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Role of mucus trails and trail-following in the behaviour and nutrition of the periwinkle *Littorina littorea*

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ABSTRACT: Gastropod locomotion typically involves the deposition of a mucus trail. This trail can be energetically costly, and its producer could defray this cost if the trail were to perform some post-deposition function. Here we report laboratory experiments aimed at assessing the potential role in nutrition of the mucus trail of the common intertidal periwinkle *Littorina littorea* (L.). Mucus trails bound more microalgal cells from suspension than did a glass substratum, microalgal densities increasing with period of exposure to the suspension up to ~5–8 h. After this time adhesion was less (but still greater than to glass). *Amphora coffeaeformis* (pennate diatom) adhered in greater densities than did *Tetraselmis suecica* (flagellate prasinophyte). Mucus trails containing microalgae of both species (~ 50 to 100 cells mm^{-2}; approximatly the conditions on-shore) induced a greater degree of trail-following than did bare trails. Individuals followed conspecific trails longer than they did their own trails. Winkles moved significantly slower on bare mucus trails (mean = 0.35 mm s^{-1}) than on glass (0.68 mm s^{-1}), though the addition of microalgae to mucus increased the speed of the winkles, *A. coffeaeformis* significantly so. Feeding rate (rate of radular rasping) was also significantly increased on trails containing *A. coffeaeformis* (mean = 17.8 bites min^{-1}) and *T. suecica* (12.9 bites min^{-1}) in comparison to control trails (4.3 bites min^{-1}), where a form of searching behaviour occurred. Microalgae embedded in mucus were seen to enter the mouth. Only 3 out of 40 periwinkles showed any radular activity on glass. Following the passage of a winkle, the density of *A. coffeaeformis* was reduced by ~38% and that of *T. suecica* by 43%. Winkles can clearly exploit food (microalgae) in conspecific mucus trails and in doing so modify their trail-following and feeding behaviour. Thus trail-following seems inextricably linked to nutrition. Since much of the intertidal is likely to be covered with a layer of mucus—or its degradation products—those experiments on trail-following that used 'clean' substrata were not representative of the field. Distribution patterns of both *L. littorea* and benthic microalgae might be shaped by the ability of mucus trails to bind microalgae and by their subsequent exploitation by the grazer.

KEY WORDS: Trail-following · Nutrition · Mucus · *Littorina littorea* · Microalgae · *Amphora coffeaeformis* · *Tetraselmis suecica*

INTRODUCTION

Locomotion by gastropod molluscs involves a secretion of mucus which is deposited on the substratum as a mucus or slime trail. The production of this mucus is energetically costly (e.g. Denny 1980, Davies et al. 1990a), to the point where it might place constraints on foraging (Davies & Hawkins 1998). For example, in the intertidal prosobranch periwinkle *Littorina littorea* (L.), common on many shores of the north-east and north-west Atlantic, mucus production during locomotion is ~35 times more expensive in energy terms than the metabolic cost (Davies et al. 1992b). However, the mucus trail might continue to be of benefit to its producer post-deposition, thus helping to defray its cost of production. Conspecific mucus trails may aid navigation (Denny 1989), homing (e.g. Chelazzi 1990), aggregation behaviour (e.g. Branch & Barkai 1987), mating (e.g. Erlandsson & Kostylev 1995) and accelerate the utilisation of patchy food resources (Hawkins & Hartnoll 1983). Calow (1979) proposed that mucus might form a site of organic enrichment (by microbes) and hence be a potential food source. Herndl & Peduzzi (1989) and Peduzzi & Herndl (1991) demonstrated

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colonisation by bacteria and protoctistans of the mucus of herbivorous intertidal gastropods. For littorinids, Davies et al. (1992b) and Imrie (1992) first suggested that mucus trails might constitute a source of nutrition, following organic enrichment. These authors were referring to the colonisation of the mucus by microalgae and macroalgal sporelings, rather than its degradation by microbial attack. Their work stemmed from that of Connor (Connor & Quinn 1984, Connor 1986), who demonstrated that the mucus of 3 species of Californian limpet could adhesively trap their primary food resource: microalgae. Further, the mucus of 2 territorial homing species (*Lottia gigantea* and *Collisella [Macclintockia] scabra*) stimulated the growth of microalgal populations. Clearly, then, if mucus trails are universally sticky and can concentrate the organic particles that marine gastropod grazers eat, enriched mucus trails could be a very important component of nutrition for benthic grazers, and it might be expected that grazers show variations in behaviour on encountering such trails.

We hypothesised that the mucus trails of *Littorina littorea* could trap food particles and that the foraging and feeding behaviour of these snails would be modified on encountering a trail that contained food, such that individuals would maximise their energy input (through feeding) and thus be able to offset the high cost of producing locomotory mucus. *L. littorea* was chosen as the test species because of its widespread distribution, its ease of handling in the laboratory, the wealth of knowledge already accrued about its biology and ecology, and because of its perceived importance as a structuring agent on many rocky shores. *L. littorea* has been shown to follow mucus trails both in the laboratory (Dinter & Manos 1972, Gilly & Swenson 1978, Erlandsson & Kostylev 1995) and in the field (Davies pers. obs.), although the purpose is still unclear. Mating is one possibility, because sexes are separate and fertilisation internal; Erlandsson & Kostylev (1995) showed that trail-following increased in male tracker-female marker combinations during the mating season. Aggregation is another: winkles are often found in dense aggregations in shelter (Newell 1958a), for example, under boulders. This is thought to provide some degree of protection from the action of waves and increases localised humidity (Lalli & Parsons 1993), and might also reduce an individual snail's chances of being preyed upon.

