

# Chemical defences in embryos and juveniles of two common Antarctic sea stars and an isopod

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**Abstract:** The brooded embryos and/or juveniles of the sea stars *Neosmilaster georgianus* (Studer, 1885) and *Lysasterias perrieri* (Studer, 1885) and the isopod *Glyptonotus antarcticus* (Eights, 1853) were examined for their acceptability using the sympatric sea star *Odontaster validus* (Koehler, 1906) as a predator. Organic extracts were prepared from embryos of both sea stars and juveniles of *Lysasterias perrieri* and *Glyptonotus antarcticus* and tested in alginate food pellets to confirm whether lack of acceptability was chemically based. We found both intact whole embryos and juveniles of the sea star *Neosmilaster georgianus* were not acceptable to *Odontaster validus*. A methanol extract of the embryos was palatable. This could be the result of either the sequestration of deterrent chemicals within embryos or the presence of noxious compounds that were not extractable in methanol. Embryos and juveniles of the sea star *Lysasterias perrieri* were not acceptable to sea stars. Food pellets containing methanol extracts of unacceptable embryos were deterrent against sea stars, suggesting a chemical defence. Juvenile brooded isopods (*Glyptonotus antarcticus*) were also found to be unacceptable in sea star feeding bioassays. Significant rejection of alginate pellets containing a lipophilic dichloromethane methanol extract of juveniles indicated that this lack of acceptability was chemically based. Our study provides further support for chemical defences in the offspring of brooding lecithotrophic Antarctic marine invertebrates.

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## Introduction

Finite resources available to an organism must be allocated strategically to a variety of important life processes. The Optimal Defence Theory (ODT; Rhoades 1979) examines within-organism variations in defensive chemistry in the context of the competing relationship between growth and the production of chemical defences, and predicts that organisms should allocate their chemical defences so as to maximize fitness. The ODT predicts that defences should be differentially allocated to those tissues or structures most valuable in terms of fitness and that there should be a correlation between energetic investment and defence in specific tissues. Reproductive processes including the production of offspring are key determinants of fitness in both marine plants and animals and as such may be under strong selection for the production or sequestration of chemical defences (Cronin 2001, McClintock & Baker 2001). This may be particularly the case when fecundity is low and thus per-individual investment of resources is high.

The most common modes of reproduction in marine invertebrates include the production of copious numbers of small eggs that develop as feeding planktotrophic larvae in the water column, or, in contrast, the production of smaller numbers of large embryos that develop as non-feeding lecithotrophic larvae either released into the water column,

imbedded in egg masses or cases, or brooded by the parent (Vance 1973, Strathmann 1985, Young & Chia 1987, McEdward 1995). While both planktotrophy and lecithotrophy occur in Antarctic marine invertebrates, historically brooding has been considered the principal reproductive mode (Thorson 1950). More recent studies have shown that while brooding is indeed common, many Antarctic marine invertebrates also release large lecithotrophic embryos or larvae into the water column, and a smaller percentage produce planktotrophic larvae (Pearse *et al.* 1991).

Marine invertebrates that produce large yolky embryos are investing considerable resources per offspring. On this basis they would be predicted by the ODT to invest significantly in the defence of each individual progeny. In some cases protection may be enhanced by physical protection of the developing embryos, such as through the production of egg cases or brooding behaviours. Nonetheless, some predators may be capable of dislodging brooded embryos and juveniles from adults thus making a chemical defence valuable in offspring. Moreover, offspring provisioned with chemical defences may retain these defences on becoming a juvenile, a particularly vulnerable life history stage often characterized by the ontogeny of increased morphological defences including an exoskeleton

or skeletal elements.

