Annual variation in the biochemical composition of newly hatched larvae of *Maja brachydactyla* in captivity

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Abstract

Quality of newly hatched larvae (NHL) of *M. brachydactyla* in captivity has been characterized throughout the year to evaluate their availability for mass production. Spawning took place every month and NHL were collected and analyzed to estimate individual dry weight (DW) and proximate biochemical composition (protein, carbohydrate and lipids). Lipid class, fatty acid composition, amino acid profile, mineral and vitamin A, E and C contents were analyzed seasonally. NHL obtained throughout the year are a potential source for aquaculture purposes, since the increment in the relative protein and lipid (especially phospholipids and n-3 PUFA) content might compensate the decrease in DW of larvae hatched from broodstock kept during one year in captivity. However, the decrease in vitamin A and E as well as in certain essential amino acids (Lys, Val, His) and trace elements (Cu, Fe) of NHL at the end of the year might be indicative of a nutritional deficiency in broodstock diets.

Keywords: *Maja brachydactyla*; larval quality; mass production; biochemical composition; broodstock condition.

1 Introduction

Larval production is a major concern for aquaculture purposes. Not only the quantity but also the quality of the larvae obtained under captivity is of paramount importance to ensure a profitable commercial harvest of juveniles and/or adults. Larval physiological condition and performance during decapod culture has been commonly referred as larval quality, which has been studied under five general criteria: biochemical composition, morphology, behavior, production yields and survival to stress (reviewed by Racotta et al., 2003). Spider crab eggs are lecithotrophic and thus their development

depends on the reserves transferred from the female. As a consequence, the initial level of these reserves in newly hatched larvae can determine their quality, and can be considered as predictive quality criteria. Recent studies in crustacean culture have been directed to improve larval quality and to establish its relationship with broodstock condition, especially in shrimps (Racotta et al., 2003). Broodstock condition or maternal effects (including nutrition, environmental conditions and reproductive exhaustion) have been proven to affect egg and larval quantity and quality in decapod crustaceans, both in the wild (Anger, 2006) and in captivity (Palacios et al., 1999; Wickins et al., 1995; Xu et al., 1994).

The spider crab *Maja brachydactyla* has a high economical value, supporting commercial captures in different countries through the NE Atlantic coasts (Spain, Portugal, France, Ireland and UK). The high fishing pressure hold up by this crab (Freire et al., 2002), together with its adequate growth and reproductive characteristics (Alaminos and Domingues, 2008; Figueiredo and Narciso, 2008; González-Gurriarán et al., 1995; Guerao and Rotllant, 2009; Iglesias et al., 2002; Palma et al., 2008; Rotllant et al., 2007; Simeó et al., 2009) have contributed to define the species as potential for aquaculture. Seminal receptacles in the female play a key role in the reproductive behavior of spider crab as a storage place of sperm from one or more copulations by several males, allowing the subsequent fertilization of consecutive oocyte batches without carrying any new copulation (reviewed by González-Gurriarán et al., 1998). Larval culture of *M. brachydactyla* has been optimized in recent years through the study of several aspects of its rearing, including its zootechny (Andrés et al., 2007) and the study of the biochemical changes occurring during ontogeny (Andrés et al., 2008, 2010a, 2010b). However, obtaining good quality newly hatched larvae all the year round is still of paramount importance in order to establish the basis for mass

production. The aim of this work was to study the variation in the composition of *M. brachydactyla* larvae obtained along a whole year under intensive culture in order to evaluate the effects of broodstock captivity.

2 Materials and methods

2.1 Broodstock capture and maintenance

Adult *M. brachydactyla* were captured with commercial fishery boats off the coast of Galicia, northwestern Spain (November 2004), and transported to IRTA (Sant Carles de la Ràpita, Tarragona, Spain) in high humidity containers at 8±2 ºC. Upon their arrival, a total of 28 adults (24 females, carapace length: CL=161.6±6.7 mm and 4 males, CL=151.8±4.2 mm) were acclimated in 2000-L tanks as follows: two tanks containing only females (6 females per tank) and two tanks each containing 6 females and 2 males. The tanks were connected to a recirculation unit (renewal rate = 65 m^3 h^{-1}) with constant conditions of salinity and temperature (35±1 ‰ and 18±1 ºC, respectively) and natural photoperiod, being the broodstock fed a combination of fresh and frozen mussels (*Mytilus* sp.) and frozen crab (*Liocarcinus depurator)*. In order to check and maintain the water quality of the recirculating system, temperature, oxygen and pH data were recorded daily whereas nitrite and ammonia concentration were evaluated on a weekly basis. These experimental conditions were held throughout a natural year (i.e. from 1st January to 31st December 2005), being the first batch recorded on the 31st of January. Spawning took place spontaneously all year round in each tank.

2.2 Source and sampling of larvae

A batch was defined as the appearance of newly hatched larvae (NHL) in one tank, considering all the females in a tank as a whole unit of reproduction. Every batch was recorded and actively swimming larvae were collected from broodstock tanks within maximally ca. 12 hours. The quantity of larvae per batch was estimated volumetrically

and a triplicate sample of 200 larvae taken for dry weight (DW) determinations after washing with distilled water. After 24 h oven-drying at 60 ºC, DW was determined on a Sartorius BP211D (Sartorius, Germany) balance to the nearest 0.01 mg. The ash content was measured after 4 h combustion at 450 ºC in a muffle furnace (NABERTHERM L5/R, Lilienthal, Germany). The remaining larvae were gently rinsed in distilled water, dried on a filter paper and kept frozen at -80 ºC for later biochemical analysis.

2.3 Biochemical analysis

2.3.1 Proximate biochemical content

Tissue homogenization was attained after 5 min disruption with an Ultra turrax T-25 (IKA ® WERKE, Germany) and 1 min sonication (Vibra-cell, Sonics, USA). Protein (Pr) and carbohydrate (Ch) content was estimated in triplicates by colorimetric analysis following the methods by Bradford (1976) and Dubois et al. (1956), respectively. Samples for the protein analysis were previously digested with NaOH (40 mg mL^{-1}) at 60 ºC for 30 min to allow a better performance of the test. Total lipid content (Lip) was quantified gravimetrically after extraction in chloroform/methanol (2:1) and evaporation of the solvent under a stream of nitrogen followed by vacuum desiccation overnight (Folch et al., 1957). Total lipids were stored (10 mg mL^{-1}) in chloroform/methanol (2:1 in volume) containing 0.01% butylated hydroxytoluene at -20ºC for later lipid class and fatty acid analysis. The individual content (µg $larva^{-1}$) of each biochemical component was calculated on a DW basis by subtracting the average water content of the samples obtained from the wet and DW measurements (see above). The proximate biochemical composition was analyzed for each batch and grouped in months.

