



The feeding behaviour of a deep-sea holothurian, *Stichopus tremulus* (Gunnerus) based on in situ observations and experiments using a Remotely Operated Vehicle

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Abstract

Using a Remotely Operated Vehicle (ROV) to deploy an in situ cage experiment incorporating fluorescent Luminophore particle tracers, the gut throughput time of the deposit feeding holothurian, *Stichopus tremulus* (Gunnerus) was determined as 23.73 h (S.D. \pm 2.3). For a range of individuals examined at different depths (350–500 m) and locations, throughput times varied between 19 and 26 h irrespective of animal size or gut tract length. In situ video observations of feeding behaviour showed that this species uses fine oral papillae in a ‘sweeping’ motion to target particles on the seafloor. Following detection of a food source fine-branched digitate tentacles collect a large range of sediment fragments from the seabed. The main types of particles ingested include silica fragments (<20>500 μ m), pelagic foraminifera, benthic foraminifera, fine phytodetrital remains and occasional larger rock fragments (\sim 1 cm). Ingested sediment consisted mainly of very fine silica fragments (\sim 50 μ m) accounting for over 50% of the total gut contents. Frame-by-frame video analysis revealed that the particle handling time (i.e. the time taken for a tentacle insertion and the subsequent collection of food) was found to be \sim 54 s. Only 10 of the 20 feeding tentacles were simultaneously employed during feeding. Use of tentacles appeared to be in sequence, alternating between the reserve and active tentacles. Estimating the rate of movement over the seabed and the total effective capture area of each tentacle, the impact of this animal on the turnover and quality of surface sediment at this deepwater site is potentially substantial. The in situ experiments provided a significant improvement over

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previous methods used to investigate deep-sea deposit feeders and represent a useful concept for further in situ deep-sea research using an industrial ROV.

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1. Introduction

Within the deep-sea, deposit feeding invertebrates, such as the Holothuroidea, dominate the benthic community, reworking particles at or immediately below the sediment–water interface (Gray, 1974; Rowe et al., 1974). Much of the sediment ingested by deposit feeders consists of sedimented material of low nutritional value (Lopez and Levinton, 1987). A lack of significant quantities of organic matter within the seafloor sediment (Santos et al., 1994) means that deposit feeders need to ingest large amounts of sediment in order to gain a net input of energy. This requirement means that the level of impact they have on the sediment surface can be substantial, in terms of both physical bioturbation and biochemical reworking (Sibuet and Lawrence, 1981; Sibuet, 1987; Roberts et al., 2000). Understanding the rate, mechanism and selectivity of feeding is of fundamental importance if organism–sediment dynamics are to be fully understood.

The residence time of particles in the digestive tract are of particular importance because digestion is significantly affected by gut residence times (Penry and Jumars, 1987). Holothurians are convenient taxa to investigate ingestion and digestion in deposit feeders as they exhibit a wide range of deposit feeding strategies and are relatively easy to identify and collect (Massin, 1982; Billett, 1991). It has been suggested that alternate feeding strategies co-vary with environmental variables such as pressure, salinity and physiological effects (Roberts et al., 2000). For the Holothuroidea variables such as these may influence the feeding strategy employed and in turn the digestion mechanism and gut residence time. Much of the work carried out on holothurian digestion has focussed on shallow water species. These species are more accessible than their deep-sea relatives and provide an easier target for investigative studies.

One of the simplest approaches to investigate holothurian feeding is to estimate their bulk diet, through the examination of the gut contents. Using light microscopy, the size and type of ingested particles can be ascertained for a variety of holothurian species (Hauksson, 1979; Levin, 1999), although most work has predominately taken place in the shallow water environment. Similar studies in the deep sea are lacking undoubtedly because they are logistically much more problematic (Sokolova, 1972; Khrpounoff and Sibuet, 1980; Tyler et al., 1992; Manship, 1995).

Perhaps one of the most studied species of deposit-feeding aspidochirotid holothurian is *Stichopus japonicus*. The gut contents of this species are dominated by shell fragments from molluscs and barnacles, algal debris, echinoderm ossicles, many pelagic and benthic foraminifera and diatoms (Hauksson, 1979). This seems to be consistent with the types of particles found within many deposit-feeding holothurian guts, termed by Sokolova (1972) as a “roughly sorting deposit feeder diet”; as been observed elsewhere; Sibuet (1987) who

found nine taxa contained within the guts of abyssal holothurians and Roberts and Moore (1997) listed 14 different taxa.

When considering the size of ingested particles, the available data from the literature suggests that habitat type is very important. For example, abyssal species ingest finer particles between 20 and 60 μm when compared to shallow water species (Sokolova, 1972; Khripounoff and Sibuet, 1980). Particle sizes of ingested material in some reef dwelling holothurians (e.g. *Holothuria atra*) can reach sizes of $>500 \mu\text{m}$, especially where species feed almost exclusively on faecal casts (Hauksson, 1979; Uthicke, 1999; Uthicke and Karez, 1999).

Ultimately, the sizes of particles ingested affects the time needed for the animal gut to extract efficiently the labile organic matter on which it relies. Gut residence time can vary in aspidochirotrids with latitude, perhaps indicating the presence of a metabolic effect (Hammond, 1982). Although the available data supporting this supposition are largely confined to species inhabiting shallow waters, values range from 7.5 h in tropical species to 10+ h in temperate species. When considering deep-sea species, estimates of gut residence time are generally higher (Sibuet, et al., 1989), a conclusion based upon limited numbers of studies (Roberts et al., 2000).

