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Temporal variation in the fatty acid composition of ovigerous females and embryos of the squat lobster *Pleuroncodes monodon* (Decapoda, Munididae)

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Pleuroncodes monodon, *an important fishery resource and key species in the Humboldt Current Large Marine ecosystem, has a prolonged reproductive period from winter until end of summer, and during this time females incubating their embryos are exposed to seasonal variation in food availability and in temperature. Additionally, in order to ensure successful reproduction and survival of embryos, changes occur in the main internal reserves and/or sources of energy of* P. monodon. *The aim of this study was to determine the extent of seasonal variation (winter* vs *summer) in the lipid content and fatty acid composition of ovigerous females and their embryos. The results show that a higher percentage of saturated and polyunsaturated fatty acids are found in females in winter. Similarly, the composition of fatty acids in embryos found here indicates that winter embryos have more saturated fatty acids and essential fatty acids (C18:2n6cis, C18:3n6 and C22:6n3) than do summer embryos. According to PCA analysis of fatty acid profile, samples from summer may be distinguished into two isolated groups with conspicuous variations in fatty acids profile of embryo and hepatopancreas. While in winter, the opposite pattern occurs in the fatty acid profile of embryo and hepatopancreas. These variations may be related to relevant physiological processes (reproduction and growth) and of their ontogeny (development and survival of offspring). Seasonal variation in the lipid content and composition of fatty acids of* P. monodon *could directly impact this species' reproduction and survival and subsequently could have consequences on the food web and fishery exploitation.*

Keywords: HCLME, crustacean, reproduction, life cycle, food web, energy investment, lipid, fishery

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INTRODUCTION

In marine invertebrates with complex life cycles, such as those composed of a benthic juvenile-adult phase and a prolonged pelagic embryonic/ larval phase (Pechenik, 1999; McEdward, 2000), the environmental conditions experienced by adult individuals can influence reproduction and subsequently the survival and successful development of offspring (Calado & Leal, 2015). For example, in Pleocyemata decapod crustaceans, embryos carried beneath the female's pleon are brooded and cared for maternally until larval hatching occurs (Meusy & Payen, 1988; Fernandez *et al.*, 2006; Hartnoll, 2006). Thus, embryos experience similar environmental conditions to their mothers.

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In temperate coastal environments, temporal variation in environmental conditions (e.g. productivity and/or food availability, temperature, etc.) is common (Medellin-Mora *et al.*, 2016). Because of this, decapod crustaceans in temperate regions must adopt strategies that allow them to invest energy in reproduction to ensure successful survival of their broods (Urzúa *et al.*, 2012; Guzmán *et al.*, 2016). Consequently, decapod crustacean mothers provide recently laid embryos with all of the energy reserves necessary for successful development (Wenner & Kuris, 1990). Additionally, the subsequent use of these energy reserves during the incubation period can be influenced by environmental factors (e.g. temperature: Fischer *et al.*, 2009; García-Guerrero, 2010; oxygen: Taylor & Leelapiyanart, 2001; Alter *et al.*, 2015; salinity: Giménez & Anger, 2001; Taylor & Seneviratna, 2005), and this, in turn, can affect larval biomass at the time of hatching (Andrés *et al.*, 2010; Rotllant *et al.*, 2014).

In decapod crustaceans, lipids are known to be the main source of energy reserves used during starvation (Sánchez-Paz *et al.*, 2006). Fatty acids, which are one component of

lipids, play a fundamental role in the reproductive cycle of decapods (Glencross, 2009). Fatty acids are important in many life phases, from reproductive maturation to vitellogenesis, both requiring large amounts of polyunsaturated fatty acids (Middleditch *et al.*, 1980). Additionally, fatty acids are components of tissues and structures (e.g. cellular membranes, nervous system) of vital importance during ontogeny (Kayama *et al.*, 1980; Bell & Dick, 1990; Beltz *et al.*, 2007). Also, during the initial stages of the life cycle, individuals are often exposed to periods of famine and/or planktonic food shortages, and lipid reserves accumulated internally are crucial for survival and successful growth (Kattner *et al.*, 1994, 2003; Rosa *et al.*, 2007; Urzúa & Anger, 2013).

In crustaceans, the hepatopancreas is the main site of lipid storage and processing (Chang & O'Connor, 1983). This organ is responsible for synthesizing enzymes for food digestion, and also hepatopancreas absorbs and stores large quantities of energy, especially fats (Vogt, 1994). During reproduction and growth, this energy is then transferred to gonads and muscles, respectively (Yamaguchi, 2004; Ying *et al.*, 2006; Fátima *et al.*, 2013). The hepatopancreas is also involved in important physiological processes associated with reproduction, for example vitellogenesis, and the synthesis of vitellogenin and sex hormones (Li *et al.*, 2006).

The model study organism, red squat lobster *Pleuroncodes monodon* from the South-eastern Pacific, reproduces up to four times per year and has an extended reproductive cycle (from winter, throughout spring and until summer) (Thiel *et al.*, 2012; Guzmán *et al.*, 2016). This reproductive cycle is characterized by production of multiple broods or clutches (i.e. eggs laid): 3–4 different broods during an annual cycle with 'larger but few winter eggs' *vs* 'smaller and numerous summer eggs' (Guzmán *et al.*, 2016). Consistently, compared with larvae originating from summer eggs, those hatching from winter eggs show reduced nutritional vulnerability and contain high reserves of energy to face planktonic food limitation (Espinoza *et al.*, 2016). Also, a recent study (Bascur *et al.*, 2017) indicated that seasonal variation in the biochemical composition of the females reflects seasonal differences in reproductive output/investment of this species.

The red squat lobster *Pleuroncodes monodon*, which in the Humboldt Current Large Marine Ecosystem (HCLME) is distributed from Isla Lobos de Afuera in Peru to Ancud in Chile, is a key species and important fishery resource (Yannicelli *et al.*, 2012; Kiko *et al.*, 2015). The HCLME is characterized by spatial-temporal variation in oceanographic phenomena (i.e. upwelling) that is highly seasonal (Thiel *et al.*, 2007; Escribano & Morales, 2012). Consequently, there is temporal variation in temperature and quantity/quality of planktonic food (i.e. phyto- and zooplankton); higher temperatures and greater availability of planktonic food have been registered in spring-summer compared with winter (Daneri *et al.*, 2000; Escribano & Schneider, 2007; Escribano *et al.*, 2012). Of course, this, in turn, directly affects quantity and quality of food consumed by the red squat lobster, as well as the quantity of energy reserves (i.e. fats) that can be stored. In this way, environment has a direct impact on the reproduction and growth of this species. Thus, the aim of this study was to determine the extent of seasonal variation in the primary energy reserves (i.e. lipids and fatty acids) of *P. monodon* females and their embryos. Additionally, the potential implications of female and embryo survival on the HCLME ecosystem are discussed.

MATERIALS AND METHODS

Sampling of squat lobster females from the field

Pleuroncodes monodon females were captured in summer (January–March 2015) and winter (July–September 2015) near Concepción, Chile (35°34′S 72°52′W). The Altaír vessel of Camanchaca Pesca Sur S.A. was used for sampling lobsters from a depth of ~100 m. In both summer and winter, a total of N = 60 ovigerous females per season were selected, measured with a vernier calliper, and transported live to the Hydrobiological Resources laboratory of the Universidad Católica de la Santísima Concepción. Upon arrival, lobsters were immediately frozen at −80 °C until later analyses were performed. Additionally, periodical data of environmental parameters (temperature, chlorophyll and day length) from sites (i.e. fishing grounds) near Concepción, Chile were obtained from the long-term data series of National Marine Fisheries Services of NOAA (www.st.nmfs.noaa.gov).

Collection and preparation of samples for analyses

Embryo masses on the pleopods of adominal area of the ovigerous females were observed using a stereo-microscope (Motic-102 M). Masses were separated according to their initial development characterized by bright orange colouration, using descriptions of Palma & Arana (1997). Then, embryo masses were washed with distilled water for 5 s, dried with filter paper, and transferred to 1.5 ml Eppendorf tubes. Subsequently, hepatopancreases (the main energy storage organ used in reproduction; Nagaraju, 2011) of the same ovigerous females were extracted via dissection using a scalpel; hepatopancreases were stored in 1.5 ml Eppendorf tubes. Embryo and hepatopancreas samples were dried at −80 °C for 48 h in a lyophilizer (Operon, FDU-7012). Then, dry weight was measured using a precision balance (Precisa, model 120A) to the nearest 0.01 mg; the standard method of Anger & Harms (1990) was followed.

Analyses were performed in triplicate (i.e. analytical replicates): three 20 mg of dry weight (DW) hepatopancreas subsamples were taken from each of total female hepatopancreas (total N = 120). These three sub-samples were used for measurements of total lipid and fatty acid. Similarly, three 25 mg (DW) embryo samples (summer 25 mg of embryos was equivalent to ~1666 embryos; winter 25 mg of embryos was equivalent to ~1315 embryos) were separated from total embryo masses of each female (total N = 120). These three sub-samples were used for lipid and fatty acid analyses. For statistical analyses the mean composition per female of the lipid content and fatty acid profile of hepatopancreas and embryo clutch were considered.

Lipid and fatty acid analyses

To obtain the best possible results, 5 ml of a solution of dichloromethane: methanol (2:1) was added to the preweighed embryo and hepatopancreas samples. Samples were incubated in this solution in a 3 l ultrasonic bath (MRC, AC-120H) for 10 min at 6 °C.

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Total lipid content of the hepatopancreases and embryos was quantified gravimetrically after dichloromethane/methanol (2:1) extraction and solvent evaporation using a digital rotary evaporator (DragonLab, model RE100-Pro) at 80 rpm and 45 °C (Folch *et al.*, 1957; Cequier-Sánchez *et al.*, 2008; Urzúa & Anger, 2013). The lipid extract was determined to the nearest 0.01 mg, using a balance (Precisa model 120A) and stored at −20 °C in dichloromethane/methanol (2:1) containing 0.01% butylated hydroxytoluene for further fatty acid composition analyses.

The composition of fatty acids was determined using methods presented by Urzúa & Anger (2011). In short, fatty acid methyl esters (FAMEs) were measured after preparation using the total lipid extracts (total lipids). Total lipid extracts were esterified using methanolic sulphuric acid incubations at 70 °C for 1 h in a Thermo-Shaker (MRC model DBS-001). Then, fatty acids were rinsed using 6 ml of *n*-hexane. Finally, fatty acids were concentrated using a rotary evaporator (DragonLab model RE100-Pro). The measurement of FAMEs was performed using a gas chromatograph (Agilent, model 7890A) at set temperature equipped with a DB-225 column (J&W Scientific, 30 m in length, 0.25 internal diameter, and 0.25 mm film). Using chromatograph software (Agilent ChemStation, USA), individual FAMEs were identified by comparison to known standard fatty acids of marine origin (certificate material, Supelco 37 FAME mix 47885-U; Malzahn *et al.*, 2007; Urzúa & Anger, 2013) and quantified by means of the response factor to internal standard (23:0 FA added prior to transmethylation; Malzahn *et al.*, 2007; Urzúa & Anger, 2011).

Statistical analysis

The statistical analyses were conducted using the software STATISTICA 8 (StatSoft), PRIMER 6 (Plymouth Routines In Multivariate Ecological Research) and FactoMineR program (R package dedicated to multivariate Exploratory Data Analysis) employing standard methods (Sokal & Rohlf, 1995; Zuur *et al.*, 2007; Le *et al.*, 2008) with 95% confidence levels ($P < 0.05$). Additionally, graphics were made using the program SigmaPlot 12 (Systat Software Inc., Chicago, USA). As a first step to compare female size (carapace length, CL) among different seasons, a Student's *t*-test was calculated. Given that no significant differences were found between size of females between summer and winter, comparisons of the energy reserves (lipids and fatty acids) of each season were made. In turn, seasonal variation in environmental parameter were evaluated with a one-way ANOVA. The seasonal variation in the fatty acids composition of female hepatopancreas and embryo were evaluated with a Student's *t*-test. Normality of the data was evaluated using a Kolmogorov–Smirnov test, and homogeneity of the variance was evaluated using Levene's test. Multivariate analyses (i.e. Principal Component Analysis, PCA; Analysis of Similarity, ANOSIM; Similarity Percentage, SIMPER) were performed to compare fatty acids profile of hepatopancreas and embryos between seasons.

