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Chemically induced predator avoidance behaviour in the burrowing bivalve *Macoma balthica*

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Abstract

The responses of the burrowing bivalves *Macoma balthica* and *Cerastoderma edule* to chemical cues emitted by feeding shore crabs *Carcinus maenas* were investigated. *M. balthica* held in the laboratory and exposed to chemical signals in effluent water discharging from tanks containing *C. maenas* fed 20 *M. balthica* day^{-1} reacted by increasing their burial depths from approximately 30 mm to depths of >60 mm, over a period of several days. When the signal was removed the bivalves gradually returned to their original depth over 5 days. *C. edule* similarly exposed to effluent from crabs feeding on conspecifics showed no response. In an attempt to identify the signal inducing this burrowing response, *M. balthica* were exposed to a variety of chemical signals. Crabs fed *M. balthica* elicited the strongest response, followed by crabs fed *C. edule*. There were also small responses to effluent from crabs fed on fish, crabs previously fed on *M. balthica* and to crab faeces, but no responses to starved crabs, crushed *M. balthica*, or controls. We conclude that increased burrowing depth of *M. balthica* is induced by some as yet unidentified chemical cue produced by feeding crabs and is strongest when the crabs were fed on *M. balthica*. Unexpectedly, neither the presence of crabs themselves, nor of damaged conspecifics, was effective in eliciting a burrowing response. The mortality rates of *M. balthica* and *C. edule* selected by crabs when burrowed at normal depths and after exposure to effluent from feeding crabs were different. Crabs selected 1.5 times more *C. edule* than *M. balthica* when both species were burrowed at their normal depths, but 15 times more after the tanks had been exposed to effluent from feeding crabs for 5 days. The burrowing response of *M. balthica* thus appears to reduce mortality significantly by displacing predation pressure on to the more accessible *C. edule*.

Keywords: Alarm signals; Burrowing behaviour; *Cerastoderma edule*; *Macoma balthica*; Predator avoidance

1. Introduction

Chemically mediated defence responses are widespread in both freshwater and marine ecosystems (for review see Harvell, 1990; Tollrian and Harvell, 1999). Such responses are triggered by water-borne alarm substances, which can be released by the predator, or by damaged prey, and may result in either morphological or behavioural responses by the prey species, such that their vulnerability to predation is reduced (see Wisenden, 2000). The advantage of such inducible responses, over purely genetic ones, is that of flexibility, since the costs associated with defence are only incurred when the threat of predation is high (Harvell, 1990; Behrens Yamada et al., 1998). Many marine

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invertebrates exhibit such responses, including the induction of protective spines in bryozoans (Harvell, 1984), extension and opening of pedicellaria in sea urchins (Phillips, 1978) and changes in shell morphology in barnacles (Lively, 1986). The greatest range of responses has, however, been reported amongst molluscs. Chemically mediated defence responses within the Gastropoda include morphological adaptations, such as reduced growth or reproductive output and thickening of the shell lip in whelks (Appleton and Palmer, 1988; Palmer, 1990; Rawlings, 1994) and littorinid snails (Behrens Yamada et al., 1998), as well as behavioural ones, such as predator avoidance behaviour in periwinkles (Phillips, 1978; Behrens Yamada et al., 1998; Jacobsen and Stabell, 1999; Keppel and Scrosati, 2004) and mud snails (Ashkenas and Atema, 1978).

To date the only work on chemically mediated defence responses in bivalves has focussed on the mussel, *Mytilus edulis*. Responses reported in this species include the development of enlarged adductor muscles and thickening of the shell (Reimer and Tedengren, 1996; Leonard et al., 1999), production of additional byssus (Cote, 1995; Dolmer, 1998; Leonard et al., 1999; Smith and Jennings, 2000) and clumping behaviour (Cote and Jelnikar, 1999). To our knowledge no studies have demonstrated chemically mediated predator avoidance responses in soft sediment bivalves, despite the fact that such species are heavily exploited by a variety of predators, notably crabs, birds and fish (for review see Seed, 1993). Burrowing bivalves can reduce the risks of predator-induced mortality in a variety of ways (Vermeij, 1978), including the colonisation of spatial refuges, such as the high intertidal, or regions of extreme or fluctuating salinity, the development of structural defences, such as large body size, thickened or ornamented shells or by employing avoidance responses (Seed, 1993). The most commonly used avoidance response is to increase burrowing depth, which is known to provide protection against both bird and crab predators (Blundon and Kennedy, 1982; Haddon et al., 1987; Zwarts and Wanink, 1989).