The preponderance of benthic marine invertebrates with lecithotrophic modes of development in Antarctica provides an opportunity to examine the incidence of chemical defences in species with lecithotrophic development. McClintock & Baker (1997) examined chemical defences in the early life history stages of seven common species of shallow water Antarctic marine invertebrates from McMurdo Sound, Ross Sea, Antarctica (77°S, 164°E). While the eggs or larvae of two species with planktotrophic modes of development were acceptable to a suite of sympatric predators, all five lecithotrophic species, whether brooders or broadcasters, had eggs or embryos that were unacceptable to at least one species of the three sympatric predators tested, strongly suggesting chemical defences. This suggests that chemical defences may be valuable regardless of whether lecithotrophic eggs and embryos are brooded or broadcasted. These initial findings support the hypothesis that chemical defences may be common in the offspring of Antarctic marine invertebrates with lecithotrophic modes of reproduction, as already suggested for temperate and tropical species (Lindquist 1996, Lindquist & Hay 1996, McClintock *et al.* 2001).

The present study extends the evaluation of chemical defences in the offspring of Antarctic marine invertebrates with lecithotrophic, brooding modes of development to three additional species, including two common West Antarctic species of sea stars and a circumpolar brooding isopod. Our study also extends the evaluation to a very different region of Antarctica by examining organisms collected at Anvers Island off the Antarctic Peninsula (64°S, 64°W).

## Materials and methods

The sea stars *Neosmilaster georgianus*, *Lysasterias perrieri* and *Odontaster validus* and the isopod *Glyptonotus antarcticus* were collected subtidally from several sites within 3.5 km of Palmer Station on Anvers Island, Antarctica (64°46'S, 64°04'W; cf. Amsler *et al.* 1995). Collections were made and bioassays conducted both during the autumn 2000 (March–May) and early summer 2001 (November–December). The sea star *Neosmilaster georgianus* is a very abundant year-round brooder in nearshore subtidal communities of the Antarctic Peninsula (Fisher 1940, Dearborn & Fell 1974, Slattery & Bosch 1993). *Lysasterias perrieri* is less common than *N. georgianus* on the Antarctic Peninsula (Dearborn & Fell 1974), but sufficient numbers were available to locate several brooding individuals. Both sea stars brood their embryos and early juveniles by becoming stationary and assuming a humped up posture while holding the brood against the oral opening. The large relatively common isopod *Glyptonotus antarcticus* has a circumpolar distribution, is known to have populations predominantly

comprised of females (Dearborn 1965), and carries its brooded embryos and juveniles against the ventral carapace (Pearse & Giese 1966). The sea star *Odontaster validus* is ubiquitous in shallow nearshore waters of Antarctica (McClintock *et al.* 1988). Its high abundance, circumpolar distribution, and voracious omnivorous feeding habits have contributed to it being chosen as a model predator in studies of chemical deterrence in Antarctic benthic communities (McClintock & Baker 1997, Amsler *et al.* 2001a, 2001b, Iken *et al.* 2002).

For all embryo and juvenile extracts, alginate pellets (2% alginate by weight; ca. 2–3 mm pellet diameter) containing dry krill powder (5% by weight) were prepared. Dried extracts were dissolved in a minimum volume of appropriate solvent carrier and dried onto the krill powder with a rotary evaporator (Hay *et al.* 1994). The extracts were imbedded in alginate pellets at “tissue-level” concentrations based on the ratio of the mass of the extract yield from a known mass of embryos or juveniles. The same volume of the solvent carrier was added to krill powder used for controls and likewise dried. The powders were then mixed into cold alginate solution which was then immediately polymerized using 1 M CaCl<sub>2</sub>. As the eggs and juveniles of Antarctic marine invertebrates with lecithotrophic development are high in energy-rich organic constituents (McClintock & Pearse 1986), alginate pellets containing a 5% krill powder are apt to be less rich in energy, yet they contained sufficient levels of stimulant to induce a consistent feeding response (McClintock & Baker 1997).

