The bioenergetic fuel for non-feeding larval development in an endemic palaemonid shrimp from the Iberian Peninsula, *Palaemonetes zariquieyi*

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Abstract

Palaemonetes zariquieyi, an endemic palaemonid species of shrimp that lives in freshwater and brackish coastal habitats in eastern Spain, shows an abbreviated, non-feeding larval development comprising only three zoeal stages. To identify the endogenous bioenergetic fuel that allows for food-independent development from hatching to metamorphosis, larvae were reared under controlled laboratory conditions, and ontogenetic changes in dry weight (W), elemental (CHN) and lipid composition (total lipids, principal lipid classes, fatty acids [FA]) were quantified at the onset of each zoeal stage and in the first juvenile. Values of W, C and H per larva and per mass unit of W decreased throughout the time of larval development, while the N content showed only a weak decline (suggesting strong lipid but only little protein degradation). Correspondingly, directly measured values of total lipids (both in µg/larva and in % of W) decreased gradually, with neutral lipids consistently remaining the predominant and most strongly used fraction; sterol esters and waxes were not detected. In contrast to the neutral lipids, the fraction of polar lipids per larva remained stable and, as a consequence, tended to increase as a percentage of total lipids. Likewise, other important lipid fractions such as free fatty acids and cholesterol remained stable throughout the time of larval development. Among the FA, palmitic (16:0), oleic (18:1n-9), linoleic (18:2n-6) and eicosapentaenoic (20:5n-3) acid were predominant, showing a significant decrease during larval development; stearic (18:0), vaccenic (18:1n-7) and arachidonic acid (20:4n-6) were found only in small amounts. Our results indicate that the lecithotrophic development of *P. zariquieyi* is primarily fuelled by the utilization of lipids (especially triacylglycerides and other neutral lipids), which is reflected by a decreasing carbon content. Proteins and polar lipids, by contrast, are preserved as structurally indispensable components (nerve and muscle tissues, cell membranes). The abbreviated and non-feeding mode of larval development of *P. zariquieyi* may have an adaptive value in land-locked freshwater habitats, where planktonic food limitation is likely to occur. The patterns of reserve utilization are similar to those previously observed in other palaemonid shrimps and various other groups of decapod crustaceans with lecithotrophic larvae. This suggests a multiple convergent evolution of bioenergetic traits allowing for reproduction in food-limited aquatic environments.

Keywords: Caridea; freshwater; lecithotrophy; ontogeny; chemical composition; lipid

Introduction

Reproductive and developmental adaptations that allow for invasions of limnic environments by marine crustaceans are among the top issues in evolutionary ecology (e.g. Lee and Bell 1999; Anger et al. 2007). Among the caridean shrimps, Palaemonidae Rafinesque, 1815 have been particularly successful invaders of brackish and freshwater habitats (Ashelby et al. 2012). Within this family, most estuarine and limnic species belong to the genera *Macrobrachium* Spence Bate, 1868 and *Palaemonetes* Heller, 1869 (Jalihal et al. 1993; Murphy and Austin 2005; Anger 2013).

Most palaemonid shrimps pass through complex life cycles (Bauer 2004). These comprise (1) embryogenesis inside the eggs, which are attached underneath the female abdomen, (2) a free-living pelagic, in most cases planktotrophic larval development, and (3) a benthic juvenile - adult phase that gradually leads to maturation and reproduction. In the early life-history stages, different reproductive strategies such as larval export towards the sea or retention within the adult habitat are associated with ontogenetic changes in the tolerance of variations in environmental conditions including changes in salinity and food availability (Anger and Hayd 2009; Charmantier and Anger 2011).

Studies of life history adaptations to non-marine conditions with low salinities and unpredictable planktonic food availability contribute significantly to the understanding of transitions and subsequent speciation of originally marine animals in limnic and terrestrial environments (Anger 1995). Compared to marine and estuarine species, fully freshwater-adapted clades show significant shifts in the salinity optimum as well as tendencies towards larger egg size, a prolonged embryonic incubation period, an abbreviated mode of larval development, and facultative or complete lecithotrophy (Lee and Bell 1999; Vogt 2013). These reproductive traits have generally been considered as adaptations to limited or unpredictable plankton production in freshwater environments (Anger 2001). Abbreviated modes of larval development and lecithotrophy have evolved in numerous palaemonid shrimps living in food-limited freshwater habitats (Bauer 2004; Murphy and Austin 2005; Anger 2013). These ontogenetic traits involve various biochemical and physiological adaptations such as an enhanced initial energy storage (Urzúa and Anger 2011) or energy saving mechanisms (McNamara et al. 1983; Faria et al. 2011).

The subject of the present study, the palaemonid shrimp *Palaemonetes zariquieyi* Sollaud 1939, is an endemic species of the Mediterranean coast of the Iberian Peninsula, inhabiting aquatic environments ranging from pure freshwater habitats to oligohaline channels, pools and lagoons along the Spanish provinces of Alicante and Tarragona

(Zariquiey 1968; Sanz Brau 1983). Due to its restricted distribution, *P. zariquieyi* is considered as a potentially endangered species, and thus, is under conservation management (Valencia Decreto 259/2004). It shows an abbreviated and lecithotrophic larval development with only three stages (Guerao 1993), which has been observed to occur within the parental habitat (Sanz 1980; Guerao 1993), where planktonic food may be scarce (Sanz Brau 1986).

While the ecology and physiology of adult *Palaemonetes zariquieyi* has been studied in some detail (Sollaud 1938; Margalef 1953; Sanz Brau 1986), there is very little information on the larval phase. This includes poor knowledge of the endogenous bioenergetic substrate that allows for food-independent development. In the present investigation, changes in larval biomass and chemical composition occurring during the lecithotrophic development from hatching to the first juvenile stage were studied under controlled laboratory conditions.

Materials and methods

Sampling and maintenance of ovigerous females, larval rearing

Adult *Palaemonetes zariquieyi* were collected by hand net in February 2008 from the "Marjal del Senillar", which connects to Moraira beach (Alicante; 38.68ºN, 0.11ºE). They were then transported in cooling boxes equipped with ice packs and aeration to the IRTA laboratory (Sant Carles de la Ràpita, Tarragona), keeping the conditions of temperature and salinity as similar as possible to those observed at the collection site (~18°C, 1 PSU). Another sample of adult shrimps was collected in May 2009 from the "Ullals de Baltasar" near Amposta (Tarragona; 40.67ºN, 0.59ºE). They were transported under similar conditions of temperature and salinity to the Marine Biological Station Helgoland (BAH), Germany. "Ullals" are natural ponds (about 5-50 m diameter, up to 6 m deep) filled with upwelling water from aquifers originating in the coastal mountain range (in the case of the Ullals de Baltasar, the Serres del Montsiá i dels Ports; for geology and hydrology of the "Ullals", see Bayó Dalmau et al. 1997; for chemical and biological characterization, see Rodrigo et al. 2001; Durán Valsero 2003).

The ovigerous females transported to the IRTA (body length = 39 ± 3 mm; n = 7) were maintained in recirculating 40 L aquaria with aerated oligohaline water (1.2 ± 0.2 PSU), constant temperature (18 ± 1°C), and a 12:12 h light:dark photoperiod. The shrimps were fed daily with frozen pieces of mussel meat (*Mytilus* sp.) and live *Artemia* sp. metanauplii. The aquaria were checked daily for the occurrence of larvae, and newly hatched larvae were transferred, using wide-bore pipettes, to rearing beakers with 100 mL filtered water (1.2 ± 0.2

PSU). They were subsequently reared individually at 18 ± 1°C and a 12:12 h light:dark cycle. Water was changed and larval moults were recorded in daily intervals. The ovigerous females transported to the BAH (body length = 38 ± 2 mm; n = 2) were maintained at the same conditions of food, temperature, salinity, and light, and larvae were obtained and reared with the same techniques and conditions as described above.

