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Presence of a seawater-filled caecum in *Echinocardium cordatum* (Echinoidea: Spatangoida)

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Heart urchins (Echinoidea: Spatangoida) are considered infaunal, deposit feeding sea urchins that utilize the surrounding sediment as a source of nutrients. Sediment occupies most of the digestive tract lumen but never enters the gastric caecum, a prominent structure that is filled with a transparent fluid. The aim of this study was to shed light on the nature of the fluid found inside the gastric caecum of a well-studied spatangoid species, Echinocardium cordatum. *Our conclusions are based on a three-step-approach: firstly, by following the movement of dyed seawater from the mouth up to the caecal lumen; secondly, by comparing the osmolarity of various body fluids; and thirdly, by describing the particulate content of the gastric caecum. In addition, we employed magnetic resonance imaging (MRI) to reveal the absence of sediment within the gastric caecum. Our osmolarity measurements show that the coelomic fluid is significantly more concentrated than the caecal fluid, which in turn has an osmolarity similar to seawater. MRI reveals that the gastric caecum, in contrast to the rest of the digestive tract, is always devoid of sediment. Light and electron microscopy observations reveal the presence of a variety of detrital particles suspended in the caecal fluid that are identical to those occurring in seawater sampled over the seafloor. We argue that the fluid filling the gastric caecum must be predominantly seawater, and we propose a scenario that explains seawater circulation in* E. cordatum. *In this context, the gastric caecum could act as an internal trap for suspended particulate organic matter. We hypothesize that spatangoid sea urchins could have adopted internal suspension feeding as a secondary feeding mode in addition to deposit feeding.*

Keywords: Echinoidea, Irregularia, Spatangoida, *Echinocardium*, gastric caecum, gut content, trophic status, suspension feeding, deposit feeding

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INTRODUCTION

Two basic modes of feeding that correlate with their epifaunal or infaunal lifestyles have been adopted by sea urchins (Echinodermata: Echinoidea). ‘Regular’ sea urchins use Aristotle’s lantern to rasp or bite algae, seagrasses, encrusting organisms, sessile animals, or carrion. Some ‘regular’ sea urchin species are exclusively herbivorous, while others are generalists (De Ridder & Lawrence, 1982). In contrast, irregular sea urchins are deposit feeders that feed on organic matter contained within the underlying or surrounding sediment. Those irregular sea urchins still possessing Aristotle’s lantern use it to rasp or crush biofilm-coated sediment grains, whereas those lacking it collect sediment grains using mechanisms such as ‘podial particle picking’ (Telford & Mooi, 1996).

In Spatangoida, an abundant and distinctive taxon among echinoids, sediment is carried to the mouth in a number of ways through the action of specialized spines and tube feet, usually following particular pathways along the body surface (Chesher, 1963; De Ridder *et al.*, 1987). In these animals, sediment occupies most of the digestive tract lumen but is presumably never present in the siphon and the gastric caecum, structures that remain fluid-filled. The siphon is a slender tube that bypasses the stomach by opening at the distal part of the oesophagus and by rejoining the digestive tract at the proximal part of the intestine. The siphon actively pumps seawater from the oesophagus to the intestine, most likely in order to provide it with oxygenated water (Stott, 1955) and to avoid dilution of the enzyme-rich contents of the stomach (Buchanan, 1969), that is the main site of digestive and absorptive processes (De Ridder *et al.*, 1985). It has also been suggested that the siphon could facilitate advancement of the digestive tract content (Ferguson, 1969). In contrast to the siphon, the gastric caecum, a large, non-muscular pouch (Figure 1), has only a single, slit-like opening which branches off the anterior part of the stomach (De Ridder & Jangoux, 1993; Ziegler *et al.*, 2010).

Despite its prominent aspect, the gastric caecum has never been the focus of physiological research and its precise function remains unclear. It has been considered to be a glandular structure (Agassiz, 1872–1874), an absorptive organ (Cuénot, 1948; De Ridder & Jangoux, 1993), or a fermentation chamber filled with symbiotic microorganisms (Thorsen, 1998, 1999). Three macroscopically visible features of the gastric caecum

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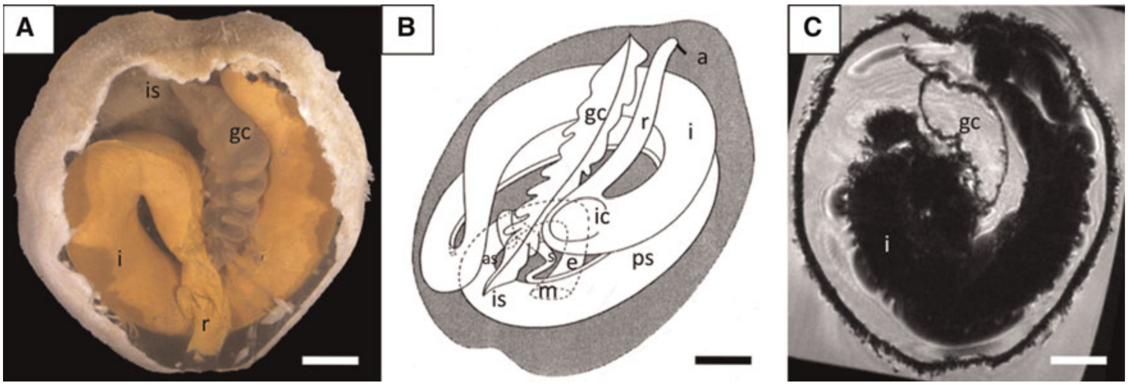

