THE BURROWING BEHAVIOUR OF SYMBIOTIC AND ASYMBIOTIC THYASIRID BIVALVES

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Abstract *The bivalve family Thyasiridae includes species living in symbiosis with chemoautotrophic, sulphur-oxidizing bacteria and others that are asymbiotic. Chemosymbiotic thyasirids create extensive ramifying burrows (pedal tracts), presumably to acquire reduced sulphur for their symbionts. Here, we investigate whether asymbiotic thyasirids may also form pedal tracts. We compared the behaviour of asymbiotic (*Parathyasira *sp. and* Thyasira *cf.* gouldi *operational taxonomic unit 3) and symbiotic (*Thyasira *cf.* gouldi *operational taxonomic units 1 and 2) thyasirids from Bonne Bay, Newfoundland Canada, maintained in thin tanks in flow-through aquaria. Photographs and X-radiographs of thin tanks showed that all thyasirids established pedal tracts, with no discernable difference in the depth, total length or number of pedal tracts among taxa. We interpret thyasirid pedal tract formation as an early adaptation for pedal feeding, likely combined with the farming of chemosynthetic bacteria along burrow walls. Pedal tract formation could also be a precursor to chemosymbiosis establishment in the Thyasiridae.*

Key words *Thyasiridae, symbiosis, burrows, Thyasira, Parathyasira*

INTRODUCTION

Bivalves of the superfamily Thyasiroidea are predominantly of small size (< 10mm), found worldwide in various cold-water habitats including organically enriched sediments in fjords and oil fields (Oliver & Killeen, 2002). Some thyasirid species establish nutritional symbioses with sulphur-oxidizing, chemoautotrophic gammaproteobacteria maintained at the surface of gill epithelial cells, while others are asymbiotic (Dando & Southward, 1986; Southward, 1986; Dufour, 2005). Chemosymbiosis has appeared multiple times in the evolutionary history of the Thyasiridae (Taylor *et al.*, 2007; Duperron *et al.*, 2013), and the factors that have allowed such associations to develop and persist are unknown. Investigating and comparing morphological, physiological or behavioural traits of symbiotic and asymbiotic thyasirid species could inform us on how bivalve chemosymbioses originated and evolved.

In order for chemosymbioses to function, the host must provide its bacterial symbionts with the compounds they require for their metabolism. In marine sediments, reduced sulphur may be patchy and occur in sub-mm scale microniches (Stockdale *et al.*, 2009), and symbiotic thyasirids can be abundant in sediments with relatively low concentrations of measurable free sulphide (Dando & Southward, 1986). Symbiotic thyasirids are thought to acquire reduced sulphur compounds by establishing long, ramifying burrows in surrounding sediments using their superextensile foot (Dando & Southward, 1986; Dufour & Felbeck, 2003). These biogenic structures, herein referred to as pedal tracts, are distinct from the mucus-lined inhalant tube that extends from the anterior end of the shell to the sediment-water interface and serves as a conduit for bioirrigation in these asiphonate bivalves (Allen, 1953, 1958; Fig. 1a). The inhalant tube of thyasirids is established through the probing activity of their vermiform foot (Allen, 1953), and root-like pedal tracts are likely also formed by sequential extension and withdrawal of the bivalve's foot in sediments. In experiments, the total length and number of pedal tracts were inversely related to the concentration of porewater sulphide (Dufour & Felbeck, 2003). Similar sedimentary structures have been observed beneath lucinid bivalves (Stanley, 1970); as lucinids and thyasirids are not closely related (Taylor *et al.*, 2007), pedal tract formation likely evolved independently in those two families.

The evolution of pedal tract formation in chemosymbiotic bivalves is enigmatic. A previous study reported that only symbiotic thyasirids formed pedal tracts (Dufour & Felbeck, 2003); however, both asymbiotic species examined in that study, *Thyasira obsoleta* and *T. ferruginea* (syn. *Mendicula ferruginosa*), possess a relatively shorter foot (especially *T. ferruginea*) compared to that of the symbiotic species examined (Payne & Allen, 1991; Oliver & Killeen, 2002; Fig. 1b). Other

