survival rate and stress resistance in *A. latus*. However, the above-mentioned authors only replaced rotifer with MD for two-weeks and they did not use *Artemia*; thus, the above-mentioned study may be considered as incomplete as not all types of LF were included during weaning, whereas a proper time for the onset of weaning was not indicated. General feeding protocol for *A. latus* larvae at 21–23 ºC consists of onset of feeding with rotifer (*Brachionus rotundiformis*) from mouth opening up to 20 days post-hatching (dph), *Artemia* (newly hatched and metanauplii) between 18 to 40 dph, followed by

weaning with MD between 35 and 40 dph (Sarvi et al., 2010). In the current research, the best WS for *A. latus* larvae from 2-30 dph was determined by considering the growth, survival rate, stress resistance, digestive enzymes activity and whole body fatty acid profile.

## 2. Materials and Methods

### *2.1. Experimental design*

Eight WS were carried out by applying different combinations of a commercial MD (Skretting® GemmaMicro™; particle sizes: 75 and 150 µm) and LF, including super small type marine rotifer (*B. rotundiformis*) and *Artemia* (Table 1), as well as different weaning times. The MD composition contained fish meal (native protein and protein hydrolysate), lecithin, wheat gluten, dried seaweed, fish oil, maize starch, vitamins and minerals (data provided by manufacturer; Table 2). Each feeding protocol was tested in triplicate from 2 (mouth opening and first feeding) to 30 dph:

1) 100 LF. Larvae were fed only LF (100%), including rotifers (from 2 to 20 dph) and *Artemia* (nauplii + enriched metanauplii, from 18 to 30 dph). Nauplii were introduced into tanks from 18 to 20 dph followed by enriched metanauplii from 21-30 dph. Live prey density in larval rearing tanks is shown in Table 1.

2) 75LF-25MD. Larvae were fed the same protocol as 100 LF, but 25% of LF was replaced by the MD as indicated in Table 1.

3) 50LF-50MD. Considering the 100 LF, 50% of LF was replaced by the MD as indicated in Table 1.

4) 25LF-75MD. Considering the 100 LF, 75% of LF was replaced by the MD.

5) 100 MD. Larvae fed only with the MD for the entire experimental period (2-30 dph). Changes in MD particle size are detailed in Table 1.

6) Sudden weaning (SW): co-feeding of MD with rotifers from 2 to 5 dph followed by feeding only with the MD from 6 to 30 dph.

7) Early weaning (EW): co-feeding of MD with rotifers from 2 to 14 dph followed by feeding only with the MD from 15 to 30 dph.

8) Late weaning (LW): co-feeding of MD with rotifers from 2 to 14 dph and inclusion of *Artemia* nauplii from 12 to 20 dph, followed by feeding only with the MD from 20 to 30 dph.

The amount of supplied MD were fixed between 0.4 to 2.0 g $tank^{-1}$ $day^{-1}$ from 2 to 30 dah according to the larval developmental stage, following the MD manufacturer's recommendations (Table1). The proximate composition and main ingredients of MD is reported in Table 2. The fatty acid profile of LF and the MD were presented in Table 3.

### *2.2. Larval rearing*

The current study was run in the Aquatic Research Laboratory of the Persian Gulf University, Bushehr (Iran). Fertilized eggs were obtained from wild-caught brooders (0.25–0.45 kg) including 10 males and 10 females (1:1 ratio) that were stocked in a 4000-L circular fiberglass tank. The broodstock tank was supplied with sand-filtered and disinfected seawater (temperature: 20 °C; salinity: 40) and it was kept under artificial photoperiod (12 light:12 darkness). Broodfish were fed with chopped shrimp, squid and fish supplemented with vitamin premix and fish oil. Broodfish naturally spawned (without hormone injection) in the early spring season and every morning floating fertilized eggs (*ca.* 80% fertilization rate) were collected using a 300 μm net. The fertilized

eggs (100 eggs $L^{-1}$) were incubated in 220-L cylindrical polyethylene tanks that supplied with gentle aeration in darkness. About 75-80% of fertilized eggs hatched after 18 to 24 h depends on water temperature (21-23ºC).

One hundred and twenty thousand newly hatched larvae (1 dph) were randomly collected from hatching tanks and carefully stocked into twenty four 220-L cylindrical polyethylene tanks (*ca.* 5,000 larvae per tank). The illumination was provided by white-light fluorescent lamps (40 W) that were fixed at 1.5 m above the tanks' surface, reaching a light intensity of 700 lux at water surface. Tanks were in a flow through system and each tank equipped with an air-stone to supply oxygen and keep LF in suspension in the water column. During the first days (1 to 5 dph), water was not exchanged, but from 6 to 30 dph, 10-50% of the water volume in each tank was exchanged (flow rate = 1 L $min^{-1}$). Tanks were supplied with sand filtered, disinfected (chlorine 20 ppm for a day and neutralized with sodium thiosulfate 10 ppm) and UV-treated seawater (40 ‰). Water temperature, pH and dissolved oxygen were 22.9 ± 1.0 ºC (mean ± standard deviation), 7.9 ± 0.2 and 6.8 ± 0.3 mg $l^{-1}$, respectively during the trial.

The green water technique was applied for rearing larvae from 1 to 20 dph by using *Nannochloropsis occulata* (0.3-0.5 × $10^6$ cell $mL^{-1}$). The exogenous feeding of larvae started from 2 dph according to prescribed weaning strategies. Rotifer (*B. rotundiformis*) was propagated with *N. oculata* in a batch culture system. *Artemia* nauplii (*Artemia franciscana*, Artemia Cysts; Iran Artemia, Kerman, Iran) were hatched at 30 °C and 30‰ after 24 h of cyst incubation. Both live preys were enriched with a commercial enrichment (S.presso, INVE, Belgium) according to the instructions provided by the manufacturer. Regarding 100LF, 75LF-25MD, 50LF-50MD and 25LF-75MD groups, *Artemia* nauplii (Instar I) were administered from 18 to 20 dph, then the enriched *Artemia* metanauplii (Instar II) were used during the rest of the feeding trial (21 to 30

dph). In the LW group, only *Artemia* nauplii were used during larval weaning. Live preys were supplied into larval tanks two times a day (09:00 and 16:00). Larvae were hand-fed with the MD 11 times a day from 08:00 h to 18:00 h at 1-hour intervals. The uneaten feed and dead larvae from the bottom of the tanks were siphoned and oil film on the water surface was cleaned by a soft clean sponge daily.

*2.3. Sampling*

At the beginning of the experiment, *ca*. 3,000 (three replicates, n = 1,000 larvae) newly-hatched larvae (0 dph) were sampled from the hatching tanks for determining their size in weight and length, digestive enzyme activities and body fatty acid profile. For determining the growth rate of larvae under different WS, 20 larvae were sampled from each tank at 5, 10, 15, 25, 25 and 30 and euthanized with an overdose of phenoxyethanol to determine their total length (TL, mm), wet (WW, mg) and dry weight (DW, µg). A stereomicroscope equipped with a micrometric eyepiece (0.1 mm) was used for measuring larval TL. The WW of larvae was determined by a laboratory microbalance (Sartorius, TE214S, Germany) (precision: 1 µg). For measuring larval DW, larvae were dried in an oven at 110 ºC for 24h, and then weighted. For evaluating digestive enzymes activity patterns and their relationship with WS, larvae (n = 1,000 to 20, depending on their WW) were sampled from each tank at 7 (n = 700), 15 (n = 80), 22 (n = 45) and 30 dph (n = 20). Larvae were euthanized with the same procedure, rinsed in distilled water and frozen at −80 °C. At the end of the trial (30 dph) 20 larvae per each tank were sampled for evaluating fatty acid profile of the whole body.

*2.4. Larval stress challenge tests*

At the end of the feeding trial, larvae reared from different WS were challenged with osmotic (Eryalcın et al., 2013) and air exposure stress (Sarvi et al., 2010) tests. In particular, thirty larvae

(10 larvae of each tank; 3 replicates per feeding strategy) were exposed to air for 35 s using a 300 µm mesh net; then, they were returned into a conical flask filled with 1 L of aerated water. Mortality was monitored at 5-min intervals during 1 h after the stress challenge. For the osmotic challenge test, larvae were transferred into a 2000-mL conical flask filled with fresh water for 1 minute; then, they returned into a conical flask contained 1 L of aerated sea water. Mortality was monitored at 5-min intervals during 1 h.

*2.5. Digestive enzyme analyses*

For evaluation of digestive enzymes activities, samples were frozen and shipped to IRTA (Sant Carles de la Rápita, Spain) for analyses. Pools of larvae were homogenized (30: 1, v/w) on ice (0–4 °C) in Tris-mannitol buffer (50 mM Mannitol, 2 mM Tris-HCl; pH 7.5) for 30 s. After homogenization, 100 µL of $CaCl_2$ (0.1 M) were added to the homogenate and sonicated for 1 min. Some aliquots of homogenate were kept at −80 °C for measuring the activity of pancreatic and cytosolic enzymes and the rest was prepared for intestinal brush border (BB) purification following the two-centrifugation step protocol described by Gisbert et al. (2018). In particular, larval homogenates were centrifuged (9,000 x *g*; 10 min; 4 °C), then the supernatant was recovered and centrifuged again (34,000 x *g*; 30 min; 4 °C). The precipitated pellet was suspended in 1 mL of Tris-mannitol buffer and kept at −80 °C for further enzyme quantification by means of spectrophotometric procedures. The activities of digestive enzymes including the intestinal cytosolic leucine-alanine peptidase (LAP; Nicholson and Kim, 1975), the brush border alkaline phosphatase (ALP; Gisbert et al., 2018), and the pancreatic total alkaline proteases (García-Careño and Haard, 1993) and α-amylase (Métais and Bieth, 1968) were determined by standard methods. The ratio of alkaline phosphatase to leucine-alanine peptidase was calculated as an indicator of the shift between intracellular and luminal protein digestion, and intestine maturation (Gisbert et al.,

2018). All enzymatic activities were measured using a microplate scanning spectrophotometer (Synergy HT, Bio-Tech, Germany) and expressed as specific (mU mg protein$^{-1}$) and total (mU larva$^{-1}$) enzyme activities. Soluble protein in enzyme extracts was quantified by the Bradford technique (Bradford, 1976) using bovine serum albumin as a standard. All the assays were made in triplicate (methodological replicates).