All three larval stages of *P. zariquieyi* are non-feeding (Guerao 1993; confirmed by preliminary feeding experiments and behavioral observations at the BAH; Anger, unpubl. data). Therefore, they were routinely reared without food. Unlike the larval stages, first-stage juveniles always accepted and ingested food (*Artemia* nauplii) when it was offered. Juvenile growth in the presence of food, however, was not studied in our experiments. While the larvae reared at the IRTA were exclusively used for analyses of lipid composition (total lipids, lipid classes, fatty acid profiles), those reared at the BAH were used for preliminary tests of possible larval feeding activity, micro-photographical documentation of lipid droplets in the hepatopancreas region, measurements of body dry weight (W), and analyses of elemental composition (contents of carbon, hydrogen, nitrogen; collectively referred to as CHN).

Dry weight and elemental composition

In total, 30 zoea I (ZI), 15 zoea II (ZII), 30 zoea III (ZIII), and 15 first-stage juveniles (JI) from the "Ullals de Baltasar" population were used for determinations of dry weight (W) and elemental composition (CHN). Samples for W and CHN were taken in daily intervals throughout larval development from hatching (day 0) to metamorphosis (day 8), and later measured with standard techniques (Anger and Harms 1990). Analyses comprised five replicate samples with three individuals each. For each analysis, larvae were briefly rinsed in distilled water, blotted on fluff-free Kleenex paper, transferred to pre-weighed tin cartridges, and stored at –20°C. Later, the samples were freeze-dried for 48 h in a vacuum dryer (Christ Alpha 1-4 LSC), W was determined to the nearest 0.1 µg on a Sartorius SC2 ultra micro balance, and CHN with an Elemental Vario Micro CHN Analyser using Sulphanilamide as a standard.

Lipid composition

Larvae obtained from ovigerous females collected from the "Marjal del Senillar" population were reared at the IRTA from hatching (ZI) to metamorphosis (JI). In total, 269 ZI, 241 ZII, 235 ZIII and 187 JI were taken for analyses of lipid composition (total lipids, lipid classes,

fatty acid profiles). Samples for lipid analyses were taken only near the beginning of each successive stage (i.e. within a few hours after hatching or moulting, respectively).

Total lipids, lipid classes, and fatty acid concentrations in each larval stage were measured at the IRTA, using standard methods (Andrés et al. 2010) with four replicate determinations and sixty individuals per analysis. Total lipid content was quantified gravimetrically after an extraction in chloroform/methanol (2:1) and evaporation of the solvent under nitrogen gas (Folch et al. 1957). The lipid extract was determined to the nearest 0.01 mg on a Sartorius BP211D balance and stored at -20°C in chloroform/methanol (2:1) containing 0.01% butylated hydroxytoluene for subsequent analyses of lipid class and fatty acid composition.

Lipid class determination and separation was performed by high-performance thin-layer chromatography (HPTLC) following the method described by Olsen and Henderson (1989). After separation, bands were identified by charring the plates at 100°C for 30 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% H_3PO_4 and quantified by scanning densitometry using a GS 800 Calibrated Densitometer (Bio-Rad Laboratories Inc, USA). Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transmethylation using 2 ml of 1% H_2SO_4 in methanol plus 1 mL toluene (Christie 1982) and thereafter extracted twice using isohexane/diethyl ether (1:1) (Ghioni et al. 2002) and purified on TLC plates. FAME were separated and quantified by gas–liquid chromatography on a Trace GC (Thermo Fisher Scientific Inc, USA) using a flame ionization detector and column injection. Individual methyl esters were identified by comparison to known standards (Supelco 37 FAME mix 47885-U) and quantified by means of the response factor to the internal standard (21:0 fatty acid added prior to transmethylation), using a Chrompack software (Thermo Electron, UK).

Micro-photographical documentation of lipid droplets

The occurrence of lipid droplets in the hepatopancreas region was microscopically observed and documented using a stereo microscope (Olympus SZX2- ILLB) equipped with a calibrated eyepiece micrometer and a digital camera. Photos were digitalized with a CELL (Olympus) image analysis software to quantify the average area of the lipid droplets.

Statistical analyses

Statistical analyses were performed with standard methods (Sokal and Rohlf 1995) using the statistic software package STATISTICA 8 (StatSoft). Differences in dry weight, elemental composition and lipid composition between stages (or development time) were tested by one-way ANOVA. Significant differences were analyzed with a multiple comparison test (Student-Newman-Keuls). All tests were run on the 95 % confidence level ($p < 0.05$). Normality and homogeneity of variances were tested with Kolmogorov–Smirnov and Levene's tests, respectively. When the data did not meet the assumptions, the non-parametric Kruskal–Wallis and Dunn´s multiple comparison test were applied.

Results

Larval development, dry weight (W), and elemental composition (CHN)

Palaemonetes zariquieyi developed within 8-10 d from hatching through three zoeal stages (ZI-III) to the first juvenile (JI). The larvae showed generally benthic rather than freely swimming (planktonic) behaviour. The first two zoeal moulting cycles (ZI, ZII) lasted for 1-2 d each; most larvae reached the ZIII stage 3 d after hatching. The remaining period of larval development (5-7 d, corresponding to 62-70% of total development time) was spent in the ZIII stage alone. Changes in larval W and CHN are shown here for the shortest development, which took 8 d from hatching to the beginning of the first juvenile stage (JI). Since larvae taking longer probably utlized higher proportions of their initially stored energy, this implies that the biomass losses shown in Figure 1a are minimum estimates.

Larval W and elemental composition changed conspicuously during the non-feeding development of *P. zariquieyi*. In particular, the absolute values of W, C and H per individual decreased significantly (Figures 1a, b, d). The N content, by contrast, changed only very little (statistically insignificant; Figure 1c). When the biomass measured at hatching is compared with that remaining in newly metamorphosed juveniles, the average C content decreased from 278 to 201 µg per individual (by 28%; Figure 1b), and a similar loss (25%) was observed in H (Figure 1d). Total W decreased during the same period by 15% (Figure 1a), and N by only 9% (Figure 1c).

As the decrease in W was weaker than the losses in C and H, the relative contents of these two elements (in % of W) showed similar tendencies as the absolute values, i.e. they

decreased significantly (Figures 2a, c). As a consequence of strongly decreasing C and almost constant N values (Figure 2b), the C/N mass ratio decreased significantly (Figure 2d).

Total lipid content

Total lipid content both per individual and per unit of W decreased gradually during the course of larval development. As a consequence, newly metamorphosed juveniles contained 40% less lipids than newly hatched larvae (Figure 3a). As a consequence of this strong lipid degradation, lipid droplets in the hepatopancreas region of the larval cephalothorax tended to become smaller, with average size (measured in microphotographs) decreasing significantly from 0.70 ± 0.08 µm² in the ZI to 0.38 ± 0.06 µm² in the ZIII ($F_{2,44}$ = 6.429; $p < 0.001$; Figure 4). As total W decreased to a lesser extent than the lipid fraction, the relative lipid content (in % of W) decreased significantly, from maximum values of 17% at hatching to a minimum of 10% in the JI ($p < 0.05$; Figure 3b).

Lipid classes

Neutral lipids (NL) were always more abundant than polar lipids (PL) ($p < 0.05$). While the PL fraction per larva remained fairly stable, NL showed a substantial decline (Table 1). Consequently, the percentage of PL within total lipids increased during larval development from 22% at hatching to 36% in the JI, while NL decreased from 78 to 64% ($p < 0.05$; Table 1).

Within the neutral lipids, triacylglycerides (TAG) and cholesterol (CHOL) were identified as predominant fractions (Table 1). The percentage of TAG decreased significantly during larval development (from 54% at hatching to 33% at metamorphosis), whereas CHOL increased from 18 to 24% (Table 1). Other neutral lipids such as sterol esters and waxes were not detected in any developmental stage of this species (Table 1). Free fatty acids (FFA) occurred only in low quantities, remaining stable around 6%.