2.3.2 Lipid class and fatty acid analysis

Lipid extracts resulting from the proximate analysis were used for the subsequent analysis of lipid class and fatty acid composition. Lipid class separation was performed

by high-performance thin-layer chromatography (HPTLC) following the method by Olsen and Henderson (1989). After separation, bands were identified by charring the plates at 100ºC for 30 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid and quantified by scanning densitometry using a GS 800 Calibrated Densitometer (Bio-Rad, Bio-Rad Laboratories, Inc, Hercules, CA, USA). Lipid class composition was analyzed for each batch and grouped in seasons in order to simplify the interpretation of the results.

Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalysed 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, v:v) (Ghioni et al., 2002) and purified on TLC plates. FAME were separated and quantified by gas-liquid chromatography on a Trace GC (Thermo, Thermo Fisher Scientific Inc., Waltham, MA, USA) using a flame ionization detector and on column injection. Individual methyl esters were identified by comparison to known standards (Supelco 37 FAME mix 47885-U) and a well characterized fish oil, and quantified by means of the response factor to the internal standard 21:0 fatty acid, added prior to transmethylation, using a Chrompack program for Windows (Thermo Electron, Winsford, UK). The fatty acid composition was analyzed in pure lipid extracts from 5 batches (n=5, randomly chosen) per season.

2.3.3 Amino acid, vitamins and minerals

Due to the large amount of material required, total amino acid (AA, except tryptophan), vitamin and mineral analysis was performed after pooling samples of larvae belonging to different batches within the same season and was carried out by an external laboratory (OFICE, S. L., Barcelona, Spain). The analysis of vitamin A and E was performed by high-performance liquid chromatography (HPLC)-UV, following the

136 procedures outlined in AOAC methods 974.29 and 970.64 (AOAC, 2007a, b) whereas
137 for vitamin C the method by Wimalasiri and Wills (1983) was used. AA content was
138 analyzed by HPLC-fluorescence based on the methods by Davies and Thomas (1973)
139 and Pfeifer et al. (1983). The analyses of Na, K, Ca, Fe, Cu, Mg and Mn were
140 performed by atomic absorption spectrophotometry (AAS) following the official
141 methods of AOAC (AOAC, 2007c, d, e, f, g, h). Chlorides were analyzed
142 volumetrically following the method described in the official method 937.09 (AOAC,
143 2007i) whereas P was analyzed spectrophotometrically (AOAC, 2007j) and S by means
144 of the sodium peroxide method (AOAC, 2007k). Sr was estimated by inductively
145 coupled plasma (ICP) analysis.

146 *2.4 Statistics*

147 Data sets were analyzed and plotted using the SigmaPlot 9 and SigmaStat 3 software
148 packages (Systat Software Inc., USA). Monthly changes in biomass (DW, ash and
149 proximate biochemical contents), and seasonal changes in lipid class, fatty acid, AA,
150 mineral and vitamin content were evaluated by means of one-way ANOVA analysis.
151 Post-hoc comparisons among groups (using either months or seasons as independent
152 variables) were performed by Holm-Sidak test. Statistical significant differences
153 ($p<0.05$) were indicated in tables and figures by different letters.

154 **3 Results**

155 After three months of broodstock captivity the first batch occurred by the end of January
156 and a total of 76 batches were collected to December, being the period from February to
157 May when more batches were observed. No differences in fecundity nor in biochemical
158 quality were found between tanks with and without males (Simeó, personal
159 communication). No significant differences could be found in the number of larvae per
160 batch and female obtained in different months (Table 1), being in average 4,846±4,304

161 the larvae per batch and female. Monthly changes in DW, ash content and proximate
162 biochemical composition of newly hatched larvae (NHL) are also shown in Table 1 (in
163 µg larva^{-1}). Dry weight of NHL gradually decreased along the year, from 108±0 µg
164 larvae^{-1} in January to 75±14 µg larvae^{-1} in November. Ash content represented in
165 average 27% of the larval DW and showed a significant decrease in the batches at the
166 end of the year in parallel with the decrease observed in DW. Individual Pr, Lip and Ch
167 content of NHL showed no significant changes along the year (Table 1) and Lip/Pr ratio
168 was maintained all the year round at 0.2. On the other hand, a gradual and significant
169 increase was detected in the relative Pr and Lip content (%DW) of NHL along the year
170 (Fig 1).
171 Significant changes in lipid class composition were detected between spring and autumn
172 batches (Table 2). Thus the increase in total lipid content observed in the larvae
173 obtained in autumn was the consequence of a significant increase in phospolipids (i.e.
174 phosphatidylcholine-PC, phosphatidylserine-PS and phosphatidylethanolamine-PE) and
175 cholesterol. Triacylglycerids (TG) also increased from spring (10.00±4.10 µg mg DW^{-1})
176 to autumn (12.65±5.50 µg mg DW^{-1}) but this increase was not statistically significant.
177 Total fatty acid (µg FA mg Lip^{-1}) content of NHL did not change significantly among
178 seasons (Table 3). Saturated FA accounted for 27 to 30% of total FA whereas monoenes
179 ranged from 17 to 20% of FA. Polyunsaturated fatty acids (PUFA) represented the main
180 FAs in NHL of *M. brachydactyla*, accounting for 49% of total FA in the larvae obtained
181 at spring and up to 53% of total FA in summer. Eicosapentaenoic acid (EPA, 20:5n-3)
182 content of larvae hatched in autumn (12.23±4.45 µg mg DW^{-1}) was significantly higher
183 than in spring (6.63±3.27 µg mg DW^{-1}) and summer (6.96±1.76 µg mg DW^{-1}) that
184 resulted in an increase in EPA/ Docosahexaenoic acid (DHA, 22:6n-3) ratio (EPA/
185 DHA) from 1.33±0.07 in spring to 1.85±0.31 in autumn. The relative increase in n-3

PUFA as seasons advanced was also reflected in the n-3/n-6 ratio, increasing significantly from 5.34±0.76 in spring to 7.53±0.99 and 7.39±1.12 in summer and autumn, respectively.

Amino acid (AA) profile of the larvae hatched at different seasons is shown in Table 4. Glutamic acid (Glu) was the most abundant AA found in NHL of *M. brachydactyla*, accounting for a maximum of 18.5±0.1% of the total AA in summer. In general, a decrease in AA content (μg AA mg DW^{-1}) occurred as time in captivity advanced, in both essential AA (Lys, Val, Ile and His) and non-essential AA (Glu, Asp, Gly, Ala and Ser) ($p<0.05$). A decrease in vitamins A and E was observed at autumn larvae (Table 5) whereas a significant increase in vitamin C was detected throughout the year.

Mineral content of NHL changed significantly through the year (Table 6). Major essential elements such as calcium and magnesium decreased significantly in larvae hatched in summer whereas others such as sulphur reached its maximum levels (14.80±2.03 g kg DW^{-1}) in larvae hatched in this season. Potassium levels remained constant in larvae throughout the year (around 19 g kg^{-1}) whereas sodium, chloride and phosphorus levels decreased in larvae as time in captivity advanced. Regarding trace elements, copper, iron and strontium levels decreased significantly on larvae hatched in autumn whereas manganese showed its maximum levels in the larvae hatched in summer (0.06±0.00 g kg DW^{-1}).