### 2.6. *Fatty acid profile analysis*

For determining the fatty acid (FA) profile of the larval whole body, lipid extracted by the method of Folch et al. (1957) and fatty acid methyl esters (FAME) were processed by acidic methanolysis (2.5% sulfuric acid in methanol) of lipid extracts at 80 °C (Christie, 1993). The body FA composition of larvae was determined by an auto sampler gas chromatography (GC, Agilent technologies 7890 N, USA), equipped with aflame ionization detector (FID) and a cyanopropyl–phenyl capillary column (DB-225MS, 30 m × 0.250 mm ID × 0.25μm Film thickness, USA) as described by Agh et al. (2014).

### *2.7. Calculations and statistics*

For determining specific growth rate (SGR), the following standard equation was used: SGR (% initial body weight / day) = ((ln final body weight– ln initial body weight) / t) × 100, where t is experimental period = 30 days. Final survival was evaluated by counting the animals surviving at the end of the experiment and calculated according to Buckley et al. (1984), which considers the number of sampled individuals during the experiment. Data are expressed as mean ± standard error of the mean (SEM.) The SPSS ver. 16.0 (Chicago, IL, USA) was used for data analyses. Normality and homogeneity of data were assessed by Kolmogorov-Smirnov and Leven tests, respectively. In

addition, the effects of weaning time (factor 1), the proportion of LF and MD (factor 2) and their interactions on growth performance, digestive enzymes activity and fatty acid profile were analyzed using a Two-way ANOVA. If effects of independent factors were significant, then these effects were evaluated separately by a one-way ANOVA and the Duncan's multiple-range as post-hoc test were performed. Data expressed as percentage were arcsine square root transformed prior to the ANOVA analysis. The $P < 0.05$ was considered as significant for all statistical tests.

## 3. Results

### *3.1 Larval performance in terms of survival and growth*

At the end of the trial, WS significantly affected larval performance in terms of survival. In particular, the lowest survival rates were found among larvae from 100MD (1.5 ± 0.8%) and SW (1.9 ± 1.2%) groups, whereas the highest survival rates were found in larvae from 100LF (31.0 ± 1.7%), 75LF-25MD (34.7 ± 4.5%) and 50LF-50MD (36.2 ± 4.0%). The rest of experimental groups (25LF-75MD and, early weaning and late weaning) showed intermediate values (Fig. 1; $P < 0.05$).

Regarding larval growth performance, larvae from 100LF, 75LF-25MD, 50LF-50MD and LW groups had the highest TL and WW values at the end of the trial (Tables 4 and 5; $P < 0.05$). Furthermore, gradual replacement of LF with the MD over 50% (*i.e.*, 25LF-75MD and 100MD groups) significantly reduced TL and WW values in *A. latus* larvae ($P < 0.05$). Moreover, WS based on SW or EW compromised larval size in terms of TL and WW compared to the LW group ($P < 0.05$). The lowest size in TL and WW were also observed in larvae from the 100MD and SW treatments (Tables 4 and 5; $P < 0.05$). In contrast, larvae from 100LF and LW groups showed the

highest values in DW, whereas the lowest ones were found in 100MD and SW groups (Table 6; $P < 0.05$). In addition, the gradual replacement of LF with the MD remarkably reduced DW in 75LF-25MD, 50LF-50MD and 25LF-75MD groups compared to the 100LF, whereas the EW treatment also resulted in a decrease in larval DW compared to the LW strategy (Table 6, $P < 0.05$).

Depending on the WS evaluated, values of $SGR_{TL}$ ranged from 2.92 to 4.70% in 100MD and 100LF, respectively during the experimental period (Table 7; $P < 0.05$). In addition, values of $SGR_{TL}$ pronouncedly lower in larvae reared using 25LF-75MD, 100MD and SW weaning strategies compared to other treatments (Table 7; $P < 0.05$). During the experimental trial, $SGR_{WW}$ ranged from 16.52 to 20.25% in 100MD and 100LF, respectively. Larvae from 100MD and SW groups had the lowest values in $SGR_{DW}$ (Table 7).

Regarding the results from the stress challenge tests, *A. latus* larvae from 50LF-50MD group showed the highest resistance against the air exposure challenge (70.0 ± 0.3%), whereas larvae from the 100MD (20.0 ± 4.3%), SW (8.3 ± 6.9%) and EW (20.0 ± 4.4%) groups had the lowest survival rates, and the other treatments had intermediate values (Fig. 2; $P < 0.05$). Regarding the osmotic stress challenge, larvae from the 50LF-50MD group showed the highest survival rate (100%), whereas larvae from the SW group had the lowest survival rate (35.0 ± 2.3%). The other experimental groups showed intermediate values (Fig. 2; $P < 0.05$).

*3.2 Activity of digestive enzymes*

The specific activity of the LAP was detected in newly hatched larvae and it generally increased in all groups up to 22 dph, while it declined in all treatments at 30 dph (Fig. 3a). Total activity of LAP increased in all treatments during the feeding trial, whereas the highest increase in total

activity (10-fold increase) was found in all groups between 7 to 15 dph (Table 8). There were not significant differences in the specific activity of LAP among 100LF, 75LF-25MD, 50LF-50MD and 25LF-75MD groups at different sampling times ($P < 0.05$, Fig. 3a). However, the complete substitution of LF with MD in the 100MD group or the application of SW or EW strategies resulted in an increase of LAP specific activities at 22 dph (Fig. 3a). There were not significant differences in specific activity of LAP between 100LF and LW groups at 7 and 15 dph ($P > 0.05$), whereas larvae from the LW group had higher LAP specific activity than the 100LF group at 22 and 30 dph (Fig. 3a).

The activity of the intestinal brush border ALP was detected in newly hatched *A. latus* larvae (Fig. 3b) and its specific and total activities (Table 8) gradually increased in all treatments during the study. At the end of the trial (30 dph), larvae from the LW strategy had the highest ALP activity, whereas those from SW and EW groups had the lowest values in ALP activity ($P < 0.05$). In addition, except for the 75LF-25MD group, the gradual replacement of LF with the MD reduced ALP activity in 50LF-50MD, 25LF-75MD and 100MD groups (Fig. 3b; $P < 0.05$). At 30 dph, larvae from the LW group had the highest level of ALP/LAP ratio, while those from the 100MD, SW and EW treatments showed the lowest ALP/LAP ratio values, and the other groups showed intermediate values (Fig. 3c). Total activity of total alkaline proteases (TAP) produced by the exocrine pancreas increased in all treatments during the trial (Table 8), although the specific activity of TAP showed some fluctuations depending on the dietary treatment (Fig. 3c). At the 30 dph, the specific activity of TAP in larvae from LW and 75LF-25MD treatments was higher than in the other groups, whereas larvae from 100MD and EW groups had the lowest specific TAP values ($P < 0.05$).

Regarding the activity of α-amylase, the specific (Fig. 3e) and total (Table 8) activities of α-amylase in larvae from 100LF and 75LF-25MD groups increased from 0 to 15 dph, decreased at 22 dph and then increased again at 30 dph. The specific activity of α-amylase in larvae from 100LF and 75LF-25MD groups increased from 0 to 15 dph, and then gradually decreased to 30 dph. On the other hand, the specific and total activities of α-amylase in 50LF-50MD, 100MD and SW groups gradually increased during the feeding trial, whereas specific and total activities in the LW group increased from 0 to 22 dph, even though activity values sharply decreased at 30 dph (Fig. 3e; $P < 0.05$). At 30 dph, larvae from 75LF-25MD and EW groups displayed the highest and the lowest specific activity of α-amylase, respectively (Fig. 3e; $P < 0.05$).

*3.3 Larval fatty acid composition*

The fatty acid profiles (µm fatty acid/larva) of the whole body of larvae from different experimental groups are presented in Table 9 (these results when expressed as mg fatty acid/mg total lipid are shown in Supplementary File 1). At the end of feeding trial, time of weaning and its interaction with proportion of LF and MD pronouncedly affected total saturated fatty acids (SFA) ($P < 0.05$). Larvae weaned following the 25LF-75MD and 100MD feeding strategies had lower total monounsaturated fatty acids (MUFA) levels than the other groups and it was significantly affected by time of weaning and proportion of LF and MD. The highest concentration of linoleic acid (LA; 18:2n-6) and n-6 polyunsaturated fatty acids (PUFA) were observed in larvae from the 100MD group. The highest level of arachidonic acid (ARA, 20:4n-6) in larvae was observed in 50LF-50MD and 25LF-75MD groups, whereas larvae from 100MD and SW groups had the lowest ARA content ($P < 0.05$). Furthermore, the highest and the lowest concentrations of α-linolenic acid (ALA, 18:3n-3) were observed in larvae from the SW and 25LF-75MD groups, respectively

($P < 0.05$). Larvae from 100MD and SW groups had generally lower levels of n-3 PUFA and LC-PUFA compared to the other treatments. The lowest values of eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6 n-3) were found in larvae from 100MD and SW groups. The highest n-3/n-6 PUFA ratios were observed in 100LF and EW groups, whereas larvae from the 100MD group showed the lowest ratio ($P < 0.05$).

## 4. Discussion

Different studies have shown that a standard WS can adapt fish larvae to MD by considering its nutritional requirements and environmental conditions (Hamre et al., 2013; Kandathil Radhakrishnan et al., 2020), even though such approach is not so evident and requires considerable workload and fine tuning at a species-specific level. In particular, several authors have suggested that the optimum time for of the onset of weaning is species-specific and mostly related to the digestive system development and the larval capacity to ingest, digest and absorb nutrients from MD (Cahu and Zambonino-Infante, 2001; Hamre et al., 2013). In the present study, the replacement of LF with the MD (Gemma Micro™, Skretting) up to 50% did not compromise performance and survival in *A. latus* larvae. In this context, a plethora of studies have reported successful larval rearing under co-feeding strategies by the simultaneous application of both LF and the MD. The above-mentioned success is generally attributed to nutritional factors (*e.g.* free amino acids) in LF that stimulate pancreatic enzyme secretions, or enhance ingestion by synergistic visual or chemical stimulations of the larvae, allowing a gradual adaptation to MD's properties (Holt, 1993; Jones et al., 1993; Person Le Ruyet et al., 1993; Rosenlund et al., 1997; Kolkovski, 2001; Alves et al., 2006; Engrola et al., 2007; 2009; Kolkovski, 2013). In agreement with the results of our study, Duy Khoa et al., (2019a) demonstrated that red seabream (*Pagrus*

*major*) larvae fed with a MD only survived for 15 days, but co-feeding of the MD with LF not only enhanced the digestibility of the MD, but also promoted larval growth and survival similarly to those only fed LF. Under current experimental conditions, larvae reared under the 50LF-50MD strategy had higher resistance to osmotic and air exposure stress tests compared to other treatments, which indicated that this co-feeding strategy was able to meet the nutritional requirements in *A. latus* larvae, as well as promoted their stress tolerance. It should be mentioned that high stress tolerance of larvae reared under the 50LF-50MD feeding strategy may be attributed to high level of whole body ARA. Furthermore, the lowest whole body ARA concentration was observed in larvae from 100MD and SW treatments, which showed the lowest survival rates and the worse tolerance to osmotic or air exposure stresses. These results are in agreement with previous studies that revealed the importance of ARA in improving stress resistance in different marine fish larval species (Koven et al., 2003; Atalah et al., 2010). In contrast, Sarvi et al. (2010) reported that the replacement of rotifer over 50% by the MD remarkably reduced survival rate after air exposure challenge test in *A. latus* larvae, although the former authors did not related their findings to the fatty acid profile of larvae, rather to the poorer condition of larvae early weaned with a MD. Furthermore, Curnow et al. (2006) demonstrated that prolonging weaning period with rotifers for 12 days, pronouncedly enhanced survival rates after an air exposure challenge in Asian seabass (*Lates calcarifer*) larvae compared to a negative control (without the administration of rotifers) or those groups that only were fed rotifers for short period of time (*i.e.*, 3 or 7 days). In addition, the above-mentioned authors reported that the inclusion of *Artemia* for 9 days in the WS enhanced larval survival rates (over 15%) compared to the experimental group that was weaned with only rotifers for 12 days, although this trend was not significant. These results indicated the co-feeding strategy by applying the appropriate level of LF during a convenient period is required for

guaranteeing and promoting stress resistance in marine fish larvae, which may be considered as a larval quality trait.

