

Differential reproductive investment in females of *Lithodes santolla* (Decapoda: Anomura) from different regions of southern South America

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Summary: The southern king crab *Lithodes santolla* experiences low temperatures and unpredictable food conditions, and is under variable fishing pressure. To compare the reproductive investment of females, crabs were captured from three sites (Beagle Channel 55°S, Tierra del Fuego Atlantic coast 52-54°S and San Jorge Gulf 45-47°S). Fecundity, water and lipid contents were measured in midgut gland, ovary and muscle. To assess the maternal investment in the offspring, egg masses were analysed on lipid, protein, water and inorganic matter contents, egg volume and embryo size. Although fecundity was similar among sites, San Jorge Gulf females showed higher water and lower lipid contents in somatic and reproductive organs. The egg mass energy reserves varied among sites, showing the highest lipid values in the Beagle Channel, and the highest protein and inorganic matter content in San Jorge Gulf. Though water content was similar, egg volume and embryo size were higher in San Jorge Gulf. Moreover, maternal size did not correlate with egg volume, embryo size or lipid, protein and inorganic matter content of the eggs. This study suggests that reproductive investment of *L. santolla* varies among regions exposed to different environmental conditions, such as the quality of food available, denoting a female compensatory mechanism.

Keywords: southern king crab; Lithodidae; energy reserves; maternal investment; energy allocation; lecithotrophy.

Inversión reproductiva diferencial en hembras de *Lithodes santolla* (Decapoda: Anomura) en distintas áreas del sur de Sudamérica

Resumen: La centolla, *Lithodes santolla*, experimenta bajas temperaturas, condiciones de alimentación impredecibles y distintas presiones pesqueras. Para comparar la inversión reproductiva de las hembras, se capturaron centollas de tres sitios (Canal Beagle 55°S, Costa Atlántica de Tierra del Fuego 52-54°S y Golfo San Jorge 45-47°S). Se midió la fecundidad, y el contenido de agua y lípidos en la glándula digestiva, ovario y músculo. Para comparar el aporte materno a la descendencia, se determinó el contenido de lípidos, proteínas, agua y materia inorgánica en la masa de huevos, el volumen del huevo y el tamaño del embrión. Aunque la fecundidad fue similar entre sitios, las hembras del Golfo San Jorge mostraron mayor contenido de agua y lípidos en los órganos somáticos y reproductivos. Las reservas energéticas de la masa de huevos variaron entre sitios, mostrando los mayores valores de lípidos en Canal Beagle y de proteínas y materia inorgánica en el Golfo San Jorge. Aunque el contenido de agua en el huevo fue similar, su volumen y el tamaño del embrión fueron mayores en el Golfo San Jorge. Además, el tamaño materno no se correlacionó con el volumen del huevo, el tamaño del embrión, ni con el contenido de lípidos, proteínas y materia inorgánica de los huevos. Este estudio sugiere que la inversión reproductiva de *L. santolla* varía entre regiones expuestas a diferentes condiciones ambientales, como la calidad de alimentos disponibles, lo cual denota un mecanismo compensatorio en las hembras.

Palabras clave: centolla; Lithodidae; reservas energéticas; aporte materno; translocación de energía; lecitotrofia.

Citation/Como citar este artículo: Díaz-Arce N., Di Salvatore P., Gowland-Sainz M.F., Lovrich G.A. 2019. Differential reproductive investment in females of *Lithodes santolla* (Decapoda: Anomura) from different regions of southern South America. Sci. Mar. 83(4): 327-336. <https://doi.org/10.3989/scimar.04974.31A>

Editor: Ch. Zeng.

Received: July 3, 2019. **Accepted:** October 24, 2019. **Published:** November 13, 2019.

INTRODUCTION

The southern king crab, *Lithodes santolla*, is a lithodid crab inhabiting cold waters of southern South America. It is distributed in both the Pacific and Atlantic oceans, including fjords and channels, the Magellan Strait and the Beagle Channel (Lovrich and Tapella 2014, Stevens and Lovrich 2014). In the Atlantic Ocean, *L. santolla* occurs in the shallow waters around Isla de los Estados (54°S) and San Jorge Gulf (45-47°S). In the Atlantic coastal waters near the eastern entrance to the Magellan Strait, another species occurs, *Lithodes confundens*, which is morphologically very similar to *L. santolla*, hence its name. Current genetic studies indicate that *L. confundens* and *L. santolla* may be the same species (Pérez-Barros et al. 2015). The mating season occurs from December to January in the Beagle Channel and is approximately one month earlier in San Jorge Gulf (Vinuesa 1985, Gowland-Sainz 2018).

Fishing for *L. santolla* and other lithodid crabs in southern South America has been carried out in Chile and Argentina since the 1920s and 1930s, respectively (Lovrich and Tapella 2014). In Argentina, the fishery has been historically located in the Beagle Channel and has been conducted on an artisanal scale (Lovrich and Tapella 2014). In the San Jorge Gulf, fishing of *L. santolla* started during the 1990s as a bycatch of other trawling fisheries (the shrimp *Pleoticus muelleri* and the hake *Merluccius hubbsi*), but is now done by an industrial fleet using baited traps (Wyngaard et al. 2016). This fishery over the southern continental shelf is rapidly expanding southwards, with catches occurring near the eastern entrance to the Magellan Strait (Mauna et al. 2017). An exploratory fishery has found areas of high yields, and it is believed that it may be an alternative ground that could help to lower the fishing effort in the San Jorge Gulf (Firpo et al. 2018). Fisheries of *L. santolla* and other king crabs are regulated with the so-called “3S” rule: sex, size, and season (Lovrich and Tapella 2014). This regulation specifies that landed animals are restricted to males larger than a legal size captured during a fishing seasons of variable duration, according to the location and jurisdiction.

