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Suspended mesh-bags enclosures for Southern King Crab *Lithodes santolla* (Molina 1782) larvae and juvenile culture in the sea

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ABSTRACT

The potential for using suspended mesh-bags for larval/juvenile southern king crab (*Lithodes santolla*) aquaculture was assessed. Three early stages (Zoea, Megalopa and first juvenile, or C1) were confined for 45–65 days in mesh-bags near the sea-bottom. As *L. santolla* starts feeding when it reaches the first juvenile stage, the effect of biofouling presence was also tested in the C1 culture. Extreme vulnerability of the first larval stage was observed, as no live larvae were recovered when Z1 were used as seeds. On the other hand, survival of Megalopa and C1 reached 19.76 ± 1.08 and 30.00 ± 3.50%, respectively, and both C1 juvenile survival and growth were enhanced significantly by the presence of biofouling on the bags. These results indicate that suspended mesh-bags have potential for *L. santolla* juvenile culture in the sea. We suggest a 2-step culture process: indoor culturing of the three Zoea stages, and subsequently sea culture starting at the Megalopa stage. Once Megalopa molt to the C1 stage in the field, the already fouled culture system (mesh-bags) would allow newly molted crabs to feed. Further research should be directed towards sea culture optimization, including feeding requirements for bigger crabs, to implement a future stock enhancement program for king crabs in the Beagle Channel.

1. Introduction

King crabs sustain some of the world's most valuable crustacean fisheries (Otto, 2014). Mainly due to their size, historical abundance, and commercial demand, king crabs have developed as an important target species for the fishery industry. These temperate-cold water animals are harvested in both hemispheres, although at very different scales. There are three commercial species in North America's arctic and subarctic waters; the red king crab *Paralithodes camtschaticus*, the blue king crab *P. platypus* and the golden king crab *Lithodes aequispinus*, while the southern king crab (SKC) *L. santolla* is the main species captured in the Atlantic and Pacific waters of southern South America.

King crab populations around the globe have fluctuated greatly over the last decades, and almost every fishery has been considered overfished (Otto, 2014). The *Lithodes santolla* (Molina 1782) fishery is no exception. The *Lithodes santolla* fishery in the Beagle Channel started in the mid 1930's, and reached maximum landings by the mid-1970's. Largely due to the deterioration of population stocks, an area of about 180 km^2 near the city of Ushuaia, Argentina remained closed to the fishery between 1994 and 2013. This fact, in addition to the decrease in landings, led the fishery to expand northwards along the Atlantic to San Jorge Gulf. Today, the Beagle Channel supports an important regional artisanal fishery in both Chilean and Argentine areas. Although the banned area in the Argentine portion of the Beagle Channel was closed to the fishery for 19 years, the last stock survey performed in 2016 revealed that the population continues to be vulnerable (i.e. decreasing trends in catches per unit effort, landings, percentage of legal sized males and proportion of ovigerous females)(Lovrich and Tapella, 2017). This evidence suggests that former fishery restrictions were not enough to sustainably manage this species. Thus, a stock enhancement program stands an alternative and/or complementary tool for conventional management.

The enhancement of natural crab stocks, through the release of cultured juveniles to revert declining abundance, is gaining attention among scientists around the world as a result of depleted natural populations (Stevens et al., 2014), but large numbers of juveniles must be produced. This approach has been applied repeatedly in Japan, where 90 species of coastal fish and invertebrates (Bell et al., 2005), including oysters, prawns and shrimps, have been released into the wild to enhance stocks with different degree of success. In general terms, the costs of aquaculture facilities (i.e. hatcheries) include financial investments mainly in infrastructure, high technology equipment, a specialized

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workforce and supplies. Among basic supplies, feed typically represents one of the largest annual operating costs (Pillary and Kutty, 2005). In this sense, SKC has great potential as candidate for aquaculture, due to its lecitotrophic larval development. Independence of food during the larval phase represents a major advantage because of cost reduction and for water quality maintenance in culture.

