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The physiological consequences of delaying metamorphosis in the marine ornamental shrimp *Lysmata seticaudata* and its implications for aquaculture

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Abstract

The timing of metamorphosis has recently been referred as a more suitable proxy to evaluate postlarval quality than size. Indeed, while displaying a larger size at settlement, late settlers that originate from larvae that have delayed metamorphosis commonly display poorer growth performances This delay in metamorphosis is possibly at the expenses of larval endogenous energetic reserves that, once allocated to facilitate the delayed transition to benthic life, will no longer be available to help fuelling early juvenile somatic growth. To further advance our knowledge on this topic, we evaluated the physiological status (energy reserves and allocation, aerobic and anaerobic energy consumption), along with biochemical responses related with detoxification processes, antioxidant defences, oxidative damage, neuromotor activity in early settlers (ES), middle settlers (MS) and late settlers (LS) of postlarvae of the marine ornamental shrimp *Lysmata seticaudata*. Our results revealed that LS postlarvae presented a higher weight compared to MS and ES, likely related with a lower metabolism and neuromotor activity. Yet, the low metabolism allied with diminished detoxification and antioxidant capacities seemed to result in an increased oxidative stress condition that may negatively condition the growth performance of LS postlarvae. Conversely, ES postlarvae presented a lower weight, likely because of high metabolic costs associated with increased neuromotor activity, detoxification, and antioxidant capacities to avoid oxidative damage. The present study highlights how the physiological, metabolic and biochemical status of *L. seticaudata* postlarvae is shaped by the timing of their metamorphosis, as well as how this event will shape their early benthic life and confirms that a larger size or weight at metamorphosis may not be good proxies to select premium seedlings for grow-out. Overall, metamorphosis is not a new beginning and does not reset larval history. Crustacean farmers should avoid decoupling larviculture history from grow-out, as only by knowing larval performance to

metamorphosis will be possible to enhance survival and growth performances to commercial size.

Keywords: caridean shrimps, decapod crustaceans, energy budget, oxidative stress status

1. Introduction

Metamorphosis is a critical stage in the development of invertebrates with bi-phasic life cycles, such as decapod crustaceans (Anger, 2001). In decapod crustaceans, as for other marine invertebrates, metamorphosis can be triggered by biological and physical factors, acting as cues that safeguard developing larvae to find a suitable habitat for their juvenile and adult life (Gebauer et al., 2002; Campos et al., 2016; Hinojosa et al., 2018; Natin and Lee, 2018). The larval stages of decapod crustaceans may vary in number and duration and even when larvae appear to be fully developed, they can still delay their metamorphosis through terminal additive moulting (Gore, 1985). The mechanisms triggering the delay in larval metamorphosis remain elusive, with this "flexibility" on timing metamorphosis being considered a selective advantage by enhancing the survival of subsequent life stages (juveniles and adults)(Pechenik, 1990; Gebauer et al., 2003; Anger, 2006).

The caridean shrimp *Lysmata seticaudata*, a marine ornamental decapod prized for marine aquariums (Calado et al., 2003), is used in the present study as a model species. *Lysmata seticaudata* displays a long larval development with multiple larval stages (nine zoeas and a decapodid) (Calado, 2004) and exhibits facultative secondary lecithotrophic (it can metamorphose by catabolising energy acquired during larval life when deprived of exogenous food) (Calado et al., 2007a). Moroever, this caridean shrimp species is well known to be able to delay metamorphosis for several weeks

(Calado, 2008). A previous study by Carvalho and Calado (2018) confirmed that juveniles originating from larvae that have delayed metamorphosis display a poorer growth performance, although they metamorphose with larger sizes. As such, at least for marine caridean shrimps, postlarval size at metamorphosis is likely an unsuitable proxy to infer the quality of postalarvae and how they will perform during grow-out. To advance our current knowledge on this topic, one can assess energy reserves and its allocation in newly metamorphosed shrimp (postlarvae), along with the evaluation of their neuromotor activity, aerobic and anaerobic energy, and stress-related parameters. Indeed, by doing so one can better understand important species-specific physiological traits that can be paramount to successful grow cultured specimens to commercial sizes. The present work aimed to identify the physiological consequences of delaying metamorphosis by late-stage larvae of the marine ornamental shrimp *L. seticaudata*. This goal was pursued by surveying the oxidative stress status, energetic metabolism and neurotransmission of organisms that have just undergone a dramatic shift in their lifecycle – metamorphosis to benthic postlarval life. We hypothesised that early settlers (ES) (postlarvae that displayed a shorter larval life), middle settlers (MS) and late settlers (LS) (postlarvae originating from larvae that delayed their metamorphosis the most) display different energy production rates and neuromotor activity that shapes their energy reserves, feeding and growth performance due to oxidative stress. As such, several parameters associated with these important physiological aspects were determined in ES, MS, and LS postlarvae, namely: energy reserves (total levels of proteins, lipids, and carbohydrates), energy available (Ea), aerobic energy production (via electron transport system - ETS), cellular energy allocation (CEA) and anaerobic energy production (lactate dehydrogenase – LDH). Moreover, parameters related to oxidative stress, such as phase II biotransformation enzyme glutathione S-transferase

