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Comparative study of the covering reaction of the purple sea urchin, *Paracentrotus lividus*, under laboratory and field conditions

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Abiotic and biotic aspects of the covering behaviour of the purple sea urchin, *Paracentrotus lividus* (Echinodermata: Echinoidea) were systematically examined *in situ* and in the laboratory to analyse potential intra-population differences in the nature of this behaviour. In the field, significant differences in the occurrence of covering behaviour were found between age groups. Smaller *P. lividus* covered at higher frequencies than larger individuals. Higher proportions of individuals were also observed to cover during afternoon sampling periods. Significant differences in the types of covering material used were found between *P. lividus* observed in the laboratory and in the field. These findings suggest that the benefits and/or opportunities of covering behaviour may change with size and habitat.

INTRODUCTION

The purple sea urchin, *Paracentrotus lividus* (Lamarck), is a common inhabitant of the shallow waters of the Mediterranean and the Atlantic coasts of Ireland, Spain and France (Southward & Southward, 1975). It generally inhabits shallow waters and it is in these conditions that they can be most commonly found at Lough Hyne Marine Nature Reserve, County Cork, Ireland (Barnes et al., 1999). Studies of *P. lividus* at Lough Hyne started as long ago as 1931 and have continued to the present (Renouf, 1931; Kitching & Thain, 1983; Barnes et al., 1999). These have found massive population fluctuations and have led to the suggestion that *P. lividus* may be a key species by controlling macro-algae. Consequently, current knowledge of *P. lividus* at this site is extensive, particularly with regards to their population dynamics. Despite this, there have been few quantitative behavioural studies of *P. lividus* in this locality.

Many marine organisms, particularly those with hard exteriors, are covered by organisms, such as algae and encrusting animals. Available space is often rare in the sublittoral zone and competition for this resource may be intense. Thus non-burrowing shelled animals are hard substratum oases. Echinoderms have generally been found to be highly resistant to fouling by epibiota (Emson, 1999), although rarely holothurians may be colonized (Barnes & Clarke, 1995). Certain benthic dwellers (e.g. many crabs) are, however, known to actively encourage colonization of shells such that the relationship is mutualistic; the colonizers providing crypsis and presumed protection from potential predators (e.g. Maldonado & Uriz, 1992). Furthermore, live organisms may be collected and actually placed on their exteriors (Ross, 1971). In extreme cases, unwilling organisms may even be abducted and held for protection (McClintock & Janssen, 1990). Collection of non-living material is a much rarer phenomenon and is most characteristic of echinoids (Millott, 1956).

Covering behaviour has been reported in representatives from a number of regular sea urchin (echinoid) families such as the Echinidae: *Psammechinus miliaris* (Milligan, 1915), Echinometridae: *Evechinus chloroticus* (Dix, 1970), Strongylocentridae: *Hemicentrotus pulcherrimus* (Mortensen, 1943), Temnopleuridae: *Temnopleurus toreumaticus* (Yoshida, 1966) and Toxopneustidae: *Lytechinus variegatus* (Millott, 1956). Covering behaviour has also been readily observed in populations of *Paracentrotus lividus* including those present at Lough Hyne (Ebling et al., 1966). Within the scientific literature, this behaviour has also been called 'masking behaviour', 'heaping' and 'dressing' (Millott, 1975). It involves sea urchins placing items from their environment, such as shells and stones, onto their dorsal surface using their tube feet and spines to lever the material(s) into position (Millott, 1956; Figure 1). Despite the apparent ubiquitous nature of this behaviour, its functional significance remains a source of considerable controversy, and a number of theories to explain it exist. These include increasing body mass to reduce the likelihood of displacement by wave action (Millott, 1975), providing protection against desiccation (Orton, 1929), and functioning as an accessory feeding mechanism (Dix, 1970). However, the most popular theories fall into two principal groups: (i) covering to protect from over-exposure to light (von Uexküll, 1897; Millott, 1956; Sharp & Gray, 1962) and (ii) covering to camouflage individuals from predators (Milligan, 1915). Although represented in numerous publications, many of these theories are based solely on qualitative observations. It was therefore the objective of the current study to conduct a preliminary investigation into this behaviour using a systematic and quantitative approach with the following aims: (1) To quantitatively compare the covering behaviour of *P. lividus* in the field and under laboratory conditions. (2) To determine whether individuals of different sizes displayed differences in covering behaviour tendencies. (3) To establish whether individuals showed preferences for the types of material used for covering themselves under laboratory conditions.

