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# Cannibalism among *Lithodes santolla* (Molina 1782) juveniles: effect of stocking density, stage and molt condition

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**Abstract** Losses of king crabs (deaths) in massive rearing conditions are frequently attributed to cannibalism. As several factors could influence cannibalism intensity during culture, the aim of the present study was to determine the effect of molt condition, stage and density on cannibalism among *Lithodes santolla* juveniles. Comparisons between intermolt and molt conditions and between C1 and C2 stages during intermolt were performed at three densities (6, 12 and 18 individuals per flask; that is equivalent to 236, 472 and 708 individuals  $m^{-2}$ ). After 16 days of juvenile coexistence, cannibalism (injured + dead) was higher during molting ( $33.9 \pm 4.5$  %) than intermolt ( $21.7 \pm 3.9$  %), even though only 36 % of crabs had molted. Although cannibalism was similar in C1 and C2 stages, the proportion of dead animals was higher by a factor of three in C1 stage, probably due to the fact that animals reach the first juvenile stage with low energetic reserves, after the lecithotrophic larval development. Density was the main factor influencing cannibalism in our experiments. Higher densities promoted higher deaths, while agonistic interactions evidenced by injured crabs remained constant. Walking appendages were lost more often than chelipeds during first agonistic encounters. We recommend the use of intermediate densities (450 crabs  $m^{-2}$  at most), the early detection of exuviae and the sorting of crabs after molting, for *L. santolla* rearing.

**Keywords** Cannibalism · Culture · Juveniles · Southern king crab · Stock enhancement

## Introduction

The southern king crab *Lithodes santolla* is the most valuable commercial species among the lithodids inhabiting the sub-Antarctic waters of Southern South America. *L. santolla* occurs off the Pacific coast from the Chiloe Island (42°S 74°W) to Cape Horn (55°S 67°W)

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including the Strait of Magellan and fjords around Tierra del Fuego island (Boschi et al. 1992; Macpherson 1988; Retamal 2000). In Atlantic waters, its distribution extends north from Cape Horn to the continental slope of Buenos Aires province (37°S 54°W) (Sotelano et al. 2012, 2013). Particularly in the Beagle Channel (54°S 67°W), the southern king crab is an important economic resource, and along with the false king crab *Paralomis granulosa*, it constitutes the main crustacean fishery at the southern tip of South America. The southern king crab fishery in the Beagle Channel began during 1920–1930s (Vinueza et al. 1991), and its maximum landings were obtained during the 1970s. The extent of overfishing in the past was reflected in an alarming decline of landings during the 1990s. Therefore, a banned area for this fishery was implemented in 1994, as a complementary rule of management (Lovrich and Tapella 2014). However, *L. santolla* population has not shown a trend of recovery of its abundance after ca. 14 years of fishing restrictions (Iorio et al. 2008). Despite controversial opinions, regulations changed in 2013, and the banned zone was reopened to fishing (Provincial Law 931). Stock enhancement program has been proposed as an alternative strategy to revert local depletion of the southern king crab population.