The way in which the animal feeds will also have a significant effect on gut residence time. The selection of a particle for ingestion takes place during particle capture, where the adhesive forces and shape of the tentacle determine which particles are taken in to the mouth (Hyman, 1955; Massin, 1982). The type of tentacle is inherently linked to the mode of feeding employed by each species (Billett, 1991; Roberts and Moore, 1997). For example, a 'sweep strategy' feeder is likely to use stubby peltate tentacles, whereas a 'rake feeder' is more likely to use branched digitate tentacles (Roberts and Moore, 1997). Large variations in tentacle morphology exist within holothurians. It seems likely then, that if a holothurian feeds in a specific way this will affect the particles taken in and thus the amount of time needed to utilise labile organic matter.

Important factors leading to ambiguity in gut residence times, including deep-sea species, are differences in experimental methodology. Studies dominated by shallow water tropical species suggest that gut residence times can range from 3 to 18 h (Roberts et al., 2000). These perceived differences could be a result of differences in methodology. Using animals in captive aquarium and observing faeces production is a common method used to assess gut residence (Yingst, 1982; Jones and Jago, 1993; Manship, 1995). One primary problem with this type of study is that if deep-sea animals are brought to the surface, the effects of pressure and changes in temperature may change metabolic rates, affect the fluid membrane consistency and pressure ultimately result in death of the animal. To overcome this, Sibuet et al. (1989) used a method of in situ observations in the deep sea of faeces production in abyssal holothurians and estimated a gut residence of ~ 24 h. A similar observational approach was also used with time-lapse photography, where the appearance of large faecal strings was observed, with one produced after ~ 16 h (Heezen and Hollister, 1971). This estimate agrees with a method using bacterial population doubling times within holothurian gut sediments (Deming and Colwell, 1982).

Stichopus tremulus was previously investigated by Hauksson (1979) using aquarium and shallow water cages (9 m depth), but to date no experiments of this kind have been carried out within the deep-sea. To this end, there is a need to carry out shallow-water

based experiments in the deep-sea. Using new technology based around a Remotely Operated Vehicle (ROV) platform, this study aims to carry out in situ incubations in a controlled manner to manipulate the diet of a common deep-sea species, *S. tremulus*, which can be found down to 1500 m (Billett, 1991) examining in situ feeding rates, feeding strategy and gut throughput time within a deep-water environment.

2. Materials and methods

2.1. Study area

This study took place within the *Schiehallion* and *Foinaven* area, two of British Petroleum's (BP) most productive oil fields. Within this area are an array of subsea structures that are continually serviced and range in depth from 350 to 500+ m. These fields are located on the edge of the United Kingdom Continental Shelf, off the western coast of the Shetland Isles (Fig. 1).

BP operates several contractors within *Schiehallion* and *Foinaven* to maintain and survey the subsea pipelines, involving a high degree of ROV intervention work. During

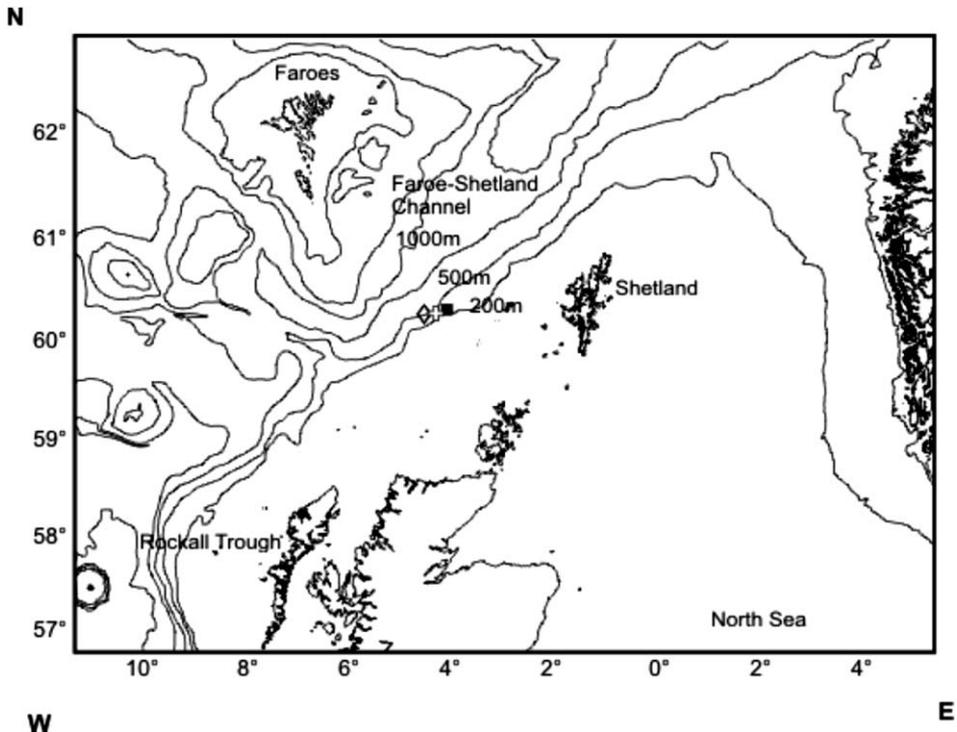


Fig. 1. Map of the West of Shetland area, showing the three sampling sites from the 2002 Regalia cruise. Black cross = Site 1 (374 m), Black square = Site 2 (474 m), Black diamond = Site 3 (505 m).

these activities, there are frequent periods of stand-by time. These periods provided a unique opportunity to carry out in situ incubation experiments and video observations, otherwise not possible in deep water. Using the ROV support vessel MSV Regalia three similar sites in terms of depth and sediment type were sampled (Table 1).

