NOTE / NOTE

Predator chemical cues affect prey feeding activity differently in juveniles and adults

Bradley R. Johnston, Markus Molis, and Ricardo A. Scrosati

Abstract: Nonconsumptive predator effects on prey behaviour are common in nature, but the possible influence of prey life-history stage on such responses is poorly known. We investigated whether prey life-history stage may be a factor affecting prey feeding activity responses to predator chemical cues, for which we used dogwhelks (*Nucella lapillus* (L., 1758)) and their main prey, barnacles (*Semibalanus balanoides* (L., 1758)), as a model system. Barnacles use their modified legs (cirri) to filter food from the water column. Through a manipulative laboratory experiment, we tested the hypothesis that the presence of dogwhelks affects the frequency of leg swipes differently in juvenile and adult barnacles. Juveniles showed a similar feeding activity with and without nearby dogwhelks, but adults exhibited a significantly lower frequency of leg swipes when dogwhelks were present. Such an ontogenetic change in the response of barnacles to predatory cues might have evolved as a result of dogwhelks preferring adult barnacles over juvenile barnacles, as found previously. Alternatively, barnacles could learn to recognize predator cues as they age, as shown for other prey species. Overall, our study indicates that the nonconsumptive effects of predators on prey need to be fully understood under consideration of the possible ontogenetic changes in prey responses to predator cues.

Résumé : Les effets des prédateurs, autres que la consommation de la proie, sur le comportement des proies sont courants en nature, mais l'influence possible du stade du cycle biologique de la proie sur ces effets reste mal connue. Nous examinons si le stade du cycle biologique de la proie peut être un facteur qui affecte les réactions d'alimentation des proies à la présence de signaux chimiques de prédateurs en utilisant des pourpres de l'Atlantique (*Nucella lapillus* (L., 1758)) et leur proies principales, les balanes communes (*Semibalanus balanoides* (L., 1758)) comme système modèle. Les balanes utilisent leurs pattes modifiées (cirres) pour extraire leur nourriture par filtration de la colonne d'eau. Lors d'une expérience de manipulation en laboratoire, nous avons testé l'hypothèse selon laquelle la présence de pourpres affecte la fréquence de balayage des cirres différemment chez les balanes jeunes et adultes. Les jeunes ont une activité alimentaire semblable, que les pourpres soient présents à proximité ou non, mais les adultes ont une fréquence de balayage des pattes significativement réduite en présence des pourpres. Une tel changement ontogénique dans la réaction des balanes aux signaux de prédation des pourpres peut s'être développé du fait que les pourpres préfèrent les balanes adultes aux jeunes, comme on l'a démontré antérieurement. D'un autre côté, les balanes pourraient avoir appris avec l'âge à reconnaître les signaux des prédateurs, comme c'est le cas chez d'autres espèces de proies. Globalement, notre étude indique que pour pleinement comprendre les effets (autres que la consommation de la proie) des prédateurs sur les proies, il est nécessaire de tenir compte des changements ontologiques possibles dans les réactions des proies aux signaux des prédateurs.

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Introduction

Predator–prey interactions are often important in structuring communities. Most research on predator influences has focused on the consumption of prey, which generally leads to the mortality of prey organisms (Abrams 2000). Recent studies, however, have indicated that nonconsumptive effects are also common between predators and prey (reviewed in Ferrari et al. 2010). Many prey species can detect nearby predators before consumption is attempted by detecting chemicals released to the environment by the predators (Kats and Dill 1998). The detection of such chemical cues in the air or water serves the prey as an indication of predation risk, which may trigger behavioural changes to avoid predation. Such behavioural responses may include evasive action, reduced activity, habitat selectivity, or use of protective body

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structures (Keppel and Scrosati 2004; Trussell et al. 2006; Creel et al. 2007; Fraker 2008; Urriago et al. 2011).

Recent research has indicated that nonconsumptive predator effects may be more important than consumptive effects in structuring prey populations and, through indirect effects, populations at lower trophic levels (Dill et al. 2003; Werner and Peacor 2003; Trussell et al. 2006). Thus, it is necessary to understand the variety of ways in which prey traits are affected by predation risk. Feeding activity is one of such traits (Trussell et al. 2003; Matassa 2010; Molis et al. 2011). A simplifying view might assume that such effects would remain similar throughout the life time of prey organisms. However, different life-history stages are often subjected to different ecological and evolutionary pressures (Cockburn 1991; Freedman et al. 2011). Thus, different stages should not be expected to necessarily react to predator cues similarly, particularly if different probabilities of predator-induced mortality apply to different prey stages. To investigate whether prey life-history stage may be a factor affecting feeding activity responses to predator cues, we used dogwhelks (*Nucella lapillus* (L., 1758)) and barnacles (*Semibalanus balanoides* (L., 1758)) as an experimental predator–prey system. Dogwhelks show a feeding preference for adult barnacles over juvenile barnacles (Dunkin and Hughes 1984), indicating a stage-dependent predation risk. Therefore, we tested the hypothesis that adult barnacles would exhibit a stronger feeding activity response than juvenile barnacles to the nearby presence of dogwhelks.