Stock enhancement efforts have been implemented for other crab species and have focused on larval and juvenile production and sources of natural mortality (Borisov et al. 2007; Daly et al. 2009, 2012b, 2013; Long et al. 2012; Paul and Paul 2001; Persselin and Daly 2010; Pirtle and Stoner 2010; Stevens 2003, 2006; Stevens et al. 2008; Sotelano et al. 2012; Swiney et al. 2012; Stoner 2009; Tapella et al. 2009a, b). For such studies, large numbers of larvae or juveniles must be produced first and, only afterward, work should be directed toward both the optimization of release strategies (e.g., suitable habitat, animal release period and density), and the success of survival of cultured animals in the field after being liberated. Massive culture can promote cannibalism, especially during production of early crab stages under conditions such as high animal density, lack of shelter, and poor quality of food (Brodersen et al. 1989; Daly et al. 2009; Johnston et al. 2006; Luppi et al. 2001; Ryer et al. 1997; Van Olst et al. 1975). Although mortality rates in culture systems are frequently attributed to predation upon post-molt animals (Borisov et al. 2007; Crear et al. 2000; Daly et al. 2009; Johnston et al. 2006; Marshall et al. 2005; Paul and Paul 2001), cannibalism during intermolt period was also reported for some decapod species such as *Portunus pelagicus* (Marshall et al. 2005) and *Paralithodes camtschaticus* (Borisov et al. 2007). In the southern king crab *L. santolla*, cannibalism during intermolt period has been observed in early juvenile stages, starting during the post-larval stage (megalopa) (Sotelano et al. 2012). As with other decapod crustacean species, stocking southern king crab juveniles at high densities requires dealing with intraspecific interactions during molting and intermolt periods mainly due to: (1) increased molt frequency at younger ages (i.e., first juveniles molt every ca. 2 months, whereas adults molt once a year) (Calcagno et al. 2005) and (2) ecdysis of a cohort may increase with time, causing a temporal overlap between hard shell and molting crabs.

Despite the economic importance of crustaceans in aquaculture, few studies have succeeded in discriminating the vulnerability of intermolt and post-molt individuals during cannibalistic interactions (Marshall et al. 2005). The lack of this information may be due to the difficulty in quantifying injuries in each individual when animals are reared in populations. Therefore, the aim of the present study was to determine the effect of molt condition or stage and density in cannibalism among *L. santolla* juveniles in order to improve large-scale culture for the implementation of a stock enhancement program.

## Materials and methods

### Larval rearing and crab stocking

Thirty egg-bearing *L. santolla* females with embryos in an advanced developmental stage were captured in the Beagle Channel (54°51'S 67°30'W) with commercial traps and taken to the Centro Austral de Investigaciones Científicas (CADIC) during August 2010. Females were individually kept in flow-through seawater containers (35 l) in an indoor facility at  $7.1 \pm 1.1$  °C. Water quality was maintained through mechanical (20 µm) and biological filters, and a UV sterilizer. Salinity, pH, ammonia, nitrite and nitrate were kept at  $31 \pm 1$  psu,  $8.1 \pm 0.3$ ,  $<0.25$ ,  $<0.3$  and  $<12.5$  mg l<sup>-1</sup>, respectively. Females were fed squid three times a week, and containers were cleaned and checked for larval hatching daily. In order to obtain a large number of same-age crabs for experimentation, Zoea I (Z1) larvae were randomly selected during the peak of the hatching period. Larvae were reared in 3.8-l bottom-meshed containers at a maximum density of 600 individuals container<sup>-1</sup> in the above-mentioned recirculation system. They were not fed since *L. santolla* larval development is lecithotrophic (Calcagno et al. 2005; Lovrich et al. 2003). Once the first juvenile (Crab I, C1 stage) was attained, crabs were stocked individually in 100-ml plastic containers to avoid injuries from agonistic interactions or cannibalism until they were used for experimentation (C1 or C2 stages). We ensured that crabs used for experimentation were vigorous, healthy and with all their legs. Containers were stored in a cold room at  $7 \pm 0.5$  °C with 12:12 dim light (0.5 lx) and dark photoperiod, respectively. Crabs were fed ad libitum with live *Artemia salina* nauplii three times a week after water changing. Crabs received 100 µl ( $\approx 270$  nauplii) of newly hatched *Artemia* nauplii (1 g *Artemia*/50 ml sea water), at each feeding.

### Experimental design

An experiment consisting of three treatments was performed in order to evaluate the effect of molt condition, stage and density on cannibalism among coexisting juveniles of southern king crabs, as follows:

Treatments 1 and 3—Cannibalism during intermolt period (C1 int and C2 int, respectively): We used crabs 3–6 days after molting to C1 or C2 stages.

