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Settlement behavior and substrate preference of the coconut crab *Birgus latro* megalopae on natural substrata in the laboratory

Katsuyuki Hamasaki *, Naoki Ishiyama, Shuichi Kitada

Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Konan, Minato, Tokyo 108-8477, Japan

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ABSTRACT

Although they are land dwellers, coconut crabs, *Birgus latro*, spend planktonic larval development in the sea and immigrate to the land as megalopae carrying gastropod shells. Better knowledge of settlement behavior and habitat requirements during the early life stages can assist in conservation of depleted coconut crab populations. Our objectives were to evaluate settlement behavior of coconut crab megalopae from 0 to 6 d post-metamorphosis (dpm), and determine the substrate preference (open sand, coral fragments, or brown algae) of 0–12 dpm megalopae. Early megalopae (0–3 dpm) were active swimmers during the night. The walking activity of naked megalopae was directly correlated with shell-inspection behavior during the day, peaking at 2–3 dpm. Almost all megalopae wore shells by 5 dpm and the walking activity of megalopae wearing shells was higher during the night than the day. Based on our observations, we hypothesize that megalopae migrate to the coast by nocturnal flood-tide transport and emigrate from the sea during the nocturnal full tidal phase to minimize predation risk. In the substrate choice experiment, early megalopae exhibited no preference for any substrata, and shell-less megalopae experienced the highest mortality, probably because of cannibalism. The survival rate plateaued at 9 dpm associated with an increase in the proportion of individuals wearing shells and of the onset of active habitat selection for complex substrata (coral fragments or brown algae). This suggests that shells and structural complex habitats are important for survival during the initial benthic phase of the coconut crab.

1. Introduction

Many benthic decapod crustaceans have a complex life cycle that includes embryonic, meroplanktonic larval, and sedentary benthic juvenile–adult phases (Anger, 2001, 2006). Individuals spend their pelagic larval life as a planktonic zoea before metamorphosing into a megalopa, during which time the transition from the pelagic zone to the benthos takes place. Thus, the zoeae and megalopae have significant roles in dispersal and recruitment processes, respectively. Larvae exhibit a positive or a negative directional movement in response to physical stimuli such as light intensity and wavelength, barometric pressure, gravity, temperature, salinity, and currents (Anger, 2001; Forward, 1988, 2009; Forward and Tankersley, 2001; Sulkin, 1984). Behavioral responses and/or endogenous swimming activity rhythms timed to a particular tidal phase allow larvae to migrate during the dispersal and recruitment periods by regulating their vertical distribution in relation to the different directions and strengths of water currents between depths and tidal cycles (Anger, 2001; Forward and Tankersley, 2001; López-Duarte and Tankersley, 2007, 2009; Ogburn et al., 2013; Sulkin, 1984; Zeng and Naylor, 1996a,b). Megalopae actively select a habitat suitable for settlement by detecting abiotic and biotic environmental stimuli such as changes in temperature and salinity, and chemical cues derived from conspecific adults and/or nursery areas (Anger, 2001, 2006; Forward et al., 2001). Furthermore, megalopae often select structurally complex habitats for settlement, including sea grass, algae, gravel, cobbles, and mollusk shells (Dittel et al., 1996; Fernandez et al., 1993; Stevens and Kittaka, 1998; Stevens and Swiney, 2005; Stoner, 2009; Tapella et al., 2009, 2012; van Montfrans et al., 2003). Complex habitats reduce predation on megalopae and juveniles by fish and crustacean predators (Dittel et al., 1996; Fernandez et al., 1993; Stevens and Swiney, 2005; Stoner, 2009). Therefore, settlement behavior is a critical trait for survival from settlement through early post-settlement stages in decapod crustaceans as it involves the selection of an adequate substrate that provides shelter and food.

