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Bioenergetics of early life-history stages of the brachyuran crab *Cancer setosus* in response to changes in temperature

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ABSTRACT

In many marine invertebrates, a latitudinal cline in egg size is considered an adaptive response to a decrease in temperature, and enhances the energetic fitness of their larvae at hatching. However, the amount of energy carried over from the egg to the larval stage depends on the metabolic efficiency of egg development. In the present study, eggs of the brachyuran crab *Cancer setosus* were sampled for their dry mass (*DM*), carbon (*C*), nitrogen (*N*), and fatty acid (*FA*) content throughout development from blastula stage until hatching of zoea 1-larvae at Antofagasta (23°S) and Puerto Montt 41°S (Chile) under different temperature treatments (12, 16 and 19 °C). Hatching zoea 1 larvae contained 60 ± 3% of the initial blastula egg *C* content, regardless of site or temperature. However, the ontogenetic decrease in egg *C* content was to a significantly higher extend based on the utilization of energy-rich *FA* at 12 °C (−1.16 µg/egg) compared to the 19 °C treatments in Antofagasta and Puerto Montt (−0.63 to −0.73 µg *FA* per egg). At 19 °C egg-metabolism was based to a substantial extend on protein, which allowed for the saving of energy-richer lipids. We conclude that the production of larger eggs with high *FA* content appears to be adaptive not only to fuel the larval development, but is also a response to the prolonged egg developmental times at lower temperatures.

1. Introduction

Intraspecific variability in egg energy provision along (latitudinal) temperature clines has been reported for a broad variety of marine invertebrates (Clarke, 1992; Hadfield and Strathmann, 1996). The prevalent pattern, namely the production of larger eggs at higher latitudes (Pandian, 1994; Yampolsky and Schreiner, 1996), is discussed to be an adaptive response to the mismatch of unpredictable food availability and prolonged development faced by the emerging larvae at lower temperatures (Thatje et al., 2005). In the brachyuran crab *Cancer setosus* (Molina, 1782), which spans in distribution from Southern Ecuador to Central Southern Chile (2°S, 079°W–46°S, 075°W) (Rathbun, 1930) energetic investment per egg (measured as dry mass (*DM*), carbon (*C*) and nitrogen content (*N*), volume (*V*)) is negatively correlated to the temperature experienced by the female crab in the time prior egg-laying (Fischer et al., 2009). Eggs produced close to the species lower temperature limit at ~11 °C in Puerto Montt (Central Southern Chile, 41°S) were about one third higher in *DM*, *C*, *N*, and *V* than eggs produced by equal sized females under conditions representative for the species upper temperature range at ~19 °C in Antofagasta (Northern Chile, 23°S) (Fischer et al., 2009). In support of these patterns, *C. setosus* larvae from Antofagasta were successfully reared until their fifth zoea stage at 16 and 20 °C, but failed to successfully complete development at lower temperatures representative for Puerto Montt (12 °C) (Weiss et al., in press). However, to assess the adaptive significance of both latitudinal and intraspecific differences in egg energy provision one has to define to which extend these differences are carried over into later life-history stages. With regard to this, traits of emerging larvae are not exclusively determined by maternal egg energy provision, but can furthermore be constrained by the physico-chemical environment (e.g. salinity, oxygen, temperature) experienced throughout egg incubation period (Kunisch and Anger, 1984; Pandian, 1994; Giménez, 2002, 2006). Egg development is hastened with temperature rise, but often at the cost of an altered metabolic efficiency (Heming, 1982; Pandian, 1994). This energetic cost may lead to a size reduction of the zoea 1 larvae, as shown for the spider crab *Hyas araneus*, the Dungeness crab *Cancer magister*, the caridean shrimps *Nauticaris magellanica*, *Betaeus emarginatus*, and *Pandalus borealis*, which in laboratory cultures hatched significantly smaller at higher temperatures of egg development (Kunisch and Anger, 1984; Shirley et al., 1987; Wehrtmann and Kattner, 1998; Wehrtmann and Lopez, 2003; Brillon et al., 2005). Such pattern has also been supported by field collected larvae of *Nauticaris magellanica* (Thatje and Bacardit, 2000). In the spiny lobster *Jasus edwardsii* and the crayfish *Cherax quadricarinatus* an increase in egg incubation temperature led to a higher consumption of lipids and certain fatty

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acids (mainly 20:5(*n*-3)) (Smith et al., 2002; García-Guerrero et al., 2003). On the other hand, in the American lobster *Homarus americanus* less lipids were utilized with higher egg incubation temperature (Sasaki et al., 1986). Lipids and fatty acids contribute about 20–30% to egg-composition (protein 15–25%, salts 2–5%, remainder water), and frequently form the most important source of metabolic energy in decapod crustacean eggs (Pandian, 1994). Fat oxidization contributes 67, 75, and 88% of the metabolic energy in *Eupagurus bernhardus*, *Crangon crangon* and *Homarus gammarus* eggs, respectively (Pandian and Schumann, 1967; Pandian, 1967, 1970). However, in the egg development of two boreo-arctic cirripede species (*Balanus balanoides*, *B. balanus*) and the deepwater giant crab *Pseudocarcinus gigas* protein was utilized in preference over lipid (Barnes, 1965; Gardner, 2001).

The aims of the present study are to

(i) compare fatty acid (*FA*) content of blastula eggs produced in the field at conditions close to the species upper and lower temperature limit (Antofagasta and Puerto Montt, respectively) and in captivity in Antofagasta
(ii) assess changes in egg traits (*DM*, *C*, *N*, *V*, *FA*) throughout embryogenesis until larvae hatching at both locations
(iii) elucidate the adaptive importance of latitudinal variation in egg traits in biogeography and evolution of marine invertebrates.

