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ORIGINAL PAPER

Glass sponges arrest pumping in response to sediment: implications for the physiology of the hexactinellid conduction system

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Abstract The hexactinellid sponge *Rhabdocalyptus dawsoni* propagates electrical signals to arrest its feeding current in response to mechanical stimuli and sediment. The deepwater habitat of other species of glass sponge, and the difficulty of working with the tissue in vitro have so far prevented confirmation of electrical signaling in other members of the Class. Here we show in laboratory experiments (ex situ) that mechanical and sediment stimuli trigger immediate arrest in *R. dawsoni* and in a second species of hexactinellid, *Aphrocallistes vastus*, suggesting that rapid signaling may be a general feature of glass sponge tissue. Further, responses of the two species differed, suggestive of underlying physiological differences in the conduction system. *R. dawsoni* and *A. vastus* were sensitive to sediment but arrests were often prolonged in *R. dawsoni*, whereas in *A. vastus* pumping resumed immediately following each arrest. Fine sediment (<25 μm) caused immediate arrests in *R. dawsoni* and *A. vastus*, but with a higher stimulus threshold in *A. vastus.* Large amounts of sediment triggered repeated arrests in both species, and prolonged exposure to sediment (over 4 h) caused a gradual reduction in pumping, with recovery taking up to 25 h. During recovery, both species of sponge carried out repeated arrests, which had a precise periodicity indicative of pacemaker activity. Scanning electron microscopy of the tissue of these specimens showed many chambers were clogged. The results suggest that the glass sponge conduction system generates arrest of the feeding current that prevent uptake of small amounts of sediment, and that each species has different threshold sensitivities. However, ongoing exposure to sediment can clog the filtration apparatus.

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Introduction

Many suspension-feeding animals avoid being clogged with sediment by temporarily stopping or reducing feeding (e.g., Mackie et al. 1974; Bock and Miller 1996; Ellis et al. 2002). Sensory systems, coordinated by the nervous system, allow quick responses to changes in water quality, such as cilary arrest and constriction of incurrent siphons in the ascidian *Corella* (Mackie et al. 1974). Sponges lack neurons, yet they too selectively remove food from the water column (Leys and Eerkes-Medrano 2006; Yahel et al. 2006) so they have evolved other ways of dealing with excessive particulates in their feeding current. Some cellular sponges (Demospongiae and Calcarea) can close the openings to their incurrent canals (ostia), constrict the size of their intake canals, and even carry out a series of slow contractions that expel unwanted material (Nickel 2004; Ellwanger et al. 2006; Leys and Meech 2006; Elliott and Leys 2007). Syncytial sponges (Porifera, Hexactinellida), known as glass sponges for their vitreous skeleton, have a different solution. Although they lack contractile tissues that would permit closure of ostia and canals to prevent the intake of suspended particulates (Mackie et al. 1983; Mackie and Singla 1983), their syncytial tissues allow action potentials initiated at single or multiple sites to propagate through the entire animal, arresting the feeding current (Leys and Mackie 1997). The impulses travel at

0.27 cm s^{-1} (Leys and Mackie 1997; Leys et al. 1999), one order of magnitude slower than electrical conduction in cnidarian epithelia (Mackie 1979). Thinness and fragility of the tissue have prevented intracellular recordings so far, but extracellular recordings suggest the action potential is Ca^{2+}-based (Leys et al. 1999).

The feeding current arrest in hexactinellids is similar to ciliary arrest in some suspension feeders, including the arrest of lateral cilia in lamellibranch gills of bivalves and the branchial cilia of ascidians (Mackie et al. 1974; Walter and Satir 1978; Bergles and Tamm 1992). In these cases particulate matter may trigger membrane depolarization; ensuing calcium influx into ciliated cells causes cilia to arrest. In the hexactinellid *R. dawsoni*, arrests are thought to result from calcium influx into branched choanocytes (Mackie et al. 1983; Leys et al. 1999). The initial observation of arrests are credited to G. Silver who noticed that pumping stopped in the presence of divers, but in situ observations suggest that natural stimuli are fish that disturb or resuspend sediment (Yahel et al. 2007). The physiology of feeding arrests has only been described for *R. dawsoni* because this species can be collected by SCUBA with some populations living as shallow as 20 m (Leys and Lauzon 1998; Leys et al. 2004). Other species of hexactinellid are found in deeper water and are difficult to collect in good condition (Bett and Rice 1992; Barthel 1995; Beaulieu 2001), but all hexactinellids have syncytial tissues (Leys et al. 2007) and all are therefore presumed to propagate electrical signals that arrest the feeding current.

The relationship between hexactinellid sponges and sediment is complex. Few species are known to settle on soft substrates; most require something hard to attach to, but nevertheless, the deep environment of glass sponges is sediment rich from the slow accumulation of marine snow (Bett and Rice 1992; Conway et al. 2004; Leys et al. 2004). Specimens of *R. dawsoni* were found to arrest pumping in the presence of sediment (Leys et al. 1999), yet at the same time it has been implied that this species may extract organic matter from sediment resuspended in bottom currents and along fjord walls (Bett and Rice 1992; Yahel et al. 2007). Furthermore, observations of 9000-year-old reefs suggest that sediment is an important factor in cementing the reef structure together over time (Conway et al. 1991; Whitney et al. 2005). Thus there appears to be a conundrum between the sensitivity to and tolerance of the stimuli produced by sediment. Presumably this has shaped the properties of the glass sponge conduction system.

In this study we examine the ubiquity of the arrest response in hexactinellids by testing whether the cloud sponge *A. vastus* (Order Hexactinosida) also arrests its feeding current in response to experimental and natural stimuli, including sediment. In *R. dawsoni* (Order Lyssacinosida) arrests are elicited immediately upon arrival of action potentials and therefore provide a convenient means of monitoring electrical activity (Leys et al. 1999). Here we examine the effect of short-term sediment exposure on pumping sensitivity, and test the effects of long-term exposure to sediment to determine whether hexactinellids eventually tolerate sediment and pump continuously. Our findings have implications for the physiology of the hexactinellid conduction system, as well as for the sensitivity of hexactinellids and suspension feeders in general, to sediment.

Materials and methods

In situ observations

The boot sponge *R. dawsoni* (Lambe, 1892) (Order Lyssacinosida) and the cloud sponge *A. vastus* (Schultz, 1886) (Order Hexactinosida) were observed in situ on vertical or near vertical walls at San Jose Islets and Hosie Islands, Barkley Sound, BC using the remote operated vehicle ROPOS (Canadian Scientific Submersible Facility, Sidney, BC). During a field study of feeding, fluorescein dye was squirted onto specimens to test for pumping. Specimens that were pumping took in the dye and exhaled it through the osculum (Fig. 1a, b). Fluorescein was also squirted on dead sponges. Dye was not drawn through these specimens. Small (<20 cm) *R. dawsoni* were collected by SCUBA from 30 m at San Jose Islets and small (<30 cm) *A. vastus* were collected by ROPOS at 160 m. *Aphrocallistes vastus* has a fused siliceous skeleton that is fragile, but small specimens were detached from the wall intact by applying gentle pressure from below with a manipulator arm (Fig. 1c). Specimens were brought to the surface in water from depth and transferred to darkened flow-through seawater tanks at the Bamfield Marine Sciences Centre, British Columbia, Canada, where water is pumped directly from 30 m depth. Water temperature was 10°C but reached 12°C on several occasions in early August, reflecting warming of inlet waters from 9 to 11°C. While Leys and Meech (2006) found that specimens often did not pump below 7°C and were less responsive to experimental stimuli at temperatures >12°C, subsequent experiments have shown that many specimens will in fact continue to respond even at temperatures as high as 19°C. Thus tank temperatures (10–12°C) were well within the range of tolerance of the animals.

