Journal of Experimental Marine Biology and Ecology 411 (2012) 70–77

Contents lists available at SciVerse ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Experimental natural substrate preference of southern king crab *Lithodes santolla* larvae

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ARTICLE INFO

Article history:
Received 11 August 2011
Received in revised form 2 November 2011
Accepted 3 November 2011
Available online 30 November 2011

Keywords:
Density
Habitat selection
Lithodid
Megalopa
Zoea

ABSTRACT

Notwithstanding the commercial importance of *Lithodes santolla* in the southern tip of South America, little is known about settlement habitat and behavior of their larvae. Such information is relevant for understanding its life history and for management and development of fishery-stock enhancement programs. The aims of this study were to determine the natural substrata that larval stages, zoeae and megalopa select for recruitment, and the effects of megalopa density and diurnal–nocturnal phase on such selection. Different laboratory experiments with durations of 8 h to 4 weeks were performed in 6-L round containers with their bottoms divided in four equal portions, each filled with sand, gravel, cobble and broken shell as substrata. Containers were kept in a cold room at 7.1 ± 0.5 °C and under 12:12 h light and dark photoperiod. Trials began with the release of larvae of different stages in the center of the containers. After different time periods, proportions of larvae swimming or settled on each substrate were determined. Larvae selected and settled on natural substrata immediately after being placed into the containers. Experiments showed that all larval stages (zoeae and megalopa) preferred complex substrata such as broken shell, cobble and gravel over sand which was rejected. The megalopa selects the substrate even during night period. Selection seems to be density-dependent since at the lowest density broken shell was the preferred substrate. Selection of complex substrata (i.e. mussel beds and/or shell fragments in nature) by all larval stages, even as early as the first zoea stage, provides a cryptic habitat which may reduce mortality by predation and/or cannibalism. Knowledge on habitat preference is useful for fishery management and also for assessing the different habitats in a potential stock enhancement program of southern king crab.

1. Introduction

In many marine decapod crustaceans the transition from planktonic larvae to benthic juveniles plays a key role during recruitment. This transition involves many factors (e.g. current, tide, salinity, temperature, settling behavior, cannibalism, predation, or competition) (Fernández et al., 1993; Forward, 1990; Hasek and Rabalais, 2001; Heck et al., 2001; Moksnes et al., 2003; Phillips et al., 1991; Stevens, 2003; Sulkin and Epifanio, 1984; Van Montfrans et al., 2003) which may affect the recruitment success and reduce significantly the number of individuals surviving to adulthood (Rabalais et al., 1995; Wahle, 2003; Wahle and Steneck, 1991). This demographic bottleneck effect is of special relevance for management in species of fishery and aquaculture interest (Rabalais et al., 1995; Wahle and Steneck, 1991). For example, the density estimation of young juvenile lobsters is a good indicator of interannual variations in settlement and is a good proxy of future harvests (Incze et al., 2003).

Settlement behavior is one of the most important factors affecting recruitment since it involves the selection of an adequate substrate that provides shelter and food during the critical early juvenile stages. Several studies have demonstrated that megalopae actively select substrate to settle before they metamorphose to the first juvenile crab (Stevens, 2003; Van Montfrans et al., 2003; Wahle and Steneck, 1992). Such selection can be made on the basis of adult conspecific presence (e.g. *Petrolisthes cinctipes* (Randall, 1839); Jensen, 1991, *Petrolisthes laevigatus* (Guérin, 1835); Gebauer et al., 2011 and *Uca pugilator* (Bosc, 1802); O'Connor, 1993), or in response of chemical cues which orientate megalopae towards nursery areas (e.g. *Callinectes sapidus* Rathbun, 1896; Forward et al., 2003). Also, many studies suggest that megalopae do such selection on the basis of settlement habitat complexity. For example, the fourth larval stage of the American lobster *Homarus americanus* Milne Edwards, 1937 selects rapidly sheltered habitat such as macroalgal-covered rocks, and delays settlement when sand substrate are offered (Botero and Atema, 1982). Megalopae of the intertidal Dungeness crab *Cancer magister* Dana, 1852 settle near shore and select shell habitat over mud (Fernández et al., 1993). Particularly, megalopae of the red and blue king crabs (*Paralithodes camtschaticus* (Tilesius, 1815) and *Paralithodes platypus* Brandt, 1850) actively select complex natural substrata (Stevens, 2003; Stevens and Kittaka, 1998; Stevens and Swiney, 2005; Tapella et al., 2009).