Treatment 2—Cannibalism during molting period (C1 molt): Stage I crabs (C1) near to molt to C2 were used. Experiment was started with C1 crabs that were kept in individual containers and began only after 20 % of the co-aged crabs had molted to C2 stage (Crabs that molted were discarded because the crabs were meant to molt during the treatment). Since molting to C2 stage occurs within 4–5 days at 6 or 9 °C, respectively (Calcagno et al. 2005), we assumed that molting for the experimental C1 crabs was imminent.

All three treatments were tested at three different densities [6 (D6), 12 (D12) and 18 (D18) crabs container<sup>-1</sup> equivalent to 236, 472 and 708 individuals m<sup>-2</sup>]. The nine treatments (C1 intermolt, C1 molt, C2 intermolt  $\times$  three densities) were performed with five replicates each.

Treatments were performed in cylindrical white PVC tubes (9 cm diameter and 11 cm height) with a 1-mm polyethylene mesh attached to their bottoms. Each cylinder had three 1-cm plastic legs for separating them from the bottom. The mesh allowed crabs to grab and move freely over the whole tube bottom. Tubes ( $n = 45$ ) were randomly sorted in three containers (40  $\times$  25  $\times$  30 cm) filled with 10 liters of filtered and sterilized seawater. Containers were set up in a single rack inside the cold room at  $7 \pm 0.5$  °C under uniform light condition to avoid any possible effects on crab behavior. Water of each container was

changed three times a week by syphoning ca. 75 % of water and re-filling the container with new filtered and sterilized seawater. Thus, the disturbance over the crabs was minimized since they were not directly manipulated and remained under water. Immediately after water changes, crabs were fed ad libitum with *A. salina* nauplii to minimize the effect of starvation on cannibalistic behavior (Luppi et al. 2001).

Treatments started when crabs were placed in PVC tubes. Each replicate was checked daily, and the number of both live and dead crabs with signs of cannibalism (i.e., crabs with at least one missing appendage) was recorded. Also, the occurrence of exuviae was observed to determine the end of intermolt experiments and as an indicator of the percentage of animals molted in the C1 molt experiment. Crabs that disappeared from the flasks were assumed to be ingested by conspecifics. Intact dead crabs were considered as natural deaths and were replaced by undamaged animals at the same developmental stage that were previously reared in isolation.

Each day, injured and dead crabs (live and dead animals with at least one missing limb, respectively) and the number and type of lost limbs (chelipeds and/or walking legs) per injured crab were enumerated in each trial (Sotelano et al. 2012). Thus, the sum of injured plus dead crabs will be hereafter referred as cannibalism. The daily cumulative proportion of cannibalism, injured and dead crabs was calculated over the six animals in each trial.

We used two complementary criteria for defining the end of the treatments. First, the day that the first animal molted in the intermolt treatment was considered to indicate the end of the intermolt period and hence the end point for testing cannibalism during this phase. On the other hand, since ecdysis was more prolonged than expected according to Calcagno et al. (2005) (up to 5 days), we decided to end the molting treatment at the same day as the intermolt treatment, i.e., day 17. However, since our experiment considered the density as a factor of variation of cannibalism, we performed all the statistical comparisons at day 16, when molting percentage was similar in all three treatments.

## Statistical procedures

Percentage of cannibalism, injured and dead crabs are presented as cumulative means  $\pm$  SEs. Data were arcsine-transformed and tested for normality and homogeneity of variances by Kolmogorov–Smirnov and Levene tests, respectively (Sokal and Rohlf 1995). In order to test the effect of crab density, molt condition and stage on injured, dead and cannibalized crabs, two-way analyses of variances (ANOVAs) and post hoc comparisons (Tukey's HSD) were performed between: (1) molt condition and density, and (2) stage and density. When significant differences between treatments were found ( $p < 0.05$ ), post hoc comparisons were carried out (Sokal and Rohlf 1995).

## Results

Over the 540 crabs used in the trials, natural mortality of southern king crab juveniles reached an overall average of 3.3 %.