Trail-following as a means of enhancing nutrition in littorinids has not been directly assessed, although Imrie (1992) reported an enhanced feeding response by *Littorina littorea* in treatments containing conspecific mucus trails. He also observed that feeding behaviour rarely occurs in the absence of mucus, although his alternative was a substratum of glass and much of the intertidal benthos will be covered with a matrix of biofilm and mucus (Davies et al. 1992a). Imrie also reported that mucus trails were capable of binding the phytoplanktonic microalga *Tetraselmis suecica*. The pedal mucus of *L. littorea* has a half-life on-shore of ~12 d (Davies et al. 1992a), which certainly seems long enough for it to trap food particles and thus become a nutritive material.

The diet of *Littorina littorea* is mixed. There is considerable evidence that *L. littorea* routinely grazes both brown and green macroalgae, particularly ephemerals and juveniles (e.g. Watson & Norton 1985, Barker & Chapman 1990, Imrie et al. 1990), and *L. littorea* can have a considerable impact on macroalgal populations and hence on intertidal community structure and succession (e.g. Lubchenco 1983, Petraitis 1983, Vadas 1992). *L. littorea* also grazes drift algae (Watson & Norton 1985). However, they are known to feed on both epiphytic and epilithic microalgae as well (Hunter & Russell-Hunter 1983, Hawkins et al. 1989, Norton et al. 1990). Diatoms comprised a large proportion of the gut contents of one population of *L. littorea* (Hawkins et al. 1989), although the proportion might be artifactual owing to preparation for scanning electron microscopy.

In the present paper we describe the adhesion of microalgae (*Amphora coffeaeformis* and *Tetraselmis suecica*) from suspension to the mucus trails of *Littorina littorea*; snail behaviour, in terms of degree of trail-following, speed and radular activity, when presented with conspecific mucus trails containing monostands of microalgae; and the effects of the passage of a snail on the density of microalgae within a mucus trail. Our aim is to assess the role of mucus trails and trail-following in the nutrition of *L. littorea*.

MATERIALS AND METHODS

Two microalgal species were separately cultured in the ASP-2 medium of Provasoli et al. (1957) in 25 l culture flasks and diluted to give suspensions of 10^5 cells ml^{-1} for experimentation. The prasinophyte *Tetraselmis suecica* (Kylin) Butcher (10 to 15 µm) is a motile flagellate and is typically planktonic. The raphed pennate diatom *Amphora coffeaeformis* Agardh (10 to 25 µm) is typically benthic and is a common constituent of intertidal and subtidal benthic microflora around the United Kingdom. *T. suecica* was chosen because of its previous use in similar experiments (Imrie 1992) and because this genus has been found in the intertidal benthos (Davies 1991). *A. coffeaeformis* is motile, and its slow motility involves the secretion of mucus through the raphe system (Round et al. 1990). Nicotri (1977) has shown selective ingestion of diatoms by lit-

toral grazers. Experiments were conducted at room temperature (~21°C) throughout, between September and December (outside the breeding season of *L. littorea*).

Adhesion of microalgae to mucus trails. *Littorina littorea* were collected from mid-shore at Whitburn (national grid reference NZ 414 616; 54° 57' N, 1° 21' W) on the north-east coast of England and were used the same day. Six snails were allowed to crawl over individual acid-washed glass plates (70 × 70 mm) in filtered (0.2 µm) sea water. The position of the resultant mucus trail was outlined on the underside of each plate with a waterproof marker pen. The plates were then placed on the bottom of a tank containing a suspension of either *Tetraselmis suecica* or *Amphora coffeaeformis* for a specified period. As a procedural control, sets of 6 plates were similarly placed into a tank containing filtered sea water for each specified period. Tanks were vigorously aerated throughout the procedure to ensure a circulation of water and suspension. The experimental design involved suspensions of both microalgae and a cell-free control containing plates for 30 s, 1, 2, 5, 10, 30 min, 1, 2, 5, 8, 16 and 24 h. The order in which treatments were performed was randomised. Subsequently, plates were rinsed by dipping into a bath of filtered sea water for 30 s, and the number of microalgal cells observed within 3 randomly-selected fields of view (each 0.61 mm^2) under a compound microscope was recorded for each plate (n = 18 per treatment). For those plates which had been in a tank of microalgae, 3 counts were made on the mucus trail and 3 in areas off the mucus trail.

Trail-following experiments. *Littorina littorea* (12 to 16 mm length) were again collected from Whitburn and were starved for 4 d prior to experimentation. No snail was used more than once in any trial. The experimental arena consisted of a glass tank (400 × 400 × 120 mm) supported at its bottom corners only. The outside walls and the upper surface of the tank were covered with black polypropylene to exclude light. A glass plate (200 × 200 mm) was placed in the bottom of the tank and served as the experimental substratum. Filtered sea water was introduced to a depth of 30 mm above the upper surface of the plate. Movements of snails on this plate were observed by a video camera situated beneath the tank, normal to the plane of the glass plate. Illumination (~9.8 µE s^{-1} m^{-2} at plate surface) was provided by a lamp situated adjacent to the camera.