2. Materials and methods

2.1. Sampling and maintenance

Ovigerous *C. setosus* were caught by divers at 5–10 m water depth at different sites around Antofagasta, Northern Chile (23°S, 70°W; 11/2005 and 01/2006) and in Carelmapu, close to Puerto Montt in Central Southern Chile (41°S, 73°W, 09/2006 and 11/2006). Females bearing early blastula stage eggs, as identified microscopically by their uniform distribution of yolk and absence of cleavage, were transferred to aquaria at both locations.

Incubation temperatures were chosen to represent the lower and upper range of egg development in normal "non-El Niño" years at both locations: ovigerous females were held in a recirculation system at 16 °C and in a flow through system at 19 °C in Antofagasta and in recirculation systems at temperatures of 12 and 16 °C in Puerto Montt. In Puerto Montt, a third group of ovigerous crabs was transferred to a recirculation aquaria system at 19 °C in order to represent Antofagasta conditions. Salinity ranged between 30 and 34. Crabs were individually labelled with a small plastic tag glued onto their carapace and were fed *ad libitum* with living mussels *Perumytilus purpuratus*. Every 2 days eggs were taken with fine forceps from the border of the egg masses to assess their developmental stage. As described by Fischer and Thatje (2008), *C. setosus* produced several subsequent egg masses in captivity. Three females with eggs laid in captivity in Antofagasta in the flow through aquaria were sampled as ovigerous crabs from the field. Eggs in four successive developmental stages and recently hatched zoea 1 larvae were sampled for elemental analysis (*DM*, *C*, *N*) and eggs in stages I and IV for fatty acid composition (fatty acids were also sampled for stages II and III for eggs produced in captivity in Antofagasta). Samples were kept frozen at −80 °C. Egg and zoea stages were defined as:

I. Blastula — no yolk used; approximately 1 to 2 days after oviposition
II. Gastrula — 25% of the yolk used, still no eyes visible (not sampled in Puerto Montt)
III. Eye-placode — eyes are visible as kidney-shaped small dark spots, but still no chromatophores present and no heartbeat
IV. Pre-hatching — all yolk utilized, eyes completely roundish, chromatophores well developed, heart beats vigorously and embryo moves inside the egg-shell, 1 to 2 days before larvae hatching
V. Zoea 1, collected immediately after hatching in a fine sieve which was connected to the overflow of the aquaria.

To assess if aquaria had an effect on the bioenergetic traits of hatching larvae independent of temperature at both locations 3 females caught in the field with advanced stage IV eggs were transferred to the laboratory and their larvae sampled after a short incubation period.

2.2. Elemental analysis and egg volume

For elemental analysis after Anger and Dawirs (1982) five aliquot samples of 50 eggs or zoea 1 larvae per female were counted under a stereomicroscope, then briefly rinsed in distilled water and subsequently transferred to pre-weighted tin cartridges. Samples were freeze dried overnight at <0.01 mbar using a lyophilizer (Lyovac) and their dry mass (*DM*) was measured with a microbalance (Sartorius M2P) to the nearest µg. Subsequently, samples were combusted at 1020 °C in an elemental analyzer (Hekatech Euro EA) for the determination of *C* and *N* content using acetanilide as a standard. Lengths (D_1) and widths (D_2) of 20 eggs per female were measured with a microscope which was equipped with a calibrated eye-piece micrometer, and their volume was calculated based on the formula for oblate spheroids: $V = (\pi * D_1^2 * D_2)/6$ (Turner and Lawrence, 1979).

2.3. Fatty acid analysis

Fatty acid analysis was based on 200 eggs per sample (stages I and IV) to reach necessary sample size. As internal standard 19:0 methyl ester was added and the samples were crushed by ultrasonification in dichloromethane:methanol (2:1, v:v). Samples were transesterified with 3% concentrated sulphuric acid in methanol for 4 h at 80 °C. After extraction of fatty acid methyl esters with hexane, the fatty acid composition was analyzed with a gas–liquid chromatograph (HP GC6890) equipped with a capillary column (30 m × 0.25 mm (i.d.); liquid phase DB-FAB; film thickness: 0.25 µm) using temperature programming following Kattner and Fricke (1986) (for overview of samples taken see Table 1).

2.4. Data analysis

The net changes in egg traits throughout development, meaning the respective differences in *DM*, *C*, *N*, and *FA* between stages I and IV eggs of the same female, were tested for significant differences between treatments with ANOVA (based on the means of the five

Table 1
C. setosus: overview of ovigerous females sampled throughout egg development (stages: I blastula, II gastrula, III eye-placode and IV pre-hatching) until hatching of the zoea 1 at different temperatures of incubation and number of replicates used for the ANOVAs.

T (°C)	Egg stages I, III, IV *DM*, *C*, *N*, *V*	Egg stages I, IV fatty acids	Zoea 1 larvae *DM*, *C*, *N*
Antofagasta			
16 ± 0.5	3 (+II)		3
19 ± 0.8	3 (+II)	3	3
19 ± 0.8 "captivity"	3 (+II)	3 (+II, III)	3
Puerto Montt			
12 ± 0.3	6	6	3
16 ± 0.5	2		2
19 ± 0.3	3	3	1

Table 2
C. setosus: sampling days after oviposition, elemental composition *DM*, *C*, *N* (μg ind^{-1}), *C*:*N* ratio and volume of eggs (*V*, mm$^3 \times 10^{-4}$) from blastula stage (I) to pre-hatching stage (IV) until zoea 1 larvae hatching (Z1) in Antofagasta and Puerto Montt (arithmetic mean values ± SD); for number of replicates see Table 1.