Recent information concerning *L. santolla* larval culture include up to 50 and 40% of survival to megalopa and first juvenile stage (C1), respectively (Tapella et al., 2009), when massive culture is performed in recirculation water systems and when seawater parameters (including pH, salinity, nitrogen wastes, ammonium and temperature) are strictly controlled. Nevertheless, high mortality (natural and due to cannibalism), space requirements and operative costs during the early stages maintenance still constrain indoor culture, leading us to consider sea culture as an alternative or at least complementary type of culture for SKC. In this context, the main goal of the present study was to test sea culture feasibility of *L. santolla* s larvae and juveniles. In particular, we tested the survival and growth of zoea I (Z1), megalopa (M) and first crab (C1) stages in a suspended mesh-bag culture-system deployed near the sea-bottom.

2. Methods

Zoeas stage I (Z1), megalopae (M) and first crab juveniles (C1) were used as seeds and confined in plastic mesh-bags to test the feasibility of larval and juvenile sea culture. As C1 actively feed, we included this treatment in the study, testing the influence of biofouling presence in crab growth and survival.

2.1. Larval and crab rearing

In August 2014, ovigerous L. *santolla* females were captured with conical traps in Ushuaia Bay (54°49′S, 68°14′W), taken to the lab and kept individually until larvae hatching. Females were fed squid three times a week, and their containers were cleaned and checked daily for larval hatching. Newly hatched larvae (Z1) were transferred to PVC round and bottom-mesh containers (mesh size:1 mm) set in a sea water-flow recirculation system until reaching the needed stage (Z1, M or C1) for experimental use. Containers were checked daily and both dead animals and exuviae were discarded. Larvae were not feed since they are lecitotrophic (Lovrich et al., 2003, Kattner et al., 2003). When animals started to reach the first juvenile stage (C1), they were maintained in the same containers and at low densities (10–20 C1 per container) to avoid cannibalism (Sotelano et al., 2012, 2016). Controlled culture conditions included temperature (7.1 ± 1.1 °C) and 12:12 h light:dark conditions. Water quality was maintained through mechanical (20 um) and biological filters, and a UV sterilizer. Salinity, pH, ammonia, nitrite and nitrate were kept at 31 ± 1 psu, 8.1 ± 0.3, $< 0.25\ mg\ l^{-1}$ $< 0.5\ mg\ l^{-1}$ and $< 12.5\ mg\ l^{-1}$, respectively.

2.2. Culture system design deployed in the sea

The culture system consisted of an array of plastic mesh-bags placed in lines. A total of four lines were settled in the sea, each of them carrying different larval or juvenile stages (Table 1). Each line consisted of 5 (replicates) cylindrical mesh-bags (20 cm diameter, 60 cm length, mesh size: 1 mm) that could be opened at the extremes, set on a main rope and separated from each other by 1 m. Each bag had a plastic internal structure to avoid collapsing from tides and/or water currents, and they were also packed with a plastic monofilament gillnet (1 m × 4 m, mesh size: 1 cm) to provide attachment substrate and interstitial spaces for larvae and/or juveniles. Live larvae and juveniles were transported to the field in several 1 L containers set in a refrigerated box. Once in the field, animals were placed into the bags, which were immediately sealed and deployed into the water. Each line was anchored at 15 m depth, in a protected bay, near Ushuaia (54°49′S, 68°14′W) with 4 concrete ballasts (5 kg each) and a recovery line. Buoys maintained the bags between 1 and 2 m above the sea bottom to avoid the bags contacting the bottom and potential collapsing with the sediment (Figs. 1-2).

Table 1
Number of larvae or crabs initially put in each bag (Ni/bag) and number of days spent underwater in the field (days in the sea). Stage and type abbreviations: Zoea 1 (Z1), Megalopa (M), Crab 1 deployed in non-fouled bags (C1nf) and Crab 1 deployed in pre-fouled bags (C1f).

Stage	Ni/bag	Anchoring date	Days in the sea
Z1	500	10/10/14	55
M	500	11/14/14	45
C1nf	40	3/2/15	65
C1f	40	3/2/15	65

To test the effect of biofouling on C1 culture growth and survival, a line with empty bags (without crabs) was anchored for a month prior to crab deployment, allowing biofouling organisms to grow on the bags. After that time, 40 C1 crabs were put in each mesh-bag of both lines, the pre-fouled and the non-fouled one, hereafter C1f and C1nf.