A snail ('marker' snail) was introduced to the centre of the glass plate and allowed to crawl until it reached the edge of the plate. No time limit was imposed. The plate and snail were then removed, and the former placed for 15 min in a vigorously aerated suspension of either *Amphora coffeaeformis*, *Tetraselmis suecica* or filtered sea water only (control). The plate was then rinsed by dipping into a bath of filtered sea water for 30 s. In the algal treatments, a layer of microalgae is likely to have adhered to the plate: ~50 to 100 cells mm^{-2} on the mucus trail and ~5 to 10 cells mm^{-2} on the glass (see 'Results', Fig. 1). Hill & Hawkins (1991) found 0 to ~350 diatoms mm^{-2} on intertidal rock chips in Britain, depending on time of year, and Davies et al. (1992a) found ~10 to 40 diatoms mm^{-2} on control surfaces and ~30 to 100 mm^{-2} on *Patella vulgata* mucus coated surfaces that had been left on a shore in Britain for 1 wk; thus our method approximates the conditions potentially found *in situ*. The plate was returned to the glass tank, and either the original snail or a new (depending on treatment) snail ('tracker' snail) was placed in the centre of the plate, in the starting position of the marker snail, and allowed to crawl until it reached the edge of the plate. Prior to each trial the plate was cleaned with soap and water, rinsed in distilled water and dried. This procedure removed all traces of mucus and microalgae from the plate. The filtered sea water in the tank was also replaced.

An experimental design was employed in a random order using *Amphora coffeaeformis*, *Tetraselmis suecica* or sea water as the seeding suspension and either a single snail as both marker and tracker or different snails as marker and tracker. For *A. coffeaeformis* and *T. suecica* seeding, n = 10 per treatment. For sea water (control) seeding, n = 20 per treatment.

We recorded an index of trail-following, the coincidence index (*CI*), as:

$$CI = \frac{O_l}{M_l}$$

where M_l = length of the marker snail trail, and O_l = length of overlap or convergence of trails between marker and tracker. The *CI* value is 1 for a tracker that wholly follows a marker and 0 for a tracker whose trail does not coincide with that of the marker at all. We recorded the speed of movement by tracker snails both on and off the marker mucus trail using the summed distances moved during the summed periods spent on and off the trail for each individual. We recorded the feeding rate of tracker snails in terms of the number of times per minute, calculated over the entire period spent on or off the marker trail, the radula appeared from the buccal sac and browsed the substratum. For a description of the feeding process see Hawkins *et al.* (1989). We also recorded a tortuosity index (*TI*) of each marker trail as:

$$TI = \frac{D_l}{M_l}$$

where D_l = the direct (straight line) length between start and finish points of the trail. The *TI* value is 1 for a snail path described by a straight line. Lower values of *TI* indicate increasingly tortuous paths.

Effects of grazer passage on benthic microalgal density. Snails (12 to 16 mm length) were again collected from Whitburn and were starved for 4 d. An immersed (in filtered sea water) snail was induced to lay a straight mucus trail along a new glass microscope slide (76 × 26 mm) by placing the slide between 2 glass plates, along the edges of which Tree Tanglefoot Pest Barrier (The Tanglefoot Company, Grand Rapids, MI, USA) had been smeared. This is a non-toxic resinous material which littorinids do not cross (Davies et al. 1997). The slide was then placed for 15 min in a vigorously aerated suspension of either *Amphora coffeaeformis* or *Tetraselmis suecica* and rinsed by dipping into a bath of filtered sea water for 30 s. The slide was returned to its position between the glass plates and either a second snail introduced to the slide and allowed to follow the marker's trail (time taken ~5 min), or the slide was left untouched for 5 min as a procedural control. The slide was then fixed in formaldehyde for 5 min, dehydrated through a series of alcohols and air-dried. The number of microalgal cells observed on the mucus trail within 10 randomly selected fields of view (each 0.61 mm^2) under a compound microscope was recorded for each slide. A balanced design (n = 10 slides per treatment) was employed. The filtered sea water in which the procedure took place was changed between trials.

RESULTS

Adhesion of microalgae to mucus trails

On the procedural control plates no cells were observed. The pedal mucus of *Littorina littorea* bound more microalgae of both species than did bare glass (Fig. 1). For both species the numbers of cells adhering to glass increased with increasing periods of immersion. For mucus the number of adhering cells of both species increased with increasing periods of immersion for the first ~5 to 8 h (~150 to 200 cells mm^{-2}); longer periods were not as effective at promoting adherence of microalgae to mucus, but levels were still considerably greater than the bare glass treatments. More *Amphora coffeaeformis* cells adhered to the treatment surfaces, either glass or mucus, than did cells of *Tetraselmis suecica*.