Stage	days	*DM*	*C*	*N*	*C*:*N*	*V*
Antofagasta 16 °C						
I	~2.0	9.6 ± 0.2	5.1 ± 0.1	0.99 ± 0.02	5.2	176 ± 06
II	15.0 ± 1.7	9.6 ± 0.3	5.0 ± 0.2	1.02 ± 0.04	4.9	194 ± 09
III	22.3 ± 1.2	9.6 ± 0.4	4.9 ± 0.1	0.98 ± 0.03	5.0	226 ± 32
IV	37.0 ± 1.0	8.7 ± 0.2	4.0 ± 0.1	1.02 ± 0.02	4.0	330 ± 08
Z1	38.7 ± 1.5	10.0 ± 0.7	3.1 ± 0.1	0.80 ± 0.03	3.8	
Antofagasta 19 °C						
I	~2.0	10.2 ± 1.5	5.4 ± 0.7	1.13 ± 0.13	4.8	181 ± 18
II	13.0 ± 1.0	10.3 ± 1.2	5.3 ± 0.6	1.04 ± 0.12	5.1	206 ± 23
III	20.3 ± 0.6	10.2 ± 1.4	5.0 ± 0.7	1.07 ± 0.12	4.7	289 ± 03
IV	28.0 ± 1.0	9.9 ± 1.5	4.4 ± 0.7	1.09 ± 0.17	4.0	357 ± 52
Z1	30.0 ± 1.0	9.4 ± 0.4	3.2 ± 0.3	0.77 ± 0.07	4.1	
Antofagasta 19 °C "captivity"						
I	2.0	10.0 ± 0.4	5.3 ± 0.2	1.06 ± 0.06	5.0	174 ± 08
II	14.0 ± 0.0	10.1 ± 0.5	5.2 ± 0.2	1.10 ± 0.07	4.7	214 ± 21
III	20.0 ± 0.0	7.5 ± 0.7	3.8 ± 0.3	0.83 ± 0.09	3.4	197 ± 09
IV	28.0 ± 0.6	7.2 ± 0.7	3.3 ± 0.3	0.79 ± 0.06	3.1	276 ± 12
Z1	30.7 ± 0.6	8.6 ± 0.1	3.0 ± 0.3	0.75 ± 0.03	4.1	
Puerto Montt 12 °C						
I	~2.0	12.0 ± 0.1	6.4 ± 0.4	1.21 ± 0.06	5.3	219 ± 16
III	36.0 ± 5.1	11.6 ± 0.7	6.0 ± 0.4	1.22 ± 0.10	4.9	296 ± 46
IV	59.7 ± 4.3	10.9 ± 0.8	5.0 ± 0.3	1.19 ± 0.08	4.2	437 ± 30
Z1	66.0 ± 0.6	12.4 ± 1.4	4.0 ± 0.4	0.95 ± 0.08	4.2	
Puerto Montt 16 °C						
I	~2.0	13.1 ± 1.1	7.2 ± 0.6	1.37 ± 0.10	5.2	263 ± 16
III	15.0 ± 0.0	13.3 ± 1.1	7.1 ± 0.6	1.41 ± 0.09	5.0	295 ± 57
IV	29.0 ± 1.4	12.1 ± 0.9	5.8 ± 0.6	1.26 ± 0.09	4.6	418 ± 35
Z1	31.5 ± 3.5	11.5 ± 0.2	4.5 ± 0.4	0.95 ± 0.07	4.7	
Puerto Montt 19 °C						
I	~2.0	13.1 ± 1.4	7.1 ± 0.7	1.37 ± 0.13	5.2	251 ± 29
III	15.7 ± 2.5	13.0 ± 1.4	6.9 ± 0.9	1.38 ± 0.16	5.0	291 ± 19
IV	30.3 ± 5.5	12.3 ± 1.3	5.7 ± 0.7	1.28 ± 0.14	4.5	457 ± 90
Z1	26.0	10.4	4.4	1.04	4.2	

parallel *DM*, *C*, and *N* measurements with "female" as sampling level). Homogeneity of variances was tested with Levene's test and normality of residuals with the Shapiro–Wilk test (Sokal and Rohlf, 1995). Differences among treatments after a significant ANOVA were tested with the Tukey HSD test. Consecutive egg stages of females were tested for differences with a paired Student's *t*-test, or when normality was not given, with a Wilcoxon signed rank test.

3. Results

3.1. Elemental composition and volume

Within the first two developmental stages from blastula (I) to gastrula (II), covering about 2 weeks of development, no significant changes in dry mass (*DM*), carbon (*C*), and nitrogen content (*N*) were observed in eggs in Antofagasta (Table 2, data combined for all treatments, paired Student's *t*-test, $\alpha = 0.01$). Therefore, the gastrula stage was not sampled in following experiments in Puerto Montt.

In general, ontogenetic changes in egg *DM*, *C*, and *N* followed a similar temporal pattern between locations and temperature treatments (except for Antofagasta 19 °C "captivity") (Table 2). From the blastula stage (I) to the eye-placode stage (III) only minor changes in *DM* and *C*, and insignificant changes in *N* occurred (combined for all treatments, Wilcoxon signed rank test, $p = 0.812$)(Table 2). Until pre-hatching (IV), *DM* decreased by 0.3–1.1 μg ind^{-1}, *C* decreased by about 20%, while *N* remained quite stable. The net changes in *DM* and *C* from stages I to IV did not differ significantly for the three temperature treatments in Puerto Montt (ANOVA) (Table 3). From pre-hatching to the zoea 1 larvae, *C* again decreased by about 20% and *N* decreased by 20–30% (Table 3, Fig. 1).

The pattern was different for Antofagasta "captivity" produced eggs, where a pronounced decrease in *DM*, *C*, and *N* occurred as early as the eye-placode stage (III). Nevertheless, the hatching zoea 1 larvae were comparable in their quantitative *DM*, *C*, and *N* content to zoea 1 larvae from eggs produced in nature in Antofagasta (incubated at 16 and 19 °C) (Table 2). Throughout development, eggs increased by 60–100% in volume.

