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Coelomic Transport and Clearance of Durable Foreign Bodies by Starfish (*Asterias rubens*)

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Abstract. Echinoderms have excellent healing and regeneration abilities, but little is known about how they deal with the related challenge of durable foreign bodies that become lodged within their bodies. Here we report a novel mechanism for foreign body elimination in starfish. When injected into the arm of a starfish, passive integrated transponder tags and magnets of similar dimensions are eliminated at a rate approximating 10% per day. These objects are forcefully ejected through the body wall at the distal tip of an arm. Ultrasound images reveal that foreign bodies are moved within the body cavity, and tracking of magnets injected into starfish suggests that the movements are haphazard rather than directed. Constrictions of the body wall near the foreign object are the likely mechanism for this transport process. Open questions include the ecological relevance of this behavior, why clearance occurs through the distal tips of the arms, the neurological and muscular control of this behavior, what other animals use this mechanism, and the range of objects starfish can eliminate in this way.

Introduction

The regenerative abilities of echinoderms (Hyman, 1955; Carneveli, 2006), and starfish in particular (Anderson, 1965; Mladenov *et al.*, 1989; Fan *et al.*, 2011), are well documented, including regrowth of whole limbs and major organs. Regeneration, however, is slow and expensive (Lawrence and Larrain, 1994; Ramsay *et al.*, 2001; Maginnis, 2006); many breaks in the body wall can be better addressed through localized healing or the removal of foreign bodies. Invertebrate immune responses have been shown to be dependent on the size of the foreign object (Leclerc, 1996). Microorganisms are typically eliminated through cellular immune responses involving phagocytosis (Götz, 1986). However, when larger objects become lodged in the tissues, hollow organs, or body cavity of the animal, they are typically encapsulated by leukocytes (Götz, 1986; Schmid-Hempel, 1994) before being eliminated (a process called “clearance”). The encapsulation of foreign bodies was first investigated at the end of the nineteenth century using starfish (Metchnikoff, 1883). The degree to which encapsulation and subsequent elimination of the intruder succeeds in some species has been shown to depend on the physical nature of the foreign object (e.g., wettability and charge, Lackie, 1983) as well as its antigenic properties (Ghiradella, 1965).

In human and veterinary medicine, problems involving embedded (Cartee and Rumph, 1984) or ingested (Cheng and Tam, 1999) durable foreign objects (e.g., splinters, glass shards, thorns, spines) are common. If not eliminated, such foreign bodies can produce a wide variety of long-lasting problems, including internal injuries, chronic inflammation, impeded movement, and increased risk of infection. Starfish, as common benthic predators, are likely to similarly acquire potentially harmful biotic objects and rock shards. The ejection of those foreign bodies that cannot be broken down has been studied in vertebrates and can involve the intestines or the bladder—organs that already actively move wastes out of the body (Chisholm and Hubert, 1985; Tracy *et al.*, 2011). While encapsulation of foreign bodies has been studied widely, we can find only passing reference to clearance of durable objects in invertebrates (Porchet-Henneré *et al.*, 1987).
Our investigation began with a desire to mark the starfish *Asterias rubens* (Linnaeus), with passive integrated transponder tags (PIT-tags), which would have allowed automated *in situ* identification of individuals (Miller and Sadro, 2003). This technique is relatively successful in sea urchins (Hagen, 1996; Duggan and Miller, 2001). Marking individual starfish in a way that is long-lasting (external vital staining, Feder, 1970; visible implant elastomer, Martinez et al., 2013) or allows for remote data collection (archival electronic tags tied to the animal, Lamare et al., 2009) is feasible, but as with previous methods, PIT-tags fail to combine these features. Rather, we witnessed a previously undescribed mechanism of foreign body elimination: ejection of PIT-tags through the distal ends of the arms. In this article, we analyze data from these and additional experiments to better characterize this mechanism.

