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Interpreting odours in hermit crabs: A comparative study

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ABSTRACT

Odours of different sources can indicate to hermit crabs the availability of empty shells, crucial resources for the life cycle of almost all of them. Here, we compared *Clibanarius erythropus* and *Pagurus bernhardus* for the intensity of investigative behaviour exhibited towards an empty, well-fitting shell in the presence of (1) plain seawater as control and seawater conditioned by (2) dead and live snails, (3) dead and live conspecifics, (4) live predators, and (5) food. During 10 min of observation, we recorded latency (the time until the first contact with the shell), and the number and duration of shell investigation bouts. The two species behaved similarly when exposed to the odours of food, live snails, and predators, while a more intense shell investigation was induced by dead snail odour in *C. erythropus* and by dead or live conspecific odour in *P. bernhardus*. Further studies should investigate the influence of phylogeny and ecology on this interspecific difference.

1. Introduction

Since visibility is most often limited in water, important sources of information to aquatic animals are chemical cues. Aquatic decapods rely on them to avoid predators, to recognize opponents, and to find resources, such as mates and food (Bergman and Moore, 2005). The occupancy of well-fitting gastropod shells is crucial for almost all hermit crab species (for an exception see Gherardi, 1996): their survival, growth, and reproduction depend in fact on both the quality and the availability of empty shells in the habitat (Elwood, 1995). Hermit crabs are indeed unable to directly prey on live snails and so depend upon other causes of snail mortality to generate new shelters (but see Rutheford, 1977). Consequently, they have evolved complex behavioural tactics to obtain empty shells: they can exchange them with con- or heterospecifics through either shell fighting (Hazlett, 1966a, b; Elwood and Glass, 1981) or bargaining (i.e. shell exchanges in which both individuals gain in shell fit; Hazlett, 1978, 1981). They also rely on refined mechanisms of orientation to reach gastropod predation sites, i.e. sites where new empty shells, released by non-destructive predation on snails, are made available (Rittschof, 1980a; Tricarico and Gherardi, 2006; Tricarico et al., 2009). Hermit crabs gather at these sites, interact with each other, and form dominance hierarchies (Winston and Jacobson, 1978). These aggregations may function as "shell markets", in which most site attendants may take advantage of a vacancy chain process (Chase et al., 1988), thus obtaining a better-quality shell (Rittschof et al., 1992).

Previous studies have shown that shell availability is signalled by the odours released by different sources (Rittschof, 1980a, b; Rittschof et al., 1992), such as calcium ions emanated from the surface of the shell itself (Mesce, 1982), partly digested snail flesh (Katz and Rittschof, 1993), the hemolymph of dying con/heterospecifics, or live conspecifics (Rittschof et al., 1992; Small and Thacker, 1994; Gherardi and Atema, 2005). As shown by Rittschof and Hazlett (1997) and Gherardi and Atema (2005), the intensity of the investigatory acts executed by hermit crabs towards empty, well-fitting shells is an index of their responses to these odours. Previous studies have suggested the existence of an interspecific variability in these behaviours as a possible reflection of both the status of the shells most available in the habitat and the systems adopted to obtain them (Gherardi and Atema, 2005; Tricarico et al., 2009).

To test this hypothesis, in the present laboratory study, we compared two species of hermit crabs (*Clibanarius erythropus* and *Pagurus bernhardus*) for the intensity of investigation exhibited towards an empty, well-fitting shell in the presence of different odours. The diogenid *C. erythropus* (Latreille, 1818) is an intertidal shallow-water species, common in the Mediterranean and European Atlantic rocky shores. Its shell preferences and aggregation formation have been studied by Gherardi and Benvenuto (2001), but little is known about the chemical ecology of this species (except Tricarico et al., 2009). The pagurid *P. bernhardus* (Linnaeus, 1758) is a common intertidal-subtidal species on the rocky shores of North Sea and European Atlantic coasts. It has been abundantly studied in the laboratory for its refined intraspecific behaviour (e.g.

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Elwood, 1995; Briffa and Williams, 2006; Briffa et al., 2008) and for its ability to assess shell quality (Elwood et al., 1979; Elwood and Stewart, 1985; Neil and Elwood, 1986; Jackson and Elwood, 1989a, b), but not for the behavioural tactics used to obtain new shells (except Elwood and Glass, 1981; Briffa and Austin, 2009). We chose these two species because they occupy different habitats, display diverse social behaviour, and have some aspects related to shell recruitment still unexplored, being thus good model systems for investigating the issue of interspecific behavioural differences in hermit crabs.

2. Materials and methods

2.1. Subjects, collection, and housing conditions

Clibanarius erythropus (180 individuals, right chela width: 0.5–1.3 mm) and *Pagurus bernhardus* (180 individuals, major chela width: 2–5.3 mm) were collected during diurnal low tides from the rocky shores of Baia Domiziana (Southern Tuscany, Italy: N 42°26'7", E 11°9'10") and Robin's Hood Bay (UK: N 54°26'3", E 0°32'6"), respectively, in the summer 2007. We also collected 5 specimens each of the predators of the two species living in the sampled areas (the crabs *Eriphia verrucosa* in Italy and *Carcinus maenas* in UK) and 150 *Cerithium vulgatum* and *Littorina littorea* each, the snail species mostly occupied by *C. erythropus* and *P. bernhardus* and inhabiting the sampled area, respectively. Once in the laboratory at the University of Florence (Italy) and at the University of Hull (UK), the animals were maintained in temperature-controlled rooms (28 and 22 °C in Italy and UK, respectively) under a natural 14:10 light:dark cycle. Hermit crabs, snails and crabs were held in separated 20-l aquaria, different for each species, with aerated artificial (in Italy; Instant Ocean™ salts; salinity 38 as natural seawater; 26 °C) or natural seawater (in UK; salinity 25; 20 °C), and fed a diet of commercial shrimp pellets every day.