(GST), the antioxidant enzyme catalase (CAT), total glutathione levels (TG), and lipid peroxidation (LPO) were also determined. Cholinesterases activity (ChE) was also analysed to determine cholinergic neurotransmission, as a proxy to infer neuromotor activity.

2. Material and methods

2.1. Broodstock husbandry, larviculture and sampling of postlarvae

Breeding pairs of *L. seticaudata* were purchased from Tropical Marine Centre Iberia (a wholesaler of marine aquarium species committed to sustainable practices) and kept in a maturation system described by Calado et al. (2007b) until larval hatching. Newly hatched larvae were cultured using the larviculture system described by Calado et al. (2008), with culture conditions and sampling being identical to those detailed by Carvalho & Calado (2018). Briefly, salinity was maintained at 35 (± 1), pH at 8.0 (± 0.1), temperature at 26 °C (± 1), and a photoperiod of 12/12 h (light/dark). Ammonia and nitrite were monitored using colorimetric tests, and kept below detectable levels, and nitrate was maintained between 7 and 9.5 mg L^{-1}. Broodstock was fed with frozen commercial diet (Gamma® Marine Cuisine) 4 times a day *ad libitum*. Newly hatched larvae from three different ovigerous shrimp were stocked in larviculture tanks and cultured until metamorphosis, as detailed by Calado (2008). Larvae were fed newly hatched *Artemia* nauplii until metamorphosis and postlarvae were collected daily using a glass pipette immediately after settlement (as described by Carvalho and Calado, 2018). Those that metamorphosed between the 1st day that the first postlarva was recorded in a larviculture tank and the 5th day were termed as early settlers (ES), while those being recorded between the 6th and the 10th day were termed as middle settlers (MS); postlarvae collected between the 11th and the 15th day after the record of the first

postlarva in a larviculture tank were termed as late settlers (LS). Ten newly metamorphosed ES, MS, and LS postlarvae were sampled randomly at the time of settlement for biochemical analysis.

2.2. Samples processing for biochemical analysis

All samples were weighted (wet weight) to the nearest 0.01 g, flash frozen with liquid nitrogen and kept at -80 ºC until further use. Samples were individually homogenised in ice by sonication using 1600 µL of ultrapure water. Three aliquots of 300 µL were taken for the analysis of lipids, crabohydrates and proteins contents, and electron transport system (ETS) activity from each sample. An aliquot of 200 µL of each sample was used for the determination of levels of lipid peroxidation (LPO), whereas the remaining homogenate (~500 µL) was further diluted with 500 µL of 0.2 M K-phosphate buffer (pH=7.4), centrifuged for 20 min at 3,000 g (4 °C). The post-mitochondrial supernatant (PMS) was collected and divided into five microtubes. Those five samples of PMS of each sample were kept at −80 °C until further analyses of biomarkers, i.e., lactate dehydrogenase (LDH), glutathione S-transferase, catalase (CAT, EC 1.11.1.6), total glutathione levels (TG) and cholinesterases activity (ChE, EC 3.1.1.7)), as well as protein content of each sample of PMS.

2.2.1. Energy reserves and aerobic consumption measurements

The quantification of energy available (proteins lipids and carbohydrates) and energy consumption (ETS) followed the method described by DeCoen and Janssen (1997) with slight modifications.