Loose sediment that had settled on rock shelves on fjord walls was collected with specimens and kept in holding tanks for later use in experiments. Sediment was in part settled marine snow, a mixture of organic detritus, microorganisms and clay minerals (Alldredge and Silver 1988). It is this sediment that would be resuspended by passing fish,

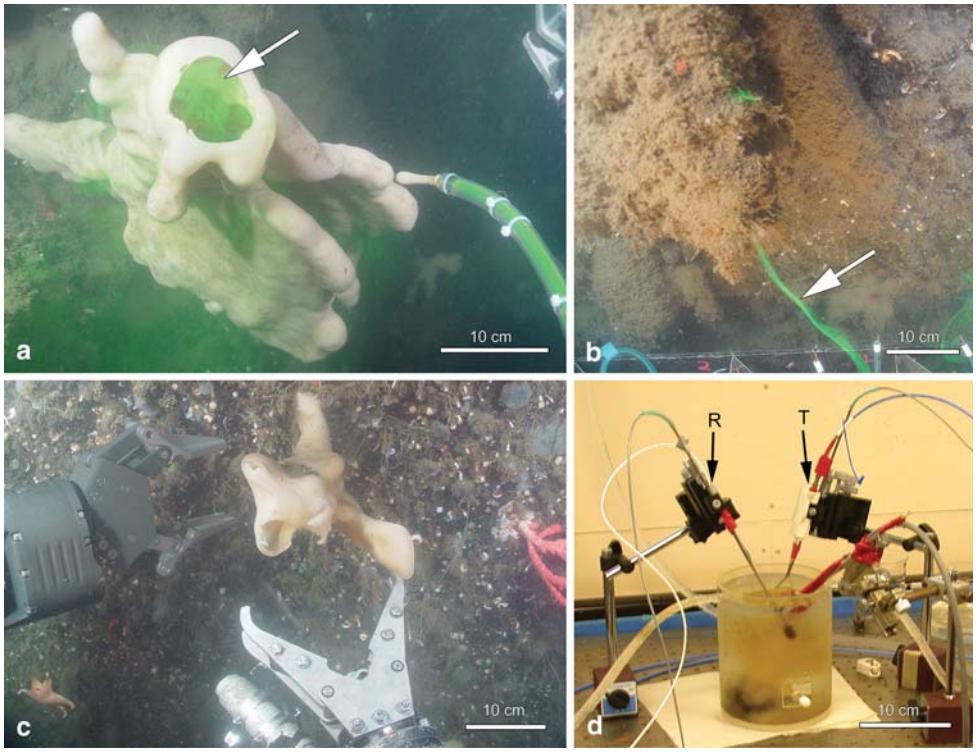

Fig. 1 Hexactinellid sponges in situ (**a**–**c**) and in flow-through experimental chambers (**d**). Fluorescein dye squirted onto **a** *Aphrocallistes vastus* and **b** *Rhabdocalyptus dawsoni* was pumped out through the osculum (*arrows*). **c** Specimens of *A. vastus* were collected with the Kraft Raptor manipulator arm (*left*). **d** In chambers a thermistor probe (*T*) recorded flow from sponges. Reference probe, *R*

or by semidiurnal tidal currents, which reach 1 m s^{-1} along the fjord walls where resuspension loops are thought to contribute the bulk of suspended material (Yahel et al. 2007). A core of sediment from the Hecate Strait BC sponge reefs that had been maintained at 4°C was obtained from K. Conway of the Pacific Geological Survey, Sidney BC, to test the difference of responsiveness of the reef-forming sponge *A. vastus* to this kind of sediment.

Ex situ experiments

Because of the fragility of *A. vastus* only three specimens of eight collected were completely undamaged and could be used for physiological work. Others lacked portions of the osculum or body wall. Ten specimens of *R. dawsoni* were used in sediment trials. Specimens were placed in 0.5–2.5 l flow-through plexiglass chambers and attached with stainless steel insect pins to the underlying Sylgard coating (Dow-Corning, Midland, MI, USA) (Fig. 1d). Unfiltered seawater was passed over bioballs (plastic spheres with high surface area for degassing) and passed through a cooling system that maintained the temperature at 9–10°C. Flow through the chamber was approximately 250 ml min^{-1}.

Excurrent flow from each specimen's osculum was recorded using a thermistor flow meter (see Mackie et al. 1983 for design) which provides a convenient means of recording small changes in flow; a reference thermistor compensated for temperature change in the chamber. Records were captured with a digital amplifier (Powerlab 8/SP, ADInstruments, Colorado Springs, CO, USA) using Chart 5. Arrests appeared as downward deflections in the flow record over 15 s–2 min. The thermistor's response at very low flow velocities was linear (see Reiswig 1971; Mackie et al. 1983). Previous use of this probe design with experiments on *R. dawsoni*, illustrated that after a stimulus to the sponge, flow takes at least 20 s to completely stop; the arrest waveform approximates slowing of water after sudden cessation of a pump driving flow through a smooth-walled pipe (Mackie et al. 1983). The lag, due to inertia of the water mass, is consistent with large Reynold's number flow ($Re \approx 100$–500 in the sponge oscula 1–5 cm diameter and approximate velocity at the osculum 1 cm s^{-1}). The duration of arrest–recovery events for each specimen was calculated by measuring the time from the start of one arrest (the first downward deflection of the trace) until the same level of pumping was recovered. The duration of the arrest phase only was considered the time from start of arrest until the lowest point in the flow record. Pumping was confirmed with fluorescein dye.

Specimens also arrested spontaneously. Because of this, experimental stimuli were applied after a minimum of 10 min, and usually 30 min, of uninterrupted pumping. The response of *A. vastus* and *R. dawsoni* to mechanical stimulation was tested by touch with a pipette and by inserting a metal probe into the body wall. Responsiveness to sediment was tested in three ways: (1) fjord sediment collected together with the sponges was added directly into the

chamber over specimens, similar to resuspension. (2) Fjord sediment was added gradually via peristaltic pump at 3 ml min^{-1} until the first arrest of feeding current was observed in order to determine sediment concentrations that trigger arrests. Large aggregates—which settle out and clog peristaltic pump tubing—were excluded by filtering sediment through 25 μm Nitex mesh. In these experiments, because it is a reef-forming species *A. vastus* was also treated with filtrate from a reef sediment core. (3) In a final set of experiments, long-term effect of sediment on pumping was tested by adding the 25 μm filtrate of fjord sediment continuously (at 3 ml min^{-1}) for 2–4 h after the first arrest of feeding current had occurred. Filtered sediment was added to the incurrent water of tanks containing each species. For each experiment only one flow record from each individual was analyzed, however, up to 20 runs were carried out with each individual, and all records showed consistent results.