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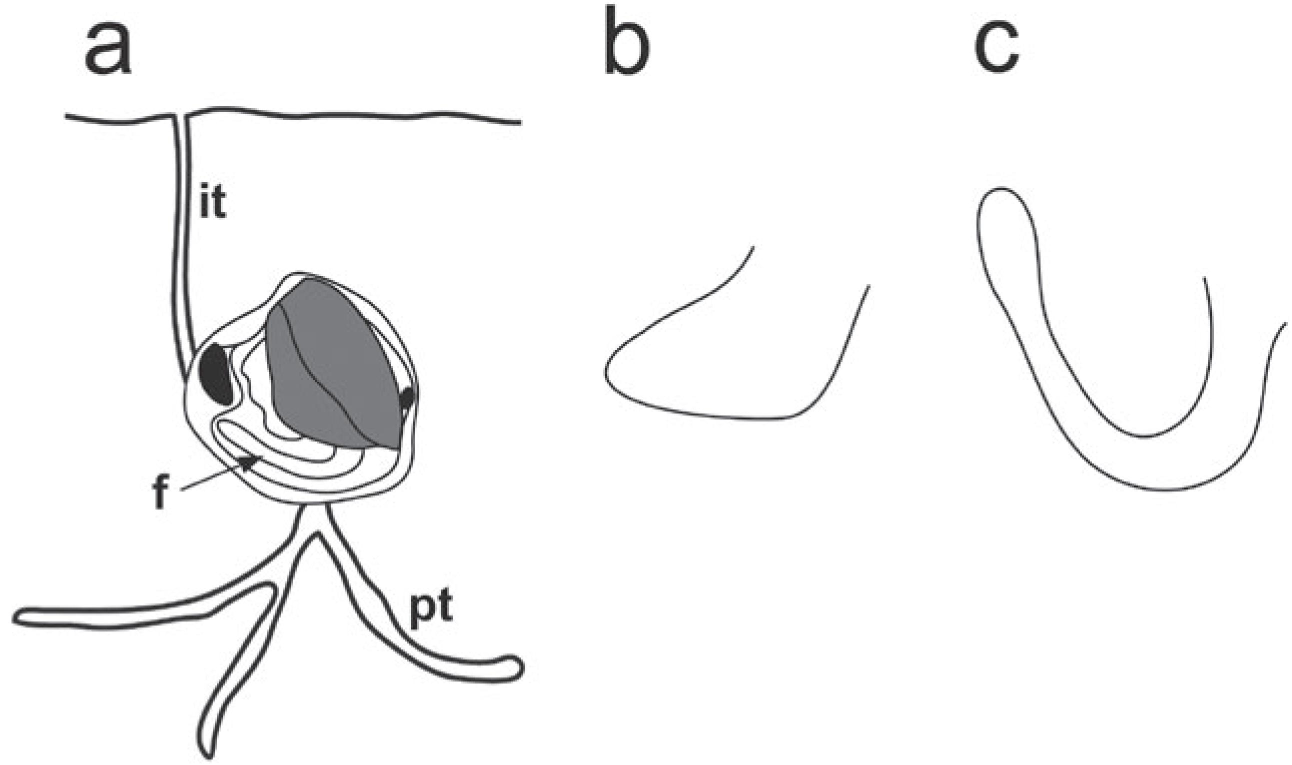

Figure 1 Thyasirid foot morphology and burrow types. a) Depiction of a thyasirid in life position, showing the location of the inhalant tube (it) and pedal tracts (pt), both constructed by the foot (f). b) A spade-shaped foot, with a heel (e.g. in *Mendicula ferruginosa*). b) A vermiform foot (e.g. in *Thyasira flexuosa*). Modified from Payne & Allen (1991).

asymbiotic thyasirids have a more elongate, vermiform foot, similar to that of symbiotic species [e.g. *Axinulus eumyaria*=*Thyasira (Axinulus) eumyaria*, see Payne & Allen, 1991 and Dufour, 2005; Fig. 1c]. All thyasirids are considered to use their foot to form their inhalant tube (Allen, 1958; Payne & Allen, 1991) and asymbiotic species with more extensible feet should, in theory, also be capable of forming pedal tracts.

In Bonne Bay, Newfoundland (Canada), the presence of symbiotic and asymbiotic thyasirids with long, vermiform feet provides an opportunity to investigate the effect of symbiont presence/absence on burrowing behaviour. Within this fjord, a complex of putative species resembling *Thyasira gouldi* was described, comprised of two symbiotic operational taxonomic units (OTUs) and one asymbiotic OTU (Batstone *et al.*, 2014). These three *T.* cf. *gouldi* OTUs are closely related but genetically distinct, and co-occur at two out of three sampling sites within Bonne Bay. Another undescribed species of *Parathyasira*, lacking symbionts (Batstone, 2012), is also found at two sampling sites [*Parathyasira* is used both as a genus and a subgenus of *Thyasira*; we use the term as a genus herein, as in Taylor *et al*. (2007), but recognize that this nomenclature is uncertain]. In this study, we introduced symbiotic and asymbiotic thyasirids into separate, sediment-filled thin aquaria and examined their burrowing behaviours using both direct observation on the wall of the aquaria and X-radiography (cf. Stanley, 1970; Dufour & Felbeck, 2003). Experiments were repeated at different dates to increase sample size, and sedimentary conditions varied slightly among those experimental periods. Given that the thyasirid taxa from Bonne Bay have similar shell sizes and foot morphologies (i.e., a vermiform foot, as in Fig. 1c), we expected that the depths of burial of symbiotic and asymbiotic specimens would be approximately equal. However, we predicted that only symbiotic thyasirids would form pedal tracts, as asymbiotic taxa should have no requirement for reduced sulphur.

MATERIALS AND METHODS

Sampling of Thyasirids and Sediments

Sediments and thyasirids were sampled from the fjord of Bonne Bay, on the west coast of

Newfoundland, in April, August and November 2013. Sediments were collected using a Peterson grab (12cm diameter/sampling depth) from three locations: South East Arm (49°27'719 N, 57°42'909 W; 30–33m depth), Deer Arm (49°33'225 N, 57°50'395 W; 29–32m depth), and Neddy's Harbour (49°31'66 N, 57°52'236; 14.5–17m depth). In April and August, sediment from South East Arm was dry sieved (1mm mesh), homogenized, and kept for use in the thin aquaria. At each date, thyasirids from the three locations were collected on a 1mm mesh sieve, pooled and sorted into three groups based on external shell morphology (cf. Oliver & Killeen, 2002; Batstone *et al.*, 2014): 1) *Thyasira* cf. *gouldi* OTU 1 or 2 (grouped in this study, and hereafter referred to as "symbiotic *T.* cf. *gouldi*"); 2) *Thyasira* cf. *gouldi* OTU 3 (hereafter referred to as "asymbiotic *T.* cf. *gouldi*"); or 3) *Parathyasira* sp. Confirmation of symbiont presence in *T.* cf. *gouldi* was performed after experiments were completed, as described below. All specimens were measured to the nearest mm and retained in filtered seawater at 4°C until experimentation began (a maximum of 3 days after collection).