A total of 5400 animals, including Z1, M and C1, were used in the experiment. As the number of newly hatched Z1 can reach 1000 individuals per day per female and almost every ovigerous female was averaging their hatching period, we were able to pool 2500 Z1 within a day. Moreover, thousands of Z1 were set up for culture to ensure the availability of another 2500 M for the experience. As large numbers of C1 stages are difficult to obtain simultaneously, and because of C1 cannibalistic behaviour (Sotelano et al., 2016, 2012), the juvenile density (C1) used in the experiments ($n = 40$ per bag, 2123 crabs•m^{-3}) was lower than the number of Z1 or M ($n = 500$ per bag, 26,539 crabs•m^{-3}) (Table 1). Two weeks were needed to gather the total amount of C1 stages (400) required for the experiment, and therefore C1 stages age varied between 0 and 15 days.

For the Z stage to reach the M stage and to guarantee for both M and C1 stages, reaching C1 and C2 stages respectively, line recovery was estimated to be performed after 45 days after settlement in the water. At 6–9 °C under laboratory conditions, the Z1 stage reaches the M stage after 19 days in average, whereas M and C1 stages last on average 33.5, and 37.5 days, respectively (Anger et al., 2004). Even though C1 stages aged between 0 and 15 days old by the time we put them in the bags, no crab was expected to molt twice during culture, according with the juvenile development time (Anger et al., 2004).

After sea culturing, bags were transported to the lab and all recovered crabs from bags were counted. Survival was estimated as the number of living crabs at the time of recovery over the initial number of larvae/crabs put in the bags ($n = 500$ for Z1 and M or $n = 40$ for C1).

To determine crab sizes, a subsample of 50 crabs recovered after megalopa culture and all crabs recovered after C1 culture were photographed, using a magnifying glass (Leica M205C). Carapace length (CL) was measured as the mid-line distance between the posterior orbital margin, excluding the rostral spine, and the posterior median margin, using Image-Pro Plus 6.0 software. Results of survival of each stage are presented as average (%) ± standard error (SE).

2.3. Data analysis

Parametric (Student's-T) tests were used to analyse survival differences ($p < 0.05$) between juvenile culture (fouled and non-fouled), as data achieved both normality and homocedasticity. No statistical analysis was performed between the two larvae culture (Z and M) because only the megalopa, and no zoea stage, actually survived. Survival analyses were performed on the previous data's angular transformation, using InfoStat software (version 2008).