The way organisms assign energy between growth, maintenance and reproduction determines their life histories. The amount of energy devoted to reproduction, and its use, may finally determine an organism's success in establishing offspring in future generations (Stancyk 1981). The production of eggs requires an optimal allocation of energy into growth and reproduction for the maximization of parental fitness (Ramirez Llodra 2002). The production of large yolky eggs is typical in animals that develop through lecithotrophic larvae (e.g. Thatje et al. 2003), in which the energy invested in the egg represents the total nutrient reserve that will sustain the larva until metamorphosis occurs

(Ramirez Llodra 2002). Thus, growth and development of these larvae are independent of an external food source and they are mainly nurtured from a large yolky mass (Thorson 1950) provided by the mother. This strategy has been understood as an adaptation to sub-Antarctic regions, low temperatures and food-limited conditions with short periods of primary production (Lovrich et al. 2003, Hall and Thatje 2009, 2011).

In crustaceans the egg is provided with large quantities of food supply as yolk, which is used as an energy source and for organ formation; the quantity and quality of the yolk vary significantly among different groups (Babu 1987). Egg traits can also vary intraspecifically within a particular “reaction norm”, which is a life-history character in itself (Ricklefs and Wikelski 2002). This phenotypic plasticity probably allows the reproductive effort to be optimized under different environmental conditions (Thatje and Bacardit 2000, Allen et al. 2007, Fischer et al. 2009). As proteins have a lower energy value than lipids (Ansell 1974, Cho et al. 1982, Beninger and Lucas 1984), their quantification in eggs reveals the energetic reserves of the mothers procured during the vitellogenesis process (Racotta et al. 2003). As larvae of *L. santolla* are fully lecithotrophic (Kattner et al. 2003, Lovrich et al. 2003), differences in egg energy provision may directly affect later life-history stages, including their fitness (Fischer et al. 2009). However, the capability of *L. santolla* to regulate the reproductive investment and egg quality to ensure larval survival remains unknown. Therefore, the main objective of this study was to compare indicators of energy content and reproductive investment among ovigerous females of *L. santolla* from different regions of its distribution.

MATERIALS AND METHODS

Sampling

Ovigerous females were collected from three different locations of the southwestern Atlantic in April and May 2012 (Fig. 1). Females of *L. santolla* were obtained in San Jorge Gulf and the Beagle Channel, while females of *L. confundens* were obtained on the Atlantic coast of Tierra del Fuego, near the eastern area of the Magellan Strait (herein “Tierra del Fuego”). In San Jorge Gulf and Tierra del Fuego, samples were taken on board the RV *Puerto Deseado* using a bottom trawl. Females from the Beagle Channel were captured with baited traps used for commercial fishing and with the help of local artisanal fishers.

In the San Jorge Gulf and Tierra del Fuego, females were measured and dissected on board. On the other hand, in the Beagle Channel females were transported alive to the laboratory facilities of the Centro Austral de Investigaciones Científicas (CADIC), where they were measured and dissected. The female size was estimated

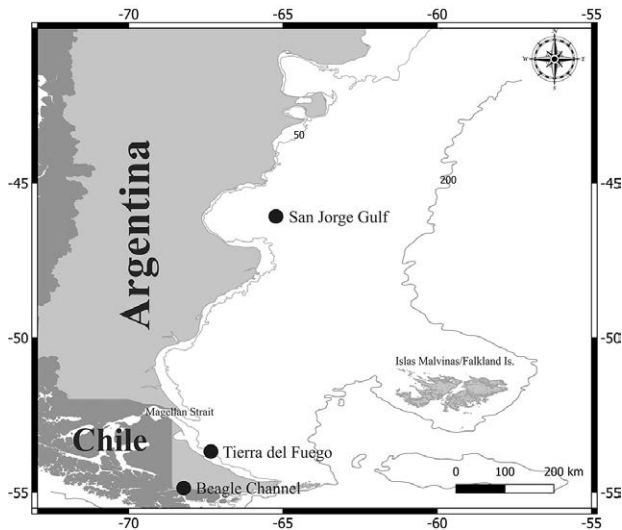


Fig. 1. – Southern tip of South America, showing the three sampling sites.

as the standard measure of the carapace length (CL), i.e. the midline distance between posterior orbital margin, excluding the rostral spine, and the posterior median margin. CL was measured to the nearest 0.1 mm with a vernier caliper. Before dissection, egg clutches from each female were inspected by visually estimating the egg mass size compared with the abdomen size. Samples of midgut gland, ovary and muscle were collected from each female in plastic bags and stored at -20°C until analysis. Pleopods and the whole egg mass were removed by cutting them from their bases. A subsample of 3 to 5 mL eggs was detached from the egg mass and measured with a small graduated test tube and later kept at -20°C in plastic bags. The rest of the egg mass was preserved in 10% formalin seawater.

Fecundity estimation and egg volume measurement

Fecundity (F) was defined as the number of eggs per clutch and was estimated following the methodology used by Lovrich and Vinuesa (1993). First, pleopods were removed from each egg mass. Then, the egg masses were blotted on absorptive paper and weighed to the nearest 0.01 g (TW). Three subsamples of around 0.5 g were weighed to the nearest 0.01 g (sw), and the eggs (sn) of each subsample were counted. As subsamples of known volume had been previously separated and kept in the freezer, the equivalent removed volume of eggs for each sample was weighed to the nearest 0.01 g and then added to correct the value of the total weight (TW) before calculating fecundity. Fecundity was calculated as

$$F = \sum_{i=1}^3 \left(\frac{\text{TW} \times \text{sn}}{\text{sw}} \right) / 3$$

To ensure that eggs were in the same stage of embryogenesis, subsamples were taken from each location and examined under a stereoscopic microscope. Egg characteristics such as colour, presence of chromatophores and appendage development were established for each sample and then catego-

rized following the developmental stages defined by Vinuesa (1987) and Balzi (2005) for *L. santolla*. The egg volume was also estimated. Maximum (d_1) and minimum (d_2) diameters of 12 eggs from each egg mass, along with the maximum embryo diameter, were measured under a binocular microscope to the nearest 0.005 mm with an ocular micrometer. Egg volume was calculated as

$$V = 1/6(\pi d_1 \times d_2^2) \quad (1)$$

Water, lipid, protein and inorganic matter content

Water contents of egg mass, midgut gland, ovaries and muscle were estimated by weight loss after lyophilization. Samples of ovary, midgut gland and muscle were weighed before and after being dried in a lyophilizer for 4 to 5 d, and their difference was considered as the water content.