The extensive intertidal sand and mudflats of Europe support dense populations of burrowing bivalves, such as the cockle, *Cerastoderma edule* and the tellinid clam *Macoma balthica*. These species are subject to intense predation pressure from birds, especially oystercatchers, which feed and select large individuals (>20 mm) high on the shore, and from shore crabs *Carcinus maenas*, which prey mostly on smaller individuals (<15 mm) at lower tidal levels (Sanchez-Salazar et al., 1987b). As a result the density, size and spatial distributions of these bivalve populations are largely determined by the seasonality and spatial pattern of predation pressure (Sanchez-Salazar et al., 1987b; Richards et al., 1999). *C. edule* has a robust and globular shell, which is relatively resistant to crab predation (Sanchez-Salazar et al., 1987a). As a result crabs selectively take only small cockles in the size range 5–15 mm and larger cockles exhibit a 'refuge in size' in which they are relatively immune to attack by *C. maenas* (Sanchez-Salazar et al., 1987a). Conversely since *C. edule* is an obligate suspension feeder, with short siphons, it is obliged to remain close to the sediment surface. By contract *M. balthica* is a smaller species, attaining a size of ~20 mm in European waters, with a slender, lightly built shell and long feeding siphons and is capable of switching between suspension and deposit feeding. Increased burrowing depth in *M. balthica* provides protection from epi-benthic predators, but imposes a cost involved in employing this strategy in that deeper burial leads to a decrease in the feeding radius of the siphons and in turn a reduction in growth rate (Zwarts and Wanink, 1989; Zwarts et al., 1994; De Goeij and Luttikhuizen, 1998). The optimal behaviour pattern in this species would thus appear to be to increase burial depth when the risk of predation is high, but return close to the surface to feed when the risk declines.

In this study we examine for the first time whether burrowing bivalves show a chemically mediated predator avoidance response and attempt to identify the nature of the signal inducing such a response. Finally, we determine whether chemically mediated increases in burrowing depth in *M. balthica* contribute to significant reductions in predation.

2. Methods

M. balthica and *C. edule* were collected during July and August from the mid to upper intertidal zone in Red Wharf Bay, Anglesey, UK, by removing the top 5–10 cm of sediment with a spade and sieving it through 2 mm mesh. Shore crabs *C. maenas* (carapace widths 50–60 mm) were collected using a hoop net baited with fish deployed from Menai Bridge pier, Anglesey. Obtaining crabs using fish bait ensured they were all actively feeding before the predation experiments were undertaken. Upon collection all the experimental animals were maintained in flowing seawater. Bivalves were held in open plastic buckets containing a 10 cm depth of sediment from the collection site, whilst the crabs were kept in similar buckets, without sediment, but with weighted perspex lids, and were fed daily with a mixture of *C. edule* and *M. balthica*.

The burrowing behaviour experiments were conducted in 6 replicate tanks (nos. 1–6) ($55 \times 35 \times 25$ cm (length, width and depth)), filled to a depth of 10 cm with sediment from Red Wharf Bay and covered with a further 10 cm depth of water. The burrowing depth of *M. balthica* was recorded by attaching a 12 cm length of thin nylon line (0.25 mm or 2.7 kg breaking strain) to the left valve of each specimen with a small drop of Araldite Rapid Adhesive. A self-adhesive plastic number (2×2 mm) was attached to this line with its bottom edge exactly 10 cm above the mid point of the shell. The bivalves were then scattered 10 to a tank, and left to burrow overnight. Any individuals that remained on the surface were replaced. Only *M. balthica* with shell lengths 13–15 mm were used in the burrowing experiments, as this size class was large enough to minimize interference by the nylon tethers, whilst at the same time excluding the largest, possibly senescent, individuals in the population. Depth of burrowing was measured daily from 09.00 to 10.00, using a 10 cm length of ruler. When the nylon tethers were gently pulled vertical and held against the ruler the position of the lower edge of the number on the scale gave a direct reading of burrowing depth, measured to the midpoint of the shell valve.