### Progression of molts

Crabs molted through time, but only  $\sim 36$  % of crabs completed the ecdysis after a period of 16 days. At the beginning of our experiment, the proportion of molted animals was



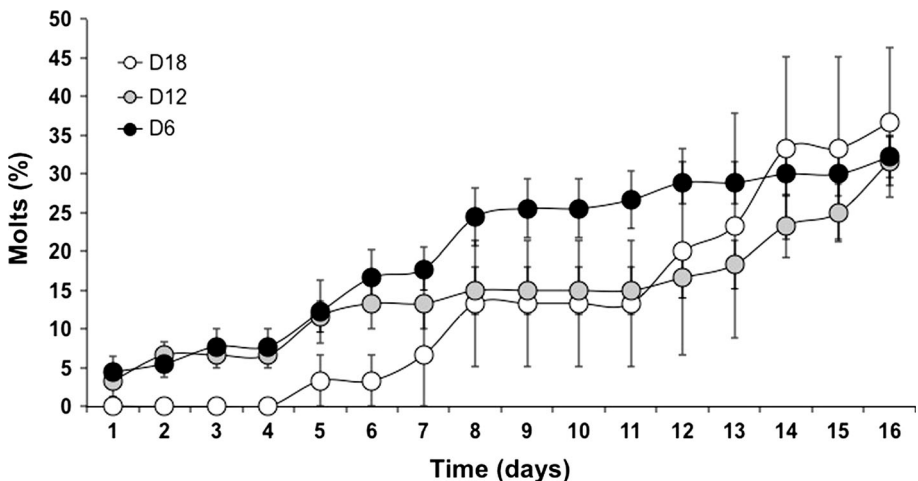
higher at the two higher densities. After day 11, the tendency slightly changed and since day 14, progression of molts was higher in D6 than in D18 and D12 (Fig. 1). Despite this trend, similar proportion of molts was found in all three densities of C1-molt treatment (ANOVA,  $F = 0.128$ ,  $p > 0.05$ ) (Fig. 1) by the end of the experiment, at day 16.

### Effect of molt condition and density on cannibalism

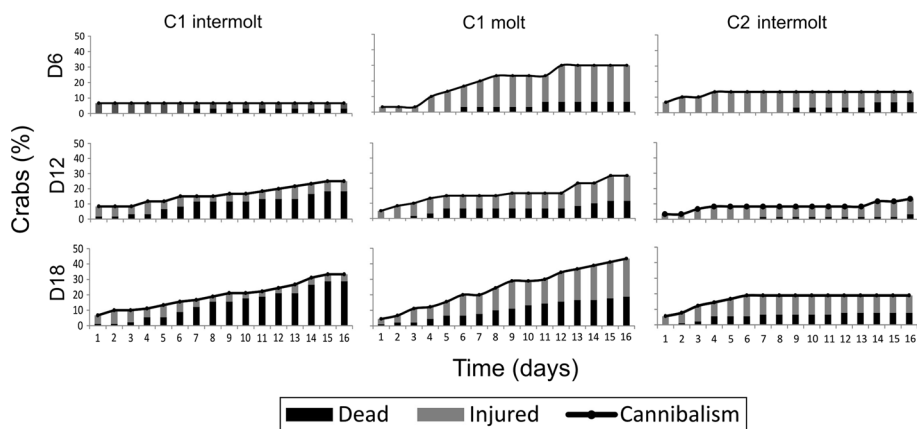
Cannibalized crabs occurred at all densities and in both molt and intermolt conditions. Damage among conspecifics (i.e., appendage loss) started as soon as the experiments began (day 1) in all treatments (Fig. 2). At the end of the experiment (day 16), the proportion of cannibalized crabs was influenced by molt condition and also by density (Fig. 2; Table 1). Cannibalistic interactions were more intense during molting ( $33.9 \pm 4.5$  %) than intermolt ( $21.7 \pm 3.9$  %) periods (Tukey test,  $p < 0.05$ ), even though only ca. 36 % of crabs had molted during the experiment (Fig. 1). Moreover, cannibalized crabs increased with density, reaching  $18.3 \pm 7.2$ ,  $26.7 \pm 3.2$  and  $38.3 \pm 3.3$  % in D6, D12 and D18, respectively. Cannibalism at D18 was significantly higher than at D6 (Tukey test,  $p < 0.01$ ).