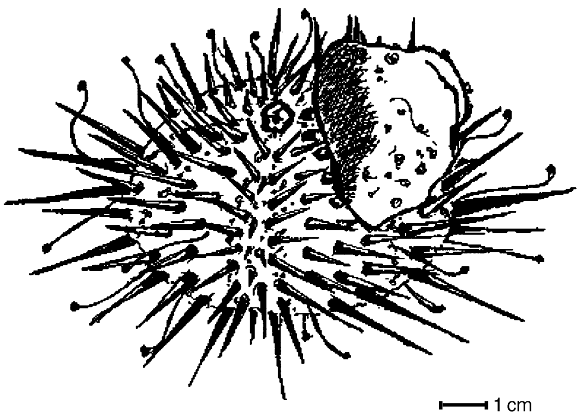

Figure 1. *Paracentrotus lividus* individual showing covering behaviour with one shell held by tube feet on its dorsal surface.

METHODS

Field observations

Observations of *Paracentrotus lividus* were intermittently made at the Lough Hyne Marine Nature Reserve, County Cork, Ireland between October 1998 and January 1999. In particular, data collection was restricted to the northern section of the Lough, called the North Wall, where *P. lividus* are most abundant (Barnes et al., 1999). This region of the Lough is characterized by boulders and cobbles with a patchy distribution of a number of algal species upon which *P. lividus* graze (Ebling et al., 1960). Since the maximum depth on this shore was typically 2 m it was possible to collect data by snorkelling. Daily sampling sessions were divided into three time periods: morning (0830–1130 h), midday/afternoon (1130–1500 h) and evening (1500–1730 h) with all data being recorded directly onto underwater slates by a single observer. During periods of low water visibility or darkness, underwater torches were used.

The proportion of *P. lividus* displaying covering behaviour at the North Wall site was estimated using a series of $3\times1\,m^2$ transects. These were positioned randomly within the site but at the same average depth of approximately 60 cm. Replicate transects were recorded for each time period. In addition, a one metre ruler was positioned perpendicular to the transect tape such that 50 cm of the ruler was showing to either side of the tape so that a one metre width was maintained. The following data were recorded for each individual within the transect area: (1) Whether or not it was displaying covering behaviour and, where appropriate, the number and type of material(s) used for cover; and (2) size of the individual (test diameter at the widest point in mm) measured using Vernier callipers.

Laboratory observations

In December 1998, 20 *P. lividus* specimens (mean diameter =45.2 mm) were selected at random from the study site. Random samples of materials that had been observed being used by *P. lividus* for covering behaviour at the study site were also brought back to the laboratory. All 20 individuals were allowed to acclimatize for a 48 h period prior to commencing experiments and were returned to these acclimation tanks in-between experiments. These tanks were provided with artificial light (65.8 W light source) for approximately 8.5 h per day to simulate the natural photoperiod experienced by *P. lividus* at this time of year. Three identical experimental aquaria ($55\times25\times25\,cm^3$) were setup containing 20 cm depth Lough Hyne seawater and an air source. Each was situated beneath the same artificial 'white' light source. The lighting was again turned on for 8.5 h per day to simulate the sea urchins' natural photoperiod. Each aquarium had a particular combination of covering material all of which had been brought back from the study site except the 'novelty' items. This enabled us to test whether *P. lividus* shows a preference for particular materials when they are presented in different abundances and combinations. All materials were weighed (wet weights) prior to the experiments. The three aquaria were set-up as follows:

Aquarium 1: 10 assorted shells +5 tree leaves
Aquarium 2: 5 assorted shells +5 tree leaves +5 'novelty' items
Aquarium 3: 10 assorted shells +5 'novelty' items

The novelty items in aquaria 2 and 3 consisted of a plastic pen lid (3.41 g), a piece of cloth (7.3 g), an air stone (6.03 g), a piece of plastic rope (3.13 g) and plastic tubing (4.42 g). Shells were used as the most numerous items in aquaria 1 and 3 because they represent the most abundant covering material at the study site (D.K.A. Barnes & A.C. Crook, unpublished data). The average mass of shells used in the experiments was 5.89 g and for tree leaves, 1.03 g.

For the experiments, covering materials were placed in each aquarium at random, followed by the addition of five *P. lividus* per aquarium from the acclimation tanks. Immediately upon placing the sea urchins into each aquarium the behaviour of individuals within each tank was recorded continuously for three hours with a digital stopwatch. These observations started at midday and were made every minute for the first 20 min since pilot studies had previously shown this to be the time period of greatest activity (E. Verling, unpublished data). Thereafter observations were made every ten min until 1730 h. Since covering behaviour can be defined as a 'behavioural state' (Lehner, 1996) observing individual behaviour at particular points in time is an appropriate sampling method. Individual behaviour was therefore recorded using focal sampling (Lehner, 1996). A sea urchin was deemed to be displaying covering behaviour when it had lifted one of the available materials so that it was no longer touching the floor of the aquarium (Figure 1). The covering item(s) deemed to have been chosen by the sea urchin were those that had been worn for the longest period during the experiment. Replicate experiments were conducted for each of the three combinations of aquaria setup with sea urchins having a minimum of 48 h in the acclimation tanks between experimental runs.

RESULTS

Field data

Chi-square analysis was used to determine whether significant differences existed between the numbers of

Table 1. *Frequency of item use by* Paracentrotus lividus *at Lough Hyne, Ireland. Individuals were classed as either single or multi-item users. The category 'other' refers to infrequently used items, such as dogfish egg cases and sea-urchin tests.*

Covering item	Frequency of single-item users	Frequency of multi-item users
Shell	90	11
Leaf	79	4
Algae	21	0
Twig	6	0
Stone	5	3
Other	1	4
Leaves and shells	—	10
Leaves and algae	—	2
Leaves and stones	—	1
Shells and stones	—	3
Shells and algae	—	1

individuals displaying covering behaviour during the three time periods (N=240). It was shown that significantly more *Paracentrotus lividus* were not displaying this behaviour than were during both the morning and evening transect samples (χ^2=15.94, $P<0.01$ and (χ^2=6.635, $P<0.01$, respectively). During the midday/afternoon-sampling period, there was no significant difference between the numbers displaying covering behaviour and those not displaying the behaviour. However, more *P. lividus* were showing covering behaviour during the midday/afternoon period than at any other time of day sampled.

Using Kruskal–Wallis analysis it was found that those individuals covering were significantly smaller than those not displaying the behaviour for all time periods sampled (H=13.16, $P<0.001$). There was no significant difference in size between sea urchins with one covering item and those with more than one item. There was, however, a significant difference in the number of covering items used by *P. lividus* with the majority having just one covering item (83.8%) and a small percentage having two items (9.5%) or more (6.7%; χ^2=15.94, $P<0.001$). Moreover, there was a significant difference between the numbers of sea urchins selecting different types of covering material (χ^2=239.39, $P<0.001$). Table 1 shows the range of covering materials used by *P. lividus* in the field that had only one type of covering material at the time of observation. It clearly shows a distinct preference for individuals to cover themselves with shells or leaves. Of those individuals covering themselves with shells, those of oysters were the most commonly observed. Similarly, of those *P. lividus* using a leaf for cover, oak leaves were the most frequently recorded. Different combinations of materials used by *P. lividus* recorded with more than one type of covering material are also given in Table 1.