The coconut crab *Birgus latro* (Linnaeus) is a terrestrial crab that inhabits oceanic islets and atolls as well as islands in the tropical Indo-Pacific coastal region (Drew et al., 2010; Hartnoll., 1988). Subtropical populations also occur in Taiwan and the Ryukyu Islands, Japan (Drew et al., 2010; Hamasaki et al., 2009; Sato et al., 2013; Wang et al., 2007). The coconut crab is an anomuran belonging to Coenobitidae (McLaughlin et al., 2010). This species, like *Coenobita*, acquires an empty gastropod shell to provide protection for the soft pleon during the megalopal and early juvenile stages. However, individuals are no longer reliant on a shell for protection after the pleon has grown and hardened (Hamasaki et al., 2014; Harms, 1938; Kadiri-Jan and Chauvet,

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* Corresponding author. Tel./fax: +81 3 5463 0538.
E-mail address: hamak@kaiyodai.ac.jp (K. Hamasaki).

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1998). Coconut crabs can reach 4 kg in weight and are easily captured by hand (Brown and Fielder, 1991). Because of these traits, the coconut crab has been an economically important resource for human consumption throughout its range. However, populations have been severely depleted on most inhabited islands owing to over-harvesting and habitat degradation (Brown and Fielder, 1991; Drew et al., 2010; Fletcher, 1993). This crab species is globally protected, and was first listed as "vulnerable" under the IUCN Red List, but was recently categorized as "data deficient" in that list — not because the species had recovered but because of the lack of available data (Drew et al., 2010; Eldredge, 1996). In Japan, the coconut crab is listed as "vulnerable" in the Red Data Book by the Ministry of the Environment.

Although the coconut crab is fully terrestrial, it depends on aquatic environments for completing its life cycle; females hatch their eggs in the sea and the larvae develop through three–five (usually four) planktonic zoeal stages in 2–3 weeks before they metamorphose into megalopae (Hamasaki et al., 2009, 2013a; Reese and Kinzie, 1968; Wang et al., 2007). After settlement, the megalopae of this species identify and co-opt gastropod shells before migrating onto land and metamorphosing into the juvenile stage. To understand the recruitment process of the coconut crab in the sea, we previously evaluated the behavior of megalopae and juveniles of the coconut crabs during the sea-to-land transition in a laboratory environment (Hamasaki et al., 2011). The environmental factors we tested included the availability of gastropod shells, substrata type (small and coarse coral sands), including shelters on land, and humidity (relative humidity, 76% and 98%). Our results suggested that megalopae developed shell acquisition behavior and emigrated from the sea at around 10 d post-metamorphosis (dpm). High humidity conditions stimulated emigration from the sea and individuals concentrated under shelters on land. In this prior study, our inference was limited to behavioral data that were collected once a day during the daytime. Substrate preference at settlement before landing has not been evaluated in the coconut crab.

Our objective with this study was to evaluate settlement behavior and substrate preference exhibited by coconut crab megalopae before they migrate from the sea. We conducted two laboratory experiments. In experiment 1, we evaluated behavior in relation to settlement by megalopae using a video recording system from 0 to 6 dpm. In experiment 2, we tested whether megalopae (0–12 dpm) exhibit a preference for any of three natural substrata with different structural complexity.

2. Materials and methods

2.1. Crab cultivation

Ovigerous females were captured by hand from late June to early July in 2010 and 2012 on Hatoma Island (24°28′N, 123°49′E) and Ishigaki Island (24°27′N, 124°07′E), Okinawa Prefecture, Japan, and transported to the laboratory at Tokyo University of Marine Science and Technology, Tokyo. The crabs were maintained in tanks following the method prescribed by Hamasaki et al. (2009) and Hamasaki (2011). After the larvae hatched, all females were released back to their natural habitat.

Larvae that hatched from two females on July 16, 2010 (brood 1) and on August 3, 2012 (brood 2) were stocked in 1-l beakers and cultured by feeding a rotifer *Brachionus plicatilis* species complex and *Artemia* sp. until they metamorphosed to megalopae, as described in our previous papers (Hamasaki et al., 2009, 2011, 2013b). Recently metamorphosed megalopae were stocked in 1-l beakers (brood 1) or 30-l tanks (brood 2) containing artificial seawater (approximately 28 °C, approximately 34–35 salinity; SEALIFE, Marine Tech Ltd., Tokyo, Japan). The megalopae were not provided with shells before use for experiments. They were fed frozen first-stage zoeae of the coconut crab and *Coenobita* spp. and frozen mysid shrimps.