**Materials and Methods**

**Experimental animals and maintenance**

Common starfish (*Asterias rubens*) were caught by local fishermen (generally as bycatch) in the waters around Kerteminde, Funen, Denmark, and transported to the University of Southern Denmark’s Marine Biological Research Centre in Kerteminde. Prior to experiments, animals were kept in 1000-l tanks supplied with unfiltered running aerated seawater in a 10 °C temperature-controlled room with a 12:12-h light/dark cycle. Salinity in the tank followed natural fluctuations of incoming water from Kerteminde Fjord and stayed around 20 psu during the experimental period. Live blue mussels (*Mytilus edulis*) were available in the tanks for *ad libitum* feeding. Individuals that were injured or visibly unhealthy at the start of the experiment were excluded.

**Experimental design and protocol**

**Experiment 1: Retention times.** To study PIT-tag retention, we injected 53 starfish with PIT-tags (rod-shaped with rounded edges, length = 12 mm, diameter = 2 mm, weight = 0.095 g) using a syringe implanter. The tag was injected through the aboral surface of the body wall of the first arm counterclockwise from the madreporite, halfway down the arm. A PIT-tag reader (Trovan LID 500) allowed daily individual identification of marked starfish and a determination of when each tag was lost. This method did not allow us to determine where within the animal the tag was located. The mass of each individual was tracked daily by allowing it to feed. Individuals that were injured or visibly unhealthy at the start of the experiment were excluded.

**Experiment 2: Tracking.** To understand how these foreign bodies were moving with the starfish, we next injected magnetic stir rods of similar dimensions (length = 8 mm, diameter = 2 mm, average weight = 0.082 g), using the same procedure as for the PIT-tags. These magnets were not individually identifying, but they allowed us to quickly and easily locate the exit of the magnets. When directly over the internal magnet, the hanging magnet would twist and rotate vigorously. The hanging magnet was kept at least 2 cm from the skin of the animal, which preliminary tests indicated would avoid moving the internal magnet. Preliminary testing revealed this method to be repeatable across independent observers. Twenty individuals were injected in this way and divided up among 10 buckets of aerated, frequently changed seawater at 10 °C, with each bucket having one starfish larger than 90 g and one smaller than 90 g. This system allowed for repeated identification of each individual based on its bucket and weight. We repeatedly recorded the location of each magnet within its starfish, whether it had come out, and (when possible) the location of the exit. These data were taken three times during the first day after injection, and once daily on days 2, 5, 6, and 10.

To gain further insight into how this transport occurs, we used our data from Exps. 1 and 2 to compare retention times of PIT-tag versus magnets. Starfish in these two experiments differed both in object type and environment, such that any differences in time to loss (tested for with Cox Regression in R package *survival*) could be due to either factor.

**Experiment 3: Focused tracking and imaging.** As internal movements of magnets were too rapid to be tracked in detail using our Exp. 2 protocols, a small number of starfish were observed in greater detail. We followed the same procedure as described for Exp. 2 except that experiments were done at 16 °C, which we expected would shorten the time to ejection, allowing for near-continuous observation. Placement of the magnets was recorded at least once per hour until loss. To better understand the anatomical details of this
transport, ultrasound observations were taken at intervals of 5 min or less on four of these individuals with a 2002 Panther B&K medical ultrasound scanner (Analogic Ultrasound, Herlev, Denmark). Simultaneously, constrictions of the body wall in the vicinity of the magnets were observed for signs of motion along the arm’s major axis.

Results

Experiment 1: Retention times

Daily hazard rate (\( h_x \), the risk of loss of each tag each day, conditional on its having been retained to that day) of PIT-tags fluctuates around 0.09 (Fig. 1). As sample size decreases and the uncertainty in the hazard rate increases, individual measurements wander further from 0.09. However, using AICc, a model that assumes a constant hazard rate is somewhat preferred (Table 1) to those that assume a linear or exponential dependence of \( h_x \) on time. No time dependence is visually apparent.