Trail-following experiments

All tracker snails followed the marker's mucus trail 'with polarity' (away from the origin of the trail), even if only for a few millimetres. Some followed the trail in its entirety, some followed from the origin of the trail and later left the trail, and some followed the trail after

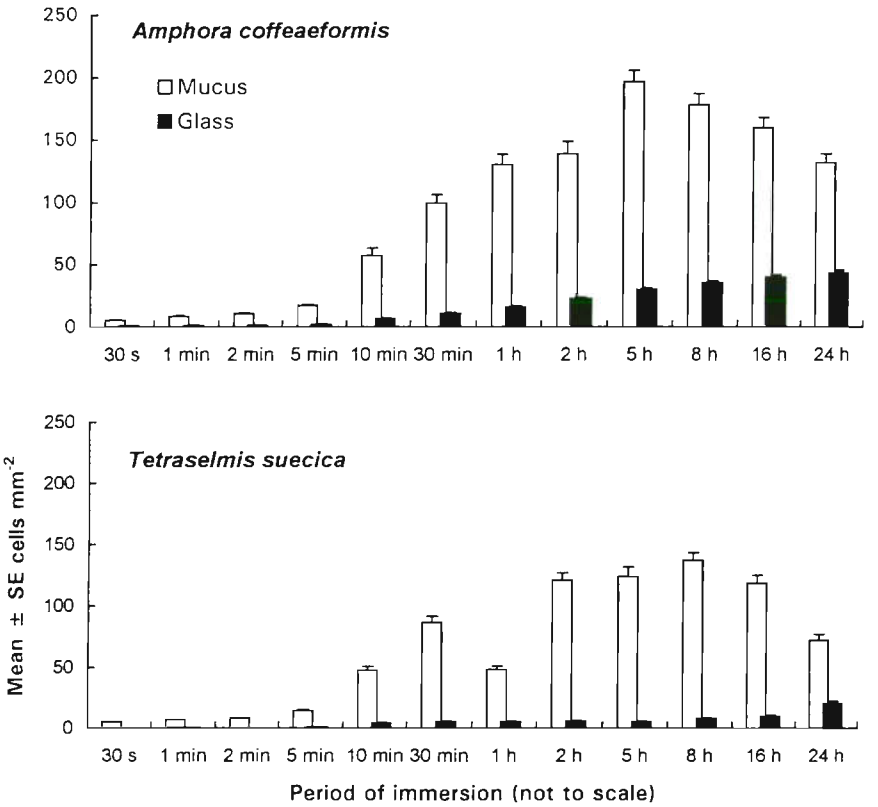

Fig. 1. Effects of period of immersion in mono-suspensions of microalgae of glass plates containing a *Littorina littorea* mucus trail on adhesion of microalgae to the mucus and to the glass

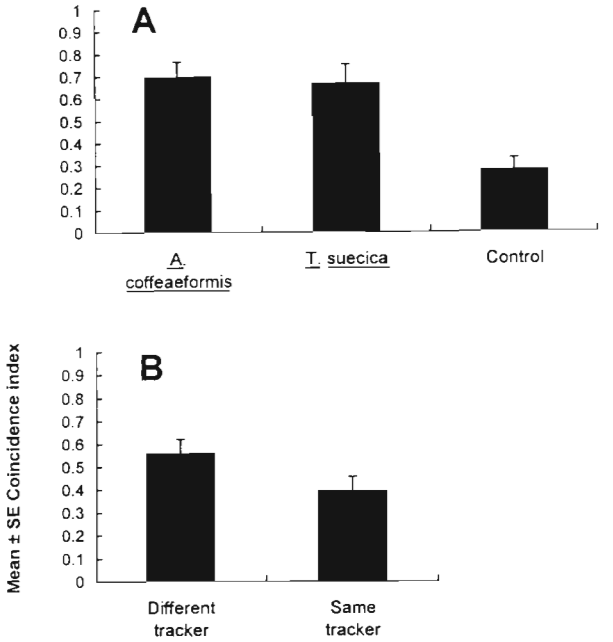

Fig. 2. Coincidence indices (see 'Materials and methods: Trail-following experiments') in a crossed experimental design of conspecific trail-following by *Littorina littorea* over mucus trails (A) dipped in suspensions of microalgae or a control; (B) where the tracker snail is different to the marker snail or is the marker snail. For analysis see Table 1

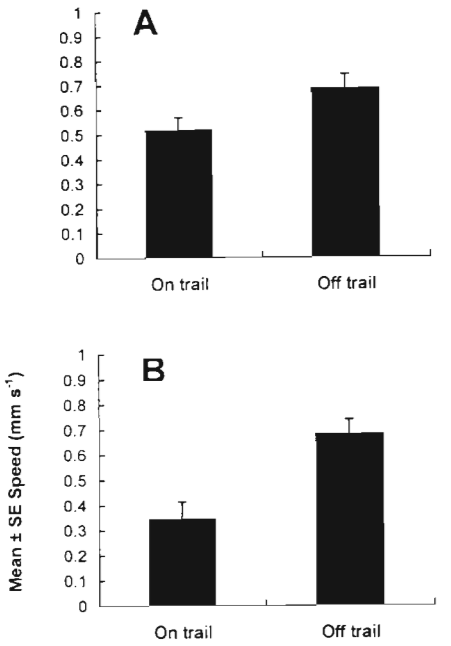

Fig. 3. Speed of movement of *Littorina littorea* on and off conspecific mucus trails. (A) Results from the entire experiment (includes data collected when trails had been dipped in suspensions of microalgae); (B) results from control treatment only (trail not dipped in a suspension of microalgae)

Table 1. Analysis of variance on coincidence indices. Data were arcsin-square-root transformed prior to analysis

Factor	df	MS	*F*	p
Tetraselmis suecica, *Amphora coffeaeformis* or control	2	3.7697	14.85	0.000
Different tracker or same tracker	1	1.0640	4.19	0.044
Interaction	2	0.2180	0.86	0.428
Error	74	0.2538		

Tukey mean separation (untransformed means). Underlined means are not significantly different

T. suecica or *A. coffeaeformis* or control

A. coffeaeformis	*T. suecica*	Control
0.697	0.662	0.274

Different tracker or same tracker

Different tracker	Same tracker
0.556	0.394

a period of crawling on the glass. Only a few snails followed 'against polarity', and this was after a long period of 'with polarity' following on trails containing microalgae. Radular rasps were always less frequent (~5 min^{-1}, though too few snails behaved thus to allow meaningful statistical comparison) when travelling 'against polarity' over a trail which had just previously been grazed.