Laboratory data

When the data for the laboratory experiments were pooled and compared with the field results it was shown that there were significant differences in the number of

Table 2. *Differences in the frequency of covering items used by* Paracentrotus lividus *observed in the laboratory and in the field.*

Covering item	Field	Laboratory
Shell	90	8
Leaf	79	12
1 item	201	17
>1 item	39	18

P. lividus wearing shells and leaves between the two environments (χ^2=7.01, $P<0.05$; Table 2). In the laboratory, individuals tended to have more than one covering item in contrast to those observed in the field where one item was most commonly observed (Table 2).

When data were pooled for the three experimental aquaria, the majority of individuals observed (75.6%) displayed covering behaviour; the remaining individuals (24.4%) were not observed to pick up any type of material throughout the observation period. Individuals were not shown to display a preference for the most numerous covering material (aquaria 1 and 3). When covering materials were presented in equal proportions (aquarium 2) the lightest (i.e. leaves) were chosen with the greatest frequency (60%). Of those individuals observed to pick up novelty items, the pen lid was the most commonly observed (38%).

DISCUSSION

The systematic protocol under both field and laboratory conditions showed that significant differences exist in covering behaviour between these two environments. In addition, this baseline data provides a basis for future detailed experiments to quantitatively analyse the potential multifunctional nature of covering behaviour in *Paracentrotus lividus*. Significantly more *P. lividus* displayed covering behaviour in the field during the midday/afternoon sampling periods. Covering behaviour of toxopneustids has also been found to be concentrated around this period of the day (Millott, 1956). This has important biological implications since during this time incident radiation is at its most intense. Displaying covering behaviour during this time may therefore be an effective strategy for reducing the actual surface area exposed to potentially damaging ultra-violet (UV) radiation. Indeed, Millott (1956) observed that covering items were more frequently picked up during afternoon periods and the role of light as a potential selection pressure for covering behaviour has been suggested by several other authors (von Uexküll, 1897; Sharp & Gray, 1962). However, the role of specific wavelengths in isolation, such as short-wave UV, has only rarely been investigated. Sea urchins exposed to these wavelengths display covering behaviour to a greater extent than in artificial 'white' light (Sharp & Gray, 1962). Of the echinoid species that display covering behaviour, *P. lividus* typically occurs in the shallowest conditions, commonly in the intertidal zone. As light and particularly UV light is substantially attenuated with depth, this species should be the most influenced by radiation and might benefit the

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most from covering behaviour. In this context, it seems likely that covering to minimize surface area exposure would be an adaptive behavioural strategy.

Since the discovery of serious seasonal ozone depletion over Antarctica (Farman et al., 1985), the hole has continued to increase in both magnitude and duration. Serious ozone depletion has, more recently, also been reported over the Arctic (Farman et al., 1994). Ultraviolet irradiation levels are consequently raised in these locations during such periods and potentially pose a serious threat to terrestrial and shallow water organisms (Woods, 1988). The monitoring of sensitive taxa such as echinoids (Häder et al., 1998), may serve an important function in early indication and warning.

It was clear from the present study that there was high individual variability in the numbers of *P. lividus* observed with covering items at different times of the day. Within the study site individuals are exposed to the same abiotic factors of tide (and therefore water depth), temperature and salinity. However, given that there have been suggestions that *P. lividus* undergoes diurnal migration (Ebling et al., 1966; Dance, 1987; Crook et al., in press) it is likely that at any one point not all individuals have equal access to covering items. When *P. lividus* are on the tops or undersurface of boulders they do not have access to covering items because items accumulate only between boulders.

Paracentrotus lividus displaying covering behaviour were significantly smaller than those not covering, suggesting that the importance of covering may change with size. In the field *P. lividus* most often covered with just a single item, usually either a shell or a leaf. Approximately one fifth of *P. lividus* at Lough Hyne were observed covering with more than one item. This might be a function of the immediate availability of covering items to certain individuals or perhaps reflects differences in ability to manipulate multiple items. In the field and in the laboratory *P. lividus* select items that cover only a small proportion of their surface area (D.K.A. Barnes & A.C. Crook, unpublished data).