Crabs, or other sources of chemical signals being investigated, were held in 5 L plastic buckets placed on wooden rails laid across the tops of the experimental tanks. Seawater flowed from the supply system into the buckets at a rate of 0.5 ± 0.2 L min^{-1}, and then overflowed through four holes around the lip of the buckets into the tanks, and from there to waste. When the signals involved crabs, these were placed in pairs in the buckets. *M. balthica* were supplied to the crabs at a rate of 20 day^{-1} and when crushed *M. balthica* were used, 20 individuals were crushed daily with a stone to break the shells and release fluids from the flesh of the bivalve and placed into the bucket. Daily, crushed and uneaten *M. balthica* from the previous day were removed. When fish was used, a single frozen sand eel *Ammodytes tobianus*, was given to the crabs daily. Crab faeces were obtained by feeding four crabs daily to satiation on a mixture of *C. edule* and *M. balthica* in a separate bucket. The contents were poured through a fine mesh net and then dipped once in clean seawater and the faeces backwashed into the experimental buckets.

A first experiment (experiment 1) investigated whether *M. balthica* and *C. edule* varied their burrowing depth when exposed to the odour of conspecifics being consumed by *C. maenas*. Tethered bivalves were placed into the 6 tanks at a density of 10 $tank^{-1}$ and burial depth measured daily for 6 days before any signal was introduced. The signal was then applied for 9 successive days, following which the signal was removed, but burial depth continued to be monitored for a further 6 days. Three replicate tanks (2, 3 and 4) (experimental) and 3 tanks (1, 5 and 6) (control—no odour) were used and the 30 depth measurements averaged daily for either the experimental or control conditions. These mean depth data were plotted against time (days) to investigate whether burial depths differed between treatments before the signal was introduced (days 1–6), after the signal had been in place for 9 days (days 7–15) and after the signal had been removed for 5 days (day 21). Daily burrowing depth data were found to have a non-normal distribution (Anderson Darling test) and heterogenous variance (Bartlett's test for homogeneity of variance). Upon log-transformation, however, the data were normally distributed, although with heterogenous variance, thus precluding the use of a nested ANOVA. Statistical differences in daily burrowing depth between the 2 treatments were therefore investigated using a two-sample T-test with unequal variance.

A second experiment (experiment 2) compared the burrowing responses of *M. balthica* exposed to a variety of chemical signals. Ten *M. balthica* were placed in each tank, but based on the results of experiment 1, only 1 day was allowed for equilibration before the signals were introduced and the experiment run for a further 9 days. When the effect of chemical signals was investigated, the physical location of the tanks was randomised and control tanks run for a comparison. The first experiment demonstrated that up to 5 days were required for burrowing depths to equilibrate after exposure to chemical signals in the effluent, therefore only the results from days 6–10 were compared statistically to ascertain the responses to the various signals. Daily burrowing depth data in the various treatments were normally distributed (Anderson Darling test) but displayed heterogenous variance (Bartlett's test for homogeneity of variance). A Kruskal–Wallis test was therefore used to identify differences in median burrowing depth between the treatments for days 6–10.

In the third experiment (experiment 3), the proportion of *M. balthica* and *C. edule* consumed by foraging *C. maenas* (50–60 mm carapace width) placed directly into the tanks was investigated. Five days prior to the introduction of the crabs, 10 bivalves of each species (length 10–15 mm) were placed in each tank and allowed to burrow to their normal depths; 3 tanks were exposed to the chemical cues in the effluent water from crabs fed 20 *M. balthica* day^{-1} inducing the *M. balthica* to burrow deeper than those placed in 3 control tanks which were not exposed to chemical cues.