Fig. 1. Anatomy of the digestive tract of the spatangoid sea urchin *Echinocardium cordatum*. (A) Aboral view of the internal anatomy of a dissected specimen. Note the semi-transparent gastric caecum between the opaque coils of the intestine (scale bar = 1 cm); (B) schematic drawing of the course of the digestive tract (modified from De Ridder & Jangoux, 1993) (scale bar = 1 cm); (C) virtual horizontal section through a 3D magnetic resonance imaging dataset of a formalin-fixed specimen at the level of the gastric caecum. Note the absence of sediment from the caecal lumen (scale bar = 1 cm). a, anus; as, anterior stomach; e, oesophagus; gc, gastric caecum; I, intestine; ic, intestinal caecum; is, intermediate stomach; m, mouth; ps, posterior stomach; r, rectum; s, siphon.

are particularly remarkable: its relatively large size, its thin, almost transparent wall, and finally, its permanently swollen aspect *in vivo*.

Differences in shape among irregular sea urchins (Ziegler *et al.*, 2008) as well as the evolutionary origin (Ziegler *et al.*, 2010) of this enigmatic structure have recently been described. According to these results, a gastric caecum is found in most sea urchin species. However, in 'regular' sea urchins its macroscopic aspect does not differ from the rest of the digestive tract and its size relative to the rest of the digestive tract is significantly smaller than in most irregular species. Therefore, a presumably more specialized gastric caecum occurs exclusively in irregular sea urchins, and might appear to be an adaptation to the infaunal lifestyle predominant in the Irregularia.

Echinocardium cordatum (Pennant, 1777) is the best-studied spatangoid species in terms of physiology and ecology (see e.g. Nichols, 1959a–c; Buchanan, 1966; Péquignat, 1970; Higgins, 1974; Bromley & Asgaard, 1975; Buchanan *et al.*, 1980; Smith, 1980; De Ridder *et al.*, 1985, 1987; De Ridder, 1987; Cramer, 1991; Kanazawa, 1992; De Ridder & Jangoux, 1993; Osinga *et al.*, 1997; Thorsen, 1998, 1999; Warnau *et al.*, 1998; De Amaral *et al.*, 2007). Specimens of this species live burrowed in fine- to medium-grained sand (Nichols, 1959a; Buchanan, 1966), and the burrow is connected to the seawater through a vertical chimney with a funnelled opening. This chimney serves as an 'umbilical cord' towards the open seawater (De Ridder *et al.*, 1987), and allows both seawater circulation and selective trapping of detritus (De Ridder *et al.*, 1985; Cramer, 1991). By focusing on the spatangoid species *E. cordatum*, the present study aims to shed light on the role of the gastric caecum.

MATERIALS AND METHODS

Fifty specimens of *Echinocardium cordatum* (Pennant, 1777) (Echinodermata: Echinoidea: Spatangoida: Loveniidae) were collected in Wimereux (Pas-de-Calais, France) by digging on a sandy beach at low tide in March 2006, July 2009 and May 2011. Sediment and seawater from the immediate surrounding area were simultaneously sampled. To measure the osmolarity, the caecal and the coelomic fluids were taken from 15 individuals kept briefly in a recirculating open-water circuit aquarium. Seawater samples from the aquaria were taken at the same time using sterile syringes. Each aquarium (25 cm height, 25 cm width and 45 cm length) contained a 15 cm layer of the collected sediment as well as five sea urchins.

In order to visually trace seawater inside the digestive tract, five sea urchins were kept in separate aquaria containing a 10 cm thick layer of sediment from the sampling site and coloured seawater (1 M neutral red) for 30 and 60 minutes, respectively, before being dissected. As a control, five more sea urchins were kept in separate aquaria filled with unstained seawater before dissection. The dissections were made from the aboral side in order to access the gastric caecum directly and to collect caecal fluid (1 ml) with sterile syringes. The optical density (at 540 nm) of these samples was then analysed with a spectrophotometer (Thermo Scientific NanoDrop 1000) in order to measure the concentration of neutral red. Since the data were not normally distributed, non-parametric tests were used to identify significant differences in the dataset. Wilcoxon rank tests were used for comparison and analyses were performed with Systat 5.2.1 (Wilkinson, 1988).