The southern king crab (SKC) *Lithodes santolla* Molina (1782) is the most valuable commercial species among the lithodids that

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doi:10.1016/j.jembe.2011.11.003

inhabits the Sub-Antarctic waters of South America. Distribution of SKC is associated with cold-temperate water, between 4 °C and 12 °C, and commonly occurs at shallow waters south to 41°S. Off the Pacific coast, *L. santolla* is distributed south from Chiloé Island (42°S 74°W) to the Cape Horn (55°S 67°W) including the Straits of Magellan (53°S 70°W) and fjords around Tierra del Fuego (Macpherson, 1988). In the Atlantic waters *L. santolla* has a disjoint distribution: mainly in the Golfo San Jorge (46°S 66°W) and the Beagle Channel (54°S 67°W) (Boschi et al., 1992; Lovrich et al., 2002; Retamal, 2000). The SKC fishery around Tierra del Fuego began during 1920s and 1930s in Chilean and Argentinean waters, respectively (Guzmán et al., 2004; Vinuesa, 1991). Maximum yields of the Chilean fisheries were at least one order of magnitude higher than in Argentina: annual landings peaked at 320 t in the Argentinean Beagle Channel and 2756 t in the Chilean fisheries. After a constant reduction in landings from the Beagle Channel, in 1994 the fishery for *L. santolla* was closed due to overfishing (Lovrich, 1997). The decreasing yields of *L. santolla* in both Argentina and Chile also encouraged retaining the sympatric less valuable species, the stone or false southern king crab *Paralomis granulosa* Jacquinot, 1847, which had been considered a bycatch species at early stages of the fishery. Currently, though *P. granulosa* is the main target of the fishery, the catch pressure on the SKC *L. santolla* remains high because of its elevated commercial value in the local and international markets.

L. santolla has an annual reproductive cycle. In late November–early December and immediately after females molt, mating occurs, oocytes are fertilized and eggs develop for approximately 9–10 months before hatching (Lovrich and Vinuesa, 1999; Vinuesa, 1991). Larval hatching extends for 4–6 weeks (Thatje et al., 2003) and larval development is completely nonfeeding (endotrophic) (Lovrich et al., 2003) from hatching to metamorphosis (3 zoea stages followed by a megalopa) (Campodonico, 1971; McLaughlin et al., 2001). Southern king crab larvae are absent from plankton samples (Lovrich, 1999) and in laboratory larval rearing they remain associated with the aquarium bottom (Anger et al., 2004; Calcagno et al., 2004; Vinuesa et al., 1985), suggesting that they have a strong epibenthic habit. There are few studies dealing with distribution of early stages of *L. santolla* and all revealed that they are associated with 3-dimensional habitats, such as the holdfast of the kelp *Macrocystis pyrifera* (L.) C. Agardh, 1820 (Brusca et al., 2000; Cárdenas et al., 2007). Juveniles of 1.5–13.5 mm carapace length (CL) occur at <40 m depth and settle in passive collectors that were also colonized by sea urchins, polychaetes and ophiurids (Tapella and Lovrich, 2006).

Despite the commercial importance of SKC *L. santolla* in the southern South America, little is known about the settlement habitat and behavior of their larvae. Thus, the aims of this study, as a part of a stock enhancement program, were to determine whether SKC larvae (zoea and megalopa stages) exhibit a preference for any of four natural substrata with different complexity level, which are commonly found in the distribution depth range of early stages (Brambatti et al., 1991; Colizza, 1991; Pineda et al., 2002). Two additional experiments were done with megalopae to test whether substrate selection was affected by their density or the schedule (day and night) at which the trials started.