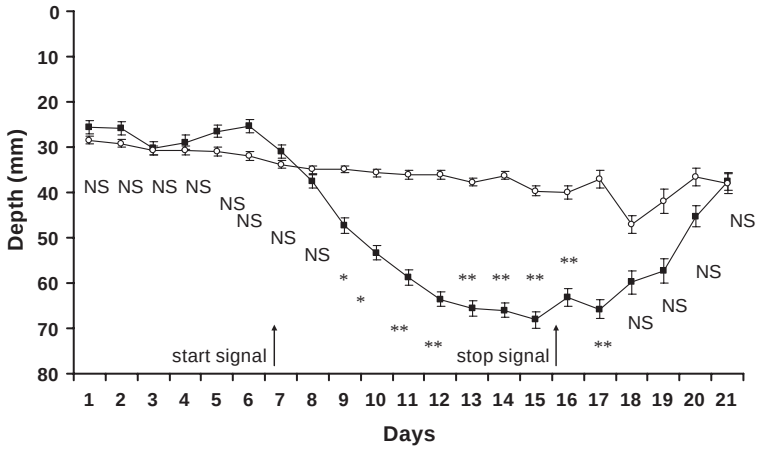

Fig. 1. Mean (± S.E.) of burrowing depth of *Macoma balthica* exposed to chemical signals from tanks containing two *Carcinus maenas* feeding on 20 *M. balthica* day^{-1}, as compared to that in control tanks. The chemical signal was introduced between days 6 and 7 and removed again between days 15 and 16. Each line is the mean of 30 individuals held in three separate tanks. Statistical differences in daily burrowing depths are shown; NS not significant, *$p < 0.002$ and **$p < 0.001$. Clear (round) and shaded (square) symbols represent the burrowing response in the control and experimental tanks respectively.

M. balthica were not tethered, as the tethers could have been used by the feeding crabs to locate or pull up the burrowed prey. Five replicate experiments were run for 24 h and at the end of each the sediment was sieved through a 2 mm mesh and the numbers of survivors recorded.

3. Results

Fig. 1 shows the mean burial depth of 30 *M. balthica* in the 3 experimental tanks, before, during and after exposure to effluent water from two *C. maenas* feeding on 20 *M. balthica* day^{-1} together with the burial depths of a similar control group (control—no odour). The daily statistical difference between the two treatments is indicated. During the initial equilibration period (days 1–7), both the experimental and control groups remained buried at fairly consistent depths of 25–33 mm, although with individual and day to day variation in the mean burial depth. The depths of *M. balthica* in all tanks became relatively stable by the time the first reading was undertaken 24 h after the start of the experiment. Mean burial depth of the experimental group was slightly shallower than that of the control group, although the difference was not statistically significant during the first 8 days of the experiment (Fig. 1).

Within 2 days of exposure to effluent water from the feeding crabs the mean burial depth of the *M. balthica*

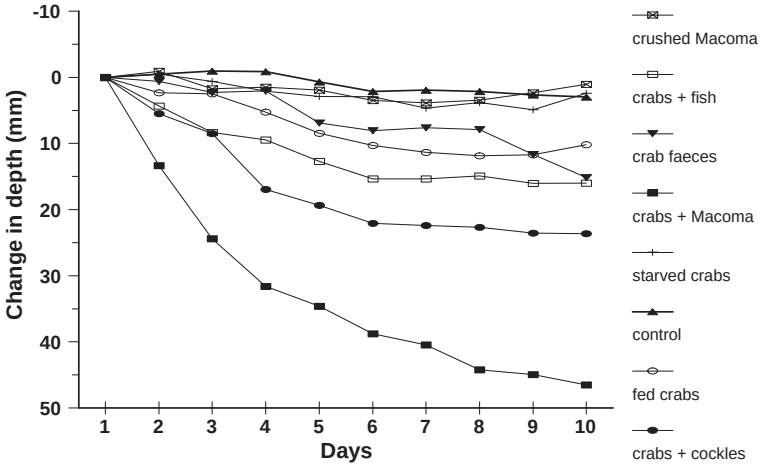

Fig. 2. Temporal change in burial depth of *Macoma balthica* following exposure to a variety of chemical signals. See text for details of how each signal was generated.