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were the predominant polar lipids. Both constituents increased, as fractions of the total lipid content, during the time of development. Hence, minimum PC percentages were recorded in the ZI, maximum values in the JI stage (10 vs. 17%; $p < 0.05$) (Table 1). Other PL such as the combined fraction of phosphatidylserine and phosphatidylinositol (PS+PI) as well as lyso-phosphatidylethanolamine (LysoPE) were found only in small amounts (ca. 2 and 1% of total

lipids, respectively), while others occurred in traces or could not be detected at all, e.g. sphingomyelins (SM) (Table 1).

Fatty acid composition

Within the total fatty acid (FA) pool, saturated (SFA) and monounsaturated fatty acids (MUFA) dominated throughout the period of larval development, followed by the fraction of polyunsaturated fatty acids (PUFA) (Table 2). All FA decreased significantly during the course of larval development. The most conspicuous FA, in general, were palmitic (PA, 16:0), oleic (OA, 18:1n-9), linoleic (LA, 18:2n-6), and eicosapentaenoic acid (EPA, 20:5n-3). Stearic (18:0), vaccenic (18:1n-7), arachidonic acid (20:4n-6), eicosanoic (20:0), and heneicosapentaenoic acid (21:5n-3) occurred only in small amounts or could not be detected (Table 2). The contents of both n-6 and n-3 PUFA decreased significantly from hatching to metamorphosis (Table 2). LA (18:2n-6) was the most abundant n-6 PUFA, while EPA (20:5n-3) was the predominant n-3 PUFA (Table 2).

Discussion

In palaemonid shrimp and other freshwater invading decapods, extended modes of larval development are generally associated with strategies of larval export to the sea, whereas abbreviated developments occur mostly in species showing larval retention in the limnic adult habitat, presumably in response to differential planktonic food availability in the larval environment (Anger 2001, Vogt 2013). In *Palaemonetes zariquieyi* as well as in several other freshwater palaemonids, larval development is abbreviated, consisting of only three larval stages prior to metamorphosis; in one species (*P. mercedae* from South America), a further abbreviation to a single larval stage has been observed (for references, see Table 3). In species with an abbreviated mode of larval development, including *P. zariquieyi*, the larvae show generally benthic crawling rather than planktonic swimming behaviour, reflecting their independence of planktonic food sources, and possibly, maternal brood care (Anger 2001, Vogt 2013).

Abbreviated development in decapod crustacean larvae is normally associated with high quantities of lipids remaining from the egg yolk, which represents an enhanced maternal energy investment per offspring (Kattner et al. 2003; Thatje and Mestre 2010). These energy reserves allow for larval independence from planktonic food sources (Anger 2001). The

results of the present study confirm that *Palaemonetes zariquieyi* has a fully lecithotrophic larval development, as suggested by Guerao (1993). Moreover, the changes in biomass and chemical composition measured in this study reveal the principal sources of endogenous energy in the early life-history stages of this species.

Some measures of biomass quantity and chemical composition (W, C, H, C/N mass ratio) decreased from hatching to metamorphosis, while others (especially the N content per individual) remained relatively stable. These results indicate a preferential utilization of lipid reserves, while proteins were largely preserved as structurally indispensable components. Similar patterns of biomass utilization during lecithotrophic development have been reported also from several other decapod species (e.g. *Lepidophthalmus louisianensis* Schmitt, 1935: Nates and Mc Kenney 2000; *Lithodes santolla* Molina, 1782 and *Paralomis granulosa* Jacquinot, 1847: Kattner et al. 2003; *Sesarma curacaoense* De Man, 1892 and *Armases miersii* Rathbun, 1897: Anger and Schultze 1995). Lipid degradation is thus a widespread pattern in species with lecithotrophic development, although some crustacean species may use different biochemical substrates for energy production during starvation (for review, see Sánchez-Paz et al. 2006).

In decapod crustacean larvae, the lipid composition reflects changes in developmental state, nutritional condition, and effects of environmental factors (Andrés et al. 2010; Urzúa and Anger 2011). TAG, PL, and free sterols usually constitute the predominant lipid fractions (Arts et al. 2009). NL, mainly TAG, are a major energy source during periods of food limitation, while phospholipids and sterols change relatively little under suboptimal nutritional conditions (Anger 2001; Arts et al. 2009). According to the results obtained in the present study, both microscopic observations and chemical analyses showed that lipid reserves are gradually utilized in the absence of food. In *P. zariquieyi*, similarly as reported in lecithotrophic larvae of other decapod crustaceans (Nates and Mc Kenney 2000; Kattner et al. 2003), the utilization of lipids was closely related to that of NL (in particular TAG), which decreased from ZI to JI, whereas PL showed the opposite pattern. In contrast to NL, PL were preserved as structural components of cell membranes. Likewise, free fatty acids (FFA) and cholesterol (CHOL) remained stable during larval development, most probably because they play vital roles in developmental processes, e.g. as constituents of cell organelles, essential precursors of the molting hormone, or structural components (see Sheen 2000; Anger 2001).

The fatty acid composition of the larval stages was characterized by a high content of palmitic, oleic, linoleic, and eicosapentaenoic acid (OA, LA, EPA), which combined comprised over 50% of total FA. These FA are common in caridean shrimps with abbreviated

larval development (Thatje et al. 2004; Calado et al. 2010). The high and largely stable content of stearic acid is explained by its predominance in membrane phospholipids (Kattner et al. 1994; Wehrtmann and Graeve 1998). High initial proportions of OA, LA and EPA, which are essential FA in crustaceans (i.e., taken up from external food sources), indicate that the larval development of this species is fuelled by lipid materials exclusively derived from the female, allowing food-independent larval survival and development (Anger 2001; Calado et al. 2005; Nghia et al. 2007). In decapod larvae, in general, high amounts of OA, LA, and EPA are known to enhance the tolerance of fluctuations in temperature, salinity and food limitation, which may occur in planktonic environments (Anger 2001; Calado et al. 2005; Nghia et al. 2007). In conclusion, PUFA (especially n-3), were largely conserved throughout larval development, while major portions of SFA and MUFA were used for energy production (see Table 2).

During larval development within the adult habitat (retention strategy, see Strathmann 1982), *P. zariquieyi* shows conspicuous life-history adaptations, which may be summarized as follows: (1) an abbreviated larval development, showing both a reduced number of stages and a shortened time of larval development (Guerao 1993); (2) benthic rather than planktonic larval behaviour; (3) high initial larval biomass, especially high total lipid and NL contents at hatching; (4), full lecithotrophy (non-feeding larval behaviour even in the presence of food). These traits allow for complete nutritional independence in all larval stages, and hence, should have an adaptive value in land-locked freshwater habitats such as "Ullals" and rivers, where planktonic food limitation may occur.

Another relevant question in this context is, which paleogeographic changes may have driven the colonization of such habitats by *P. zariquieyi*. As a testable hypothesis, we propose the following scenario: The Messinian salinity crisis (i.e. the transitory isolation and subsequent desiccation of the Mediterranean; see Krijgsman et al. 1999; García et al. 2011) separated in the Late Miocene ancestral estuarine *Palaemonetes* populations remaining in the Mediterranean from those inhabiting the Atlantic coast of the Iberian Peninsula (Cuesta et al. 2012). When the Mediterranean Sea regressed and eventually dried out, coastal shrimp may have colonized adjacent brackish and, eventually, land-locked limnic habitats in the eastern part of the Iberian Peninsula. Due to the Mediterranean regression, those ancestral shrimp could not possibly conserve an amphidromous strategy with an extended larval development in coastal marine waters. As a consequence, they could only survive through the evolution of life-history adaptations that allowed for spending the entire life cycle in land-locked fresh water habitats. In shrimp belonging to the genus *Palaemonetes* (or to a larger "*Palaemon*

clade"; Ashelby et al. 2012), evolutionary invasions of freshwater have probably occurred repeatedly in different biogeographic regions. As a consequence of allopatric divergence in reproductive and developmental traits, *P. zariquieyi* eventually became a separate species that is now endemic for the eastern coast of the Iberian Peninsula.