Thin Aquaria

Thin aquaria were assembled by placing ¼ inch tubing in a U shape between two plates of glass, clamping the plates together, and filling the inner space with sediment from South East Arm (Fig. 2). All thin aquaria were maintained in larger, flow-through tanks containing seawater continuously pumped in from Bonne Bay. Temperatures in tanks approximated those naturally experienced by thyasirids at this site, and ranged from 4 to 7°C over the period of study.

In April, sediments were deposited as layers, to mimic natural sedimentation processes, and prepared aquaria were used immediately. The aquaria used in August had been set up in May with sediments collected in April, and stored in flow-through tanks prior to experimentation. Over this prolonged resting period, a greater amount of sulphate reduction likely occurred, resulting in overall higher levels of porewater reduced sulphur in the aquaria used in August compared to those used in April. Given the expected spatial patchiness (Stockdale *et al.*, 2009), we felt that reduced sulphur would be difficult to characterize adequately over the entire sedimentary volume without the use of planar sensors (Zhu & Aller, 2013), which were not available to us, so we did not attempt to measure reduced sulphur. The thin aquaria used for experimentation in November were prepared in September (using sediment collected in August). In the final set of aquaria, sediments were not deposited as layers to improve the visibility of structures on X-radiographs.

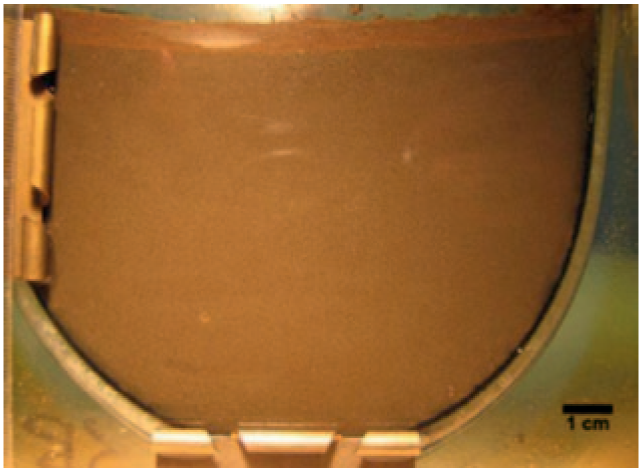

Figure 2 Sediment-filled thin aquarium: two plates of glass separated by silicone tubing, and held together by stainless steel clamps.

A single bivalve was introduced into each thin aquarium; the number of specimens of each taxon and size ranges are listed in Table 1. There was no significant difference in shell size among the three taxa based on a Kruskal-Wallis test (p-value=0.19).

Table 1 Number and size range of specimens introduced into thin tanks at each experimentation period. Values represent only the specimens whose identity was confirmed at the end of the experiment.

	April		August		November	
	Number of specimens	Size range (mm)	Number of specimens	Size range (mm)	Number of specimens	Size range (mm)
Symbiotic *Thyasira* cf. *gouldi*	6	3–5	5	3–5	4	3–5
Asymbiotic *Thyasira* cf. *gouldi*	0	–	3	2–3	4	2–3
Parathyasira sp.	6	3–4	6	2–5	6	4–5

