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Effects of visual and chemical cues on orientation behavior of the Red Sea hermit crab *Clibanarius signatus*

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Abstract Directional orientation of *Clibanarius signatus* toward different targets of gastropod shells was studied in a circular arena upon exposure to background seawater, calcium concentrations and predatory odor. Directional orientation was absent when crabs were presented with the white background alone. Each shell was tested in different positions (e.g., anterior, posterior, upside-down, lateral). Adult crabs were tested without their gastropod shells, and orientation varied with concentration and chemical cue. With calcium, orientation increased as concentration increased up to a maximum attraction percentage and then attraction became stable. In the case of predator cues, some individuals swim away from the target toward the opposite direction representing a predator avoidance response. Whenever, the blind hermit crab *C. signatus* was exposed to a shell target combined with calcium or predator cues, the majority of them stop moving or move in circles around the arena center. The others exhibited uniform orientation distribution. The responsiveness was higher with calcium cues than predator cues. Thus in the absence of vision, individual hermit crabs were able to detect both calcium and predator cues and have different response regarding them.

Introduction

Directional orientation is regarded as one of the major behavioral mechanisms required for survival of motile crustaceans living in intertidal areas (Diaz et al., 1995a). Many crustaceans use visual cues for orientation and are able to discriminate between shapes providing them with the ability to differentiate between predators, conspecifics and refuge (Woodbury, 1986; Romano et al., 1990; Chiussi, 2002) while others use chemical cues for orientation since aquatic organisms live in a complex cocktail of chemical stimuli (Brönmark and Hansson, 2000). Thus, chemical stimuli are a major source of information for many invertebrates about their aquatic environment especially those living in topographically complex habitats such as intertidal areas (Iglesias, 2007; Turra and Denadai, 2002; Dill, 1987; Chivers and Smith, 1998; Kats and Dill, 1998). Different stimuli elicit different behaviors including aggregation, spawning, attraction to mates, searching for food, homing in suitable microhabitats and appropriate responses to potential predators, such as hiding or fleeing. Therefore, at any particular moment, an individual must not only be a monitor of these diverse stimuli, but also rank the value of potential behavioral

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responses to decide which response is most appropriate (Dill, 1987; Kats and Dill, 1998; Dicke and Grostal, 2001).

Hermit crabs make an ideal model system for studying sensory capabilities and decision-making processes in crustaceans, because their shelters, food sources and mates may all potentially have the same appearance. This may necessitate the adaptation of behavioral and physiological means to differentiate between resources. To make efficient use of information, it must be sorted or prioritized (Billock and Dunbar, 2009).

Hermit crabs tend to use visual and chemical cues for orientation (Diaz et al., 1995a; Chiussi et al., 2001), agnostic interactions (Briffa and Williams, 2006), predator avoidance (Brooks, 1991; Rotjan et al., 2004; Chiussi et al., 2001), conspecific identification and shell location (Rittschof et al., 1992; Gherardi and Tiedemann, 2004; Gherardi et al., 2005). Moreover, hermit crabs are known to modulate their behavior in response to surrounding visual and chemical cues (Pezzuti et al., 2002; Mima et al., 2003; Gherardi and Atema, 2005; Briffa and Williams, 2006).

Most hermit crab species need shells for nearly all aspects of their biology (Laidre and Elwood, 2007) and use shell seeking behavior to locate gastropod shells which become available due to death of a gastropod or conspecific (Rittschof, 1980a,b). Hermit crabs can locate shells visually (Ohta, 1971; Kinosita and Okajima, 1968; Orihuela et al., 1992) and discriminate their shapes (Diaz et al., 1994, 1995a) which play an important role in shell identification and selection (Hazlett, 1975; Salmon and Hyatt, 1983; Chiussi, 2002). Also, hermit crabs use chemical cues for guidance to sites of shells that are associated with predation events (Gilchrist, 1984; Rittschof, 1980a,b), and death of a conspecific (Rittschof et al., 1992). It is believed that many species switch from chemical cues at long distances to visual cues at short distances (Bell, 1991). Although either visual or chemical cues may be used independently for orientation toward shells (Chiussi et al., 2001), integration of input from both senses may determine responses(Hazlett, 1982). Underwater, hermit crabs respond to silhouettes of shells or predators either by moving toward or away according to their odors and visual images (Mesce, 1982; Hazlett, 1982; Diaz et al., 1995a).