Lipid content of the egg mass, midgut gland, ovary and muscle was estimated by taking a subsample of eggs or tissue of 0.15 to 0.20 g (lw, weighed to the nearest 0.1 mg) followed by a double lipid extraction. Previous rehydration of the lyophilized tissues and pre-extraction of lipids in hot acetic acid was performed as described by Phillips and Privett (1979). The first lipid extraction was done following the method of Bligh and Dyer (1959), and a complementary second extraction was done as described by Folch et al. (1957). Both extracts were placed together in a pre-weighed tube (tw) and left in the oven for 24 h at 50°C for chloroform evaporation. The tubes and their contents were then weighed to the nearest 0.1 mg (fw). Lipid content was estimated as a percentage fraction of weight between the extracted lipid and the original tissue weight:

$$\text{Lipid content} = \left(\frac{\text{fw} - \text{tw}}{100} \right) lw$$

The protein content of the egg mass was estimated in subsamples of 0.005 to 0.01 g by colorimetric quantification using Lowry's method (Lowry et al. 1951). Subsamples of egg masses that weighed 300 to 500 mg were ashed in the muffle at 540°C for 12 h. The ashes were then weighed and considered as the inorganic matter content of the sample.

Statistical analyses

Fecundity and CL were linearized using a logarithmic function, and then the relationship between log CL and log fecundity was fitted to a linear regression model. The null hypotheses of equality of slopes and ordinates were tested with an ANCOVA with sampling sites as the categorical variable. All ANCOVA assumptions were tested and met prior analysis. Differences in egg volume, embryo size and water and lipid content among the three sampling sites were tested by ANOVA tests when possible. If differences in the means were significant at the $p < 0.05$ level, they were also tested with a posteriori Tukey test (HSD). Normality and homogeneity of variances were verified

Table 1. – Linear regressions between fecundity (F) and female size (CL) for *Lithodes santolla*, among the three sampling sites.

Categorical variable	n	Equation	R ²	F-value	p
Beagle Channel	30	$\log F = -2.410 + 3.272 \log CL$	0.727	78.24	<0.001
Tierra del Fuego	25	$\log F = -2.212 + 3.194 \log CL$	0.625	38.29	<0.001
San Jorge Gulf	23	$\log F = -3.097 + 3.644 \log CL$	0.781	79.36	<0.001
All three locations	78	$\log F = -2.041 + 3.098 \log CL$	0.811	327.30	<0.001
Covariate (CL)				180.95	<0.001
Comparison of slopes				0.32	0.731
Comparison of intercepts				1.27	0.286

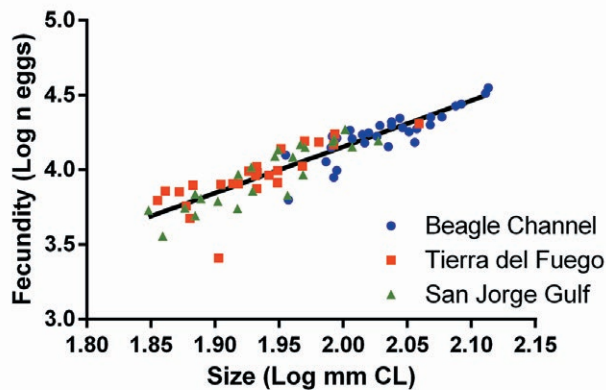


Fig. 2. – Fecundity and female size of *L. santolla* from the three sampling sites. A single regression fits the data from all locations, as no significant differences were found in the fecundity-size relation between sites (ANCOVA, $p > 0.05$). Fecundity and size values were log-transformed.

with the Shapiro-Wilk and Bartlett tests, respectively. When these assumptions were not met even after data transformation, a Kruskal Wallis test was used instead. The Dunn multiple comparison post hoc test was used to investigate differences between sampling sites. Correlations between egg volume, embryo size, fecundity, egg lipid, protein and inorganic matter content and female size were calculated by the Pearson correlation coefficient.

RESULTS

Fecundity

A total of 30, 25 and 23 females of *L. santolla* were captured from the Beagle Channel, Tierra del Fuego and San Jorge Gulf, respectively. Every female had a full clutch after the visual inspection. At all three sampling sites, fecundity increased with size (Table 1; Fig. 2). Sampled females from the Beagle Channel were generally larger (average size \pm SE: 108.2 ± 10.4 mm; min-max: 90.2–129.8 mm CL) than those from the other two sampling sites (85.85 ± 9.7 ; 71.7–114.6 and 87.27 ± 10.0 ; 70.5–106.5 mm CL for Tierra del Fuego and San Jorge Gulf, respectively). However, the ANCOVA showed that there were no significant differences between slopes ($F = 0.32$, $p = 0.731$) or between intercepts ($F = 1.27$, $p = 0.286$) from different sites. Therefore, there was no interaction between the fecundity-size relationship among sampling sites, and the three regression lines could be pooled in a single one (Table 1).