Observed size frequency distributions were fitted to the expected

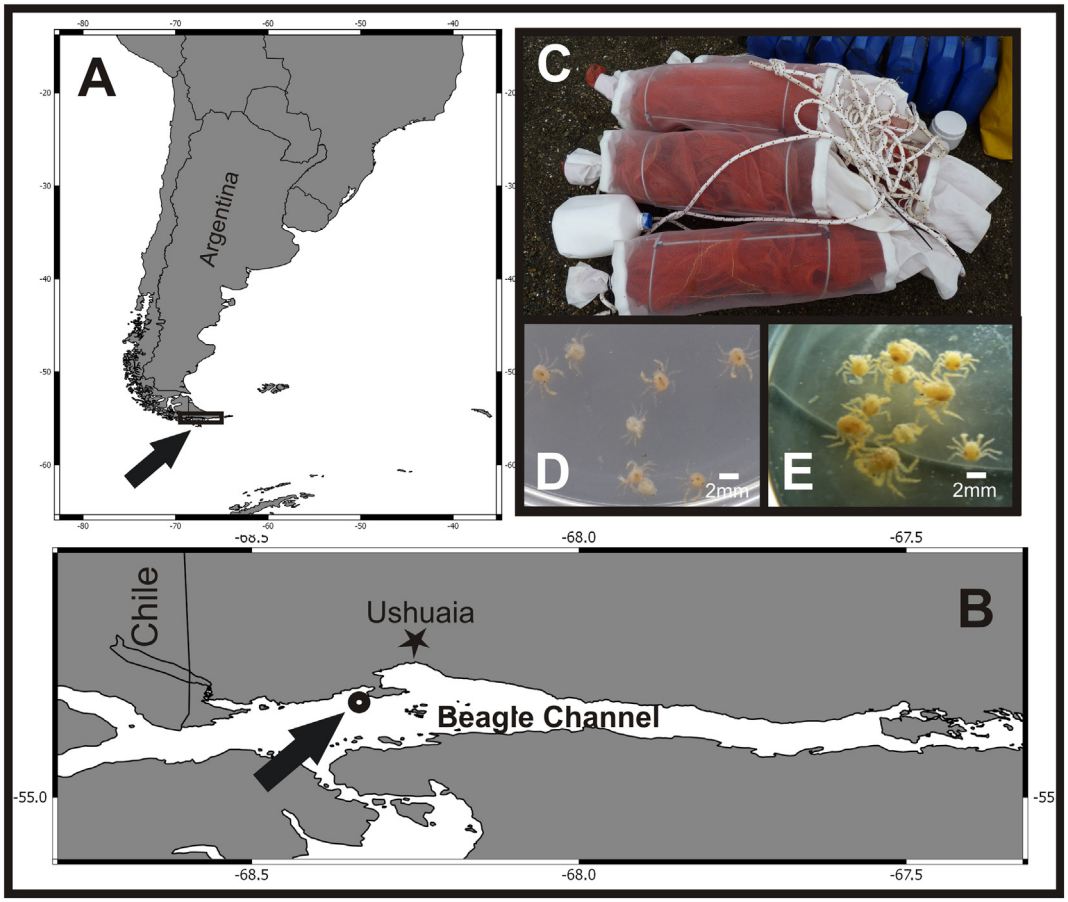

Fig. 1. Suspended mesh-bag deployment area (A-B): black circle indicates the deployment site, near Ushuaia, Tierra del Fuego, Argentina. Mesh-bag culture system (C) and C1 crabs before (D) and after sea culture (E).

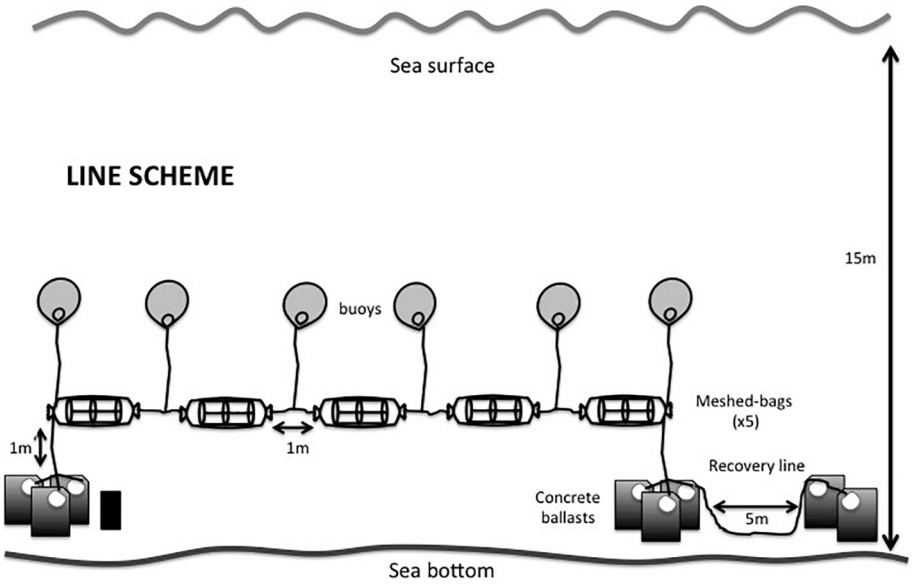

Fig. 2. Array of mesh-bags used for *Lithodes santolla* culturing. Lines were anchored to the sea-bottom as showed in the figure. Detailed information in Methods section.

values, assuming a mixture of normal distributions by the least squares method, through a modification of the procedure proposed by MacDonald and Pitcher (1979)(MIX), described by Bas et al., 2005.

3. Results

Mesh-bag cultures were maintained in the sea between October 2014 and April 2015. During this period, sea-water temperature ranged between 8.1 and 8.8 °C. Although lines were intended to last under