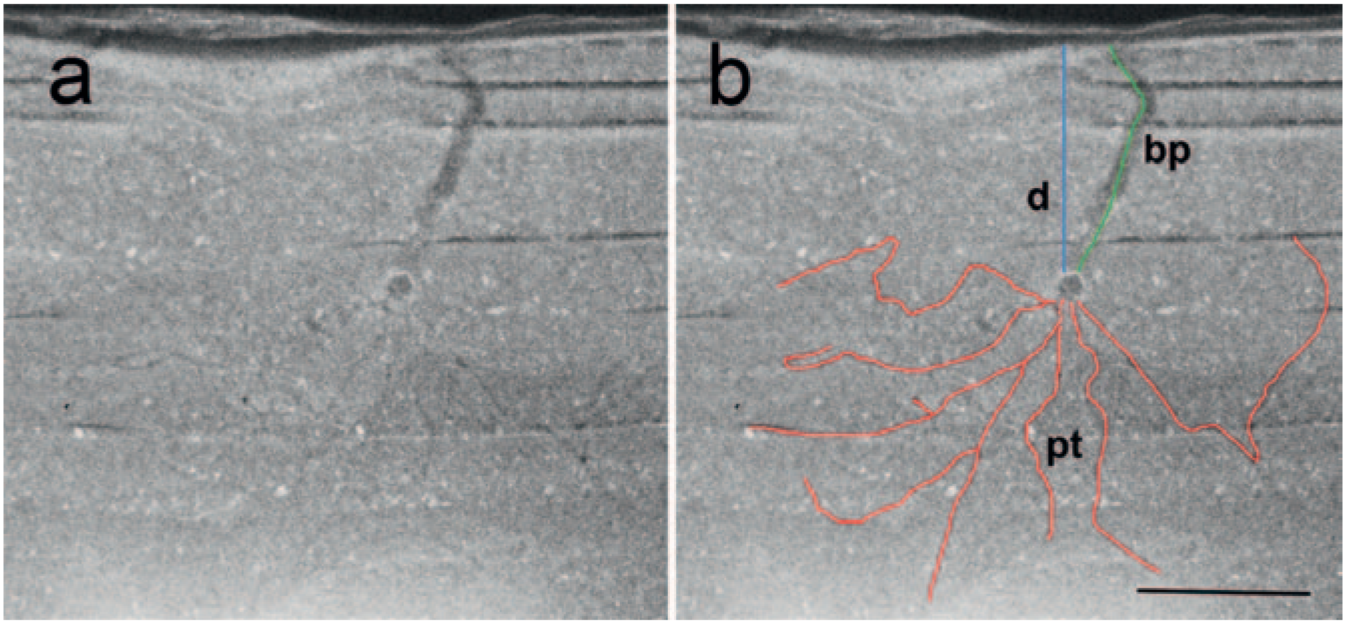

Figure 3 a) X-radiograph of a symbiotic *Thyasira* cf. *gouldi* in a tank with layered sediments. b) The burial depth (d, blue line) and pedal tracts (pt, red lines) were traced on the same X-radiograph for measurement in ImageJ. The green line represents the burial path (bp) taken from the initial placement of the bivalve at the sediment surface to its final position. Scale bar: 5cm.

Digital photographs were taken of the mini aquaria approximately every day over a 2-week period during the months of April, August, and November, to monitor the location and position of visible burrows. After an initial period of burrow establishment (approximately 2 weeks), X-radiographs were taken using an ultra-light MinXray HF 8015+ at 50 kV for 0.24 seconds. A disposable aluminium baking sheet was placed in front of the thin aquaria during X-radiography to help reduce noise and enhance the image (Van Geet *et al*., 2000).

Following X-radiography, the thin aquaria were dismantled and the bivalves retained. Because of external morphological similarities between symbiotic and asymbiotic *Thyasira* cf. *gouldi*, all individuals were further examined to confirm symbiont presence or absence (cf. Batstone *et al*., 2014). Gills were removed and individually fixed in 2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer (approximately iso-osmotic) for 24 hours, and then stored in the same buffer. Post-fixation was conducted by immersing the gills in a 2% osmium tetroxide solution in 0.1 M sodium cacodylate buffer for 15 minutes. Tissues were then dehydrated in an ascending ethanol series and embedded in Epon resin. Thin sections (3 μm) of gills were prepared using an ultramicrotome, placed on glass slides and stained with 1% toluidine blue. At this point, the determination of bacterial symbiont presence/absence was possible (cf. Batstone *et al*., 2014).

Image Analysis

X-radiographs were digitized using a scanner and images enhanced using Photoshop C5. Using ImageJ (Abramoff *et al*., 2004), the burial depth (the linear distance between the bivalve and the sediment surface), total cumulative length and number of pedal tracts (root-like, located deeper in sediments, with ramifications originating from the presumed location of the shell) were measured (Fig. 3). No measurement of the inhalant tube was undertaken as this structure was not visible on most X-radiographs. More evident were burial paths (technical artefacts due to bivalves having to burrow down from the sediment surface where they were initially placed); these paths were refilled with a slightly less dense matrix of sediment compared to surrounding sediments, and are not considered to be of functional importance to the bivalves.

Statistical analyses were performed to test four null hypotheses: 1) depth of burial does not differ significantly among the three groups, or according to experimental period (month); 2) total cumulative pedal tract lengths do not differ among the three groups, or according to experimental period (month); 3) thyasirid size does not significantly affect burial depth; and 4) thyasirid size does not significantly affect pedal tract length. We used Shapiro-Wilks and Breusch-Pagan tests to test parametric assumptions, and applied ln transformations on data that were not normal or had unequal variances (in our case, pedal tract length). Thyasirid size (either 3, 4 or 5mm) was treated as a categorical variable. ANOVA tests (hypotheses 1 and 2) and Kruskal-Wallis tests (hypotheses 3 and 4) were performed in R (R Core Team, 2013).

RESULTS

Most (90%) of the specimens studied burrowed within two days of being placed at the sediment surface. Thereafter, burrows became evident along the walls of most of the thin aquaria (generally 2–5 days later), and many burrows could be identified as either inhalant tubes or pedal tracts. Examples of burrows visible from the tank exterior are shown in Fig. 4 and examples of X-radiographs in Fig. 5. Considering only individuals whose identity as symbiotic or asymbiotic could be confirmed, we observed pedal tracts along the tank wall of 13 symbiotic *Thyasira* cf. *gouldi*, 6 asymbiotic *T.* cf. *gouldi*, and 16 *Parathyasira* sp. X-radiography allowed data collection from 15 symbiotic *T.* cf. *gouldi*, 2 asymbiotic *T.* cf. *gouldi*, and 12 *Parathyasira* sp. Complementary photographic and X-radiographic evidence of burrows were available for some, but not all individuals.

Depth of burrowing and inhalant tube

The three thyasirid taxa burrowed to depths of between 12.2 and 64.2mm (Fig. 6a). Shell size did not influence the depth of burial (p-value=0.46), and the latter did not differ significantly among taxa (p-value=0.24) or months (p-value=0.26). The shallowest burrower was a symbiotic *Thyasira* cf. *gouldi* specimen, while the deepest burrower was a *Parathyasira* sp. Colour changes in the near-burrow sediment, attributed to sediment oxygenation, were observed for 44% of the symbiotic *T.* cf. *gouldi*, 11% of asymbiotic *T.* cf. *gouldi*, and 55% of the *Parathyasira* sp. (Fig. 4). X-radiographs confirm that the zone of inferred oxidation surrounds the inhalant tube rather than the burial path. In April, at least three *Parathyasira* sp. changed their position within the sediment, after having formed pedal tracts (Fig. 7). As X-radiographs were only taken at the end of the experiment, it is not known when these individuals relocated within sediments. In November, one symbiotic *T.* cf. *gouldi* constructed two inhalant tubes, the first being