During intermolt period, cannibalism was composed mainly by dead crabs, and during the molting period, the proportion of injured crabs increased.

When the two components of cannibalism were separately analyzed, the proportion of dead crabs was influenced by density while the proportion of injured animals was affected by molt condition (Table 1; Fig. 2). The mortality pattern showed that dead crabs occurred sooner with higher densities (Fig. 2): In intermolt, dead crabs occurred after day 7 at D6, yet at D12 and D18, they appeared immediately after the experiment began (day 1). In molting period, dead crabs were observed at days 6, 3 and 1 at D6, D12 and D18, respectively. At day 16, mortality by cannibalism was affected only by density (Fig. 2; Table 1). Proportion of dead crabs was higher at both D18



**Fig. 1** Cumulative proportion ( $\pm$ SE) of first juvenile stage crabs (C1) molted during a 16-day experiment and according to the stocking density (6, 12 and 18 individuals per experimental unit (D6, D12, D18, respectively))



**Fig. 2** Cumulative proportion of dead, injured and cannibalism (dead + injured) juvenile *L. santolla* during 16-day experiment: treatment 1 (C1 stage intermolt), treatment 2 (C1 stage-molt), and treatment 3 (C2 stage intermolt), according to the stocking density (6, 12 and 18 individuals per experimental unit: D6, D12 and D18, respectively)

**Table 1** *Lithodes santolla*: two-way analysis of variance (ANOVAs) conducted to test the effect of molt condition and density on cannibalism, dead and injured stage C1 crabs

Molt condition versus density				
Source	df	MS	F	p
<b>Cannibalism</b>				
Molt condition	1	682.42	5.09	<b>0.0334</b>
Density	2	864.74	6.46	<b>0.0057</b>
Molt condition × density	2	209.73	1.57	0.2296
Error	24	133.96		
<b>Dead</b>				
Molt condition	1	64.48	0.43	0.5192
Density	2	1043.85	6.93	<b>0.0042</b>
Molt condition × density	2	127.11	0.84	0.4425
Error	24	150.66		
<b>Injured</b>				
Molt condition	1	1166.43	7.88	<b>0.0098</b>
Density	2	10.13	0.07	0.9340
Molt condition × density	2	53.85	0.36	0.6988
Error	24	147.99		

F F-statistic, df degree of freedom, MS mean square. For details on control treatments see “Materials and methods” section. Bold indicates statistical significance ( $\alpha < 0.05$ )

( $23.9 \pm 4.3$  %) and D12 ( $15.0 \pm 3.9$  %) than at D6 ( $5.0 \pm 2.5$  %) (Tukey test,  $p < 0.01$ ). Independently of density, a similar proportion of dead crabs was observed in both experiments, reaching  $16.8 \pm 4.1$  and  $12.4 \pm 2.9$  % in intermolt and molt condition respectively ( $p > 0.05$ ).

At the end of experiment, injured crabs were affected by molt condition, as higher proportion of injured animals was found in molting than in intermolt (Table 2, Tukey test,  $p < 0.01$ , Fig. 2). Injured crabs increased to  $21.5 \pm 4.2$  % in molt and  $5.9 \pm 1.5$  % in intermolt period. Appendages lost per injured crab were influenced by density, and the



analysis showed differences between D6 and D18 (ANOVA,  $F = 1.49$ ,  $p < 0.05$ ; Tukey test,  $p < 0.05$ ). Injured crabs lost on average  $0.67 \pm 0.24$ ,  $0.93 \pm 0.11$  and  $1.43 \pm 0.16$  appendages in D6, D12 and D18 respectively.