Predator avoidance has been suggested as one of the principal selection pressures for covering behaviour in a range of sea urchin species (Milligan, 1915; Ebling et al., 1966). However, this proposal was not tested in our study. For the *P. lividus* population at Lough Hyne, the more important predators are considered to be the starfish *Marthasterias glacialis* and a number of crab species such as *Necora puber*. These predators are not totally reliant upon vision to locate their prey and the relevance of covering as a means of avoiding predation seems questionable in this case. *Paracentrotus lividus* are sometimes preyed upon by birds (Ebling et al., 1966) and covering with items taken from the background may function as camouflage against these predators. However, none of us have witnessed a single bird predation event or evidence thereof in two years of study at the North Wall site. It thus seems unlikely that camouflage against aerial predation is the principal function of covering behaviour in *P. lividus* at Lough Hyne. However, this does not undermine the idea that covering could be of considerable survival value for a sea urchin population preyed upon principally by predators using visual cues.

Under laboratory conditions, *P. lividus* showed a tendency to cover with more than one item, compared with a single item being the mode (median and mean) in the field. The potential biological significance of this may be any one or a combination of the following factors: (i) there were no predators in the laboratory aquaria and individuals may have been less inhibited in their movements, thereby increasing the likelihood of encountering multiple covering items (randomly distributed within the aquaria); (ii) there may have been a higher relative abundance of covering material within the aquaria, providing individuals with increased opportunities for covering with multiple items; (iii) there were no wave or wind effects within the aquaria and individuals may have been able to hold onto multiple items more effectively than in the field where tidal action may cause item displacement.

In the laboratory, *P. lividus* displayed a preference for covering with leaves when they were available. However, in the field, shells were the most common covering item (Table 2). This difference may reflect the lack of wind and wave effects in the aquaria, thus permitting individuals to cover with lighter items. This may also help to explain why one of the lightest 'novelty' items, the pen lid, was a favoured choice of covering item. When shells were presented in the greatest density/numerical superiority to other items, significantly fewer individuals than anticipated used them. This result suggested that sea urchins might in fact exert a choice for the type of covering material they use.

We believe that covering behaviour in *P. lividus* is not a response to a single isolated factor. Future research will concentrate on covering behaviour *in situ*, in particular an analysis of the extent of individual covering in relation to predation pressures, light intensity and relative availability of covering materials.

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REFERENCES

Barnes, D.K.A. & Clarke, A., 1995. Epibiotic communities on sublittoral macroinvertebrates at Signy Island, Antarctica. *Journal of the Marine Biological Association of the United Kingdom*, **75**, 689–703.

Barnes, D.K.A., Steele, S., Maguire, D. & Turner, J., 1999. Population dynamics of the urchin *Paracentrotus lividus* at Lough Hyne, Ireland. *Proceedings of the Fifth European Echinodermata Conference, Milano, Italy. Echinoderm Research 1998*, 427–431.

Crook, A.C., Long, M. & Barnes, D.K.A., in press. Quantifying daily migration in the sea urchin *Paracentrotus lividus*. *Journal of the Marine Biological Association of the United Kingdom*.

Dance, C., 1987. Patterns of activity of the sea urchin *Paracentrotus lividus* in the Bay of Port-Cros (Var, Fance, Mediterranean). *Marine Ecology*, **8**, 131–142.

Dawkins, M.S., 1985. The scientific basis for assessing suffering in animals. In *Defence of animals* (ed. P. Singer), pp. 27–40, Oxford: Basil Blackwell.

Dix, T.G., 1970. Covering response of the Echinoid *Evechinus chloroticus* (Val.). *Pacific Science*, **24**, 187–194.

Ebling, F.J., Hawkins, A.D., Kitching, J.A., Muntz, L. & Pratt, V.M., 1966. The ecology of Lough Hyne. XVI. Predation and diurnal migration in the *Paracentrotus* community. *Journal of Animal Ecology*, **35**, 559–566.