In the present study, WS based on sudden or early weaning significantly compromised growth performance and survival rates compared to the LW group in *A. latus* larvae, which may due to the inclusion of *Artemia* nauplii for 9 days (from 12 to 20 dph). These results indicated that a successful weaning strategy for *A. latus* larvae onto a MD requires the inclusion of *Artemia* nauplii contrary to previous data from Sarvi et al. (2010). In this sense, an early co-feeding period seems to prepare the gut for accepting and processing inert diets, allowing earlier weaning and resulting in better growth performances compared to feeding strategies based on late weaning at the end of the larval stage (Conceiçao et al., 2010). On one hand, a short period of co-feeding (4 days with rotifers) in the SW treatment did not lead to the acceptance of the MD by *A. latus* larvae, which may be attributed that this period was not long enough for promoting larval development and enhance the digestive function for properly digesting the MD. On the other hand, prolonged feeding of larvae with rotifers during a period of 13 days in the EW treatment compromised larval growth compared to those larvae from the LW group, because might be attributed to a reduction to the net energy intake per prey (rotifer *vs. Artemia*) with regard to increasing larval size (Olsen, 2004). It may be hypothesized that the ingestion of MD by larvae might have led to the reduction of rotifer consumption that resulted in less energy and nutrients available for growth due to restricted capacity of larvae to digest the MD in SW and EW groups (without using *Artemia* nauplii) (Anderade et al., 2012). In addition, higher levels of taurine in *Artemia* compared to rotifers (Aragão et al., 2004) may also explain the increased growth of larvae in the LW group, as well as in those larvae were reared under different co-feeding strategies. Similarly, WS without applying *Artemia* remarkably reduced growth and/or survival rates in several sparid species like

red porgy (*Pagrus pagrus*, Aristizábal and Suárez, 2006) and white seabream (*Diplodus sargus*, Guerreiro et al., 2010) , as well as in larvae from other marine species such as Asian seabass (Curnow et al., 2006), Senegalese sole (Engrola et al., 2007), spotted sand bass larvae (*Paralabrax maculatofasciatus*, Civera-Cerecedo et al., 2008), mulloway (*Argyrosomus japonicas*, Ballagh et al. 2010), cobia (*Rachycentron cancadum*, Nguyen et al. 2011). Furthermore, results of current study revealed that larvae from the LW group had the same growth performance, but lower survival rates compared to their congeners from the 100LF group, which may be as a consequence of an earlier introduction of *Artemia* nauplii (12 *vs.* 18 dph) and difficulties in digesting *Artemia* at that early stage due to their poor gut development, as it was previously shown in red porgy (Roo et al., 2010). However, it should be mentioned that lower survival rates result in lower larval density in rearing tanks, which generally leads to higher growth rates, a hypothesis that may not be excluded for explaining current results.

The knowledge of the changes in digestive enzymes activities (*i.e.* pancreatic and intestinal enzymes) in fish larvae is used as an indicator of the maturation process of the gastrointestinal tract that may apply for defining larval nutritional condition and proper WS (Gisbert et al., 2008; 2009). The digestive enzyme activity pattern in *A. latus* larval ontogeny was in agreement with data on other sparids like sharpsnout seabream (*Diplodus puntazzo*, Cara et al., 2003), white seabream (*Diplodus sargus*, Suzer et al., 2007), blackspot seabream (*Pagellus bogaraveo*, Ribeiro et al., 2008), common dentex (*Dentex dentex*, Gisbert et al., 2009), and red seabream (Duy Khoa et al., 2019b). Similar to other marine fish larvae (Rønnestad et al., 2013), the activity of the digestive enzymes evaluated in this study were detected before first feeding of *A. latus* larvae, indicating that digestive enzyme activity is genetically programmed (Zambonino-Infante et al., 2008). In addition, total activity of all digestive enzymes increased from hatching to 30 dph larvae,

indicating that the digestive capacity of larvae progressively increased throughout development (Rønnestad et al., 2013).

The BB membrane of enterocytes develops during ontogeny of fish larvae and the activities of BB associated enzymes increase with larval development, whereas activities of cytosolic enzymes such as LAP decline; thus, the ratio of ALP/LAP can be used as a valuable indicator of digestive tract maturation (Zambonino-Infante and Cahu, 2001). In the present study, the complete substitution of LF with the MD (100MD feeding strategy) or the application of the SW or EW strategies resulted in an increase of specific activity of LAP especially at 22 dph, indicating a compensatory adaptation due to a delay in gut maturation, as previously was described when European sea bass (*Dicentrarchus labrax)* were not properly weaned (Cahu and Zambonino-Infante, 1994). Similarly, Andrade et al. (2012) reported that a 3-day period of weaning with rotifers without using *Artemia* reduced larval growth and survival rates, as well as the ALP/LAP ratio in red porgy compared to those co-fed with rotifers and *Artemia*. The results from the current study indicated the low digestive capacity and/or nutrient assimilation of *A. latus* larvae from 100MD and SW groups due to the lack of a fully functional digestive system during the first feeding period of larvae, which may be also confirmed by low gut maturation index in these groups, especially at 30 dph (Srichanun et al., 2013; Yúfera et al., 2018). At the end of the trial (30 dph), larvae from the LW feeding strategy had the highest ALP specific activity, whereas those from the SW and EW treatments had the lowest ALP activities, except for the 75LF-25MD group. Furthermore, the gradual replacement of LF with the MD reduced ALP activity in 50LF-50MD, 25LF-75MD and 100MD groups suggesting a delay in digestive system maturation. Similarly, first feeding of larvae with MD without using LF significantly reduced ALP in European seabass (Cahu and Zambonino-Infante, 1994) and red seabream (Anderade et al., 2012). It should be mentioned

that the activity of ALP was pronouncedly enhanced at 15 dph in larvae reared under the LW feeding strategy compared to the other groups, which might be attributed to the early introduction of *Artemia* nauplii that promoted gut maturation (Kolkovski et al., 1997; Salze et al., 2012). In contrast, Engrola et al. (2009) reported that co-feeding in Senegalese sole pronouncedly enhanced ALP activity, but did not affect LAP activity compared to those only fed with LF. In addition, Guerreiro et al. (2010) reported that LW in white seabream larvae at 27 dph promoted gut functionality compared to those weaned at 20 dph, as total activity values of digestive enzymes, including ALP and LAP indicated, even though these changes in enzyme activities were attributed to larger larval sizes.

During the early stages of larval development, protein digestion occurs in alkaline environment by action of alkaline proteases in the luminal zone (Zambonino Infante and Cahu, 2001). In *A. latus* larvae, TAP activity was detected before mouth opening but its activity remarkably low. Then specific activity of TAP peaked at 7 DAH in all groups that was coincided with exogenous feeding and the completion of yolk sac resorption that contains high protein and amino acids content to energy production. The specific activity of TAP continued to decrease generally in all experimental groups until the end of the experiment period due to metamorphosis of digestive system, appearance of stomach and gastric enzymes and annex organs (i.e. liver, pancreas and gall bladder) as well as increase in soluble protein in tissue as reported in meagre (*Argyrosomus regius*, Solovyev et al., 2016). An increase in protease activity during weaning from LF to MD were demonstrated in Senegalese sole (Engrola et al., 2007), red drum (*Sciaenops ocellatus*, Kolkovski, 2001); European sea bass (Skalli et al., 2014) and totoaba (*Totoaba macdonaldi*, Galaviz et al., 2015). In addition, higher ALP and TAP specific activities were recorded in *A. latus* larvae under the 100MD feeding strategy, and especially in the SW group

between 15 and 22 dph; results that were in contradiction with the low growth and survival rates observed in these groups. Such results may be attributed to a sort of compensatory digestive mechanism (*i.e.*, increase in proteolytic activity) for allowing larvae to adapt unsuitable nutritional conditions derived from the limited larval capacity to digest the MD (Hamza et al., 2007). Furthermore, a larger amount of protease activity in these groups is required to compensate the lack of acid digestion in order to properly digest the MD, activity levels that are also modulated by the protein level and its molecular form in the MD (Holt et al., 2011; Skalli et al., 2014; Moguel-Hernández et al., 2016; Engrola et al., 2018).

Different studies have suggested that dietary carbohydrate levels and their molecular form in MD and LF along with WS can result in changes in α-amylase activity (Cahu and Zambonino-Infante, 1994; Cahu et al., 2004; Zambonino-Infante et al., 2008). In the present study, α-amylase specific activity generally increased in different groups in newly-hatched larvae up to 15 dph and it remarkably decreased at 30 dph mainly due to an increase in larval growth and their protein content. This pattern of α-amylase activity also suggested as a clear indicator of the normal maturational process of pancreas maturation in different groups (Zambonino-Infante and Cahu, 2001). In the present study, total α-amylase activity in larvae reared under 100MD and SW weaning strategies progressively increased during the trial and peaked at 30 dph, which could indicate a delay in digestive system development in these groups as results from growth performance indicated. Larvae in 75LF-25MD group also showed higher total and specific α-amylase activities at 30 dph; however, α-amylase in this groups showed fluctuations (saw-type activity profile) with an increase at 15 dph and a decrease at 22 dph and a remarkable increase at 30 dph. Fluctuations in the activity of digestive enzymes similar to those observed in α-amylase activity are generally reported in different marine fish species as a result of metabolic and

physiological events during larval development, as well as due to the feeding protocol (*i.e.* changes in the quantity and composition of LF or MD) or changes in nutrients absorption for compensating nutritional deficiencies to meet larval growth and development (Zambonino-Infante et al., 2008).