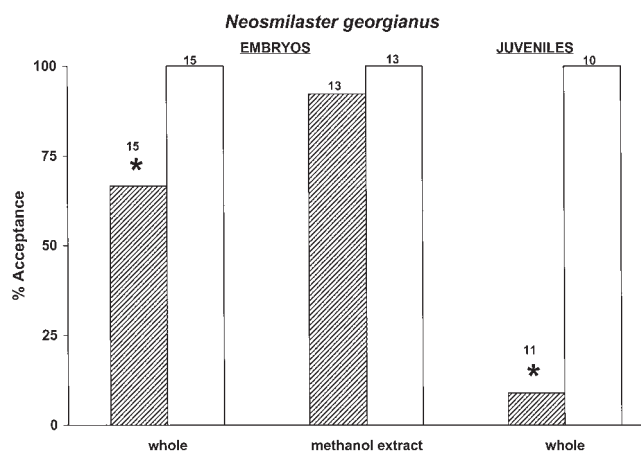
Brooded embryos and juveniles of the sea stars *Neosmilaster georgianus* and *Lysasterias perrieri* were teased apart using forceps. Bioassays were conducted using intact embryos and juveniles and then, following extraction of additional individuals, as extracts in alginate pellets. Insufficient numbers of juvenile *Neosmilaster georgianus* were available for extract preparation. No adult isopods (*Glyptonotus antarcticus*) were found carrying brooded embryos. Nonetheless, brooded juveniles from several individuals were collected and tested intact, and then extracted and subsequently tested in alginate pellets. Individual embryos and/or juveniles isolated from the three brooding species were typically about 2–3 mm in size.

Methanol extracts of the embryos of *Neosmilaster georgianus* and the embryos and juveniles of *Lysasterias perrieri* were prepared and tested in alginate pellets. Methanol was selected as a solvent of choice as it is known to be generally effective at extracting both lipophilic and hydrophilic compounds. Embryos and juveniles were weighed and then lyophilized and re-weighed. The lyophilized material was then extracted in methanol for 24 h three times. Evaporation of the methanol yielded the final extract which was then weighed.

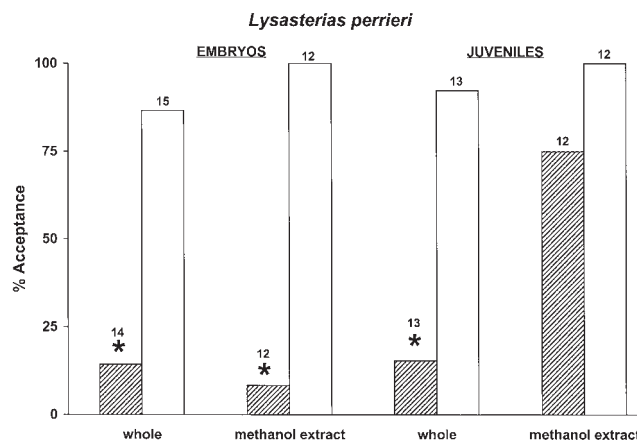
In the case of brooded juveniles from the isopod *Glyptonotus antarcticus* a separate lipophilic and

hydrophilic extract was prepared. Juveniles were first weighed, lyophilized, and re-weighed. Subsequently they were extracted first with dichloromethane/methanol (1:1) (2 x 25 ml) for 12 h twice. Evaporation of the solvents produced a lipophilic extract. The hydrophilic extract was prepared by subsequent extraction of the freeze dried material with methanol/water (1:1) in a similar fashion. Final extract dry weights were determined.

Sea star feeding deterrent bioassays were conducted using protocols similar to those described in McClintock & Baker (1997). *Odontaster validus* is a model sea star predator in the sense that it has been used in previous feeding acceptability studies (McClintock & Baker 1997, Iken *et al.* 2002), is readily available, and its feeding response lends itself particularly well to laboratory based bioassays. Approximately 400 *Odontaster validus* (mean arm radius 43.2 mm;  $n = 52$ ) were collected and placed in a large circular tank (2 m diameter; 3200 l) equipped with running seawater pumped directly from the sea (-1 to 2°C). *Odontaster validus*, when placed into a seawater tank, will typically climb to the air-water interface and then extend their arms back exposing the ambulacral feeding grooves of 2–3 arms. Intact embryos, juveniles, and experimental and control feeding pellets were placed individually on the extended tube-feet within the ambulacral groove equidistant between the oral opening and the tip of the arm. An embryo, juvenile or algininate pellet was considered accepted when individuals used their tube feet to move the embryo, juvenile or pellet to the oral opening. Rejection was considered to be when an individual 1) dropped the embryo, juvenile or pellet, or 2) moved the embryo, juvenile or pellet



**Fig. 1.** Percentages of whole embryos, algininate pellets with embryo extract, or whole juveniles of the sea star *Neosmilaster georgianus* carried along the ambulacral groove by tube feet to the oral opening of the sea star *Odontaster validus* (hatched bars). In all assays, controls consisted of algininate krill pellets (open bars). Numbers of replicates are shown at the top of each bar. Asterisks indicate experimental treatments that are significantly different from controls ( $P < 0.05$ ).