2.2. Experiment 1: settlement behavior

Megalopae from brood 1 were individually housed in small transparent plastic containers (0.6 cm wide, 4.6 cm long, and 5.8 cm high) containing seawater (approximately 28 °C, 35 salinity, approximately 15 ml) and a hard 2.8 cm^2 substrate consisting of black sand (1.7 mm mean diameter, approximately 5 mm thickness). A cleaned gastropod shell of *Littoraria* sp. (mean shell length ± SD, 4.4 ± 0.4 mm, $n = 70$) was placed at the bottom of each container. Ten containers were placed in a temperature-controlled dark room (28 °C ± 0.5 °C) equipped with a fluorescent lighting system. The photoperiod was regulated at a 12L:12D cycle and the light intensity was 4 μmol s^{-1} m^{-2} around the observation containers during the light period (6 a.m.–6 p.m.). Megalopal behavior was recorded for 6 h per day: 3–4 p.m., 7–8 p.m., 11–12 p.m., 3–4 a.m., 7–8 a.m., and 11–12 a.m. using a video recording system with CCD cameras (WM-N041DNR f = 3.6 mm, Sony Co., Ltd., Tokyo, Japan) and an infrared light source (850 nm). Coenobitid crabs have no spectral sensitivity to this wavelength (Cronin and Forward, 1988). Behavioral observations were conducted on megalopae from 0 to 6 dpm. To avoid the possible effects of prey organisms on animal behavior, megalopae were not fed during the observation period. To avoid the influence of starvation on animals, individual test animals were exchanged daily with new ones from the same cohort between 1 and 2 p.m. Thus, we assigned a change in the age of animals at 1–2 p.m. each day. No megalopa was used more than once.

We quantified the behavior of individual megalopae using the video records. The individuals were treated as replicates. The following six behavioral events were documented: resting state on the substrate; swimming using the pleopods in the upper, mid, or lower layers of the water column (each layer was approximately 1.9 cm high) (swimming activity in the respective layers); walking using the thoracic legs on the substrate (walking activity); inspecting a shell using the chelipeds (shell-inspection activity); wearing a shell (shell-wearing activity); and walking using the thoracic legs while wearing a shell (walking activity with a shell). These events excluding resting state on the substrate were quantified as follows: swimming activity or walking activity was represented by the total number of events from counts every 5 s (720 total counts per h); shell-inspection activity, shell-wearing activity or walking activity with a shell was represented by the total number of times (s) an individual engaged in the designated event during 1 h (3600 s) instead of the number of events because shell-inspection activity was a very short event and it was not easy to count the events for shell-wearing activity and walking activity with a shell simultaneously. Observations were not performed for animals that had died after the onset of the experiments. We used 6, 10, 10, 8, 8, 8, and 8 individuals for the 0–6 dpm megalopae replicates, respectively.

2.3. Experiment 2: substrate preference

We compared three substrates with different structural complexity, open sand, dead coral fragments, and macro-algae. These were chosen based on substrates commonly found in the natural coral reef environment where coconut crab megalopae migrate (Hamasaki et al., personal observation). Small coral sand (approximately 0.5 mm diameter) and dead coral fragments (approximately 2–6 cm length, approximately 0.5–1 cm width), both obtained commercially (Mango International Corp., Kyoto, Japan, and Stone Dealer Shinsei Corp., Osaka, Japan, respectively), were boiled with fresh water, dried at room temperature, and washed in artificial seawater (35 salinity) before use. To unify the quality of macro-algae used for experiments, we purchased the commercially cultivated brown algae *Cladosiphon okamuranus*, which has a string-like branched form (Horiuchi Corp., Fukuoka, Japan). The algae were washed in fresh water and rinsed in artificial seawater (35 salinity). Algal bodies of *C. okamuranus* are found from October to early August in the coral reef environment in subtropical Japan (Touma, 1996). Therefore, taking the zoeal duration of the coconut crab into account (Hamasaki et al., 2009),