RESULTS

Environmental parameters

Environmental parameters displayed seasonal variation. Higher temperatures were observed in summer (13.8 ± 0.2 °C; months 1–3 of the year, January–March) than winter (10.1 ± 0.2 °C; months 7–9 of the year, July–September) (ANOVA, $F_{11,132} = 1209.3$; $P < 0.001$). Meanwhile, chlorophyll also presented a higher average in summer (5.07 ± 0.2 μg l^{-1}) than winter (3.65 ± 0.3 μg l^{-1})

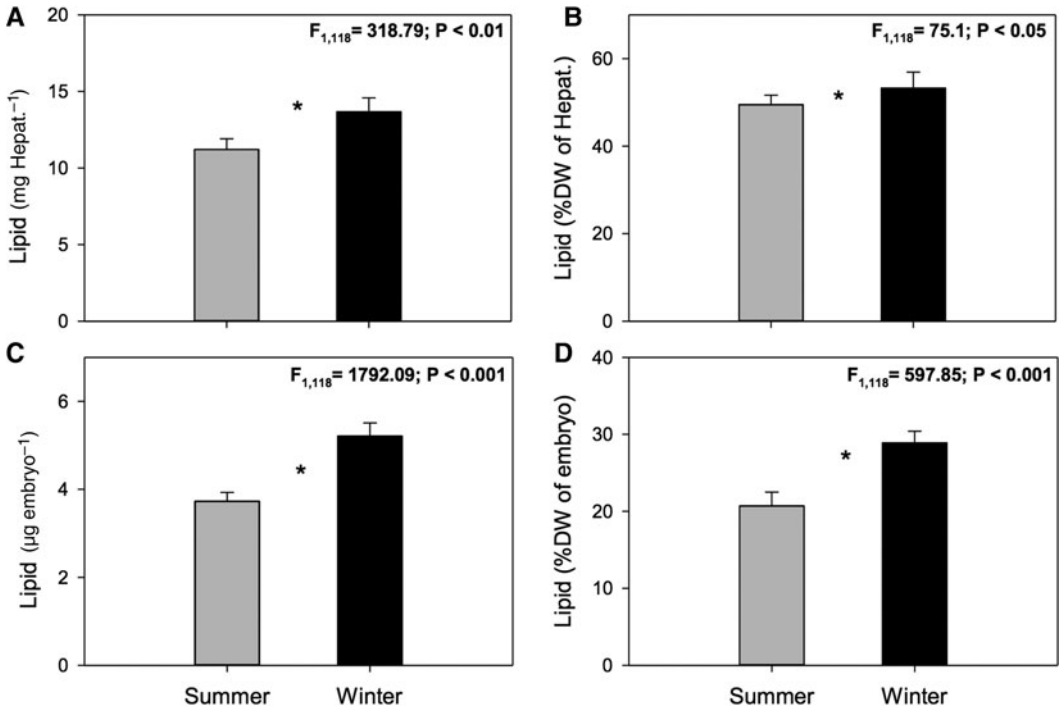

Fig. 1. *Pleuroncodes monodon*. Seasonal variation in: (A) hepatopancreases total lipids (TL) (mg Hepat.$^{-1}$), (B) hepatopancreases total lipids (TL) (%DW), (C) total lipids of embryos (μg embryo^{-1}), (D) total lipids of embryos (%DW); mean values ± S.D. Asterisks indicate significant differences between seasons. In all cases N = 120.

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Table 1. Environmental variables of capture site near Concepción, Chile during 2015.

Season		Summer		Autumn		Winter		Spring	
Variable	**Unit**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Temperature	°C	13.8^{a}	0.31	12^{b}	0.65	10.1^{c}	0.4	11.4^{b}	1.06
Salinity	psu	33.8^{a}	0.03	33.8^{a}	0.08	33.7^{a}	0.02	33.8^{a}	0.04
Chlorophyll a	$\mu g\ l^{-1}$	5.07^{a}	0.31	3.7^{b}	0.7	3.65^{b}	0.51	5.6^{a}	0.62
Day length	h	13.7^{a}	0.8	10.6^{b}	0.4	10.6^{b}	0.76	13.7^{a}	0.8

Temperature (°C), salinity (psu), chlorophyll a ($\mu g\ l^{-1}$) and day length (h) (mean values ± SD) from capture site. Summer = January–March; Autumn = April–June; Winter = July–September; Spring = October–December. In all cases N = 144.
Different lower case letters in a row: significant differences between seasons (ANOVA, $P<0.05$).

(ANOVA, $F_{11,132} = 681.7$; $P < 0.001$) (Figure 1B). Finally, day length was significantly longer (ANOVA, $F_{11,132} = 1044.8$; $P < 0.001$) in summer (13.7 ± 0.8 h) than winter (10.6 ± 0.8 h) (Table 1).

Size of ovigerous females

No significant seasonal differences were found in female carapace length (CL) (summer: 43.38 ± 0.21 mm *vs* winter: 43.84 ± 0.57 mm; ($t_{1,118} = 0.81$; $P = 0.42$).

Seasonal variation in the amount of lipids in hepatopancreases and embryos

Significant seasonal differences were observed in total lipid content (absolute values, mg Hepat.$^{-1}$) of ovigerous female hepatopancreas (ANOVA, $F_{1,118} = 318.79$, $P < 0.01$). Hepatopancreas of females sampled in summer had a lower amount of lipids than hepatopancreas of females sampled in winter (11.21 ± 0.05 mg *vs* 13.68 ± 0.07 mg; summer and winter, respectively) (Figure 1A). Significant differences were also observed in the percentage of lipids (%DW) in the hepatopancreas (summer: 49.47 ± 2.19% *vs* winter: 53.27 ± 3.64%; ANOVA, $F_{1,118} = 75.1$; $P < 0.05$) (Figure 1B).

In turn, highly significant seasonal differences were found in the amount of lipids (absolute values, μg embryo^{-1}) in embryos (ANOVA, $F_{1,118} = 1792.09$, $P < 0.001$). Embryos laid in summer had fewer lipids than embryos laid in winter (3.73 ± 0.2 μg *vs* 5.21 ± 0.3 μg; summer and winter, respectively) (Figure 1C). Similarly, significant seasonal differences were also seen in the percentage of lipids as a function of dry embryo weight (%DW) (ANOVA, $F_{1,118} = 597.85$, $P < 0.001$); embryos laid in summer had a lower percentage of lipids than winter embryos (20.7 ± 1.8% *vs* 28.9 ± 1.5%; summer and winter, respectively) (Figure 1D).

Multivariate analysis of FAs profiles of hepatopancreas and embryos

HEPATOPANCREAS FAS DATA TO COMPARE DIFFERENCES BETWEEN WINTER AND SUMMER

Based on multivariate analysis of PCA, PC1 axis explains 63.8% of seasonal variability and PC2 axis 15.8% (Figure 2). These seasonal tendencies were significant, for example in summer palmitic FA (C16:0) was predominant while in winter stearic FA was (C18:0) (ANOSIM, $R_{ANOSIM} = 0.81$; $P = 0.001$; 999 R permutations). According to SIMPER test, in the summer group the FAs palmitic (C16:0), oleic (C18:1*n*-9) and palmitoleic (C16:1*n*-7) contributed together with 73.83% of variability. In turn, in the winter group the FAs EPA (20:5*n*-3) and stearic (C18:0) contributed with 40.47% of variability (Table 2).

EMBRYOS FAS DATA TO COMPARE DIFFERENCES BETWEEN SUMMER AND WINTER

Considering the data set of embryos FAs, in summer stearic FA (C18:0) was predominant whereas in winter DHA FA was (C22:6*n*-3). 71.5% of this seasonal variability is explained by PC1 axis and 21.3% of the variability by PC2 axis (Figure 3), and according to ANOSIM analysis these trends were significant ($R_{ANOSIM} = 0.56$; $P = 0.001$; 999 R permutations). In turn, results of the SIMPER test indicated that for the summer group the FAs stearic (C18:0) and oleic (C18:1*n*-9) contributed together with 67.45% of cumulative variability, whereas in the winter group the FAs stearic (C18:0) and DHA (C22:6*n*-3) contributed with 56.05% of variability (Table 3).

FAS DATA OF HEPATOPANCREAS AND EMBRYOS ONLY FROM SUMMER

In the comparison between fatty acids of hepatopancreas and embryos only from summer, the PCA plot shows two completely separated groups, with conspicuous variations in fatty acids profile recorded in hepatopancreas and embryos (Figure 4). While PC1 axis explains 90.4% of fatty acid profile variations between 'structures or organs' (i.e. summer

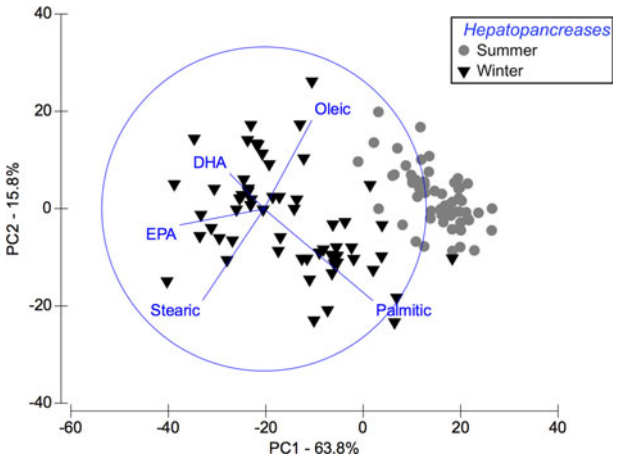

Fig. 2. *Pleuroncodes monodon*. Principal component analysis (PCA) plot of hepatopancreas fatty acids data to compare differences between winter and summer. N = 120.

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Table 2. *Pleuroncodes monodon*. Similarity percentage (SIMPER) analysis used to assess the contribution of each fatty acid (FA) recorded in hepatopancreas of both seasons.

Factor	Similarity	FA	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Summer	76.05	**Palmitic (C16:0)**	**30.86**	**26.64**	**3.83**	**35.03**	**35.03**
		Oleic (C18:1*n*-9)	**22.13**	**18.55**	**3.42**	**24.39**	**59.42**
		Palmitoleic (C16:1*n*-9)	13.16	10.95	3.69	14.40	73.83
		Stearic (C18:0)	5.90	4.86	3.87	6.39	80.21
		Myristic (C14:0)	5.83	4.57	2.76	6.01	86.22
		Gadoleic (C20:1*n*-9)	2.84	2.24	2.42	2.94	89.17
		Heneicosylic (C21:0)	5.54	1.97	0.51	2.59	91.75
Winter	63.71	**EPA (C20:5*n*-3)**	**17.44**	**12.96**	**2.81**	**20.34**	**20.34**
		Stearic (C18:0)	**18.63**	**12.83**	**1.59**	**20.14**	**40.47**
		DHA (C22:6*n*-3)	**10.78**	**7.53**	**1.96**	**11.82**	**52.29**
		Palmitic (C16:0)	14.59	7.49	0.84	11.75	64.05
		Oleic (C18:1*n*-9)	9.95	7.25	1.95	11.39	75.43
		Palmitoleic (C16:1*n*-9)	10.17	6.33	1.21	9.94	85.37
		Myristic (C14:0)	5.87	4.11	2.00	6.45	91.82

Av. Abund, average abundance of each FA present in each season; Av. Sim, the average similarity contributed by the fatty acid; Sim/SD, the ratio of similarity to standard deviation; Contrib%, the contribution to the fatty acid to the overall similarity; Cum.%, additive overall similarity. The fatty acids with the highest contribution in the Principal Component Analysis (PCA) in Figure 2 are marked in bold.