Levels of essential fatty acids including EPA, DHA and ARA in the larval tissues correlate well with larval performance in response to weaning strategies (Anderade et al., 2012; Hauville et al., 2014). Essential fatty acids, particularly DHA, play important roles in developing visual and neural tissues and many studies have demonstrated that DHA has a more dominant role in growth and membrane structure than EPA (Innis 2008; Glencross, 2009; Tocher, 2010). In this study, larvae from 100MD and SW groups had the highest levels of LA and ALA, but the lowest values of EPA and DHA. It should be mentioned that despite the high levels of LC-PUFA, especially DHA, in Gemma Micro$^{TM}$ microdiet, the concentrations of these FA were pronouncedly reduced in larvae reared under 100MD and SW strategies that consequently resulted in the lowest survival rate, stress resistance and growth performance compared to the other treatments. It may be hypothesized that lower feed intake in 100MD and SW groups may have resulted in lower LC-PUFA retention, and consequently, this had detrimental effects on larvae performance. Similarly, it has been reported that retention of DHA decreased with starvation in gilthead seabream (Rodriguez et al., 1998). On the other hand, the decrease in n−3 PUFA content in the 100MD group and the increase of n−6 PUFA levels as consequence of high dietary levels of LA may have disrupted the balance between n−3 and n−6 PUFA. In this sense, the imbalances between n−3 and n−6 PUFA has been reported in larvae displaying starvation signs and growth reduction in red seabream (Tandler et al., 1989) and in gilthead seabream (Rainuzzo et al., 1994). Under current experimental conditions, excessive levels of EPA accumulated in tissues of larvae reared by co-feeding protocols strategy were mainly due to *A. latus* like most of marine fish species unable to

elongate and desaturate them into DHA (Morais et al., 2004), as well as *Artemia* nauplii metabolism responsible for retro-converting DHA to EPA and accumulating EPA at higher concentrations than DHA, and reducing the DHA/EPA ratio (McEvoy et al., 1995). It has been reported that ARA has a pivotal role in growth and stress resistance in gilthead seabream larvae (Koven et al., 2001). In the present study, the lowest levels of ARA were found in larvae reared under 100MD and SW feeding strategies that might result in a reduced growth, survival and stress resistance in larvae from these groups. It can be postulated that the highest stress resistance observed in 50LF-50MD group could be related to high levels of ARA and suitable proportion of ARA to EPA in the whole body of larvae reared under this WS.

## 5. Conclusion

In summary, results of the present study indicated that WS through schedule, time of beginning of weaning, as well as dietary items, including quantity of MD or LF, profoundly affected the growth, survival and stress resistance in *A. latus* larvae by modifying their digestive enzyme activity and FA profile. Results indicated that the direct administration of the MD after mouth opening not only reduced growth and survival, but it also remarkably reduced the digestive function in larvae and pronouncedly decreased their levels of LC-PUFA mainly EPA, DHA, ARA and n-3/n-6 PUFA ratio. Furthermore, using only rotifers for short or long term periods as used in SW and EW strategies compromised growth and survival rates in *A. latus* larvae, especially in the SW group, which may be attributed to the single use of rotifers during the co-feeding period. In addition, early inclusion of *Artemia* nauplii in LW strategy from 12 dph, remarkably reduced survival rates in *A. latus* larvae due to poor nutritional quality of *Artemia* nauplii or difficulties in ingestion and digestion of nauplii at early stages. The replacement of LF with MD up to 50% neither have detrimental effects on growth, survival and digestive enzymes activities, meanwhile enhanced

stress resistance of *A. latus* larvae. Thus, the implementation of the co-feeding regime by replacement of 50% of LF with MD could result in greater economic viability of *A. latus* propagation by reducing labor and *Artemia* cyst consumption.

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**Table 1**. Description of weaning strategies evaluated in *A. latus* larvae during 30 days.

| **Days post hatch** | *Microalgae* ($0.5 \times 10^6$ cell $ml^{-1}$) | Rotifer (individual $ml^{-1}$) | *Artemia* (individual $ml^{-1}$) | Gemma-Micro 75 (g $tank^{-1}$ $day^{-1}$) | Gemma-Micro 150 (g $tank^{-1}$ $day^{-1}$) |
|---|---|---|---|---|---|
| | | | **100 LF** | | |
| 2-5 | + | 5 | - | - | - |
| 5-10 | + | 10 | - | - | - |
| 10-20 | + | 20 | 0.5 | - | - |
| 20-30 | - | - | 1-3 | - | - |
| | | | **75LF-25MD** | | |
| 2-5 | + | 3.75 | - | 0.4 | - |
| 5-10 | + | 7.5 | - | 0.6 | - |
| 10-20 | + | 15 | 0.375 | 0.8-1 | - |
| 20-30 | - | - | 0.75-2.25 | - | 1.6-2 |
| | | | **50LF-50MD** | | |
| 2-5 | + | 2.5 | - | 0.4 | - |
| 5-10 | + | 5.0 | - | 0.6 | - |
| 10-20 | + | 10 | 0.25 | 0.8-1 | - |
| 20-30 | - | - | 0.5-1.5 | - | 1.6-2 |
| | | | **25LF-75MD** | | |
| 2-5 | + | 1.25 | - | 0.4 | - |
| 5-10 | + | 2.5 | - | 0.6 | - |
| 10-20 | + | 5.0 | 0.125 | 0.8-1 | - |
| 20-30 | - | - | 0.25-0.75 | - | 1.6-2 |
| | | | **100 MD** | | |
| 2-5 | + | - | - | 0.4 | - |
| 5-10 | + | - | - | 0.6 | - |
| 10-20 | + | - | - | 0.8-1 | - |
| 20-30 | - | - | - | - | 1.6-2 |
| | | | **SW** | | |
| 2-5 | + | 5-10-15-20 | - | 0.4 | - |
| 6-10 | + | - | - | 0.6 | - |
| 10-14 | + | - | - | 0.8 | - |
| 15-20 | + | - | - | 1 | - |
| 20-25 | | | | | 1.6 |
| 25-30 | | | | | 2 |
| | | | **EW** | | |
| 2-5 | + | 5-10-15-20 | - | - | - |
| 6-10 | + | 20-20-20-20-20 | - | - | - |
| 10-14 | + | 20-15-10-5 | - | 0.8 | - |
| 15-20 | + | - | - | 1 | - |
| 20-25 | - | - | - | - | 1.6 |
| 25-30 | - | - | - | - | 2 |
| | | | **LW** | | |
| 2-5 | + | 5-10-15-20 | | - | - |
| 6-10 | + | 20-20-20-20-20 | | - | - |
| 10-14 | + | 20-15-10-5 | From day 12 to 20 | 0.8 | - |
| 15-20 | + | | 0.5-1-1-2-2-2-3-2-1 | 1 | - |
| 20-25 | - | | - | - | 1.6 |
| 25-30 | - | | - | - | 2 |

Abbreviations: 100 LF. larvae were fed only live food (LF) (100%); 75LF-25MD, 25% of LF was replaced by the microdiet (MD); 50LF-50MD, 50% of LF was replaced by the MD; 25LF-75MD; 75% of LF was replaced by the MD; 100 MD. larvae fed only with the MD; SW (Sudden weaning); EW (Early weaning) and LW (Late weaning).

**Table 2.** Nutritional composition (% of dry matter) of the microdiet used for weaning *A. latus* larvae (Gemma Micro, Skretting®). The nutritional content was the same for all particle sizes. Information was provided by feed manufacturer.

| Proximate composition (%) | |
|---|---|
| Protein | 55 |
| Lipid | 15 |
| Fiber | 5 |
| Ash | 13.5 |
| Phosphorous | 2 |
| Dry weight | 91.4 |
| Main ingredients | Fish meal (native protein and protein hydrolysate), lecithin, wheat gluten, dried seaweed, fish oil, maize starch, vitamins and minerals |

**Table 3.** Fatty acid composition (mg $g^{-1}$ extracted lipid) of enriched live food and microdiets (mean ± SE, n= 3).

| | | | Diets |
|---|---|---|---|
| *Fatty acids* | Rotifer | *Artemia* | Gemma Micro |
| 14:0 | 14.1 ±2.6 | 18.7 ±0.3 | 10.6 ±0.8 |
| 16:0 | 197.0 ± 10.1 | 16.07 ± 3.3 | 212.2 ±4.8 |
| 18:0 | 39.5 ±0.9 | 4.70 ±0.3 | 78.0 ±1.5 |
| SFA[a] | 259.0 ±3.5 | 226.3 ±5.1 | 304.1 ±4.4 |
| 18:1n-9 | 115.8 ± 10.3 | 142.4 ±2.9 | 114.8 ±1.0 |
| MUFA[b] | 265.5 ± 43.3 | 382.6 ±3.3 | 246.1 ± 14.8 |
| 18:2n-6, LA[c] | 64.7 ± 10.2 | 73.6 ±3.1 | 97.0 ±8.6 |
| 20:4n-6, ARA[d] | 49.4 ± 4.3 | 16.0 ±1.1 | 49.5 ±1.4 |
| n-6 PUFA[e] | 114.1 ± 14.3 | 89.5 ±4.2 | 146.5 ± 10.5 |
| 18:3n-3, LNA[f] | 121.6 ±6.5 | 15.5 ±0.6 | 12.6 ± 2.0 |
| 20:5n-3, EPA[g] | 94.8 ± 12.7 | 130.5 ±2.9 | 79.5 ± 6.2 |
| 22:6n-3, DHA[h] | 126.8 ± 3.2 | 82.6 ±2.6 | 198.8 ± 11.6 |
| n-3 PUFA[i] | 343.2 ± 22.3 | 228.2 ±0.9 | 291.0 ±7.4 |
| LC-PUFA[j] | 271.2 ± 20.0 | 228.4 ±1.4 | 327.8 ±6.8 |
| n-3 / n-6 | 3.05 ± 0.19 | 2.56 ± 0.11 | 2.00 ± 0.09 |
| ARA / EPA | 0.52 ± 0.03 | 0.12 ± 0.01 | 0.63 ± 0.07 |
| DHA / EPA | 1.37 ± 0.15 | 0.63 ± 0.03 | 2.55 ± 0.35 |

[a] SFA: saturated fatty acids includes: 20:0 and 22:0.
[b] MUFA: monounsaturated fatty acids also includes: 14:1n−5, 18:1n−7, 20:1n−9 and 22:1n−9.
[c] LA; linoleic acid.
[d] ARA; arachidonic acid.
[e] n-6 PUFA: n-6 polyunsaturated fatty acids also includes: LA, ARA and 20:2n−6.
[f] LNA; linolenic acid.
[g] EPA; eicosapentaenoic acid.
[h] DHA; docosahexaenoic acid.
[i] n-3 PUFA: n-3 polyunsaturated fatty acids also includes: LNA and EPA.
[j] LC-PUFA; long chain polyunsaturated fatty acids: 20:2n−6 + 20:3n−6 + ARA+ 20:3n-3 + EPA + 22:5n−3 + DHA.