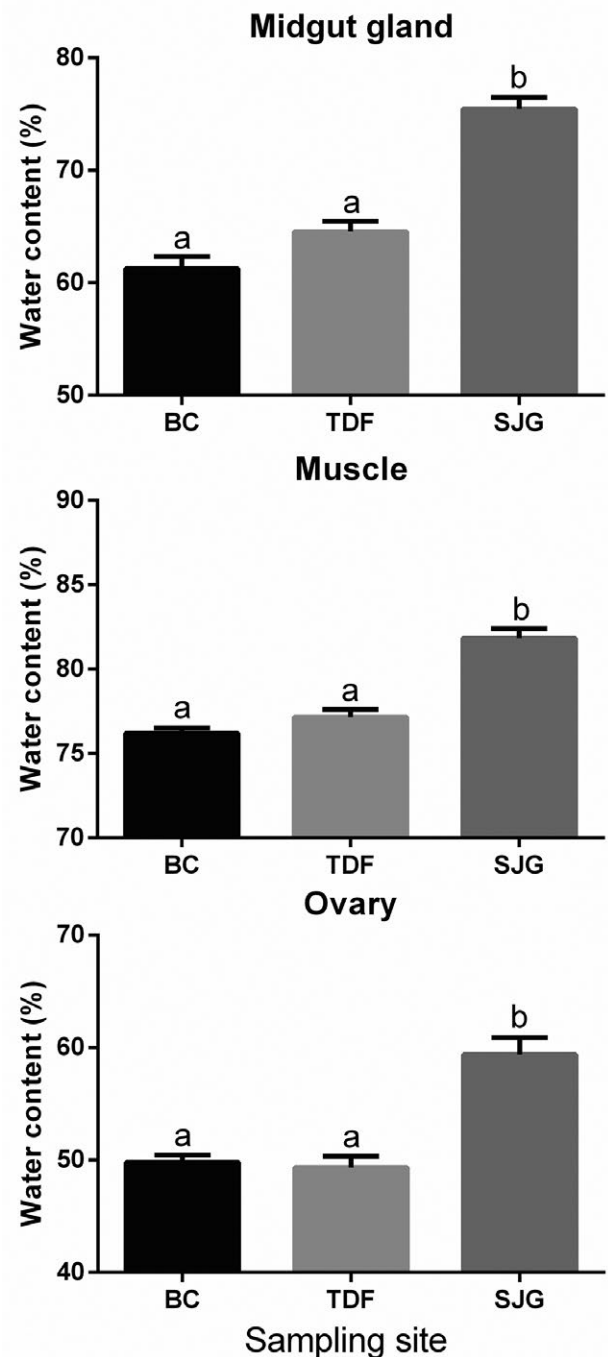


Fig. 3. – Water content (mean \pm SE) in midgut gland, muscle and ovary of *L. santolla* from the three sampling sites: Beagle Channel (BC), Tierra del Fuego (TDF) and San Jorge Gulf (SJG). Different letters indicate significant differences between sites (ANOVA and Tukey, $p < 0.05$; or Kruskal Wallis and Dunn, $p < 0.05$).

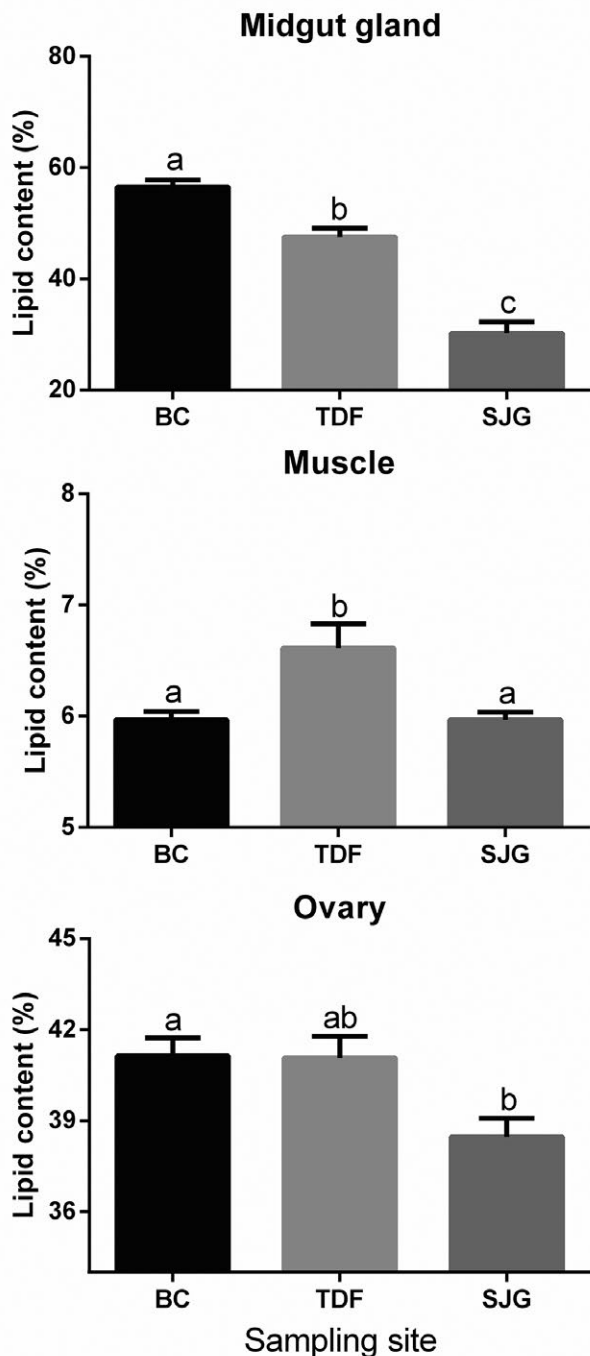


Fig. 4. – Lipid content (mean±SE) in midgut gland, muscle and ovary of *L. santolla* from the three sampling sites: Beagle Channel (BC), Tierra del Fuego (TDF) and San Jorge Gulf (SJG). Different letters indicate significant differences between sites (ANOVA and Tukey, $p < 0.05$; or Kruskal Wallis and Dunn, $p < 0.05$).