To determine the concentration of sediment in the chamber when the specimens first arrested, 20–60 ml water samples were collected by syringe. Small volumes were taken so as to avoid altering flow in the chamber, but as the amount of sediment in these samples was in fact too little to weigh (<0.001 g), the concentration of sediment in the chamber was determined indirectly by comparing percent transmittance (660 nm on a Spectronic 20) of water samples taken at the time of arrest to that of a dilution series of stock sediment suspension of known concentration. Stock sediment concentration was determined by suction filtering samples through pre-combusted glass fiber filters (Whatman GF/F). Filters were rinsed with distilled water, dried to constant mass in a 70°C oven and weighed prior to use. Concentrations and numerical trends in pumping were expressed as mean ± standard error.

Sediment from the fjords and the reef core was also fixed in 2% glutaraldehyde in seawater for microscopy. Fixed sediment was suction filtered onto 0.45 μm Millipore filters and rinsed with 70% ethanol. Pieces of air-dried filters were mounted on aluminum stubs, coated with gold and viewed in a JOEL JSM-6301FXV scanning electron microscope (SEM). Composition of sediment in randomly selected regions was determined by Energy Dispersive X-Ray (EDX) analysis. Reef sediment was found to be finer with >75% of particles smaller than 3 μm; <50% of fjord sediment particles were smaller than 3 μm in diameter.

Scanning electron microscopy

To look for evidence of possible clogging of the aquiferous system, specimens were fixed after 20, 40 and >60 min in tanks made opaque with fjord sediment (<50% transmittance). By this time sediment had caused repeated arrests and a gradual decline in overall pumping level. Pieces of tissue (5–10 pieces, each <1 cm^3) were cut from different portions of the body wall and fixed in 1% OsO_4, 2% glutaraldehyde, 0.45 M sodium acetate buffer pH 6.4, with 10% sucrose, overnight (Leys 1995). Samples were dehydrated to 70% ethanol, desilicified with 4% hydrofluoric acid in 70% ethanol for 2 days, dehydrated further to 100% ethanol and fractured in liquid nitrogen. Fractured pieces were dried at critical point, mounted on aluminum stubs, coated with gold and examined by SEM. Control tissue was from specimens not treated with sediment. Tissue was desilicified because the glass skeleton does not fracture; for comparison, non-desilicified tissue was cut open with a razor blade and prepared as above for SEM, and EDX to determine the presence, location and composition of sediment inside specimens. To see where inert particles would lodge in the aquiferous system, a 10% solution of 0.1, 0.5 and 1 μm latex beads (Molecular Probes, Eugene, OR, USA) in seawater (approximately 1×10^{10}, 1×10^{9} and 1×10^{6} beads, respectively) was poured into the chamber with both species. Tissue became pink (the color of the beads) within 5 min, and at that time pieces of the specimens were fixed and prepared for SEM as above.

To determine if any canals bypass the flagellated chambers, where the narrowest channels occur, liquid plastic (Batson's No. 17 Plastic Replica Corrosion Kit, Polysciences, Warrington, PA, USA) was injected in to either incurrent or excurrent sides of live and Bouins-fixed tissue. Plastic was injected gently until it was just visible under the tissue at the side opposite injection; despite great care not to force the plastic into the sponge, in some instances the polymer traversed the entire body wall. After the casts had hardened, the sponge skeleton was dissolved in 4% hydrofluoric acid overnight, and tissue digested with sodium hydroxide. Hardened replicas were fractured with a razor blade, mounted on stubs, coated with gold, and examined by SEM as described previously (Leys 1999; Bavestrello et al. 2003).

Results

In situ observations

Pumping of both *A. vastus* and *R. dawsoni* was tested with fluorescein dye at 160 m depth at San Jose Islets and Hosie Islands (Fig. 1a, b). Here, both species grow on almost vertical (80–90°), brachiopod-covered walls. Suspended sediment largely in the form of 'marine snow' falls onto sponges, settles on abundant shelves on fjord walls and can reduce visibility to a few meters. On several occasions the rapid movements of fish attracted by lights of the ROV resuspended sediment that had settled on the wall.

Despite the continual fallout of sediment, the surface tissue of all specimens of *A. vastus* was largely clear of debris.

In contrast, the outer spicule coat of *R. dawsoni* was invariably cloaked with brown debris. Crabs and fish were usually in the oscula of both species, but despite these possible mechanical disturbances, all specimens of *A. vastus* that were squirted with fluorescein dye in situ drew the dye in and pumped it out of the osculum within 30 s. In contrast, four of nine individuals of *R. dawsoni* tested with fluorescein did not take up dye. One specimen was repeatedly tested and only took up dye 30 min after the arrival of the ROV. Dye flow from the osculum of one specimen of *R. dawsoni* ceased abruptly when it was purposefully tapped with the manipulator arm. Similar knocks to *A. vastus* did not affect dye flow. Dye did not flow from the osculum of dead specimens.

Ex situ (tank) experiments

Spontaneous arrests

Thermistor records showed that both *A. vastus* and *R. dawsoni* could pump continuously for several hours; however, both species also arrested periodically in the absence of experimental stimuli (Fig. 2). Because of this, sediment and mechanical stimuli were delivered only after a period of uninterrupted pumping (typically 30 min). As previously reported for *R. dawsoni* (Leys et al. 1999), neither species showed diurnal rhythmicity in pumping, nor were sensitive to changes in ambient light. In the interval 30–60 min after putting a new specimen in the chamber, arrests occurred at mean intervals of 6.4 ± 2.0 min ($n = 3$) in *A. vastus* and 10.3 ± 3.6 min ($n = 8$) in *R. dawsoni*. Despite the lower frequency of arrests in *R. dawsoni* arrests were longer and therefore these sponges were arrested a greater percentage of the time. Individual arrest–recovery events—from start of arrest until return to full pumping level—typically lasted 1–4 min (2.7 ± 0.3 min, $n = 3$) in *A. vastus* (Fig. 2a, b) and 1–10 min (4.1 ± 0.5 min, $n = 7$) in *R. dawsoni* (Fig. 2c–e),

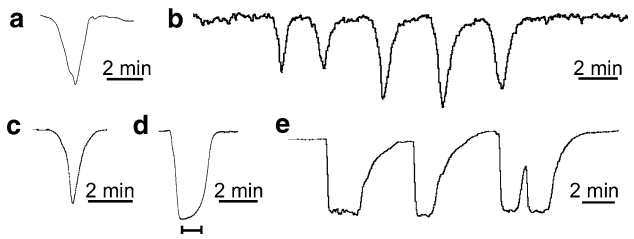

Fig. 2 Spontaneous arrests of pumping. Single or intermittent arrests appear as downward deflections in pumping records. **a**, **b** Arrest–recovery events in *A. vastus* were brief and V-shaped. **c**, **d** Arrests could be brief in *R. dawsoni* (**c**) but were often longer (**d**, **e**) and the initial phase of the recovery (**d**, *bracketed*) was more gradual than in *A. vastus*

however, *R. dawsoni* could remain arrested up to 6 h. The arrest phase of each event (from start of arrest to base of arrest–recovery curve) was usually 0.5–1.5 min (71 ± 11 s, $n = 3$ and 57 ± 12 s, $n = 7$ for *A. vastus* and *R. dawsoni,* respectively).