Table 1
Summary from the Kruskal–Wallis test of median burrowing depth of *Macoma balthica* exposed to eight different treatments in experiment 2

Treatment	*Z* value
Carcinus fed *Macoma*	10.43
Carcinus fed *Cerastoderma*	7.96
Carcinus fed *Cerastoderma* and faeces	1.47
Carcinus fed fish	0.43
Carcinus faeces	−1.41
Starved *Carcinus*	−4.46
Crushed *Macoma*	−5.91
Control (no odour)	−8.61

Test statistic $H=267.15$, $df=7$, $p<0.001$.

significantly increased (day 9). *M. balthica* continued to burrow deeper throughout the remaining 6 days of exposure, until a mean depth of 68 mm had been achieved, more than twice the original mean depth of 28 mm (days 1–6). Burrowing was most rapid initially and showed a tendency towards stabilisation after about 5 days of exposure with a gradual increase in mean depth throughout the experimental period. A high degree of variability between the responses of individual *M. balthica* was observed. Instead of all individuals progressively increasing their burrowing depth during the experiment, some showed a rapid reaction, whilst others remained at shallow depths for several days before responding. There was thus a gradual shift, over several days, from almost all individuals being shallow to most being deep, although one or two individuals had still not responded when the signal was terminated. Upon removal of the signal, *M. balthica* gradually migrated towards the sediment surface, but again this took several days and occurred more rapidly in some individuals than in others. By day 18, however, 3 days after the signal was removed, the experimental group had returned to a depth which was not significantly different from the control group (see Fig. 1). Throughout the experiment, burial depth of *M. balthica* in the control tanks remained relatively stable, although they did show a gradual increase over 21 days to a depth of ~30–40 mm. In a similar experiment using *C. edule* as the experimental animal and crabs feeding on *C. edule* as the signal, no response was observed (results not depicted). Mean burrowing depth remained at about 20 mm ± 8 mm throughout the equilibration period and remained unchanged when the signal was applied.

Fig. 2 displays the responses of *M. balthica* in the second experiment to different chemical signals. Since experiment 1 had shown that long equilibration periods were unnecessary, *M. balthica* were only allowed to burrow for 1 day before the signals were introduced. As the intention was to compare the strengths of the various reactions, all the results are shown as deviations from the starting depth. This eliminated slight variations between the means of burrowing depth of the various groups on Day 1, the day before the signals were introduced. The results of the Kruskal–Wallis test (see ranked *Z* values in Table 1) demonstrated that the strongest burrowing response was obtained by exposure to effluent water from tanks containing crabs feeding on *M. balthica*, with a maximum increase in burial depth of 46.5 mm. Crabs feeding on cockles, *C. edule*, also elicited a strong response, but of only half this amplitude (23.7 mm). Increases in mean burrowing depth also occurred in response to effluent water from tanks containing crabs fed on fish, crab faeces and crabs that had been fed the previous day, but these were of smaller final amplitude (10–16 mm). No responses were recorded to effluent from starved crabs and to crushed

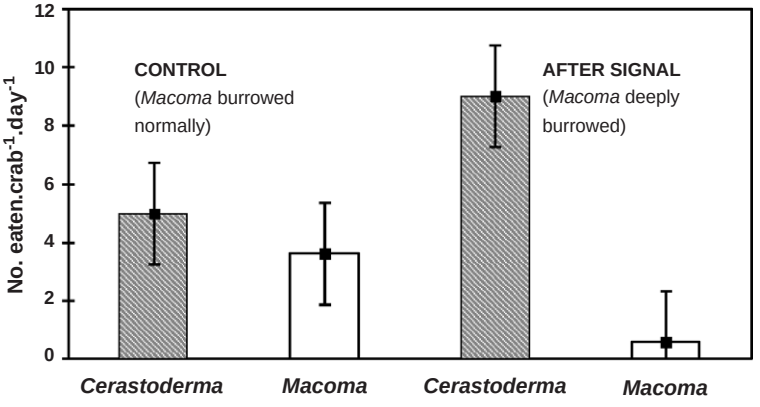

Fig. 3. Proportions of *Cerastoderma edule* to *Macoma balthica* consumed by crabs *Carcinus maenas*, when buried at their normal depths, and after the tanks had been exposed to the odour from two crabs feeding on 20 *M. balthica* day^{-1} for 5 days. Each reading is the mean (± S.E.) of five experiments.