Water, lipid, protein and inorganic matter content

From the total of the captured individuals, 30, 10 and 23 females (Beagle Channel, Tierra del Fuego and San Jorge Gulf, respectively) were used to measure water, lipid, protein and inorganic matter content. The water content (Fig. 3) of the midgut gland (ANOVA, Tukey, $p < 0.05$), ovary (Kruskal Wallis, Dunn, $p < 0.05$) and muscle (Kruskal Wallis, Dunn, $p < 0.05$) were significantly higher in females from San Jorge

Gulf (water [mean±SE]: 75.46 ± 1.03 , 59.40 ± 1.50 , and $81.84 \pm 0.57\%$, respectively) than in those from the Beagle Channel (61.29 ± 1.06 , 49.82 ± 0.61 , and $76.21 \pm 0.31\%$, respectively) and Tierra del Fuego (64.56 ± 0.93 , 49.35 ± 1.00 , and $77.17 \pm 0.44\%$, respectively). Furthermore, no differences in water content were found in those organs between females from the Beagle Channel and Tierra del Fuego (ANOVA and Tukey or Kruskal Wallis and Dunn, $p > 0.05$).

Lipid content in the midgut gland was significantly higher in the Beagle Channel (lipid [mean±SE]: $56.55 \pm 1.21\%$) than Tierra del Fuego ($47.58 \pm 1.56\%$) and San Jorge Gulf ($30.21 \pm 2.07\%$), the latter being significantly the lowest (ANOVA, Tukey, $p < 0.05$; Fig. 4). Lipid content in the ovary showed significant differences only between the Beagle Channel ($41.14 \pm 0.59\%$) and San Jorge Gulf ($38.47 \pm 0.61\%$), with females from Tierra del Fuego having intermediate values ($41.07 \pm 0.71\%$) (ANOVA, Tukey, $p < 0.05$). On the other hand, in Tierra del Fuego muscle lipid content was the highest value ($6.61 \pm 0.22\%$) (Kruskal Wallis, Dunn, $p < 0.05$), whereas there were no differences between the Beagle Channel and the San Jorge Gulf (both $5.97 \pm 0.07\%$).

In the eggs there were significant differences in the lipid content only between the Beagle Channel (lipid [mean±SE]: $48.66 \pm 0.31\%$) and Tierra del Fuego ($46.11 \pm 0.95\%$), with the San Jorge Gulf showing intermediate values ($47.68 \pm 0.45\%$) (ANOVA, Tukey, $p < 0.05$; Fig. 5). Eggs from the Beagle Channel had a lower protein content (protein [mean±SE]: $45.75 \pm 0.83\%$) than those from San Jorge Gulf ($49.12 \pm 0.91\%$) (ANOVA, Tukey, $p = 0.02$), but no differences were found between egg masses from Tierra del Fuego ($46.84 \pm 1.31\%$) and the other sampling sites (ANOVA, Tukey, $p > 0.05$). The egg water content was similar at the three sampling sites (water [mean±SE]: Beagle Channel $57.21 \pm 0.46\%$, Tierra del Fuego $57.81 \pm 1.08\%$ and San Jorge Gulf $56.33 \pm 0.84\%$) (ANOVA, $p > 0.05$). Moreover, inorganic matter content of the eggs from the Beagle Channel was the lowest (mean±SE: $7.89 \pm 0.13\%$) (ANOVA, Tukey, $p < 0.05$), but no differences were recorded between Tierra del Fuego ($9.37 \pm 0.17\%$) and the San Jorge Gulf ($9.56 \pm 0.14\%$) (ANOVA, Tukey, $p = 0.74$). Lipid, protein and inorganic matter content in the eggs were not correlated with female size (Pearson, $p > 0.05$; Table 2).

Egg volume and embryo size

From the captured individuals, 30, 10 and 23 females (from the Beagle Channel, Tierra del Fuego and the San Jorge Gulf, respectively) were used to analyse both egg and embryo sizes. Neither egg volume nor embryo size was correlated with female size (Pearson, $p > 0.05$; Table 2). As there was no correlation between these parameters, a comparative analysis of egg and embryo sizes was made between sites.

Eggs from the three sites were at the same stage of embryo development. Egg volume ranged between 2.5 and 4.86 mm^3 , and both the highest and lowest values