The two species differed in the way arrests occurred. While for both species the shape of the arrest–recovery record could be V-shaped (i.e., flow arrested and began immediately again) (Fig. 2a–c), in *R. dawsoni* pumping normally resumed very gradually after an arrest, as described previously by Mackie et al. (1983) (Fig. 2d, e). Given that the oscula though which flow was recorded were 1–5 cm in diameter, the Reynolds number in the atrium and osculum would be large and the thermistor would normally record a small lag in the time between the arrest of the chambers and the arrest of flow through the osculum. V-shaped records were more typical in *A. vastus*, suggesting that either pumping resumed before flow from the osculum had come to a full halt, or that not all of the chambers in the sponge arrest pumping.

Responses to mechanical and sediment stimuli

Mechanical and sediment stimuli both triggered arrests in *A. vastus* and *R. dawsoni* (Fig. 3). Sponges stopped pumping when a metal probe was inserted into the body wall. However, consistent with in situ observations, light touches or knocks to the outer body wall caused arrests in *R. dawsoni* but not in *A. vastus*. Arrest–recovery events in response to mechanical stimuli were of similar duration in both species: 1.7 ± 0.6 min in *A. vastus* and 1.8 ± 0.2 min in *R. dawsoni* ($n = 3$) with arrest phases of 26 ± 7 s and 21 ± 3 s, respectively. In one specimen of *A. vastus* in particular the recovery phase of the record was often, but not always, marked by a temporary plateau, which could reflect variations in the internal morphology of the sponge, or perhaps a second arrest that occurred during the recovery phase (Fig. 3a).

Sediment stimuli induced arrest–recovery events were slightly longer for both species: 2.4 ± 0.1 min, $n = 3$ in *A. vastus* and 2.4 ± 0.5, $n = 5$ in *R. dawsoni* with arrest phases of 47 ± 10 s, $n = 3$ and 36 ± 5 s, $n = 5$, respectively. Small and large additions of sediment (i.e., 10–1,000 ml of 0.5–1 g l^{-1}) applied to the body wall caused one or more arrests in *A. vastus* (Fig. 3e) and *R. dawsoni* (Fig. 3f). Seawater alone poured onto the sponges did not cause arrests.

Acclimation to small amounts of sediment

Addition of gradually greater amounts of sediment produced an apparent reduction in sensitivity of the arrest response (Fig. 4). The first small additions of sediment (2–10 ml) triggered arrests, suggesting that even a few parti-

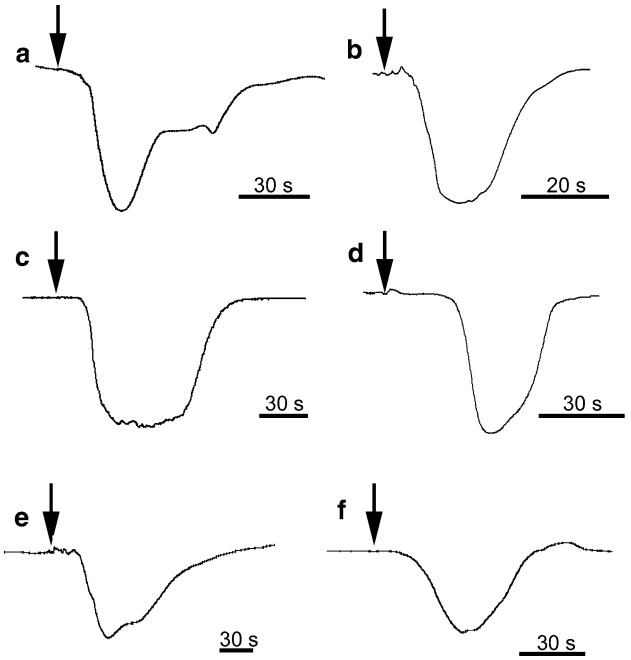

Fig. 3 Arrest responses to mechanical and sediment stimuli. Probes advanced into the body wall (*arrows*) of *A. vastus* (**a**, **b**) and *R. dawsoni* (**c**, **d**) induced arrests. Topical applications of sediment caused both *A. vastus* (**e**) and *R. dawsoni* (**f**) to arrest

cles may be a sufficient stimulus; however, subsequent additions of 10–100 ml for *A. vastus* and 2–20 ml for *R. dawsoni* had no effect on pumping. Larger sediment doses (50–200 ml for *R. dawsoni* and 200–500 ml for *A. vastus*) triggered arrests and repeated 500 ml doses applied over 10–20 min caused a series of arrest–recovery events of 2–4 min duration that lasted several hours. Many of these events had a marked periodicity (e.g., Fig. 4b inset). Periodicities of these long trains of repeated arrest–recovery events were 3.4 ± 0.9 min ($n = 3$) in *A. vastus* and 3.3 ± 0.3 min ($n = 5$) in *R. dawsoni*.

Response to continuous sediment

Fjord sediment added by peristaltic pump to the incurrent flow of experimental chambers caused both species to arrest. The first arrests occurred at sediment concentrations of 15 ± 5 mg l^{-1}, $n = 9$ for *R. dawsoni* and 36 ± 12 mg l^{-1}, $n = 3$ for *A. vastus*. Initial arrest responses were distinct in the two species. In *R. dawsoni* the arrest phase was consistently abrupt: 25–60 s (34 ± 6 s, $n = 6$; Fig. 5a). In *A. vastus* pumping declined over 50 s–4.5 min (143 ± 66 s, $n = 3$), often in steps (Fig. 5b, c). Fine sediment from reef cores had a similar effect on pumping in *A. vastus* but reduction in flow rate took even longer (7.7 ± 4.8 min, $n = 3$); in one instance flow took 17 min to completely arrest (Fig. 5d). If sediment was only added until the first arrest, pumping resumed within minutes; however, sequential arrest–recovery events persisted for up to an hour afterwards in both species (Fig. 5d, e). Arrest–recovery events were 1–3 min in duration (2.2 ± 0.3 min, $n = 3$) at 1–4 min intervals (3.0 ± 0.6 min between arrests, $n = 3$) in *A. vastus* and 1–7 min in duration (2.7 ± 0.3 min, $n = 5$) at 1–10 min intervals (3.9 ± 0.8 min between arrests, $n = 5$) in *R. dawsoni*.