The patterns of reserve utilization in larval *P. zariquieyi* are similar to those previously observed in various other palaemonid shrimp and further groups of decapod crustaceans with lecithotrophic development (Anger 2001; Bauer 2004; Ituarte et al. 2005; Calado et al. 2007; Anger and Hayd 2009). This suggests a multiple convergent evolution of developmental and bioenergetic traits allowing for reproduction and development in food-limited non-marine environments. Future comparative studies of adaptive physiological and biochemical mechanisms in the context of evolutionary colonizations of new environments such as fresh water habitats will enhance our understanding of life-history evolution in crustaceans, in general.

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References

Andrés M, Estévez A, Hontoria F, Rotllant G. 2010. Differential utilization of biochemical components during larval development of the spider crab *Maja brachydactyla* (Decapoda: Majidae). Mar Biol. 157:2329–2340.

Anger K. 2013. Neotropical Macrobrachium (Caridea: Palaemonidae): On the biology, origin, and radiation of freshwater-invading shrimp. J Crust Biol. 33:151–183.

Anger K, Hayd L. 2009. From lecithotrophy to planktotrophy: ontogeny of larval feeding in the Amazon River prawn *Macrobrachium amazonicum*. Aquat Biol. 7:19–30.

Anger K, Torres G, Nettelmann U. 2007. Adaptive traits in ecology, reproduction and early life history of *Sesarma meridies*, an endemic stream crab from Jamaica. Mar Freshwater Res. 58:743–755.

Anger K. 2001. The biology of decapod crustacean larvae. Crustacean Issues No. 14. Lisse (The Netherlands): A.A. Balkema. p. 420.

Anger K. 1995. The conquest of freshwater and land by marine crabs: adaptations in life-history patterns and larval bioenergetics. J. Exp Mar Biol Ecol. 193:119–145.

Anger K, Schultze K. 1995. Elemental composition (CHN), growth, and exuvial loss in the larval stages of two semiterrestrial crabs, *Sesarma curacaoense* and *Armases miersii* (Decapoda: Grapsidae). Comp Biochem Physiol A. 111:615–623.

Anger K, Harms J. 1990. Elemental (CHN) and proximate biochemical composition of decapod crustacean larvae. Comp Biochem Physiol B. 97:69–80.

Arts MT, Brett MT, Heinz MJ. 2009. Lipids in Aquatic Ecosystems. Heidelberg (Germany): Springer. p. 377.

Ashelby CW, Page TJ, De Grave S, Hughes JM, Johnson ML. 2012. Regional scale speciation reveals multiple invasions of freshwater in Palaemoninae (Decapoda). Zool Scr. 41:293–306.

Bauer RT. 2004. Remarkable shrimps: adaptations and natural history of the Carideans. Norman (Oklahoma): University of Oklahoma Press. p. 282.

Bayó Dalmau A, Custodio E, Loaso C. 1997. Las aguas subterráneas en el Delta del Ebro. Revista de Obras Públicas. 3368:47–65.

Bray DM. 1976. Larval development of two Western Australian shrimps, *Palaemonetes australis* Dakin and *Palaemonetes atrinubes* Bray (Decapoda, Palaemonidae) reared in the laboratory. Rec West Aust Mus. 4:145–162.

Broad AC, Hubschman JH. 1963. The larval development of *Palaemonetes kadiakensis* M.J. Rathbun in the laboratory. Trans Amer microsc Soc. 82:185–197.

Broad AC. 1957. Larval development of *Palaemonetes pugio* Holthuis. Biol Bull. 112:144–161.

Calado R, Pimentel T, Cleary D, Dionísio G, Nunes C, Lopes da Silva T, Dinis M, Reis A. 2010. Providing a common diet to different marine decapods does not standardize the fatty acid profiles of their larvae: a warning sign for experimentation using invertebrate larvae produced in captivity. Mar Biol. 157:2427–2434.

413 Calado R, Dionisio G, Dinis MT. 2007. Starvation resistance of early zoeal stages of marine
414 ornamental shrimps *Lysmata spp*. (Decapoda: Hippolytidae) from different habitats. J
415 Exp Mar Biol Ecol. 351:226–233.
416 Calado R, Rosa R, Morais S, Nunes ML, Narciso L. 2005. Growth, survival, lipid and fatty
417 acid profile of juvenile Monaco shrimp *Lysmata seticaudata* fed on different diets.
418 Aquac Res. 36:493–504.
419 Charmantier G, Anger K. 2011. Ontogeny of osmoregulatory patterns in the South American
420 shrimp *Macrobrachium amazonicum*: loss of hypo-regulation in a land-locked
421 population indicates phylogenetic separation from estuarine ancestors. J Exp Mar Biol
422 Ecol. 396:89–98.
423 Christie WW. 1982. Lipid analysis: isolation, separation, identification, and structural
424 analysis of lipids. New York: Pergamon. p. 446.
425 Cuesta JA, Drake P, Martínez-Rodríguez G, Rodríguez A, Schubart CD. 2012. Molecular
426 phylogeny of the genera *Palaemon* and *Palaemonetes* (Decapoda, Caridea,
427 Palaemonidae) from a European Perspective. Crustaceana. 85:877–888.
428 Dobkin S. 1971. The larval development of *Palaemonetes cummingi* Chace, 1954 (Decapoda,
429 Palaemonidae) reared in the laboratory. Crustaceana. 20:285–297.
430 Dobkin S. 1963. The larval development of *Palaemonetes paludosus* (Gibbes, 1850)
431 (Decapoda, Palaemonidae), reared in the laboratory. Crustaceana. 6:41–61.
432 Durán Valsero JJ. 2003. Presencia de aguas de diferente salinidad y origen en los humedales
433 del litoral mediteráneo Español. In: Geta JAL, de Dios Gómez J, de la Orden JA, Ramos
434 G, Rodríguez L (eds) Tecnología de la Intrusión de Agua de Mar en Acuíferos Costeros:
435 Paises Mediterráneos. Publicaciones del Instituto Geológico y Minero de España. Serie:
436 Hidrología y Aguas Subterráneas No 8, Book 2. Ministerio de Ciencia y Tecnología,
437 Instituto Geológico y Minero de España, Madrid
438 Faria SC, Augusto A, McNamara J. 2011. Intra- and extracellular osmotic regulation in the
439 hololimnetic Caridea and Anomura: a phylogenetic perspective on the conquest of fresh
440 water by the decapod Crustacea. J Comp Physiol B Biochem Syst Environ Physiol.
441 181:175–186.
442 Falciai L, Palmerini E. 2001. Larval development of the freshwater shrimp, *Palaemonetes*
443 *antennarius* (H. Milne Edwards, 1837) (Decapoda, Palaemonidae) reared in the
444 laboratory. Crustaceana. 74:1315–1333.