To measure osmotic concentrations of body fluids, specimens were dissected as described above and the caecal and coelomic fluids were obtained with sterile syringes. In addition, seawater was sampled from the aquarium. Each sample (1 ml) was centrifuged for 10 minutes at 2000 G using a microcentrifuge (Tomy HF120). After centrifugation, the osmotic concentration of the supernatant was measured with an osmometer (Vapro 5520). After normality of the data was verified, analyses of variance (one-way ANOVA) were performed using Systat 5.2.1. Directions of significant differences were obtained with a Tukey–Kramer *post-hoc* test ($\alpha = 0.05$).

In order to characterize the particles suspended in the caecal fluid and in seawater, contrast phase microscopy (CPM) and scanning electron microscopy (SEM) were employed. Using CPM, seawater sampled over the seafloor in the surroundings of the animals as well as the caecal fluid was observed. The caecal fluid (1 ml) was sampled with sterile syringes after aboral dissection. Both samples (i.e. seawater and caecal fluid) were put on sterile microscope slides covered by sterile coverglasses and observed with a contrast phase microscope (Leitz Diaplan). Diatoms found in these samples were identified, and four species were counted in order to compare their abundance. After normality of the data was verified, a Student's *t*-test was performed using Systat 5.2.1. For SEM, only the caecal fluid was sampled with sterile syringes and filtered through Durapore (Millipore) filters with a pore size of 0.22 μm and a filter diameter of 25 mm in order to capture suspended particles and microorganisms. Subsequently, filters were fixed in Bouin's fluid for 24 hours, rinsed with 70% ethanol until picric acid elimination, dehydrated in a graded ethanol series and finally coated with gold before SEM observation (JEOL-6100). For magnetic resonance imaging (MRI), fifteen *E. cordatum* were dredged at depths between 40 and 50 m in the North Sea off Helgoland in April 2006. Four individuals were fixed in 7% formaldehyde solution and transferred to 70% ethanol for storage. For MRI, these specimens were gradually immersed in distilled water and placed inside 50 ml Falcon tubes. Magnevist (Bayer-Schering) was added at a final concentration of 2 mM. Imaging was conducted on a 7 T Pharmascan 70/16 AS pre-clinical scanner with a ^{1}H-resonance frequency of 300 MHz (Bruker Biospin). The maximum strength of the gradient system was 300 mT/m. A linear birdcage resonator with an inner diameter of 38 mm was used for excitation and signal detection. A 3D FLASH protocol with (81 μm)^3 spatial resolution, (384)^3 pixel matrix size, 6.7 ms echo time, 30 ms repetition time, 12 averages, and a scan time of ~15 hours was employed. Data acquisition was carried out with Paravision 4.0 (Bruker Biospin). Image processing was carried out using ImageJ 1.42q (NIH) and its Volume Viewer plugin.

RESULTS

In all the individuals studied by dissection (N = 50) and by MRI (N = 15), the gastric caecum was always completely free of sediment and was filled with a transparent fluid (Figure 1).

In sea urchins kept in dyed seawater (N = 5), the fluid of the gastric caecum turned red after approximately 30 minutes. The dyed seawater has been observed only inside the foregut and within the gastric caecum, but never in the hindgut. Absorbance measurements of the caecal fluid reveal a significant increase in colour over time (Figure 2). In the beginning ($t = 0$ minute), the absorbance reaches 0.12 ± 0.021, while 30 minutes later, it has been raised to 0.282 ± 0.043 ($P = 0.0121$). Finally, at the end of the experiment, it reaches 0.53 ± 0.035 ($P = 0.0088$).

Osmolarity measurements of the coelomic and the caecal fluids (N = 15) indicate that the osmolarity of the caecal fluid (943.76 ± 6.37 mOsm, $P = 0.6612$) and that of the surrounding seawater (940.45 ± 5.68 mOsm, $P = 0.6809$) did not differ significantly and that both of them were significantly lower than the osmolarity of the coelomic fluid (956.21 ± 5.69 mOsm, $P = 0.0038$; Figure 3).

A variety of detrital particles do occur in the caecal fluid. These particles were identified as plant (Figure 4A) or animal (Figure 4B) fragments, flocculent organic aggregates of undetermined origin (Figure 4C), and pipe-like structures resembling fungal sporangia *sensu* Egglishaw (1972) (Figure 4D). Egg capsules of parasitic platyhelminthes with an undulating extremity were also regularly observed (Figure 4E). The most abundant particles were diatoms. Four species, namely *Actinoptychus senarius* (Ehrenberg) Ehrenberg, 1843, *Biddulphia pulchella* Gray, 1821 (Figure 4F), *Raphoneis amphiceros* (Ehrenberg) Ehrenberg, 1844 (Figure 4G) and *Asterionellopsis glacialis* (Castracane) Round, 1990 (Figure 4H) were systematically present in all the samples, but were significantly less abundant in the caecal fluid as compared to the seawater samples (N = 15; Table 1).