A microplate reader MultiSkan Spectrum (Thermo Fisher Scientific, USA) was used for all measurements required. Total lipid content was separated by centrifugation of samples (300 µL of homogenate) with the addition of 500 µL of chloroform (119.38 M; ACS spectrophotometric grade, ≥99.8%) and methanol (32.04 M; ACS reagent, ≥99.8%). The organic phase of each sample was transferred to clean glass tubes, 500 µL of H_2SO_4 was added and incubated at 200 °C for 15 min. After cooling down to room temperature, 1500 µL of ultra-pure water was added to each tube and the absorbance measured in the microplate at 375 nm. Tripalmitin was used as a lipid standard. The quantification of carbohydrate content was performed by adding 100 µL of 15% TCA to 300 µL of homogenate followed by an incubation of 10 min at −20 °C. After centrifugation at 1000 g for 10min at 4 °C, 200 µL of 5% phenol and 800 µL of H_2SO_4 were added to the supernatant.

The samples and glucose used as standard were incubated at 20 °C for 30 min and the absorbance read at 492 nm in the microplate reader. Bradford's method (Bradford, 1976) was used for total protein content quantification in homogenate using bovine serum albumin as a standard. Absorbance was measured after 30 min incubation in the microplate at 520 nm.

For the ETS activity measurement 150 µL of homogenization buffer (0.3 M Tris base; 0.45% (w/v) Poly Vinyl Pyrrolidone; 459 µM MgSO4; 0.6% (v/v) Triton X-100 at a pH of 8.5) was added to 300 µL homogenate samples and centrifuged (1000 g, 10 min, 4 °C). Supernatant was transferred to a microplate (50 µL per replicate), and 150 µL of buffered solution 0.13MTris base; 0.27% (v/v) Triton X-100; 1.7mM NADH; 274 µM NADPH; and 100 µL of INT solution (p-iodonitrotetrazolium; 8 mM) were added. The absorbance was measured kinetically at 490 nm over a 3 min period.

The conversion into energetic values of the fractions of energy available was calculated using the corresponding energy of combustion: 39,500 mJ/g lipid, 17,500 mJ/g glycogen, and 24,000 mJ/g protein (DeCoen and Janssen, 1997). The cellular oxygen consumption rate was calculated based on the stoichiometrical relationship in which for 2 μmol of formazan formed, 1 μmol of oxygen is consumed. The quantity of oxygen consumed was determined by the formula of Lambert-Beer: $A=\varepsilon \times l \times c$ (A=absorbance; ε for INT-formazan=15,900/(M·cm); l=0.9 cm; c=oxygen consumed in M). The final Ec value was obtained by converting to energetic values using the specific oxyenthalpic equivalent for an average lipid, protein and carbohydrate mixture of 480 kJ/mol O_2. The sum of total lipids, carbohydrates and proteins constitutes the available energy (Ea). All the values obtained were adjusted to the weight of the organisms using the following allometric equation: $Z=Y(M-0.71)$, where Y is the energetic value of each measured content, M is the fresh weight of the sample and Z the final value corrected to the weight of analysed organisms (Pentinen and Holopainen, 1995). The final CEA value is calculated by the formula: CEA = La/Ec (Verslycke et al., 2003).

2.2.2. Biomarkers of stress, neurotransmission, and anaerobic energy consumption

Tissue homogenate (200 μL) was separated into a microtube with 4 μL of 4% butylated hydroxytoluene (BHT) in methanol for endogenous LPO determination by measuring thiobarbituric acid-reactive substances (TBARS) at 535 nm (Bird and Draper, 1984). TG content was determined with PMS fraction at 412 nm using a recycling reaction of reduced glutathione (GSH) with 100 μL 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) in the presence of glutathione reductase (GR) excess (25 μL from stock with 1 U/mL) (Tietze, 1969; Baker et al., 1990). GST activity (EC 2.5.1.18) was determined in PMS following the conjugation of GSH with 1-chloro-2,4-dinitrobenzene at 340 nm (Habig

et al., 1974). CAT activity was determined in PMS by measuring decomposition of the substrate H_2O_2 at 240 nm (Claiborne, 1985). ChE activity was measured by following Ellman's method (Ellman et al., 1961) adapted to a microplate (Guilhermino et al., 1996). The method uses acetylthiocholine as a substrate following the increase of absorbance at 412 nm.

2.2.3. Protein content

Protein content was determined according to the method developed by Bradford (1976), adapted from BioRad's Bradford micro-assay setup in a 96 well flat bottom plate, using bovine γ-globulin as a standard.