2.2. Experimental trap design and methodology

We designed an experimental cage to facilitate operation by ROV manipulation arms, comprising a robust steel handle and back support, a hinged lid with a grab handle and a solid base which is spiked into the sediment for stability within a high current environment (~ 30 cm/s) (Bett, pers comm.) (Fig. 2). Each trap had a food tray at the base, which was loaded to a depth of ~ 2 cm with a pre-determined sediment food source made up of natural sediment taken from each site mixed with different sized glass beads and luminophores (fluorescent sand based tracer particles). The glass beads were 150–200 μm in tracer batch #1 and 600 μm in tracer batch #2. The luminophores (ETS Tracers) were sized between 200 and 300 μm and fluoresced pink under UV light. To each food tray 10 g of #1, 20 g of #2 and 10 g of tracer were added prior to ROV deployment.

2.3. ROV design and deployment of traps

The ROV used for this experiment was a Centurion 30 work-class system developed and operated by SUBSEA7 (UK). The ROV is capable of operating up to 3000 m depths with one 7-function manipulator arm and one 5-function manipulator arm. Each vehicle is fitted with a high-resolution zoom-pan-tilt video camera system, with a live feed and record facility, and a 3-mega-pixel digital stills camera. The experimental cages were crane-lowered to the sea floor in a workbasket. On reaching the seabed the ROV would ‘pick up’ a cage, search for a suitable specimen of the common sea cucumber, *S. tremulus*, before placing the cage down next to the animal. Using the 7-function manipulator arm, a mesh scoop is used to collect the individual. The specimen is placed into the trap onto the sediment and tracer batches supplied and the lid closed. The animal was then observed via

Table 1
Site details and sampling locations for Regalia cruise 2002

Specimen no.	Site	Date	Depth (m)	Latitude (N)	Longitude (W)
1	1	25/7/02	374	60°41.4	4°24.0
2	1	25/7/02	374	60°41.4	4°24.0
3	1	26/7/02	373	60°41.5	4°24.0
4	2	29/7/02	505	60°41.4	4°25.2
5	2	29/7/02	505	60°41.4	4°25.2
6	1	30/7/02	374	60°41.4	4°24.0
7	1	30/7/02	374	60°40.2	4°24.0
8	1	30/7/02	374	60°40.2	4°24.0
9	3	31/7/02	475	60°41.4	4°26.4
10	3	31/7/03	476	60°41.5	4°26.4

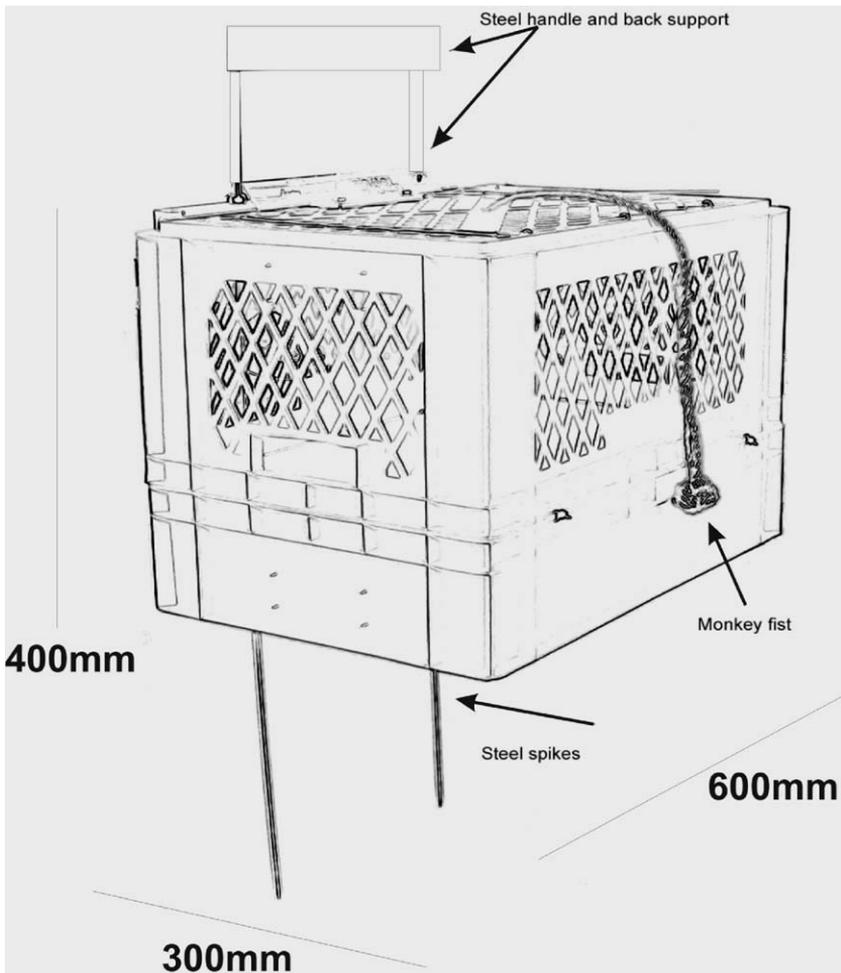


Fig. 2. Schematic diagram of the benthic incubation trap used for all experimental deployments, showing functional parts and dimensions.

live video telemetry until feeding resumed on the supplied food and the start time recorded. A total of 10 successful deployments were made in three locations (Table 1).