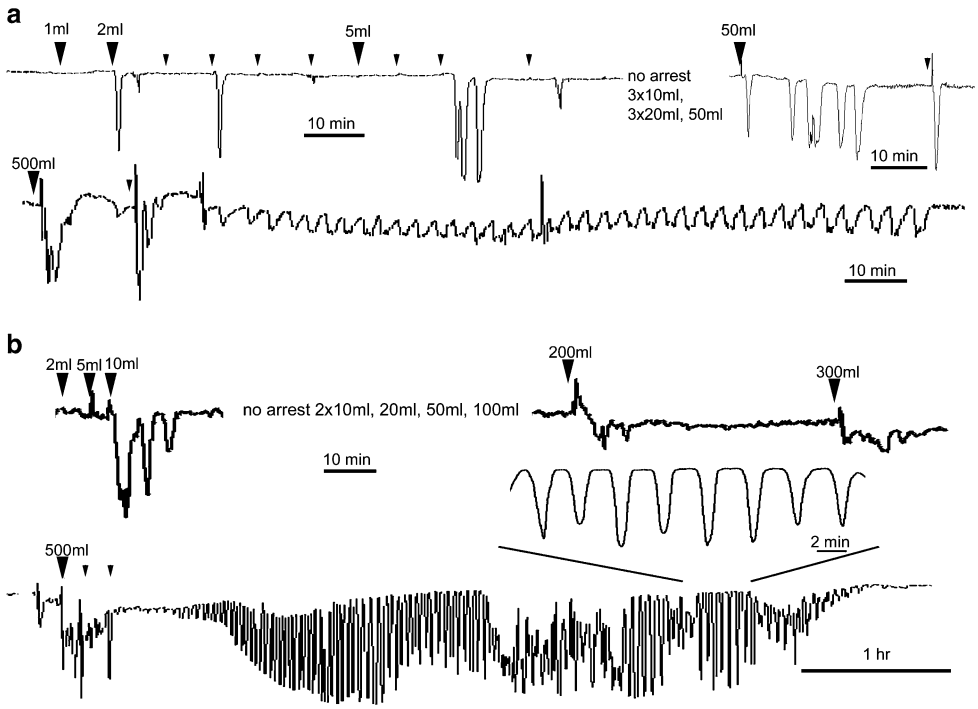

Fig. 4 Incremental addition of 0.5–1 g l^{-1} sediment to *R. dawsoni* (**a**) and *A. vastus* (**b**). Initial applications were more likely to trigger arrests than subsequent doses of the same or larger volumes. Much larger doses (here, repeated doses of 500 ml) caused prolonged arrest–recovery cycles with marked periodicity (e.g., *inset* in **b**). *Small arrowheads* indicate repeated additions of the same volume

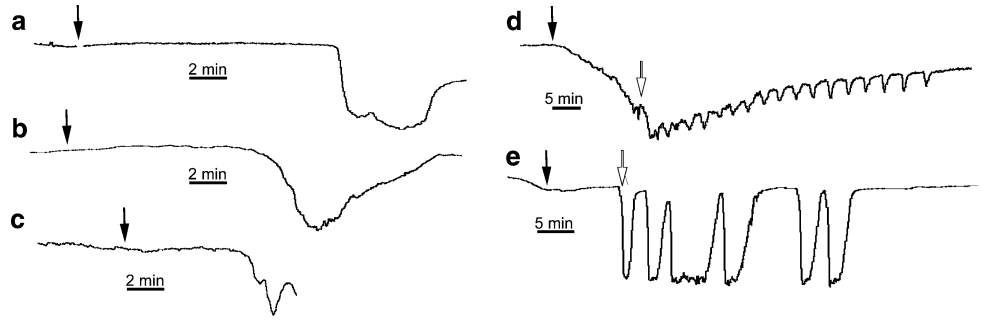

Fig. 5 Gradual addition (3 ml min^{-1}) of 10 g l^{-1} filtered (<25 µm) fjord sediment (*black arrows*) to *R. dawsoni* (**a**, **e**) and *A. vastus* (**b**, **c**) and reef sediment to *A. vastus* (**d**). **a** The arrest phase (time from start of arrest to full arrest) of *R. dawsoni* was 25–60 s, whereas in *A. vastus* (**b**, **c**) similar 'abrupt' arrests were not obvious, rather pumping level gradually declined over 50 s–4.5 min in fjord sediment and up to 17 min in reef sediment. Recovery after sediment addition stopped (*white arrows*) was marked by repeated arrest–recovery events in *A. vastus* (**d**) and *R. dawsoni* (**e**)

Addition of fjord sediment (<25 µm) continuously for 2–4 h reduced pumping to 50–80% of original pumping levels in *A. vastus* ($n = 3$) and 5–70% in *R. dawsoni* ($n = 7$) (Fig. 6). In all runs with *A. vastus* and five of seven trials with *R. dawsoni*, pumping levels recovered only after sediment addition stopped. Responses by *R. dawsoni* were variable. Some animals arrested less than once an hour (Fig. 6a), others arrested every 2–3 min (6 ± 1.7 arrests h^{-1}, $n = 7$). In *R. dawsoni* arrests became less frequent with ongoing sediment addition. In Fig. 6a a single arrest was triggered at the start of sediment addition, but after recovering from the initial arrest the sponge continued pumping for some hours at a much-reduced level. Return to normal pumping was drawn out (3–25 h); pumping was minimal for hours after sediment addition (Fig. 6a, b), and often arrest–recovery events interrupted return to normal pumping.

In *A. vastus*, continued addition of sediment triggered either a series of arrest–recovery events 1 s–3 min in duration or gradual oscillations in flow over 8–12 min. Arrests were never prolonged as they were in *R. dawsoni*. Pumping resumed immediately following each arrest, and the sponge arrested every 2–3 min until original pumping levels were reached 6 or more hours later (Fig. 6c).

Composition of sediment and fine structure of the aquiferous system

Scanning electron microscope analysis showed fjord sediment consisted of clays, diatom fragments and calcareous and siliceous sponge spicules, often bound in an organic matrix (Fig. 7). Large aggregates were over 100 µm in diameter (Fig. 7a); however, more than 75% of the sedi-