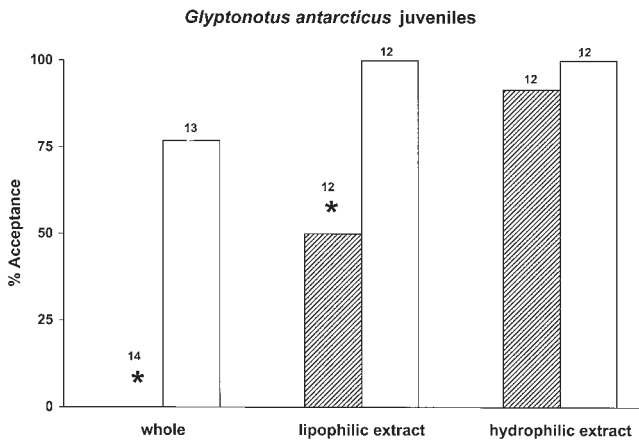


**Fig. 2.** Percentages of whole embryos, whole juveniles, and algininate pellets with embryo and juvenile extracts of the sea star *Lysasterias perrieri* carried along the ambulacral groove by tube feet to the oral opening of the sea star *Odontaster validus* (hatched bars). In all assays, controls consisted of algininate krill pellets (open bars). Numbers of replicates are shown at the top of each bar. Asterisks indicate experimental treatments that are significantly different from controls ( $P < 0.05$ ).

along the ambulacral groove away from the mouth and towards the tip of the arm. Following an experimental feeding trial the same sea star was presented a control algininate pellet. Individuals generally completed each feeding assay within a 30 min period. As sea stars survive and behave normally for extended periods of time without regular feeding (Jangoux 1982), consumption of control pellets by sea stars that were routinely used in assays was considered a sufficient maintenance diet. Following each feeding assay, individual sea stars were displaced from the sea tank wall and allowed to settle to the bottom of the tank. This prevented individuals from being used more than once in a given experimental feeding trial. The non-parametric Fisher's Exact test (Zar 1999) was used to compare the acceptability of experimental tissues or extracts imbedded in krill algininate pellets with control krill pellets.

## Results

The sea star *Odontaster validus* displayed significant feeding deterrence to whole embryos ( $P = 0.04$ ) and whole juveniles ( $P = 0.0003$ ) of the sea star *Neosmilaster georgianus*, while methanol extracts of embryos of *N. georgianus* incorporated in algininate pellets were not deterrent ( $P = 1.0$ ) (Fig. 1). Moreover, significant sea star feeding deterrence was detected against whole embryos ( $P = 0.0001$ ) and whole juveniles ( $P = 0.0002$ ) of the sea star *Lysasterias perrieri* (Fig. 2). Algininate pellets containing methanol extracts of embryos of *Lysasterias perrieri* were significantly rejected by sea stars ( $P = 0.0001$ ), while methanol extracts of juveniles were not deterrent ( $P = 0.22$ ). *Odontaster validus* also displayed significant feeding



**Fig. 3.** Percentages of whole juveniles and alginates pellets with lipophilic or hydrophilic extracts of juveniles of the isopod *Glyptonotus antarcticus* carried along the ambulacral groove by tube feet to the oral opening of the sea star *Odontaster validus* (hatched bars). In all assays, controls consisted of alginates krill pellets (open bars). Numbers of replicates are shown at the top of each bar. Asterisks indicate experimental treatments significant different from controls ( $P < 0.05$ ).

deterrence to whole juveniles of the isopod *Glyptonotus antarcticus* ( $P = 0.00003$ ) as well as alginates pellets containing the lipophilic juvenile extract ( $P = 0.014$ ) (Fig. 3). In contrast, no sea star feeding deterrence was detected against alginates pellets containing a hydrophilic extract of juvenile isopods ( $P = 1.0$ ).