2.3. Statistical analyses

Kolmogorov–Smirnov and Barlett's tests were used to check for normality of data and homogeneity of variances, respectively. A one-way analysis of variance (ANOVA) was then performed, followed by Tukey's multiple comparison test to determine the differences between ES, MS, and LS postlarvae. Results were considered statistically significant when $p < 0.05$. The software SigmaStat 3.5 was used to perform all statistical analysis.

3. Results

3.1 Settlement pattern

The first postlarva was recorded 25 days after hatching, with the number of postlarvae peaking at the 10th day after recording the first postlarva. By the 10th day of settlement, approximately 50% of all larvae had already metamorphosed, with the highest proportion of settlements being recorded between the 5th and 10th day corresponding to

MS ($F_{2, 42} = 8.25$, $p < 0.001$). The other 50% of postlarvae recorded were approximately equally distributed between ES (~25%) and LS (~25%).

3.2. Wet weight, energy reserves, and production available for postlarvae

Postlarvae's wet weight was significantly different between ES, MS and LS periods ($F_{2, 27} = 4.091$, $p = 0.028$), with wet weight of LS being higher than that of MS and ES exhibiting the lowest values (Table 1).

The levels of carbohydrates were significantly higher ($F_{2, 27} = 12.757$, $p < 0.001$) on LS postlarvae than in ES ($p = 0.001$) and MS ($p < 0.001$) postlarvae, showing the following pattern LS>ES~MS (Table 1). No significant differences were observed for the levels of proteins ($F_{2, 26} = 2.12$, $p = 0.14$), lipids ($F_{2, 27} = 2.723$, $p = 0.084$), and total levels of energy available ($F_{2, 26} = 1.753$, $p = 0.193$) when comparing ES, MS, and LS (Table 1). The aerobic energy production (ETS activity) was significantly lower ($F_{2, 26} = 16.131$, $p < 0.001$) on LS when compared to the ES ($p = 0.003$) and MS ($p < 0.001$) postlarvae (Figure 1A). Concomitantly, CEA was significantly higher ($F_{2, 25} = 17.042$, $p < 0.001$) on LS compared to the ES ($p < 0.001$) and MS ($p < 0.001$) postlarvae (Figure 2A). LDH activity was significantly lower ($F_{2, 27} = 4.789$, $p = 0.017$) on LS compared to ES ($p = 0.018$) postlarvae (Figure 1B).

3.3. Detoxification capacity, antioxidants, oxidative stress, and neurotransmission

The GST activity was significantly ($F_{2, 27} = 7.426$, $p < 0.003$) higher in ES postlarvae compared to LS ($p = 0.003$) and MS ($p < 0.019$) postlarvae (Figure 2A). No significant differences were observed for CAT activity ($F_{2, 27} = 2.222$, $p = 0.128$) in ES, MS, and LS postlarvae (Figure 2B). The levels of total glutathione were significantly ($F_{2, 27} = 13.721$, $p < 0.001$) higher in ES postlarvae compared to LS ($p < 0.001$) and MS ($p = 0.002$)

postlarvae (Figure 2C). In contrast, levels of LPO were significantly ($F_{2, 27} = 13.488$, $p < 0.001$) higher in LS ($p < 0.001$) and MS ($p = 0.01$) postlarvae than in ES postlarvae (Figure 2D).

ChE activity of LS postlarvae was significantly lower ($F_{2, 27} = 4.970$, $p < 0.015$) than that recorded for ES postlarvae ($p = 0.011$) (Figure 3).

4. Discussion

Our results revealed that LS postlarvae of *L. seticaudata* presented significantly higher weight values than ES conspecifics. This finding agrees with a previous study performed with the same shrimp species under similar experimental settings by Carvalho and Calado (2018). As highlighted by those authors, larger LS postlarvae of *L. seticaudata* failed to display a better growth performance during early benthic life, as juvenile shrimp originating from ES and MS always outperformed them. As highlighted by Pechenik (2006), metamorphosis is not a new beginning, as delaying this key event in marine organisms with biphasic life cycles commonly comes at a cost. Carryover effects can occur between different life-history stages, driving non-lethal interactions between distinct periods of an organism's lifetime (O'Connor et al., 2014), with decapod crustaceans being no exception (Gebauer, et al., 2003). Indeed, Carvalho and Calado (2018) suggested that *L. seticaudata* larvae delaying metamorphosis unequivocally experience a trade-off, as once their endogenous energetic reserves are allocated to prolong larval life, they will no longer be available to help fuelling juvenile growth once the shrimp shift from a pelagic to a benthic environment.