445 Fincham AA. 1979. Larval development of British prawns and shrimps (Crustacea:
446 Decapoda: Natantia). 2. *Palaemonetes (Palaemonetes) varians* (Leach, 1814) and
447 morphological variation. Bull Br Mus Nat Hist. 35:127–200.
448 Folch J, Lees M, Stanley GHS. 1957. A simple method for the isolation and purification of
449 total lipids from animal tissues. J Biol Chem. 226:497–509.
450 García M, Maillard A, Aslanian D, Rabineau M, Alonso B, Gorini C, Estrada F. 2011. The
451 Catalan margin during the Messian Salinity Crisis: Physiography, morphology and
452 sedimentary record. Mar Geol. 284:158–174.
453 Ghioni C, Porter AEA, Taylor GW, Tocher DR. 2002. Metabolism of 18: 4n–3 (stearidonic
454 acid) and 20: 4n–3 in salmonid cells in culture and inhibition of the production of
455 prostaglandin F-2 alpha (PGF(2 alpha)) from 20: 4n–6 (arachidonic acid). Fish Physiol
456 Biochem. 27:81–96.
457 Guerao G. 1993. The larval development of a fresh-water prawn, *Palaemonetes zariquieyi*
458 Sollaud, 1939 (Decapoda, Palaemonidae), reared in the laboratory. Crustaceana. 64:
459 226–241.
460 Ituarte RB, Spivak ED, Anger K. 2005. Effects of salinity on embryonic development of
461 *Palaemonetes argentinus* (Crustacea: Decapoda: Palaemonidae) cultured in vitro.
462 Invertebr Reprod Dev. 47:213–223.
463 Jalihal DR, Sankolli KN, Shenoy S. 1993. Evolution of larval developmental patterns and the
464 process of freshwaterization in the prawn genus *Macrobrachium* Bate, 1868 (Decapoda,
465 Palaemonidae). Crustaceana. 65:365–376.
466 Kattner G, Graeve M, Calcagno JA, Lovrich GA, Thatje S, Anger K. 2003. Lipid, fatty acid
467 and protein utilization during lecithotrophic larval development of *Lithodes santolla*
468 (Molina) and *Paralomis granulosa* (Jacquinot). J Exp Mar Biol Ecol. 292:61–74.
469 Kattner G, Wehrtmann IS, Merck T. 1994. Interannual variations of lipids and fatty acids
470 during larval development of *Crangon spp*. in the German Bight, North Sea. Comp
471 Biochem Physiol B. 107:103–110.
472 Krijgsman W, Hilgen FJ, Raffi I, Sierro FJ, Wilson DS. 1999. Chronology, causes and
473 progression of the Messinian salinity crisis. Nature. 400:652–655.
474 Lee CE, Bell MA. 1999. Causes and consequences of recent freshwater invasions by saltwater
475 animals. Trends Ecol Evol. 14:284–288.
476 Magalhães C. 1988. The larval development of palaemonid shrimps from the Amazon region
477 reared in the laboratory. III. Extremely abbreviated development of *Palaemonetes*

(*Palaemonetes*) *mercedae* Pereira, 1986 (Crustacea, Decapoda). Stud Neotrop Fauna E. 23:1–8.

Magalhães C. 1986. The larval development of palaemonid shrimps from the Amazon Region reared in the laboratory. IV. Abbreviated development of *Palaemonetes ivonicus* Holthuis, 1950 (Crustacea, Decapoda). Amazoniana. 10:63–78.

Margalef R. 1953. Los crustáceos de las aguas continentales ibéricas. Madrid: Ministerio de Agricultura, Dirección General de Montes, Caza y Pesca Fluvial, Instituto Forestal de Investigaciones y Experiencias. p. 243.

McNamara JC, Moreira GS, Moreira PS. 1983. The effect of salinity on respiratory metabolism, survival and moulting in the first zoea of *Macrobrachium amazonicum* (Heller) (Crustacea, Palaemonidae). Hydrobiologia. 101:239–242.

Menú-Marque SA. 1973. Desarrollo larval de *Palaemonetes argentinus* (Nobili, 1901) en el laboratorio (Crustacea, Caridea, Palaemonidae). Physis Sec B. 32:149–169.

Murphy NP, Austin CM. 2005. Phylogenetic relationships of the globally distributed freshwater prawn genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae): biogeography, taxonomy and the convergent evolution of abbreviated larval development. Zool Scr. 34:187–197.

Nates SF, McKenney CL. 2000. Ontogenetic changes in biochemical composition during larval and early postlarval development of *Lepidophthalmus louisianensis*, a ghost shrimp with abbreviated development. Comp Bioch Physiol B. 127:459–468.

Nghia TT, Wille M, Vandendriessche S, Vinh QT, Sorgeloos P. 2007. Influence of highly unsaturated fatty acids in live food on larviculture of mud crab *Scylla paramamosain* (Estampador, 1949). Aquac Res. 38:1512–1528.

Olsen RE, Henderson RJ. 1989. The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. J Exp Mar Biol Ecol. 129:189–197.

Pereira GAS, García DJV. 1995. Larval development of *Macrobrachium reyesi* Pereira (Decapoda: Palaemonidae), with a discussion on the origin of abbreviated development in Palaemonids. J Crust Biol. 15:117–133.

Rodrigo MA, C. Rojo C, Armengol X, Mañá M. 2001. Heterogeneidad espacio-temporal de la calidad del agua en un humedal costero: El Marjal de la Safor (Valencia). Limnetica. 20:329–339.

510 Rodríguez-Almaraz GA, Muñiz-Martinez R, Millán-Cervantes A. 2010. Larval development
511 of *Palaemonetes mexicanus* and *P. hobbsi* (Caridea: Palaemonidae) reared in the
512 laboratory. Rev Mex Biodivers. 81:73–97.
513 Sánchez-Paz A, García-Carreno F, Muhlia-Almazán A, Peregrino-Uriarte AB, Hernández-
514 López J, Yepiz-Plascencia G. 2006. Usage of energy reserves in crustaceans during
515 starvation: status and future directions. Insect Biochem Mol Biol. 36:241–249.
516 Sanz A. 1980. Biología y ecología de *Pa1aemonetes zariquieyi* Sollaud, 1939 (Crustacea,
517 Decapoda, Palaemonidae). Tesis doctoral. Universidad de Valencia.
518 Sanz Brau A. 1983. Localidades y sus caracteristicas ambientales del camarón Palaemonetes
519 zariquieyi Sollaud, 1939 (Crustacea: Decapoda). Actas del I Congreso Ibérico de
520 Entomologia (Leon), pags. 737–742.
521 Sanz Brau A. 1986. Biología del camarón de agua dulce *Palaemonetes zariquieyi* Sollaud,
522 1939 (Crustacea: Decapoda: Palaemonidae). Limnetica. 2:293–304.
523 Sheen S.S. 2000. Dietary cholesterol requirements of juvenile mud crab *Scylla serrata*.
524 Aquaculture. 189:277–285.
525 Sokal R, Rohlf J. 1995. Biometry. 3rd ed. New York: WH Freeman. p. 887.
526 Sollaud E. 1938. Sur un *Palaemonetes* endemique, *P. zariquieyi*, n. sp. localisé dans la plaine
527 littorale du Golfe de Va1ence. Trav Sta Zool Wimereux. 13:635–645.
528 Sollaud E. 1923. Le développement larvaire des "Palaemoninae". 1. Partie descriptive. La
529 condensation progressive de l'ontogénèse. Bull biol France Belgique. 57:509–603.
530 Strathmann RR.1982. Estuarine comparisons. San Diego (CA): edited by VS Kennedy,
531 Academic Press. Selection for retention or export of larvae in estuaries; p. 521–535.
532 Strenth NE. 1976. A review of the systematics and zoogeography of the freshwater species of
533 *Palaemonetes* Heller of North America (Crustacea: Decapoda). Smithson Contrib Zool.
534 228:1–27.
535 Thatje S, Mestre NC. 2010. Energetic changes throughout lecithotrophic larval development
536 in the deep-sea lithodid crab *Paralomis spinosissima* from the Southern Ocean. J Exp
537 Mar Biol Ecol. 386:119–124.
538 Thatje S, Lovrich GA, Torres G, Hagen W, Anger K. 2004. Changes in biomass, lipid, fatty
539 acid and elemental composition during the abbreviated larval development of the
540 subantarctic shrimp *Campylonotus vagans.* J Exp Mar Biol Ecol. 301:159–174.
541 Urzúa Á, Anger K. 2011. Larval biomass and chemical composition at hatching in two
542 geographically isolated clades of the shrimp *Macrobrachium amazonicum*: intra-or
543 interspecific variation?. Invertebr Reprod Dev. 55:236–246.