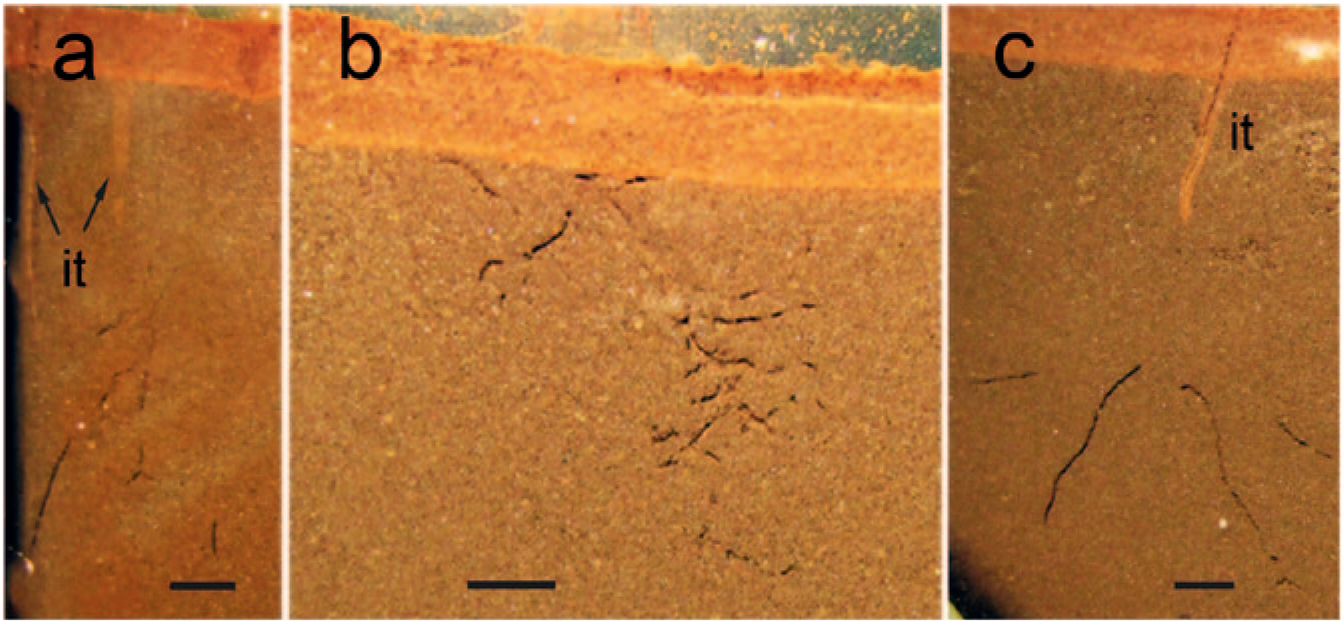

Figure 4 Examples of structures visible on tank walls throughout the experimentation period. Note evidence of sediment oxidation around the inhalant tube (it). a) Symbiotic *Thyasira* cf. *gouldi*. The inhalant tube on the right appeared nine days after the experiment began; the one on the left appeared five days later. b) Asymbiotic *T.* cf. *gouldi*. c) *Parathyasira* sp. Scale bars: 5mm.