2.4. Sample analysis and data collection

After each cage was retrieved from the seabed, the animal was removed and the whole gut dissected out and frozen intact at $-25\text{ }^{\circ}\text{C}$. Each specimen was measured for total body length and gut length. Each gut was sectioned at 1 cm intervals from anterior to posterior. Each 1 cm section was placed into a glass petri dish, distilled water was added and the contents viewed and recorded under a dissecting microscope. Total gut contents were examined for the type, size and number of particles of glass beads and/or

luminophores. For enumeration of luminophores, a UV light source was used to aid visual location. The procedure was repeated for each of the gut sections examined. A digital image of each gut section examined was used to ensure comparative estimates remained consistent.

We measured the time at which particles moved through the holothurian gut as:

$$G_t = \frac{G_L x t}{D_{\max}}$$

where G_t = gut throughput time h^{-1} , G_L = gut tract length, t = time, D_{\max} = maximum distance moved by a tracer particle.

Measuring each specimen allowed a comparative scale to be used for each observed feeding sequence. Using this scale, it was possible to examine the total distance moved by each individual over the duration of a sequence and to calculate a mean locomotion speed. Effective particle capture area of a typical tentacle was calculated by measuring the diameter of each tentacle, to give an estimate of total effective tentacle area of the whole tentacular crown. From these parameters the total reworking capability of this species was derived as follows:

$$TR = T_n \times Mc \times Ti$$

where TR = total reworking value, T_n = no. of tentacles on seafloor at any one time, Mc = mean effective capture area cm^2 , Ti = tentacle insertion rate m^{-1} .

2.5. Video analysis

Video footage was stored on S-VHS video format. This was transferred via a digital signal onto MPEG-2 DVD format to enable frame-by-frame analysis of the footage. Each sequence was analysed for the number of tentacles in view, the rate at which feeding tentacles were placed onto the seafloor sediment (tentacle insertion) ($n = 100$), rate of particle pick-ups by each tentacle ($n = 100$), rate of sediment passed to the mouth ($n = 100$), tentacle feeding action, feeding strategy and rate of movement. Each individual filmed at site 1 was brought back to the surface, and the actual number of tentacles recorded and the total effective capture area of each tentacle was calculated.

This method provides an opportunity to observe natural holothurian feeding behaviour in an undisturbed manner, a considerable advantage over previous invasive methods.

3. Results

3.1. Gut content analysis for *S. tremulus*

3.1.1. Particle type observations

Using light microscopy, each section of gut from each animal was examined for the dominant particle type and size, along with any other characteristic objects, and then enumerated for the total animal (Table 2). The dominant particle types were silica fragments, accounting 90% of the total gut across all 10 animals observed (Fig. 3). Next, the most

Table 2
Gut contents and particle size measurements

Gut contents	Specimen no.										Mean %
	1	2	3	4	5	6	7	8	9	10	
<hr/> % Mean abundance of gut contents <hr/>											
<i>Particle types</i>											
Pelagic Foraminifera	4	5	5	2	5	1	3	4	2	5	<i>3.6 ± 1.51</i>
Benthic Foraminifera	1	1	1	1	1	2			1	1	<i>1.13 ± 0.35</i>
Diatom/Dinoflagellate fragments	1	1	1		1		1				<i>1 ± 0.00</i>
Faecal pellets									1		<i>1 ± 0.00</i>
Silica particles	91	87	80	95	89	95	89	90	91	91	<i>89.8 ± 4.26</i>
Irregular stones	1	1	5	2		1	1	1		1	<i>1.63 ± 1.41</i>
Calcareous ossicles			1		1		1				<i>1 ± 0.00</i>
<i>Particle size µm</i>											
>50	30	30	30	45	30	35	25	30	30	30	<i>31.5 ± 5.30</i>
50–100	44	27	35	15	33	26	20	30	32	30	<i>29.2 ± 1.00</i>
100–200	10	23	5	10	20	30	25	24	25	20	<i>19.2 ± 8.12</i>
200–500	10	10	15	25	7	5	15	10	5	10	<i>11.2 ± 5.96</i>
500–1000	3	5	10	3	5	3	10	5	5	8	<i>5.7 ± 2.71</i>
1000+	3	5	5	2	5	1	5	1	3	2	<i>3.2 ± 1.69</i>

Standard deviation in italics.

dominant particle types were pelagic foraminifera (3.6%). Other particles were benthic foraminifera (1.13%), phytoplanktonic remains (1%), faecal casts from other deposit feeders including holothurians (1%), irregular stones (1.63%) and calcareous ossicles (1%).

3.1.2. Particle size observations

For particle size distribution, the % abundance of each particle size class was calculated for each gut section analysed (Table 2). Of all the holothurian gut tracts examined, the dominant ingested particle size class was < 50 µm (31%), followed by the 50–100 µm

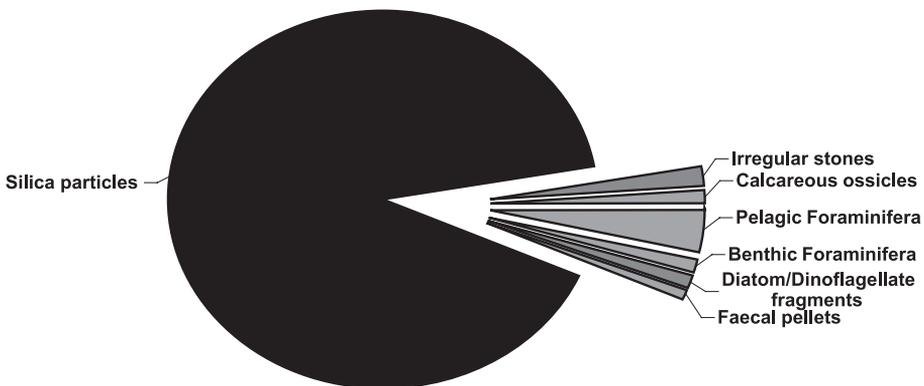


Fig. 3. Mean % abundance pie chart of the gut contents found within the digestive tracts of *S. tremulus*.