**Table 4.** Changes in total length (mm, mean ± SE, n= 3 tank) of *A. latus* larvae fed with different weaning strategies. Different superscript letters within a column indicate significant differences ($P < 0.05$).

| | Age (day) | | | | | | |
|---|---|---|---|---|---|---|---|
| Weaning strategies | 0 | 5 | 10 | 15 | 20 | 25 | 30 |
| 100 LF | 2.0 ± 0.03 | 3.0 ± 0.1[a] | 4.5± 0.08 [a] | 4.8 ± 0.1[a] | 6.4 ± 0.08[a] | 7.3 ± 0.12[a] | 8.2 ± 0.1[a] |
| 75 LF-25MD | 2.0 ± 0.03 | 3.1 ± 0.06[a] | 4.4 ± 0.1[a] | 4.8 ± 0.07[a] | 6.2 ± 0.2[a] | 7.2 ± 0.1[a] | 7.9 ± 0.25[a] |
| 50 LF-50MD | 2.0 ± 0.04 | 3.1 ± 0.06 [a] | 4.4 ± 0.6[a] | 4.8 ± 0.1[a] | 6.2 ± 0.1[a] | 7.3 ± 0.1[a] | 7.9 ± 0.06[a] |
| 25 LF-75MD | 2.0 ± 0.04 | 3.0 ± 0.07[a] | 4.1± 0.06 [b] | 4.5 ± 0.48 [a] | 5.8 ± 0.05[b] | 6.4 ± 0.11[b] | 6.9 ± 0.15[b] |
| 100 MD | 2.0 ± 0.04 | 2.4 ± 0.05[b] | 3.0 ± 0.05[c] | 3.5 ± 0.05[b] | 3.9 ± 0.1[d] | 4.2 ± 0.22[c] | 4.8 ± 0.05[c] |
| Sudden weaning | 2.0 ± 0.04 | 2.9 ± 0.05[a] | 3.6 ± 0.06[bc] | 3.8 ± 0.1[b] | 4.1 ± 0.16[c] | 4.5 ± 0.1[c] | 5.0 ± 0.1[c] |
| Early weaning | 2.0 ± 0.04 | 2.9 ± 0.06[a] | 4.2 ± 0.07 [a] | 4.6 ± 0.03[a] | 6.2 ± 0.1[a] | 6.8 ± 0.1[b] | 7.3 ± 0.05 [b] |
| Late weaning | 2.0 ± 0.04 | 2.9 ± 0.8[a] | 4.2 ± 0.01[a] | 4.5 ± 0.1[a] | 6.1 ± 0.06[ab] | 7.2 ± 0.08[a] | 7.8 ± 0.05[a] |
| | Two-Way ANOVA | | | | | | |
| Time of weaning | 1.000 | 0.285 | 0.010 | 0.041 | 0.055 | 0.001 | 0.032 |
| Proportion of LF and MD | 1.000 | 0.031 | 0.042 | 0.017 | 0.039 | 0.001 | 0.037 |
| Interactions | 1.000 | 0.406 | 0.143 | 0.072 | 0.083 | 0.427 | 0.001 |

**Table 5.** Changes in wet weight (mg, mean ± SE, n= 3 tank) of *A. latus* larvae fed with different weaning strategies. Different superscript letters within a column indicate significant differences ($P < 0.05$).

| Weaning strategies | Age (day) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 |
| 100 LF | 0.009 ± 0.0 | 0.0921 ± 0.0[ab] | 0.40 ± 0.05 [a] | 0.44 ± 0.10[a] | 0.64 ± 0.10[a] | 3.10 ± 0.12[a] | 3.92 ± 0.20[a] |
| 75 LF-25MD | 0.009 ± 0.0 | 0.0770 ± 0.0[b] | 0.29 ± 0.01[b] | 0.33 ± 0.03[a] | 0.72 ± 0.04[a] | 3.00 ± 0.36[a] | 3.68± 0.10[a] |
| 50 LF-50MD | 0.009 ± 0.0 | 0.0726 ± 0.0[b] | 0.38 ± 0.01[a] | 0.47± 0.02 [a] | 0.78 ± 0.06[a] | 2.70 ± 0.23[a] | 3.64± 0.16[a] |
| 25 LF-75MD | 0.009 ± 0.0 | 0.0678 ± 0.0[b] | 0.29 ± 0.03[b] | 0.30 ± 0.02[bc] | 0.62± 0.02[a] | 2.10 ± 0.40[b] | 2.53± 0.22[b] |
| 100 MD | 0.009 ± 0.0 | 0.0686 ± 0.0[b] | 0.10 ± 0.0[c] | 0.14 ± 0.06[d] | 0.25± 0.02[b] | 0.87± 0.1[c] | 1.28± 0.05[c] |
| Sudden weaning | 0.009 ± 0.0 | 0.1250 ± 0.0[a] | 0.16 ± 0.01[c] | 0.25 ± 0.01[c] | 0.33± 0.01[b] | 0.93± 0.05[c] | 1.37 ± 0.20[c] |
| Early weaning | 0.009 ± 0.0 | 0.086 ± 0.0[b] | 0.32 ± 0.02[a] | 0.36 ± 0.01[a] | 0.66 ± 0.01[a] | 1.90 ± 0.01[b] | 2.42 ± 0.03[b] |
| Late weaning | 0.009 ± 0.0 | 0.072 ± 0.0[b] | 0.34 ± 0.08[a] | 0.43 ± 0.05[a] | 0.77± 0.10[a] | 2.90 ± 0.20[a] | 3.39± 0.18[a] |
| | Two-Way ANOVA | | | | | | |
| Time of weaning | 1.000 | 0.845 | 0.001 | 0.010 | 0.002 | 0.001 | 0.001 |
| Proportion of LF and MD | 1.000 | 0.008 | 0.002 | 0.008 | 0.088 | 0.030 | 0.017 |
| Interactions | 1.000 | 0.674 | 0.178 | 0.124 | 0.013 | 0.372 | 0.478 |

**Table 6.** Changes in dry weight (µg, mean ± SE, n= 3 tank) of *A. latus* larvae fed with different weaning strategies. Different superscript letters within a column indicate significant differences ($P < 0.05$).

| Weaning strategies | Age (day) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 |
| 100 LF | 1.5 ± 0.0 | 29.5 ± 1.4$^{ab}$ | 65.3 ± 8.3$^{a}$ | 85.2 ± 11.0 $^{a}$ | 141.5 ± 14.9$^{a}$ | 471.5 ± 19.6$^{a}$ | 835.8 ± 61.3$^{a}$ |
| 75 LF-25MD | 1.5 ± 0.0 | 28.1 ± 1.2$^{ab}$ | 63.0 ± 5.5$^{a}$ | 76.1 ± 9.5$^{a}$ | 137.8 ± 23.8$^{a}$ | 386.1 ± 7.8$^{a}$ | 661.4 ± 62.3 $^{b}$ |
| 50 LF-50MD | 1.5 ± 0.0 | 20.5 ± 4.2$^{b}$ | 68.7 ± 7.7 $^{a}$ | 83.0 ± 3.1$^{a}$ | 147.6 ± 6.7$^{a}$ | 423.8 ± 35.7$^{a}$ | 624.9 ± 22.7$^{b}$ |
| 25 LF-75MD | 1.5 ± 0.0 | 13.4 ± 1.0$^{c}$ | 45.5 ± 3.6$^{b}$ | 55.0 ± 24.1$^{b}$ | 130.7 ± 7.2$^{ab}$ | 397.9 ± 35.9 $^{a}$ | 579.7 ± 22.6$^{b}$ |
| 100 MD | 1.5 ± 0.0 | 13.0 ± 2.1$^{c}$ | 33.4 ± 9.5$^{c}$ | 51.1 ± 4.1$^{b}$ | 107.7 ± 5.6$^{c}$ | 158.6 ± 34.4$^{c}$ | 191.7 ± 33.7$^{d}$ |
| Sudden weaning | 1.5 ± 0.0 | 33.6 ± 2.4$^{a}$ | 30.7 ± 4.2$^{c}$ | 40.7 ± 4.1$^{b}$ | 104.0 ± 6.4$^{c}$ | 176.7 ± 37.4$^{c}$ | 216.0 ± 16.0$^{d}$ |
| Early weaning | 1.5 ± 0.0 | 26.3 ± 3.5$^{ab}$ | 67.9 ± 6.7 $^{a}$ | 76.6 ± 1.1$^{a}$ | 114.0 ± 17.1$^{bc}$ | 251.3 ± 46.2$^{b}$ | 376.2 ± 11.5$^{c}$ |
| Late weaning | 1.5 ± 0.0 | 25.2 ± 3.3$^{ab}$ | 72.0 ± 7.0$^{a}$ | 91.6 ± 3.6$^{a}$ | 146.0 ± 13.5$^{a}$ | 473.7 ± 27.9 $^{a}$ | 796.0 ± 45.2$^{a}$ |
| | Two-Way ANOVA | | | | | | |
| Time of weaning | 1.000 | 0.040 | 0.006 | 0.002 | 0.001 | 0.001 | 0.001 |
| Proportion of LF and MD | 1.000 | 0.565 | 0.009 | 0.008 | 0.008 | 0.035 | 0.009 |
| Interactions | 1.000 | 0.866 | 0.798 | 0.478 | 0.471 | 0.044 | 0.034 |

**Table 7.** Changes in SGR in total length (% initial TL day$^{-1}$, mean ± SE, n= 3 tank), wet weight (% initial WW day$^{-1}$, mean ± SE, n= 3 tank) and dry weight (% initial DW day$^{-1}$, mean ± SE, n= 3 tank) of *A. latus* larvae with different weaning strategies. Different superscript letters within a column indicate significant differences ($P < 0.05$).