Where mucus trails did not contain microalgae, they were followed for about 1/4 of their length, but where they contained microalgae, tracker snails followed them for a significantly longer distance, about 2/3 of their length (Fig. 2A, Table 1). There was no significant difference in coincidence indices between trails containing *Amphora coffeaeformis* and trails containing *Tetraselmis suecica* (Table 1). Over all microalgal and control treatments, significantly more of the trail (~16%) was followed when the tracker was different from the marker snail than when the tracker and the marker were the same individual (Fig. 2B, Table 1), winkles preferring to follow the trails of conspecifics over their own.

Over all treatments winkles moved significantly faster (Student's *t*-test: $t = 2.14$, $p = 0.034$), by ~30%, on glass (all values given as mean ± SE: 0.68 ± 0.06 mm s^{-1}, n = 80) than on mucus trails (0.52 ± 0.05 mm s^{-1}, n = 80) (Fig. 3A). Comparison of movement on glass with movement on mucus trails not containing microalgae (Fig. 3B) revealed a greater significant difference ($t = 3.72$, $p < 0.001$), again winkles moving faster (by ~2 times) on glass (mucus, 0.35 ± 0.07 mm s^{-1}, n = 40;

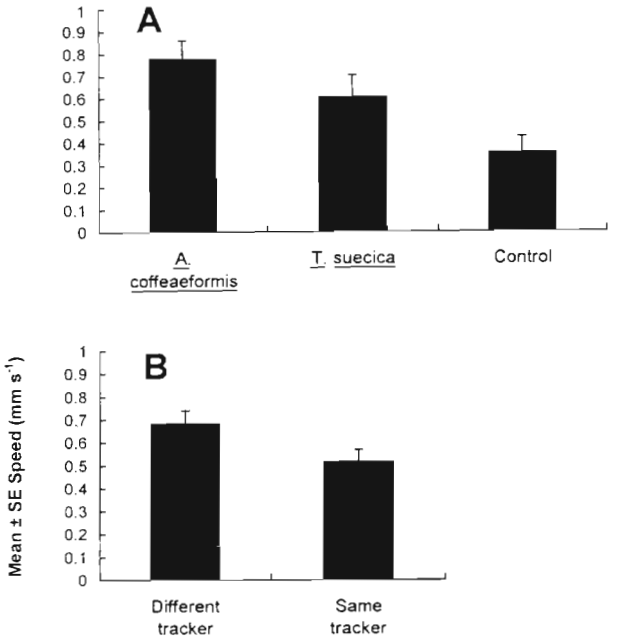

Fig. 4. Speed of movement of trail-following *Littorina littorea* in a crossed experimental design. (A) Mucus trails dipped in suspensions of microalgae or a control; (B) where the tracker snail is different to the marker snail or is the marker snail. For analysis see Table 2

glass, 0.68 ± 0.06 mm s^{-1}, n = 80). Mucus thus appears to slow down the movement of *Littorina littorea*, but when the mucus trail contains microalgae (food) snails show an increase in speed over that on bare mucus. This is illustrated in Fig. 4A where the winkles move significantly faster (again by ~2 times) on *Amphora coffeaeformis* treated trails (0.78 ± 0.08 mm s^{-1}, n = 20) than on control trails (0.35 ± 0.07 mm s^{-1}, n = 40), though there was no significant difference between *Tetraselmis suecica* treated trails (0.60 ± 0.10 mm s^{-1}, n = 20) and control trails (Table 2). The speed of tracker snails was not dependent on whether the tracker was the same snail or different to the marker snail (Fig. 4B, Table 2).

The feeding rate (as radular rasps or bites) of tracker snails was significantly greater on trails containing microalgae than on control trails (by at least 3 times), but there was no significant difference between trails containing the 2 species of microalgae (*Amphora coffeaeformis*, 17.8 ± 2.5 bites min^{-1}, n = 20; *Tetraselmis suecica*, 12.9 ± 2.8 bites min^{-1}, n = 20; control, 4.26 ± 1.4 bites min^{-1}, n = 40) (Fig. 5A). On control trails where the tracker and marker were not the same individual, the tracker paused to take 2 or 3 bites, moved a centimetre or so and paused again, the process repeating until the snail moved off the trail or reached its end. On other trails bites were more regular and *Littorina littorea* individuals characteristically swept their heads

Table 2. Analysis of variance on speed of *Littorina littorea* tracker snails on a conspecific mucus trail previously dipped in a suspension of microalgae

Factor	df	MS	*F*	p
Tetraselmis suecica, *Amphora coffeaeformis* or control	2	1.285	7.41	0.001
Different tracker or same tracker	1	0.027	1.2	0.278
Interaction	2	0.227	1.31	0.275
Error	74	0.173		