2. Materials and methods

2.1. Animals captured and maintenance

During August 2006, 25 SKC ovigerous females were caught with commercial traps in the Beagle Channel at the proximity of Ushuaia city (54°S 67°W) and were taken to the wet laboratory of the Centro Austral de Investigaciones Científicas (CADIC). Throughout the two-month-hatching period, females were maintained in an increasing temperature regime from 7 to 10 °C, in individual 20-L containers which were set up in a recycling 3000-L seawater system. Water quality was maintained with mechanic (20 μm) and biological filters, and UV-sterilizer. Nitrogen wastes were measured twice a week and ammonia (NH_3/NH_4^+), nitrite (NO^{-2}) and nitrate (NO^{-3}) levels were kept under 0.25, 0.8 and 12.5 mg·l^{-1}, respectively by the replacement of ~1000 l of seawater (commonly every ~10 days). Females were fed ad libitum twice a week with squid (*Illex* spp.) and the leftovers were removed the following day.

2.2. Larval cultivation

During the peak of the hatching period (*ca.* 400–1800 larvae·day^{-1}; Tapella, unpublished results), stage 1 zoeae (Z1) were collected by siphoning with a tube of 10 mm diameter, thus avoiding larval damage. Massive larval cultivation allowed us to obtain a large number of stage zoeae 2 and 3 (Z2 and Z3) and megalopae for substrate preference experiments. Larval cultures were performed in a cold room at 7.1 ± 0.5 °C with 12:12 h dim uniform light (0.5 lx) and dark photoperiod. Larvae were reared at a density of 30 individuals·l^{-1} in round containers of 11.5 l (24×25.5 cm of diameter and height, respectively). Containers were filled with 10 l of seawater previously filtered to 5 μm and UV-sterilized and aerated continuously to maintain oxygen at saturation levels and keep larvae suspended in the water column. Water was changed three times per week (Monday, Wednesday and Friday) and larvae were not fed since they are lecitotrophic (Lovrich et al., 2003). Dead larvae were removed every time that water was changed, and close to each molting stage periods daily observations were made to determine the occurrence of different larval stages. Substrate selection experiments were performed with freshly hatched Z1, with Z2 and Z3 that molted in the previous 24 h, and with megalopae that molted 96 h before the start of the trials.

2.3. Zoea and megalopa substrate preference

In order to determine natural substrate that SKC zoea and megalopa stages prefer to settle and whether they redistribute among substrata over time, two experiments of 24 h and 4 week duration were performed for each zoea stage (Z1, Z2 and Z3) and megalopa, respectively. Experiments were conducted in 6 L-round containers (22×16 cm of diameter and height, respectively) with their bottoms divided in four equal portions by a cross-shaped white plastic sheet of 5 cm height. Each section of the container was filled with one of the following natural substrata: 150 cm^3 of beach sand (<0.1 cm), 220 g of gravel (0.1–0.34 cm), 250 g of cobble (0.96–1.9 cm), and 110 g of broken shells of 0.34–0.96 cm length of several bivalves as mussels *Mytilus chilensis* (Hupé, 1854), *Perumytilus purpuratus* (Lamarck, 1797) and *Aulacomya atra* (Molina, 1782) and clams *Eurhomalea exalbida* (Chemnitz 1795) and *Tawera gayi* (Hupé, 1854). Particle size for each experimental substrate was controlled by sieving. Before setting up the substrata into round containers, they were rinsed three times with fresh water to remove naturally occurring organisms and dried at 50 °C. Substrata were randomly sorted in each portion of the round container to avoid location effect. The surface of each substrate was *ca.* 3–4 cm below the upper lip of the white plastic divider. This design allowed larvae to settle in any substrate and change among them only by an actively swimming over dividers. Experiments utilized a total of 40 round containers (4 for each zoea stages and 28 for megalopa) filled with 5 l of filtered (5 μm) and UV-sterilized seawater. Containers were randomly distributed in a single rack inside the cold room where larvae were cultured. During the course of experiments, all containers were covered with a white plastic sheet in order to avoid any possible external perturbation.

Experiments began with the release of 36 larvae belonging to the corresponding stage in the center of each container at water surface by means of a pipette. During the course of the experiments four replicate containers of each larval stage were analyzed by removing them from the experiments after periods of 24 h, 2, 4, 7, 14, 21 and