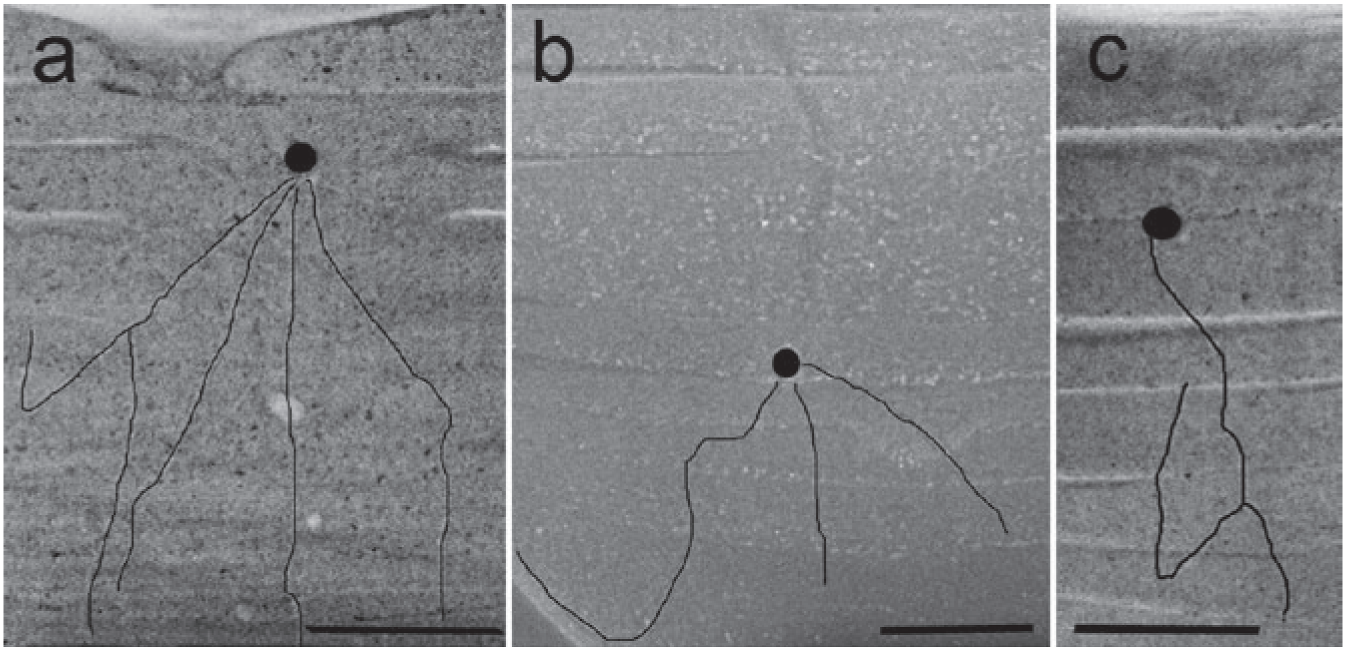

Figure 5 Examples of X-radiographs taken after 2 weeks of experimentation. Black circles show the location of the shell, and black lines trace the pedal tracts. a) Symbiotic *Thyasira* cf. *gouldi*; b) asymbiotic *T.* cf. *gouldi*; c) *Parathyasira* sp. Scale bars: 5cm.

evident on the 9th day, and the second on the 14th day (Fig. 4a); this individual appeared to have also moved to a deeper location over the course of the experiment.

Pedal tracts

Pedal tracts were formed by all three thyasirid taxa, during all experimentation periods (Figs 3–5, 7). A greater number of pedal tracts were observed in the thin aquaria established in April, with some individuals of symbiotic *Thyasira* cf. *gouldi* and *Parathyasira* sp. having more than seven pedal tracts. All thyasirids in experiments run in August and November formed less than five pedal tracts. In August and November, most individual pedal tracts were > 40mm in length, with only a few measuring < 10mm (range: 8.3mm to 61.5mm), whereas many of the pedal tracts in the April experiment measured < 20mm. The longest pedal tract recorded (76.6mm) was formed by a symbiotic *T.* cf. *gouldi*. There were no obvious morphometric differences between the pedal tracts established in layered sediments and in non-layered sediments.

Total (summed) pedal tract lengths ranged between 28.3–258.9mm in symbiotic *Thyasira* cf. *gouldi*, between 54.4–59.9mm in asymbiotic *T.* cf. *gouldi* and between 46.4–157.2mm in *Parathyasira* sp. (Fig. 6b). Total pedal tract length did not differ significantly among the three taxa (p-value=0.12), among sampling dates (p-value=0.46) or according to shell size (p-value=0.44).

DISCUSSION

Stanley (1970) used X-radiography of thin aquaria to investigate burrowing in 63 species of bivalves, including chemosymbiotic species such as *Lucina pensylvanica* and *Solemya* sp. Observations of burrowing behaviour in thyasirids are sparse and focus on symbiotic species (Dando & Southward, 1986; Dufour & Felbeck, 2003, Dando *et al*., 2004); this study provides the first evidence of pedal tract formation in asymbiotic thyasirids.

Depth of Burial

We found no significant difference in depth of burial among the three groups examined (Fig. 6a), possibly because shell sizes (and likely foot lengths) were comparable among the specimens from which data were obtained. It should be noted that symbiotic *Thyasira* cf. *gouldi* can attain larger sizes than asymbiotic *T.* cf. *gouldi*, and that there are some subtle differences in shell shape between these taxa (Batstone *et al*., 2014). Anderson (2014) found that the size and shell shape of lucinid and thyasirid individuals

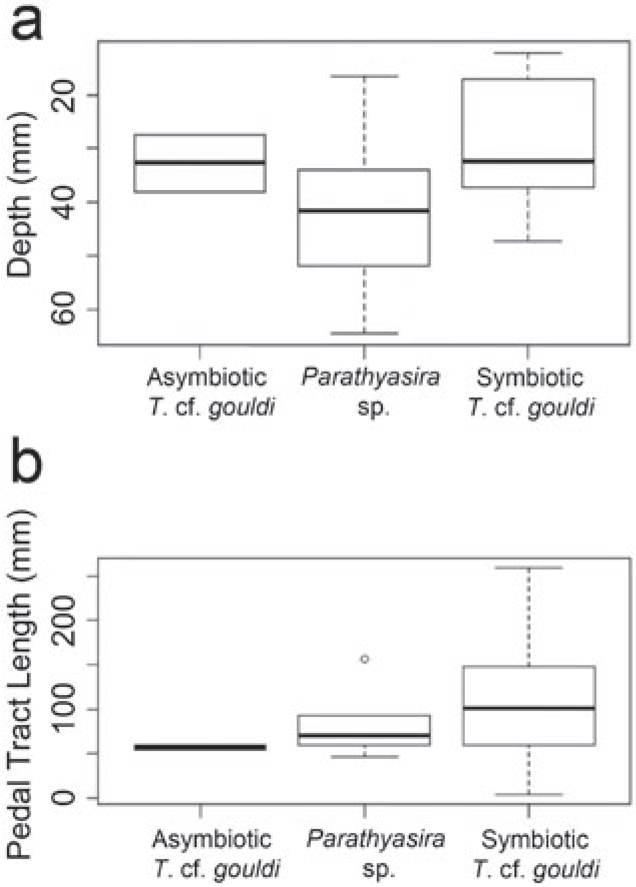

Figure 6 a) Burial depth of thyasirids, all experimental periods combined (symbiotic *Thyasira* cf. *gouldi*, n=15; asymbiotic *T.* cf. *gouldi*, n=2; *Parathyasira* sp, n=12). Values represent the linear distance between the sediment surface and the location of the bivalve at the end of the experiment. b) Total pedal tract length per specimen, all experimental periods combined (symbiotic *T.* cf. *gouldi*, n=11; asymbiotic *T.* cf. *gouldi*, n=2; *Parathyasira* sp., n=6). Boxes show average values along with 25th to 75th percentiles, whiskers represent the maximum and minimum values, and the dot is an outlier.