3.2. Fatty acid composition

The quantitatively most important fatty acids in the blastula stage were the saturate (SAT) 16:0, the monounsaturates (MUFA) 16:1(*n*-7) and 18:1(*n*-9), and the polyunsaturates (PUFA) 20:5(*n*-3) and 22:6(*n*-3) contributing to about 2/3 of the total of fatty acids (Tables 4 and 5). Despite for palmitic acid (16:0), which was the most abundant fatty acid in all treatments, small differences between fatty acids at both locations were found. In descending order, in Puerto Montt 18:1(*n*-9), 20:5(*n*-3), 16:1(*n*-7) and 22:6(*n*-3) were the quantitatively most prominent fatty acids following 16:0. In "field-produced" eggs in Antofagasta the order was identical with the exception of 20:5(*n*-3) showing higher quantities than 18:1(*n*-9) and for the "captivity eggs" 22:6(*n*-3) was quantitatively the second most important fatty acid. Overall, PUFA were the most abundant fatty acids in blastula eggs (37–40%), followed by MUFA (32–37%) and SAT (25–28%) (Table 5). As indicated by the *C* content, little ontogenetic changes in fatty acid content occurred until the eye-placode stage (III) (see Antofagasta "captivity"; Tables 3 and 4).

From blastula to pre-hatching SAT changed little on the percentage basis, MUFA decreased over-proportionally, and the percentage share of polyunsaturated fatty acids increased. The marked decrease in MUFA is largely explained by a pronounced utilization of 16:1(*n*-7) (decrease of 58–84%), and to a lesser extend of 18:1(*n*-9) and 18:1(*n*-7) (Table 4). Within PUFA, especially 20:5 (*n*-3) and 22:6 (*n*-3) were less utilized than other fatty acids, and thus showed an increase in their relative contribution in the pre-hatching stage (IV) (Table 5).

Fatty acids made up between 13.3 and 15.5% of the blastula egg *DM* and 25.0 to 28.9% of blastula *C* content (Table 6). In the pre-hatching stage (IV) the percentage share of fatty acids dropped to 14.6–15.7% of *C*, except for Puerto Montt (19 °C incubation), where fatty acids still contributed 20.3% to the *C* content. Overall, 60% of the initial fatty acid was consumed throughout development at Puerto Montt at 12 °C, 55 and 48% for "field" and "captivity eggs" in Antofagasta at ~19 °C, respectively, and no more than 35% in Puerto Montt at 19 °C. The

Table 3
C. setosus: percentage changes in elemental composition (*DM*, *C*, *N*), and volume (*V*) during egg development from stage I (blastula) to stage IV (pre-hatching) and from stage I to freshly hatched zoea 1 larvae (Z1) (calculated solely for females with zoea 1 sampled).

T	Stage IV as % of stage I				Z1 as % of stage I		
°C	*DM*	*C*	*N*	*V*	*DM*	*C*	*N*
Antofagasta							
16	90^{A}	78^{ABC}	103^{C}	187	104	60	81
19	96^{BC}	81^{BC}	96^{ABC}	197	92	59	69
19 "captivity"	72^{C}	62^{C}	75^{BC}	158	87	57	70
Puerto Montt							
12	91^{A}	78^{A}	98^{BC}	200	101	61	77
16	92^{A}	82^{AB}	92^{A}	159	87	62	69
19	94^{AB}	81^{AB}	94^{AB}	182	75	58	72

The results of ANOVA, testing for the differences (μg ind^{-1}) between stages I and IV eggs as shown by raised letters. Different letters indicate significant differences (Tukey HSD at $\alpha = 0.05$). For number of replicates see Table 1.

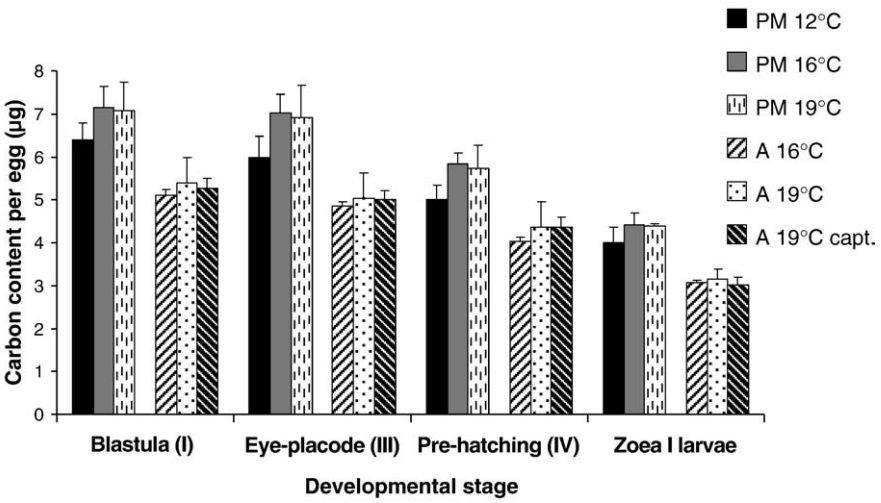

Fig. 1. *C. setosus*. Carbon content throughout egg development and in freshly hatched zoea 1 larvae in Puerto Montt (PM) and Antofagasta (A) at different incubation temperatures. With the exception of "A 19 °C capt." all eggs were produced in the field.

absolute decrease in *FA* was significantly larger at 12 °C than at 19 °C in Puerto Montt and 19 °C in Antofagasta "captivity" (ANOVA).

