

**An investigation into the behavioural ecology and
management of the common garden snail (*Cornu
aspersum*) and its role in the transmission of canine lung
worm (*Angiostrongylus vasorum*)**

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Abstract

Gastropods are both agricultural pests and vectors of the parasitic canine lung worm *Angiostrongylus vasorum* (Morgan *et al.* 2005; Amiri-Besheli 2009). While attempts are made to manage their numbers with molluscicides, there is a risk of damage to non-target species (Amiri-Besheli 2009). Despite human intervention gastropods are highly abundant. In this thesis I investigate the adaptations that may fuel the success of the common garden snail (*Cornu aspersum*). I investigate the use of a physical exclusion device in limiting gastropod damage and investigate the prevalence and impact of *A. vasorum* infection on defensive behaviour. I further investigate the use of conspecific trails as a form of information sharing and the metabolic impact of following these trails. I found the physical exclusion device significantly reduced gastropod number and crop damage. No conclusions could be drawn on the behavioural impact of *A. vasorum* infection as no infected individuals were found. *C. aspersum* were found to use trails to glean information on the foraging success of conspecifics and used this information to guide their own behaviour. Unfed individuals were significantly more likely to follow trails left by fed individuals, while fed individuals showed no preference. Travelling on mucus indirectly increased locomotor efficiency as travelling on slime allowed individuals to travel faster for no added metabolic effort.

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An investigation into the behavioural ecology and management of the common garden snail (*Cornu aspersum*) and its role in the transmission of canine lung worm (*Angiostrongylus vasorum*)

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Summary

1. Gastropods, such as the common garden snail (*Cornu aspersum*) can cause damage to agriculture and garden plants and may act as an intermediate host to the parasitic nematode, *Angiostrongylus vasorum*.
2. This study investigates the efficiency of a spiked barrier in preventing gastropods from accessing a valuable food resource in a field setting.
3. This study further investigates the use of conspecific trails as a form of social information transfer and the metabolic implication of using trails for locomotion.
4. This study concludes with an investigation into prevalence and behavioural implication of *Cornu aspersum* infection by *Angiostrongylus vasorum*
5. A spiked exclusion device significantly reduced the number of individuals feeding and reduced the number of damaged leaves.
6. Unfed snails were more likely to follow a trail left by a fed individual and fed individuals were less likely to follow any individual of either feeding status.

7. Travelling on mucus significantly increased the speed at which an individual travelled at no extra metabolic cost.
8. No individuals were found to be infected with *Angiostrongylus vasorum* which may be due to physiological mechanisms preventing maturation of larvae within *Cornu aspersum* or vastly improved management of *Angiostrongylus vasorum* in domestic dogs from the sampled location.

Introduction

Understanding the ecology of species that act as vectors of disease and damage crops, is important to ensure they are managed appropriately. While lethal control measures may be effective, it is understood that their use can cause environmental damage, such as inadvertently killing non-target species (Amiri-Besheli 2009) and there is pressure to develop less damaging means of control.

While much of the damage caused to agriculture by pests goes unseen by the layperson, the common garden snail (*Cornu aspersum*) damages both agriculture and horticulture. There is a large range of molluscicide products available for the home user and their use by the layperson is of concern, due to the potential risk posed to domestic animals and wildlife; of veterinary poisoning cases involving dogs reported to veterinarians in Australia, 82% were due to metaldehyde, a common chemical used in home and commercial molluscicides (Studdert 1985) .

While the protection of garden plants has long been the reason for managing gastropod populations in a domestic setting, in recent years the threat of disease spread by gastropods to domestic dogs (*Canis lupus*) has become of pressing concern. Canine lungworm, sometimes referred to as French heartworm (*Angiostrongylus vasorum*), is a tropically transmitted parasite that infects a range of canids in Great Britain, specifically the red fox (*Vulpes vulpes*) and the domestic dog, via a gastropod intermediate host (Morgan *et al.* 2008).

Preventing *A. vasorum* infection in domestic dogs requires administration of anthelmintic medication. However, of equal importance to dog owners, is limiting the potential exposure their pet may receive to infectious gastropods through population

control, while recognising the concern about the use of lethal control methods and the risk this may pose.

The development of new methods of controlling *C. aspersum* and managing domestic habitat to minimise the risk of parasite transmission relies upon understanding its ecology. *C. aspersum* is widespread in a range of habitats and despite current control methods it remains abundant, therefore, it may be beneficial to investigate the adaptations that underpin this success and subsequently consider how this knowledge may be exploited to manage its prevalence.

Gastropods have evolved a unique form of locomotion upon a trail of mucus using a ventral foot, which while not conducive to great speed, is thought to have allowed greater access to resources in a three dimensional environment (Denny & Gosline 1980). However, despite moving in such a manner, they appear to be extremely efficient at consuming plant matter, even if resources have a patchy distribution.