As it can be perceived from the present study, LS, MS, and ES postlarvae did present significant differences on energy consumption and allocation, neuromotor activity and

oxidative stress levels at metamorphosis. The lower metabolism can be inferred from a lower energy consumption and a higher energy allocation, while a reduced neuromotor activity can be inferred by ChE activity displayed by LS postlarvae. These features are a direct consequence of their prior larval life (e.g., performing mark-time moulting and delaying metamorphosis) and it can explain their considerable higher weight when compared to ES and MS conspecifics. As suggested by Knowlton (1974), when zoea enter such a "maintenance mode" as mark-time moulting, they will delay metamorphosis by prioritizing "staying alive", eventually undergo somatic growth, but not morphogenesis. Whenever maintenance requirements are fulfilled (e.g., through proper feeding), morphogenesis may proceed, and metamorphosis will occur (Gore, 1985). It is worth highlighting that LS postlarvae also presented a lower detoxification and antioxidant ability (GST, TG) that seem to be associated with an increased oxidative stress condition that will negatively impact the health status of their subsequent benthic life stages. Conversely, ES postlarvae presented a lower oxidative stress condition, which indicates that a higher metabolic rate, along with an increased detoxification and antioxidant ability, were sufficient to control/impair oxidative stress. Yet, such high energetic costs associated with increased neuromotor activity may also be the drivers shaping the lower weight displayed by ES postlarvae when compared to MS and LS conspecifics.

LS, MS, and ES postlarvae exhibited different proportions of energy reserves (e.g., lipids, proteins, and carbohydrates). The levels of proteins, lipids and carbohydrates recorded are in line to the proportions previously reported for decapod crustaceans, with proteins and lipids acting as the main energy reserves to fuel development and somatic growth (Anger, 2001). In our study, the pattern of energy reserves recorded in postlarvae of *L. seticaudata* varied significantly with the timing of metamorphosis. For

instance, the levels of carbohydrates recorded for LS postlarvae were significantly higher than those recorded for ES or MS. While LS postlarvae exhibited a pattern of energy reserves (in decreasing order) of proteins > carbohydrates > lipids, ES and MS postlarvae displayed a more "classical" pattern for marine decapods (proteins > lipids > carbohydrates). The energy reserve pattern exhibited by LS postlarvae may have been shaped by the competition by food they may have experienced during larviculture. When prolonging their larval life, developing late stage zoea (zoea 9) will have to compete for food with conspecifics that have metamorphosed and are much more agile when foraging for newly hatched *Artemia* nauplii. In the present work, as newly settled postlarvae were sampled daily and removed from the larviculture tank, the coexistence between zoea 9 and newly metamorphosed conspecifics lasted for lower periods (up to 23 h per day) than what will most likely occur under commercial aquaculture set-ups. Allocating human resources to collect postlarvae daily may not be possible and/or economically feasible, thus zoea 9 delaying metamorphosis will have to endure a much more prolonged and intense competition for food during larviculture, where several days old (and not hours old) postlarvae will certainly be present. The last zoeal stage of *L. seticaudata* and its postlarval stage display a dramatically different morphology (Calado et al., 2004), with these shifts being even more pronounced in other species in the same genus (Bartilotti et al., 2012). While zoea swim sluggishly in the water column being thrusted backwards from the beating of their feather-like exopods and perform loops stretching their paddle-like enlarged fifth pair of pereiopods to capture live prey, postlarvae swim forward by vigorously beating their pleopods and feed both on the water column as in the larviculture tank surfaces (Calado, 2008). Zoea that metamorphose as LS postlarvae, will have to endure at least 10 days of feeding competition by postlarvae settling before them, even if these are removed daily from the