4. Discussion

4.1. Elemental composition and volume

The temporal sequence of egg energy-utilization by the early life stages of *C. setosus* followed the pattern of low losses in carbon until the beginning of the heartbeat (transition stages III–IV) and an accelerated utilization of egg-nutrients thereafter (Gardner, 2001). The observed increase in egg volume (58–100%) throughout embryogenesis is a consequence of osmotic water uptake and, to a lesser extend of the retention of metabolic water (Pandian, 1970; Amsler and George, 1984; Rosa et al., 2007). Egg *DM* is negatively affected by utilization of energy reserves through respiration and positively, at least for marine Crustacea, by the uptake of salts and minerals (Green, 1965; Pandian, 1967). Because of these two antagonistic processes, which accelerate with development, egg *DM* and energy measures based on *DM* (e.g. fatty acid content/*DM*), are not the most suitable

Table 4
C. setosus: absolute fatty acid composition (µg/200 eggs) in egg stages I–IV in Antofagasta captivity (19 °C) and in stages I and IV for field-produced eggs in Antofagasta (19 °C) and Puerto Montt (12 and 19 °C) ± SD.

Fatty acid	A (capt.) ~19 °C ($n=3$)				A (field) ~19 °C ($n=3$)		PM 19 °C ($n=3$)		PM 12 °C ($n=6$)	
	I	II	III	IV	I	IV	I	IV	I	IV
14:0	3.9 ± 1.0	3.4 ± 0.8	2.5 ± 1.0	1.0 ± 0.1	6.5 ± 1.7	1.0 ± 0.6	6.1 ± 0.9	2.6 ± 0.2	6.4 ± 1.0	0.9 ± 0.3
15:0	3.3 ± 0.7	3.0 ± 0.4	2.3 ± 0.5	1.1 ± 0.1	1.8 ± 0.3	0.5 ± 0.3	3.0 ± 0.9	1.5 ± 0.5	1.8 ± 0.5	0.4 ± 0.1
16:0	48.4 ± 6.0	45.6 ± 2.5	37.3 ± 3.9	21.9 ± 1.1	49.1 ± 11.3	19.0 ± 10.2	59.0 ± 4.4	36.1 ± 5.8	62.3 ± 7.8	21.2 ± 2.7
17:0	3.0 ± 0.5	2.9± 0.3	2.6 ± 0.3	1.8 ± 0.2	1.5 ± 0.1	0.5 ± 0.5	2.4 ± 0.4	2.0 ± 0.7	1.7 ± 0.5	1.0 ± 0.2
18:0	13.8 ± 1.7	14.0 ± 1.1	12.9 ± 1.3	11.6 ± 0.9	15.1 ± 2.2	9.0 ± 1.3	17.4 ± 0.3	15.3 ± 2.8	19.6 ± 6.2	9.3 ± 0.9
20:0	0.5 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	1.1 ± 0.2	1.0 ± 0.2	1.6 ± 0.7	1.6 ± 0.4	1.7 ± 0.6	1.1 ± 0.2
∑ SFA	73.0	69.7	58.5	38.2	75.0	31.0	89.5	59.1	93.5	33.9
16:1(*n*-7)	26.3 ± 6.3	20.6 ± 3.8	14.8 ± 3.2	5.4 ± 1.1	36.7 ± 12.7	7.6 ± 7.0	42.9 ± 10.2	18.2 ± 5.3	35.4 ± 8.9	5.5 ± 1.4