4 Discussion

The present study provides information about the variation in the biochemical composition of *M. brachydactyla* newly hatched larvae obtained under captivity conditions during an entire year. February to May was the period when more batches were observed, although the number of newly hatched larvae showed no significant fluctuation throughout the period studied. Larval biomass was clearly affected by the

time that females remained in captivity before hatching occurred, being the larvae obtained at the end of the year smaller and lighter than those from the beginning. In *Chasmagnatus granulata*, initial larval size at hatching was correlated with the initial egg size at the onset of embryogenesis (Giménez et al., 2004). Hence, decreasing egg size may probably cause the decrease in biomass of NHL of *M. brachydactyla* as seasons advanced. A similar effect was observed in *Portunus trituberculatus* (Hamasaki et al., 2006), although in this case the decrease in the size of first zoea as breeding season advanced was correlated with increasing temperatures. Up to date, no work has been published concerning variations in biomass of NHL of *M. brachydactyla* under natural environmental conditions, but data of berried females collected from the wild showed that egg biomass decreased after successive spawning (Verísimo, 2000). Thus, the reduction in larval size might be explained as an adaptation, reducing the energy investment in egg/larval size through consecutive spawning, as observed in the wild. In addition, we cannot discard a potential reproductive exhaustion of broodstock caused by forced-spawning under the constant temperature conditions used in the present study. The effect that a reduction in the initial size of the larvae might have in further development and culture performance has been recently investigated in other decapod species. Thus the size of newly hatched nauplii from different batches of *Litopenaeus vannamei* was not indicative of their quality (Palacios et al., 2001; as *Penaeus vannamei*), and was not correlated to survival during culture (Palacios et al., 1999; as *P. vannamei*). On the contrary, larvae of *C. granulata* that hatched with a biomass lower than the average tended to develop slower, resulting in larger juveniles with a significantly higher tolerance to starvation (Giménez et al., 2004).

Although a significant reduction in larval weight was observed along consecutive spawning, NHL had a similar absolute individual protein and lipid content (μg larva^{-1})

236 along the year, resulting in significantly higher protein and lipid content (%DW) in
237 larvae at the end of the year than the larvae obtained at the beginning of the spawning
238 season. Up to date, reports on the variation in protein content of the offspring
239 throughout the spawning season are contradictory. In wild populations of *M.*
240 *brachydactyla* no differences were detected in the protein content of eggs from
241 consecutive spawns (Verísimo, 2000). In *L. vannamei*, although total protein levels in
242 the eggs did not change significantly from the beginning to the end of the spawning
243 period, protein content in the nauplii decreased significantly and concomitant with a
244 reduction in body size and weight (Palacios et al., 1999). In the same species, no further
245 evidences of the relationship between total protein levels and spawn or larval quality
246 have been found (Racotta et al., 2003).
247 Relative lipid content increased significantly in batches from spring to autumn. The
248 association between lipid concentration and the physiological condition or performance
249 of decapod larvae is supported by several studies as reviewed by Racotta et al. (2003).
250 Furthermore, TG are considered the most important energy reserve during
251 embryogenesis and early larval development in *L. vannamei*, in which a positive
252 correlation between TG content in the nauplii and larval survival to stress was found
253 (Palacios et al., 1999). Also in the lobster, *Homarus gammarus*, high TG content in the
254 larvae increase the chances for survival to stressors such as low salinity, pollution or
255 starvation (Wickins et al., 1995). In addition to TG, phospholipids (PL) have specific
256 functions during larval development (Coutteau et al., 1997) and their contents can also
257 be related to larval quality. Specifically, a growth-promoting effect was found in
258 *Marsupenaeus japonicus* larvae (Kanazawa et al., 1985; as *Penaeus japonicus*) and a
259 survival-enhancing effect was also found in *Homarus americanus* (Conklin et al., 1980;
260 D'Abramo et al., 1981) and attributed to the role of PL in the absorption and transport of

dietary cholesterol. In the wild, spider crab's main spawning season occurs in spring, however, some berried females have been reported during autumn (González-Gurriarán et al., 1998), thus NHL are expected to face environmental adverse conditions, such as low temperatures and prey scarcity. Therefore, the high lipid content (together with the increase in proteins) found in autumn batches of *M. brachydactyla* might be explained as a species adaptative strategy, compensating the decrease in DW. As in our conditions broodstock diet and temperature were constant throughout the year, seasonal variation in larval composition in terms of lipid content suggests that photoperiod might be a key factor controlling the female reproductive cycle. Further studies would be needed to test this hypothesis.

The fatty acid profile of NHL reflected the changes observed in total lipid levels from spring to autumn, as clearly shown by the significant increase in EPA and in EPA/DHA and n-3/n-6 ratios. As in the case of TG, n-3 PUFA have been cited to increase the chances for survival of lobster larvae (Wickins et al., 1995). According to Figueiredo and Narciso (2008), EPA plays a key role in the development of *M. brachydactyla*, being highly required during the embryogenesis. In the shrimp *L. vannamei* EPA has been found to be positively correlated with egg survival suggesting a specific role of EPA in certain PLs (Palacios et al., 2001). Thus, the higher EPA content observed in the spider crab larvae obtained in autumn might increase their individual chances to survive in the wild.

Amino acid profiles (AA) of NHL of *M. brachydactyla* described here are in agreement to those reported previously by Villanueva et al. (2004) in this species. Most of the AA defined as essential (EAA) for crustaceans (reviewed by Guillaume, 1997) were kept constant in NHL composition along the seasons, although Lys, Val and His levels were reduced form spring to autumn. A strong correlation between the EAA in the diet and in

286 the whole body tissue has been cited in crustacean nutritional studies (Guillaume,
287 1997). However, Peñaflorida (2004) found no relationships between the lack of certain
288 AA in the diet and their presence in the female ovary, eggs or zoeas of the mud crab,
289 *Scylla serrata*. Due to the little knowledge concerning EAA dynamics and requirements
290 in brachyuran larvae, it remains unclear whether the important reduction (especially in
291 Lys) observed in our study, might indicate a deficiency in the broodstock diet or
292 reproductive exhaustion, and as a consequence, a reduction in the culturing performance
293 of their offspring.
294 Levels of fat-soluble vitamins (A and E) decreased significantly in NHL from spring to
295 autumn. Among the vitamins, vitamin A (VA) and vitamin E (VE) play a key role in
296 growth, reproduction and embryonic development. Primary non-enzymatic antioxidants
297 in fish eggs are VA and VE as well as provitamin A carotenoids. The content of these
298 lipid-soluble vitamins in fish eggs has been related to higher larval size and survival
299 (Lavens et al., 1999). VA is required for vision being concentrated in the eyes of
300 crustaceans and not distributed throughout the body as in vertebrates (Dall, 1995).VA
301 has also been known as a ‘growth vitamin’ (Gouillou-Coustans and Guillaume, 2001)
302 because it stimulates new cell growth inducing messenger RNA and specific protein
303 synthesis. However, Dall (1995) suggested that carotenoids instead of VA (retinoids)
304 are essential in the early development of decapod crustaceans because retinoids could
305 not be detected in eggs, naupliar stages and protozoea I of *Penaeus semisulcatus*
306 whereas carotenoids were present and metabolized exponentially. Similarly, Villanueva
307 et al. (2009) did not detect retinol in newly hatched zoea of *M. brachydactyla*. In the
308 present study significant levels of VA were found in newly hatched zoea of *M.*
309 *brachydactyla* that decreased as seasons advanced. These contradictory results in the
310 same species and time of development might be attributed to the different sensibility of