The present work aims to; (1) examine visual orientation of *Clibanarius signatus* when confronted with targets subtended at different angles, (2) test if *C. signatus* can visually discriminate between shells from different species of gastropods, (3) determine if orientation response of *C. signatus* changed in the presence of chemical cues of calcium and a predator and (4) and to determine whether olfaction or visual has greater influence on hermit crab response and orientation.

Materials and methods

Study site

Field work took place on a mangrove site located 17 km south of Safaga city (26°21′N and 33°48′E). The site represents a mangrove swamp protected area that is monitored by the Nature Conservation Sector at the Egyptian Environmental Affairs Agency (NCS/EEAA) and characterized by a high diversity of marine life. The areas near the shore have monospecific forests of mangrove trees (*Avicennia marina*) that are intersected by channels of different sizes. At this site, the mangroves are mixed with the impressive remains of fossilized corals.

Hermit crab collection and maintenance

The hermit crab, *C. signatus* (Heller, 1861), is a common species within the mangrove site and has a spatial distribution in the intertidal zone (El-Wakeil et al., 2009; Ismail, 2010, 2011).

In July 2009, large numbers of hermit crabs were randomly hand-collected around mangrove roots at daylight and low tide. The selected crabs were occupied in different shell types (see Ismail, 2010). The hermit crabs were separated into small groups and transferred to the laboratory. In the laboratory, they were maintained in aerated seawater arenas (20 L) in groups of about 40 individuals under a natural 14:10 light:dark cycle and a water temperature of about 31 ± 2 °C. Hermit crabs were extracted from their original shell by gently breaking it with a bench vise, sexed and measured to the nearest 0.1 mm. Only sexually mature males with cephalothorax lengths ranging between 11.3 and 12.5 mm were used in the present investigation to avoid any bias in results due to size, maturity variations and sex (to avoid the possibility of chemical cues associated with reproduction) (Rodrigues et al., 2002; Briffa and Williams, 2006). The hermit crabs were fed with a diet of commercial fish pellet every day, and water was changed every 2 days.

Experimental design

Five series of experiments were performed in the present investigation; (1) orientation of the crabs toward a solid target in ambient clean sea water, (2) orientation of the crab individuals toward different views of varying shell species subtended at a fixed angle in ambient sea water, (3) orientation of the crab individuals toward a fixed-angle target in the presence of calcium cues, (4) orientation of the crab individuals toward a fixed-angle target in the presence of predator cues and (5) orientation of the blinded-eyes crabs toward a fixed-angle target in the presence of different previous two chemical cues.

All experiments were done in translucent circular plastic arenas (54 cm diameter) surrounded by an opaque white wall of 16 cm height. The arenas were held within a small indoor room to ensure similar activity levels of test species (Iglesias, 2007). The arenas were filled to a depth of 7 cm with ambient sea water (salinity about 39.5%) or ambient sea water conditioned by two chemical cues. All experiments were conducted between 08:00am and 18:00pm. Illumination during observations was provided by a 75-W incandescent light, 50 cm above the water level. After each experiment, crabs were caged individually in running sea water. Since different experiments were conducted on different days, crabs were never tested to the same conditions if they were recollected, but may be used in the other experiments of different conditions.

In each experimental condition, 30 individual hermit crabs, without a shell, were used. Crabs were found to increase their orientation behavior activity if they are removed from their shells (Diaz et al., 2001). The test was initiated by placing crabs individually within a short polyvinylchloride cylinder (PVC) (4 cm height, 5 cm diameter) in the center of the arena. The individual hermit crab was removed by hand from its holding arena and this handling caused the hermit crab to expose