were not related to the depth of burial, with smaller species such as *T. flexuosa* often burrowing to similar depths as larger species (e.g. *Lucina pensylvanica* and *Phacoides pectinatus*); however, her work concerned only symbiotic species. We observed that asymbiotic thyasirids can burrow to at least 6cm depth, while a maximum depth of 1.5cm was reported previously for asymbiotic thyasirids of smaller size (Dufour & Felbeck, 2003). Burrowing depth is likely related to the morphology of the foot: Payne & Allen (1991) thought that thyasirids with elongate, vermiform feet are likely deeply buried, while those with a shorter foot and well-differentiated heel most likely remain close to the sediment surface.

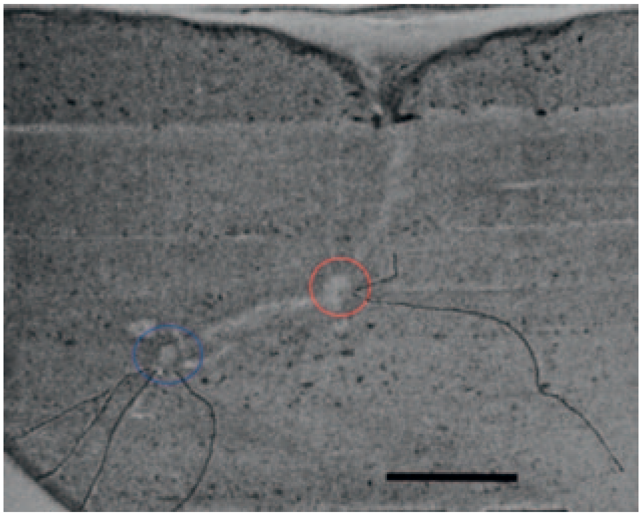

Figure 7 X-radiograph of *Parathyasira* sp., showing relocation after an initial phase of pedal tract generation. The red circle shows the bivalve's previous location within the sediment, and the blue circle shows the final position at the end of the 2-week period. Scale bar: 5cm.

Inhalant tube

Formation of the semi-permanent inhalant tube is characteristic of thyasirids, which lack an inhalant siphon and continually probe the tube with their foot to keep it open and intact (Allen, 1958). In our tanks, inhalant tubes were often readily apparent due to a surrounding layer of paler, presumably oxidized sediment (Fig. 4a,c), as described previously in microcosms containing *Thyasira sarsi* (Dando *et al*., 2004). Comparisons of X-radiographs and tank photographs confirmed that the oxidized structures did not correspond to the burial paths, the latter being particularly evident on X-radiographs due to slight differences in sediment density. Sediment oxidation was more commonly observed around the inhalant tubes of *Parathyasira* sp. and symbiotic *Thyasira* cf. *gouldi* than around those of asymbiotic *T.* cf. *gouldi*, possibly due to differences in ventilation behaviour (periodicity, duration of irrigation events) among taxa. A longer period of experimentation might have led to a greater proportion of oxidized inhalant tubes, as well as visible oxidation along the pedal tracts. No chimney or curvature of the inhalant tube at the sediment surface (as in Allen, 1958; Oliver & Killeen, 2002) was observed.

Pedal tracts

Symbiotic and asymbiotic thyasirids examined created long, branching pedal tracts in both layered and non-layered sediments, at all experimental periods. In contrast, asymbiotic species examined previously (*Thyasira obsoleta* and *T. ferruginea*; Dufour & Felbeck, 2003) formed no evident pedal tracts. *Thyasira obsoleta* and *T. ferruginea* possess a shorter, thicker foot (Oliver & Killeen, 2002) than asymbiotic thyasirids from Bonne Bay; therefore, the form of the foot may be a good predictor of the likelihood of pedal tract formation in thyasirids. We did not expect asymbiotic thyasirids to form pedal tracts, and propose two alternative hypotheses to explain this behaviour: 1) these structures serve no role and their formation is a vestige of a previously symbiotic state; and 2) these structures are used for pedal feeding.

Symbiosis appears to have evolved multiple times in different thyasirid lineages (Taylor *et al.*, 2007; Duperron *et al.*, 2013). Chemosymbiotic relationships may have broken down in some species, possibly as a secondary loss due to habitat expansion into areas with fewer free-living bacterial symbionts (there is evidence that thyasirids acquire their symbionts from surrounding sediments; Dufour *et al.*, 2014). Asymbiotic *Thyasira* cf. *gouldi* OTU 3 in Bonne Bay may be more derived than the symbiotic OTUs 1 and 2 (Batstone *et al.*, 2014), and could have retained the pedal tract-forming behaviour despite the loss of symbionts. Similarly, *Parathyasira* sp. may be derived from symbiotic ancestors that formed pedal tracts as a sulphur mining mechanism. However, as phylogenetic relationships among thyasirids remain unresolved (Taylor *et al.*, 2007), it is difficult to further evaluate whether pedal tract formation is a vestigial behaviour in the asymbiotic species studied here.