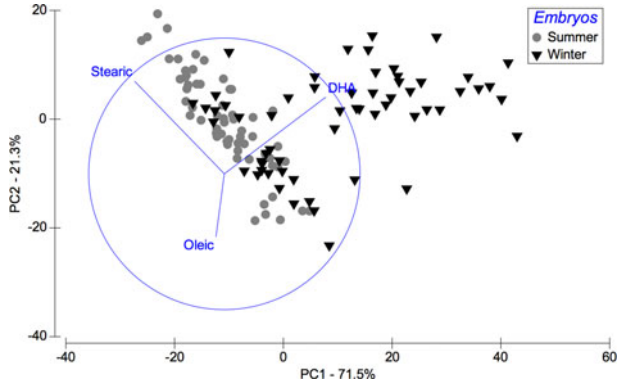

Fig. 3. *Pleuroncodes monodon*. Principal component analysis (PCA) plot of embryos fatty acids data to compare differences between winter and summer. N = 120.

hepatopancreas *vs* summer embryos), PC2 axis explains only 5.8% of this variability (Figure 4). Following the ANOSIM test, this indicated significant differences ($R_{ANOSIM} = 0.99$; $P = 0.001$; 999 R permutations). In turn, SIMPER test showed that in the hepatopancreas group, oleic (C18:1*n*-9) and palmitic (C16:0) fatty acids contributed with 59.42% of this cumulative variability (Table 4), whereas for the embryos group the stearic (C18:0) and oleic (C18:1*n*-9) fatty acids contributed together with 67.45% of this variability (Table 4).

FAS DATA OF HEPATOPANCREAS AND EMBRYOS ONLY FROM WINTER

In PCA analysis of fatty acids of hepatopancreas and embryos only from winter, PC1 axis explained 45.6% of variability between them (i.e. winter hepatopancreas *vs* winter

Table 3. *Pleuroncodes monodon*. Similarity percentage (SIMPER) analysis used to assess the contribution of each fatty acid (FA) recorded in embryos of both seasons.

Factor	Similarity	FA	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Summer	73.93	**Stearic (C18:0)**	**47.55**	**41.51**	**4.96**	**56.16**	**56.16**
		Oleic (C18:1*n*-9)	**12.00**	**8.35**	**1.77**	**11.30**	**67.45**
		Myristic (C14:0)	7.10	5.26	2.25	7.11	74.56
		Palmitoleic (C16:1*n*-9)	9.31	4.91	0.82	6.64	81.20
		Arachidic (C20:0)	5.02	3.82	2.91	5.17	86.37
		Palmitic (C16:0)	6.18	3.76	1.69	5.09	91.46
Winter	64.05	**Stearic (C18:0)**	**33.00**	**27.52**	**3.72**	**42.97**	**42.97**
		DHA (C22:6*n*-3)	**16.94**	**8.38**	**0.74**	**13.08**	**56.05**
		Oleic (C18:1*n*-9)	8.51	5.41	1.34	8.45	64.50
		Palmitoleic (C16:1*n*-9)	6.29	3.51	1.11	5.48	69.98
		Arachidic (C20:0)	2.82	2.11	2.68	3.29	73.27
		Myristic (C14:0)	3.37	2.04	1.40	3.19	76.46
		Behenic (C22:0)	3.33	1.96	1.52	3.06	79.52
		Gadoleic (C20:1*n*-9)	2.51	1.70	2.17	2.65	82.17
		Alpha linolenic (C18:3*n*-3)	1.93	1.41	2.75	2.20	84.37
		DH-G-Linolenic (C20:3*n*-6)	3.19	1.38	0.63	2.15	86.52
		Palmitic (C16:0)	2.12	1.35	2.11	2.11	88.63
		Heptadecanoic (C17:0)	1.63	1.23	2.89	1.91	90.54

Av. Abund, average abundance of each FA present in each season; Av. Sim, the average similarity contributed by the fatty acid; Sim/SD, the ratio of similarity to standard deviation; Contrib%, the contribution to the fatty acid to the overall similarity; Cum.%, additive overall similarity. The fatty acids with the highest contribution in the Principal Component Analysis (PCA) in Figure 3 are marked in bold.

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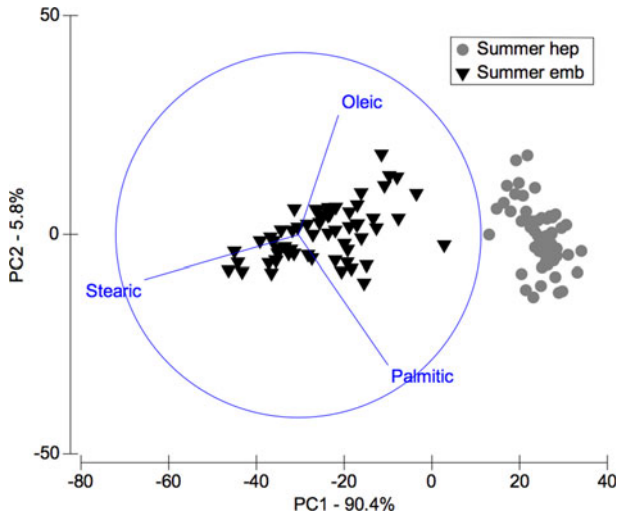

Fig. 4. *Pleuroncodes monodon*. Principal component analysis (PCA) plot of fatty acids data of hepatopancreas (hep) and embryos (emb) only from summer. N = 120.

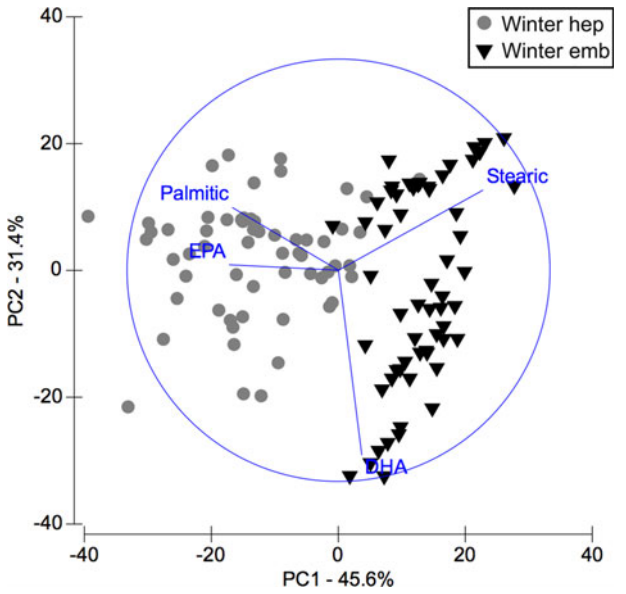

Fig. 5. *Pleuroncodes monodon*. Principal component analysis (PCA) plot of fatty acids data of hepatopancreas (hep) and embryos (emb) only from winter. N = 120.

embryos), while PC2 explains 31.4% of fatty acid variability between 'structures or organs' (Figure 5). According to the analysis of ANOSIM, this showed significant variations in fatty acid profile 'structures or organs' in winter (R_{ANOSIM} = 0.73; P = 0.001; 999 R permutations). In turn, SIMPER test indicated that for hepatopancreas group, the fatty acids EPA (20:5*n*-3), stearic (C18:0), oleic (C18:1*n*-9), DHA (C22:6*n*-3) and palmitic (C16:0) contributed together with 75.43% of variability (Table 5). While for embryo group, stearic acid (C18:0) and DHA (C22:6*n*-3) contributed 56.05% of cumulative variability (Table 5).

Seasonal variation in the composition of hepatopancreas fatty acids

Significant seasonal differences were found in the composition and content of hepatopancreas fatty acids (FA) ($t_{1,118}$ = 12.33, P < 0.001; Table 6). For example, a greater amount of saturated (SFA) and polyunsaturated fatty acids (PUFAs) were found in samples from winter than those from summer; the SFA value for summer samples was 57.69 mg g DW^{-1} while 92.11 mg g DW^{-1} was recorded for winter samples. Correspondingly, the PUFA value for summer samples was 18.51 mg g DW^{-1} while 60.4 mg g DW^{-1} was found for winter samples. Despite this, no significant differences in monounsaturated fatty acids (MUFAs) were found between seasons; values close to 44.6 mg g DW^{-1} were found for summer samples, and values near 44 mg g DW^{-1} were found for winter (Figure 6A).

In relation to the percentage of fatty acids found between seasons, summer SFA and summer MUFA values represented 47.76% and 36.92% of the total hepatopancreas FA, respectively. Winter values were slightly lower with SFA and MUFA representing 46.87 and 22.39% of total FAs, respectively. Conversely, an inverse relationship was found for PUFA; PUFA represented 15.32% of total summer FAs

Table 4. *Pleuroncodes monodon*. Similarity percentage (SIMPER) analysis used to assess the contribution of each fatty acid (FA) recorded in hepatopancreases and embryos of summer.

Factor	Similarity	FA	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Summer hepatopancreases	76.05	**Palmitic (C16:0)**	**30.86**	**26.64**	**3.83**	**35.03**	**35.03**
		Oleic (C18:1*n*-9)	**22.13**	**18.55**	**3.42**	**24.39**	**59.42**
		Palmitoleic (C16:1*n*-9)	13.16	10.95	3.69	14.40	73.83
		Stearic (C18:0)	5.90	4.86	3.87	6.39	80.21
		Myristic (C14:0)	5.83	4.57	2.76	6.01	86.22
		Gadoleic (C20:1*n*-9)	2.84	2.24	2.42	2.94	89.17
		Heneicosylic (C21:0)	5.54	1.97	0.51	2.59	91.75
Summer embryos	73.93	**Stearic (C18:0)**	**47.55**	**41.51**	**4.96**	**56.16**	**56.16**
		Oleic (C18:1*n*-9)	**12.00**	**8.35**	**1.77**	**11.30**	**67.45**
		Myristic (C14:0)	7.10	5.26	2.55	7.11	74.56
		Palmitoleic (C16:1*n*-9)	9.31	4.91	0.82	6.64	81.20
		Arachidic (C20:0)	5.02	3.82	2.91	5.17	86.37
		Palmitic (C16:0)	6.18	3.76	1.69	5.09	91.46

Av. Abund, average abundance of each FA present in each season; Av. Sim, the average similarity contributed by the fatty acid; Sim/SD, the ratio of similarity to standard deviation; Contrib%, the contribution to the fatty acid to the overall similarity; Cum.%, additive overall similarity. The fatty acids with the highest contribution in the Principal Component Analysis (PCA) in Figure 4 are marked in bold.

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Table 5. *Pleuroncodes monodon*. Similarity percentage (SIMPER) analysis used to assess the contribution of each fatty acid (FA) recorded in hepatopancreases and embryos of winter.

Factor	Similarity	FA	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Winter hepatopancreases	63.71	**EPA (C20:5*n*-3)**	**17.44**	**12.96**	**2.81**	**20.34**	**20.34**
		Stearic (C18:0)	**18.63**	**12.83**	**1.59**	**20.14**	**40.47**
		DHA (C22:6*n*-3)	**10.78**	**7.53**	**1.96**	**11.82**	**52.29**
		Palmitic (C16:0)	**14.59**	**7.49**	**0.84**	**11.75**	**64.05**
		Oleic (C18:1*n*-9)	9.95	7.25	1.95	11.39	75.43
		Palmitoleic (C16:1*n*-9)	10.17	6.33	1.21	9.94	85.37
		Myristic (C14:0)	5.87	4.11	2.00	6.45	91.82
Winter embryos	64.05	**Stearic (C18:0)**	**33.00**	**27.52**	**3.72**	**42.79**	**42.97**
		DHA (C22:6*n*-3)	**16.94**	**8.38**	**0.74**	**13.08**	**56.05**
		Oleic (C18:1*n*-9)	8.51	5.41	1.34	8.45	64.50
		Palmitoleic (C16:1*n*-9)	6.29	3.51	1.11	5.48	69.98
		Arachidic (C20:0)	2.82	2.11	2.68	3.29	73.27
		Myristic (C14:0)	3.37	2.04	1.40	3.19	76.46
		Behenic (C22:0)	3.33	1.96	1.52	3.06	79.52
		Gadoleic (C20:1*n*-9)	2.51	1.70	2.17	2.65	82.17
		Alpha Linoleic (C18:3*n*-3)	1.93	1.41	2.75	2.20	84.37
		DH-G-Linolenic (C20:3*n*-6)	3.19	1.38	0.63	2.15	86.52
		Palmitic (C16:0)	2.12	1.35	2.11	2.11	88.63
		Heptadecanoic (C17:0)	1.63	1.23	2.89	1.91	90.54

Av. Abund, average abundance of each FA present in each season; Av. Sim, the average similarity contributed by the fatty acid; Sim/SD, the ratio of similarity to standard deviation; Contrib%, the contribution to the fatty acid to the overall similarity; Cum.%, additive overall similarity. The fatty acids with the highest contribution in the Principal Component Analysis (PCA) in Figure 5 are marked in bold.

while it almost doubled to represent 30.74% of total winter FAs. In this way, the percentage of PUFA in the hepatopancreases of winter females surpassed the percentage of MUFA (~22.39%) (Table 6).