## Discussion

Antarctic marine invertebrates possess both planktotrophic and lecithotrophic modes of development (reviewed by Pearse *et al.* 1991). Nonetheless, the preponderance of Antarctic benthic marine invertebrates are lecithotrophic, producing small numbers of large yolky embryos that are either broadcast into the water column, encased in protective structures such as egg ribbons, or brooded by the parent. The only study to date to have examined feeding acceptability and chemical defences in a suite of planktotrophic and lecithotrophic Antarctic marine invertebrates found planktotrophic larvae to be acceptable to ecologically relevant predators, whereas all lecithotrophic species had embryos that were unacceptable and, where tested, chemically defended (McClintock & Baker 1997). Two of the benthic marine invertebrates with unacceptable lecithotrophic embryos were the brooding sea star *Diplasterias brucei* and the brooding sponge *Isodictya setifera*. Our present study extends the list of brooding benthic species with unacceptable embryos and/or juveniles by an additional three species.

Our additional evidence for the lack of acceptability and presence of chemical defences in the offspring (embryos and juveniles) of brooding lecithotrophic species of

Antarctic marine invertebrates lends further support to the predictions of the ODT. Lecithotrophic embryos of Antarctic marine invertebrates, whether brooded, in egg ribbons, or broadcasted, are rich in energy due to their high levels of lipids and proteins (McClintock & Pearse 1986), making them attractive food items. This is probably a contributing factor to the high incidence of chemical defences detected to date in the offspring of marine invertebrates with lecithotrophic modes of reproduction (Lindquist *et al.* 1992, Lindquist 1996, Lindquist & Hay 1996, McClintock & Baker 1997; reviewed by McClintock *et al.* 2001). Selection for defences in brooded embryos and larvae may also be particularly strong in Antarctica where the brooding period in marine invertebrates is on the order of months instead of weeks as is more typical in temperate and tropical marine systems (Pearse *et al.* 1991). This extended development period increases the likelihood of predation.

The highly mobile omnivorous sea star *Odontaster validus*, employed in the present study as a model predator, may project its arms into the water column as a "larval filter" (Dayton *et al.* 1974). It also forms dense feeding aggregations to attack and consume larger adult sea stars (e.g. *Acontaster conspicuus* which broadcasts its lecithotrophic larvae, Dayton *et al.* 1974). It is likely that brooding species of sea stars would be even more susceptible to capture by *O. validus*, as their immobile brooding posture makes them vulnerable. While a number of studies have reported on the feeding habits of Antarctic Peninsula sea stars (Brand 1974, Dearborn & Fell 1974, Dearborn *et al.* 1982, Dearborn & Edwards 1984), there are no reports of *O. validus* preying on the common sea stars *Neosmilaster georgianus* and *Lysasterias perrieri* (McClintock 1994). This suggests that adults of these two sea stars may possess a chemical defence (e.g. McClintock 1989) against predation by *Odontaster validus*. The unacceptable nature of the embryos and juveniles of *Neosmilaster georgianus* and *Lysasterias perrieri* may ensure that *O. validus* does not dislodge and consume the broods of immobile brooding adults.

The basis of the rejection response for embryos and juveniles of *Neosmilaster georgianus* remains unknown. A methanol extract of the unacceptable embryos incorporated in alginates pellets did not elicit a significant feeding deterrent response in sea stars. While it is possible that these large yolky embryos do not possess a chemical defence, it seems unlikely given their conspicuous lack of structural defence. There are several potential reasons why deterrent chemistry may have gone undetected. First, while methanol is a good solvent of choice to extract many secondary metabolites, it is possible that feeding deterrent compounds that are strongly lipophilic or hydrophilic might not be extracted in methanol. Second, if the deterrent chemistry in these embryos were sequestered, such as in the outer surfaces of the embryos where it might be most effective,