We suggest herein that some asymbiotic thyasirids form pedal tracts as a feeding adaptation. Pedal feeding is a primitive mode of deposit feeding that is common in juvenile bivalves and retained into maturity in some small bivalve species (Reid *et al.*, 1992; Morton, 1996). Pedal feeders collect organic material on the mucociliary surface of the foot, which then transports particles into the mantle cavity (Reid *et al.*, 1992). Thyasirids from Bonne Bay may use this approach to acquire organic matter and sediment-dwelling bacteria, and pedal tracts could be evidence of this mode of feeding. The chemical conditions along the lining of pedal tracts are likely to be particularly suitable for microbial growth: the burrow linings of several infaunal organisms are hotspots of bacterial activity, particularly when the burrows are irrigated (Papaspyrou *et al.*, 2006). Large pH gradients have been reported around both the inhalant tube and the pedal tracts of *Thyasira sarsi*, suggesting active irrigation throughout all burrow structures and the establishment of redox boundaries in the near-burrow environment (Hakonen *et al.*, 2010). The irrigation of asymbiotic *T.* cf. *gouldi* or *Parathyasira* sp. burrows could enhance the growth of bacteria, as observed in other microbial gardening infauna (Lopez & Levinton, 1987; Vasquez-Cardenas *et al.*, 2016). Investigations of the diet of asymbiotic thyasirids are needed to test the likelihood and relative importance of pedal feeding in those bivalves.

Although accessing sulphides may be the primary function of pedal tracts in symbiotic thyasirids, these bivalves may also use pedal feeding to gain nutrients. Chemosymbiotic thyasirids are mixotrophs, nutritionally relying on more than just their symbionts, as evidenced by differences in stable isotope composition among conspecifics (Spiro *et al.*, 1986; Dando & Spiro, 1993) and variability in symbiont abundance (Dufour & Felbeck, 2006). In populations of the symbiotic *Thyasira* cf. *gouldi* from Bonne Bay, symbiont abundance fluctuates temporally (Laurich *et al.*, 2015), and pedal feeding could be useful during periods of low symbiont abundance.

The length of individual pedal tracts was highly variable in this study, possibly due to differences in sediment reduced sulphur concentrations within the thin aquaria. Dufour & Felbeck (2003) found that in low porewater sulphide conditions, the length and number of pedal tracts created by symbiotic thyasirids was greater than in high sulphide conditions. In our experiment, although pedal tract length did not vary significantly among sampling periods, we observed a greater number of shorter pedal tracts in the aquaria established in April. Sediments in the first set of tanks had been freshly sieved and had not experienced a prolonged settling time prior to experimentation; therefore, sulphur concentrations may have been relatively low in April tanks, as in the low sulphide treatments of Dufour & Felbeck (2003). Additionally, nutrient depletion may have occurred in the sediments over the winter (Rozan *et al*., 2002), resulting in lower reduced sulphur concentrations in the spring. Concentrations of porewater dissolved sulphide in sediments from our Bonne Bay sampling sites were significantly lower in April 2011 than in August 2011 (Laurich *et al*., 2015). A lower sulphide content in April would explain the greater number of pedal tracts observed at that experimental period, but the relatively shorter length of pedal tracts in April remains enigmatic. The change in location of some of the *Parathyasira* sp. (Fig. 7) in April may be related to a greater search effort for nutrients; relationships between sediment chemistry, the number and length of pedal tracts, and the efficacy of microbial farming should be explored.

Maintenance of pedal tracts

In most cases, pedal tracts were visible on the exterior of the mini aquaria within a few days after bivalves were introduced. Over the course of experimental periods, some of the pedal tracts appeared to collapse; similarly, Dufour & Felbeck (2003) reported the disappearance of some pedal tracts from one week to the next, likely from disuse. It remains unclear whether thyasirids maintain their pedal tracts, or consistently form new ones, particularly in natural environments where burrowing organisms may disrupt such structures. Further, pedal tracts did not show any discernible spatial pattern within the sediment and did not appear to follow the redox layers that were visible within the mini aquaria, or the boundaries of sediment layers. It is not known if the foot is able to sense sulphides, or how the directionality of thyasirid pedal tracts is determined. Additional work on burrow formation, maintenance, and irrigation, coupled with fine-scale microbial distribution and sedimentary redox oscillation patterns are needed to better understand the controls on pedal tract formation and their interactions with surrounding sediments.

Pedal tracts and the Evolution of Chemosymbiosis in Thyasirids

The thyasirid burrow irrigation/putative microbial gardening behaviour might be a precursor to the establishment of chemosymbiosis in this group. During the transfer of food particles from the foot to the pallial organs, sediment-dwelling bacteria could become trapped and retained at the surface of the gill epithelium, initiating the extracellular symbiotic relationship observed in many modern thyasirids. Intriguingly, the symbionts of *Thyasira* cf. *gouldi* in Bonne Bay contain magnetosomes, supporting the hypothesis that free-living, magnetotactic, symbiosis-competent bacteria become attracted to pedal tracts or inhalant tubes, and are eventually acquired by hosts (Dufour *et al*., 2014). Indeed, many magnetotactic bacteria are sulphur-oxidizers that are attracted to oxic/anoxic interfaces (Lefèvre & Bazylinski, 2013).

This study demonstrates that at least some asymbiotic thyasirids burrow and create pedal tracts in a similar manner to symbiotic thyasirids. Other asymbiotic thyasirids may show similar burrowing behaviours; the form of the foot may be a good predictor of the likelihood of pedal tract formation. Additional research on feeding mechanisms and ventilation patterns in various thyasirid species would help clarify the role of pedal tracts in both asymbiotic and symbiotic thyasirids, and the evolution of these behaviours in relation to chemosymbiosis. In particular, the possibility that pedal tracts might be associated with microbial gardening/pedal feeding and symbiosis acquisition should be investigated.

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