Materials and methods

We conducted a manipulative laboratory experiment using barnacles and dogwhelks from rocky intertidal habitats from Helgoland Island, off the coast of Germany on the North Sea, northeast Atlantic (54°11′N, 7°53′E). These species commonly occur sympatrically on North Atlantic rocky shores (Scrosati and Heaven 2007; Scrosati et al. 2011), with *N. lapillus* being one of the main predators of *S. balanoides* (Largen 1967).

In June 2010, we collected dogwhelks from a wave-sheltered section of Helgoland's shoreline (mean (SE) daily maximum water velocity of 2.0 ± 0.2 $m{\cdot}s^{-1}$; Scrosati et al. 2011), kept the organisms in a tank with running seawater at 16 °C, and fed them barnacles and mussels for 20 days prior to experimentation. We collected the experimental barnacles by chipping off small pieces of rocks to which barnacles were attached on the same shore, using a hammer and a chisel. We kept the barnacles in seawater tubs and fed them a culture of the genus *Dunaliella* Teodoresco, 1904 also for 20 days prior to experimentation. To simulate intertidal conditions, we kept the organisms immersed 12 h and emerged 12 h every day. We used a 12 h day and 12 h night lighting regime.

The adult barnacles used for the experiment had an opercular diameter of approximately 5 mm, whereas the juvenile specimens had an opercular diameter smaller than 3 mm. Settlement and recruitment of *S. balanoides* occurs between winter and spring every year (Luther 1987; MacPherson et al. 2008). Thus, differentiating juvenile organisms (those resulting from settlement in the year of visualization) from adults (those that overwintered at least once) is easily done in June because of the clear size differences between both stages (Cole et al. 2011). The dogwhelks used for the experiment were approximately 3 cm in length from the shell apex to the end of the siphonal canal.

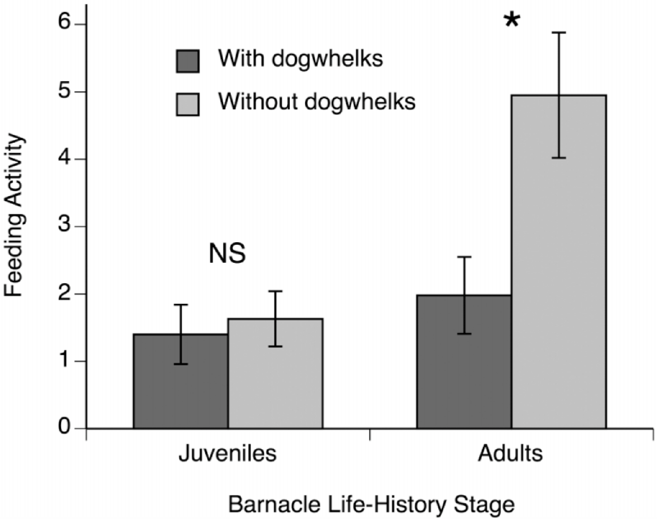
Sessile individuals of *S. balanoides* feed by filtering plankton from the water column by repeatedly swiping their modified legs, known as cirri (Rainbow 1984). Barnacles can modify their feeding activity in response to a variety of chemicals occurring in seawater (Crisp 1967). To test whether feeding activity of barnacles is affected differently in juveniles and adults by the presence of predator chemical cues, we compared the number of leg swipes between barnacles with and without nearby dogwhelks for each life-history stage. We used the same experimental procedure for juvenile and adult barnacles. A replicate of the no-dogwhelk treatment consisted of a single barnacle placed in a 10 cm × 10 cm × 5 cm, white plastic tub with 500 mL of still seawater at 16 °C. A replicate of the dogwhelk treatment consisted of the same materials plus the addition of five nonfeeding dogwhelks, which were placed in the tub 30 min before the barnacle. The dogwhelks were kept separate from the barnacle using a plastic mesh with 5 mm × 5 mm windows. For both treatments of predation risk, barnacles were allowed to acclimate to the water for 10 min after being placed in the tubs. For each replicate, we counted leg swipes during 10 s after this acclimation period. We used 20 different barnacles of each life-history stage for each predation-risk treatment. The five dogwhelks placed in each replicate of the dogwhelk treatment were also different. The use of animals for our experiment followed standard protocols accepted by the institution where this project was done (Biologische Anstalt Helgoland, Germany).

We analyzed our results using a two-way analysis of variance (ANOVA), for which the two factors were barnacle life-history stage (with two levels: juvenile and adult) and predation risk (with two levels: presence and absence of dogwhelks). Because a significant interaction between both factors was detected, we ran tests of simple effects to evaluate whether predation risk affected feeding activity differently for juvenile and adult barnacles. For each simple-effect test, we used the error mean squares from the two-way ANOVA (Quinn and Keough 2002). Statistical analyses were conducted in SYSTAT version 5.2 for Macintosh (Wilkinson et al. 1992).