M. balthica. The negative *Z* values in Table 1 suggest that there were no differences between these treatments. The response to crabs eating *M. balthica* and to crabs eating cockles was greater than those to the other signals. The burrowing response to effluent from starved crabs and from crushed *M. balthica* was similar to the controls. The remaining signals fall into a third, intermediate group, which differ from the controls and from crabs eating both *M. balthica* and cockles, but cannot be clearly distinguished from one another. The most inconsistent of these trends was that obtained to crab faeces.

In the final experiment we investigated whether increasing burial depth influenced the daily consumption of *C. edule* and *M. balthica* by *C. maenas* and from these data the relative proportion of *M. balthica* to *C. edule* eaten was estimated (Fig. 3). When offered similar-sized individuals of the two prey species buried at their normal depths, crabs selected 1.4 times as many *C. edule* as *M. balthica* (Fig. 3 control). However after the *M. balthica* had increased their burrowing depth in response to the odour of feeding crabs, the ratio of *C. edule* to *M. balthica* consumed increased to 15 (Fig. 3 after signal). Thus indicating that *M. balthica* are able to significantly reduce their exposure to crab predation by retreating to greater depths within the sediment.

4. Discussion and conclusions

The initial burrowing experiment showed that in the laboratory *M. balthica* (length 13–15 mm) normally burrowed to a depth of ~25–30 mm. This burrowing depth is similar to that observed by previous authors, such as Zwarts et al. (1994), who recorded that *M. balthica* occurred at depths of ~30 mm. However, it is known that in their natural environment *M. balthica* may vary their burial depth seasonally, burying to depths of ~80 mm in the autumn and winter, but ascending again to shallow depths of 20–40 mm in the early spring, prior to the growing season (Zwarts and Wanink, 1989; De Goeij and Luttikhuizen, 1998).

When exposed to the effluent from crabs crushing conspecifics *M. balthica* doubled their burial depth over a period of several days. This is the demonstration of a chemically induced predator avoidance response in a burrowing bivalve. A similar increase in burrowing depth by the clam *Mya arenaria* in the presence of foraging *C. maenas* has been demonstrated by Whitlow and Dochtermann (2001). A variety of similar chemically induced behavioural and morphological responses have, however, been reported in mussels and in other marine invertebrates. Morphological adaptations demonstrated to occur in mussels include development of predator-resistant features, such as thicker shells, larger adductor muscles and stronger byssal attachments when exposed to odours from either predatory crabs (Cote, 1995; Leonard et al., 1999), or starfish (Reimer and Tedengren, 1996; Dolmer, 1998), while behavioural adaptations include aggregation behaviour in response to the threat of lobster predation (Cote and Jelnikar, 1999) and barnacle hiding behaviour (Palmer et al., 1982; Dill and Gillett, 1991).

The observation that *C. edule* failed to show any increase in burial depth in response to the odour of crabs feeding on conspecifics is perhaps not surprising since this species is an obligate suspension-feeder, with short siphons, and is thus obliged to remain close to the surface in order to feed and respire (Zwarts and Wanink, 1989). By contrast *M. balthica* have elongate siphons and can alternate between deposit feeding, when close to the surface and suspension feeding, when buried at greater depths (Zwarts and Wanink, 1989). Movement to greater burial depth in this and related bivalve species has long been known to provide a refuge from predation by crabs (e.g. Blundon and Kennedy, 1982; Haddon et al., 1987; Zwarts and Wanink, 1989; Zwarts et al., 1994), as a response to the presence of parasites (Swennen and Ching, 1974; Huxham et al., 1995) and intraspecific competition between the siphons of adjacent deposit feeders (Bouma et al., 2001). However, an increase in burial depth results in a decrease in the feeding radius of the siphons (Zwarts et al., 1994) and in turn reduced growth and condition (De Goeij and Luttikhuizen, 1998). There are thus conflicting demands between foraging efficiency and predator avoidance in deposit feeding bivalves, making it adaptive for them to remain shallowly buried when the threat of predation is low and to return to these depths once any threat has passed. The behaviour of the *M. balthica* observed in this study is consistent with such an interpretation.