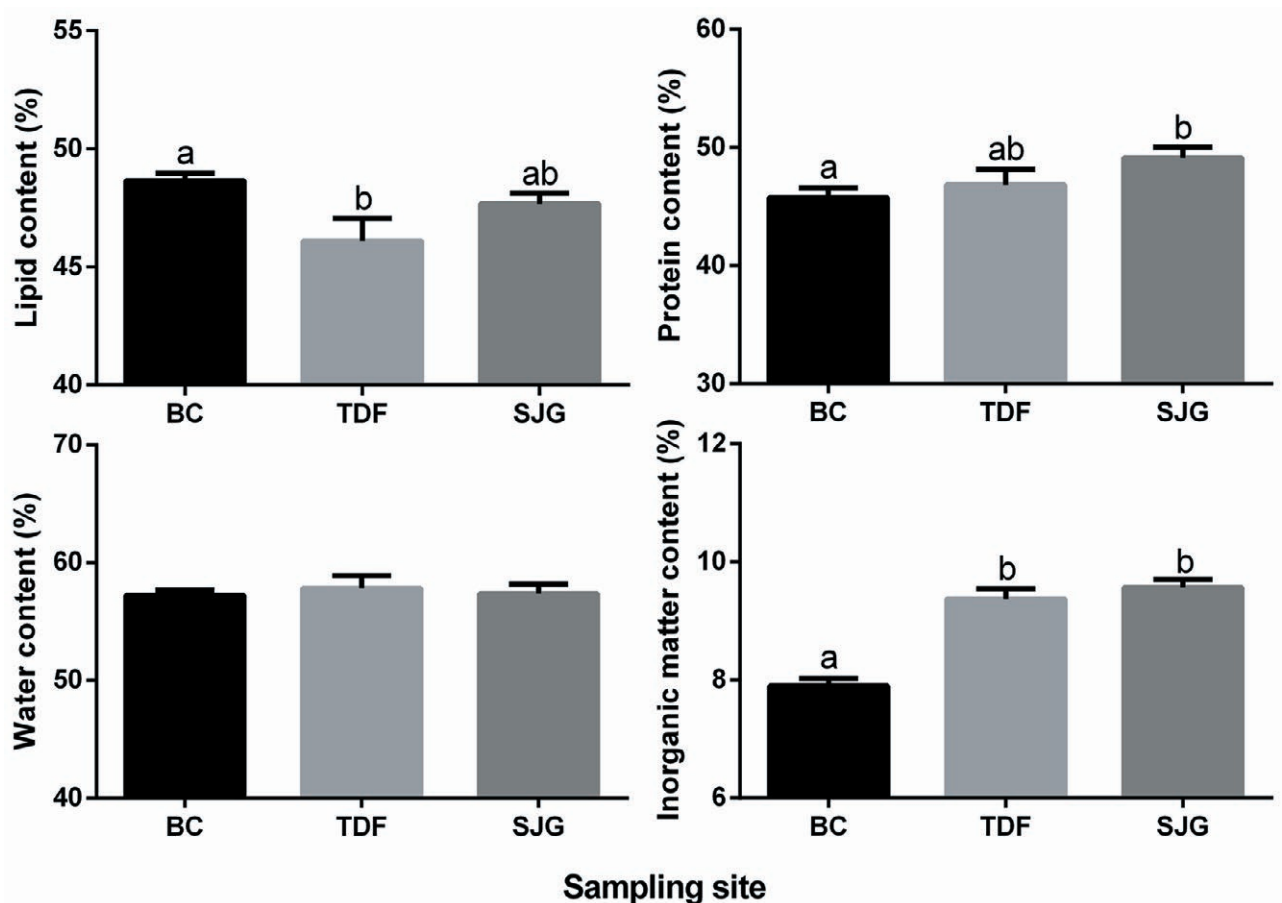


Fig. 5. – Lipid, protein, water and inorganic matter content in egg mass of *L. santolla* (mean±SE) from the Beagle Channel (BC), Tierra del Fuego (TDF) and San Jorge Gulf (SJG). Different letters indicate significant differences between sites (ANOVA and Tukey, $p < 0.05$; or Kruskal Wallis and Dunn, $p < 0.05$).

were recorded in San Jorge Gulf, where the egg volume was particularly variable (Table 3). Eggs from San Jorge Gulf were significantly bigger than those from the other two sites (ANOVA, Tukey, $p < 0.05$; Fig. 6.A). As for egg volume, embryo size in San Jorge Gulf was highly variable, fluctuating between 1.70 and 2.04 mm (Table 3). Embryo size was significantly smaller in the Beagle Channel (ANOVA, Tukey, $p < 0.05$), and there

were no differences in embryo size between Tierra del Fuego and San Jorge Gulf (ANOVA, Tukey, $p = 0.94$; Fig. 6.B).

DISCUSSION

Understanding the reproductive biology of commercial species is critical for stock assessments and

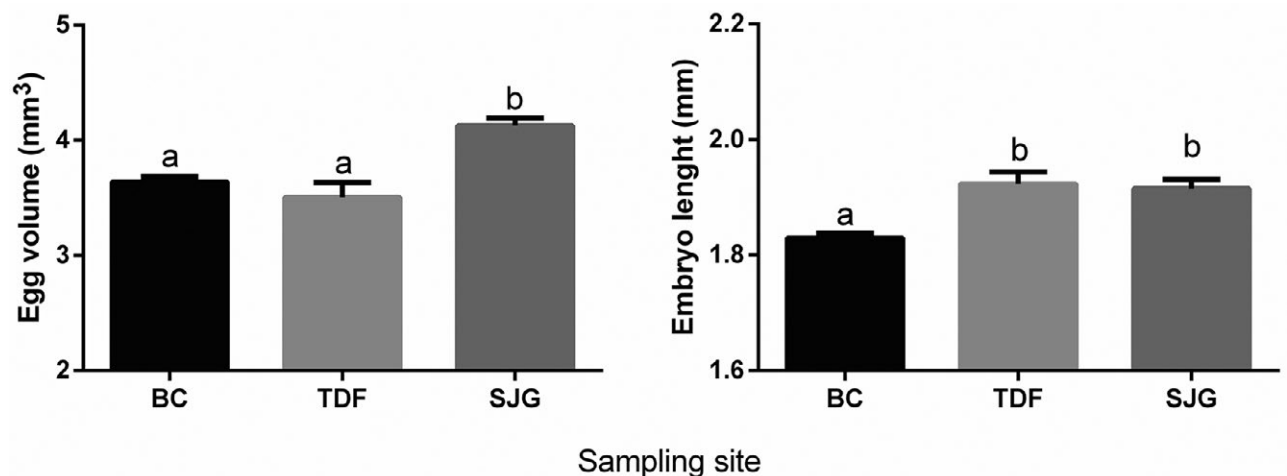


Fig. 6. – Egg volume (A) and embryo size (B) of *L. santolla* (mean±SE) from three sampling sites: Beagle Channel (BC), Tierra del Fuego (TDF) and San Jorge Gulf (SJG). Different letters indicate significant differences between sites (ANOVA, and Tukey, $p < 0.05$).