Ebling, F.J., Sleigh, M.A., Sloane, J.F. & Kitching, J.A., 1960. The ecology of Lough Hyne. VII. Distribution of some common plants and animals of the littoral and shallow sublittoral regions. *Journal of Ecology*, **48**, 29–53.
Emson, R.H., 1999. Making the best of it. The ecology of deep sea echinoderms. *Proceedings of the Fifth European Conference on Echinoderms, Milano, Italy, 1998*, in press.
Farman J.C., Gardiner B.G. & Shanklin J.D., 1985. Large losses of total ozone in Antarctica reveal seasonal ClOx/NOx interaction, *Nature*, *London*, **315**, 207–210.
Farman J.C., O'Neill, A. & Swinbank, R., 1994. The dynamics of the Arctic polar vortex during the EASOE campaign. *Geophysical Research Letters*, **21**, 1195–1198.
Häder, D.P., Kumar, H.D., Smith, R.C. & Worrest, R.C., 1998. Effects on aquatic ecosystems. *Journal of Photochemistry and Photobiology* B, **46**, 53–68.
Kitching, J.A. & Thain, V.M., 1983. The ecological impact of the sea urchin *Paracentrotus lividus* in Lough Ine, Ireland. *Philosophical Transactions of the Royal Society* B, **300**, 513–552.
Lehner, P., 1996. *Handbook of ethological methods*, 2nd ed. Cambridge: Cambridge University Press.
McClintock, J.B. & Janssen, J., 1990. Pteropod abduction as a chemical defence in a pelagic Antarctic amphipod. *Nature*, *London*, **346**, 462–464.
Maldonado, M. & Uriz, M.J., 1992. Relationships between sponges and crabs; patterns of epibiosis on *Inachus aguarii* (Decapoda: Majidae). *Marine Biology*, **113**, 281–286.
Milligan, H.N., 1915. Observations on the foreign objects carried by the purple sea urchin. *Zoologist*, **894**, 441–453.
Millott, N., 1956. The covering reaction of sea urchins. I. A preliminary account of covering in the tropical echinoid *Lytechinus variegatus* (Lamarck), and its relation to light. *Journal of Experimental Biology*, **33**, 508–523.
Millott, N., 1975. The photosensitivity of echinoids. *Advances in Marine Biology*, **13**, 1–52.
Mortensen, T., 1943. *A monograph of the Echinoidea. Vol. III.* 2. Camarodonta I. Copenhagen: Reitzel.
Orton, J.H., 1929. On the occurrence of *Echinus esculentus* on the foreshore of the British Isles. *Journal of the Marine Biological Association of the United Kingdom*, **16**, 289–296.
Renouf, L.P.W., 1931. Preliminary work of a new biological station (Lough Ine, Co. Cork, I.F.S.). *Journal of Ecology*, **XIX**, 410–438.
Ross, D.M., 1971. Protection of hermit crabs (*Dardanus* spp.) from octopus by commensal sea anemones (*Calliactis* spp.). *Nature*, *London*, **230**, 401–402.
Sharp, D.T. & Gray, I.E., 1962. Studies on factors affecting the local distribution of two sea urchins, *Arbacia punctulata* and *Lytechinus variegatus*. *Ecology*, **43**, 309–313.
Southward, A. & Southward, E., 1975. Endangered urchins. *New Scientist*, **66**, 70–72.
Uexküll, J. von, 1897. Ueber reflexe bei den Seeigeln. *Zeitschrift für Biologie*, **34**, 298–318.
Woods, C., 1988. Life without a sunscreen: the ozone layer shields the surface of the earth from the sun's most harmful rays. What will happen if we lose that protection? *New Scientist*, **120**, 46.
Yoshida, M., 1966. Photosensitivity. In *Physiology of Echinodermata* (ed. R.A. Boolootian), pp. 435–463. New York: John Wiley & Sons.

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