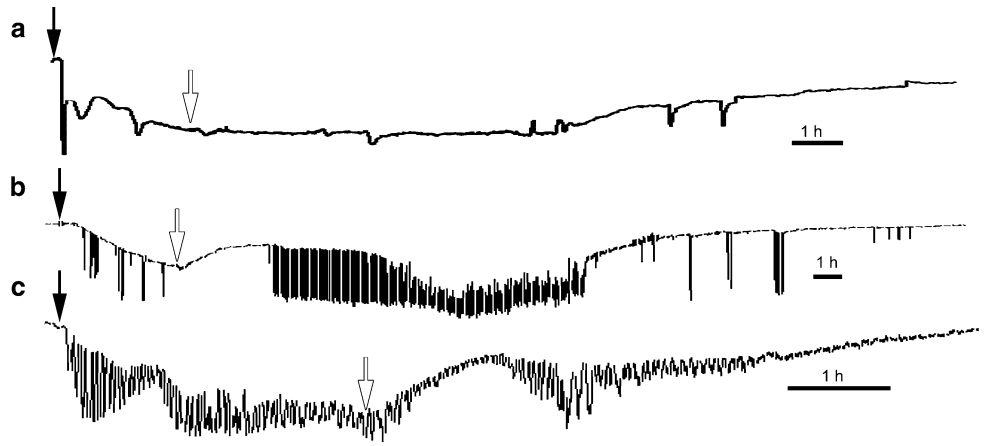

Fig. 6 Long-term (2–4 h) addition of filtered (<25 µm) fjord sediment. Sediment (10 g l^{-1}) was added at 3 ml min^{-1} (*black arrow start*, *white arrow stop*) to *R. dawsoni* (**a**, **b**) and *A. vastus* (**c**) triggered a gradual decline in pumping. **a**, **b** Arrests in *R. dawsoni* were less frequent with ongoing sediment addition. In (**a**), a single arrest was triggered at the onset of sediment addition; thereafter pumping level fluctuated and reached negligible levels within 1.5 h of start of sediment addition. Return to normal pumping was delayed by prolonged periods of negligible pumping (**a**) or repeated arrests lasting several hours (**b**, **c**). **c** In *A. vastus* pumping resumed immediately after each arrest during both sediment addition and return to normal pumping. Pumping levels remained low in both sponges as long as sediment was added

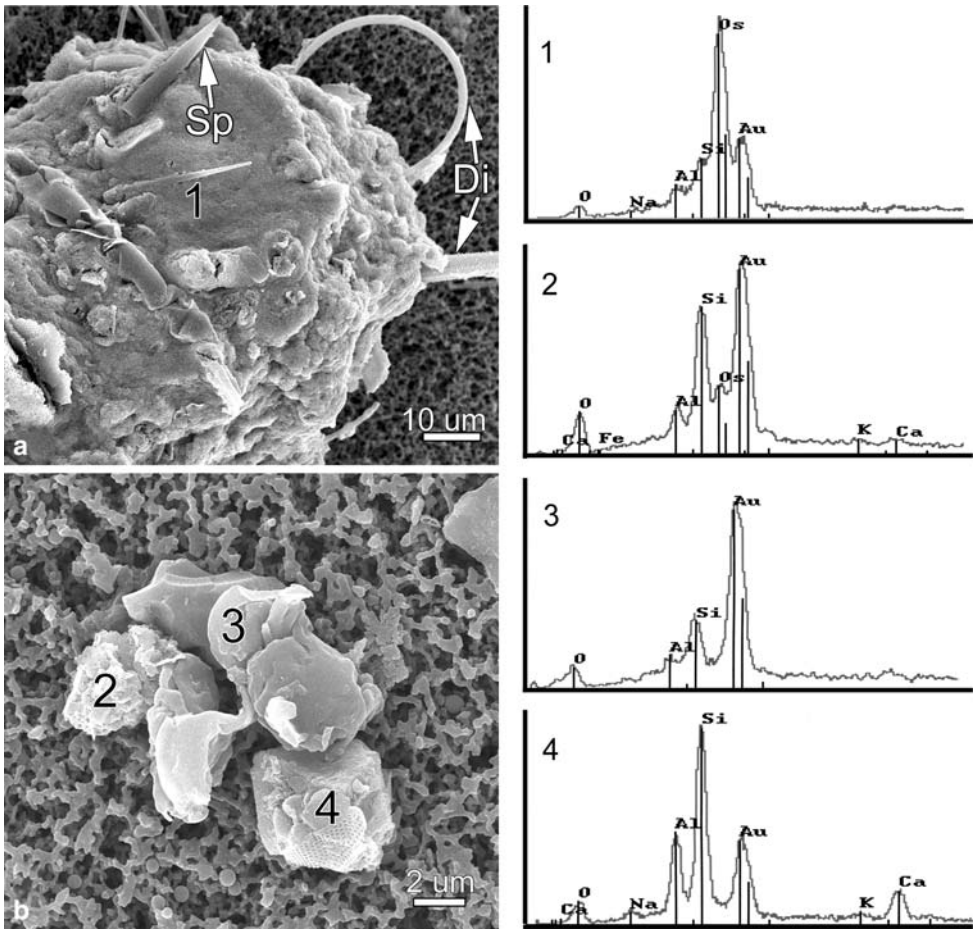

Fig. 7 Particles of fjord sediment from sponge collection sites viewed by SEM. **a** Aggregates of organic and inorganic material. **b** Small inorganic particles. EDX traces show the elemental composition of regions 1–4. Gold (Au) peaks are due to a gold coating applied in preparation for SEM. Osmium (*Os*) (applied during fixation) indicates organic material. Aluminum (*Al*) and silica (*Si*) indicate clays; (*Si*) could indicate sponge spicules (Sp) or diatom fragments (Di)

ment pieces were smaller than 20 µm (Fig. 7b), and most pieces of sediment were smaller than 10 µm, small enough to fit through the pores in the dermal membrane of the sponges (10–20 µm in diameter).

Clay (sediment containing aluminum and silica) was found in the aquiferous system of specimens of both species fixed after more than 40 min exposure to sediment, but chambers were not clogged by clay if only exposed to sediment for less than 40 min. In these specimens ($n = 8$) neither incurrent nor excurrent surfaces of the flagellated chambers appeared to be obscured by debris (i.e., Fig. 8a–e). Individual pieces of clay were found on the excurrent surface of fewer than 10% of flagellated chambers examined (e.g., Fig. 8d); only bacteria—no clays—were lodged against the surface of the collar microvilli (Fig. 8e). Latex microspheres were fed to sponges for 5 min to determine where in the aquiferous system particles of comparable size would lodge. Examination of fixed specimens showed these attached to the incurrent side of the microvilli collars; 1 µm spheres and most 0.5 µm spheres did not pass through the collar microvilli to the excurrent stream (Fig. 8f).

In contrast, in specimens subjected to long-term exposure to sediment, the excurrent surface of flagellated chambers were covered in clays. Clay was found in 20% of chambers of *R. dawsoni* and in some areas of the tissue 10 or more chambers were completely clogged with clays and other sediments (Fig. 8g).

Discussion

We have confirmed that the hexactinellid sponges *A. vastus* and *R. dawsoni* arrest pumping in response to sediment. Small discrete doses of sediment from the natural habitat, added as though resuspensed by ambient currents or fish, caused immediate feeding arrests in both species, although sponges appeared to arrest less frequently if at all to subsequent small doses. However, prolonged perfusion of sediment caused reduced pumping in all specimens of both species, as though the canal system became clogged. Recovery was marked by repeated on–off events similar to those described by Mackie et al. (1983) and considered indicative of pacemaker activity triggered by "unsatisfactory" conditions.