Within the SFAs, palmitic acid (C16:0) and stearic acid (C18:0) were the most abundant components making up 14.76% and 18.32% of total FAs of winter hepatopancreases, respectively. The following fatty acids also varied seasonally: meristic acid (C14:0), stearic acid (C18:0) and arachidic acid (C20:0) ($t_{1,118} = 11.06$, $P < 0.001$). Greater values of these acids were found in winter rather than summer (meristic: 10 *vs* 6 mg g DW^{-1}, stearic: 36 *vs* 6 mg g DW^{-1}, arachidic: 5 *vs* 0.7 mg g DW^{-1}; winter *vs* summer, respectively) (Figure 7A). On the other hand, caprylic (C8:0), tridecylic (C13:0), pentadecylic (C15:0) and heneicosylic (C21:0) fatty acids were only present in hepatopancreases of summer females, and they were present only in small quantities representing only 0.33%, 0.25%, 0.65% and 8.28%, respectively of total FA pool (Table 6).

Regarding the *n*-3 PUFA, significant seasonal differences were only found in certain types of acids such as docosahexaenoic acid (DHA; C22:6*n*-3) and α-linolenic acid (C18:3*n*-3); higher values were found for winter samples than for summer samples (DHA: 18 *vs* 11 mg g DW^{-1}; α-linolenic: 2.7 *vs* 1.1 mg g DW^{-1}, respectively) ($t_{1,118} = 9.76$, $P < 0.001$; Table 6). Moreover, some *n*-3 PUFAs were only found during one season. For example, eicosatrienoic acid (C20:3*n*-3) was only found in summer and was only found in small amounts (3 mg g DW^{-1}) while eicosapentaenoic acid (EPA; C20:5*n*-3) was found in large amounts (31 mg g DW^{-1}) but only in winter samples (Figure 8A).

In turn, the *n*-6 PUFAs such as linoleic acid (C18:2*n*-6c), eicosadienoic acid (C20:2*n*-6) and dihomo-gamma-linolenic (DGLA; C20:3*n*-6) varied seasonally with greater values being registered for winter rather than for summer samples (linoleic acid: 1.4 *vs* 0.6 mg g DW^{-1}, eicosadienoic acid: 1.8 *vs* 1 mg g DW^{-1}, DGLA: 5 *vs* 0.81 mg g DW^{-1}, winter *vs* summer respectively). Significant differences in γ-linolenic acid (C18:3*n*-6) were also found between seasons, but the relationship was opposite that for the previously mentioned *n*-6 PUFAs; a greater amount was found in the hepatopancreases of summer females than in hepatopancreases of winter females (1 *vs* 0.5 mg g DW^{-1}) (Figure 8B). Within the *n*-6 PUFAs, DGLA acid was most abundant and represented 2.54% of total FA pool of winter samples. As a whole, EPA and DHA acids represented 15.78% and 9.16% of total FA pool, respectively (Table 6).

Seasonal variation in the amount of fatty acids in embryos

Highly significant differences were seen in fatty acid profiles of embryos between seasons ($t_{1,118} = 10.27$, $P < 0.001$; Table 7). The content (mg g DW^{-1}) of saturated acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids was consistently higher in winter embryos than in summer embryos (Figure 6B). In turn, the respective values of these types of FAs also varied between seasons. For example, in summer there was a tendency of SFA > MUFA > PUFA with these types of fatty acids representing close to 66.47%, 27.29% and 6.24% of total fatty acids, respectively. On the other hand, in winter the tendency was SFA > PUFA > MUFA with these fatty acids representing close to 40.04%, 33.17% and 26.79% of total FA, respectively (Table 7).

Within the SFAs, stearic acid (C18:0) was the most abundant; 7 mg g DW^{-1} was found in summer embryos while 11 mg g DW^{-1} was found in winter embryos (*t*-test, $t_{1,118} = -7.55$, $P < 0.001$; Table 7) (Figure 7B). However, no significant seasonal differences were detected in myristic (C14:0), palmitic (C16:0) or arachidic (C20:0) SFAs; values for these fatty acids were 1, 0.9 and 0.7 mg g DW^{-1} for

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Table 6. *Pleuroncodes monodon*. Fatty acid (FA) content and profile (given in mg FA g dry weight^{-1} (DW) and mg FA g total lipids^{-1} (TL)) of female hepatopancreases between seasons (summer *vs* winter); mean ± SD.

Fatty acids, FA	mg FA g DW^{-1}				mg FA g TL^{-1}			
	Summer	Winter	*t*-value	*P*-value	Summer	Winter	*t*-value	*P*-value
C8:0	0.4 ± 0.03 (0.33)	0	–	–	0.8 ± 0.1 (0.35)	0	–	–
C12:0	0.5 ± 0.03^{a} (0.41)	0.7 ± 0.3^{a} (0.36)	−1.43	0.32	1 ± 0.1^{a} (0.43)	2 ± 1.3^{a} (0.57)	−1.69	0.11
C13:0	0.3 ± 0.04 (0.25)	0	–	–	0.52 ± 0.1 (0.23)	0	–	–
C14:0	6 ± 0.6^{a} (4.97)	10 ± 1^{b} (5.09)	−6.41	<0.001	11 ± 1^{a} (4.78)	20 ± 4^{b} (5.7)	−4.21	<0.001
C15:0	0.79 ± 0.2 (0.65)	0	–	–	1.4 ± 0.5 (0.61)	0	–	–
C16:0	31 ± 3^{a} (25.66)	29 ± 8^{a} (14.76)	0.66	0.51	58 ± 5^{a} (25.21)	46 ± 13^{a} (13.1)	0.37	0.46
C17:0	1 ± 0.1^{a} (0.83)	2 ± 0.3^{b} (1.02)	−3.14	<0.01	1.4 ± 0.1^{a} (0.61)	4 ± 1^{b} (1.14)	−2.08	<0.01
C18:0	6 ± 0.4^{a} (4.97)	36 ± 6^{b} (18.32)	−10.05	<0.001	11 ± 1^{a} (4.78)	63 ± 13^{b} (17.95)	−8.03	<0.001
C20:0	0.7 ± 0.03^{a} (0.58)	5 ± 1^{b} (2.54)	−3.62	<0.001	1.4 ± 0.1^{a} (0.61)	8 ± 2.1^{b} (2.28)	−3.12	<0.001
C21:0	10 ± 3 (8.28)	0	–	–	19 ± 7 (8.26)	0	–	–
C22:0	1 ± 0.1^{a} (0.83)	2.41 ± 1^{b} (1.23)	−2.90	<0.01	2.25 ± 0.2^{a} (0.98)	4 ± 1.6^{b} (1.14)	−1.21	<0.01
C24:0	0	7 ± 2 (3.56)	–	–	0	14 ± 6 (3.99)	–	–
Total SFA	57.69 ± 2.9^{a} (47.76)	92.11 ± 0.6^{b} (46.87)	−9.45	<0.001	107.77 ± 5.3^{a} (46.84)	161 ± 5.3^{b} (45.86)	−10.54	<0.001
C14:1*n*-5	0.4 ± 0.02^{a} (0.33)	0.4 ± 0.1^{a} (0.2)	−0.21	0.61	0.7 ± 0.1^{a} (0.3)	1 ± 0.3^{a} (0.28)	−0.13	0.47
C16:1*n*-7	13.3 ± 1^{a} (11.01)	19 ± 4^{b} (9.67)	−2.22	<0.01	26 ± 2^{a} (11.3)	34 ± 7^{b} (9.69)	−1.97	<0.05
C18:1*n*-9 (OLA)	22.3 ± 2^{a} (18.46)	17 ± 2^{b} (8.65)	3.12	<0.01	43 ± 3^{a} (18.69)	31 ± 4^{b} (8.83)	3.54	<0.001
C20:1*n*-9	3 ± 0.3^{a} (2.48)	4.6 ± 0.6^{b} (2.34)	−2.41	<0.001	5.1 ± 0.5^{a} (2.22)	8.55 ± 1.2^{b} (2.44)	−2.62	<0.001
C22:1*n*-9	1.3 ± 0.2^{a} (1.08)	2 ± 0.6^{b} (1.02)	−1.32	<0.05	3 ± 0.2^{a} (1.3)	3.5 ± 1^{a} (1)	−1.49	0.31
C24:1*n*-9	4.3 ± 0.7^{a} (3.56)	1 ± 0.4^{b} (0.51)	2.64	<0.001	8.5 ± 1.3^{a} (3.69)	3 ± 1.7^{b} (0.85)	3.14	<0.001
Total MUFA	44.6 ± 4.1^{a} (36.92)	44 ± 4.4^{a} (22.39)	0.28	0.79	86.3 ± 7.2^{a} (37.51)	81.05 ± 8.7^{a} (23.09)	0.97	0.17
C18:2*n*-6c (LOA)	0.6 ± 0.04^{a} (0.5)	1.4 ± 0.4^{b} (0.71)	−1.93	<0.01	1.3 ± 0.1^{a} (0.57)	3 ± 0.5^{b} (0.85)	−2.08	<0.01
C18:3*n*-6	1 ± 0.1^{a} (0.83)	0.5 ± 0.1^{b} (0.25)	2.31	<0.05	2 ± 0.3^{a} (0.87)	1.4 ± 0.3^{a} (0.4)	0.99	0.08
C20:2*n*-6	1 ± 0.2^{a} (0.83)	1.8 ± 0.1^{b} (0.92)	−2.48	<0.01	2 ± 0.6^{a} (0.87)	3.1 ± 0.2^{b} (0.88)	−2.11	<0.05
C20:3*n*-6	0.81 ± 0.1^{a} (0.67)	5 ± 0.6^{b} (2.54)	−4.83	<0.001	1.7 ± 0.1^{a} (0.74)	8.5 ± 1.3^{b} (2.42)	−3.19	<0.001
Total *n*-6 PUFA	3.41 ± 0.1^{a} (2.82)	8.7 ± 0.7^{b} (4.43)	−5.34	<0.001	7 ± 0.2^{a} (3.04)	16 ± 2^{b} (4.56)	−5.87	<0.001
18:3*n*-3	1.1 ± 0.1^{a} (0.91)	2.7 ± 0.6^{b} (1.37)	−3.44	<0.001	2 ± 0.1^{a} (0.87)	5 ± 1^{b} (1.42)	−3.09	<0.001
22:6*n*-3 (DHA)	11 ± 2^{a} (9.11)	18 ± 2.4^{b} (9.16)	−3.68	<0.001	22 ± 0.1^{a} (9.56)	33 ± 5^{b} (9.40)	−4.13	<0.001
20:3*n*-3	3 ± 0.7 (2.48)	0	–	–	5 ± 2 (2.17)	0	–	–
20:5*n*-3 (EPA)	0	31 ± 2.3 (15.78)	–	–	0	55 ± 13 (15.67)	–	–
Total *n*-3 PUFA	15.1 ± 3^{a} (12.5)	51.7 ± 8.2^{b} (26.31)	−10.26	<0.001	29 ± 6^{a} (12.60)	93 ± 15^{b} (26.49)	−11.20	<0.001
Total PUFA	18.51 ± 5.6^{a} (15.32)	60.4 ± 22^{b} (30.74)	−9.21	<0.001	36 ± 9^{a} (15.65)	109 ± 19^{b} (31.05)	−10.31	<0.001
TOTAL FA	120.80 ± 12^{a} (100)	196.51 ± 12^{b} (100)	−10.64	<0.001	230.07 ± 3^{a} (100)	351.05 ± 4^{b} (100)	−12.67	<0.001

Fatty acids are expressed in % of total FA pool for both DW and TL (in parentheses, below). Significantly higher values observed in pairwise comparisons are highlighted as bold numbers.