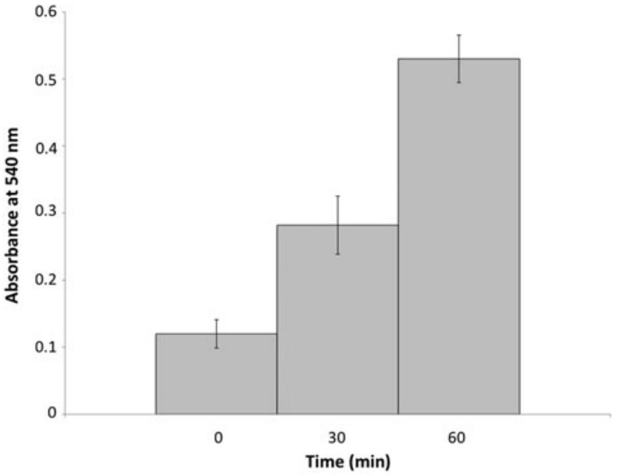

Fig. 2. Absorbance measurements at 540 nm of samples from the caecal fluid of *Echinocardium cordatum* for specimens placed in seawater (0 minute) and in seawater with neutral red (1 M) for 30 minutes and 60 minutes. According to the Wilcoxon rank test, each series is significantly different from the other series (N = 5, α = 0.05).

DISCUSSION

Numerous burrowing deposit feeders have developed mechanisms to collect sediment from the seafloor surface. In *Echinocardium cordatum*, surface sediment reaches the animal by falling down the chimney of the burrow. Once located on top of the animal, it is carried to the mouth by specialized spines (De Ridder *et al.*, 1987). Within the oesophagus, the sediment with its load of organic matter is moulded into a compact cylinder owing to intensive mucus secretion and to the peristalsis of the oesophagus and anterior

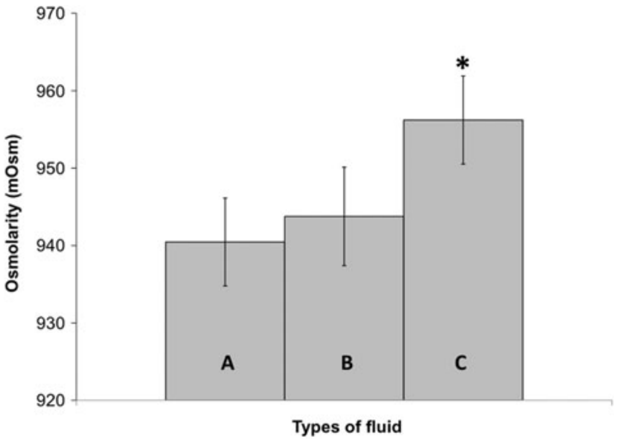

Fig. 3. Osmolarity measurements in mOsm of seawater (A), the caecal fluid (B), and the coelomic fluid (C). The asterisk (*) signifies a significant difference according to a Tukey–Kramer *post-hoc* test (N = 15, α = 0.05).