Tukey mean separation. Underlined means are not significantly different

T. suecica or *A. coffeaeformis* or control (mm s^{-1})

A. coffeaeformis	*T. suecica*	Control
0.776	0.601	0.352

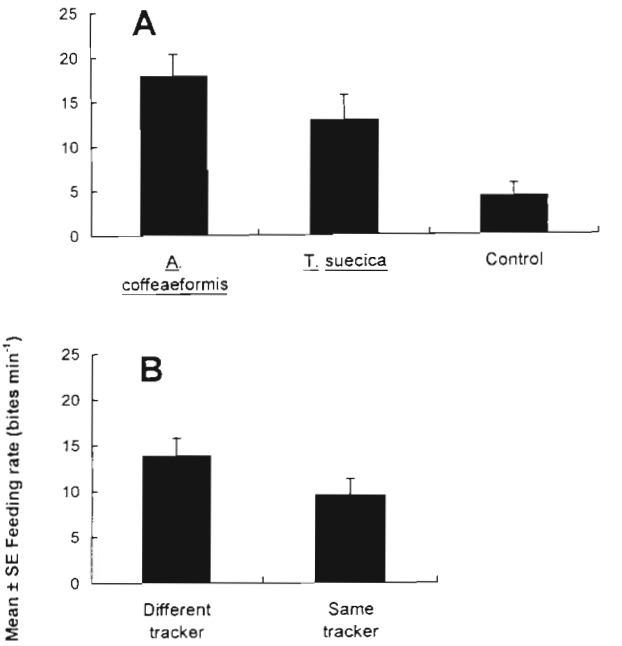

Fig. 5. Feeding rate (radular rasps, or bites, min^{-1}) of trail-following *Littorina littorea* in a crossed experimental design. (A) Mucus trails dipped in suspensions of microalgae or a control; (B) where the tracker snail is different to the marker snail or is the marker snail. For analysis see Table 3

from side-to-side as they grazed, although this lateral head movement was not observed on the control trails. The feeding rate of tracker snails was not dependent on whether the tracker was the same snail or different to the marker (Fig. 5B, Table 3). Microalgae of both species embedded in the mucus trail were seen, both by eye and on video recordings, entering the mouths of

Table 3. Analysis of variance on feeding rate of *Littorina littorea* tracker snails on a conspecific mucus trail previously dipped in a suspension of microalgae

Factor	df	MS	*F*	p
Tetraselmis suecica, *Amphora coffeaeformis* or control	2	1357.1	13.60	0.000
Different tracker or same tracker	1	334.7	3.36	0.071
Interaction	2	473.6	4.75	0.011
Error	74	99.8		

Tukey mean separation. Underlined means are not significantly different

T. suecica or *A. coffeaeformis* or control (bites min^{-1})

A. coffeaeformis	*T. suecica*	Control
17.9	12.9	4.3

tracker snails. Only 3 trackers showed radular activity on a substratum of glass, indicating that *L. littorea* can distinguish between surfaces covered with mucus and those not so covered.

The mean tortuosity index of marker snails was 0.749 ± 0.023 (n = 80), indicating that the snails traversed a path that was ~50% longer than the direct path between its start and finish points. Tortuosity indices of trackers were not described as these would be dependent on the path of the marker snails.

Effects of grazer passage on benthic microalgal density

Within each treatment there were no significant differences between the mean densities of microalgae recorded from each slide. Both *Amphora coffeaeformis* and *Tetraselmis suecica* were significantly reduced in density following the passage of a tracker *Littorina littorea* over a mucus trail to which the microalgae were adhering (Fig. 6, Table 4). *A. coffeaeformis* adhered to the mucus in significantly greater numbers than *T. suecica* (as in the adhesion experiments) and was reduced in density by ~38% (from a mean ± SE of 330.0 ± 32.2 to 206.8 ± 23.4 cells mm^{-2}) after the passage of a tracker snail. Though adhering in significantly fewer numbers than *A. coffeaeformis*, *T. suecica* was reduced in density by ~43% (from a mean ± SE of 19.25 ± 2.22 to 11.03 ± 2.09 cells mm^{-2}) after grazer passage. Interestingly, *A. coffeaeformis* adhered to the mucus trails in greater numbers than in the adhesion experiments, but *T. suecica* adhered in fewer numbers than in the adhesion experiments (compare Figs. 1 & 6).

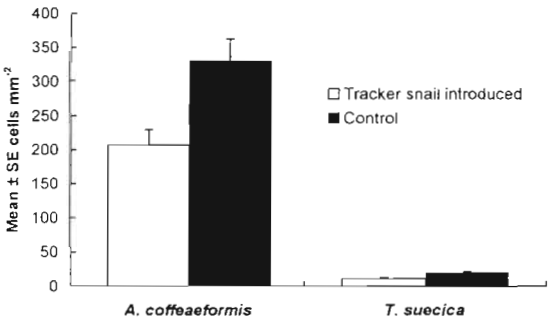

Fig. 6. Microalgal densities on control mucus trails of *Littorina littorea* and on trails that had been crawled over by a conspecific (tracker snail). For analysis see Table 4

Table 4. Analysis of variance on effects of *Littorina littorea* tracker snail passage on density of microalgae in mucus trails

Factor	df	MS	*F*	p
Tetraselmis suecica or *Amphora coffeaeformis*	1	64.1×10^5	161.4	0.000
Tracker snail introduced?	1	4.3×10^5	10.9	0.001
Interaction	1	3.3×10^5	8.3	0.004
Error	396	0.4×10^5		