All ejected tags but two were found, undamaged, in the bottom of the tank at the end of the experiment. The remaining two tags may have been lost in a drain hole. Following injection, glands of the pyloric caecum often briefly protruded from the entry wound, indicating that the tag was entering the coelom. Newly ejected tags often had minute bits of soft tissue adhering to them; these were loosely attached and appeared randomly distributed.

Experiment 2: Tracking

All injected magnets moved repeatedly within the starfish, and in none was the movement solely distal. While most movement was in the injected arm, four magnets were at some point observed in the central disc of the starfish, and two of these later moved into a different arm. One magnet that was retained to the end of the experiment (Day 10) moved between three different arms. We witnessed four magnets being ejected, three from the injected arm (Arm #1) and one from an adjacent arm (Arm #2). The inclusion of bucket-ID and individual size as random effects had no influence on preliminary statistical models of retention time and therefore were not further considered.

Comparing Experiments 1 and 2

We observed a much earlier loss of magnets than of PIT-tags (Fig. 2). On the first day, 70% of the magnets were lost, compared to a little more than 20% of the PIT-tags. Only one magnet lasted 10 days, while one PIT-tag was retained beyond 21 days. The hazard of ejection for magnets was more than twice that of PIT-tags (Cox regression, hazard ratio = 2.11, \( z = 2.7, P = 0.007 \)).

Table 1

<table>
<thead>
<tr>
<th>Model</th>
<th>Function</th>
<th>AICc</th>
<th>( \Delta \text{AICc} )</th>
<th>AICcWt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null model</td>
<td>( h_x = 0.086 )</td>
<td>-43.14</td>
<td>0.00</td>
<td>0.55</td>
</tr>
<tr>
<td>Linear time model</td>
<td>( h_x = -0.00089x + 0.095 )</td>
<td>-40.44</td>
<td>2.70</td>
<td>0.14</td>
</tr>
<tr>
<td>Exponential time model</td>
<td>( h_x = 9.32 \times 10^{-2} \times (4.97 \times 10^{-10})^x )</td>
<td>-41.92</td>
<td>1.22</td>
<td>0.30</td>
</tr>
</tbody>
</table>

AICc calculations and Akaike weights. AICc: estimated information loss, AICcWt: Akaike weights, the relative probability that each model is best.
Experiment 3: Focused tracking and imaging

Movements of magnets varied in both pace and direction (see Fig. 3 for an example). All seven magnets injected at 16 °C were lost within the first 3 h. All such losses were via the tip of one of the arms (Fig. 4). The magnet was squeezed out through the skin after roughly 1 min of increasing stretching followed by a rupture and a few seconds of ejection, leaving a tiny tear barely detectable upon microscopic inspection. Cross-sectional ultrasound images of starfish arms containing magnets (Fig. 5) show that the magnet is transported within the coelom, or body cavity, of the arm. In all of dozens of ultrasound views of four individuals, magnets were clearly visible within the coelomic space, and were generally well away from other spaces, such as the water vascular system. An externally visible constriction of the arm (Fig. 6) was often associated (roughly half the time, persisting for several minutes) with the location of the magnet, and ultrasound (Fig. 7) demonstrates that this constriction generally narrows the coelomic space just proximal or distal to the magnet. However, we observed no movement of these constrictions, even when the associated

Figure 3. The movements of a magnet in a starfish over time. Numerals correspond to location at the following number of minutes post-injection: (1) 0, (2) 15, (3) 23, (4) 42, (5) 66, (6) 84, (7) 121, (8) 137, (9) 154, (10) 162, (11) 191. (M) is the madreporite. The magnet wandered, sometimes distally, sometimes proximally, and briefly moved into the base of another arm. It was ejected from the end of the injected arm (12) 201 min after injection.

Figure 4. Ejection of a magnet (white object in front of arrow) from the aboral side of the distal end of an arm just above the sensory cluster. Across experiments, we directly observed 12 ejections of foreign bodies, all from this same location.