311 the methodology employed for detection. The units employed in the present study were
312 UI, which can be transformed in µg of retinol by a multiplying factor of 0.3 (AOAC
313 official method 974.29, 2007a). Hence, the equivalent levels of retinoids detected in the
314 present study would range from 1.5 µg retinol g DW^{-1} in spring to 0.5 µg retinol g DW^{-1}
315 in autumn, which are low enough to be overlooked by a standard method of analysis.
316 Furthermore, the different origins of the larvae used for both studies (especially in terms
317 of broodstock condition) could be the cause of the differences observed in the VA
318 content.
319 Some studies have suggested the essentiality of VE (α-tocopherol) in crustaceans due to
320 their antioxidant protection to membrane-bound polyunsaturated fatty acids (for review
321 see Conklin, 1997). As only plants synthesize α-tocopherol, algae, either direct or
322 indirectly, are the ultimate source of this compound for crustaceans. In the present
323 study, the significant decrease of VE in NHL from spring to autumn might be indicative
324 of a nutritional deficiency of the broodstock. Ascorbic acid (VC) is involved in a variety
325 of biochemical reactions within cells due to its ability to undergo reversible oxidation
326 and reduction (Conklin, 1997). The increasing levels of this vitamin in the NHL of *M.*
327 *brachydactyla* as seasons advanced, suggest that requirements on this important vitamin
328 are fulfilled with the broodstock diet.
329 The concentration of minerals observed in this study are within the range of values
330 reported previously for spider crab (Villanueva and Bustamante, 2006) with the
331 exception of Ca, K and P contents, that are substantially higher in our study than those
332 found previously (44.5 g Ca kg DW^{-1}, 15.0 g K kg DW^{-1} and 12.1 g P kg DW^{-1}). In
333 aquatic animals minerals may serve as components of hard-tissue matrices, soft tissues
334 and as cofactors and/or activators of a variety of enzymes. The more soluble minerals
335 (Ca, P, Na, K and Cl^-) also function in osmoregulation and the maintenance of acid-base

balance and membrane potentials (Davis and Lawrence, 1997). Macro elements such as Ca, Mg, Na, K and Cl^- can be taken up directly from the surrounding water (across the epithelia of the gill chamber and the intestine) and hence are considered non limiting factors for aquatic decapods living in the marine environment (Anger, 2001). Furthermore, during embryonic development, a certain degree of mineral exchange between the egg and the medium is expected to occur, as reported for fish (Zeitoun et al., 1976) and is likely affected by the mineral concentration in the environment (Harrison, 1990). There is some evidence that under culturing conditions, decapod diet needs to be supplemented with certain minerals in order to optimize growth and reproduction (reviewed by Davis and Lawrence, 1997). From the elements analyzed in this study, Na, Cl^-, P, Fe, Sr and Cu clearly showed a significant decrease in newly hatched larvae as the time in captivity advanced. The decrease observed in Na, Cl^- and Sr cannot be attributed to a deficiency in the broodstock since seawater contains sufficient amounts of these minerals to satisfy their physiological needs and, additionally, these ions can be found in substantial amounts in most diets. Copper is an essential element for crustaceans as it works as a respiratory pigment in haemocyanin among other important metabolic functions. Studies on *L. vannamei* (Mendez et al., 2001) had revealed that Cu and Fe levels were depleted in broodstock as a result of continuous intensive reproduction. Thus, the significant decrease observed in Cu levels of NHL of *M. brachydactyla* obtained in autumn might be the consequence of a broodstock depletion of this element, due to continuous spawning or a dietary deficiency. The same may apply for the decrease observed in Fe. On the other hand, Ca and Mg reduction detected in summer might be correlated with the decrease in the ash content observed in July.

5 Conclusions

1. Overall, newly hatched larvae obtained throughout the year seem to be an adequate source for spider crab mass production.
2. The maintenance of *M. brachydactyla* broodstock under intensive culturing conditions throughout a whole year resulted in the gradual reduction of larval weight.
3. Although the larvae showed a reduction in weight at the end of the year, their biochemical composition was maintained with an increase in the relative protein and lipid content as seasons advanced, indicating a certain adaptation of the larvae to face winter adverse conditions of temperature and prey availability.
4. Decrease in Vitamins A and E as well as in certain essential amino acids (Lys, Val, His) and trace elements (Cu, Fe) of the larvae at the end of the year might be indicative of a nutritional deficiency in broodstock diets.

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7 References

Alaminos, J., Domingues, P., 2008. Effects of different natural or prepared diets on growth and survival of juvenile spider crabs, *Maja brachydactyla* (Balss, 1922). Aquac. Int. 16, 417-425.

Andrés, M., Estévez, A., Rotllant, G., 2007. Growth, survival and biochemical composition of spider crab *Maja brachydactyla* (Balss, 1922) (Decapoda:

385 Majidae) larvae reared under different stocking densities, prey: larva ratios and
386 diets. Aquaculture 273, 494-502.
387 Andrés, M., Estévez, A., Anger, K., Rotllant, G., 2008. Developmental patterns of larval
388 growth in the edible spider crab, *Maja brachydactyla* (Decapoda: Majidae). J.
389 Exp. Mar. Biol. Ecol. 357, 35-40.
390 Andrés, M., Estévez, A., Hontoria, F., Rotllant, G., 2010a. Differential utilization of
391 biochemical components during larval development of the spider crab *Maja*
392 *brachydactyla* (Decapoda: Majidae). Mar. Biol. 157, 2329-2340.
393 Andrés, M., Gisbert, E., Díaz, M., Moyano, F.J., Estévez, A., Rotllant, G., 2010b.
394 Ontogenetic changes in digestive enzymatic capacities of the spider crab, *Maja*
395 *brachydactyla* (Decapoda: Majidae). J. Exp. Mar. Biol. Ecol. 389, 75-84.
396 Anger, K., 2001. Vitamins and minerals. In: Balkema, A.A. (Ed.), The Biology of
397 Decapod Crustacean Larvae, Crustacean Issues 14, Rotterdam, pp. 117-118.
398 Anger, K., 2006. Contributions of larval biology to crustacean research: a review.
399 Invertebr. Reprod. Dev. 49, 175-205.
400 AOAC, 2007a. Official Method 970.64: Carotenes and xanthophylls in dried plant
401 materials and mixed feeds. In: Horwitz, W. (Ed.), Official Methods of Analysis.
402 AOAC International, Gaithersburg (USA).
403 AOAC, 2007b. Official Method 974.29: Vitamin A in mixed feeds, premixes, and
404 human and pet foods. In: Horwitz, W. (Ed.), Official Methods of Analysis.
405 AOAC International, Gaithersburg (USA).
406 AOAC, 2007c. Official Method 945.04: Calcium (acid soluble) in fertilizers. In:
407 Horwitz, W. (Ed.), The Official Methods of Analysis. AOAC International,
408 Gaithersburg (USA).