DISCUSSION

The mucus of littorinids effectively serves to concentrate food items which are preferentially exploited. *Amphora coffeaeformis* appears to adhere in greater numbers to glass and to mucus than does *Tetraselmis suecica* (Fig. 1). *A. coffeaeformis* is typically benthic and immotile in suspension and may sink to a substratum, while *T. suecica* swims in suspension. Also, *A. coffeaeformis* secretes mucus (Edgar 1980) which is used in locomotion and may serve to bind the organism to a substratum while *T. suecica* may be able to release itself. Davies et al. (1992a) provided data suggesting that diatoms might preferentially move onto molluscan mucus. *A. coffeaeformis* can move at speeds approaching 3 μm s^{-1} on glass (Davies et al. 1998) and in experiments lasting hours could move distances of the order of millimetres. Prosobranch mucus is composed of 10 to 20% carbohydrate (Davies et al. 1990b), and Cooksey & Cooksey (1988) reported chemotaxis towards carbohydrates in *A. coffeaeformis*. After ~5 to 8 h the mucus of *Littorina littorea* becomes less effective at binding microalgae (in contrast to glass, Fig. 1), despite the greater period available for adhesion. Davies et al. (1992a) showed that *L. littorea* pedal mucus has a half-life on-shore of ~12 d, much shorter than that of the limpet *Patella vulgata*. After 5 to 8 h of immersion the mucus may begin decaying. If tracker winkles are deliberately profiting nutritionally from the microalgae

in trails, then grazing while the trail is young would seem appropriate. Chapman (1998) reports that the Australasian *Nodilittorina unifasciata* follows fresh conspecific trails much more readily than ones 2 to 3 h old. However, the present results concern adhesion only, and a more nutritious assemblage containing, for example, growing macroalgal propagules might succeed over time. Imrie (1992) performed a similar experiment to ours in which *T. suecica* in 'agitated' sea water adhered in greater numbers to the pedal mucus trails of *L. littorea* than to glass. Unfortunately, the density of the microalgal suspension was not recorded, and so quantitative comparison with the present work cannot be made.

Winkles are more likely to follow a mucus trail if it has microalgae within it (Fig. 2) and thus appear, in this instance, to use trail-following for grazing purposes. Trail-following, then, might be a response to food availability rather than any other consideration (see discussion below). However, during the non-breeding season winkles prefer to follow the trails of conspecifics over their own trails (described for other gastropods by: Townsend 1974, Trott & Dimock 1978, Tankersley 1989); this provides evidence (1) that snails can detect some component of 'self' in a trail and (2) that trail-following has evolved to facilitate aggregation and hence as a stress-reduction device. On the other hand, *Littorina littorea* can detect conspecifics through pheromones (Dinter & Manos 1972), and trail-following in order to aggregate might only occur when the source of the pheromones is masked, e.g. when water movements are great. Cook (1992) reached a similar conclusion for airborne versus trail-bound cues to aggregation in the slug *Limax pseudoflavus*. Although littorinids use the mucus trails of others as a foraging resource, the evolution of trails for this purpose is unlikely to be evolutionarily stable: individuals cannot guarantee a return on their investment of mucus (see Davies et al. 1992a). Hence the adhesive properties of mucus, though exploited through provendering, are likely to be a by-product of locomotory function, rather than adaptive in their own right.

Townsend (1974) reported a positive correlation between coincidence index (calculated differently) and period of starvation for tracker *Biomphalaria glabrata*. Townsend appears to regard increased coincidence indices as reflecting an increased desire to aggregate, but this might be accounted for by snails searching for food in mucus trails. Tracking requires less force than marking in *Littorina irrorata* (Tankersley 1989) and has thus been suggested as an energy-saving device.

Locomotion over a layer of mucus appears to slow winkles, in comparison to their speed on glass (Fig. 3). Interestingly this was not found in similar experiments on *Littorina littorea* conducted by Imrie (1992), although the experimental design used by Imrie did not clearly distinguish whether locomotion took place on mucus. Hall (1973) found that marker *L. irrorata* were slower than tracker snails, though this was over a substratum of sand which might place constraints on movement. Tankersley (1989) and Dimock (1985) found no such relationship, but noted that the speed of *L. irrorata* and *Ilyanassa obsoleta*, respectively, was much reduced on sand in comparison to glass. These authors also noted no significant difference in speed between marker and tracker snails over a variety of substrata. This could imply a difference in function of the mucus between *L. irrorata* and *I. obsoleta*, and *L. littorea*. In the present study, winkles increased speed over mucus trails containing microalgae to approximately the same speed as that on glass (Figs. 3 & 4). Thus the mucus cannot be mechanically impeding locomotion (owing to its sticky properties). If *L. littorea* usually encounter food on mucus trails (which perhaps *L. irrorata* and *I. obsoleta* do not), the slowing might constitute part of a methodical search for food on the trail, as seen here; once the snail finds food, its speed increases. Similar rates of locomotion by *L. littorea* were recorded by Gowanloch & Hayes (1926), Newell (1958a), Innes & Houlihan (1985), Imrie (1992) and Erlandsson & Kostylev (1995).