18:1(*n*-9)	30.0 ± 1.1	28.7 ± 0.9	23.4 ± 1.3	13.6 ± 0.7	38.5 ± 11.7	14.7 ± 8.7	51.7 ± 9.5	30.0 ± 6.7	56.3 ± 10.9	17.2 ± 4.6
18:1(*n*-7)	14.4 ± 0.7	14.0 ± 1.0	11.9 ± 1.1	7.7 ± 0.2	17.6 ± 2.1	8.3 ± 3.1	24.1 ± 6.1	16.9 ± 5.3	29.0 ± 2.9	12.1 ± 1.5
20:1(*n*-7)	4.1 ± 0.2	4.0 ± 0.2	3.3 ± 0.2	1.8 ± 0.1	3.5 ± 0.6	1.2 ± 0.7	5.2 ± 1.8	2.3 ± 0.7	6.1 ± 1.5	1.6 ± 0.5
20:1(*n*-9)	8.5 ± 0.3	8.3 ± 0.5	7.2 ± 0.5	3.9 ± 0.3	2.8 ± 0.3	1.2 ± 0.4	3.7 ± 1.4	3.5 ± 0.9	5.0 ± 0.9	1.8 ± 0.8
20:1(*n*-11)	2.7 ± 0.4	2.6 ± 0.2	2.1 ± 0.1	0.9 ± 0.1	3.2 ± 1.7	1.0 ± 1.0	5.5 ± 2.9	1.4 ± 1.2	4.9 ± 2.4	1.0 ± 0.5
22:1(*n*-7)	1.1 ± 0.1	1.1 ± 0.0	0.9 ± 0.2	0.6 ± 0.1	4.0 ± 1.6	2.3 ± 1.6	2.4 ± 0.7	1.7 ± 0.3	4.6 ± 2.6	1.6 ± 1.0
∑ MUFA	87.0	79.3	63.6	33.9	106.3	36.2	135.6	74.0	141.4	40.8
16:2(*n*-4)	0.6 ± 0.3	0.5 ± 0.4	0.4 ± 0.3	0.1 ± 0.0	1.7 ± 1.1	0.2 ± 0.1	1.5 ± 0.1	1.2 ± 1.0	1.2 ± 0.5	0.2 ± 0.0
16:3(*n*-4)	10.4 ± 6.4	10.7 ± 6.7	5.3 ± 5.5	4.0 ± 0.8	4.3 ± 2.7	1.5 ± 1.9	2.9 ± 0.3	1.5 ± 0.4	2.4 ± 0.8	0.5 ± 0.2
18:2(*n*-6)	3.9 ± 0.2	3.7 ± 0.2	2.9 ± 0.2	1.5 ± 0.1	3.0 ± 0.5	1.0 ± 0.4	2.3 ± 0.4	1.2 ± 0.4	4.9 ± 3.2	1.0 ± 1.0
18:3(*n*-3)	2.2 ± 0.3	1.9 ± 0.2	1.4 ± 0.2	0.5 ± 0.0	6.1 ± 1.3	4.7 ± 0.9	6.6 ± 1.4	5.4 ± 1.1	5.9 ± 0.5	4.7 ± 0.5
18:4(*n*-3)	1.0 ± 0.1	0.8 ± 0.1	0.6 ± 0.2	0.2 ± 0.0	1.0 ± 0.2	0.2 ± 0.2	1.2 ± 0.3	0.6 ± 0.3	1.6 ± 0.3	0.3 ± 0.1
20:2(*n*-6)	1.3 ± 0.1	1.3 ± 0.3	1.2 ± 0.2	1.0 ± 0.2	2.3 ± 1.0	1.6 ± 0.9	2.5 ± 0.9	2.3 ± 0.8	3.3 ± 0.5	1.9 ± 0.4
20:4(*n*-6)	11.2 ± 0.8	10.9 ± 0.3	9.3 ± 0.2	7.2 ± 0.5	12.4 ± 3.5	7.0 ± 4.4	9.2 ± 4.3	6.8 ± 3.7	7.3 ± 2.3	3.9 ± 0.9
20:4(*n*-3)	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	1.3 ± 0.1	0.2 ± 0.1	1.8 ± 0.8	0.8 ± 0.3	2.8 ± 1.5	0.4 ± 0.2
20:5(*n*-3)	29.1 ± 3.9	28.6 ± 2.9	25.1 ± 2.5	23.9 ± 2.0	42.5 ± 15.5	23.8 ± 1.9	50.4 ± 14.6	39.4 ± 9.3	54.8 ± 8.8	31.9 ± 4.0
22:5(*n*-3)	5.0 ± 0.8	4.7 ± 0.6	3.9 ± 0.4	2.3 ± 0.4	7.4 ± 4.6	5.5 ± 2.4	16.4 ± 1.8	11.0 ± 3.6	15.0 ± 5.0	7.2 ± 3.2
22:6(*n*-3)	39.5 ± 2.3	37.6 ± 3.0	32.8 ± 1.9	23.4 ± 1.0	21.6 ± 4.2	14.1 ± 2.3	35.7 ± 8.1	29.5 ± 9.9	36.0 ± 7.9	20.0 ± 4.5
∑ PUFA	*105.1*	*101.4*	*83.5*	*64.6*	*103.5*	*59.7*	*130.6*	*99.9*	*135.1*	*71.9*
∑ Total	265.1	250.3	205.5	136.7	284.9	127.0	355.7	232.9	370.0	146.5
% of the total stage I *FA* left in stage IV				51.6		44.6		65.5		39.6