Concentrations of sediment that caused instantaneous arrest were similar in the two species and were comparable

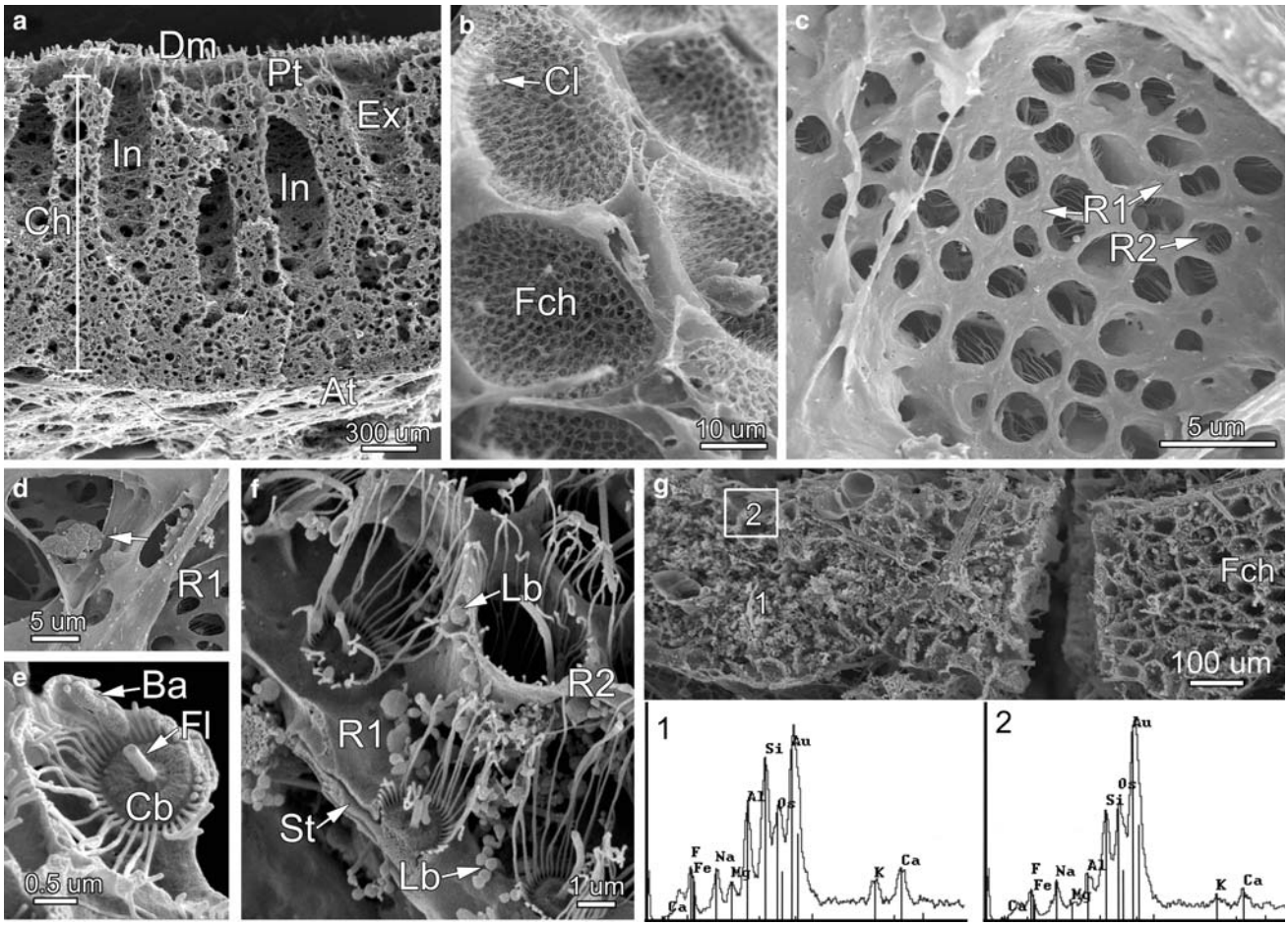

Fig. 8 Scanning electron microscopy of *A. vastus* (**a**–**f**) and *R. dawsoni* tissue (**g**) after either exposure to a heavy sediment suspension (**a**–**e**, **g**) or a mixture of 0.1 μm, 0.5 μm and 1 μm latex beads diluted in seawater (**f**). **a** A fracture through the body wall shows incurrent dermal (*Dm*) and excurrent atrial (*At*) surfaces, peripheral trabecular space (*Pt*) and juxtaposed incurrent (*In*) and excurrent (*Ex*) canals spanning the choanosome (*Ch*, *bracket*). **b** Inside of several flagellated chambers (*Fch*) containing organic debris and clay (*Cl*). **c** Incurrent surface of a flagellated chamber showing prosopyles (*pores*) in the primary trabecular reticulum (*R1*). The secondary reticulum (*R2*) can be seen arising from the primary reticulum. **d** Clay particle (*arrow*) on the trabecular tissue (*R1*) at the incurrent surface of a flagellated chamber. **e** Single collar body (*Cb*) of the branched choanocyte with a central flagellum (*Fl*) and rod-shaped bacteria (*Ba*) on the inner surface of the collar microvilli. **f** Branched choanocytes within the primary reticulum. Collar bodies arise from stolons (*St*) and collar microvilli are surrounded and supported by the secondary reticulum (*R2*). Latex beads (*Lb*, 1 μm and 0.5 μm) were found on the primary reticulum (*R1*) but were excluded from the inside of chambers by the microvilli mesh. **g** Clogged (*1*) and clean (*2*) flagellated chambers (*Fch*) of *R. dawsoni*. High aluminum content (*Al*) in EDX scan **1** indicates clogging by clays. *Si* indicates silica from clay or the glass skeleton

to levels that caused reduced pumping in the demosponge *Verongia lacunosa* (Gerrodette and Flechsig 1979). This concentration was only slightly higher than the range of suspended particulate concentrations found during the summer months in the fjords when the specimens were collected (7 mg l^{-1}) (Yahel et al. 2007).

Physiological significance of feeding current arrests

The arrest of pumping in *R. dawsoni* is due to spreading of an action potential (AP) throughout animal at 0.27 cm s^{-1} (Leys and Mackie 1997). The AP is thought to be calcium-based, and its arrival at any region would cause entry of calcium into the branched choanocytes, causing the arrest of flagella (Leys et al. 1999). Thus a point stimulus would generate an AP that would reach the length of a 20-cm long animal in approximately 1 min, arresting all choanocytes in that time.

The immediate nature of the arrest response in *A. vastus*, both to mechanical stimuli and to sediment applied topically, and the similarity in duration of the arrest and recovery phases in both species of glass sponge, suggest that arrests in *A. vastus* are responses to electrical events as in *R. dawsoni*. However, arrest–recovery responses were distinct in the two species, which can be taken to reflect underlying differences in the physiology of the action potential. Presumably this ultimately reflects different strategies for coping with sediment.