then the decision to base our calculations for loading extracts into alginate pellets on the basis of total yield of crude extract from the embryos may have been too conservative. The lack of acceptability of juvenile *Neosmilaster georgianus* does not rule out a physical defence such as that seen in small spinated crustaceans (e.g. Morgan 1990, 1995). However, it is difficult to imagine that spination would be an effective deterrent against *Odontaster validus* which can extrude its cardiac stomach around prey (McClintock 1994). As insufficient numbers of juvenile *N. georgianus* were available to prepare a chemical extract, their potential to harbour chemical deterrents remains unknown. While it is possible that the small size and low nutrient levels of juveniles make them unattractive prey, the inclusion of small prey and a variety of sea stars in the omnivorous diet of *Odontaster validus* argues against this hypothesis (McClintock 1994).

Both the embryos and juveniles of the sea star *Lysasterias perrieri* were found to be unacceptable to the sea star *Odontaster validus*. Moreover, methanol extracts of embryos were deterrent in alginate pellets at ecologically relevant concentrations indicating that rejection was chemically based. Lucas *et al.* (1979) detected chemical defences in the eggs, larvae and juveniles of the sea star *Acanthaster planci*. McClintock (1989) examined the toxicity of a variety of Antarctic sea stars, but did not include embryos or juveniles. It is likely that if juvenile *L. perrieri* possess a chemical defence, that the deterrent chemistry is different to that detected in embryos, as methanol extracts of juveniles did not elicit a rejection response as seen in methanol extracts of embryos. The chemical(s) responsible for deterrence in the embryos and possibly juveniles of both sea stars examined in the present study are unknown, but may be steroidal saponins, a group of compounds known to play a key, but not exclusive, defensive role in echinoderms (Lucas *et al.* 1979, Cimino & Ghiselin 2001, McClintock & Baker 2001). It should be noted, however, that the lack of feeding deterrence in sea stars offered alginate krill pellets containing the methanol extract of juveniles of the sea star *Lysasterias perrieri* may suggest that, if a chemical defence is present, it may be a rare non-steroidal defensive metabolite, as asterosaponins are generally stable products that can be extracted in methanol.

Brooded juveniles of the common giant isopod *Glyptonotus antarcticus* were rejected in feeding assays by the sea star *Odontaster validus*. Field observations indicate that *O. validus* is capable of capturing adult *Glyptonotes antarcticus* (Peckham 1964). Juvenile isopods were soft and flexible, but had a well defined exoskeleton with serrated lateral edges of the posterior carapace and walking legs bearing small spines. It is possible that feeding deterrent properties of juvenile *Glyptonotus antarcticus* are related in part to the presence of an exoskeleton with serration and spination as seen in a variety of small planktonic larval

crustaceans which use their spines to deter fish predators (Morgan 1990, 1995). However, as noted above, spination is unlikely to deter extraoral feeding in *Odontaster validus*. We found alginate pellets containing lipophilic extracts of juvenile isopods were unacceptable to sea stars. Thus, it appears that in the case of juvenile *Glyptonotus antarcticus*, a chemical defence complements any protection afforded by the exoskeleton.

There are very few examples of chemical defences among the Crustacea. Luckenbach & Orth (1990) found that the free living fourth larval stage of the pea crab *Pinnotheres ostreum* was consistently rejected by several species of sympatric fish. Live pea crabs were rejected significantly more often than dead crabs by the mummichog fish *Fundulus heteroclitus*. The investigators concluded that fourth stage *Pinnotheres ostreum* larvae, which lack defensive spines, employ a noxious antipredator defence, perhaps an extracellular mucus-like material found in regular rows on the carapace. McClintock *et al.* (2001) found the large black pelagic copepod *Candacia ethiopica* to be rejected by the sympatric planktivorous red ear sardine *Harregula humeralis*. Moreover, homogenates of this copepod incorporated in alginate pellets containing a feeding stimulant were deterrent suggesting that defence is chemically derived. No information is currently available about the specific chemical compounds responsible for these chemical defences in crustaceans.

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