Figure 5. Cross-sectional images through Arm #1 with magnet in coelom. (A) Ultrasound image. (B) Interpretive cartoon of same view. In all our observations, the magnet (black and white stripes) was parallel to the arm and is therefore seen end-on in the coelom (gray) between the body walls (white).
magnet moved quickly. Again we observed a seemingly undirected transport resulting in removal of magnets through the tips of arms in all four starfish examined by ultrasound.

Comparing Experiments 2 and 3

Patterns of movement observed at 16 °C, as compared to 10 °C (Fig. 8) are quite similar, except that starfish held at 16 °C lose their tags rapidly. The small sample size at 16 °C (n = 7) and short retention of tags at the higher temperature make Cox regression uninformative for this temperature contrast.

Discussion

Starfish have the ability to transport durable foreign bodies through their coelom (Figs. 5 and 7) and eject them through the distal ends of their arms (Fig. 4). That the hazard of ejection is constant over time (Fig. 1) indicates both that the PIT-tag is not coming back out through the entrance wound, and that movement toward arm-tips involves a somewhat haphazard wander. A direct, coordinated distal movement would likely result in an increase in clearance rate with time as an increasing proportion of tags reached the tips of arms. Tracking of individual magnets (e.g., Fig. 3) confirms this wandering pattern. Despite this random aspect, the comparison of ejection times of PIT-tags versus magnets (Fig. 2) indicates that object type, the environment to which the animal is subjected (temperature, container, etc.), or their interaction can influence how fast an object is ejected. Further experiments will be needed to determine what controls rate of clearance. The magnets, in addition to being harder, denser, and 4-mm shorter than the PIT-tags (and therefore potentially easier to transport), may be more disturbing to starfish if they, like many other animals, can sense magnetic fields (Wiltschko and Wiltschko, 1995). Additionally, the surface properties of the object can influence the immune response to it, as previously observed in insects by Lackie (1983). The degree to which starfish encapsulate different...
types of objects could perhaps affect the triggering and frequency of constrictions of the arms around the object, because muscle contractions have been shown to be involved in immune responses of other invertebrates (Leclerc, 1996). Similarly, ejection of magnets at different temperatures (Fig. 8) suggests that temperature influences the time to ejection. Temperature likely speeds transport by increasing the physiological and physical activity of the echinoderm. The hypothesis of active but loosely directed transport is confirmed by our ultrasound observations of magnets within starfish. These images revealed that the magnets were moving within the coelom (Fig. 5).

We frequently observed externally visible constrictions of arms (Fig. 6) close to the location of magnets, and the magnets seemed to move most rapidly when pushing away from the internal manifestations of these constrictions (Fig. 7). Constrictions observed over several minutes of continuous observation did not appear to move along the length of an arm as would be expected in peristalsis, although our experiments were not specifically designed to detect this. As we did not expect these contractions, we do not have good data on their prevalence, persistence, etc. Our impression based on *ad hoc* observation is that they persist for several minutes and occur in all injected individuals roughly half the time.

This previously undescribed behavior functions to transport and clear durable foreign bodies, raising several questions.

*What is the ecological relevance to Asterias rubens?* The ability to clear durable foreign objects is useful to organisms only if such objects occur in the coelom frequently enough to impose a significant selective cost on those lacking this ability. Endoparasites may grow in starfish’ coeloms (Stone, 1987), and forcible expulsion could serve where encapsulation fails. Durham (1887), who studied the clearance of microscopic particles from the coelom of this species, suggested that this ability was most needed when an arm is shed. Extrapolating, it seems likely that most durable foreign bodies enter the coelom through injuries to the arms. A compilation by Lindsay (2010) indicates that depending on species and environment, 0 to 69% of individuals bear such injuries, with most populations in the 20% to 40% range. A survey of *A. rubens* reported that a mean of 26% of individuals had arm damage (Marrs *et al.*, 2000). Given the efficiency with which objects are ejected from the coelom and the fact that this aspect of organismal maintenance has rarely been investigated, it is not surprising that nonparasite durable intracoelomic foreign objects have not been reported in starfish. Ultimately, our results suggest that only internal examination of thousands of freshly caught wild individuals from a variety of environments would reveal the frequency with which such objects occur.