In situ observations showed that *R. dawsoni* was most often arrested (not pumping) after the arrival of the ROV which stirred up sediment; ex situ experiments showed that *R. dawsoni* arrested readily in response to light touch, small amounts of sediment added both topically and to incoming seawater, and to vibrations transmitted through the seawater from knocks to the aquaria. Furthermore, arrests were usually long. In contrast, pumping in *A. vastus* was more robust. In situ, all specimens took up dye and pumped it immediately out of the osculum; none were arrested. In ex situ experiments *A. vastus* arrested only in response to very strong mechanical stimuli, but not when touched with a pipette or when the aquarium was tapped. Mechanical stimuli from fine (<25 μm) sediment from the reef core also failed to elicit immediate arrests, instead pumping levels slowly diminished as sponges became clogged. *Rhabdocalyptus dawsoni* and *Aphrocallistes vastus* also responded differently to long periods of exposure to sediment. Although this treatment caused reduced pumping in both species, *R. dawsoni* spent longer off, or barely pumping both during and after sediment addition, that is, arrests could last up to several hours. In contrast, specimens of *A. vastus* began to pump immediately after each arrest, during and after sediment addition.

The fused skeleton of *A. vastus* could account in part for differences in sensitivity. Whereas the spicules of *R. dawsoni* are loosely held together by tissue and vibration of a long spicule can cause an arrest (Mackie et al. 1983), in *A. vastus*, all but very small spicules in the skeleton are fused into a rigid scaffold. It is likely, however, that conductive properties of the tissue differ. In earlier work Mackie et al. (1983) suggested that slow recovery from arrests in *R. dawsoni* could be either because flagella beat weakly following to start or because some beat earlier than others. The fact that *A. vastus* began to pump immediately after each arrest may indicate that flagella all begin to beat strongly and in unison. This difference between the two species could reflect differences in the ability to resequester Ca^{2+} after an arrest.

The function of the arrest response

Rapid arrests coordinated by electrical signaling presumably protect hexactinellids from taking in noxious or excessive particulates (Lawn et al. 1981; Mackie et al. 1983). It has been suggested that arrests serve as an early warning system to enable distant parts of the specimens to stop pumping before noxious water reaches them (Leys et al. 1999). Since uptake of sediment is slow (fluorescein-dyed water traverses the body wall in 30–40 s, considerably slower than for most demosponges) an arrest spreading throughout the tissue in 30–60 s could prevent sediment reaching pristine flagellated chambers deeper in the body wall. However, records showed that even when sponges were in continually murky water, after initial arrests they kept on pumping, albeit at a weaker level. Thus, although hexactinellids can arrest their feeding current in response to small irritations, large amounts of sediment clearly clog the feeding apparatus.

Clogging may be a wide-spread problem among sponges. In the demosponges *Petrosia ficiformis* (Bavestrello et al. 1988) and *Tethya wilhelma* (Nickel et al. 2006) and in another hexactinellid (*Scolymastra joubini*, Bavestrello et al. 2003) it has been suggested that water and sediment may bypass flagellated chambers by special canals. In the hexactinellids *R. dawsoni* (Leys 1999) and *Sericolophus hawaiicus* (Reiswig, pers. comm.) prosopyles (entrances to the flagellated chambers) can line up directly opposite perforations in the secondary reticulum, providing a direct 5 μm diameter passage into the flagellated chamber, but it is unlikely that enough sediment would pass through these to adequately clear the sponge. No direct connections between incurrent and excurrent canals could be found in any of 15 plastic replicas of the aquiferous system of *A. vastus* (data not shown).

Alternatively, if sediment is ingested the inorganic component of sediment could be expelled into the excurrent stream after processing by the tissue. It is even possible that arrests could facilitate processing of sediment. Ca^{2+} influx into the trabecular tissue could trigger endocytosis as in adrenal chromaffin cells (Chan et al. 2003).

Feeding experiments on the hexactinellid *S. hawaiicus* show relatively low retention of bacteria (47%) and autotrophic eukaryotes (54%) (Pile and Young 2006), however, similar experiments on *A. vastus* and *R. dawsoni*, show that small heterotrophic protists and up to 99% of the most abundant bacterial cells were retained, and inorganic sediment was excreted (Yahel et al. 2006). The high retention of bacteria by *A. vastus* and *R. dawsoni* is inconsistent with bypass routes; without a bypass system how do hexactinellids cope with clogging?

One possibility is the use of repeated on/off events. The very regular arrests that occur after prolonged exposure to sediment are reminiscent of the rhythmic contractions seen in demosponges that flush material out of the aquiferous system in *T. wilhelma* and *Ephydatia muelleri* (Nickel 2004; Elliott and Leys 2007). The on/off events in the glass sponges occurred during a gradual increase in pumping level during prolonged sediment addition, which suggests they may be effective in clearing sediment from the animal, but exactly how stopping and starting flow in the sponge would aid in clearing sediment is still unclear.

Ecological implications of sensitivity to sediment

Several demosponges have special adaptations for habitats with high sediment loads. *Biemna ehrenbergi* lives buried

in coarse sand through which it draws in nutrient rich water (Ilan and Abelson 1995); other sponges grow on shells of mobile invertebrates, which periodically dislodge sediment (van Soest 1993; Burns and Bingham 2002). Corals and bivalves have even been shown to adopt more efficient particle feeding in a high sediment regime (Urrutia et al. 1997; Anthony 2000). The distinct pumping patterns shown for the two hexactinellid species may reflect specializations for coping with sediment.

Aphrocallistes vastus is one of the three sponges that form reefs at 100–250 m depths on the Pacific Coast of North America. Sponge reefs are exposed to chronic low levels of sediment and the reefs are thought to act as baffles trapping suspended sediment, which then cements the reef framework together (Conway et al. 2004; Whitney et al. 2005). Immediate attempts to pump after each arrest could allow this species to continue pumping in the presence of ongoing sediment, making it well adapted for reef habitats such as the Fraser Ridge reef where sedimentation from the Fraser River plume exceeds 2 cm $year^{-1}$ (Conway et al. 2004). *Rhabdocalyptus dawsoni* is notably absent at Fraser Ridge. In contrast, the low threshold of arrests and their prolonged nature in *R. dawsoni* may be suitable for preventing clogging during temporary sediment loads, such as by resuspension events along the fjord walls.

Nevertheless, the progressive decline in overall pumping level, presumably due to clogging, indicates that sustained exposure to sediment is likely to be detrimental to both species. While short-term adaptation, a phenomenon known from many species including cnidarians (Josephson 1985; Grigoriev and Spencer 1995), could explain the sustained pumping under continued sediment exposure, under these conditions pumping levels are so low (and thus ineffective for feeding) that glass sponges living under higher sediment conditions might be expected to be energetically stressed in comparison to those areas less affected by sediment. It has been noted that sponges in sediment-rich habitats have narrower oscula (Conway et al. 2004). Future studies should investigate the relative health of sponges at sediment rich and poor habitats, in relation to sediment, ambient current, and biotic factors including competition and predation.

The response of different species of glass sponge to particulates in the natural environment can tell us much about the unique electrical conduction system that these animals have evolved for controlling their feeding current.

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