544 Valencia Decreto 259/2004, de 19 de noviembre. Plan Rector de Uso y Gestión del Parque
545 Natural de l'Albufera. Diari Oficial de la Comunitat Valenciana, 24 de noviembre de
546 2004, num. 4890, p. 29930.
547 Vogt G. 2013. Abbreviation of larval development and extension of brood care as key
548 features of the evolution of freshwater Decapoda. Biol Rev. 88:81–116.
549 Wehrtmann IS, Graeve M. 1998. Lipid composition and utilization in developing eggs of two
550 tropical marine caridean shrimps (Decapoda: Caridea: Alpheidae, Palaemonidae). Comp
551 Bioch Physiol B. 121:457–463.
552 Zariquiey R. 1968. Crustáceos Decápodos Ibéricos. Invest Pesq. 32:1–510.
553

Legend of figures and tables

Figure 1. *P. zariquieyi*. Changes in dry weight (W) and elemental composition (CHN) during development from hatching through three larval stages (Zoea I-III) to the first juvenile (JI): absolute values ($\mu g \cdot ind^{-1}$) of (a) dry weight; (b) carbon; (c) nitrogen; (d) hydrogen content. ANOVA (*F*-values) and significance level (*p*), mean values ± SD. Different lower case letters indicate significant differences between stages (or development time) after SNK test

Figure 2. *P. zariquieyi*. Changes in relative chemical composition during development from hatching through three larval stages (Zoea I-III) to the first juvenile (JI): percentage W values of (a) carbon; (b) nitrogen; (c) hydrogen; (d) C/N mass ratio. ANOVA (*F*-values), Kruskal–Wallis (*H*) and significance level (*p*), mean values ± SD. Different lower case letters indicate significant differences between stages (or development time) after SNK or Dunn´s test

Figure 3. *P. zariquieyi.* Changes in the lipid content during development from hatching through three larval stages (Zoea I-III) to the first juvenile (JI): (a) absolute values ($\mu g \cdot ind^{-1}$); (b) percentage W values. ANOVA (*F*-values) and significance level (*p*), mean values ± SD. Different lower case letters indicate significant differences between stages (or development time) after SNK test

Figure 4. *P. zariquieyi.* Changes in the size and density of lipid droplets in the hepatopancreas region of the cephalotorax during the larval development (Zoea I-III)

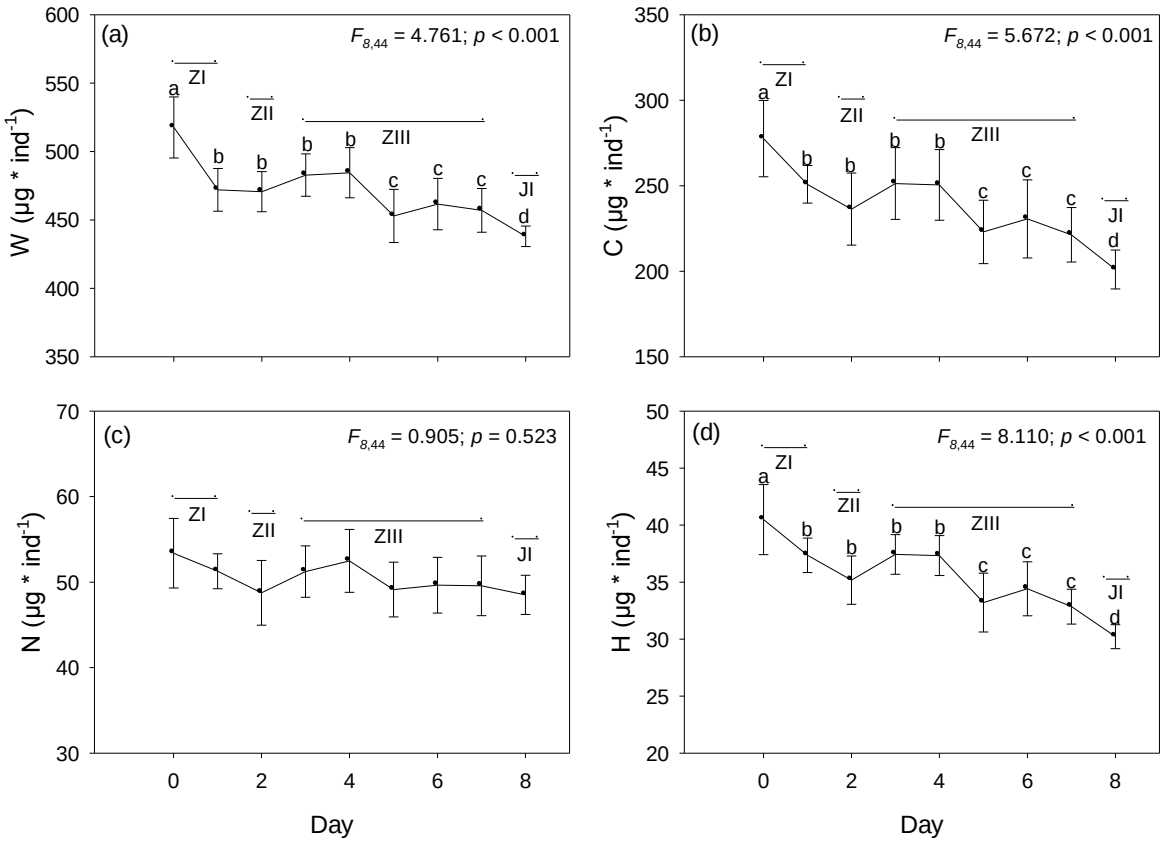
Table 1. *P. zariquieyi.* Changes in total lipid (TL) content and lipid composition during larval development (Zoea I-III) to the first juvenile stage (JI); all values are given in $mg \cdot g\ W^{-1}$, lipid classes also % of TL (in parentheses, below); mean values ± SD. Different lower case letters in a row: significant differences between stages (ANOVA, SNK test, $p < 0.05$). Total polar lipids (Total PL): sum of sphingomyelins (SM), phosphatidylcholine (PC), phosphatidylserine + phosphatidylinositol (PS+PI), phosphatidylethanolamine (PE), and lysophosphatidylethanolamine (LysoPE); total neutral lipids (total NL): sum of cholesterol (CHOL), free fatty acids (FFA), and tryacylglycerides (TAG)

Table 2. *P. zariquieyi.* Changes in the fatty acid (FA) content and profile (all values are given in $mg\ FA \cdot g\ TL^{-1}$) during larval development (Zoea I-III) to the first juvenile stage (JI); mean ± SD. Different lower case letters in a row: significant differences between stages (ANOVA, SNK test, $p < 0.05$). SFA (Saturated FA): sum of 14:0, 15:0, 16:0, 18:0 and 20:0; MUFA (Monounsaturated FA): sum of 16:1n-9, 18:1n-9, 18:1n-7 and 20:1n-9; total n-6 PUFA

585 (polyunsaturated n-6 FA): sum of 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:5n-6; total n-3
586 PUFA (polyunsaturated n-3 FA): sum of 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-
587 3, 22:6n -3; TOTAL PUFA: sum of n-3 and n-6 PUFA

588 **Table 3**. Comparison between habitat and number of larval stages in *Palaemonetes* species
589 (listed by stage number, habitat, and geographic region)
590