*Why push an object out through the skin rather than the mouth?* Starfish can evert their stomachs and will eliminate digestive waste this way. While it is possible that some of the objects we injected were eliminated through the mouths of starfish, we saw no indication of this, and elimination generally followed the magnet being close to the tip of an arm. Ghiradella (1965) observed that bits of foreign tissue injected into the coelom of two starfish species could sometimes be ejected through the body wall by means of the dermal brachiae—small projections of the coelom that communicate with the exterior seawater and function in waste removal and respiration. While this mechanism is likely related to what we report here, clearance of these small, soft bits of tissue was not through the arm tips and did not rupture the body wall as tags did; no constriction of the arm was reported. The same author hypothesized that some of the foreign tissue could have been transported into the stomach and either digested or ejected from there. It may be that while soft tissues could be eliminated in this way, no mechanism exists for transporting hard, larger objects from the coelom across the stomach wall, a necessary step for elimination through the mouth. A breach of the barrier between coelom and stomach could risk coelom infection by digestive microbes.

*How is this behavior controlled?* The observed constrictions used to move the foreign bodies through the coelom clearly involve the circular muscles of the body wall (Hyman, 1955). The radial nerves within each arm have direct control of that arm’s movements (Dale, 1999) with little input from the central nerve ring (Migita *et al.*, 2005). The activity and coordination of these muscles remain poorly understood in starfish, but it appears that they can be activated locally by sensing a foreign body within the coelom.

*What range of objects can be eliminated?* We have investigated only two types of objects of similar shapes and sizes, both plastic-coated. Whether this same mechanism would serve to eject soft-bodied endoparasites, small sand-grains, or a large and complex fragment such as a piece of arthropod exoskeleton is unknown.

*How wide a range of organisms beyond Asterias rubens employs this mechanism?* Although encapsulation has been studied in a wide range of organisms, clearance of durable foreign bodies has not. Starfish and most other echinoderms inhabit and feed in benthic habitats where sharp detritus, prey-remains (*e.g.*, molluscan shells), pieces of predators, and potential endoparasites could end up inside their tissues or cavities. How other echinoderms, let alone more disparate taxa, clear durable foreign bodies is unknown. As sea urchins do not have this ability (Hagen, 1996) it may be unique to Asterozoa (starfish and brittle stars), or some subgroup thereof.

We propose the following crude model to explain our observations: (1) An object gains entry to the coelom. (2) The animal detects the object, perhaps through immune-mediated irritation. (3) The radial nerves cause a contraction of the circular muscles in the location of the irritation. (4) The resulting pressure causes the object to move, either proximally or
distally. (5) This relieves the irritation at the initial location, allowing the constriction to relax, but moves the irritant to a new location. (6) Steps 2 through 5 are repeated (accompanied by movement of the object caused by the normal behavioral movements of the animal) until the object reaches an arm tip. (7) A contraction just proximate to the object can now trap it against the distal tip of the coelom, causing stretching of the body wall at that point. (8) When this stretching exceeds the tensile strength of the weakest part of the affected tissue, the object erupts and is cleared.

The ability to transport and clear durable foreign bodies would seem to be useful for a wide range of animals, but how most invertebrates accomplish this remains unknown. Starfish have the ability to move objects through the coelom to the distal ends of the arms, apparently by muscular contraction of the body wall, and thence to eject them. This behavioral mechanism, discovered here accidentally, represents another skill in the already impressive repertoire that echinoderms have for maintaining and repairing themselves.

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Literature Cited