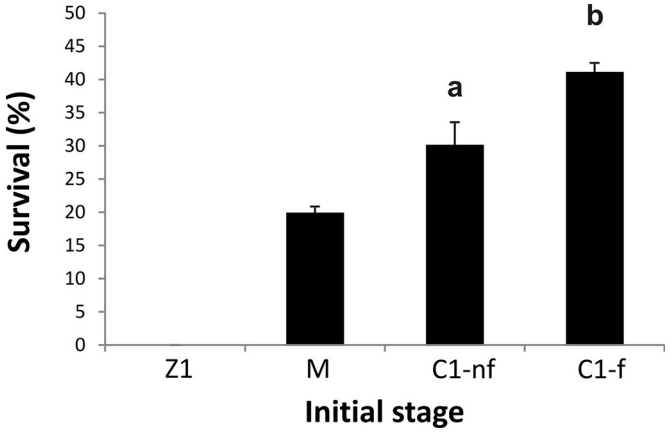

Fig. 3. Average (± SE) percent survival of zoea 1 (Z1), megalopa (M), crab 1 deployed in non-fouled bags (C1nf) and Crab 1 deployed in pre-fouled bags (C1f) of *L. santolla* after sea culture. Different letters indicate statistical significance (Student *t*-test, $p < 0.05$).

water for 45 days bad weather interfered and line recovery was delayed, as shown in Table 1.

We observed a temporal assemblage pattern of the biofouling community, although the analysis of successional development of biofouling communities was not the aim of the present study. We observed the presence of different organisms in fouled and non-fouled bags after culture. The main organisms found in non-fouled bags (65 days under water) were green algae, while juvenile bivalves, ascidians, polychaetes and hydrozoans prevailed in fouled bags (95 days under water). Amphipods were also found in fouled bags, but in lower proportion.

3.1. Animal survival

All recovered crabs were found alive and with all their appendages (i.e. no crab was found dead inside the bags). Survival was related to the animal's development stage at the time it was placed in the bags, with more developed stages having higher survival over the experimental period (Fig. 3). No animal was recovered after 55 days for the Zoea stage culture (100% mortality). In contrast, we registered 19.76 ± 1.08% survival after 45 days in the megalopa culture, and all animals recovered correspond to the C1 stage. Among juveniles, survival was enhanced by the presence of biofouling (Student's *t*-Test $p < 0.01$). Survival reached 30 ± 3.50% and 41 ± 1.50% in C1nf and C1f culture, respectively (Fig. 3).

3.2. Animal size

Crabs recovered from the megalopa culture ranged between 1.65 and 2.53 mm CL, and all corresponded to C1 stages. In particular, the analyses of size frequency distributions revealed a unimodal distribution for crabs recovered after M cultured in the sea, with a modal group mean of 1.55 ± 0.05 mm CL (Fig. 4A).

On the other hand, crabs recovered from the juvenile culture, showed a wider range of sizes and a polymodal type of size frequency distribution. Sizes ranged from 2.14 to 3.73 and 2.35 to 4.10 mm CL in non-fouled and fouled bags, respectively. In our study, 3 modal groups explained the size frequency distributions for juveniles obtained from both fouled and non-fouled bags (Fig.4 B–C). We use the term “modal group,” mainly because these groups could be composed by individuals of different stages (see ‘Discussion’ for a further details). The three fitted modal groups (1, 2 and 3) explained the size frequency distribution with a good fit ($p > 0.99$, Fig. 4) in both fouled and non-fouled bags. The mean size of each three modal groups observed in fouled mesh-bags was higher than those found in non-fouled bags.

4. Discussion

This study provides the first evidence for sea culture feasibility of early life stages of *L. santolla*. These results indicate that massive rearing of southern king crab megalopa and early juveniles in suspended mesh-bags is viable and constitutes a promising culture option, mainly if efforts are directed towards sea culture technology improvement.

An unexpected finding was that newly hatched larvae (Z1 stage) did not survive in the field. We suggest their mortality was likely the consequence of physical damage rather than natural mortality. If compared with megalopa and C1 stages, zoea stages lack of a well-developed chela, and instead have rudimentary pereiopods (Campodonico, 1971) to hold onto the culture materials (i.e. gillnet). Also the Z1 stage requires three molts and 19 days to reach the Megalopa stage at 8 °C (Anger et al., 2004). Soon after each molt event, zoeae remain in a vulnerable physical condition (i.e. soft carapace) and would be susceptible to injuries as a consequence of larvae hitting the mesh-bags structure due to the absence of grip appendages and flowing currents (Balestrini et al., 1998). Thus, zoea larvae could have died soon after they were placed in the sea and/or after molting events and remains could have disintegrated with time.