Different lower case letters in a row: significant differences between seasons (Student's *t*-test, $P < 0.05$). SFA (Saturated FA): sum of 8:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0 and 24:0; MUFA (Monounsaturated FA): sum of 14:1*n*-5, 16:1*n*-7, 18:1*n*-9, 20:1*n*-9, 22:1*n*-9 and 24:1*n*-9; Total *n*-6 PUFA (Polyunsaturated *n*-6 FA): sum of 18:2*n*-6c, 18:3*n*-6, 20:2*n*-6, 20:3*n*-6; Total *n*-3 PUFA (Polyunsaturated *n*-3 FA): sum of 18:3*n*-3, 22:6*n*-3, 20:3*n*-3, 20:5*n*-3; Total PUFA: sum of *n*-3 and *n*-6 PUFA; TOTAL FA: sum of Total SFA, Total MUFA and Total PUFA.

summer samples and 1, 0.6 and 0.9 mg g DW^{-1} for winter samples respectively. Lignoceric (C24:0) SFA was only found in embryos laid in winter; 1.6 mg g DW^{-1} of this fatty acid was found (Table 7).

Seasonal variation was also found in *n*-3 and *n*-6 PUFAs. For example, in the case of the *n*-3 PUFAs, the proportion of DHA (C22:6*n*-3) and α-linolenic acid (C18:3*n*-3) was greater in winter embryos than in summer embryos ($t_{1,118} = 3.47$, $P < 0.01$) (Figure 9A). Within the *n*-3 PUFAs, DHA was consistently the most abundant, representing 22.27% of the total FA pool of winter embryos. Regarding *n*-6 PUFAs, linoleic acid (C18:2*n*-6c) varied seasonally with greater amounts of this acid found in winter. Conversely, other *n*-6 PUFAs, including γ-linolenic (C18:3*n*-6), eicosadienoic (C20:2*n*-6) and DGLA (C20:3*n*-6) PUFAs, were not found in summer embryos (Figure 9B). Overall, combination of these types of *n*-6 FAs represented 3.08%, 0.71% and 4.74% of the total FA pool found in winter embryos, respectively (Table 7).

Significant seasonal variation in MUFAs such as heptadecanoic (C17:1), oleic (C18:1*n*-9), gaddoleic (C20:1*n*-9), erucic (C22:1*n*-9) and nervonic (C24:1*n*-9) acids was detected ($t_{1,118} = 9.84$, $P < 0.001$). Overall, a greater amount of acids was found in winter samples than in summer samples (Figure 10). Within the MUFAs, oleic FA was the most abundant; 3 mg g DW^{-1} was found in winter samples while 1.6 mg g DW^{-1} was found in summer samples (Figure 10).

DISCUSSION

Seasonal variation in the lipid and fatty acid content of females studied here could represent an adaptive response of female squat lobsters to marked seasonal variation in the environmental conditions of their surrounding habitat. During unfavourable and unpredictable periods (i.e. winter: low primary productivity, cold temperature), the composition of stored energy of *P. monodon* females is related to production

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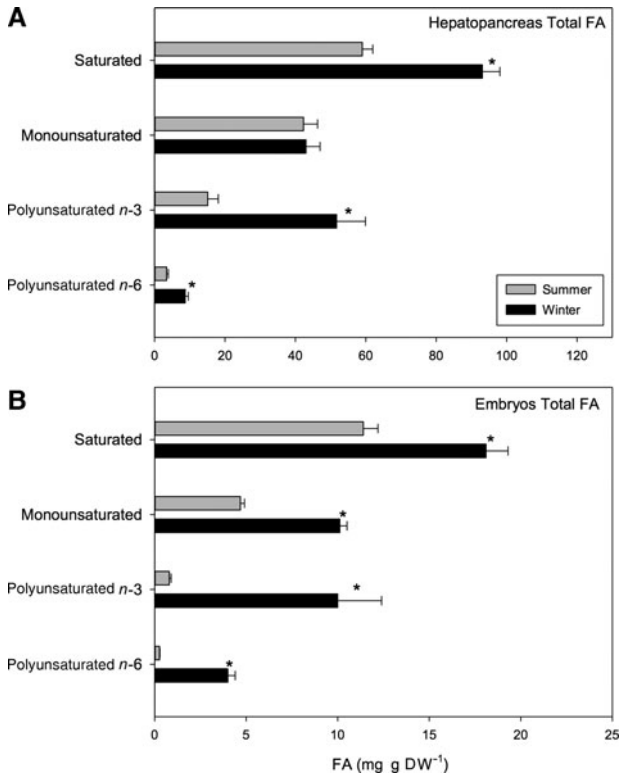

Fig. 6. *Pleuroncodes monodon*. Seasonal variation in: (A) hepatopancreases total fatty acids (mg g DW^{-1}), (B) total fatty acids of embryos (mg g DW^{-1}); mean values ± S.D. Asterisks indicate significant differences between seasons. In both N = 120.

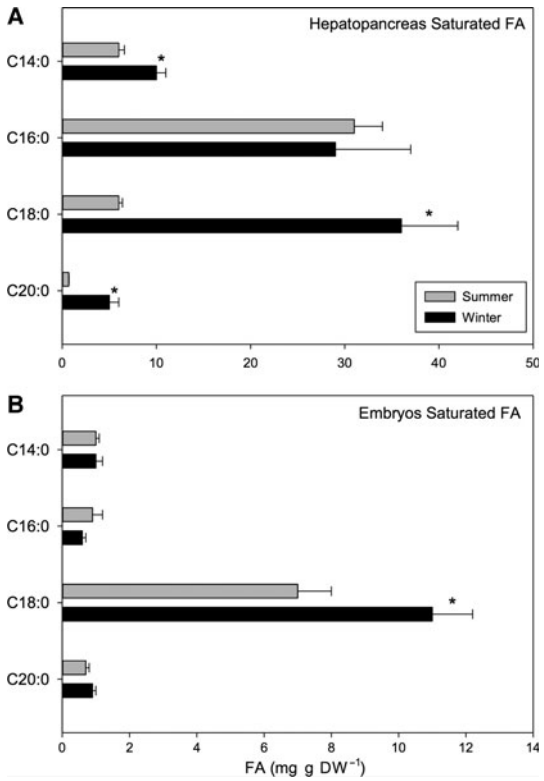

Fig. 7. *Pleuroncodes monodon*. Seasonal variation in: (A) hepatopancreases saturated fatty acids (mg g DW^{-1}), (B) saturated fatty acids of embryos (mg g DW^{-1}); mean values ± S.D. Asterisks indicate significant differences between seasons. In both N = 120.

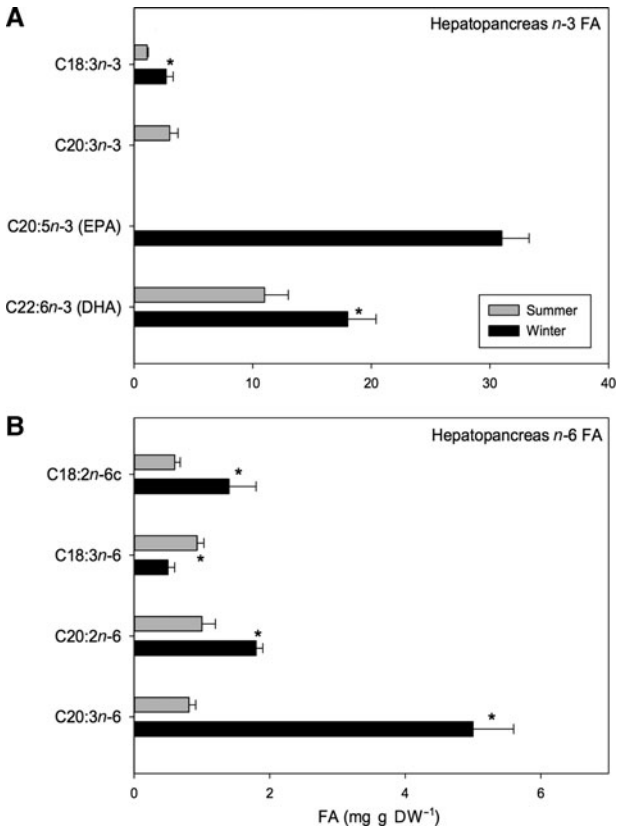

Fig. 8. *Pleuroncodes monodon*. Seasonal variation in: (A) hepatopancreases *n*-3 polyunsaturated fatty acid (mg g DW^{-1}), (B) hepatopancreases *n*-6 polyunsaturated fatty acid (mg g DW^{-1}); mean values ± S.D. Asterisks indicate significant differences between seasons. In both N = 120.

of a single embryo laying; in summer (high primary productivity, warm temperature), energy is likely used for physiological processes of growth (e.g. moulting) and also for the numerous different embryo production events (2–3 embryo laying) during the main reproductive period. In this context, according to PCA, analysis of fatty acid in summer clearly distinguished two isolated groups and with conspicuous variations in fatty acids profile recorded in hepatopancreas and embryos. In winter, the opposite pattern occurs in the fatty acid profile of embryo and hepatopancreas. These seasonal variations in fatty acids profile of *P. monodon* may be related to relevant physiological processes of adult individuals (e.g. for 'winter hepatopancreas' (stearic, palmitic, EPA): reproduction and growth) and also for their ontogeny (e.g. for 'winter embryos' (DHA): successful development and survival of offspring) (Glencross, 2009).

This study reveals that there is a greater content of lipids and saturated fatty acids in female hepatopancreases in winter, which could be closely linked to an extension of reproduction. This is reflected in the production of embryos (1–2 broods) (Thiel *et al.*, 2012; Guzmán *et al.*, 2016) during winter with large amounts of lipids and essential fatty acids (EPA and DHA) (present study). In this context, winter embryos containing high lipid content would be useful for both embryonic development at low temperatures and for larvae hatching (during late winter) when planktonic food availability is poor or unpredictable (low primary productivity).

In turn, lipid content of females in summer is probably related to physiological processes (i.e. moulting and

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Table 7. *Pleuroncodes monodon*. Fatty acid (FA) content and profile (given in mg FA g dry weight^{-1} (DW) and mg FA g total lipids^{-1} (TL)) of initial stage eggs between seasons (summer *vs* winter); mean ± SD.