(29%) and 100–200 μm (19%) size classes (Fig. 4). Overall, 80% of the total particles ingested were between 0 and 200 μm . The largest particle ingested was >1 mm, although these occurred relatively infrequently (3%) (Fig. 4).

3.2. Video sequences and feeding behaviour

3.2.1. Feeding behaviour observations of *S. tremulus*: sensory papillae

Frame-by-frame analysis of the video footage ($n = 10$) showed a clear pattern of specific feeding mechanisms. The first observation revealed that the sensory papillae surrounding the oral crown (Fig. 5) were directly involved in feeding. These papillae were observed making contact with the seafloor sediment in an alternate ‘sweeping’ motion. We interpreted this behaviour as a means of searching the sediment surface for food prior to insertion of the feeding tentacles (Fig. 5). The same motion was consistent across all the individuals observed.

3.2.2. Tentacle action

All individuals observed and returned to the surface had a total of 20 feeding tentacles. Close-up ROV video footage allowed each tentacle to be clearly observed. Each tentacle

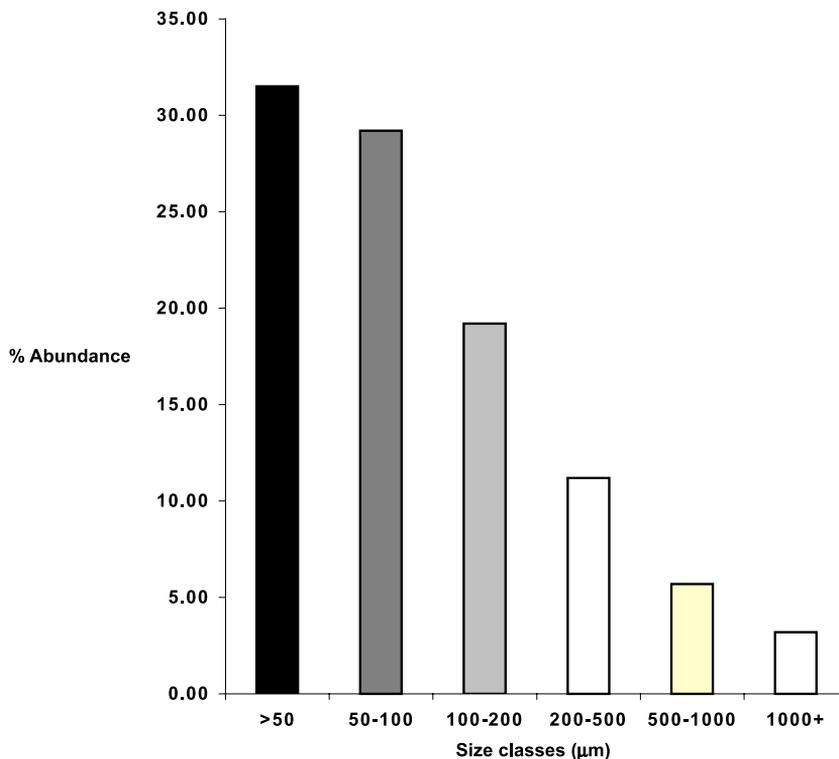


Fig. 4. Mean % abundance bar chart showing the size classes of particles collected from the digestive tracts of *S. tremulus*.

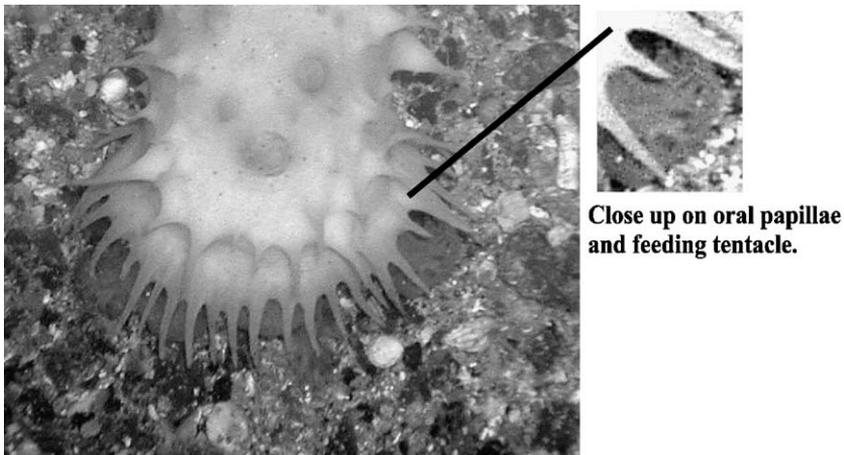


Fig. 5. ROV photograph of the oral papillae and feeding tentacles of *S. tremulus*. The close up view shows a finely branched tentacle spreading out across the seabed and the initial contact of the sensory papillae.

was placed alternately onto the seafloor sediment, so that only 50% of the total tentacle array (i.e. 10) was in contact with the seafloor and actively feeding at any one time. Similar images from the video camera systems on board the ROV allowed the fine detailed structure of a feeding tentacle to be observed (Fig. 5). Each tentacle is finely branched to form a tree-like array, which seems to facilitate dextrous function. When feeding each tentacle extends