larviculture tank, as performed in the present study. LS postlarvae did present a low energy consumption, assessed as ETS (aerobic) and LDH (anaerobic), being concomitant with a low neuromotor activity (assessed as ChE) when compared to ES and MS, which likely resulted in a decreased ability to predate live food. These findings are also in good agreement with the values record for cellular energy allocation, which exhibited a significantly higher ratio in LS than ES or MS postlarvae of *L seticaudata* due to a significantly higher level of carbohydrates and decreased ETS in LS, as well as decreased LDH activity. Gebauer et al. (2003) referred that shifts in energy usage may be expected in organisms that present complex life cycles, such as most decapod crustaceans, due to morphological, behavioural, and other developmental aspects. Moreover, previous studies pointed out that the energetic metabolic costs associated with a delayed metamorphosis commonly cascades to subsequent life stages and shape the survival and growth performance of juveniles (Gimenez, 2006; Pechenik, 2006). As such, the lower growth-rate reported by Carvalho and Calado (2018) for LS postlarvae may be directly linked to metabolic processes and energy consumption. It has already been reported that other decapod crustaceans facing nutritional stress due to suboptimal feeding seem to preferentially catabolize lipids to become competent and metamorphose (Anger, 1998). Nonetheless, this has not been the case for LS postlarvae, as no significant differences in their lipid levels were recorded when compared to ES and MS postlarvae. Concerning the growth performance of ES postlarvae, this seems to be somehow compromised (Carvalho and Calado, 2018), likely due to hyperactivity (observed as increased ChE activity) that allow organisms to achieve a good nutritional status to acquire enough food, but fosters a higher allocation of energy for behavioural and detoxification processes to avoid oxidative stress than to somatic growth. In contrast, MS postlarvae, not so active as ES, seem to allocate much of their acquired

energy to maximise somatic growth and detoxification processes, which allows them to avoid oxidative stress, as the ratio between LPO and lipids levels in MS and ES postlarvae is similar.

Identifying the physiological differences reported above for ES, MS, and LS postlarvae that will shape the survival and growth performance of this valuable marine ornamental shrimp, is paramount to help breeders select the best performing specimens to minimise costs and enhance commercial revenues. Calado et al. (2005)had already suggested that size differences observed in the total length of postlarvae of *L. seticaudata* should be used cautiously in the assessment of postlarval quality. This caveat was later confirmed by Carvalho and Calado (2018) and highlight the need for farmers of decapod crustaceans for human consumption or other high-end uses (e.g., ornamentals) to revise their selection criteria when sourcing premium seedlings for grow-out. In decapod crustaceans, as in other marine invertebrates with bi-phasic life cycles, planktonic larval life and the biochemical status at metamorphosis will shape the subsequent performance of benthic life phases and condition grow-out (Gebauer, et al., 2003; Pechenik, 2006).

5. Conclusions

The metabolic differences observed in ES, MS, and LS postlarvae of *L. seticaudata*, along with detoxification and antioxidant capacities and neuromotor activity, seem to be strongly associated with the timing of metamorphosis. As such, crustacean farmers should avoid decoupling larviculture history from grow-out, as not knowing larval performance to metamorphosis will impair the selection of premium post-larvae for grow-out and, consequently, enhancing survival and growth performances to commercial size. In aquaculture, as in natural aquatic environments, metamorphosis is not a new beginning, and it does not reset larval history. Nonetheless, assessing the

physiological status of postlarvae is not a straight-forward procedure as determining their size and/or wight. As such, monitoring the physiological parameters we reported may be a challenge on-site under aquaculture settings, unless fast and easy to use kits are made available.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure 1- (A) Electron transport system (ETS) activity and Celular energy allocation (CEA), and (B) Lactate dehydrogenase (LDH) of early settlers (ES), median settlers (MS) and late settlers (LS) *Lysmata seticaudata* postlarvae. Values are means of 10 postlarvae per settler type, with error bars representing standard deviation and different letters representing significant differences at $p < 0.05$.

Figure 2 - (A) Glutathione S-transferase (GST) activity, (B) Catalase (CAT) activity, (C) total glutathione (TG) levels, and (D) Lipid peroxidation (LPO) of early settlers (ES), median settlers (MS) and late settlers (LS) *Lysmata seticaudata* postlarvae**.** Values

are means of 10 postlarvae per settler type, with error bars representing standard deviation and different letters representing significant differences at $p < 0.05$.

Figure 3 - Cholinesterase (ChE) activity of early settlers (ES), median settlers (MS) and late settlers (LS) of *Lysmata seticaudata* postlarvae (n = 10 postlarvae per settler type)**.** Values are means of 10 postlarvae per settler type, with error bars representing standard deviation and different letters representing significant differences at $p < 0.05$.