Table 2. – Pearson correlation between egg volume, embryo size, fecundity, egg lipid, protein and inorganic matter content and female size (CL), among the three sampling sites.

	Sampling site	n	r	p
Egg volume - fecundity	Beagle Channel	30	-0.091	0.631
	Tierra del Fuego	10	0.284	0.426
	San Jorge Gulf	23	0.148	0.499
Egg volume - CL	Beagle Channel	30	-0.11	0.563
	Tierra del Fuego	10	0.095	0.795
	San Jorge Gulf	23	0.197	0.367
Embryo size - Fecundity	Beagle Channel	30	0.175	0.356
	Tierra del Fuego	10	-0.438	0.206
	San Jorge Gulf	23	0.056	0.798
Embryo size - CL	Beagle Channel	30	0.108	0.571
	Tierra del Fuego	10	-0.448	0.194
	San Jorge Gulf	23	0.161	0.462
Egg lipid content - CL	Beagle Channel	30	0.066	0.727
	Tierra del Fuego	10	0.012	0.972
	San Jorge Gulf	23	0.388	0.066
Egg protein content - CL	Beagle Channel	30	-0.223	0.234
	Tierra del Fuego	10	-0.350	0.320
	San Jorge Gulf	23	0.142	0.517
Egg inorganic matter content - CL	Beagle Channel	30	0.205	0.275
	Tierra del Fuego	10	0.376	0.283
	San Jorge Gulf	23	0.129	0.557

Table 3. – Egg volume (mm³) and embryo size (mm) at the three sampling sites.

	n	mean	SE	Min	Max
Egg volume					
Beagle Channel	30	3.64	0.05	3.17	4.26
Tierra del Fuego	10	3.51	0.13	3.04	4.18
San Jorge Gulf	23	4.01	0.07	2.50	4.86
Embryo size					
Beagle Channel	30	1.83	0.01	1.72	1.91
Tierra del Fuego	10	1.90	0.02	1.73	2.00
San Jorge Gulf	23	1.91	0.02	1.70	2.04

fishery management because reproductive biology (i.e. embryo production) largely determines productivity (Swiney et al. 2012). This study compares the reproductive investment of *Lithodes santolla* from three locations in southern South America.

Fecundity is related to female size in *L. santolla* (Lovrich and Vinuesa 1999). In this study, this relation was accomplished, but no differences among sites were found. Since fecundity in *L. santolla* is affected by fishing at both the Beagle Channel (Gowland-Sainz et al. 2015, Di Salvatore et al. 2019) and San Jorge Gulf (Firpo et al. 2017, Militelli et al. 2019, Varisco et al. 2019), we expected to find differences between sites associated with the commercial activity. We consider that a broader sampling will give further insight into this issue and allow a better understanding of the fecundity variability among populations.

The lipid and water content of tissues gives proxies of the energy reservoir of the individuals and their nutritional state (Dall 1975, Moore et al. 2000). During the process of vitellogenesis, there is an increase in the lipid content in the midgut gland, the main organ for lipid storage in crustaceans (Chang and O'Connor 1983), followed by a progressive increase in lipids in the ovary at the expense of the provisions previously stored at the midgut gland (Spaargaren and Haefner Jr 1994). At the time when samples were taken (April-May), secondary vitellogenesis, in which the lipid

storage occurs in the oocytes, was likely ongoing (Vinuesa and Labal de Vinuesa 1998), with the consequent mobilization of lipids from the midgut gland towards the ovary. Ovaries of *L. santolla* had a higher lipid content in the Beagle Channel than in San Jorge Gulf, whereas Tierra del Fuego showed intermediate values. Also, the midgut gland of females in the Beagle Channel showed the highest content, revealing a richer energy budget for reproduction. Furthermore, water content is a good indicator of nutritional status in crustaceans, presumably because metabolized tissues are replaced by water under starvation conditions (Dall 1975). For example, under starvation conditions the snow crab *Chionoecetes opilio* decreases its muscular and midgut gland masses and increases its water content (Mayrand et al. 2000, Siikavuopio et al. 2019). Since females from San Jorge Gulf showed a lower water content than those from the other sites, along with their lower lipid contents, they presumably have a worse nutritional condition.

The nutritional status of individuals and hence the quality of their offspring will depend on the amount and quality of their food. Lithodid crabs are generalist decapods with an opportunistic feeding strategy and feed on the most common prey present in the habitat. Thus, this difference in the nutritional status between *L. santolla* of San Jorge Gulf and the Beagle Channel may be due to a differential diet (Lovrich and Tapella 2014). In the Beagle Channel, *L. santolla* feeds on a wide variety of organisms, about 20 different items including snails, crustaceans such as the squat lobster *Munida gregaria*, bryozoans, and algae (Comoglio and Amin 1996). In San Jorge Gulf, this species has a more restricted diet, feeding only on five items (mostly fishes) (Balzi 1999). What is more concerning is that they may be feeding mostly on the bycatch discards of the two main fisheries of the gulf: the shrimp *Pleoticus muelleri* and the Argentine hake, *Merluccius hubbsi* (Balzi 1999), which is considered a lean fish due to

its low fat content (Picklo 2016). These discards are constituted mainly of juvenile hake (Góngora et al. 2012), which has been reported to have a reduced nutritional value, because eicosanoic acid is only detected in medium-sized hake muscle tissues (Swanepoel et al. 2016). Consequently, *L. santolla* in the San Jorge Gulf may have a deficient lipid intake and, since the scavenging habit is not energetically rewarding, it should be complemented by good quality food (Ruxton and Houston 2004).