Megalopa and first juvenile (C1) survival represents one of the major findings of this research, not only because animals survived in field enclosures, but also because they succeeded in molting several times during the experimental period. Overall the survival rates registered in our experiences for megalopae and first juveniles (20 and 40% survival) are comparable not only with our previous findings in lab culture (Tapella et al., 2009) but also with red king crab (*Paralithodes camchaticus*) cultures. Juvenile *P. camthaticus* hatchery-scale culture has been proven successful with an overall survival ranging from 23 to 60% when crab density varied between 1400 and 400 crabs m^{-2} (Daly et al., 2012). Moreover, the survival rates found in the present study supersede those observed in other commercially-important crustacean species in hatcheries and also in the field. Japan, one of the pioneers in crab aquaculture and assessment of stock enhancement programs, has centered efforts in developing hatchery technology since the 1960’s. Mainly focused on portunid crab production, they reached survival rates that range between 9.4 and 11.2% for *Portunus trituberculatus*, *P. pelagicus* and *Scylla paramamosain*, from hatching to the first crab stage (C1)(Katsuyuki et al., 2010). Higher survival rates were found for the blue crab *Callinectes sapidus,* averaging 5.2% to crab stage 6 (C6) (Zmora et al., 2005).

Also, sea culture attempts have previously been reported for other crustaceans, such as the lobsters *Homarus gammarus* and *H. americanus* and *Jasus eduardsii* (Beal and Protopopescu, 2012; Daniels et al., 2015; Jeffs and James, 2001). Daniels and co-workers (2015) reported survival rates over 56% for stage V lobsters. Similar to our work, larval stages were first raised in hatcheries and then transferred to the field, but lobsters were maintained isolated from each other in the field, in individual containers, instead of massively pooled, as in our trials. The individual stocking in the field prevented animal interaction (i.e. cannibalism) and diminished potential competition for food.

Despite the encouraging survival results, the remaining question is why did the rest of the crabs die? Predation and escapes are not possible answers, as bags were found intact and without holes meaning no predator could have entered the bags nor any crabs could have left. Thus, cannibalism was identified as the potential main source of mortality in our field enclosures, but also the mechanical action of the culture device materials over Megalopae and crabs must be considered. Cannibalism is a frequent behaviour when SKC's early stages are pooled together (Sotelano et al. 2012, 2016). Under laboratory conditions, cannibalism occurs during both intermolt and molting periods, when animals are sharing a limited space and agonistic encounters are frequent even in short experimental time lapses (16–25 days). Higher mortality during molting times suggests that molting events play an important rol as mortality enhancers because they produce: 1) soft-