| | $SGR_{TL}$ | $SGR_{WW}$ | $SGR_{DW}$ |
|---|---|---|---|
| 100 LF | 4.70 ± 0.11[a] | 20.25 ± 1.40[a] | 21.08 ± 0.85[a] |
| 75 LF-25MD | 4.58 ± 0.27[a] | 20.04 ± 0.55[a] | 20.30 ± 0.44[a] |
| 50 LF-50MD | 4.58 ± 0.18[a] | 20.0 ± 0.22[a] | 20.10 ± 0.35[a] |
| 25 LF-75MD | 4.13 ± 0.20[b] | 18.8 ± 0.61[b] | 19.76 ± 0.75[ab] |
| 100 MD | 2.92 ± 0.08[c] | 16.52 ± 0.92[c] | 16.17 ± 1.0[c] |
| Sudden weaning | 3.05 ± 0.15[c] | 16.75 ± 0.40[c] | 16.56 ± 0.30[c] |
| Early weaning | 4.31 ± 0.32[ab] | 18.64 ± 0.42[b] | 18.41 ± 0.23[b] |
| Late weaning | 4.53 ± 0.26[a] | 19.77 ± 0.36[a] | 20.91 ± 0.35[a] |
| | | Two-Way ANOVA | |
| Time of weaning | 0.037 | 0.001 | 0.001 |
| Proportion of LF and MD | 0.032 | 0.017 | 0.008 |
| Interactions | 0.276 | 0.220 | 0.206 |

**Table 8.** Total activities of digestive enzymes of *A. latus* larvae reared at different weaning strategies (mean ± SE, n= 3 tank). Different superscript letters within a column indicate significant differences ($P < 0.05$).

| Weaning strategies | Age (day) | | | | |
|---|---|---|---|---|---|
| | 0 | 7 | 15 | 22 | 30 |
| *Leucine-Alanine peptidase* (U larvae$^{-1}$) | | | | | |
| 100 LF | 0.114 ± 0.01 | 0.172 ± 0.002$^{b}$ | 1.858± 0.005$^{a}$ | 3.070 ± 0.017$^{d}$ | 7.205 ± 0.09$^{d}$ |
| 75 LF-25MD | 0.114 ± 0.01 | 0.152 ± 0.002$^{d}$ | 1.498± 0.007$^{d}$ | 2.783 ± 0.016$^{e}$ | 8.360 ± 0.10$^{b}$ |
| 50 LF-50MD | 0.114 ± 0.01 | 0.172 ± 0.002$^{b}$ | 1.580± 0.005$^{c}$ | 2.522 ± 0.21$^{f}$ | 7.922 ± 0.04$^{c}$ |
| 25 LF-75MD | 0.114 ± 0.01 | 0.148 ± 0.003$^{d}$ | 1.714± 0.009$^{b}$ | 2.434 ± 0.011$^{f}$ | 6.150 ± 0.27$^{e}$ |
| 100 MD | 0.114 ± 0.01 | 0.204 ± 0.003$^{a}$ | 1.518± 0.008$^{d}$ | 4.118 ± 0.018$^{b}$ | 8.330 ± 0.08$^{b}$ |
| Sudden weaning | 0.114 ± 0.01 | 0.158 ± 0.002$^{c}$ | 1.577± 0.008$^{c}$ | 3.000 ± 0.016$^{d}$ | 9.192 ± 0.29$^{a}$ |
| Early weaning | 0.114 ± 0.01 | 0.162 ± 0.003$^{c}$ | 1.311± 0.005$^{e}$ | 5.955 ± 0.21$^{a}$ | 5.546 ± 0.05$^{f}$ |
| Late weaning | 0.114 ± 0.01 | 0.160 ± 0.003$^{c}$ | 1.526± 0.002$^{d}$ | 3.452 ± 0.23$^{c}$ | 7.194 ± 0.011$^{d}$ |
| | | | Two-Way ANOVA | | |
| Time of weaning | 1.000 | 0.001 | 0.001 | 0.005 | 0.006 |
| Proportion of LF and MD | 1.000 | 0.001 | 0.032 | 0.546 | 0.065 |
| Interactions | 1.000 | 0.01 | 0.003 | 0.574 | 0.515 |
| *Alkaline phosphatase* (µU larvae$^{-1}$) | | | | | |
| 100 LF | 0.438 ± 0.05 | 13.57± 0.22$^{a}$ | 19.0±1.1$^{c}$ | 58.51±2.5$^{d}$ | 339.91±17.8$^{c}$ |
| 75 LF-25MD | 0.438 ± 0.05 | 2.14± 0.13$^{c}$ | 56.97±3.9$^{b}$ | 283.81±34.6$^{a}$ | 398.33±8.8$^{b}$ |
| 50 LF-50MD | 0.438 ± 0.05 | 0.13± 0.0$^{e}$ | 0.27± 0.01$^{e}$ | 123.8±2.8$^{b}$ | 484.97±42.5$^{a}$ |
| 25 LF-75MD | 0.438 ± 0.05 | 3.44± 0.54$^{b}$ | 24.73±0.13$^{bc}$ | 102.72±3.3$^{c}$ | 320.01±15.2$^{c}$ |
| 100 MD | 0.438 ± 0.05 | 0.91± 0.05$^{d}$ | 2.11±0.06$^{d}$ | 41.06±2.3$^{e}$ | 289.91±11.9$^{d}$ |
| Sudden weaning | 0.438 ± 0.05 | 1.07± 0.04$^{d}$ | 194.37±16.7$^{a}$ | 38.53±3.2$^{e}$ | 110.88±4.0$^{f}$ |
| Early weaning | 0.438 ± 0.05 | 0.70± 0.23$^{de}$ | 218.53±8.8$^{a}$ | 26.29±1.8$^{f}$ | 239.16±2.7$^{e}$ |
| Late weaning | 0.438 ± 0.05 | 1.10± 0.02$^{d}$ | 55.73±15.3$^{b}$ | 0.98±0.01$^{g}$ | 246.0±14.6$^{e}$ |
| | | | Two-Way ANOVA | | |
| Time of weaning | 1.000 | 0.001 | 0.003 | 0.005 | 0.001 |
| Proportion of LF and MD | 1.000 | 0.001 | 0.032 | 0.021 | 0.011 |
| Interactions | 1.000 | 0.001 | 0.007 | 0.091 | 0.647 |
| *Total alkaline protease* (µU larvae$^{-1}$) | | | | | |
| 100 LF | 2.236 ± 0.235 | 53.63 ± 2.63$^{a}$ | 423.75 ± 42.4$^{c}$ | 691.06±57.8$^{e}$ | 2466.3± 22.6$^{c}$ |
| 75 LF-25MD | 2.236 ± 0.235 | 41.45 ± 0.32$^{b}$ | 358.83±18.3 $^{d}$ | 669.28±14.1$^{e}$ | 3024.9 ± 23.8$^{a}$ |
| 50 LF-50MD | 2.236 ± 0.235 | 40.75 ± 1.53$^{b}$ | 526.90±29.7$^{a}$ | 776.86±21.6$^{d}$ | 2655.4 ± 28.5$^{b}$ |
| 25 LF-75MD | 2.236 ± 0.235 | 44.17 ± 0.08$^{b}$ | 522.98±14.5$^{a}$ | 693.67±6.5$^{e}$ | 2675.0 ± 17.5$^{b}$ |
| 100 MD | 2.236 ± 0.235 | 40.33 ± 0.39$^{b}$ | 417.88±15.4$^{c}$ | 846.12±16.1$^{cd}$ | 1633.3 ± 52.1$^{d}$ |
| Sudden weaning | 2.236 ± 0.235 | 45.04 ± 0.65$^{b}$ | 377.94±57.9$^{d}$ | 900.12±16.8$^{bc}$ | 3050.9 ± 24.8$^{a}$ |
| Early weaning | 2.236 ± 0.235 | 43.92 ± 0.16$^{b}$ | 419.59±45.2$^{c}$ | 929.31±45.2$^{b}$ | 2054.7 ± 118.8$^{c}$ |
| Late weaning | 2.236 ± 0.235 | 42.91 ± 1.87$^{b}$ | 478.94±66.5$^{b}$ | 1270.3±27.4$^{a}$ | 2399.6 ± 25.6$^{c}$ |
| | | | Two-Way ANOVA | | |
| Time of weaning | 1.000 | 0.001 | 0.028 | 0.001 | 0.001 |
| Proportion of LF and MD | 1.000 | 0.001 | 0.051 | 0.159 | 0.066 |
| Interactions | 1.000 | 0.020 | 0.043 | 0.078 | 0.001 |
| α-Amylase (µU larvae$^{-1}$) | | | | | |
| 100 LF | 0.77 ± 0.28 | 8.71± 0.38$^{a}$ | 119.46±11.4$^{a}$ | 55.29±4.2$^{d}$ | 168.14±4.6$^{bc}$ |
| 75 LF-25MD | 0.77 ± 0.28 | 2.87± 0.35$^{b}$ | 53.15±12.8$^{b}$ | 33.17±7.3$^{e}$ | 255.63±15.6$^{a}$ |
| 50 LF-50MD | 0.77 ± 0.28 | 1.16±0.25$^{c}$ | 105.83±9.9$^{a}$ | 142.93±2.9$^{ab}$ | 189.25±3.9$^{bc}$ |
| 25 LF-75MD | 0.77 ± 0.28 | 2.02±0.08$^{bc}$ | 109.98±3.6$^{a}$ | 88.62±1.0$^{c}$ | 110.84±14.7$^{d}$ |
| 100 MD | 0.77 ± 0.28 | 1.97±0.10$^{bc}$ | 66.32±0.91$^{b}$ | 96.42±7.8$^{bc}$ | 229.31±7.8$^{ab}$ |
| Sudden weaning | 0.77 ± 0.28 | 1.60±0.27$^{bc}$ | 35.11±3.7$^{b}$ | 54.52±4.1$^{d}$ | 233.58±7.7$^{ab}$ |
| Early weaning | 0.77 ± 0.28 | 1.10±0.28$^{c}$ | 62.21±6.7 | 56.12±23.7$^{d}$ | 92.93±19.6$^{e}$ |
| Late weaning | 0.77 ± 0.28 | 1.40±0.41$^{c}$ | 54.34±2.7$^{b}$ | 174.8±9.2$^{a}$ | 134.65±18.4$^{c}$ |

| | | | | | |
|---|---|---|---|---|---|
| | | | Two-Way ANOVA | | |
| Time of weaning | 1.000 | 0.001 | 0.001 | 0.027 | 0.030 |
| Proportion of LF and MD | 1.000 | 0.001 | 0.513 | 0.082 | 0.014 |
| Interactions | 1.000 | 0.001 | 0.043 | 0.051 | 0.310 |