Results and discussion

The two-way ANOVA detected significant main effects of barnacle life-history stage ($F_{[1,76]} = 9.81$, $P = 0.002$) and predation risk ($F_{[1,76]} = 6.61$, $P = 0.012$) on the frequency of leg swipes of barnacles. The interaction between both factors was also significant ($F_{[1,76]} = 4.88$, $P = 0.030$). Adult barnacles exhibited a significantly higher feeding activity when feeding alone than when doing so with nearby dogwhelks ($F_{[1,38]} = 11.42$, $P = 0.002$). On average, adult barnacles were 150% more active when predator chemical cues were absent than when they were present (Fig. 1). In the presence of dogwhelks, the operculum of adult barnacles was closed between successive cirral beats. Contrary to adults, juvenile barnacles did not show a significant difference in feeding ac-

Fig. 1. Feeding activity (number of leg swipes in 10 s; mean ± SE, $n = 20$ individuals) of juvenile and adult barnacles (*Semibalanus balanoides*) in the presence and absence of dogwhelks (*Nucella lapillus*). NS denotes the nonsignificant result, whereas the asterisk indicates the significant difference.



tivity between the two levels of predation risk ($F_{[1,38]} = 0.07$, $P = 0.800$; Fig. 1).

The results of this experiment indicate that there is an ontogenetic change in the reaction of barnacles to chemical cues from dogwhelks. While juvenile barnacles do not modify their feeding activity when dogwhelks are nearby, adult barnacles reduce it when predatory cues are in the water. This behavioural difference could be the result of selection. When barnacles swipe their cirri to feed, they also help disseminate their own chemical cues in the water, which may attract foraging dogwhelks. If these predators do not pose a significant predation threat to juvenile barnacles, reducing leg swipe frequency when dogwhelks are nearby may not be particularly selected for in juvenile barnacles. Current evidence lends some support to this notion. A previous experiment has reported that adult specimens of *N. lapillus* (2.5–3 cm in length) prefer consumption of adult specimens of *S. balanoides* over juvenile specimens. While barnacles between 4 and 6 mm in opercular diameter constituted approximately 60% of the dogwhelks' diet in that experiment, juvenile barnacles smaller than 3 mm in opercular diameter constituted only about 10% of the diet of these predators (Dunkin and Hughes 1984). Dogwhelks may prefer adult barnacles because juvenile barnacles may be too small to be considered to be profitable prey (Barnett 1979; Hughes and Drewett 1985). Therefore, the possible explanation for our results that is summarized above deserves to be formally evaluated.

An alternative explanation of our results is that barnacles might learn to recognize predation risk during their transition from juveniles to adults. Although no evidence supporting this idea exists for barnacles, studies have reported that some fish species (Ferrari et al. 2010) and even one marine mollusc (Rochette et al. 1998) learn to recognize an unknown predator species when their first exposure to predator odour is accompanied by exposure to cues from injured conspecifics. Experiments using dogwhelks and barnacles are thus needed to test this possibility.

Implicit in the interpretation of our results is the notion that reduced cirral activity in adult barnacles would lead to a reduced susceptibility to predation by dogwhelks (for example, by decreasing dissemination of barnacle cues in the water). At present, experimental confirmation of this notion is lacking. However, this idea is highly feasible, because foraging prey are often more conspicuous and vulnerable to predators than prey that actively avoid predators (Trussell et al. 2011). In general, prey movement is considered to increase detection and attack by predators (Ware 1973; Werner and Anholt 1993).

The lack of response of juvenile barnacles to predator presence could also be related to their energetic needs. The behaviour of young organisms is often regarded as adaptive for their age class, being affected by conflicting pressures such as avoiding predation and obtaining food (Lea and Blumstein 2011). The nutritional needs of fast-growing juvenile barnacles could be higher than for adult barnacles. Hence, decreasing cirral swipe frequency when predators are nearby might prevent juvenile barnacles from obtaining enough food to survive and grow. This is another possibility that needs further study.

It is worth noting that our experiment was done under still-water conditions. Under high water flow, barnacles show a passive feeding behaviour by holding their cirri constantly extended into the water, aiming to catch the food particles that currents bring (Crisp and Southward 1961; Trager et al. 1990). Predation risk effects on barnacle feeding behaviour have not been evaluated under high-flow conditions, but it is reasonable to expect that adult barnacles could shorten the frequency of cirral exposure to the water column when predator presence is detected. At rather high-flow velocities, however, dogwhelks might be ineffective as predators owing to strong hydrodynamic forces (Menge 1976), so adult barnacles might not alter their feeding behaviour if local selection is strong there.

In summary, our study has revealed that chemical cues from dogwhelks affect the feeding activity of barnacles differently depending on the barnacle's life-history stage. Our results are thus similar to those found for a freshwater predator–prey system, in which anuran larvae of different ages reacted differently to larval dragonfly predatory cues in terms of foraging behaviour (Peacor and Werner 2000). Although exceptions exist (Molis et al. 2011), other types of response to predation risk have also been found to vary with age for several prey species confronted with chemical cues from predators (Mathis et al. 2003; Harvey and Brown 2004; Crumrine 2006; Ferris and Rudolf 2007; Brown et al. 2011). Thus, this body of evidence suggests that the nonconsumptive effects of predators on prey need to be fully understood under consideration of the possible ontogenetic changes in prey responses to predator cues.

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