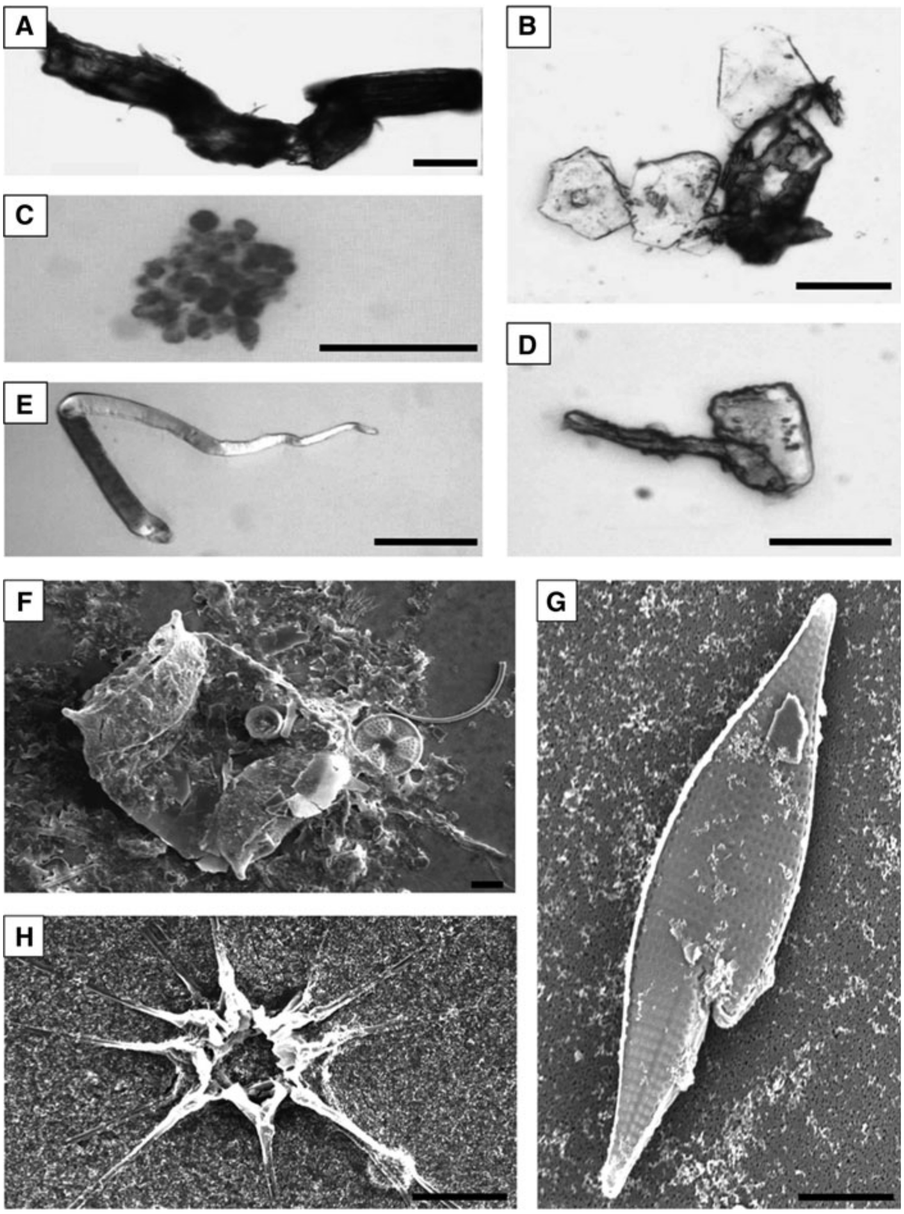

Fig. 4. Contrast phase (A–E) and scanning electron (F–H) microscopic images of detrital particles from the caecal fluid of *Echinocardium cordatum*. (A) Plant fragment (scale bar = 25 μm); (B) remains of chitinous fragments from a crustacean carapace (scale bar = 25 μm); (C) organic aggregates of undetermined origin (scale bar = 25 μm); (D) pipe-like structures, presumably fungal sporangia (scale bar = 25 μm); (E) tapered capsules with an undulating extremity, possibly egg capsules of platyhelminthes (scale bar = 100 μm); (F–H) diatoms: *Biddulphia pulchella* on the left and *Actinoptychus senarius* on the right (F, scale bar = 10 μm); *Raphoneis amphiceros* (G, scale bar = 10 μm); and *Asterionellopsis glacialis* (H, scale bar = 10 μm).

stomach (Holland & Ghiselin, 1970; De Ridder, 1987; De Ridder & Jangoux, 1993).

In addition, *E. cordatum*, like most infaunal organisms, also generates a steady influx of seawater from the seafloor into its burrow (Foster-Smith, 1978). This well-described circulation is due to currents created by ciliated spines. It conveys seawater first over the respiratory tube-feet and, later on, into the mouth and the sanitary drain (Nichols, 1959a, b; Buchanan, 1966). Owing to the even macroscopically visible peristaltic movements of the oesophagus and of the siphon, sea urchins in general are able to ingest seawater (Stott, 1955; Buchanan, 1969; Fechter, 1972; Buchanan *et al.*, 1980; Hollertz & Duchêne, 2001). In *E. cordatum*, this pumping action allows seawater to be moved from the mouth to the proximal part of the stomach and more particularly towards the siphon and the slit-like opening of the gastric caecum (De Ridder, 1987; De Ridder & Jangoux, 1993). Seawater is then carried through the siphon to the intestine where it is later eliminated through the anus. Our results

Table 1. Concentration of diatoms (/ml) within the caecal fluid as well as in the seawater originating from the area surrounding the specimen (N=15, mean ± SE), (*t*-test, $\alpha=0.05$).

Diatom species	Seawater	Caecal fluid	*P*
Actinoptychus senarius	8.8 ± 1.8	5.1 ± 1.5	0.0231
Asterionellopsis glacialis	7.4 ± 3.5	3.4 ± 2.1	0.0317
Biddulphia pulchella	9.6 ± 2.2	2.4 ± 1.9	0.0085
Raphoneis amphiceros	7.8 ± 2.4	4.3 ± 1.2	0.0175

demonstrate that seawater can also enter the gastric caecum and in fact constitutes most of its fluid content. Our conclusions have been based on various evidences. Firstly, when individuals are kept in aquaria containing coloured seawater, only the caecal fluid (as opposed to the coelomic fluid) becomes coloured. Secondly, osmolarity values of natural seawater and that of the caecal fluid are not significantly different. Thirdly, the suspended particles occurring within the caecal fluid correspond to a range of detrital particles that commonly occur in seawater over the seafloor (Egglishaw, 1972, *inter alia*). The diatoms that were identified in the surrounding seawater and within the caecum are phytoplanktonic species (Hendey, 1974; M'Harzi, 1999; Horner, 2002; Muller, 2004) that must have accumulated on the seafloor after their death.