**Table 9.** Fatty acid composition of (µg larvae$^{-1}$) *A. latus* larvae under different weaning strategies after 30 days (mean ± SE, n= 3 tank). Different superscript letters within a column indicate significant differences ($P < 0.05$).

| | Diets | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Fatty acids* | 100 LF | 75 LF-25MD | 50LF-50MD | 25LF-75MD | 100MD | SW | EW | LW |
| 14:0 | 4.60 ± 0.15$^{bc}$ | 5.28 ± 0.78$^{bc}$ | 5.53 ± 0.60$^{bc}$ | 3.55 ± 0.36$^{c}$ | 8.14 ± 0.26$^{a}$ | 6.17 ± 0.85$^{ab}$ | 3.88 ± 0.27$^{bc}$ | 6.12 ± 0.11$^{ab}$ |
| 16:0 | 81.86 ± 0.78$^{ab}$ | 70.68 ± 0.30$^{abc}$ | 78.92 ± 6.29$^{abc}$ | 84.98 ± 3.90$^{a}$ | 77.42 ± 0.75$^{abc}$ | 70.40 ± 1.78$^{bcd}$ | 66.44 ± 2.68$^{cd}$ | 63.61 ± 1.53$^{d}$ |
| 18:0 | 45.93 ± 2.79$^{a}$ | 27.31 ± 2.50$^{b}$ | 31.54 ± 3.68$^{ab}$ | 16.76 ± 8.9$^{b}$ | 15.41 ± 0.28$^{b}$ | 18.11 ± 0.13$^{b}$ | 30.19 ± 1.27$^{ab}$ | 19.88 ± 0.77$^{b}$ |
| 20:0 | 1.22 ± 0.70$^{b}$ | 1.70 ± 0.45$^{b}$ | 2.26 ± 0.41$^{b}$ | 0.63 ± 0.36$^{ab}$ | 3.37 ± 0.97$^{a}$ | 1.24 ± 0.45$^{b}$ | 1.56 ± 0.11$^{b}$ | 0.83 ± 0.09$^{b}$ |
| 22:0 | 2.90 ± 1.67$^{ab}$ | n.d$^{b}$ | n.d$^{b}$ | n.d$^{b}$ | 6.59 ± 0.01$^{a}$ | 1.30 ± 0.75$^{b}$ | 2.20 ± 1.27$^{b}$ | n.d$^{b}$ |
| SFA[a] | 136.52 ± 0.51$^{a}$ | 104.0 ± 1.77$^{b}$ | 118.26 ± 9.80$^{ab}$ | 105.92 ± 13.53$^{b}$ | 110.95 ± 2.28$^{ab}$ | 97.24 ± 0.50$^{b}$ | 104.28 ± 2.84$^{b}$ | 90.45 ± 2.51$^{b}$ |
| 14:1n-5 | 12.05 ± 4.08$^{ab}$ | 14.56 ± 4.68$^{a}$ | 9.13 ± 0.68$^{ab}$ | 13.98 ± 1.17$^{ab}$ | 0.82 ± 0.01$^{b}$ | 14.83 ± 3.68$^{a}$ | 3.87 ± 0.06$^{ab}$ | 11.90 ± 1.98$^{ab}$ |
| 16:1n-7 | 28.69 ± 4.04$^{a}$ | 32.52 ± 1.73$^{a}$ | 25.57 ± 7.19$^{ab}$ | 10.32 ± 0.78$^{b}$ | 8.88 ± 0.16$^{b}$ | 30.12 ± 5.86$^{a}$ | 28.72 ± 0.74$^{a}$ | 35.83 ± 2.15$^{a}$ |
| 18:1n-7 | 62.55 ± 2.77$^{a}$ | 43.60 ± 2.04$^{ad}$ | 33.64 ± 5.95$^{bc}$ | 18.01 ± 1.80$^{c}$ | 8.96 ± 0.37 $^{d}$ | 31.63 ± 8.44$^{bc}$ | 45.42 ± 0.42$^{ad}$ | 39.13 ± 1.58$^{d}$ |
| 18:1n-9 | 60.26 ± 6.22$^{a}$ | 58.71 ± 1.30$^{ab}$ | 55.56 ± 1.64$^{ab}$ | 50.42 ± 2.06$^{b}$ | 52.78 ± 2.14$^{ab}$ | 61.96 ± 0.28$^{a}$ | 56.80 ± 0.20$^{ab}$ | 51.85 ± 0.54$^{b}$ |
| 20:1n-9 | 1.20 ± 0.21$^{b}$ | 1.21 ± 0.01$^{b}$ | 12.08 ± 5.96$^{a}$ | 12.08 ± 6.25$^{a}$ | 6.97 ± 0.21$^{ab}$ | 1.31 ± 0.17$^{b}$ | 1.77 ± 0.37$^{b}$ | 0.61 ± 0.19$^{b}$ |
| 22:1n-9 | 7.45 ± 4.30$^{ab}$ | 5.64 ± 3.26$^{ab}$ | n.d.$^{b}$ | n.d$^{b}$ | 2.28 ± 0.07$^{ab}$ | 1.87 ± 1.07$^{ab}$ | 10.31 ± 0.07$^{a}$ | n.d$^{b}$ |
| MUFA[b] | 172.21 ± 7.92$^{a}$ | 156.26 ± 0.2$^{ab}$ | 136.0 ± 9.50$^{bc}$ | 104.82 ± 9.71$^{cd}$ | 80.67 ± 2.07$^{d}$ | 141.75 ± 11.2$^{ab}$ | 146.9 ± 1.80$^{ab}$ | 139.3 ± 2.45$^{ab}$ |
| LA[c] | 26.0 ± 0.13$^{b}$ | 35.31 ± 4.03$^{b}$ | 33.66 ± 2.50$^{b}$ | 27.85 ± 2.73$^{b}$ | 120.41 ± 0.29$^{a}$ | 36.92 ± 5.84$^{b}$ | 22.87 ± 0.59$^{b}$ | 31.37 ± 3.29$^{b}$ |
| ARA[d] | 15.92 ± 0.03$^{ab}$ | 14.53 ± 0.12$^{ab}$ | 28.53 ± 8.05$^{a}$ | 28.38 ±3.58$^{a}$ | 3.21 ± 0.24$^{b}$ | 8.71 ± 1.67$^{b}$ | 12.76 ± 0.39$^{b}$ | 13.19 ± 0.09$^{ab}$ |
| n-6 PUFA[e] | 41.93 ± 0.16$^{bc}$ | 49.85 ± 3.90$^{bc}$ | 62.19 ± 10.55$^{b}$ | 56.22 ± 0.85$^{bc}$ | 123.56 ± 0.54$^{a}$ | 45.64 ± 7.50$^{bc}$ | 35.63 ± 0.98$^{c}$ | 44.56 ± 3.40$^{bc}$ |
| LNA[f] | 7.52 ± 0.44$^{ab}$ | 9.36 ± 1.40$^{ab}$ | 7.07 ± 1.55$^{ab}$ | 4.29 ± 0.02$^{b}$ | 14.7 ± 0.50$^{ab}$ | 31.20 ± 14.53$^{a}$ | 6.17 ± 0.58$^{ab}$ | 7.97 ± 0.41$^{ab}$ |
| EPA[g] | 68.09 ± 2.68$^{ab}$ | 71.88 ± 2.62$^{a}$ | 55.56 ± 16.4$^{abc}$ | 28.59 ± 0.29$^{cd}$ | 23.87 ± 0.33$^{d}$ | 39.83 ± 7.4$^{bcd}$ | 57.39 ± 1.2$^{abc}$ | 74.81 ±1.54$^{a}$ |
| DHA[h] | 78.23 ± 1.54$^{a}$ | 66.37 ± 2.95$^{abc}$ | 74.08 ± 6.32$^{ab}$ | 75.81 ± 9.04$^{ab}$ | 54.54 ± 0.72$^{cd}$ | 42.57 ± 2.66$^{d}$ | 60.79 ± 1.1$^{abcd}$ | 56.32 ± 0.7$^{bcd}$ |
| n-3 PUFA[i] | 153.85 ± 3.78$^{a}$ | 147.62 ± 1.7$^{a}$ | 136.72 ± 11.6$^{ab}$ | 108.70 ± 8.76$^{bc}$ | 93.13 ± 1.57$^{c}$ | 113.6 ± 9.76$^{bc}$ | 124.36 ± 1.6$^{ab}$ | 139.11 ± 0.40$^{ab}$ |
| LC-PUFA[j] | 162.25 ± 4.19$^{a}$ | 152.79 ± 0.5$^{ab}$ | 158.18 ± 2.0$^{ab}$ | 132.78 ± 5.16$^{c}$ | 81.63 ± 1.31$^{d}$ | 91.12 ± 3.10$^{d}$ | 130.95 ± 2.72$^{c}$ | 144.32 ± 0.72$^{bc}$ |
| n-3 / n-6 | 3.67 ± 0.1$^{a}$ | 3.0 ± 0.27$^{ab}$ | 2.40 ± 0.62$^{ab}$ | 1.93 ± 0.19$^{bc}$ | 0.75 ± 0.01$^{c}$ | 2.55 ± 0.21$^{ab}$ | 3.49 ± 0.47$^{a}$ | 3.16 ± 0.25$^{ab}$ |
| ARA / EPA | 0.23 ± 0.0$^{ab}$ | 0.20 ± 0.0$^{b}$ | 0.75 ± 0.40$^{ab}$ | 1.0 ± 0.1$^{a}$ | 0.13 ± 0.0$^{b}$ | 0.25 ± 0.09$^{ab}$ | 0.22 ± 0.02$^{ab}$ | 0.18 ± 0.0$^{b}$ |
| DHA / EPA | 1.15 ± 0.02$^{bc}$ | 0.93 ± 0.08$^{bc}$ | 1.73 ± 0.71$^{abc}$ | 2.66 ± 0.34$^{a}$ | 2.28 ± 0.01$^{ab}$ | 1.18 ± 0.30$^{bc}$ | 1.06 ± 0.0$^{bc}$ | 0.75 ± 0.03$^{c}$ |

Abbreviations: SW: sudden weaning; EW: early weaning; LW: late weaning, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, LA: linoleic acid, ARA: arachidonic acid, PUFA: polyunsaturated fatty acids, LNA: linolenic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, n.d: not detected.