Fatty acids, FA	mg FA g dry weight^{-1} (DW)				mg FA g total lipids^{-1} (TL)			
	Summer	Winter	*t*-value	*P*-value	Summer	Winter	*t*-value	*P*-value
C12:0	0.3 ± 0.02^{a} (1.75)	0.3 ± 0.01^{a} (0.71)	−1.26	0.21	0.6 ± 0.2^{a} (1.37)	1 ± 0.1^{b} (0.73)	−1.72	<0.05
C13:0	0.4 ± 0.01^{a} (2.33)	0	–	–	0.6 ± 0.05 (1.37)	0	–	–
C14:0	1 ± 0.1^{a} (5.83)	1 ± 0.2^{a} (2.37)	0.10	0.82	2.6 ± 0.4^{a} (5.94)	3.62 ± 0.8^{a} (2.63)	−1.27	0.07
C16:0	0.9 ± 0.3^{a} (5.25)	0.6 ± 0.1^{a} (1.42)	1.08	0.31	2.1 ± 0.5^{a} (4.8)	2.32 ± 0.5^{a} (1.68)	−0.30	0.76
C17:0	0.4 ± 0.1^{a} (2.33)	0.4 ± 0.1^{a} (0.95)	1.37	0.17	1 ± 0.2^{a} (2.28)	1.6 ± 0.3^{b} (1.16)	−2.09	<0.01
C18:0	7 ± 1^{a} (40.82)	11 ± 1.2^{b} (26.06)	−4.16	<0.001	18 ± 2.3^{a} (41.11)	38 ± 4^{b} (27.56)	−9.15	<0.001
C20:0	0.7 ± 0.1^{a} (4.08)	0.9 ± 0.1^{a} (2.13)	−1.20	0.22	2 ± 0.2^{a} (4.57)	3.1 ± 0.3^{b} (2.25)	−2.18	<0.05
C22:0	0.7 ± 0.05^{a} (4.08)	1.1 ± 0.2^{b} (2.61)	−2.37	<0.01	1.7 ± 0.2^{a} (3.88)	3.6 ± 0.6^{b} (2.61)	−2.62	<0.01
C24:0	0	1.6 ± 0.7 (3.79)	–	–	0	4.4 ± 2.1 (3.19)	–	–
Total SFA	11.4 ± 0.8^{a} (66.47)	16.9 ± 0.8^{b} (40.04)	−5.14	<0.001	28.6 ± 2.1^{a} (65.33)	57.64 ± 3.5^{b} (41.81)	−11.36	<0.001
C14:1*n*-5	0.28 ± 0.04^{a} (1.63)	0.3 ± 0.04^{a} (0.71)	−1.16	0.11	0.81 ± 0.1^{a} (1.85)	1.61 ± 0.4^{b} (1.17)	−1.92	<0.05
C15:1	0.3 ± 0.04^{a} (1.75)	0.3 ± 0.05^{a} (0.71)	0.95	0.27	0.8 ± 0.1^{a} (1.83)	1.1 ± 0.2^{a} (0.8)	−0.87	0.22
C16:1*n*-7	1.5 ± 0.3^{a} (8.75)	2.1 ± 0.5^{a} (4.98)	−1.40	0.09	4.2 ± 1.1^{a} (9.59)	6.8 ± 2^{a} (4.93)	−1.23	0.11
C17:1	0.3 ± 0.03^{a} (1.75)	0.5 ± 0.05^{b} (1.18)	−1.98	<0.05	0.7 ± 0.1^{a} (1.60)	1.9 ± 0.2^{b} (1.38)	−2.80	<0.01
C18:1*n*-9 (OLA)	1.6 ± 0.2^{a} (9.33)	3 ± 0.3^{b} (7.11)	−2.21	<0.01	4.18 ± 0.6^{a} (9.55)	9.4 ± 2^{b} (6.82)	−3.51	<0.001
C20:1*n*-9	0.4 ± 0.04^{a} (2.33)	1 ± 0.1^{b} (2.37)	−1.33	<0.05	1 ± 0.1^{a} (2.28)	2.7 ± 0.3^{b} (1.96)	−3.94	<0.001
C22:1*n*-9	0.3 ± 0.02^{a} (1.75)	0.5 ± 0.1^{b} (1.18)	−1.65	<0.05	0.8 ± 0.1^{a} (1.83)	1.7 ± 0.2^{b} (1.23)	−2.57	<0.01
C24:1*n*-9	0	3.61 ± 0.7 (8.55)	–	–	0	11.6 ± 4.7 (8.41)	–	–
Total MUFA	4.68 ± 0.23^{a} (27.29)	11.31 ± 0.8^{b} (26.79)	−6.71	<0.001	12.49 ± 0.6^{a} (28.53)	36.81 ± 2^{b} (26.7)	−10.33	<0.001
C18:2*n*-6c (LOA)	0.27 ± 0.02^{a} (1.57)	0.4 ± 0.05^{b} (0.95)	−1.84	<0.05	0.8 ± 0.03^{a} (1.83)	1.31 ± 0.2^{b} (0.95)	−2.66	<0.05
C18:3*n*-6	0	1.3 ± 0.6 (3.08)	–	–	0	4.4 ± 2.2 (3.19)	–	–
C20:2*n*-6	0	0.3 ± 0.01 (0.71)	–	–	0	0.7 ± 0.04 (0.51)	–	–
C20:3*n*-6	0	2 ± 0.1 (4.74)	–	–	0	6 ± 1 (4.35)	–	–
Total *n*-6 PUFA	0.27 ± 0.02^{a} (1.57)	4 ± 0.4^{b} (9.48)	−5.88	<0.001	0.8 ± 0.03^{a} (1.83)	12.41 ± 1.2^{b} (9)	−9.25	<0.001
18:3*n*-3	0.3 ± 0.1^{a} (1.75)	0.6 ± 0.1^{b} (1.42)	−1.19	<0.05	0.69 ± 0.1^{a} (1.58)	2 ± 0.2^{b} (1.45)	−3.10	<0.01
22:6*n*-3 (DHA)	0.5 ± 0.04^{a} (2.92)	9.4 ± 0.7^{b} (22.27)	−7.41	<0.001	1.2 ± 0.1^{a} (2.74)	29 ± 5^{b} (21.04)	−11.03	<0.001
Total *n*-3 PUFA	0.8 ± 0.1^{a} (4.66)	10 ± 2.4^{b} (23.69)	−8.13	<0.001	1.89 ± 0.3^{a} (4.32)	31 ± 13^{b} (22.49)	−10.71	<0.001
Total PUFA	1.07 ± 0.3^{a} (6.24)	14 ± 3^{b} (33.17)	−10.77	<0.001	2.69 ± 0.6^{a} (6.14)	43.41 ± 9^{b} (31.49)	−11.06	<0.001
TOTAL FA	17.15 ± 3^{a} (100)	42.21 ± 2.5^{b} (100)	−10.15	<0.001	43.78 ± 7.5^{a} (100)	137.86 ± 8.2^{b} (100)	−11.77	<0.001

Fatty acids are expressed in % of total FA pool for both DW and TL (in parentheses, below). Significantly higher values observed in pairwise comparisons are highlighted as bold numbers.

Different lower case letters in a row: significant differences between seasons (Student's *t*-test, $P < 0.05$). SFA (Saturated FA): sum of 12:0, 13:0, 14:0, 16:0, 17:0, 18:0, 20:0, 22:0 and 24:0; MUFA (Monounsaturated FA): sum of 14:1*n*-5, 15:1, 16:1*n*-7, 17:1, 18:1*n*-9, 20:1*n*-9, 22:1*n*-9 and 24:1*n*-9; Total *n*-6 PUFA (Polyunsaturated *n*-6 FA): sum of 18:2*n*-6c, 18:3*n*-6, 20:2*n*-6, 20:3*n*-6; Total *n*-3 PUFA (Polyunsaturated *n*-3 FA): sum of 18:3*n*-3 and 22:6*n*-3; Total PUFA: sum of *n*-3 and *n*-6 PUFA; Total FA: sum of Total SFA, Total MUFA and total PUFA.

reproduction) and with the production of numerous embryos with high amounts of structural fatty acids that would confer rapid growth to the embryo. Some types of structural fatty acids may help crustacean larvae to present a greater degree of flexibility of their jaw that would allow them to capture a wide spectrum of prey in marine environments with strong seasonality of temperature and food availability (see Anger, 2001; Liddy *et al.*, 2005; Pandian, 2016). Similar responses have been described for crustacean species inhabiting coastal areas with marked seasonality of environmental conditions (Kattner *et al.*, 1994, 2007; Calado *et al.*, 2013).

Crustaceans, in general, require a large amount of long-chain polyunsaturated fatty acids (lcPUFA; FA with more than 20 carbons) for various important physiological processes (e.g. synthesis of eicosanoid hormones, neural and immune development). These lcPUFA can be directly supplied to the individual through food consumption (i.e. prey rich in lcPUFA: phyto-zooplankton, Arts *et al.*, 2009) or by enzymatic degradation and/or bio-conversion of short-chain polyunsaturated fatty acids (scPUFA; FA with less than 20 carbons, e.g. linoleic acid (C18:2*n*6cis) and alpha linolenic acid (C18:3*n*6)) to lcPUFA (e.g. docosahexaenoic (DHA; 22:3*n*6) and eicosapentaenoic (EPA; 20:5*n*3)) (Glencross, 2009). Several studies have demonstrated that marine crustaceans (e.g. *Crangon crangon*: Mika *et al.*, 2014) and freshwater crustaceans (e.g. *Macrobrachium rosenbergii*: Reigh & Stickney, 1989) are capable of enzymatically transforming scPUFA into lcPUFA. It is yet unknown whether *Pleuroncodes monodon* is capable of enzymatically bio-converting scPUFA to lcPUFA. Furthermore, more studies are needed to determine the adaptive role of reproducing during periods when food availability is of low quality and quantity.

In agreement with the fatty acid profiles in both seasons for female *Pleuroncodes monodon* hepatopancreas, evaluating fatty acid content and composition can be used to determine the type of food that red squat lobster is eating, and it can also be used to elucidate interspecific interactions within the marine food web (e.g. Connelly *et al.*, 2014; Kiyashko *et al.*, 2014; Legeżyńska

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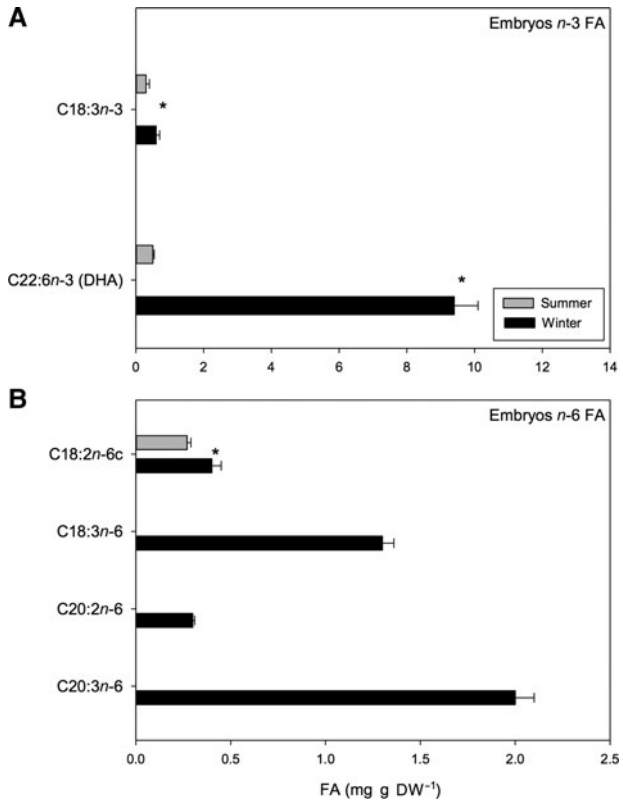

Fig. 9. *Pleuroncodes monodon*. Seasonal variation in: (A) *n*-3 polyunsaturated fatty acids of embryos (mg g DW^{-1}), (B) *n*-6 polyunsaturated fatty acids of embryos (mg g DW^{-1}); mean values ± S.D. Asterisks indicate significant differences between seasons. In both N = 120.

et al., 2014). In this context, presence of SFA, such as C15:0 and C17:0, indicates that females feed off detritus present in sediments (Volkman *et al.*, 1998). The consistent and significant amount of oleic acid (C18:1*n*-9) in hepatopancreases of winter females suggests that *P. monodon* is also a carnivore (Dalsgaard *et al.*, 2003), probably consuming other crustaceans (larvae or pieces of dead adults), polychaetes and molluscs. In turn, large amounts of EPA and DHA (~25% of total FA) present in hepatopancreases of winter females reflect the consumption of phytoplankton and zooplankton that consume primary producers (i.e. micro and macroalgae) (Rosa *et al.*, 2007). We therefore propose that female red squat lobsters are omnivores (generalist trophic niche, for concept see Lovrich & Thiel, 2011); this feeding regime has been described for other squat lobster species (e.g. *Munida quadrispina*: Burd & Brinkhurst, 1984, *Munida gregaria*: Romero *et al.*, 2004, *Munidopsis subsquamosa*: Phleger *et al.*, 2005).

In relation to the seasonal variation observed for fatty acid profiles of red squat lobster embryos, the variation detected can be explained by the differential energy input by mothers to their offspring depending on season (i.e. winter *vs* summer) (Torres *et al.*, 2008). For example, high levels of saturated fatty acids (e.g. stearic acid and myristic acid) and MUFA (e.g. palmitoleic acid and oleic acid) observed in embryos laid in winter would be used during embryogenesis as energy reserves. These would also be the precursors of structural components of cells, membranes and other types of organelles (for examples of decapods see Calado *et al.*, 2007; Figuereido & Narciso, 2008). Overall, this could indicate that embryos laid in winter will later probably hatch into larvae of larger size and with more biomass than embryos that hatch in summer.