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Table 5
C. setosus: relative fatty acid composition (% of total fatty acids) in egg stages I–IV in Antofagasta captivity (19 °C) and in stages I and IV for field-produced eggs in Antofagasta (19 °C) and Puerto Montt (12 and 19 °C) ± SD.

Fatty acid	A (capt.) ~19 °C ($n=3$)				A (field) ~19 °C ($n=3$)		PM 19 °C ($n=3$)		PM 12 °C ($n=6$)	
	I	II	III	IV	I	IV	I	IV	I	IV
14:0	1.5 ± 0.2	1.3 ± 0.2	1.2 ± 0.3	0.7 ± 0.0	2.3 ± 0.6	0.7 ± 0.1	1.7 ± 0.3	1.1 ± 0.2	1.7 ± 0.1	0.6 ± 0.2
15:0	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	0.8 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	0.8 ± 0.2	0.6 ± 0.2	0.6 ± 0.4	0.3 ± 0.1
16:0	18.3 ± 0.9	18.2 ± 0.4	18.1 ± 0.3	16.1 ± 0.1	17.1 ± 1.7	14.5 ± 2.1	16.6 ± 0.2	15.5 ± 0.4	17.2 ± 0.9	14.5 ± 0.7
17:0	1.1 ± 0.1	1.2 ± 0.0	1.2 ± 0.0	1.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.5	0.7 ± 0.1	0.8 ± 0.2	0.6 ± 0.3	0.7 ± 0.2
18:0	5.2 ± 0.1	5.6 ± 0.1	6.3 ± 0.1	8.5 ± 0.5	5.4 ± 1.0	7.5 ± 1.5	4.9 ± 0.5	6.5 ± 0.1	5.3 ± 1.0	6.4 ± 0.4
20:0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.7 ± 0.0	0.4 ± 0.0	0.8 ± 0.1	0.5 ± 0.2	0.7 ± 0.1	0.4 ± 0.2	0.7 ± 0.1
∑ SFA	27.5	27.8	28.4	28.0	26.4	24.4	25.2	25.4	25.9	23.2
16:1(*n*-7)	9.9 ± 2.3	8.2 ± 1.2	7.2 ± 1.1	4.0 ± 0.6	12.7 ± 2.8	5.3 ± 2.9	12.0 ± 0.3	7.8 ± 1.7	9.5 ± 1.7	3.7 ± 0.8
18:1(*n*-9)	11.4 ± 0.7	11.5 ± 1.0	11.5 ± 1.1	10.0 ± 0.4	13.4 ± 2.5	11.0 ± 2.3	14.5 ± 2.0	12.9 ± 1.8	14.3 ± 2.1	11.6 ± 2.1
18:1(*n*-7)	5.4 ± 0.5	5.6 ± 0.7	5.6 ± 0.6	5.6 ± 0.4	6.2 ± 0.4	6.5 ± 0.3	6.8 ± 1.4	7.2 ± 1.4	7.4 ± 1.4	8.2 ± 0.4
20:1(*n*-11)	1.0 ± 0.1	0.6 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.4	0.7 ± 0.5	1.6 ± 1.0	0.5 ± 0.5	1.4 ± 0.6	0.7 ± 0.4
20:1(*n*-9)	3.2 ± 0.3	2.9 ± 0.2	3.3 ± 0.4	3.5 ± 0.4	1.0 ± 0.2	0.9 ± 0.1	1.0 ± 0.3	1.6 ± 0.7	1.6 ± 0.6	1.2 ± 0.4
20:1(*n*-7)	1.6 ± 0.1	1.8 ± 0.2	1.6 ± 0.2	1.3 ± 0.0	1.2 ± 0.1	0.9 ± 0.2	1.5 ± 0.6	1.0 ± 0.2	1.6 ± 0.4	1.1 ± 0.4
22:1(*n*-7)	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	1.4 ± 0.4	1.7 ± 1.2	0.7 ± 0.2	0.7 ± 0.1	1.1 ± 0.6	1.0 ± 0.6
∑ MUFA	32.9	31.7	31.0	24.8	32.0	27.1	38.1	31.8	36.9	27.7
16:2(*n*-4)	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.0	0.6 ± 0.5	0.2 ± 0.1	0.4 ± 0.1	0.6 ± 0.6	0.3 ± 0.1	0.1 ± 0.0
16:3(*n*-4)	3.9 ± 2.2	4.2 ± 2.5	2.4 ± 2.3	3.0 ± 0.5	1.5 ± 0.7	0.9 ± 0.9	0.8 ± 0.0	0.6 ± 0.1	1.4 ± 1.9	0.3 ± 0.1
18:2(*n*-6)	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	0.8 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	1.4 ± 0.6	0.7 ± 0.6
18:3(*n*-3)	0.8 ± 0.0	0.8 ± 0.1	0.7 ± 0.0	0.4 ± 0.0	2.1 ± 0.1	3.9 ± 0.8	1.8 ± 0.2	2.3 ± 0.1	1.5 ± 0.4	3.3 ± 0.7
18:4(*n*-3)	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.1	0.2 ± 0.1
20:2(*n*-6)	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.8 ± 0.3	1.2 ± 0.2	0.7 ± 0.3	1.0 ± 0.3	0.8 ± 0.2	1.3 ± 0.2
20:4(*n*-6)	4.2 ± 0.2	4.4 ± 0.2	4.5 ± 0.3	5.3 ± 0.2	4.3 ± 0.9	5.2 ± 1.4	2.5 ± 1.1	2.9 ± 1.3	2.3 ± 1.0	2.6 ± 0.4
20:4(*n*-3)	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.2 ± 0.1	0.5 ± 0.3	0.4 ± 0.2	0.7 ± 0.5	0.3 ± 0.1
20:5(*n*-3)	10.9 ± 0.4	11.4 ± 0.7	12.2 ± 0.1	17.5 ± 0.7	15.2 ± 5.6	20.1 ± 5.7	14.3 ± 4.2	17.1 ± 4.0	14.0 ± 2.1	21.8 ± 1.4
22:5(*n*-3)	1.9 ± 0.2	1.9 ± 0.1	1.0 ± 0.1	1.7 ± 0.2	2.5 ± 1.4	4.2 ± 0.7	4.6 ± 0.6	4.8 ± 1.2	3.9 ± 2.1	4.9 ± 2.2
22:6(*n*-3)	14.9 ± 0.9	15.0 ± 1.2	16.0 ± 1.4	17.1 ± 0.6	7.7 ± 2.0	11.7 ± 2.3	10.0 ± 1.6	12.4 ± 2.3	10.4 ± 2.2	13.6 ± 2.4
∑ PUFA	39.6	40.5	40.6	47.2	36.6	48.5	36.7	42.9	37.2	49.1

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

measures of energy content of late stage eggs (Jaeckle, 1995). In this respect, *DM* of recently hatched zoea 1 larvae varied between 75% and 104% of that of early blastula stage eggs, while the *C* content of the zoea 1 larvae was 60 ± 3% of the blastula stage, irrespective of location and incubation temperature (Table 3). Comparably, the zoea 1 of the estuarine crab *Chasmagnathus granulata* hatches with 60–66% of their initial blastula *C* content depending on the salinity conditions encountered throughout embryogenesis (Giménez and Anger, 2001). The decreasing *C*:*N* ratio during egg development indicates that lipids, as in most other Crustacea, were preferably used as energy source over proteins (which are rich in *N*) (Holland, 1978; Clarke et al., 1990; Petersen and Anger, 1997; Giménez and Anger, 2001).

The reason why eggs produced in captivity in Antofagasta showed an earlier and more pronounced decrease in *C* and *N* than eggs of females produced in nature, both incubated parallel under the same conditions, is by no means clear and needs further investigation. Strikingly, larvae hatched with similar *DM*, *C*, and *N* content after the same duration of development (Table 2). Furthermore, zoea 1 larvae that were hatched from advanced stage IV eggs obtained from the field were highly comparable in their *DM*, *C*, and *N* content to larvae from aquaria incubated eggs (Antofagasta: *DM* 9.1 ± 1.2 μg, *C* 3.1 ± 0.1 μg and *N* 0.77 ± 0.03 μg; Puerto Montt: *DM* 11.7 ± 0.8 μg, *C* 4.3 ± 0.3 μg; and *N* 1.05 ± 0.03 μg) (see Table 2).