591 **Figures and Tables**



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594 **Figure 1**

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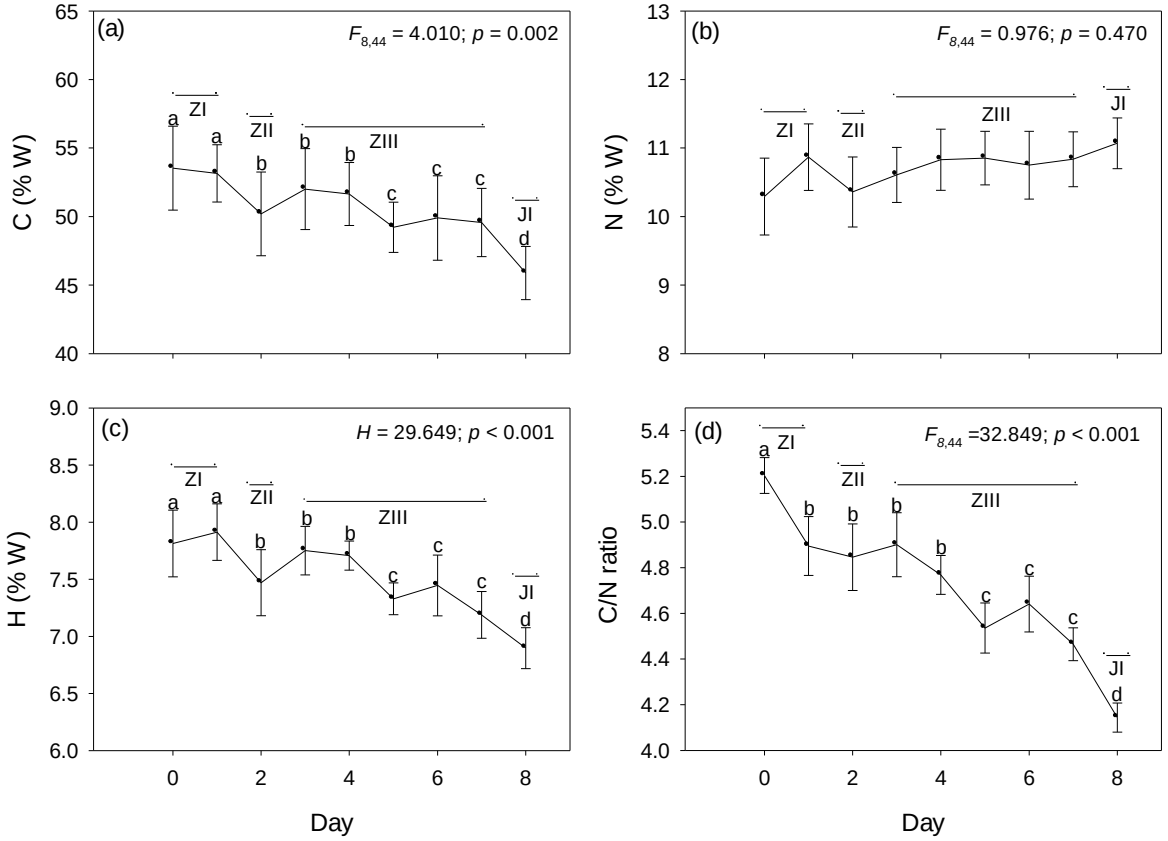

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598 **Figure 2**

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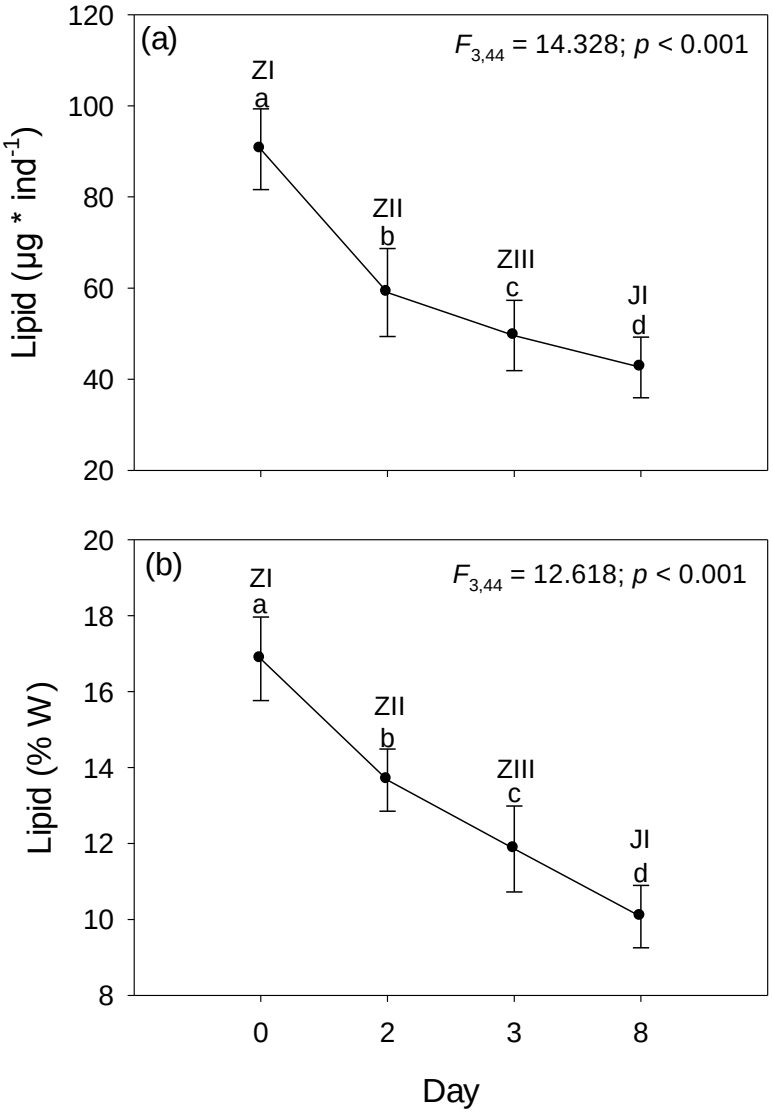

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602 **Figure 3**

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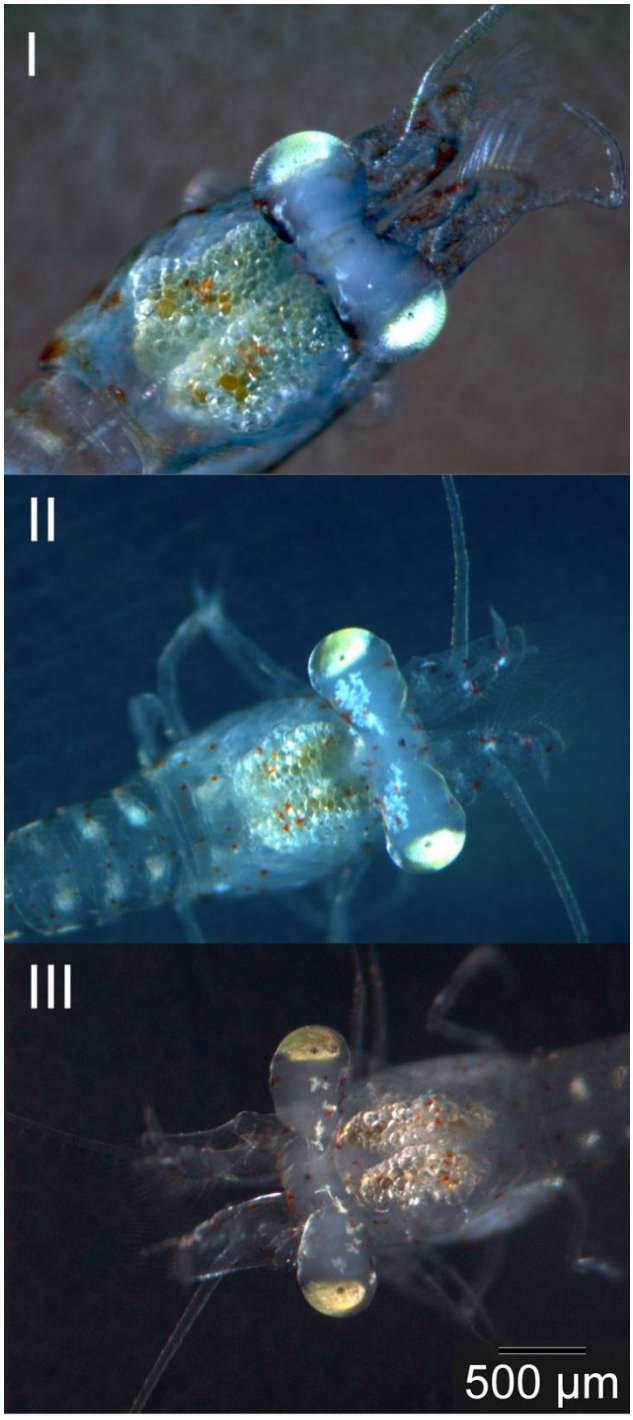