Conversely, high levels of essential fatty acids (e.g. docosahexaenoic acid (DHA), alpha linoleic (C18: 3*n*6) and linolenic

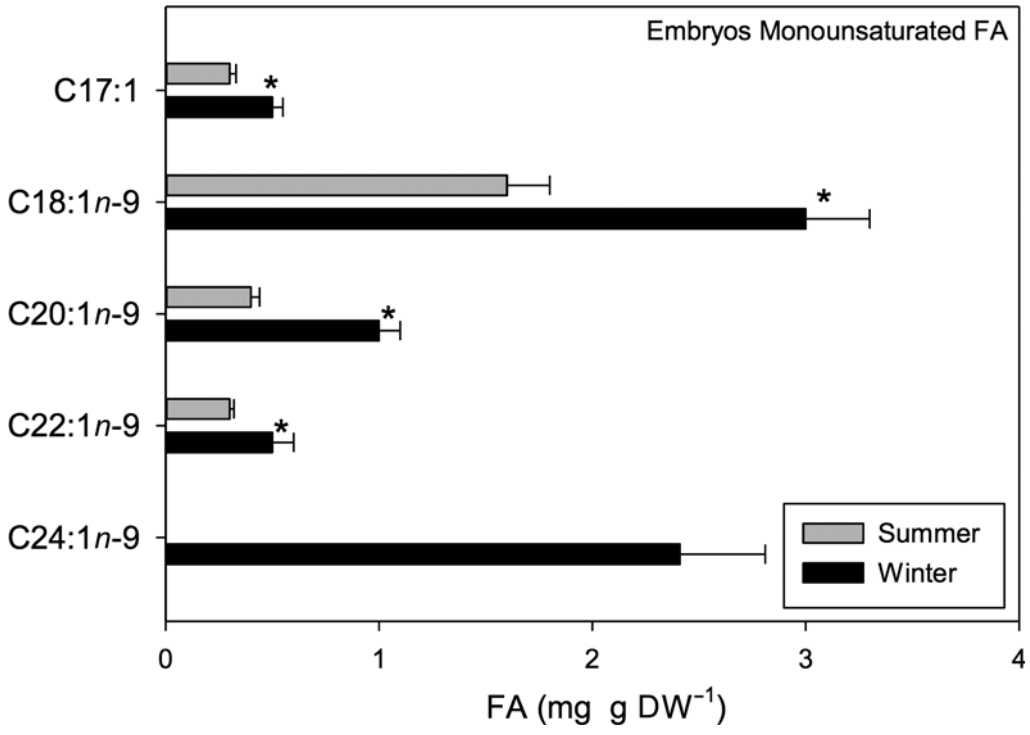

Fig. 10. *Pleuroncodes monodon*. Seasonal variation in monounsaturated fatty acids of embryos (mg g DW^{-1}); mean values ± S.D. Asterisks indicate significant differences between seasons. N = 120.

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acid (C18: 2*n*6cis)) in *P. monodon* embryos are highly related with the development of the nervous and immune systems during embryogenesis (Beltz *et al.*, 2007). Additionally, these types of fatty acids, with a high amount of DHA and only recorded in the winter season (above the EPA), are highly relevant sources of reserve energy during periods of food scarcity (Anger, 2001; Glencross, 2009). In this context, more DHA content in embryos laid in winter would decrease red squat lobster larval nutritional vulnerability at the time of hatching when planktonic food availability is scarce (Espinoza *et al.*, 2016). The newly hatched larvae (without feeding) of *P. monodon* could use this AG type (DHA) as an energy reserve, transferred directly from the mother. However, it is not known if the DHA and other essential fatty acids are self-catabolized by the larvae through alternative metabolic routes (e.g. enzymatic activities).

Pleuroncodes monodon is a key species in the Humboldt Current Large Marine Ecosystem (HCLME) because it plays an important role in the marine food web, and because it is a main resource for demersal crustacean fisheries. In the HCLME, *P. monodon* is a predator of a large variety of species spanning various trophic groups (for example, bacteria, phyto-zooplankton, detritus, polychaetes and amphipods; Gallardo *et al.*, 1994; Roa *et al.*, 1995). *Pleuroncodes monodon* is also prey to a number of fish species including *Genypterus maculatus* (Henríquez & Bahamonde, 1964; Andrade, 1986), *Genypterus chilensis* (Chong *et al.*, 2006), *Genypterus blacodes* (Bahamonde & Zavala, 1981), *Merluccius* spp. (Arancibia & Meléndez, 1987) and *Hippiglossina macrops* (Villarroel & Acuña, 2000). As a general conclusion, given the important role of this species in the trophic web and in fisheries, seasonal variation in lipid content and fatty acid composition of *P. monodon* females and embryos found in this study probably has a direct impact on reproduction, survival and recruitment during later stages of the life cycle (larvae and juveniles). Overall, this could have global consequences on the trophic marine chain and its commercial exploitation.

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REFERENCES

Alter K., Paschke K., Gebauer P., Cumillaf J.P. and Pörtner H.O. (2015) Differential physiological responses to oxygen availability in early life stages of decapods developing in distinct environments. *Marine Biology* 162, 1111–1124.

Andrade H. (1986) Observaciones bioecológicas sobre invertebrados demersales de la zona central de Chile. In Arana P. (ed) *La pesca en Chile*. Chile: Universidad Católica de Valparaíso, Editorial Universitaria, pp. 41–56.

Andrés M., Estévez A., Simeó C.G. and Rotllant G. (2010) Annual variation in the biochemical composition of newly hatched larvae of *Maja brachydactyla* in captivity. *Aquaculture* 310, 99–105.

Anger K. (2001) *The biology of decapod crustacean larvae*. Lisse: A.A. Balkema.

Anger K. and Harms J. (1990) Effects of salinity on the larval development of a semiterrestrial tropical crab, *Sesarma angustipes* (Decapoda: Grapsidae). *Marine Ecology Progress Series* 62, 89–94.

Arancibia H. and Meléndez R. (1987) Alimentación de peces concurrentes en la pesquería de *Pleuroncodes monodon* Milne Edwards. *Investigaciones Pesqueras* 34, 113–128.

Arts M.T., Brett M.T. and Kainz M.J. (2009) *Lipids in aquatic ecosystems*, 1st edition. New York, NY: Springer-Verlag.

Bahamonde N. and Zavala P. (1981) Contenidos gástricos de *Genypterus maculatus* (Tschudi) y *Genypterus blacodes* (Schneider) capturados en Chile entre 31° y 37°S (Teleostomi, Ophidiidae). *Boletín del Museo Nacional de Historia Natural* 38, 53–59.

Bascur M., Guzmán F., Mora S. and Urzúa Á. (2017) Seasonal changes in the biochemical composition of females and offspring of red squat lobster, *Pleuroncodes monodon* (Decapoda, Munididae) from the Southeastern Pacific. *Marine Ecology* 38, e12419. doi: 10.1111/maec.12419.

Bell M.V. and Dick J.R. (1990) The fatty acid composition of phospholipids from the eyes of the northern deepwater prawn *Pandalus borealis*. *Biochemical Society Transactions* 18, 907–908.

Beltz B., Tlusty M., Benton J. and Sandeman D. (2007) Omega-3 fatty acids upregulate adult neurogenesis. *Neuroscience Letters* 415, 154–158.

Burd B.J. and Brinkhurst R.O. (1984) The distribution of the galatheid crab *Munida quadrispina* (Benedict 1902) in relation to oxygen concentrations in British Columbia fjords. *Journal of Experimental Marine Biology and Ecology* 81, 1–20.

Calado R., Dionisio G. and Dinis M.T. (2007) Starvation resistance of early zoeal stages of marine ornamental shrimps *Lysmata* spp. (Decapoda: Hippolytidae) from different habitats. *Journal of Experimental Marine Biology and Ecology* 351, 226–233.

Calado R., Guerao G., Gras N., Cleary D. and Rotllant G. (2013) Contrasting habitats occupied by sibling spider crabs *Maja squinado* and *Maja brachydactyla* (Brachyura, Majidae) can influence the biochemical variability displayed by newly hatched larvae. *Journal of Plankton Research* 35, 684–688.

Calado R. and Leal M.C. (2015) Trophic ecology of benthic marine invertebrates with bi-phasic life cycles: what are we still missing? *Advances in Marine Biology* 71, 1–70.

Cequier-Sánchez E., Rodríguez C., Ravelo A. and Zárate R. (2008) Dichloromethane as a solvent for lipid extraction and assessment of lipid classes and fatty acids from samples of different natures. *Journal of Agricultural and Food Chemistry* 56, 4297–4303.

Chang E.S. and O'Connor J.D. (1983) *Metabolism and transport of carbohydrates and lipids. Internal anatomy and physiological regulation*. New York, NY: Academic Press.

Chong J., Sepúlveda K. and Ibáñez C. (2006) Variación temporal en la dieta del congrio colorado, *Genypterus chilensis* (Guichenot, 1881) frente al litoral de Talcahuano, Chile (36°32′S–36°45′S). *Revista de Biología Marina y Oceanografía* 41, 1–8.

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Connelly T.L., Deibel D. and Parrish C.C. (2014) Trophic interactions in the benthic boundary layer of the Beaufort Sea shelf, Arctic Ocean: combining bulk stable isotope and fatty acid signatures. *Progress in Oceanography* 120, 79–92.

Dalsgaard J., St John M., Kattner G., Muller-Navarra D. and Hagen W. (2003) Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology* 46, 225–340.

Daneri G., Dellarossa V., Quiñones R., Jacob B., Montero P. and Ulloa O. (2000) Primary production and community respiration in the Humboldt Current System off Chile and associated oceanic areas. *Marine Ecology Progress Series* 197, 41–49.

Escribano R., Hidalgo P., Fuentes M. and Donoso K. (2012) Zooplankton time series in the coastal upwelling and coastal transition zones off central-southern Chile (35–40°S). *Progress in Oceanography* 97, 174–186.

Escribano R. and Morales C.E. (2012) Spatial and temporal scales of variability in the coastal upwelling and coastal transition zones off central-southern Chile (35–40°S). *Progress in Oceanography* 92–95, 1–7.

Escribano R. and Schneider W. (2007) The structure and functioning of the coastal upwelling system off central/southern Chile. *Progress in Oceanography* 75, 343–347.

Espinoza E., Guzmán F., Bascur M. and Urzúa A. (2016) Effect of starvation on the nutritional condition of early zoea larvae of the red squat lobster *Pleuroncodes monodon* (Decapoda, Munididae). *Invertebrate Reproduction and Development* 60, 137–144.

Fátima H., Ayub Z., Ali S. and Siddiqui G. (2013) Biochemical composition of the hemolymph, hepatopancreas, ovary, and muscle during ovarian maturation in the penaeid shrimps *Fenneropenaeus merguiensis* and *F. penicillatus* (Crustacea: Decapoda). *Turkish Journal of Zoology* 37, 334–347.

Fernandez M., Calderon R., Cifuentes M. and Pappalardo P. (2006) Brooding behaviour and cost of brooding in small body size brachyuran crabs. *Marine Ecology Progress Series* 309, 213–220.

Figuereido J. and Narciso L. (2008) Egg volume, energy content and fatty acid profile of *Maja brachydactyla* (Crustacea: Brachyura: Majidae) during embryogenesis. *Journal of the Marine Biological Association of the United Kingdom* 88, 1401–1405.

Fischer S., Thatje S., Graeve M., Paschke K. and Kattner G. (2009) Bioenergetics of early life history stages of the brachyuran crab *Cancer setosus* in response to changes in temperature. *Journal of Experimental Marine Biology and Ecology* 374, 160–166.

Folch J., Lees M. and Stanley S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 276, 497–509.

Gallardo V., Cañete J., Roa R., Enríquez-Briones S. and Baltazar M. (1994) Recruitment of squat lobster *Pleuroncodes monodon* of the continental shelf off Central Chile. *Journal of Crustacean Biology* 14, 665–669.

García-Guerrero M. (2010) Effect of temperature on consumption rate of main yolk components during the embryo development of the prawn *Macrobrachium americanum* (Crustacea: Decapoda: Palaemonidae). *Journal of the World Aquaculture Society* 41, 84–92.

Giménez L. and Anger K. (2001) Relationships among salinity, egg size, embryonic development, and larval biomass in the estuarine crab *Chasmagnathus granulata* Dana, 1851. *Journal of Experimental Marine Biology and Ecology* 260, 241–257.