Egg lipid content was higher in the Beagle Channel than in Tierra del Fuego, with the egg mass from San Jorge Gulf showing intermediate values. Hence, due to the worse nutritional condition assumed for the San Jorge Gulf females (discussed in the previous section), they will probably exhibit a compensatory mechanism to produce eggs of similar lipid content to those with a better nutritional status. However, although lipids are the primary source of energy used by *L. santolla* during its larval development, proteins can also constitute a significant complementary source (used as a metabolic substrate and in lesser quantity), because 40% of the initial proteins are consumed when the juvenile stage is reached (Kattner et al. 2003, Lovrich et al. 2003). Egg masses from San Jorge Gulf showed higher protein and inorganic matter content than those from Beagle Channel, supporting our hypothesis on a compensatory mechanism in which the former may compensate for the lipid intake deficiency by allocating more protein to the egg mass.

In several decapod crustaceans, the egg organic content correlates with the egg size (Shakuntala and Reddy 1982). Moreover, the average size of the eggs may vary among conspecific populations, and such differences have been related to latitudinal temperature gradients, interannual temperature variation, regional variation in salinity and other environmental factors (Bas et al. 2007). For example, the shrimp *Crangon crangon* shows both seasonal and interannual egg size variations, as an ability to adapt to different temperatures and feeding conditions (Urzúa et al. 2012). However, although larger eggs are usually considered to reflect higher maternal investment, this parameter by itself is not always a good indicator of energy content if it is not complemented with others (Moran and McAlister 2009). Such is the case of the *L. santolla* females from San Jorge Gulf, in which egg volume showed the highest values but the lipid content did not, supporting the idea that egg size is not necessarily associated with higher egg energy reserves. It is important to note that these volume variations were not due to differences in the egg mass water content.

Female *L. santolla* in San Jorge Gulf had larger embryos than the ones from the Beagle Channel, although embryos were at the same development stage at all sampling sites. Smaller embryo sizes from the Beagle Channel could be explained by sea temperature differences between sites. Temperature significantly influences the duration of embryonic development in the decapod crustaceans (Hamasaki et al. 2003, Webb et al. 2007), and lithodid crabs in particular (Stevens et al. 2008). Thermal regime ranges

between 4.2°C and 9.8°C (in winter and summer, respectively) in the Beagle Channel (Pérez-Barros et al. 2004) and between 5.1°C and 13.4°C in San Jorge Gulf (Vinuesa 2005). Eggs from the Beagle Channel are thus exposed to lower temperatures, potentially slowing down embryo growth. Eggs of females from Tierra del Fuego were smaller than those from the Beagle Channel, but embryo size was larger in females from San Jorge Gulf. Females from Tierra del Fuego showed an intermediate situation, in which environmental conditions may be the main explanatory factor.

Among marine decapods, the relationship between maternal size and the quality of larvae and embryos is variable (Militelli et al. 2019). In lithodid crabs, evidence shows that female size is not correlated with egg quality or with egg dry mass in the red king crab *Paralithodes camtschaticus* (Hjelset et al. 2012, Swiney et al. 2013) or with egg size (measured as diameter) in *L. santolla* (Militelli et al. 2019). However, Militelli et al. (2019) found a positive correlation of the egg dry mass with female size and suggested a “maternal effect”, which proposes that larger females have better-quality eggs. Our results in females of *L. santolla* from all sampling sites did not show such a correlation of maternal size with egg energy content (measured as lipid, protein and inorganic matter content) or with egg volume or embryo size. This finding indicates that in San Jorge Gulf females from the sampling size range have a similar compensatory capacity of a low nutritional status in order to ensure an adequate energy reserve for the embryo.

Intraspecific variability in the number and size of eggs produced under different environmental conditions may help to optimize the relative allocation of female energy resources to growth and reproduction, in addition to survival, dispersal and recruitment of the offspring (Hadfield and Strathmann 1996). Our study shows differences in the energetic quality of female organs and eggs of *L. santolla*, indicating variability in the reproductive investment among regions of its distribution. The fact that females in San Jorge Gulf had a higher water content and lower lipid content in the midgut gland and ovaries suggests that they have a lower-quality diet than females in the Beagle Channel. We attribute these differences to a more scavenging habit in San Jorge Gulf during the year previous to our sampling, when oogenesis occurs. Furthermore, though eggs and embryos were larger in San Jorge Gulf, the energy reserves showed a similar amount of lipid content in the egg mass but higher protein and inorganic matter content (considered as less energetic) than in those from the Beagle Channel. Thus, we suggest a compensatory mechanism for *L. santolla* from San Jorge Gulf to cope with the deficient lipid intake due to the scavenger habit and poor-quality food. A further study of the site-specific egg composition would be necessary to determine the nature and quality of different types of lipids present in the egg mass from the different regions, in order to better evaluate this proposed hypothesis and its efficiency for guaranteeing larval survival.

ACKNOWLEDGEMENTS

We are indebted to the captain and crew of the RV *Puerto Deseado* for access to the samples in Tierra del Fuego and San Jorge Gulf and to M. Pérez and L. Pagnossin for their help in sampling in the Beagle Channel. O. Florentín assisted in the laboratory work, and C.P. Fraysse provided suggestions to improve this manuscript. We wish to thank J.M. Txurruka (EHU/UPV) for technical assistance and lending laboratory facilities for the chemical analysis of protein contents. We thank the two anonymous reviewers for their contributions to improve this paper. This study was funded by the Argentine State through grants from FONCyT PICTs 12-0554 and 16-0142. N. Díaz-Arce was supported by the FiDEX scholarship granted by the Bordeaux University to do her MSc thesis at CADIC. P. Di Salvatore has a postdoctoral fellowship from the Argentine CONICET.

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