Radular rasping appears to occur only where there is a suitable surface to rasp on (see also Imrie 1992). Feeding then appears to be biphasic: a detection phase (probably involving tentacular sensing) and an ingestion phase. Attempted ingestion (radular activity) does not occur while *Littorina littorea* are involved in the detection of a suitable substratum on which to graze, although radular activity (tasting?) and the 'searching' behaviour of snails on bare mucus trails suggests that the periwinkles are expecting to find food there. While winkles will feed on a bare mucus trail, their radular activity (and presumably ingestion rate) increases dramatically on trails containing microalgae (Fig. 5). Values recorded are similar to those recorded by Newell et al. (1971) for *L. littorea* feeding on epiphytes on an aquarium wall and by Imrie (1992) for *L. littorea* feeding on mucus, though they are much lower than rates recorded by Petraitis & Sayigh (1987) for *L. littorea* feeding on natural substrata. This discrepancy might be owing to increased temperatures during the observations of Petraitis & Sayigh (1987) or to their complete lack of handling of the specimens. The few snails that turned and followed trails 'against polarity' may have been satiated after their travels and grazing over mucus trails containing microalgae.

It is possible that the reduction in microalgal densities following tracker passage (Fig. 6) is owing to the displacement of microalgae onto the foot and shell of

the tracker as it passes along the mucus trail. Post-experiment examination of the snails revealed no adhering microalgae, and it is our conclusion that the reduction in microalgal density is solely due to ingestion by the tracker snail, as was seen during the trail-following experiments. The accompanying ingestion of some of the mucus trail will be inevitable. The anomaly in rates of microalgal adhesion between the experiments on adhesion and effects of grazer passage might be accounted for by the preservation technique used in the latter experiment, although no loss of microalgae was observed during either experiment. In an experiment by Hunter & Russell-Hunter (1983), *Littorina littorea* feeding on a biofilm that was allowed to develop on glass selectively avoided the ingestion of large pennate diatoms, including those identified as members of the genus *Amphora*. This may have been because the diatoms were able to adhere tightly to the glass using mucoid extracellular polymers (Hoagland et al. 1993), and so escape grazing. Adhesion to mucus might be difficult, and so mucus trails might facilitate the ingestion of microalgal species that would otherwise escape grazing. Nevertheless, *A. coffeaeformis* appeared more refractory than *Tetraselmis suecica* in our experiment (Fig. 6). Other examples of mucus being grazed by gastropods are sparse (though ingestion of mucus in other molluscan groups is widespread, see Davies & Hawkins 1998), but may be more common than has been thought. *Hydrobia ulvae* consumes its own pedal mucus after organic enrichment, as it floats between ripple marks on the beach (Fenchel et al. 1975). *H. ventrosa* similarly consumes its own mucus which presumably contains bacteria (see Herndl & Peduzzi 1989). Growth of *H. ventrosa* is enhanced when bacteria are consumed (Kofoed 1975).

Although it has long been speculated that mucus trails might provide a source of nutrition for grazing molluscs (e.g. Calow 1979), the reduction in microalgal density following the passage of a grazer and the observations of mucus plus microalgae entering the mouth of grazers are the first pieces of direct evidence. Grazers are clearly able to detect the presence of microscopic food items in mucus trails and modify their behaviour upon such detection. Given that much of the intertidal will be covered with mucus trails (see Davies & Hawkins 1998) which incorporate food items (Davies et al. 1992b), results of experiments on trail-following behaviour that used 'clean' trails, generated in the laboratory, will not be representative of behaviour *in situ*. From our search of the literature, we could not find 1 experiment performed on trails that were likely to have a composition similar to that found *in situ*, although the observations of Newell (1958a,b) were made on-shore. Nevertheless, we are aware that our control substratum (glass) may differ considerably from natural substrata. We regard its use as justified as it provided a uniform surface *through which* observations could be made.

Littorinids can have a considerable impact on diatom populations *in situ* (Castenholz 1961, Nicotri 1977), and trail-following behaviour seems to be inextricably linked with nutrition, a much closer association than has been postulated previously (i.e. that aquatic snails follow others, usually conspecifics, to more profitable patches of food: see Hawkins & Hartnoll 1983, Deneubourg et al. 1988). If snails derive a considerable proportion of their diet from mucus trails, this would alter our views on: the sources of nutrition for grazers; grazer life-history strategies; microalgal population dynamics; and autotroph/herbivore interactions; and could explain why littorinids have softer radulae than other grazers, such as limpets (Hawkins et al. 1989, Fretter & Graham 1994). Such grazing behaviour would also have evolutionary and ecological consequences for both the mollusc and the microalgae. The latter may benefit from being cradled in a mucus gel which might provide some nutrition, assuming they are fortunate enough to escape grazing (see Davies et al. 1992a). The distribution patterns of the former might be a response to food availability in mucus trails. The former also expends a considerable proportion of its consumed energy (Davies et al. 1992a) on mucus production which was hitherto thought of as being lost to the environment and functioning only as a locomotory coupler. It was thought that the mucus might have an additional role as a provendering agent, helping to offset the high cost of mucus production, but until now this has only been speculation. *Littorina littorea* are cosmopolitan in their diet and consume a range of both microalgae and macroalgae (see Norton et al. 1990 for review). We are not proposing that winkles exist solely on a diet of organic particles bound in mucus trails, but that a significant proportion of their diet might be thus derived. In any case microalgae on British shores are seasonal in abundance (Hill & Hawkins 1991), and a switch to macroalgae is probably inevitable at certain times of the year.

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