Glencross B.D. (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. *Reviews in Aquaculture* 1, 71–124.

Guzmán F., Olavarría L. and Urzúa Á. (2016) Seasonal variation in reproductive parameters of the squat lobster *Pleuroncodes monodon* from a South Pacific population. *Invertebrate Reproduction and Development* 60, 137–144.

Hartnoll R.G. (2006) Reproductive investment in Brachyura. *Hydrobiologia* 557, 31–40.

Henríquez G. and Bahamonde N. (1964) Análisis cualitativo y cuantitativo del contenido gástrico del congrio negro (*Genypterus maculatus* Tschudi) en pescas realizadas entre San Antonio y Constitución (1961–1962). *Revista Universitaria* 49, 139–158.

Kattner G., Graeve M., Calcagno J.A., Lovrich G.A., Thatje S. and Anger K. (2003) Lipid, fatty acid and protein utilization during lecithotrophic larval development of *Lithodes santolla* (Molina) and *Paralomis granulosa* (Jacquinot). *Journal of Experimental Marine Biology and Ecology* 292, 61–74.

Kattner G., Hagen W., Lee R.F., Campbell R., Deibel D., Falk-Petersen S., Graeve M., Hansen B.W., Hirche H.J., Jonasdottir S.H., Madsen M.L., Mayzaud P., Muller-Navarra D., Nichols P.D., Paffenhofer G.A., Pond D., Saito H., Stubing D. and Virtue P. (2007) Perspectives on marine zooplankton lipids. *Canadian Journal of Fisheries and Aquatic Sciences* 64, 1628–1639.

Kattner G., Wehrtmann I.S. and Merck T. (1994) Interannual variations of lipids and fatty acids during larval development of *Crangon* spp in the German bight, North Sea. *Comparative Biochemistry and Physiology B* 107, 103–110.

Kayama M., Hirata M., Kazanawa A., Tokiwa S. and Saito M. (1980) Essential fatty acids in the diet of prawn-III. Lipid metabolism and fatty acid composition. *Bulletin of the Japanese Society of Scientific Fisheries* 46, 483–488.

Kiko R., Hauss H., Dengler M., Sommer S. and Melzner F. (2015) The squat lobster *Pleuroncodes monodon* tolerates anoxic 'dead zone' conditions off Peru. *Marine Biology* 162, 1913–1921.

Kiyashko S.I., Kharlamenko V.I., Sanamyan K., Alalykina I.L. and Würzberg L. (2014) Trophic structure of the abyssal benthic community in the Sea of Japan inferred from stable isotope and fatty acid analyses. *Marine Ecology Progress Series* 500, 121–137.

Le S., Josse J. and Husson F. (2008) FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software* 25, 1–18.

Legezżyńska J., Kędra M. and Walkusz W. (2014) Identifying trophic relationships within the high Arctic benthic community: how much can fatty acids tell? *Marine Biology* 161, 821–836.

Li K., Chen L.Q., Zhou Z.L., Li E., Zhao X.Q. and Guo H. (2006) The site of vitellogenin synthesis in Chinese mitten-handed crab *Eriocheir sinensis*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 143, 453–458.

Liddy G.C., Kolkovski S., Nelson M.M., Nichols P.D., Phillips B.F. and Maguire G.B. (2005) The effect of PUFA enriched *Artemia* on growth, survival and lipid composition of western rock lobster, *Panulirus cygnus*, phyllosoma. *Aquaculture Nutrition* 11, 375–384.

Lovrich G. and Thiel M. (2011) Ecology, physiology, feeding and trophic role of squat lobster. In Poore G., Ahyong S. and Taylor J. (eds) *The biology of squat lobster*. Collingwood, Australia: CSIRO Publishing, pp. 183–222.

Malzahn A.M., Aberle N., Clemmesen C. and Boersma M. (2007) Nutrient limitation of primary producers affects planktivorous fish condition. *Limnology and Oceanography* 52, 2062–2071.

McEdward L.R. (2000) Adaptive evolution of larvae and life cycles. *Seminars in Cell and Developmental Biology* 11, 403–409.

Medellin-Mora J., Escribano R. and Schneider W. (2016) Community response of zooplankton to oceanographic changes (2002–2012) in the central/southern upwelling system of Chile. *Progress in Oceanography* 142, 17–29.

Meusy J.J. and Payen G.G. (1988) Female reproduction in Malacostracan Crustacea: a review. *Zoological Science* 5, 217–265.

https://doi.org/10.1017/S002531541700145X Published online by Cambridge University Press

Middleditch B.S., Missler S.R., Hines H.B., McVey J.P., Brown A. and Lawrence A.L. (1980) Metabolic profiles of penaeid shrimp: dietary lipids and ovarian maturation. *Journal of Chromatography* 195, 359–368.

Mika A., Skorkowski E. and Stepnowski P. (2014) Effect of seasonal and experimental temperature on de novo synthesis of fatty acids in *C. crangon*. *Bioscience, Biotechnology, and Biochemistry* 78, 1529–1536.

Nagaraju G.P. (2011) Reproductive regulators in decapod crustaceans: an overview. *Journal of Experimental Biology* 214, 3–16.

Palma S. and Arana P. (1997) Aspectos reproductivos del langostino Colorado (*Pleuroncodes monodon* H. Milne Edwards, 1837) frente a la costa de Concepción, Chile. *Investigaciones Marinas* 25, 203–221.

Pandian T.J. (2016) *Reproduction and development in crustacea. Series reproduction and development in aquatic invertebrates*. Boca Raton, FL: CRC Press.

Pechenik J.A. (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series* 177, 269–297.

Phleger CF., Nelson M.M., Groce A.K., Cary S.C., Coyne K.J. and Nichols P.D. (2005) Lipid composition of deep-sea hydrothermal vent tubeworm *Riftia pachyptila*, crabs *Munidopsis subsquamosa* and *Bythograea thermydron*, mussels *Bathymodiolus* sp. and limpets *Lepetodrilus* spp. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 141, 196–210.

Reigh R.C. and Stickney R.R. (1989) Effects of purified dietary fatty acids on the fatty acid composition of freshwater shrimp, *Macrobrachium rosenbergii*. *Aquaculture* 77, 157–174.

Roa R., Gallardo V., Ernst B., Baltazar M., Cañete J. and Enríquez-Briones S. (1995) Nursery ground, age structure and abundance of juvenile squat lobster *Pleuroncodes monodon* on the continental shelf off central Chile. *Marine Ecology Progress Series* 116, 47–54.

Romero M., Lovrich G., Tapella F. and Thatje S. (2004) Feeding ecology of the crab *Munida subrugosa* (Decapoda: Anomura: Galatheidae) in the Beagle Channel, Argentina. *Journal of the Marine Biological Association of the United Kingdom* 84, 359–365.

Rosa R., Calado R., Narciso L. and Nunes M.L. (2007) Embryogenesis of decapod crustaceans with different life history traits, feeding ecologies and habitats: a fatty acid approach. *Marine Biology* 151, 935–947.

Rotllant G., Simeó C.G., Guerao G., Sastre M., Cleary D, Calado R. and Estévez A. (2014) Interannual variability in the biochemical composition of newly hatched larvae of the spider crab *Maja brachydactyla* (Decapoda, Majidae). *Marine Ecology* 35, 298–307.

Sánchez-Paz A., García-Carreño F., Muhlia-Almazán A., Peregrino-Uriarte A.B., Hernández-López J. and Yepiz-Plascencia G. (2006) Usage of energy reserves in crustaceans during starvation: status and future directions. *Insect Biochemistry and Molecular Biology* 36, 241–249.

Sokal R.R. and Rohlf F.J. (1995) *Biometry. The principles and practice of statistics in biological research*, 3rd edition. New York, NY: W.H. Freeman.

Taylor H.H. and Leelapiyanart N. (2001) Oxygen uptake by embryos and ovigerous females of two intertidal crabs, *Heterozius rotundifrons* (Belliidae) and *Cyclograpsus lavauxi* (Grapsidae): scaling and the metabolic costs of reproduction. *Journal of Experimental Biology* 204, 1083–1097.

Taylor H.H. and Seneviratna D. (2005) Ontogeny of salinity tolerance and hyper-osmoregulation by embryos of the intertidal crabs *Hemigrapsus edwardsii* and *Hemigrapsus crenulatus* (Decapoda, Grapsidae): survival of acute hyposaline exposure. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 140, 495–505.

Thiel M., Espinoza-Fuenzalida N., Acuña E. and Rivadeneira M. (2012) Annual brood number and breeding periodicity of squat lobsters (Decapoda: Anomura: Galatheidae) from the continental shelf of the SE Pacific – implications for fisheries management. *Fisheries Research* 129–130, 28–37.

Thiel M., Macaya E.C., Acuña E., Arntz W.E., Bastías H., Brokordt K., Camus P., Castilla J.C., Castro L.R., Cortés M., Dumont C.P., Escribano R., Fernández M., Gajardo J.A., Gaymer C.F., Gomez I., González A.E., González H.E., Haye P.A., Illanes J.E., Iriarte J.L., Lancellotti D.L., Luna-Jorquera G., Luxoro C., Manriquez P.H., Marín V., Muñoz P., Navarrete S.A., Pérez E., Poulin E., Sellanes J., Sepúlveda H.H., Stotz W., Tala F., Thomas A., Vargas C.A., Vásquez J.A. and Vega J.M.A. (2007) The Humboldt Current System of northern and central Chile oceanographic processes, ecological interactions and socioeconomic feedback. *Oceanography and Marine Biology: An Annual Review* 45, 195–344.

Torres P., Penha-Lopes G., Narciso L., Macia A. and Paula J. (2008) Fatty acids dynamics during embryonic development in genus *Uca* (Brachyura: Ocypodidae), from the mangroves of Inhaca Island, Mozambique. *Estuarine, Coastal and Shelf Science* 80, 307–313.

Urzúa Á. and Anger K. (2011) Larval biomass and chemical composition at hatching in two geographically isolated clades of the shrimp *Macrobrachium amazonicum*: intra or interspecific variation? *Invertebrate Reproduction and Development* 55, 236–246.

Urzúa Á. and Anger K. (2013) Seasonal variations in larval biomass and biochemical composition of brown shrimp, *Crangon crangon* (Decapoda, Caridea), at hatching. *Helgoland Marine Research* 67, 267–277.

Urzúa Á., Paschke K., Gebauer P. and Anger K. (2012) Seasonal and interannual variations in size, biomass and chemical composition of the eggs of North Sea shrimp, *Crangon crangon* (Decapoda: Caridea). *Marine Biology* 159, 583–599.

Villarroel J.C. and Acuña E. (2000) Consumption of deep-sea shrimp by bigeye flounder *Hippoglossina macrops* in fishing grounds off northern Chile. *Journal of Fish Biology* 57, 1280–1289.

Vogt G. (1994) Life-cycle and functional cytology of the hepatopancreatic cells of *Astacus astacus* (Crustacea, Decapoda). *Zoomorphology* 114, 83–101.

Volkman J.K., Barrett S.M., Blackburn S.I., Mansour M.P., Sikes E.L. and Gelin F. (1998) Microalgal biomarkers: a review of recent research developments. *Organic Geochemistry* 29, 1163–1179.

Wenner A. and Kuris A. (1990) *Crustacean egg production*. Rotterdam: A.A. Balkema.

Yamaguchi T. (2004) Seasonal changes in the energy content of females of the fiddler crab, *Uca lactea*, especially during the reproductive period. *Crustaceana* 76, 1371–1397.

Yannicelli B., Castro L., Parada C., Schneider W., Colas F. and Donoso D. (2012) Distribution of *Pleuroncodes monodon* larvae over the continental shelf of south-central Chile: field and modeling evidence for partial local retention and transport. *Progress in Oceanography* 92–95, 206–227.

Ying X.P., Yang W.X. and Zhang Y.P. (2006) Comparative studies on fatty acid composition of the ovaries and hepatopancreas at different physiological stages of the Chinese mitten crab. *Aquaculture* 256, 617–623.

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Zuur A.F., Ieno E.N. and Graham S.M. (2007) *Analysing ecological data (statistics for biology and health)*. New York, NY: Springer.

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