When the burrowing responses of *M. balthica* exposed to a variety of chemical cues were compared, the greatest response was obtained to effluent water from predatory *C. maenas* feeding on *M. balthica*. A significant increase in burial depth was, however, also obtained to crabs feeding on *C. edule*, and a smaller one to crabs feeding on sand eel (*A. tobianus*). An unexpected result was that neither starved crabs alone, nor *M. balthica* deliberately crushed, elicited any burrowing response, whereas crab faeces, and crabs which had been fed in a separate tank immediately preceding the experiment, did produce intermediate levels of response. The exact relative magnitudes of these

responses should not be over interpreted as signal strengths may differ between experiments (e.g. exact quantities of food supplied were not equivalent when crabs were fed fish and bivalves). The rather erratic response to crab faeces is a particular case in point here, since faeces production could have varied considerably in both quantity and timing from day to day and soluble components of the faeces could have been lost by leaching before the solids were transferred to the experimental tanks. The results nevertheless clearly suggest that the signal to which the *M. balthica* are responding is a breakdown product of crab feeding and that the breakdown products from related species produced a stronger response than those from more distantly related species. The failure of damaged conspecifics and of starved crabs to produce a burrowing response can be interpreted as having adaptive value, since there would be no benefit in responding to the death of conspecifics if this has not resulted from predation, nor indeed is there value in responding to the presence of crabs which are not feeding. *Macoma* that had been mechanically damaged by a stone to break their shells also had their flesh damaged and this may have resulted in the release of an alarm signal(s) which stimulated the buried *Macoma* to burrow deeper. Further research is needed to ascertain whether the response is dose dependent or is a response to a mixture of multiple cues.

The discovery that there is a graduated array of chemical cues producing these responses is a novel finding but also makes comparison with earlier literature more difficult to interpret. Earlier authors studying chemical mediation of defence behaviour in marine invertebrates have obtained positive responses to a variety of chemical cues and used various experimental designs, involving field or laboratory experiments and static or flow through systems (which in turn may result in very different signal strengths). The most frequently recorded positive responses are those elicited by exposing the target organism to water in which predators have been feeding on conspecific prey (e.g. Rawlings, 1994; Reimer and Tedengren, 1996; Jacobsen and Stabell, 1999). However, other researchers have obtained positive responses by exposing prey to effluent from unfed or deliberately starved predators (e.g. Phillips, 1978; Cote, 1995; Cote and Jelnikar, 1999), from predators feeding on prey other than the test species (Leonard et al., 1999) or from manually broken conspecifics (Leonard et al., 1999). This perhaps suggests that the nature of the signals differ between receptor species, but the different responses obtained by various workers have also have been influenced by the very different signal strengths used, especially in static relative to flow through experimental protocols. Few previous studies have directly compared the responses elicited by different signals. Behrens Yamada et al. (1998), for example, demonstrated that the presence of feeding crabs *Cancer productus*, elicited a variety of escape behaviours in the intertidal snail *Littorina sitkana*, whereas non-feeding crabs did not. Similarly Appleton and Palmer (1988) demonstrated that phenotypic changes in shell morphology (smaller apertures and larger teeth) in the whelk *Nucella lamellosa* could be induced by exposing them to effluent from predators feeding on conspecifics, but they did not occur if the predators were fed on non-gastropod prey. Both these findings are consistent with the observation that a graded series of responses may be obtained from different signal types.

In our last experiment we demonstrated that the increased burial depth of *M. balthica* exposed to effluent from feeding crabs was sufficient to confer considerable protection from crab predation, at least when alternative prey are available; a situation that normally occurs in nature. This observation is entirely consistent with earlier papers which have demonstrated that increased burial depth in bivalves is associated with reduced predation (Blundon and Kennedy, 1982; Haddon et al., 1987; Zwarts and Wanink, 1989; Zwarts et al., 1994).

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