Figure 4

610 **Table 1**

Lipid class	ZI	ZII	ZIII	JI
Total lipids (TL)	168 ± 11[a]	136 ± 8[b]	118 ± 7[c]	100 ± 9[d]
***Polar lipids* (PL)**				
SM	0 -	0 -	0.49 ± 0.002[a] (0.41)	0.43 ± 0.001[a] (0.42)
PC	16.2 ± 0.009[a] (9.7)	12.1± 0.004[a] (8.9)	14.5 ± 0.001[b] (12.2)	17.2 ± 0.003[c] (17.1)
PS+PI	4.47 ± 0.007[a] (2.6)	3.66 ± 0.001[b] (2.7)	2.87 ± 0.002[b] (2.4)	4.09 ± 0.001[c] (4.1)
PE	13.8 ± 0.002[a] (8.2)	11.5 ± 0.004[a] (8.5)	10.1 ± 0.001[a] (8.5)	12.7 ± 0.009[b] (12.6)
LysoPE	2.07 ± 0.001[a] (1.2)	1.75± 0.003[a] (1.3)	0.75 ± 0.001[b] (0.6)	1.68 ± 0.003[c] (1.7)
Total PL	36.9 ± 0.021[a] (21.9)	29.4 ± 0.014[b] (21.6)	28.7 ± 0.001[b] (24.2)	36.1 ± 0.016[c] (35.9)
***Neutral lipids* (NL)**				
CHOL	30.3± 0.002[a] (18.0)	26.6 ± 0.003[b] (19.5)	26.7 ± 0.022[c] (22.5)	24.6 ± 0.008[c] (24.5)
FFA	9.96 ± 0.004[a] (5.9)	8.86 ± 0.002[b] (6.5)	7.98 ± 0.004[b] (6.7)	6.60 ± 0.001[b] (6.5)
TAG	91.3± 0.006[a] (54.2)	71.7 ± 0.009[b] (52.3)	55.1 ± 0.005[c] (46.5)	33.2 ± 0.025[d] (33.0)
Total NL	131 ± 0.01[a] (78.1)	107 ± 0.01[a] (78.4)	89.8 ± 0.03[b] (75.8)	64.5 ± 0.03[c] (64.1)

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615 **Table 2**

Fatty acids, FA	ZI	ZII	ZIII	JI
Total FA	90.2 ± 6[a]	101.8 ± 8[b]	69.4 ± 7[c]	53.1 ± 5[d]
14:0	1.31 ± 0.1[a]	1.68 ± 0.09[b]	1.27 ± 0.11[a]	0.91 ± 0.07[c]
15:0	0.80 ± 0.09[a]	1.09 ± 0.06[b]	0.64 ± 0.04[c]	0.57 ± 0.1[c]
16:0	16.4 ± 1.2[a]	18.5 ± 0.9[b]	12.9 ± 0.8[c]	10.1 ± 1.1[d]
18:0	3.06 ± 0.1[a]	2.93 ± 0.12[a]	2.8 ± 0.9[b]	2.72 ± 0.8[b]
20:0	0	0	0.29 ± 0.01[c]	0.27 ± 0.06[c]
Total SFA	21.6 ± 1.8[a]	24.2 ± 1.6[b]	18.0 ± 1.2[c]	14.6 ± 1.1[d]
16:1n-9	7.40 ± 0.9[a]	9.23 ± 0.7[b]	4.0 ± 0.9[a]	3.24 ± 1.2[a]
18:1n-9	18.4 ± 1.8[a]	25.6 ± 2.6[b]	13.2 ± 3.2[c]	8.21 ± 1.4[d]
18:1n-7	4.70 ± 1.9[a]	3.90 ± 0.8[a]	5.30 ± 1.2[a]	5.27 ± 0.8[a]
20:1n-9	0.25 ± 0.1[a]	0.30 ± 0.1[b]	0.10 ± 0.06[c]	0.09 ± 0.05[c]
Total MUFA	30.8 ± 2.8[a]	39.0 ± 2.1[b]	22.6 ± 1.8[c]	16.8 ± 3.1[d]
18:2n-6	13.7 ± 1.2[a]	12.9 ± 0.9[a]	11.1 ± 1.1[b]	7.83 ± 0.6[c]
18:3n-6	0.50 ± 0.1[a]	0.55 ± 0.1[a]	0.27 ± 0.2[b]	0.20 ± 0.1[c]
20:3n-6	0.12 ± 0.1[a]	0.17 ± 0.2[b]	0	0
20:4n-6	1.63 ± 0.2[a]	2.26 ± 0.3[b]	1.27 ± 0.1[c]	1.24 ± 0.1[c]
22:5n-6	0	0	0.26 ± 0.09[a]	0.18 ± 0.02[b]
Total n-6 PUFA	16 ± 1.6[a]	15.8 ± 1.4[a]	13 ± 0.9[b]	9.45 ± 1.4[c]
18:3n-3	2.81 ± 1.2[a]	2.2 ± 1.1[a]	1.55 ± 0.8[b]	1.02 ± 0.4[c]
18:4n-3	0.19 ± 0.04[a]	0.14 ± 0.08[b]	0.05 ± 0.01[c]	0.04 ± 0.01[c]
20:4n-3	0	1.27 ± 0.9[a]	0.13 ± 0.04[b]	0.10 ± 0.01[b]
20:5n-3	13.4 ± 1.6[a]	14.5 ± 1.8[a]	10.1 ± 0.9[b]	7.82 ± 1.5[c]
21:5n-3	0	0	0.11 ± 0.01[a]	0.07 ± 0.02[b]
22:5n-3	0.81 ± 0.2[a]	1.26 ± 0.6[b]	0.55 ± 0.1[c]	0.18 ± 0.09[d]
22:6n-3	4.58 ± 1.2[a]	3.45 ± 0.9[b]	3.37 ± 0.7[b]	2.93 ± 0.6[b]
Total n-3 PUFA	21.8 ± 1.6[a]	22.8 ± 1.2[a]	15.8 ± 0.9[b]	12.1 ± 1.4[c]
TOTAL PUFA	37.7 ± 2.2[a]	38.6 ± 1.9[a]	28.8 ± 2.9[b]	21.7 ± 2.4[c]

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618 **Table 3**

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Species	Distribution	Habitat	Larval stages	Reference
P. pugio Holthuis, 1949	North America (Atlantic coast)	E	10	Broad (1957)
P. vulgaris Say, 1818	North America (Atlantic coast)	E	10	Sollaud (1923)
P. argentinus Nobili, 1901	South America (Atlantic and Caribbean coasts)	E	9	Menú-Marque (1973)
P. kadiakensis Rathbun, 1902	North America (Pacific coast)	E	5-8	Broad and Hubschman (1963)
P. atrinubes Bray, 1976	Western Australia (Western Australia, Swan River)	E	7	Bray (1976)
P. varians Leach, 1813	Europe, North Africa (Atlantic, Mediterranean)	E	5	Fincham (1979)
P. australis Dakin, 1915	Western Australia (Western Australia, Swan River)	F	3	Bray (1976)
P. carteri Gordon, 1935	South America (Amazon and Orinoco basins)	F	3	Pereira and García (1995)
P. ivonicus Holthuis, 1950	South America (Amazon basin)	F	3	Magalhães (1986)
P. antrorum Benedict, 1896	North America (Texas)	F, T	3	Strenth (1976)
P. cummingi Chace, 1954	North America (Florida, West Indies)	F	3	Dobkin (1971)
P. paludosus Gibbes, 1850	North America (South Carolina, USA)	F	3	Dobkin (1963)
P. hobbsi Strenth, 1994	North America (Mexico)	F	3	Rodríguez-Almaraz et al. (2010)
P. mexicanus Strenth, 1976	North America (Mexico)	F	3	Rodríguez-Almaraz et al. (2010)
P. antennarius H. Milne Edwards, 1837	Europe (Mediterranean lagoons)	F	3	Falciai and Palmerini (2001)
P. zariquieyi Sollaud, 1939	Europe (eastern Spain)	F	3	Guerao (1993)
P. mercedae Pereira, 1986	South America (Amazon and Orinoco basins)	F	1	Magalhães (1988)

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