Movement of seawater into the non-muscular and blind-ending gastric caecum appears at first sight rather improbable. However, since the coelomic fluid surrounding the gastric caecum has a significantly higher osmolarity than that of the caecal fluid (Figure 3), an osmotic gradient exists across the relatively thin, almost transparent caecal wall. This leads us to hypothesize that osmotic pressure drives water molecules across the caecal wall into the coelomic cavity. In turn, seawater from the lumen of the anterior stomach flows into the gastric caecum in order to replace water lost to the coelomic cavity (Figure 5). In order to balance this influx into the coelomic cavity, a similar volume of fluid must return to the digestive tract lumen (Fechter, 1972). This process could take place via the rectum and the intestinal caecum. In these parts of the digestive tract, molecules from the coelomic cavity have been shown to move from the coelomic cavity towards the digestive tract lumen, before escaping through the anus (Warnau *et al.*, 1998).

Detrital particles are abundant in coastal waters and are used as a source of nutrients by a well-represented feeding guild in the marine realm—the suspension feeders (see, for example, Margalef, 1967; Turquier, 1989). In order to catch suspended particles, these animals have developed a variety of specialized mechanisms that involve two basic mechanical processes: use of water currents (whether generated or not by the organism itself) and particle interception (the organism places specialized organs or appendages across these water currents). Based on the observations presented here, we hypothesize that the gastric caecum in *E. cordatum*, and probably also the gastric caecum found in other irregular sea urchins, acts as an internal trap that intercepts suspended organic matter in seawater. Being trapped in the caecal lumen, these particles could then undergo digestion by bacterial activity (Thorsen, 1998, 1999) and/or the secretions of glandular enterocytes that are abundant in the gastric caecum (De Ridder & Jangoux, 1993). The general morphology of the gastric caecum fits an absorptive function. The well-developed microvilli, the occurrence of primary and secondary lysosome-like vacuoles in the enterocytes, and the extensive network of haemal lacunae within the caecal wall in spatangoids (De Ridder & Jangoux, 1993; Ziegler *et al.*, 2010) are indicative of nutrient exchange in this part of the digestive tract.

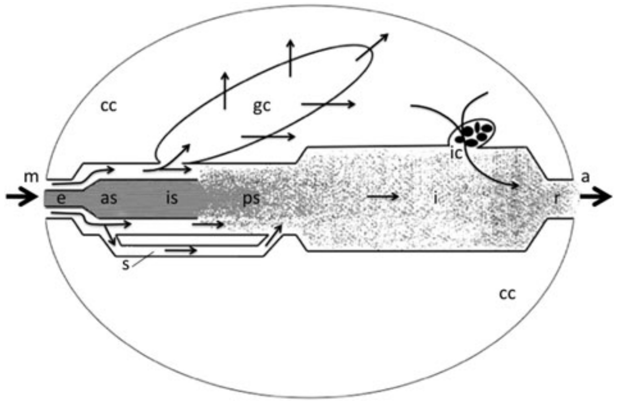

Fig. 5. Schematic drawing of the hypothetical trajectory of water in *Echinocardium cordatum*. The digestive tract is drawn uncoiled. Small arrows correspond to internal seawater pathways, large arrows to water flowing into and out of the animal. The ingested sediment is represented in grey: dark grey corresponds to the mucus-moulded sediment column and light grey to scattered sediment. a, anus; as, anterior stomach; cc, coelomic cavity; e, oesophagus; gc, gastric caecum; I, intestine; ic, intestinal caecum; is, intermediate stomach; m, mouth; ps, posterior stomach; r, rectum; s = siphon.

Many irregular sea urchins and almost all spatangoids possess a well-developed gastric caecum (Ziegler *et al.*, 2010). Since it is precisely these sea urchin taxa that have acquired a predominantly infaunal lifestyle, we hypothesize that the development of the gastric caecum could be an adaptation of the irregular sea urchin digestive tube to optimize detritus exploitation, presumably as a response to restricted access to food sources. Through the gastric caecum acting as an internal trap, partially as well as fully infaunal spatangoids could gain additional access to suspended particulate organic matter in seawater. Spatangoid species could consequently have adopted a generalized diet made of two complementary feeding strategies (i.e. deposit and suspension feeding) and would therefore be particularly well adapted to exploit a wide range of detritus. Suspended organic particles in the water column constitute a complementary food source in a clypeasteroid species (*Dendraster excentricus*) as demonstrated by Chia (1969) and Timko (1976), and in two spatangoid species as suggested by Hollertz (1999) for *Brissopsis lyrifera* and by Cramer (1991) for *E. cordatum*.