2.2. Experimental design

Hermit crabs were randomly assigned to one of seven odour treatments (20 replicates per treatment): (1) live and (2) dead conspecifics, (3) live and (4) dead snails, (5) food, (6) predators, and (7) plain seawater as control. Solutions of odours from live organisms were obtained by keeping, immediately prior to use, 5 live *Clibanarius erythropus* or *Pagurus bernhardus*, 5 live *Cerithium* or *Littorina* (Gherardi and Atema, 2005), and 1 live *Eriphia verrucosa* or *Carcinus maenas* for 12 h in 100 cc seawater. Stock solutions of hermit crab flesh were generated by crushing, macerating for 1 h at room temperature, and then filtering with coarse filter paper 5 hermit crabs in 100 cc seawater. To generate stock solutions of snail flesh, we incubated (and then filtered following Rittschof 1980b) frozen and then thawed pieces (ca. 2 g dry weight) of 5 *Cerithium* or 5 *Littorina* flesh in seawater for 1 h at room temperature in 100 cc seawater. Food solution was made by macerating and filtering with coarse filter paper 2 g of dried shrimp pellets (the same used to feed the test individuals) in 100 cc seawater. Experiments were staged in opaque plastic bowls (diameter: 10 cm), containing 160 ml unfiltered standing seawater (salinity 38 or 25) at 26 or 20 °C with 1 cc of each of the seven test solutions. Bowls were illuminated during observations with a 75-W incandescent light, 50 cm above the water level. The experimental bowl was provided with an empty *Cerithium* or *Littorina* shell placed with its apex upwards (test shell). Its size was optimal for the size of the test hermit crab. The optimal length of shells for crabs of a given size was determined from the regression line $y = 5.81x + 13.10$ in *C. erythropus* and $y = 3.00x + 7.54$ in *P. bernhardus*, where y is the shell length (SL) and x is the chela width (CW), both in mm. These equations were obtained from a preliminary free-choice experiment in which 30 crabs of each species (CW: 0.3–1.2 mm in *C. erythropus*; 2–4.8 mm in *P. bernhardus*) were separately allowed to choose among three empty undamaged and unfouled *Cerithium* or *Littorina* shells of different size (SL: 10–22.8 mm in *Cerithium*; 11–25 mm in *Littorina*).

The shells were prepared by boiling and removing the flesh, rinsing the shells several times in seawater, and air-drying them to eliminate any possible odour; their apertures were partially blocked with a resin to avoid it being occupied by hermit crabs. Preliminary observations had shown that resin and its odour had no effect on shell attractiveness.

Tests started with the introduction of an individual into the bowl at the opposite side of the shell. As shown by Gherardi and Tiedemann (2004) and Gherardi and Atema (2005), the behaviour that hermit crabs exhibit towards this target shell can be taken as an assay revealing their detection of the offered odours. So, during 10 min of observation, we recorded: (1) latency (the time taken for the hermit crab to first come in contact with the shell; when an individual never investigated the shell, we arbitrarily assigned a latency of 605 s); and the (2) number and (3) total duration of bouts of shell investigation in *s*. Observations were conducted between 09:00 and18:00 h. All the tested crabs were in suboptimal shells (average shell adequacy index, as defined by Gherardi and Vannini 1993: -15.3% in *Clibanarius erythropus*; −15.5% in *Pagurus bernhardus*).

Data were first checked for normality using one-sample Kolmogorov–Smirnov test and for homogeneity of variance using the Levene test. A two-way and a one-way ANOVA (statistic: F) were used to compare responses to different factors (species, odour treatments, and species × odour treatments). When we obtained significant F-ratios, Student-Newman-Keuls multiple comparisons tests (SNK) or independent samples Student's *t*-tests were applied; for the SNK, α levels were adjusted by a Bonferroni correction to reduce type I error. Independent samples Student's *t*-tests were conducted to compare the two species within each odour treatments. For predator odour, we only analyzed latency, because hermit crabs never explored the experimental shell in their presence. Figures give means and SE. The level of significance at which the null hypothesis was rejected was $\alpha = 0.05$ ($\alpha = 0.008$ or 0.01 after Bonferroni corrections).

3. Results

A two-way ANOVA shows a significant interaction between the two species for latency only (Table 1a). Investigation bouts were more numerous and longer in *Pagurus bernhardus* than in *Clibanarius erythropus* (Table 1a). In the latter species, latency was the shortest and the total duration of shell investigation was the longest in the presence of the dead snail odour (Table 1b; Fig. 1); on the contrary, in *P. bernhardus* it was the odour of dead and live conspecifics that induced earlier shell investigation and its longer duration (Table 1b; Fig. 1).

Overall *Pagurus bernhardus* was quicker than *Clibanarius erythropus* in responding to the offered shell in the control and in the presence of the odours of food, dead, and live conspecifics, but not in the presence of the odours of dead snails, live conspecifics, and predators (Table 2; Fig. 1a). In addition, *P. bernhardus* always explored the shell for a longer time than the other species, except in the presence of the dead snail odour (Table 2; Fig. 1b). In both species, no significant difference was found among odours in the number of the investigation bouts (Table 1b).

4. Discussion

Our study shows the existence of significant differences between *Clibanarius erythropus* and *Pagurus bernhardus* in the intensity of the target shell investigation when exposed to different