### Effect of stage and density on cannibalism

During intermolt period cannibalism also occurred as soon as the experiment started (day 1) in both analyzed stages (C1 and C2) and at all densities (Fig. 2). Higher cannibalism was found at higher densities in both stages. In the C1 stage, cannibalism was mainly constituted by dead crabs rather than injured ones. At day 16, the proportion of cannibalism was affected only by density and not by the stage (Table 2). Higher cannibalism was found in D18 ( $26.1 \pm 3.3 \%$ ) than at D6 ( $10.0 \pm 3.7 \%$ ) (Tukey test,  $p < 0.01$ ).

Dead crabs were found in both stages and at all three densities and, as in the molting experiment, they occurred earlier in time as density increased. Data showed that both stage and density have an impact on dead crabs (Table 2). Regardless of density, cannibalism among C2 stage was higher by a factor of three than among C1 stage reaching  $16.8 \pm 4.1 \%$  and  $5.9 \pm 1.5 \%$ , respectively (Tukey test,  $p < 0.05$ ). Data also revealed differences between extreme densities (Tukey test,  $p < 0.01$ ), independently of stage: While the proportion of dead crabs in D6 reached  $5 \pm 2.5 \%$ , it reached to  $18.3 \pm 4.4 \%$  in D18.

On the contrary, percentage of injured crabs was similar between stages or densities (Table 1). Injured crabs varied between  $3.3 \pm 3.3$  and  $11.1 \pm 2.0 \%$  in C1int (D6) and C2int (D18) respectively. Condition of injured crabs by day 16 was affected only by density and differences were found between extreme densities (ANOVA,  $F = 3.70$ ;  $p < 0.05$ , Tukey test,  $p < 0.05$ ). Injured crabs lost on average  $0.60 \pm 0.26$ ,  $0.87 \pm 0.15$  and  $1.37 \pm 0.12$  appendages in D6, D12 and D18, respectively.

**Table 2** *Lithodes santolla*: two-way analysis of variance (ANOVAs) conducted to test the effect of stage and density on cannibalism, dead and injured crabs

Stage versus density				
Source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>p</i>
<b>Cannibalism</b>				
Stage	1	145.88	1.20	0.2837
Density	2	751.52	6.20	<b>0.0068</b>
Stage × density	2	247.12	2.04	0.1523
Error	24	121.31		
<b>Dead</b>				
Stage	1	596.22	5.27	<b>0.0307</b>
Density	2	707.62	6.26	<b>0.0065</b>
Stage × density	2	353.92	3.13	0.0620
Error	24	113.11		
<b>Injured</b>				
Stage	1	152.27	2.61	0.1190
Density	2	37.45	0.64	0.5345
Stage × density	2	11.37	0.20	0.8239
Error	24	58.24		

*F* *F*-statistic, *df* degree of freedom, *MS* mean square. For details on control treatments see “Materials and methods” section. Bold indicates statistical significance ( $\alpha < 0.05$ )

## Type of appendage lost

Crabs lost their walking appendages more frequently than chelipeds. If first successful attacks are counted (=first appendage lost in each crab), crabs lost walking appendages in more than 80, 88 and 63 % of cases in C1 int, C1 molt and C2 int respectively. If density increases, the proportion of chelipeds lost also increases.

## Discussion

The present study demonstrates that juvenile *L. santolla* vulnerability is influenced by the two inherent factors of molt condition and stage as well as an external factor of density.

### Molt condition

The widespread vulnerability of molting crabs over intermolts was shown in our experiments as expected. Crabs exhibited greater cannibalism during molting than during intermolt. We could detect that during the ecdysis of the C1 stage, higher cannibalism was largely driven by the agonistic component, as injured crabs were more common than dead crabs.