AOAC, 2007d. Official Method 969.23: Sodium and potassium in seafood. In: Horwitz, W. (Ed.), Official Methods of Analysis. AOAC International, Gaithersburg (USA).

AOAC, 2007e. Official Method 975.03: Metals in plants and pet foods In: Horwitz, W. (Ed.), Official Methods of Analysis. AOAC International, Gaithersburg (USA).

AOAC, 2007f. Official Method 975.01: Copper in fertilizers. In: Horwitz, W. (Ed.), Official Methods of Analysis. AOAC International, Gaithersburg (USA).

AOAC, 2007g. Official Method 972.02: Manganese (acid-soluble) in fertilizers. In: Horwitz, W. (Ed.), Official Methods of Analysis. AOAC International, Gaithersburg (USA).

AOAC, 2007h. Official Method 980.01: Iron in fertilizers. In: Horwitz, W. (Ed.), Official Methods of Analysis. AOAC International, Gaithersburg (USA).

AOAC, 2007i. Official Method 937.09: Salt (chlorine as sodium chloride) in seafood. In: Horwitz, W. (Ed.), Official Methods of Analysis. AOAC International, Gaithersburg (USA).

AOAC, 2007j. Official Method 958.01: Phosphorus (total) in fertilizers. In: Horwitz, W. (Ed.), Official Methods of Analysis. AOAC International, Gaithersburg (USA).

AOAC, 2007k. Official Method 920.10: Sulfur in plants. In: Horwitz, W. (Ed.), Official Methods of Analysis. AOAC International, Gaithersburg (USA).

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

Conklin, D.E., 1997. Vitamins. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition, Advances in World Aquaculture. The World Aquaculture Society, Baton Rouge, Lousiana (US), pp. 123-149.

434 Conklin, D.E., D'Abramo, L.R., Bordner, C.E., Baum, N.A., 1980. A succesful purified
435 diet for the culture of juvenile lobsters: The effect of lecithin. Aquaculture 21,
436 243-249.
437 Coutteau, P., Geurden, I., Camara, M.R., Bergot, P., Sorgeloos, P., 1997. Review on the
438 dietary effects of phospholipids in fish and crustacean larviculture. Aquaculture
439 155, 149-164.
440 Christie, W.W., 1982. Lipid analysis: isolation, separation, identification, and structural
441 analysis of lipids. Pergamon Press, New York, 207 pp.
442 D'Abramo, L.R., Bordner, C.E., Conklin, D.E., Baum, N.A., 1981. Essentiality of
443 dietary phosphatidylcholine for the survival of juvenile lobsters. J. Nutr. 111,
444 425-431.
445 Dall, W., 1995. Carotenoids versus retinoids (Vitamin A) as essential growth factors in
446 penaeid prawns (*Penaeus semisulcatus*). Mar. Biol. 124, 209-213.
447 Davies, M.G., Thomas, A.J., 1973. An investigation of hydrolytic techniques for the
448 amino acid analysis of foodstuffs. J. Sci. Food Agric. 24, 1525-1540.
449 Davis, D.A., Lawrence, A.L., 1997. Minerals. In: D'Abramo, L.R., Conklin, D.E.,
450 Akiyama, D.M. (Eds.), Crustacean Nutrition, Advances in World Aquaculture.
451 The World Aquaculture Society, Baton Rouge, Lousiana (US), pp. 150-163.
452 Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric
453 method for determination of sugars and related substances. Anal. Chem. 28,
454 350-356.
455 Figueiredo, J., Narciso, L., 2008. Egg volume, energy content and fatty acid a profile of
456 *Maja brachydactyla* (Crustacea: Brachyura: Majidae) during embryogenesis. J.
457 Mar. Biol. Assoc. U. K. 88, 1401-1405.

458 Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and
459 purification of total lipides from animal tissues. J. Biol. Chem. 226, 497-509.
460 Freire, J., Bernárdez, C., Corgos, A., Fernández, L., González-Gurriarán, E., Sampedro,
461 M.P., Verísimo, P., 2002. Management strategies for sustainable invertebrate
462 fisheries in coastal ecosystems of Galicia (NW Spain). Aquat. Ecol. 36, 41-50.
463 Ghioni, C., Porter, A.E.A., Taylor, G.W., Tocher, D.R., 2002. Metabolism of 18: 4n-3
464 (stearidonic acid) and 20: 4n-3 in salmonid cells in culture and inhibition of the
465 production of prostaglandin F-2 alpha (PGF(2 alpha)) from 20: 4n-6
466 (arachidonic acid). Fish Physiol. Biochem. 27, 81-96.
467 Giménez, L., Anger, K., Torres, G., 2004. Linking life history traits in succesive phases
468 of a complex life cycle: effects of larval biomass on early juvenile development
469 in an estuarine crab, *Chasmagnatus granulata*. Oikos 104, 580-580.
470 González-Gurriarán, E., Fernández, L., Freire, J., Muiño, R., 1998. Mating and role of
471 seminal receptacles in the reproductive biology of the spider crab *Maja squinado*
472 (Decapoda: Majiidae). J. Exp. Mar. Biol. Ecol. 220, 269-285.
473 González-Gurriarán, E., Freire, J., Parapar, J., Sampedro, M.P., Urcera, M., 1995.
474 Growth at moult and moulting seasonality of the spider crab, *Maja squinado*
475 (Herbst) (Decapoda: Majidae) in experimental conditions: implications for
476 juvenile life history. J. Exp. Mar. Biol. Ecol. 189, 183-203.
477 Gouillou-Coustans, M.F., Guillaume, J., 2001. Vitamin nutrition. In: Guillaume, J.,
478 Métailler, R., Kaushik, S., Bergot, P. (Eds.), Nutrition and feeding of fish and
479 crustaceans. Springer-Praxis Publishing Ltd., Chichester (UK), pp. 145-166.
480 Guerao, G., Rotllant, G., 2009. Survival and growth of post-settlement juveniles of the
481 spider crab *Maja brachydactyla* (Brachyura: Majoidea) reared under individual
482 culture system. Aquaculture 289, 181-184.