However, this additional feeding mode and its importance in terms of food supply remain to be demonstrated. The evaluation of the quantitative importance of the uptake of suspended organic matter, observation of its extracellular and intracellular digestion within the caecum, investigation on the absorption of nutrients by enterocytes, and the identification of the bacterial community found within the caecal fluid as compared to the bacterial community found in the surrounding seawater and the sediment are aspects that remain to be studied in order to fully demonstrate that *E. cordatum* uses suspended particles as a complementary food source.

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REFERENCES

Agassiz A. (1872–1874) Revision of the Echini. *Illustrated Catalogue of the Museum of Comparative Zoology* 7.

Bromley R.G. and Asgaard U. (1975) Sediment structures produced by a spatangoid echinoid: a problem of preservation. *Bulletin of the Geological Society of Denmark* 24, 261–281.

Buchanan J.B. (1966) The biology of *Echinocardium cordatum* (Echinodermata: Spatangoidea) from different habitats. *Journal of the Marine Biological Association of the United Kingdom* 46, 97–114.

Buchanan J.B. (1969) Feeding and control of volume within the tests of regular sea-urchins. *Journal of Zoology* 159, 51–64.

Buchanan J.B., Brown B.E., Coombs T.L., Pirie B.J.S. and Allen J.A. (1980) The accumulation of ferric iron in the guts of some spatangoid echinoderms. *Journal of the Marine Biological Association of the United Kingdom* 60, 631–640.

Chesher R.H. (1963) The morphology and function of the frontal ambulacrum of *Moira atropos* (Echinoidea: Spatangoida). *Bulletin of Marine Science of the Gulf and Caribbean* 13, 549–573.

Chia F.S. (1969) Some observations on the locomotion and feeding of the sand dollar *Dendraster excentricus* (Eschscholtz). *Journal of Experimental Marine Biology and Ecology* 3, 162–170.

Cramer A. (1991) *Observations on spatial distribution, metabolism and feeding strategy of* Echinocardium cordatum *(Pennant) (Echinodermata) and the implications for its energy budget, in Metabolic activity at frontal systems in the North Sea*. PhD thesis. Universiteit Amsterdam, The Netherlands.

Cuénot L. (1948) Anatomie, éthologie et systématique des échinodermes. In Grassé P.P. (ed.) *Traité de Zoologie, Volume 11*. Paris: Masson, pp. 1–363.

De Amaral P., Nunes C.D.A.P. and Jangoux M. (2007) Larval growth and perimetamorphosis in the echinoid *Echinocardium cordatum* (Echinodermata): the spatangoid way to become a sea urchin. *Zoomorphology* 126, 103–119.

De Ridder C. (1987) Mécanique digestive chez l'échinide fouisseur *Echinocardium cordatum* (Echinodermata). *Bulletin de la Société des Sciences Naturelles de l'Ouest de la France Supplement HS*, 65–71.

De Ridder C., Jangoux M. and Van Impe E. (1985) Food selection and absorption efficiency in the spatangoid echinoid, *Echinocardium cordatum* (Echinodermata). In Keegan B. and O'Connor B. (eds) *Proceedings of the 5th International Echinoderm Conference, Galway 1984*. Rotterdam: A.A. Balkema Press, pp. 245–251.

De Ridder C., Jangoux M. and De Vos L. (1987) Frontal ambulacral and peribuccal areas of the spatangoid echinoid *Echinocardium cordatum* (Echinodermata): a functional entity in feeding mechanism. *Marine Biology* 94, 613–624.

De Ridder C. and Jangoux M. (1993) The digestive tract of the spatangoid echinoid *Echinocardium cordatum* (Echinodermata): morphofunctional study. *Acta Zoologica* 74, 337–351.

De Ridder C. and Lawrence J. (1982) Food and feeding mechanisms: Echinoidea. In Jangoux M. and Lawrence J. (eds) *Echinoderm nutrition*. Rotterdam: A.A. Balkema Press, pp. 97–115.

Egglishaw H.J. (1972) An experimental study of the breakdown of cellulose in fast-flowing streams. In Melchiorri-Santolini U. and Hopton J.W. (eds) *Detritus and its role in aquatic ecosystems. Proceedings of an IBP-UNESCO symposium*. Memorie dell'Istituto Italiano di Idrobiologia 29, pp. 406–428.

Fechter H. (1972) Untersuchung über den Wasserwechsel der Seeigel und seine Bedeutung für Atmung und Exkretion. *Helgoländer Wissenschaftliche Meeresuntersuchungen* 23, 80–99.

Ferguson J.C. (1969) Feeding, digestion and nutrition in Echinodermata. In Florkin M. and Scheer B.T. (eds) *Chemical Zoology* 3, 71–100.

Foster-Smith R.L. (1978) An analysis of water flow in tube-living animals. *Journal of Experimental Marine Biology and Ecology* 34, 73–95.

Hendey N.I. (1974) A revised check-list of British marine diatoms. *Journal of the Marine Biological Association of the United Kingdom* 54, 277–300.