Our results for *L. santolla* are consistent with those found in other crab species, in which post-molting individuals were especially susceptible to predation by conspecifics (Borisov et al. 2007; Crear et al. 2000; Johnston et al. 2006; Marshall et al. 2005; Paul and Paul 2001). Yet these studies did not continuously monitor cannibalism throughout the duration of the experiment. In the snow crab *Chionoecetes opilio*, molting crabs in the same stage were not particularly vulnerable to cannibalism (Sainte-Marie and Lafrance 2002). Although mortality by cannibalism registered in *Portunus pelagicus* affected 70 % of post-molt juveniles compared to the 10–40 % of death during intermolt (Marshall et al. 2005), high values of cannibalism could probably be due to a size differential. Size variation increases cannibalism rates (i.e., 60–75 %) in *L. santolla* during intermolt, even when the interactions occur between consecutive stages (C1—Megalopa or C2–C1) (Sotelano et al. 2012). Moreover, the complete ingestion of several megalopae or first juvenile stage occurs within 96 h when they are combined with conspecifics of 1 year old (Sotelano et al. 2012) suggesting that, during the juvenile phase, higher cannibalism corresponds with greater size differences. Hence, we hypothesize that the higher cannibalism during molting is due to size variations, rather than the vulnerability of molting (soft) crabs.

Presence of conspecific animals and/or signs of cannibalism in crabs (i.e., limb loss) could have modified the molting period among our molting crabs. First, in our molting treatment, <40 % of crabs molted during a 16-day period, when ecdysis was expected to occur within 5 days (c.f. Calcagno et al. 2005) since they came from a cohort in which 20 % of specimens had already molted. We attribute the delay of molting to the presence of conspecifics, as they may function as a stressor, enhancing production of ecdysone and inhibiting the ecdysis of other crabs. Similarly, this delay of molting in the presence of conspecific has been shown for the first juvenile instars of *C. opilio* (Sainte-Marie and Lafrance 2002) and in juvenile lobsters of *Homarus americanus* (Cobb and Tamm 1974). Notwithstanding, molt-accelerating factor in several species such as *Palaemonetes kadiakensis* or *Gecarcinus lateralis* was a phenomenon noticed when numerous limbs (four or

more) are lost in each individual. Since the average number of limb lost per injured animal in the present study was low ( $1.31 \pm 0.10$ ), no precocious molting should be expected.

## Stage

During intermolt period, total cannibalism was similar between both tested stages C1 and C2. However, percentage of dead animals was higher in C1 stage implying that total or partial ingestion of conspecifics is higher in this first juvenile stage. We attribute these higher cannibalistic deaths to the fact that animals arrive to the first juvenile stage with low energetic reserves. Initial lipid and protein storage is progressively consumed during the ca. 60 days of lecithotrophic larval development (Kattner et al. 2003; Lovrich et al. 2003), and therefore, the first feeding stage is expected to have higher energetic requirements. On the one hand, cannibalism has been suggested to supplement a diet when low food quality is available (Barki et al. 1997; Brodersen et al. 1989; Polis 1981), and hence, it is plausible that conspecific ingestion could operate as a complement to the provided diet during our experiment. Nevertheless, *Artemia* nauplii was proved to fulfill the energetic requirements of early stages of *L. santolla* that were individually held for 254 days, and successfully molted at least to C6 stage (Calcagno et al. 2005). On the other hand, the first feeding stage likely needs to acquire some experience for feeding. For example, for capturing a single copepod nauplius per day, the Zoea I stage of *P. camtschaticus* needs to be provided with a minimum of 200 nauplii  $l^{-1}$  (Paul et al. 1989). If food items require experience and effort to be caught, then for our first stage juveniles it would be easier to grab a crab of their same size than a swimming nauplius. Moreover, our experimental design may have limited the access of crabs to the nauplii, since they distributed all over the water column of the experimental container, while the crabs were restricted to its bottom. Finally, if the relatively low deaths due to cannibalism found in C2 stage at the three experimental densities persist in the subsequent juvenile stages remains subject of further research, since predation rates and functional predator–prey responses change ontogenetically (Long and Whitefleet-Smith 2013).