Table 3
Video analysis of feeding and movement sequences

Specimen no.										
1	2	3	4	5	6	7	8	9	10	Mean rate
<i>(a) Mean tentacle insertion rate (s)</i>										
35.45	32.93	40.54	36.60	35.09	38.23	34.45	34.91	38.36	36.55	36.31
<i>1.51</i>	<i>1.45</i>	<i>1.06</i>	<i>0.79</i>	<i>0.81</i>	<i>0.71</i>	<i>0.74</i>	<i>0.86</i>	<i>0.55</i>	<i>0.69</i>	<i>0.92</i>
<i>(b) Mean transfer time of sediment to mouth (s)</i>										
16.23	14.98	16.87	15.72	17.83	13.87	16.03	15.74	14.63	13.97	15.59
<i>0.78</i>	<i>0.85</i>	<i>0.79</i>	<i>0.66</i>	<i>0.87</i>	<i>0.98</i>	<i>0.25</i>	<i>0.65</i>	<i>0.12</i>	<i>0.53</i>	<i>0.65</i>
<i>(c) Total time of tentacle feeding sequence (s)</i>										
51.68	47.91	57.41	52.32	52.92	52.10	50.48	50.65	52.99	50.52	51.90
<i>(d) Rate of movement (cm min⁻¹)</i>										
1.43	1.65	1.89	1.56	1.77	1.62	1.46	1.56	1.65	1.98	1.66
										<i>0.18</i>
<i>(e) Mean effective tentacle particle capture area (cm²)</i>										
1.3	0.98	1.1	1.2	1.5	1.6	1.3	1.4	1.4	1.2	1.30
										<i>0.19</i>

Standard deviation in italics.

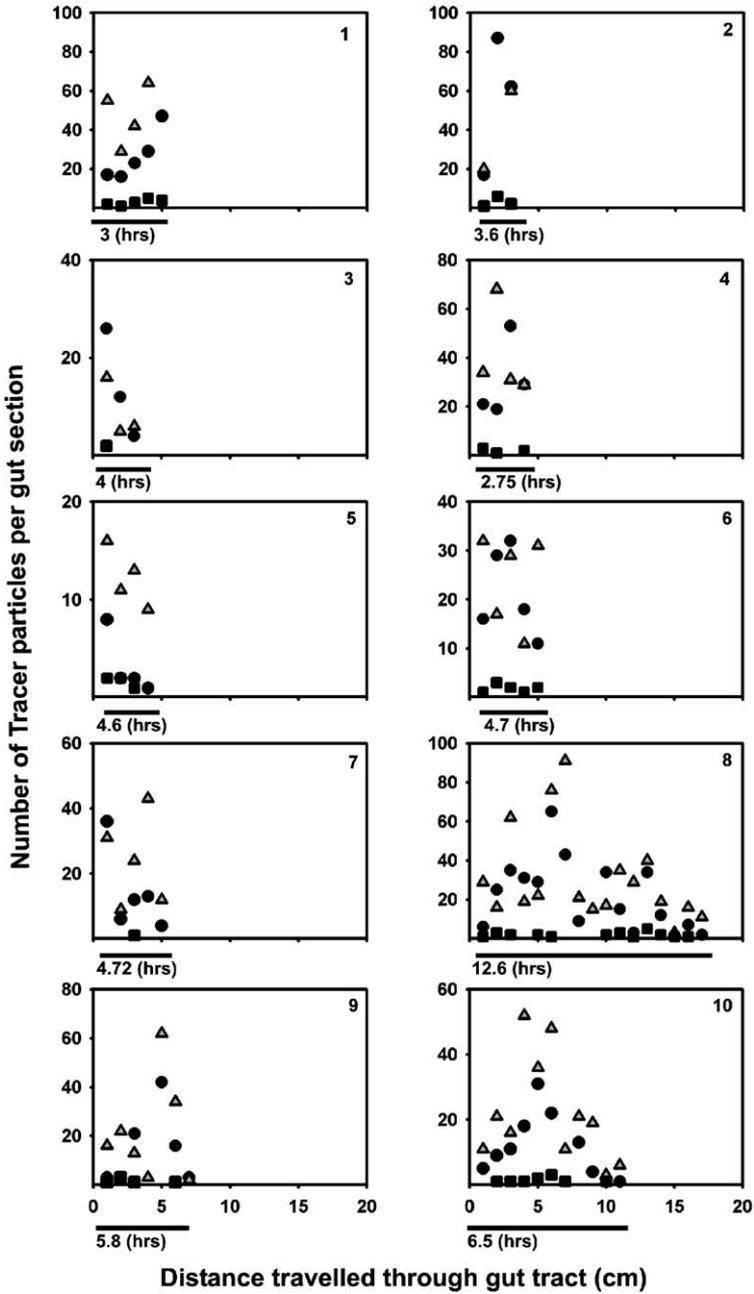


Fig. 6. Montage of 10 specimens showing the actual number of the three tracer particles used in the in situ incubation experiments. Grey triangle=#1 (150–200 μm), Circle=Luminophore tracers (200–300 μm) and Square=#2 (600 μm). The black bar indicates the duration of the incubation in relation to the distance travelled down the gut tract.

from the oral crown and is moved towards the sediment surface. As it approaches the seafloor, each branch is outstretched further to achieve maximum surface coverage for feeding. Each tentacle is spread open across the seafloor sediment where it makes contact with the sediment and ‘picks up’ a ‘handful’ of particles in a grasping motion. Each ‘handful’ of sediment is passed upwards towards the mouth.

3.2.3. Tentacle insertion rates

During 10 separate feeding observations of individual holothurians a mean tentacle insertion rate was calculated based on 100 separate tentacle movements. This showed that an individual tentacle was placed onto the seafloor every 36 s (S.D. ± 0.92 , $n = 100$) (Table 3). Each tentacle collected sediment in the manner described above before passing it to the mouth and returning to the seafloor to feed again. The entire sequence took an average of ~ 52 s per individual tentacle (Table 3).