4.2. Fatty acid composition

MUFA were utilized at a higher rate in *C. setosus* than SAT and PUFA, confirming the previous described pattern for the hippolytidid shrimp *Lysmata seticaudata*, the European lobster *Homarus gammarus*, and the grapsid crab *Armases cinereum* (Morais et al., 2002; Calado et al., 2005; Rosa et al., 2005; Figueiredo et al., 2008b). The 16:1(*n*-7) fatty acid declined particularly strongly (by 79% in Antofagasta; 58 and 84% in Puerto Montt at 19 and 12°) (Fig. 2), as also reported for *Nauticaris magellanica* (Wehrtmann and Kattner, 1998). SAT and MUFA are usually major fatty acids of triacylglycerols which are storage lipids in most organisms. Their decrease during egg development shows clearly the utilization for energetic requirements. In contrast, PUFA are necessary for cell differentiation and thus for the membrane formation during embryogenesis. This is in agreement with the observed quantitative retaining of the *n*-3 PUFA, eicosapentaenoic acid (EPA, 20:5(*n*-3)) and docosahexaenoic acid (DHA, 22:6 (*n*-3)). In addition, the importance of these fatty acids has been attributed to maintaining membrane flexibility (Chapelle, 1986; Morais et al., 2002; Figueiredo et al., 2008a). For copepods a seasonal increase in body DHA content was interpreted as mechanism to maintain membrane fluidity at lower temperatures (Farkas, 1979). In the present study, eggs sampled in the field had higher proportions of EPA than DHA. Eggs produced in Puerto Montt (~11 °C) showed a slightly higher percentage of DHA (10%) than eggs produced under warmer conditions in Antofagasta (~19 °C; 8% DHA). The elevated proportions of EPA and moreover of 16:1(*n*-7) reflect a dietary input based primarily on a diatom source (Dalsgaard et al., 2003). However, eggs produced in captivity at 19 °C had the highest proportion of DHA (15%) and thus do not fit into this latitudinal pattern (Table 5). The

Table 6
C. setosus: Fatty acid content (*FA* μg/egg) and as percentage of *DM* and *C* in egg stages I and IV.

T (°C)	*FA* μg/egg		*FA* as % of *DM*		*FA* as % of *C*	
	I	IV	I	IV	I	IV
Antofagasta						
19 "captivity"	1.33	0.68	13.3	7.1	25.2	15.7
19	1.42	0.63	13.9	6.4	26.5	14.6
Puerto Montt						
19	1.78	1.16	13.6	9.5	25.0	20.3
12	1.85	0.73	15.5	6.7	28.9	14.6

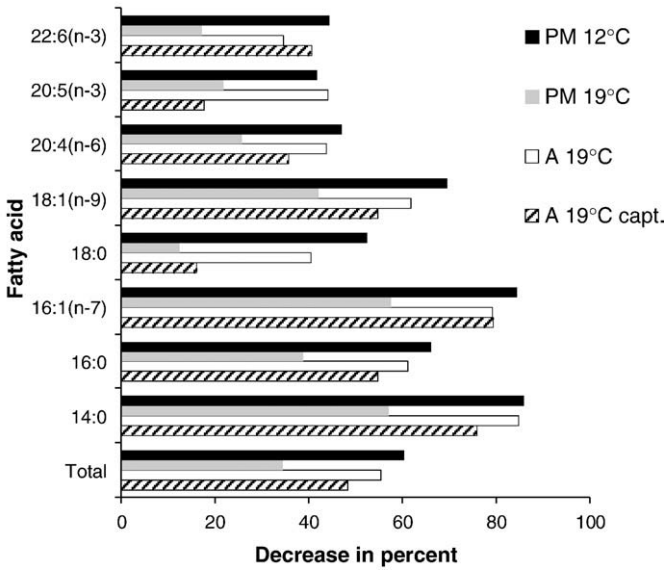

Fig. 2. *C. setosus*. Percentage change in the total of fatty acids and in the most abundant fatty acids from the start (stage I) to the end of the incubation period (stage IV) at 12 and 19 °C in Puerto Montt (PM) and at 19 °C in Antofagasta (A; field and captivity produced eggs). Decrease in percent refers to changes in the absolute mass (µg) per fatty acid.

eggs produced in captivity originated from females that prior to egg-laying, were fed exclusively on *Perumytilus purpuratus*, which may be the source of DHA. These PUFA are essential in Crustacea since they cannot be synthesised *de novo* (Anger, 2001), and thus are a reflection of the female food intake. They are considered an important part of crustacean and finfish-broodstock diet, leading to enhanced egg-quality (Harrison, 1990; Wiegand, 1996; Brooks et al., 1997).

4.3. Adaptive importance of latitudinal variation in egg traits

Although eggs at all three temperatures in Puerto Montt showed a similar decrease of 1.4 µg carbon from blastula to pre-hatching (Table 4; Fig. 1), almost twice as much fatty acids were utilized at 12 °C (−1.15 µg/egg) compared to 19 °C (−0.62 µg/egg) (Table 6). Since lipids composed of fatty acids make up about 80% of the total lipid fraction, the remaining decrease in carbon content must largely be based on utilization of proteins. On a mass-basis protein contains about 40% less energy than lipids. The utilization of protein may lead to a saving of the energetically richer lipids.

The most pronounced utilization of fatty acids took place at 12 °C in Puerto Montt (−60%), which is a typical temperature of egg incubation for this location close to the species southern distributional range (Fischer and Thatje, 2008). Blastula eggs at Puerto Montt at 12 °C were provided by 30% more fatty acids as blastula eggs produced in Antofagasta at 19 °C. This advantage is largely compensated by the prolonged larval development at the lower temperature. However, when exposed to the higher Antofagasta temperatures (19 °C), eggs from Puerto Montt maintain their higher initial investment in fatty acids throughout development.

This clearly indicates an increased conversion efficiency of embryos incubated at higher temperatures in *C. setosus*, as reported also for the American lobster *Homarus americanus* (Sasaki et al., 1986). In this respect, at least for *C. setosus* the production of larger, energy-richer eggs at the colder location, is not solely an adaptation to the prolonged development faced by the hatching larvae (Weiss et al., in press), but also a necessity to meet the energetic demands of the prolonged egg development.

5. Conclusions

C. setosus produces larger, energy-richer eggs at lower temperatures. This higher investment in the single offspring appears to be an energetic necessity due to the reduced metabolic efficiency of egg development at low temperatures and thus is only partly carried over to later larval stages.

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