483 Guillaume, J., 1997. Protein and Amino Acids. In: D'Abramo, L.R., D'Abramo, L.R.,
484 Akiyama, D.M. (Eds.), Crustacean Nutrition, Advances in World Aquaculture.
485 The World Aquaculture Society, Baton Rouge, Lousiana (US), pp. 26-50.
486 Hamasaki, K., Fukunaga, K., Kitada, S., 2006. Batch fecundity of the swimming crab
487 *Portunus trituberculatus* (Brachyura: Portunidae). Aquaculture 253, 359-365.
488 Harrison, K.E., 1990. The role of nutrition in maturation, reproductioon and embryonic
489 development of decapod crustaceans: a review. J. Shellfish Res. 9, 1-28.
490 Iglesias, J., Sánchez, F.J., Moxica, C., Fuentes, L., Otero, J.J., Pérez, J.L., 2002. Datos
491 preliminares sobre el cultivo de larvas y juveniles de centolla *Maja squinado*
492 Herbst, 1788 en el Centro Oceanográfico de Vigo del Instituto Español de
493 Oceanografía. Bol. Inst. Esp. Oceanogr. 18, 25-30.
494 Kanazawa, A., Teshima, S., Sakamoto, M., 1985. Effects od dietary lipids, fatty acids,
495 and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae.
496 Aquaculture 50, 39-49.
497 Lavens, P., Lebegue, E., Jaunet, H., Brunel, A., Dhert, P., Sorgeloos, P., 1999. Effect of
498 dietary essential fatty acids and vitamins on egg quality in turbot broodstocks.
499 Aquac. Int. 7, 225-240.
500 Mendez, L., Racotta, I.S., Acosta, B., Rodriguez-Jaramillo, C., 2001. Mineral
501 concentration in tissues during ovarian development of the white shrimp
502 *Penaeus vannamei* (Decapoda : Penaeidae). Mar. Biol. 138, 687-692.
503 Olsen, R.E., Henderson, R.J., 1989. The rapid analysis of neutral and polar marine
504 lipids using double-development HPTLC and scanning densitometry. J. Exp.
505 Mar. Biol. Ecol. 129, 189-197.

506 Palacios, E., Perez-Rostro, C.I., Ramirez, J.L., Ibarra, A.M., Racotta, I.S., 1999.
507 Reproductive exhaustion in shrimp (*Penaeus vannamei*) reflected in larval
508 biochemical composition, survival and growth. Aquaculture 171, 309-321.
509 Palacios, E., Racotta, I.S., Heras, H., Marty, Y., Moal, J., Samain, J.F., 2001. Relation
510 between lipid and fatty acid composition of eggs and larval survival in white
511 pacific shrimp (*Penaeus vannamei*, Boone, 1931). Aquac. Int. 9, 531-543.
512 Palma, J., Correia, M., Andrade, J.P., 2008. Usefulness of flat bottom tanks on the
513 settlement of spider crab (*Maja squinado*, Herbst) larvae. Aquac. Res. 39, 1005-
514 1008.
515 Peñaflorida, V.D., 2004. Amino acid profiles in the midgut, ovary, developing eggs and
516 zoea of the mud crab, *Scylla serrata*. Isr. J. Aquacult. Bamidgeh 56, 113-125.
517 Pfeifer, R., Karol, R., Korpi, J., Burgoyne, R., McCourt, D., 1983. Practical application
518 of HPLC to amino-acid analysis. Am. Lab. 15, 77-84.
519 Racotta, I.S., Palacios, E., Ibarra, A.M., 2003. Shrimp larval quality in relation to
520 broodstock condition. Aquaculture 227, 107-130.
521 Rotllant, G., González-Gurriarán, E., Fernández, L., Benhalima, K., Ribes, E., 2007.
522 Ovarian maturation of the multi-spawning spider crab *Maja brachydactyla*
523 (Decapoda: Majidae) with special reference to yolk formation. Mar. Biol. 152,
524 383-394.
525 Simeó, C.G., Ribes, E., Rotllant, G., 2009. Internal anatomy and ultrastructure of the
526 male reproductive system of the spider crab *Maja brachydactyla* (Decapoda:
527 Brachyura). Tissue Cell 41, 345-361.
528 Verísimo, P., 2000. Estrategias reproductivas de la centolla, *Maja squinado* (Herbst,
529 1788), en la Ria de Arousa: caracterización de sus puestas, Departamento de

530 Bioloxía Animal, Bioloxía Vexetal e Ecoloxía. Universidade da Coruña, A
531 Coruña, pp. 74.
532 Villanueva, R., Bustamante, P., 2006. Composition in essential and non-essential
533 elements of early stages of cephalopods and dietary effects on the elemental
534 profiles of *Octopus vulgaris* paralarvae. Aquaculture 261, 225-240.
535 Villanueva, R., Riba, J., Ruíz-Capillas, C., González, A.V., Baeta, M., 2004. Amino
536 acid composition of early stages of cephalopods and effect of amino acid dietary
537 treatments on *Octopus vulgaris* paralarvae. Aquaculture 242, 455-478.
538 Villanueva, R., Escudero, J.M., Deulofeu, R., Bozzano, A., Casoliva, C., 2009. Vitamin
539 A and E content in early stages of cephalopods and their dietary effects in
540 *Octopus vulgaris* paralarvae. Aquaculture 286, 277-282.
541 Wickins, J.F., Beard, T.W., Child, A.R., 1995. Maximizing lobster, *Homarus gammarus*
542 (L.), egg and larval viability. Aquac. Res. 26, 379-392.
543 Wimalasiri, P., Wills, R.B.H., 1983. Simultaneous analysis of ascorbic acid and
544 dehydroascorbic acid in fruit and vegetables by high-performance liquid
545 chromatography. J. Chromatogr. A 256, 368-371.
546 Xu, X.L., Ji, W.J., Castell, J.D., Odor, R.K., 1994. Influence of dietary-lipid sources on
547 fecundity, egg hatchability and fatty-acid composition of Chinese prawn
548 (*Penaeus chinensis*) broodstock. Aquaculture 119, 359-370.
549 Zeitoun, I.H., Ullrey, D.E., Bergen, W.G., Magee, W.T., 1976. Mineral metabolism
550 during ontogenesis of rainbow-trout (*Salmo gairdneri*). J. Fish. Res. Board Can.
551 33, 2587-2591.
552
553

FIGURE CAPTION

Fig 1. Variation in protein (Pr), lipid (Lip) and carbohydrate (Ch) content (% of dry weight, DW) of newly hatched larvae of *M. brachydactyla* during one year of broodstock captivity. Different letters indicate significant differences in Lip and Pr content among monthly samples ($p<0.05$) (letters above bars refer to Lip, no significant differences were found in Ch).