3.2.4. Rate of movement and particle capture

The mean movement speed for this species was calculated at ~ 0.99 m h⁻¹ S.D. ± 0.11 , $n = 10$ (Table 3). Effective particle capture area of a typical tentacle (Table 3) was ~ 1.3 cm² ($n = 10$) with a total effective area per individual of >26 cm² (Table 3). Total reworking capability of an individual of this species was calculated as 11.7 cm² min⁻¹.

3.3. Experimental approach: gut throughput rates

All 10 animals ingested both the tracer glass beads from tracer batch #1 and tracer batch #2 and the luminophore tracer; however, the glass beads from batch #1 (150–200 μ m) were more abundant than the luminophores within the gut sections (Fig. 6) and proved difficult to identify from the other types of particle ingested. The larger glass beads of batch #2 (600 μ m) were also recorded, but at a much lower frequency (Fig. 5). The luminophore tracer was comparatively simple to identify within the gut contents, although it was not as abundant as the glass beads of batch #1 (Fig. 6).

The maximum distance travelled through the gut tract in the incubation period represented an accurate measurement of gut throughput time (Fig. 6, Table 4). For 10

Table 4
Experimental incubation times and gut throughput rates

Specimen no.	Incubation time (h)	Gut length (cm)	Throughput rate (h)
1	3	42	22.08
2	3.6	30	21.14
3	4	26	27
4	2.75	28	19.25
5	4.6	24	25.07
6	4.7	32	23.6
7	4.72	30	25.2
8	12.6	36	26
9	5.8	33	23.93
10	6.5	42	22.75

different animals, with varying gut length and body length and from three different sites, the mean gut throughput time was 23.60 h (S.D. \pm 2.36), ranging from 19 to 27 h (Table 4). Although the abundances of the different tracer particles differed between specimens, and within each gut section, they were consistently spread between the furthest section containing tracer (maximum distance travelled) and samples closest to the mouth and oesophagus (most recently ingested material) (Fig. 6). This provides evidence that each animal constantly ingested food material, and that the tracers were moved through the gut tract at an approximately even throughput rate over the incubation period. This allows the calculation of the total time it would take a particle to move through the entire digestive tract (gut throughput time).

4. Discussion

4.1. Gut contents

Studying gut contents to provide information on the feeding specifics of benthic animals has long since been the accepted methodology (Théel, 1882). Many studies have examined the bulk diet of holothurians to investigate their feeding preferences (Hauksson, 1979; Khripounoff and Sibuet, 1980; Tyler et al., 1992; Manship, 1995). A study by Hauksson (1979) on shallow-water specimens of *S. tremulus* showed that it had a preference for faecal pellets (500 μm) perhaps linked to their bacterial content. A broad range of particle sizes was also shown within its gut, although they were generally larger than the dominant sizes in the surrounding sediment. The present study found little evidence of faecal pellets within the gut, and the majority of particles ingested were $< 50 \mu\text{m}$. Here we found a range of particle types within the gut of *S. tremulus*, much of it in agreement with the observations of Hauksson (1979), with pelagic and benthic foraminifera, calcareous ossicles and little or no other meiofauna present in the gut.

The use of an in situ incubation experiment provided the opportunity to examine the types of particles selected by a common deep-sea holothurian and the sediment sizes preferred, in an environment as close to their natural habitat as possible. The results suggested that finer glass beads (#1, 150–200 μm) were ingested in preference to larger glass beads (#2, 600 μm), while luminophore tracers (200–300 μm) were also ingested in relatively high numbers. This result may not imply a direct size selection by this species but may highlight how a specific feeding behaviour and tentacle pattern may influence the types of particles picked up at the initial stage of tentacle contact with the seabed. Other deep-sea species investigated are shown to select for particles around 50–60 μm (Khripounoff and Sibuet, 1980), consistent with the observations in this study. The dominant mean particle size found in the gut was $> 50 \mu\text{m}$. It has been suggested that holothurians may feed upon the dominant particle sizes available at the surficial sediment layer. Many of the abyssal species sampled have been collected from fine sediments where the mean particle size is approximately 50 μm (Khripounoff and Sibuet, 1980). Although Hauksson (1979) found a larger size range in his samples, larger particles remained rare in this study.

4.2. Feeding observations

Most knowledge of feeding strategy is based upon the study of animals collected by traditional methods (trawling/dredging) where inferences are made from the gut contents, tentacle morphology, gut morphology and suspected locomotion of the species collected (Roberts and Bryce, 1982; Billett, 1991; Roberts and Moore, 1997; Roberts et al., 2000). The lack of results at the tentacle level has meant that much of what is known about feeding behaviour in the deep-sea is directly inferred from shallow-water observations. Shallow-water holothurian research suggests that they pick up particles from the seabed using the adhesive force of a tentacle or through entrapment within the tentacular nodules (Massin et al., 1978; Cameron and Fankboner, 1984; Billett, 1991). Deep-sea species are believed to select for smaller particle fractions (Khripounoff and Sibuet, 1980) in a similar way and that size selection may be linked to the level and strength of the protein-based mucus secreted from specific cells within the tentacle (Khripounoff and Sibuet, 1980).