## Density

Density was the main factor that promoted cannibalism in all of our experiments either during the intermolt and molting periods. Higher densities promoted higher deaths, while agonistic interactions evidenced by injured crabs remained constant. In our study, differences in cannibalism were found between the minimum and maximum tested densities. Density-dependant cannibalism shown in our study could be the result of the violation of a minimum inter-individual or territorial space (Polis 1981). The observed equidistance among crabs in aquaria of pooled early juveniles of *L. santolla* (C1 stages) (personal observations) and their faster response (within 2 h) selecting complex natural substrata independently of light (Tapella et al. 2012) suggest they are intolerant to conspecifics. In *P. camtschaticus*, the age-0 crabs remain isolated and in cryptic habits (Powell and Nickerson 1965) especially during the first year of life, until they express their “podding behavior” (crabs >20 mm) (Cárdenas et al. 2007; Dew 1990). Aggregations of juvenile king crabs (crabs 34–75 mm CL) *L. santolla* were also observed in southern Chile (Cárdenas et al. 2007). Moreover, it has been suggested that the release of *P. camtschaticus* in a given area every 2 years may alleviate predation of the first year class over the newly recruits (Long et al. 2012). The intracohort and intercohort cannibalism found in *L. santolla* (Sotelano et al. 2012 and this study) should encourage low release densities to ensure the ability to

use complex substrata (Long et al. 2012; Stevens and Swiney 2005; Stoner et al. 2010; Tapella et al. 2009b, 2012).

The effect of crowding has been a target of investigation for many species of crabs since the production of large numbers of individuals are needed for research, aquaculture or even population subsidy (Arnold et al. 2006; Johnston et al. 2006; Mann et al. 2007; Zmora et al. 2005). The effect of combining different year classes has been studied particularly in *P. camtschaticus* in which intraspecific predation functional response is density dependent (Daly et al. 2009; Long et al. 2012). Although some studies have been carried out varying crab density, and accounting for cannibalism after a period of coexistence, they did not track each individual. For example, the survival of *P. camtschaticus* after 42 days (from C1 to C3) was 58, 55 and 44 % at 500, 1000 and 2000 individuals  $m^{-2}$ , respectively (Daly et al. 2009). Moreover, the average survival of blue crabs from Megalopa to C6 stage was  $\sim 10.5$  % when stocking densities ranged from 6 to 10 individuals  $l^{-1}$  (Zmora et al. 2005). Our study confirms the assumption that cannibalism is responsible for much of the mortality in long-term crab culture as we were able to discriminate without doubt between deaths from natural causes or predation by cannibalism.

## Stock and release

Maximizing crab production is fundamental for enhancement programs and should consider crab stage and molt condition.

Although crowding during molting enhances cannibalism, some strategies can diminish excessive mortality under massive culture conditions. Complex substrates provide refuge and reduce agonistic interactions (Chau et al. 2009; Johnston et al. 2006; Mann et al. 2007; Moksnes et al. 1998; Nguyen et al. 2008; Stoner et al. 2010). Dietary supplementation (e.g., tryptophan) can control aggression and diminish cannibalism in the mud crab *Scylla serrata*, resulting in higher survival due to the reduction of aggressive behavior (Laranja et al. 2010), and the addition of astaxanthin to the diet can improve survival of red king crabs (Daly et al. 2012a). Grading by size can also help controlling cannibalism intensity (Daly et al. 2012c), but it requires costly labor. The ideal solution for avoiding cannibalism would be to keep crabs individually with the trade-off between the compartment size and growth rate (Swiney et al. 2012) but keeping a large number of individuals in single units is likely economically prohibitive. We suggest the maintenance of juveniles at low or medium densities (maximum of 450 crabs  $m^{-2}$ ) with periodical size grading. Future work should evaluate artificial structures to further minimize agonistic behavior.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Animal rights** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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