Figure 1

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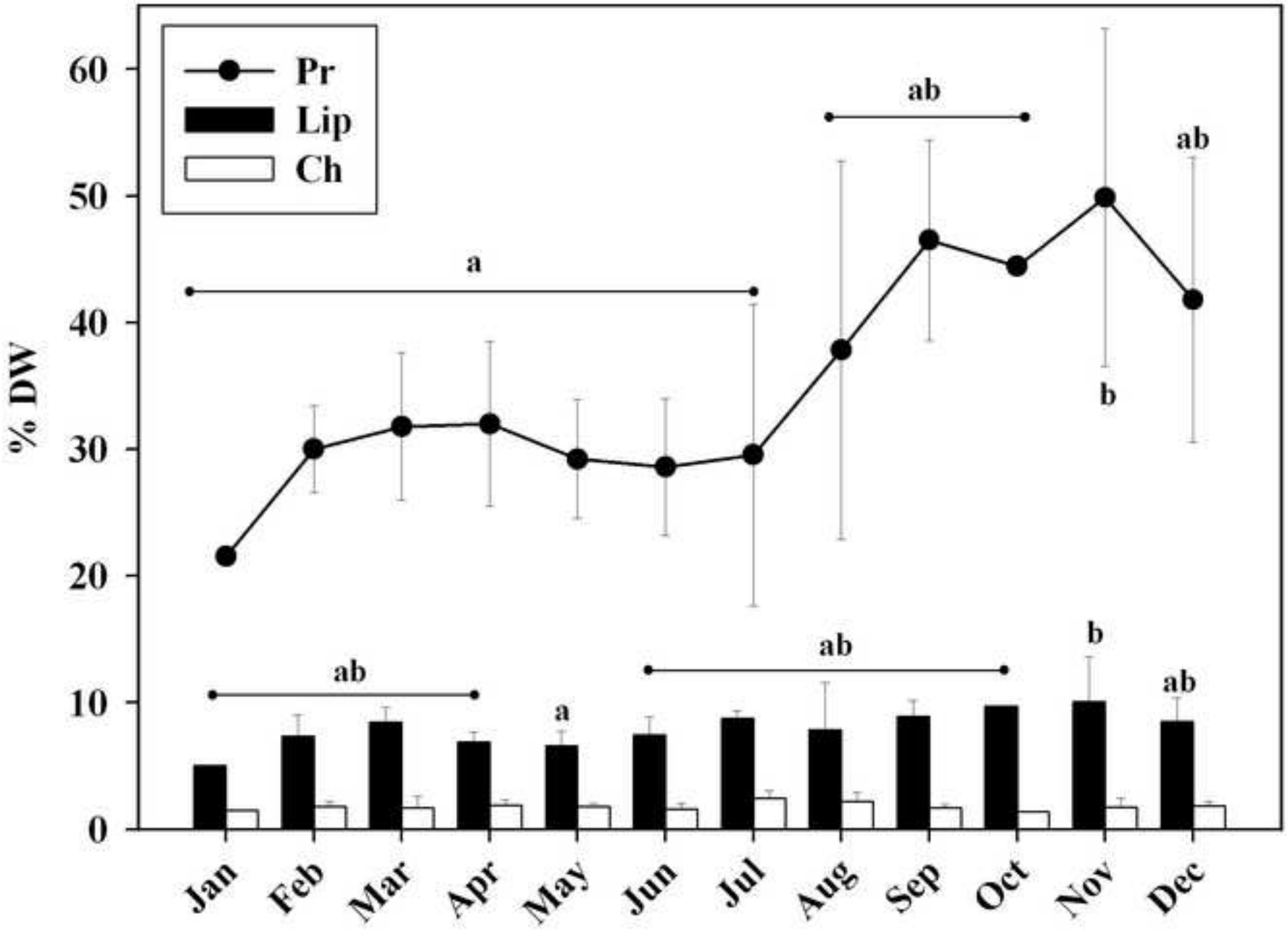

Table 1

Table 1. Number of larvae per batch and female and larval dry weight (DW), ash content and proximate biochemical composition (Prt= proteins; Lip= lipids; Ch= carbohydrates) (all in µg larva^{-1}) of *M. brachydactyla* obtained in successive months of captivity (*n*=number of batches per month).

Month	*n*	Nº larvae/batch/female	DW	Ash	Prt	Lip	Ch
January	1	4,788±0	108±0^{ab}	39±0^{a}	23±0	5±0	2±0
February	10	4,136±3,636	98±11^{a}	26±4^{abc}	30±5	7±2	2±0
March	9	3,814±2,991	98±11^{a}	25±2^{abc}	31±5	8±2	2±1
April	14	5,103±4,946	98±6^{a}	25±2^{abc}	31±7	7±1	2±0
May	17	5,122±4,345	100±8^{a}	28±5^{ab}	29±5	7±1	2±0
June	5	4,972±2,202	98±9^{a}	27±5^{abc}	28±7	7±1	2±0
July	2	2,024±1,990	95±8^{a}	19±2^{b}	28±13	8±1	2±1
August	4	4,129±3,609	84±10^{ab}	25±5^{abc}	33±15	7±3	2±1
September	2	1,958±2,369	87±5^{ab}	21±3^{b}	40±5	8±1	1±0
October	1	2,283±0	84±0^{ab}	20±0^{abc}	37±0	8±0	1±0
November	7	6,240±6,767	75±14^{b}	19±5^{c}	37±9	7±3	1±0
December	4	8,507±6,122	82±14^{ab}	24±8^{abc}	34±9	7±2	2±0

Data shown as mean±S.D. Different letters in superscript within the same column indicate significant differences among months (ANOVA, $p<0.05$).

Table 2

Table 2. Individual dry weight (DW), lipid (Lip) content and lipid class composition (µg mg DW^{-1}) of newly hatched larvae obtained in different seasons.

	SPRING	SUMMER	AUTUMN
DW (µg)	98.8±7.1[a]	92.6±10.2[a]	78.4±13.0[b]
% Lip	6.7±1.2[a]	8.1±1.9[ab]	9.2±2.6[b]
Total polar	28.23±6.80[a]	33.12±8.69[ab]	37.24±9.60[b]
Sphingomyelin	0.69±0.49	0.79±0.41	0.41±0.45
Phosphatidylcholine	11.89±3.03[a]	13.95±3.93[ab]	15.71±4.04[b]
Phosphatidylserine	1.28±0.66[a]	1.48±0.84[a]	2.15±0.71[b]
Phosphatidylinositol	2.78±0.75	3.28±0.97	3.28±0.84
Phosphatidic acid / Cardiolopin	1.22±0.83	1.37±0.97	1.51±1.71
Phosphatidylethanolamine	10.16±2.05[a]	11.93±3.08[ab]	14.18±3.42[b]
Total neutral	39.07±7.75[a]	46.82±13.02[ab]	55.08±18.32[b]
Cholesterol	13.47±2.45[a]	15.92±4.18[ab]	18.62±4.48[b]
Free fatty acids	0.86±0.74[a]	1.88±1.37[b]	3.53±2.12[c]
Triglycerides	10.00±4.10	11.59±6.10	12.65±5.50
Sterol esters / waxes	14.59±4.67	16.75±7.76	20.28±9.73

Data is shown as mean±S.D (n_{spring}=19, n_{summer}=18, n_{autumn}=13). Different letters in superscript within the same row indicate significant differences among seasons (ANOVA, p<0.05).