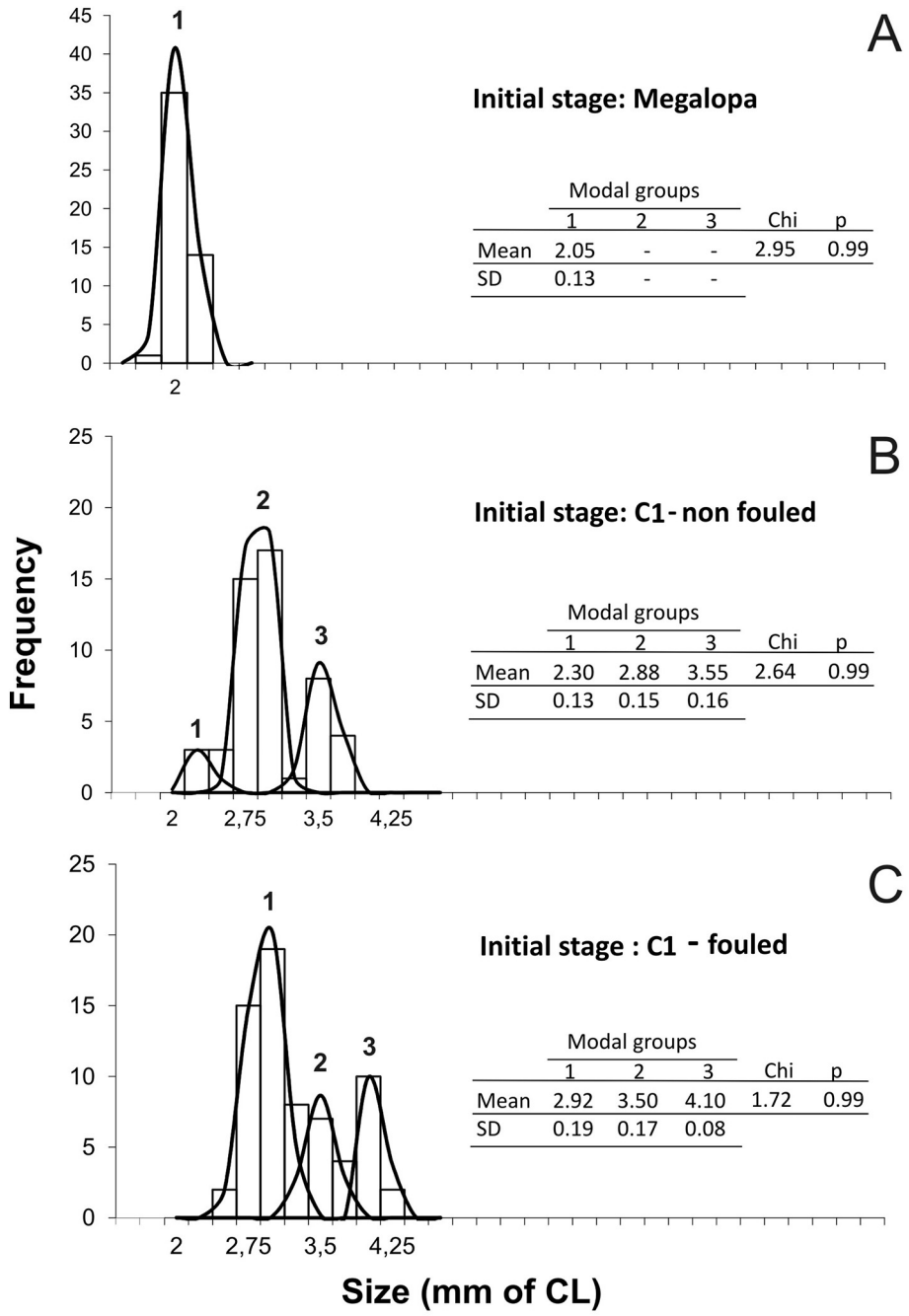

Fig. 4. Size frequency distribution of crabs recovered after megalopa (A), C1 non-fouled (C1nf) (B) and C1 fouled (C1f) culturein the sea. Each value in the X axis is the superior limit for each size class. Numbers indicate each modal group found in each distribution. Tables show the mean ± SD of each modal group and Chi^2 and *p* values. CL: carapace lenght.

shelled crabs that are prone to intraspecific predation and 2) coexistence of different stage/size crabs, which was also proven to double cannibalism rates in SKC (Sotelano et al., 2016). Therefore, we hypothesized that cannibalism in our grow-out culture occurred and was enhanced by particular conditions, such as longer culture period (up to 65 days), several molting events (1–3) and potentially suboptimal feeding during culture. In our experience, even though biofouling enhanced both survival and growth beyond 10 and 8%, respectively, very likely due to consumption (Beal, 2012), crabs could have also suffered some kind of energetic restrictions caused by suboptimal nutritional conditions of available biofouling. Consequently, suboptimal feeding (i.e. food shortage) during culture may have influenced and promoted consumption of conspecifics (Brodersen et al., 1989; Moller et al., 2008).

On the other hand, although our laboratory experience indicates that Megalopae and juveniles can firmly hold on to rugged materials, such as these mesh-bags, we suggest that the friction of animals (mechanical effects) against the mesh material could produce physical damage. Unlike Zoea stages, both Megalpae and juveniles are especially difficult to release through suction from meshed-containers while daily checked in laboratory. Thus, we expect that Megalopa/juveniles could hang on to the internal or external mesh of the bags, enduring water movements from currents but are not exempt from potential events of friction with the bag material, if they somehow collapse. Mortality from this mechanical action could have also been intensified during and shortly after molting events, when exoskeletons remain soft. Future

Table 2
Average carapace length (mm) of early *Lithodes santolla* juveniles from and field and laboratory data. Standard deviation is shown in parentheses.