Higgins R.C. (1974) Specific status of *Echinocardium cordatum*, *E. australe* and *E. zealandicum* (Echinoidea: Spatangoida) around New Zealand, with comments on the relation of morphological variation to environment. *Journal of Zoology* 173, 451–475.

Holland N.D. and Ghiselin M.T. (1970) A comparative study of gut mucous cells in thirty-seven species of the class Echinoidea (Echinodermata). *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 138, 286–305.

Hollertz K. (1999) The response of *Brissopsis lyrifera* (Echinoidea: Spatangoida) to organic matter on the sediment surface. *In Proceedings of the 5th European Conference on Echinoderms*. Rotterdam: A.A. Balkema Press, pp. 19–24.

Hollertz K. and Duchêne J.C. (2001) Burrowing behaviour and sediment reworking in the heart urchin *Brissopsis lyrifera* Forbes (Spatangoida). *Marine Biology* 139, 951–957.

Horner R.A. (2002) *A taxonomic guide to some common marine phytoplankton*. Dorchester, UK: Biopress Limited and Dorset Press.

Kanazawa K. (1992) Adaptation of test shape for burrowing and locomotion in spatangoid echinoids. *Palaeontology* 35, 733–750.

Margalef R. (1967) Laboratory analogues of estuarine plankton systems. In Lauff G.H. (ed.) *Estuaries: perspectives in ecological theory*. American Association for the Advancement of Science Publication No. 83, pp. 515–521.

M'Harzi A. (1999) *Phytoplankton community structuring in some areas of the North Sea*. PhD thesis. Vrije Universiteit Brussel, Belgium.

Muller Y. (2004) *Faune et flore du littoral du Nord, du Pas-de-Calais et de la Belgique: inventaire*. Commission Régionale de Biologie de la Région Nord Pas-de-Calais.

Nichols D. (1959a) Changes in the chalk heart-urchin *Micraster* interpreted in relation to living forms. *Philosophical Transactions of the Royal Society London* 242, 347–437.

Nichols D. (1959b) The histology of the tube-feet and clavulae of *Echinocardium cordatum*. *Quarterly Journal of Microscopical Science* 100, 73–87.

Nichols D. (1959c) Mode of life and taxonomy in irregular sea-urchins. In Nichols D. (ed.) *Taxonomy and geography, a symposium*. Systematics Association Publication No. 3, pp. 61–80.

Osinga R., Kop A.J., Malschaert J.F.P. and Van Duyl F.C. (1997) Effects of the sea urchin *Echinocardium cordatum* on bacterial production and carbon flow in experimental benthic systems under increasing organic loading. *Journal of Sea Research* 37, 109–121.

Péquignat C.E. (1970) Biologie des *Echinocardium cordatum* (Pennant) de la Baie de Seine. *Forma et Functio* 2, 121–168.

Smith A.B. (1980) The structure and arrangement of echinoid tubercles. *Philosophical Transactions of the Royal Society B* 289, 1–54.

Stott F.C. (1955) The food canal of the sea-urchin *Echinus esculentus* L. and its functions. *Proceedings of the Zoological Society of London* 125, 63–86.

Telford M. and Mooi R. (1996) Podial particle picking in *Cassidulus caribaearum* (Echinodermata: Echinoidea) and the phylogeny of sea urchin feeding mechanisms. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 191, 209–223.

Thorsen M.S. (1998) Microbial activity, oxygen status and fermentation in the gut of the irregular sea urchin *Echinocardium cordatum* (Spatangoida: Echinodermata). *Marine Biology* 132, 423–433.

Thorsen M.S. (1999) Abundance and biomass of the gut-living microorganisms in the irregular sea urchin *Echinocardium cordatum* (Spatangoida: Echinodermata). *Marine Biology* 133, 353–360.

Timko P.L. (1976) Sand dollars as suspension feeders: a new description of feeding in *Dendraster excentricus*. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 151, 247–259.

Turquier Y. (1989) *L'organisme dans son milieu. Tome 1: les fonctions de nutrition*. Paris: Editions Doin.

Warnau M., Temara A., Ameye L. and Jangoux M. (1998) The excretory function of the posteriormost part of the echinoid and holothuroid gut (Echinodermata). *Comparative Biochemistry and Physiology A* 120, 687–691.

Wilkinson L. (1988) *Systat 5.2.1: the system for statistics*. Evanton, IL: Systat.

Ziegler A., Faber C., Mueller S. and Bartolomaeus T. (2008) Systematic comparison and reconstruction of sea urchin (Echinoidea) internal anatomy: a novel approach using magnetic resonance imaging. *BMC Biology* 6, 33.

and

Ziegler A., Mooi R., Rolet G. and De Ridder C. (2010) Origin and evolutionary plasticity of the gastric caecum in sea urchins (Echinodermata: Echinoidea). *BMC Evolutionary Biology* 10, 313.

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