Table 3. Individual dry weight (DW), lipid (Lip) and fatty acid (FA) content and main FA profile (µg FA mg DW^{-1}) of newly hatched larvae obtained in different seasons.

	SPRING	SUMMER	AUTUMN
DW (µg)	97.1±10.2[a]	94.2±7.1[ab]	77.4±14.6[b]
% Lip	6.6±0.5	7.4±1.4	9.5±3.9
µg FA mg Lip^{-1}	426.9±129.8	445.7±56.8	473.2±150.3
16:0	4.14±1.33	3.95±1.28	7.48±5.14
18:0	3.84±0.86	3.36±1.09	6.22±3.18
SFA[1]	8.44±2.18	7.72±2.57	14.31±8.13
16:1	0.47±0.26	0.71±0.32	1.29±0.88
18:1n-9	3.09±0.91	2.54±0.87	3.31±1.49
MUFA[2]	5.79±1.65	5.40±2.06	8.18±4.58
18:2n-6	0.31±0.07	0.24±0.10	0.33±0.17
20:4n-6	1.73±0.60	1.37±0.13	2.21±0.79
PUFA n-6[3]	2.20±0.78	1.66±0.26	2.70±1.06
18:3n-3	0.10±0.09	0.08±0.07	0.08±0.04
20:5n-3 (EPA)	6.63±3.27[a]	6.96±1.76[a]	12.23±4.45[b]
22:6n-3 (DHA)	4.96±2.36	5.08±0.96	6.71±2.38
EPA/DHA	1.33±0.07[a]	1.32±0.09[a]	1.85±0.31[b]
PUFA n-3[4]	12.02±5.87	12.47±2.80	19.64±7.08
TOTAL PUFA[5]	14.79±7.18	14.58±2.98	23.12±8.40
n-3/n-6	5.34±0.76[a]	7.53±0.99[b]	7.39±1.12[b]

Data is shown as mean ± S.D. Different letters in superscripts within the same row denote significant differences among seasons (ANOVA; $p<0.05$, $n= 5$).[1]SFA (Saturated FA): sum of 14:0, 16:0, 18:0, 20:0 and 22:0. [2]MUFA (Monounsaturated FA): sum of 16:1, 18:1, 20:1 and 22:1. [3]PUFA n-6 (Polyunsaturated FA ω-6): sum of 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22: 5n-6. [4]PUFA n-6 (Polyunsaturated FA ω-3): sum of 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:4n-3, 22:5n-3, 22:6n-3. [5]sum of 16:2, 16:3, 16:4, n-3 and n-6

Table 4

Table 4. Total amino acid content (µg AA mg DW^{-1}) of newly hatched larvae obtained in different seasons.

	SPRING	SUMMER	AUTUMN
EAA			
Lys	46.7±1.2[a]	32.2±1.5[b]	29.3±0.6[c]
Leu	31.6±2.6	29.3±2.4	26.4±1.1
Arg	24.4±0.6	24.5±0.6	22.6±1.5
Thr	22.3±2.1	19.7±2.2	18.7±0.6
Val	19.6±0.0[a]	16.9±0.6[b]	15.2±0.6[c]
Phe	16.8±0.6	15.3±1.7	14.8±1.5
Ile	12.7±0.6	10.5±1.0	10.2±1.3
His	10.5±0.7[a]	7.6±0.6[b]	7.3±0.3[b]
Met	8.5±0.4	7.9±0.4	7.2±2.0
NAA			
Glu	80.3±0.0[a]	75.8±0.6[b]	68.7±1.0[c]
Asp	49.1±0.6[a]	45.5±0.6[b]	41.9±0.6[c]
Gly	41.5±0.6[a]	38.5±0.6[b]	35.1±1.1[c]
Ala	34.7±1.6[a]	31.5±1.7[ab]	30.0±0.0[b]
Ser	27.5±0.6[a]	24.5±1.1[b]	22.6±1.5[b]
Tyr	13.7±1.6	11.8±2.4	10.6±1.7
Pro	19.2±0.6	18.1±1.7	17.7±2.2

Data is shown as mean ± S.D. Different letters in superscript within the same line indicate significant differences among seasons (ANOVA, $p<0.05$, $n=3$).

Table 5

Table 5. Vitamin content (Vit A in UI g DW^{-1}; Vit E and C in mg Kg DW^{-1}) of newly hatched larvae obtained in different seasons.

	SPRING	SUMMER	AUTUMN
Vit A	5.15 ± 0.00^{a}	2.23 ± 0.55^{b}	1.52 ± 0.15^{c}
Vit E	326.1 ± 6.0^{a}	277.0 ± 0.0^{b}	245.0 ± 5.6^{c}
Vit C	3.09 ± 0.00^{a}	3.82 ± 0.00^{b}	4.38 ± 0.06^{c}

Data is shown as mean ± S.D. Different letters in superscript within the same line indicate significant differences among seasons (ANOVA, $p<0.05$, $n=3$).

Table 6

Table 6. Mineral content (g kg DW^{-1}) of newly hatched larvae obtained in different seasons.

	SPRING	SUMMER	AUTUMN
Macro elements			
Ca	71.16±0.00^{b}	63.36±0.55^{c}	72.53±0.97^{a}
Na	37.08±0.00^{a}	29.61±0.00^{b}	28.69±0.56^{c}
K	19.91±0.59	19.74±0.55	19.02±0.56
P	19.22±0.59^{a}	18.15±0.00^{ab}	16.76±1.12^{b}
Cl^{-}	17.51±0.00^{a}	13.85±0.68^{b}	11.61±0.00^{c}
Mg	11.33±0.00^{a}	10.51±0.00^{c}	10.64±0.00^{b}
S	9.27±0.44^{b}	14.80±2.03^{a}	2.42±0.27^{c}
Trace elements			
Sr	0.50±0.01^{a}	0.43±0.01^{b}	0.40±0.00^{c}
Fe	0.09±0.00^{a}	0.06±0.00^{b}	0.05±0.00^{c}
Cu	0.07±0.00^{b}	0.08±0.00^{a}	0.05±0.00^{c}
Mn	0.01±0.00^{c}	0.06±0.00^{a}	0.05±0.00^{b}

Data is shown as mean ± S.D. Different letters in superscript within the same row indicate significant differences among seasons (ANOVA, $p<0.05$, $n=3$).