	Initial stage	C1	C2	C3	C4	C5	C6
Present study (field data)	Megalopa	2.05 (0.13)					
	C1 non fouled		2.3 (0.13)	2.88 (0.15)	3.55 (0.16)		
	C1 fouled			2.92 (0.19)	3.50 (0.17)	4.10 (0.08)	
Sotelano (2013) (laboratory data)	C1	1.98 (0.05)	2.35 (0.08)	2.74 (0.12)	3.16 (0.17)	3.60 (0.27)	4.40 (0.29)

studies should provide both internal and external rigid structures to the bags to avoid or at least diminish mechanical damage over crabs during culture.

Since large-scale culturing functions as a "black box" where researchers know initial and final condition of crabs, without knowing what happens in between, we cannot distinguish whether the dispersion among size classes in both the non-fouled and fouled bags, may have been caused by the variability of one or both components of growth: molt frequency and/or molt increment. Juveniles that were placed into the bags aged between 0 and 15 days (in C1 stage). Sea culture lasted 65 days, thus the younger and older crabs recovered aged between 65 and 80 days. Under laboratory conditions at 8 °C it takes a minimum of 76 days for a C1 to reach C3 stage (Sotelano, 2013). Thus, considering that the oldest crabs recovered were 80 days, crabs in both fouled and non-fouled cultures, may correspond to C3 as the most developed stage and even the majority should correspond to C2 stage. Nevertheless, if crab size is taken into account, size of recovered crabs suggests that each modal group correspond to: C3, C4 and C5 in fouled bags, and C2, C3 and C4 in non-fouled ones (Table 2, Sotelano, 2013). Finally and regardless the stages attained by the crabs, it stands clear that recovered crabs attained bigger sizes if compared with those raised in the laboratory (Table 2) and more interestingly, a tendency is shown for crabs after "fouled" condition to grow bigger and especially faster.

4.1. Applicability of suspended mesh-bag enclosures

Regarding the feasibility of sea culture for L. *santolla*, we propose a combined culture system. Mortality of newly hatched zoea in sea bags suggests that early larval stages should be raised in the lab, under controlled conditions, and animals should be only transferred to the field in Megalopa or even C1 stage. Despite C1 stages had higher survival after sea culture, choosing Megalopa (instead of C1) as the "seed" stage for the bags, would reduce indoor culture periods and facilitate a new set of animals ready to be transferred to the sea every ≈19 days. Due to *L. santolla*'s extended hatching period (Thatje et al., 2003), the megalopa production would last between 1 (single female) and 3 months, depending on the number of ovigerous females available and hatching synchronization. Although the megalopa stage is lecithotrophic, by the time those megalopae molt to C1 stage and start feeding, the mesh-bags that contain them would be already fouled, allowing recently molted crabs to forage on the settled organisms on the mesh. Thus, we propose, as a next step for SKC sea culture research, to test the culture devise presented here with megalopae in advance development stage (i.e. 20–25 days old), meaning that they would be close to molt to C1 shortly after sea deployment. Therefore, we expect to considerably reduce mortality since megalopa swimming behavior declines with time (Stevens, 2003), and consequently they would spend more time griped to the gillnet or the mesh bag. Moreover, if juveniles are meant to last several molts and/or long time periods in the sea, both the potential biofouling problem and periodical external feeding should also be addressed. If biofouling overgrows, it may prevent water flowing through the bags, diminishing oxygen concentration and even causing bags to drag down (Fitridge et al., 2012; Green and Grizzle, 2007; Swift et al., 2006) and finally leading the set up device to collapse. Biofouling is typically managed by frequent net cleaning or regular net changes (Fitridge et al., 2012) and should be taken into account for optimizing sea culture.

Because of costs associated with maintaining large numbers of small crabs under controlled conditions (indoor facilities or hatcheries), the results of our short-term field experiment indicate that an adequate massive sea culture technique/system is a simple and viable option for southern king crab culture and should be considered as a complement for the indoor type of rearing. Taking advantage of the lecitotrophic larval development, and the extended hatching period of *L. santolla* (Thatje et al., 2003), we suggest a mixed type of culture for raising early stages: combined techniques of indoor (hatchery) and outdoor culture (sea culture, as here presented) may represent the best alternative to ensure both short term animal survival and growth. This culture technique represents the first step towards the production of large numbers of juveniles to implement an enhancement program for southern king crab in the Beagle Channel to support both to the natural population and for the fishery stock to recover.

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