Although this study primarily examined animals collected from 350 to 500 m depth, the same holothurian species has been observed to occur at depths exceeding 1000 m in the Porcupine Seabight, NE Atlantic (Billett, 1991), and represents a suitable deep-sea analogue species. *S. tremulus* has been studied feeding in aquaria and in shallow-water cages (9 m), and was described to have peltate tentacles (Hauksson, 1979). The present study using in situ video observations of the tentacle structure of *S. tremulus* found that the tentacles spread out in a tree-like network or with fine branches, suggesting it may conform to a more classic digitate type as described by Roberts et al. (2000).

Sweeping and raking are the two main modes of tentacle action in holothurians (Billett, 1991; Roberts et al., 2000). These modes are found across a range of elasipodid and aspidochirotid species in the deep-sea. The present study has shown that the aspidochirotid holothurian *S. tremulus* does not use the known feeding strategies for deep-sea holothurians. The in situ video footage clearly demonstrates that tentacles are placed onto the seabed, spread out like 'fingers' and then used to grasp sediment and pass it to the mouth. This behaviour was not observed by Hauksson (1979), as he describes the animal as feeding directly, with its mouth ventrally, as it 'glides' over the sediment surface. The fact that this species uses fine oral papillae to 'sense' the seafloor has also not previously been reported, in addition, Hauksson (1979) also reported that the 20 tentacles gathered food, whereas this study proposes that only 10 of these are used at any one time.

The rate of locomotion, and rate of tentacle insertion has yet to be investigated in the deep-sea and studies are few in shallow-water. The rate at which *S. tremulus* feeds is slow; 54 s for a full tentacle cycle (i.e. tentacles being placed on the seabed, ingestion, then back onto the seabed again; Table 3). When multiplied by the number of finely branched tentacles, although slow, this feeding strategy provides a large effective particle capture area, which suggests the impact of this species on the benthos, is likely to be significant. Work in the deep sea (Bett et al., 2001) using time-lapse photography showed that the locomotion rate of a common abyssal species *Oneirophanta mutabilis* allows this species to cover large areas of the seafloor over the course of a year owing to its high abundance and locomotion rate. Any estimates of area covered by such species is, however, likely to underestimate the full extent of the sediment processed by these organisms because of the movement and search patterns employed. Observations on *S. tremulus* showed that it

moves its head from side to side while feeding, before moving forward. Such observations give a truer reflection of the actual locomotion patterns of a holothurian. The use of an ROV allows not only the rates of movement of the whole animal to be recorded but to the level of each tentacle. Collection of the observed specimens allows each tentacle to be measured, allowing a more detailed and accurate account of feeding rate and potential impact on the seabed.

4.3. Gut throughput times

Much of the information available in the literature for gut residence time and throughput rate in holothurians in both shallow-water and in the deep sea is variable, with estimations ranging from 4 to 24+ h. The range of experimental approaches to this topic still remains a problem, with many different methods used and no real definitive answers as to which method is the best. Methods used include observations of faecal string production (Heezen and Hollister, 1971; Hauksson, 1979; Sibuet et al., 1984), bacterial counts (Deming and Colwell, 1982), while attempts were made by Sibuet (1987) to use carbon-labelled tracers. The results remained inconclusive, but progress was made in the use of tracer techniques in the deep-sea. A common estimated figure is between 16 and 24 h, but with large overlaps above and below has been suggested from the majority of in situ based research, although many values remain as estimates. This study uses a relatively simple incubation trap principle (Fig. 2), involving tracing a range of particles through the gut tract, with observations to support when the animal starts to feed on the provided food source, a crucial link that has been missing from other in situ experiments. The ROV approach allows these observations to take place and the accuracy obtained (23.73 h S.D. \pm 2.3) from the tracer experiment suggests that this technique is both easy and allows much greater precision. Examining animals from different depths and with different gut tract lengths still showed that the average throughout time was \sim 24 h (Table 4). Particles were shown to move through the gut at a constant rate regardless of length (Fig. 5). This was an element that feeding studies only previously assumed, and was indeed an area of possible error (see Billett, 1991). These findings suggest that the digestion time to extract a useable amount of organic material from the sediment is about 24 h. This result is slightly slower than suggested by Hauksson (1979) in aquarium studies on the same species.

Nevertheless, one fact that seems to remain constant is that each species and each habitat is likely to disproportionately influence a particular throughput time. The findings of Sibuet et al. (1984) showed that abyssal species feeding and living on fine mud-like substrates had increasing levels of finer material in their guts, and factors like this may influence the gut throughput times estimated.

5. Conclusions

This study proposes a unique way to examine gut throughput rates in any type of deposit-feeding megafauna, to a high level of accuracy and 'opens the door' to a new approach to deposit-feeding ecology. The use of ROVs for observations and experiments

is clearly important as it creates a new perspective on feeding ecology in a natural environment. This provides a previously undescribed and unique resolution on basic life-processes at the deep-sea floor (Fig. 5). This technique will provide a greater insight into 'well-understood' species and help to answer questions of how other deep-sea invertebrates, including holothurians, feed. Using a novel in situ experiment and ROV observations it has been possible to understand the feeding biology of a common and important holothurian species to a new degree. An observation of feeding ecology at the tentacle level allows a greater understanding of how an animal feeds, and what the important components of a specific feeding behaviour are. ROV-related experiments are beginning to provide a new resolution to deep-sea ecology, and the fact that an industrial work-class ROV was used to carry out a large proportion of this study outlines the fact the number of dedicated science ROVs is not sufficient to provide the levels of access required to gain basic ecological data. The present collaboration scheme between science and industry, although still in its infancy, has the potential to provide access to ROV technology across the globe to carry out simple yet important background studies on animals once thought to be well understood. The potential to place many years of traditional research in a new light is emerging with the use of relatively simple experiments and industrial ROVs.

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