Figure 1

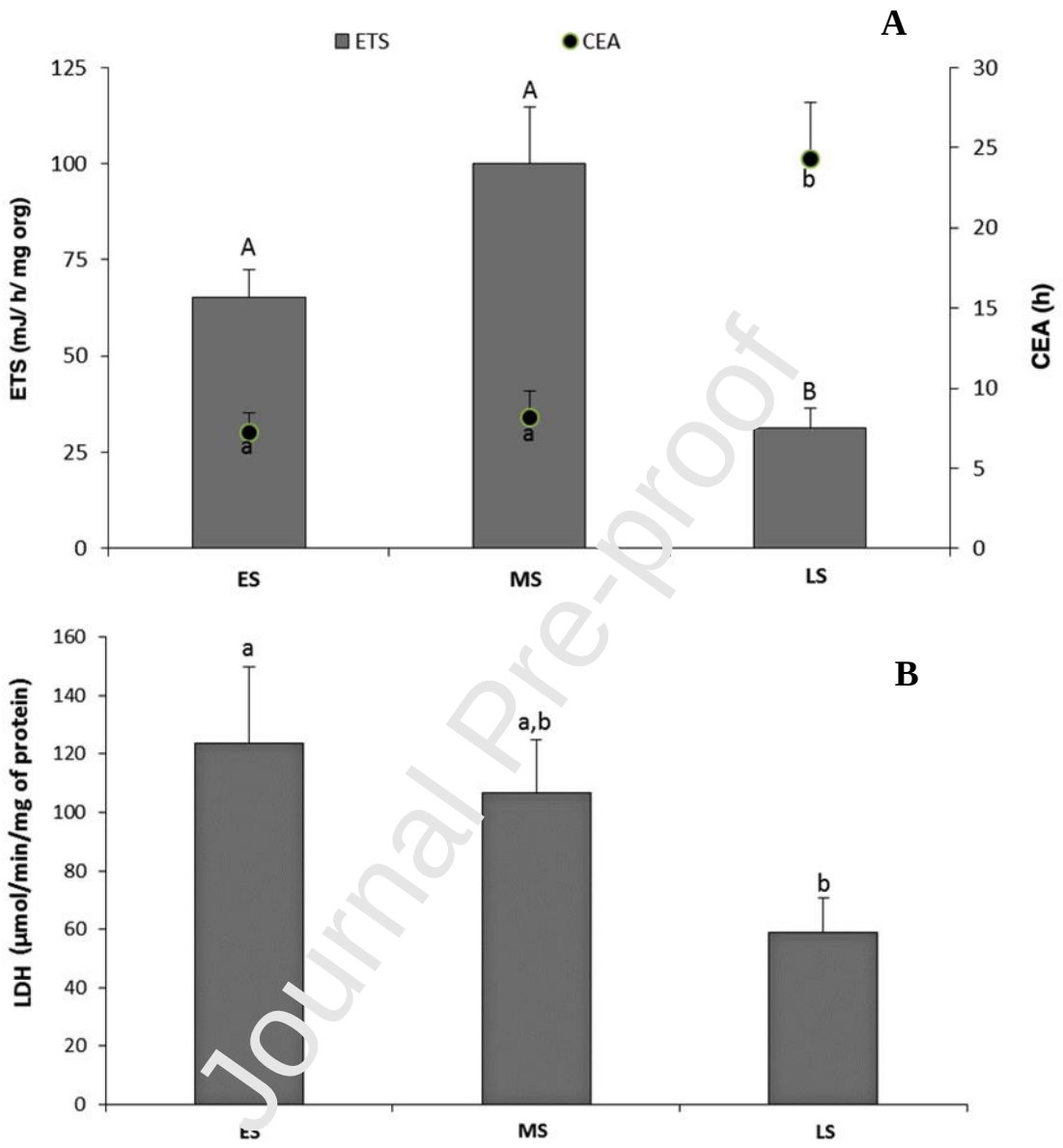

Figure 2

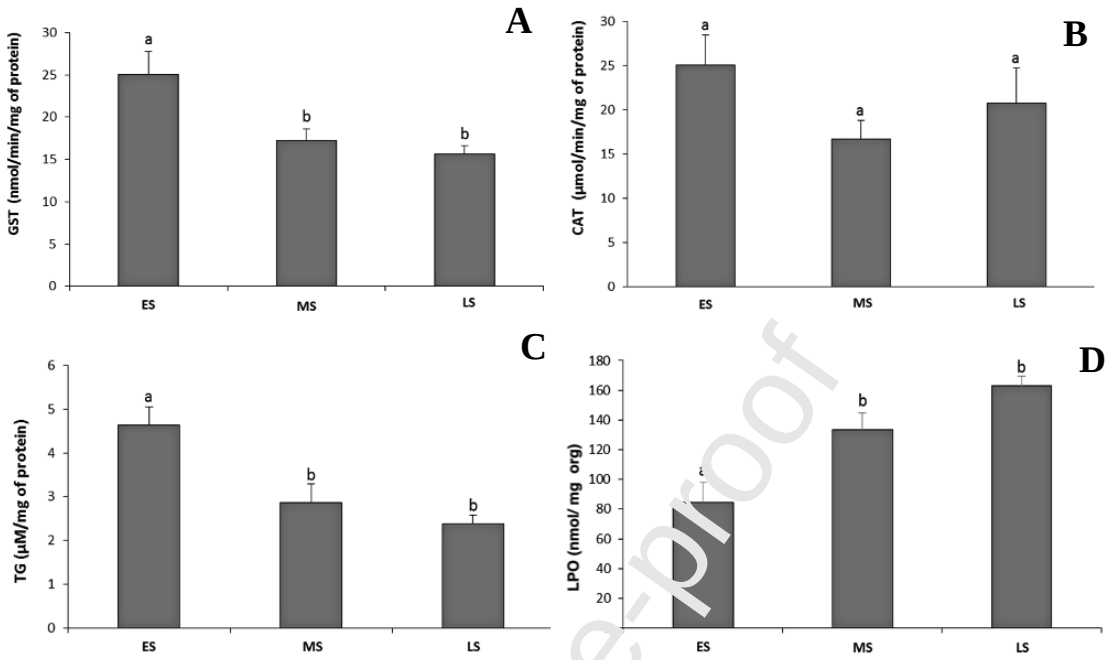

Figure 3

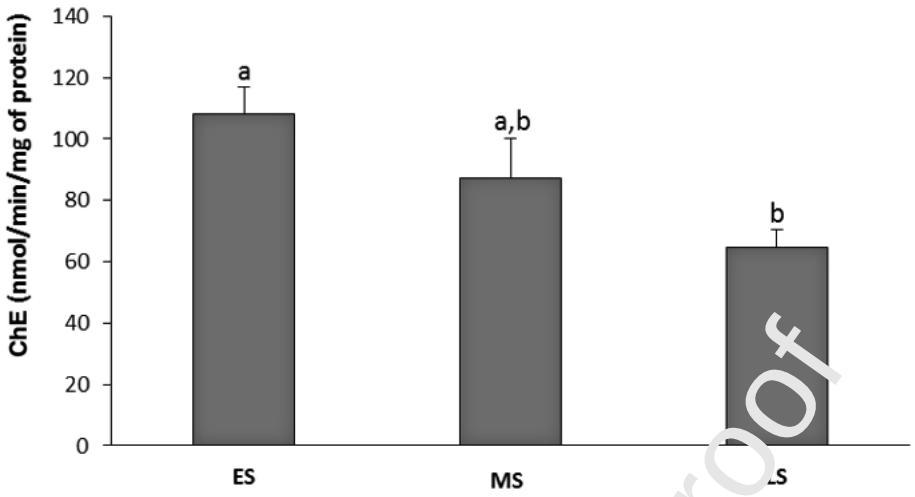

Table 1 – Wet weight (g), energy reserves (proteins, lipids and carbohydrates) and energy available (EA) (mJ/mg organism) of early settlers, middle settlers and late settlers *Lysmata seticaudata* postlarvae. Values are means (of 10 postlarvae per settler type) ± SE and different superscript letters within the same row represent significant differences at $p < 0.05$.

	Early Settlers	Middle Settlers	Late Settlers
Wet weigth	5.70±1.13[a]	6.11±1.24[a,b]	7.21±1.09[b]
Proteins	233.39±35.57[a]	299.95±24.13[a]	311.23±25.62[a]
Lipids	156.51±31.88[a]	292.76±64.29[a]	154.02±42.35[a]
Carbohydrates	90.44±21.07[a]	76.84±11.00[a]	187.01±23.00[b]
EA	480.34±77.37[a]	637.85±73.07[a]	652.25±67.36[a]

Highlights

- Postlarvae that delayed metamorphosis (late settlers) display a higher weight
- Late settlers have a lower metabolism and neuromotor activity
- Delaying metamorphosis results in an increased oxidative stress condition
- Early settlers show higher neuromotor activity and antioxidant capacity
- Physiological status is a better proxy for postlarval quality than weight