**Supplementary file.** Fatty acid composition of (mg g$^{-1}$ extracted lipid) *A. latus* larvae under different weaning strategies after 30 days (mean ± SE, n= 3 tank). Different superscript letters within a column indicate significant differences ($P < 0.05$).

| | Diets | | | | | | | | Two-Way ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Fatty acids* | 100 LF | 75 LF-25MD | 50LF-50MD | 25LF-75MD | 100MD | SW | EW | LW | Time of weaning | Proportion of LF and MD | Interactions |
| 14:0 | 8.2 ±0.3$^{c}$ | 10.8 ±1.8$^{bc}$ | 11.8 ± 0.22$^{bc}$ | 9.0 ±0.6$^{c}$ | 19.3 ±0.6$^{a}$ | 14.2 ±2.0$^{b}$ | 9.0 ±0.9$^{c}$ | 14.2 ±0.3$^{b}$ | 0.001 | 0.001 | 0.075 |
| 16:0 | 146.8 ±1.4$^{c}$ | 144.8 ±0.4$^{c}$ | 168.8 ± 13.4$^{bc}$ | 214.6 ±9.8$^{a}$ | 183.6 ±1.8$^{ab}$ | 162.0 ±4.0$^{bc}$ | 153.0 ±6.2$^{bc}$ | 148.2 ±3.6 $^{c}$ | 0.479 | 0.011 | 0.999 |
| 18:0 | 82.3 ±5.0 $^{a}$ | 56.0 ±5.1$^{ab}$ | 67.4 ±7.9$^{ab}$ | 42.3 ± 22.4$^{ab}$ | 36.6 ±0.7$^{b}$ | 41.7 ±0.3$^{ab}$ | 69.5 ±2.9$^{ab}$ | 46.3 ± 1.8$^{ab}$ | 0.008 | 0.016 | 0.503 |
| 20:0 | 2.1 ± 1.2$^{b}$ | 3.5 ± 0.1$^{ab}$ | 4.8 ± 0.9$^{ab}$ | 1.6 ± 0.1$^{b}$ | 8.0 ± 2.3$^{a}$ | 2.9 ± 1.1$^{b}$ | 3.6 ± 0.2$^{ab}$ | 1.9 ± 0.2$^{b}$ | 0.012 | 0.004 | 0.047 |
| 22:0 | 5.2 ± 3.0$^{b}$ | n.d$^{c}$ | n.d$^{c}$ | n.d$^{c}$ | 15.6 ± 0.0$^{a}$ | 3.0 ± 1.7$^{b}$ | 5.1 ± 0.3$^{b}$ | n.d$^{c}$ | 0.001 | 0.009 | 0.001 |
| SFA[a] | 244.8 ±0.9 | 215.1 ±3.6 | 253.0 ± 21.2 | 267.4 ± 34.6 | 263.2 ±5.4 | 223.8 ±1.1 | 240.7 ±6.5 | 210.8 ±5.9 | 0.043 | 0.163 | 0.170 |
| 14:1n-5 | 21.6 ± 7.3$^{b}$ | 29.8 ± 9.6$^{a}$ | 19.5 ± 1.4$^{b}$ | 35.2 ± 2.9$^{a}$ | 1.9 ± 0.3$^{d}$ | 34.1 ± 8.4$^{a}$ | 8.9 ± 0.1$^{c}$ | 27.8 ± 4.6$^{a}$ | 0.045 | 0.203 | 0.022 |
| 16:1n-7 | 51.45 ± 7.2 | 66.6 ± 3.5 | 54.7 ± 15.3 | 26.1 ± 1.9 | 21.1 ± 0.4 | 69.3 ± 13.4 | 66.1 ± 1.7 | 83.5 ± 5.2 | 0.699 | 0.004 | 0.007 |
| 18:1n-7 | 112.2 ± 4.9$^{a}$ | 89.3 ± 4.2$^{a}$ | 72.0 ± 12.7$^{ab}$ | 45.5 ± 4.5$^{bc}$ | 21.3 ± 0.9$^{c}$ | 72.8 ± 19.4$^{ab}$ | 104.5 ± 1.1$^{a}$ | 91.2 ± 3.7$^{a}$ | 0.012 | 0.415 | 0.046 |
| 18:1n-9 | 108.0 ± 11.1$^{b}$ | 120.3 ±2.7$^{ab}$ | 118.8 ±3.5$^{ab}$ | 127.3 ±5.2$^{ab}$ | 125.2 ±5.0$^{ab}$ | 142.6 ±0.6$^{a}$ | 130.7 ±0.5$^{ab}$ | 120.8 ±1.3$^{ab}$ | 0.001 | 0.001 | 0.205 |
| 20:1n-9 | 2.1 ± 0.4$^{c}$ | 2.5 ± 0.0$^{c}$ | 25.9 ± 12.7$^{a}$ | 30.5 ± 15.7$^{a}$ | 16.5 ± 0.5$^{b}$ | 3.0 ± 0.4$^{c}$ | 4.1 ± 0.8$^{c}$ | 1.4 ± 0.4$^{c}$ | 0.857 | 0.027 | 0.776 |
| 22:1n-9 | 13.3 ± 4.5$^{ab}$ | 11.5 ± 3.5$^{ab}$ | n.d.$^{b}$ | n.d$^{b}$ | 5.3 ± 0.7$^{ab}$ | 4.3 ± 1.7$^{ab}$ | 23.7 ± 7.2$^{a}$ | n.d$^{b}$ | 0.782 | 0.157 | 0.556 |
| MUFA[b] | 308.8 ± 14.2$^{a}$ | 320.2 ±0.4$^{a}$ | 290.9 ± 20.3$^{a}$ | 264.7 ± 24.5$^{ab}$ | 191.4 ±4.9$^{b}$ | 326.2 ± 25.8$^{a}$ | 338.1 ±4.2$^{a}$ | 324.8 ±5.8$^{a}$ | 0.008 | 0.001 | 0.001 |
| LA[c] | 46.6 ±0.2$^{c}$ | 72.3 ±8.3$^{c}$ | 72.0 ±5.3$^{c}$ | 70.3 ±6.9$^{c}$ | 125.6 ± 13.3$^{a}$ | 85.0 ± 13.4$^{b}$ | 52.6 ±1.4$^{c}$ | 73.1 ±7.8$^{c}$ | 0.001 | 0.001 | 0.001 |
| ARA[d] | 28.5 ±0.1$^{c}$ | 29.8 ±0.3$^{c}$ | 61.0 ± 17.2$^{a}$ | 71.6 ±9.0$^{a}$ | 7.6 ±0.6$^{d}$ | 20.0 ±3.8$^{d}$ | 29.3 ±0.9$^{c}$ | 30.7 ±0.2$^{bc}$ | 0.005 | 0.680 | 0.009 |
| n-6 PUFA[e] | 75.2 ±0.3$^{d}$ | 102.1 ±8.0$^{bc}$ | 133.0 ± 22.5 | 142.0 ± 2.2$^{b}$ | 133.3 ±1.2$^{a}$ | 105.0 ± 17.2$^{bc}$ | 82.0 ±2.3$^{cd}$ | 104.0 ±8.0$^{bc}$ | 0.001 | 0.001 | 0.001 |
| LNA[f] | 13.4 ±0.8$^{c}$ | 19.1 ±2.8$^{c}$ | 15.1 ±3.3$^{c}$ | 10.8 ±0.1$^{c}$ | 34.8 ±1.2$^{b}$ | 71.8 ± 33.4$^{a}$ | 14.2 ±1.3$^{c}$ | 18.6 ±1.0$^{c}$ | 0.103 | 0.048 | 0.950 |
| EPA[g] | 122.1 ±4.8$^{ab}$ | 147.3 ±5.4$^{ab}$ | 118.9 ± 35.0$^{ab}$ | 72.1 ±0.7$^{c}$ | 56.6 ±0.8$^{d}$ | 91.6 ± 17.0$^{c}$ | 132.1 ±2.8$^{ab}$ | 174.3 ±3.6$^{a}$ | 0.215 | 0.026 | 0.075 |
| DHA[h] | 140.3 ±2.8$^{bc}$ | 136.0 ±6.0$^{bc}$ | 158.4 ± 13.5$^{ab}$ | 191.4 ± 22.8$^{a}$ | 129.4 ± 01.7$^{bc}$ | 97.9 ±6.1$^{c}$ | 140.0 ±2.8$^{bc}$ | 1312. ±1.7$^{bc}$ | 0.046 | 0.247 | 0.522 |
| n-3 PUFA[i] | 276.0 ±6.8$^{ab}$ | 302.5 ±3.6$^{a}$ | 292.4 ± 24.8$^{a}$ | 274.5 ± 22.1$^{ab}$ | 221.0 ± 03.7$^{b}$ | 261.4 ± 22.4$^{ab}$ | 286.2 ±4.0$^{ab}$ | 324.2 ±0.9$^{a}$ | 0.066 | 0.058 | 0.007 |
| LC-PUFA[j] | 291.0 ± 7.5$^{b}$ | 313.0 ±0.3$^{b}$ | 338.3 ±4.2$^{a}$ | 335.3 ± 13.0$^{a}$ | 193.6 ± 3.1$^{c}$ | 209.7 ±7.2$^{c}$ | 301.3 ±6.3$^{b}$ | 336.4 ±1.7$^{a}$ | 0.001 | 0.171 | 0.002 |
| n-3 / n-6 | 3.66 ± 0.10$^{a}$ | 30.0 ±2.7$^{ab}$ | 2.40 ± 0.62$^{b}$ | 1.93 ± 0.19$^{bc}$ | 1.67 ± 0.39$^{c}$ | 2.56 ± 0.11 $^{b}$ | 3.49 ± 0.05$^{a}$ | 3.15 ± 0.25$^{ab}$ | 0.103 | 0.03 | 0.126 |
| ARA / EPA | 0.23 ± 0.01$^{ab}$ | 2.0 ±0.1$^{b}$ | 0.75 ± 0.41$^{ab}$ | 1.0 ± 0.11$^{a}$ | 0.13 ± 0.01$^{b}$ | 0.25 ± 0.09$^{ab}$ | 0.22 ± 0.01$^{ab}$ | 0.17 ± 0.01$^{b}$ | 0.582 | 0.007 | 0.311 |
| DHA / EPA | 1.15 ± 0.02$^{bc}$ | 9.2 ±0.8$^{bc}$ | 1.73 ± 0.71$^{abc}$ | 2.65 ± 0.34$^{a}$ | 2.28 ± 0.01$^{ab}$ | 1.17 ± 0.3$^{bc}$ | 1.05 ± 0.01$^{bc}$ | 0.75 ± 0.03$^{c}$ | 0.001 | 0.001 | 0.059 |

Abbreviations: SW: sudden weaning; EW: early weaning; LW: late weaning, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, LA: linoleic acid, ARA: arachidonic acid, PUFA: polyunsaturated fatty acids, LNA: linolenic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, n.d: not detected.