Gastropods leave a mucus trail in their wake as they travel, the production cost of which is far greater than the cost incurred through locomotion (Denny 1980a). This form of locomotion is conserved across the class despite its great cost; therefore it may offer additional functions than simply aiding the locomotion and adhesion of the individual.

In this thesis I investigated the foraging behaviour of free-living gastropods in a field setting and the efficacy of a snail exclusion barrier in protecting plants from damage on behalf of an innovations company. While the efficiency of the device was of interest, the apparent trail following behaviour to and from the food sources noted during data collection was particularly compelling.

I hypothesised that the mucus trails left by gastropods may reveal the location of food resources to conspecifics, particularly if those that had successfully found food revealed the fact in their mucus trail.

I further investigated this hypothesis through a laboratory experiment into the use of conspecific trail following as a potential means of information transfer.

Trail following has been observed in a number of gastropods and it has been suggested that this behaviour allows individuals to conserve energy. I therefore investigate this hypothesis in order to fully understand the many functions of this behaviour, through a laboratory experiment, to compare the metabolic affect of crawling on slime.

Following a change in funding provider I conclude with an investigation into the potential prevalence and impact of *A. vasorum* infection on the defensive behaviour of *C. aspersum*.

1 - Evaluation of a spiked barrier for the exclusion of terrestrial gastropods

Abstract

The effect of using a spiked barrier to exclude molluscs from foraging on commercial lettuces was investigated through monitoring the number of foraging molluscs and associated crop damage over four consecutive days. The use of a spiked barrier significantly reduced the number of molluscs by 41.7% and crop damage by 31.14% suggesting mollusc arrival and damage was slowed rather than halted.

Introduction

Land living gastropods pose a threat to agriculture and horticulture due to their consumption of plant matter. For example, when at a high density, damage caused by the great garden slug (*Deroceras reticulatum*) was found to reduce the yield of alfalfa by 46% (Barratt *et al.* 1994). Ensuring plants are protected from damage is important and there is motivation to develop new and environmentally friendly means of doing so.

One of the most prevalent gastropods, the common garden snail (*Cornu aspersum*) has been shown to eat in a selective fashion, preferring plants with a high level of protein and calcium (Iglesias & Castillejo 1999). Young plants, such as commercial lettuces have been shown to contain a greater quantity of protein than older plants and therefore may prove more attractive to foraging gastropods than surrounding vegetation (Hansen 1978).

Such a foraging preference may be of particular concern to horticulturalists who may utilise metaldehyde based molluscicide to control their numbers. Unfortunately, such pellets may cause environmental damage; potentially killing beneficial non-target species such as earthworms (Edwards *et al.* 2009), predatory carabid beetles (Purvis & Bannon 1992) and even domestic pets such as cats and dogs (Studdert 1985). Such concerns, along with a general desire to reduce pesticide use, may motivate gardeners to seek non-lethal alternatives.

Non-lethal gastropod control has traditionally relied on various means of physical exclusion and may provide an environmentally friendly alternative to lethal methods. Copper tape has been suggested as a non-chemical means of excluding gastropods (Amiri-Besheli 2009), however its mode of action is yet to be categorically determined and it is not always effective (Schüder *et al.* 2003). Such ambiguity leaves a potential niche for physical exclusion devices with a clearer mode of action.

The use of a novel, spiked barrier may offer a new means of limiting gastropod damage. Gastropods show great sensitivity in both their antenna and their exposed flesh (Srivastava 1992) and may be prevented from crossing a barrier composing of plastic bristles.

Here we investigate the efficacy of using a spiked barrier to prevent gastropod predation on commercial lettuces. We use experimental data to compare both the number of visiting gastropods and the damage caused when employing a novel spiked barrier.

Methods

Eighty 50cm x 50cm carpet tiles were individually numbered using coloured waterproof varnish. All odd numbered tiles (n=40) had 15 holes drilled into the edges and a two meter length of spiked barrier (see fig 1) was attached to the circumference using 10cm cable ties placed through the holes. Even numbered tiles (n=40) had no barrier attached (see fig 1).



Figure 1 Experimental set-up showing tile with spiked barrier (left) and control tile (right).

All eighty tiles were placed in numerical order, in pairs with approximately 10 cm between pair members and with approximately 30 cm between pairs (see fig 1), in an enclosed field containing a mixture of grassland and potted broad bean plants (*Vicia faba*). The location was known to harbour a range of gastropod species, including the common garden snail (*C. aspersum*), the common garden slug (*Arion*

horntensis), the grey field slug (*Deroceras reticulatum*) and the large red or black slug (*Arion ater*).

Eighty 'living salad' plants were obtained from a supermarket range. Each consisted of a selection of lettuce plants growing in a plastic tray, with leaves approximately 10cm in length. One lettuce tray was placed in the middle of each tile.

The number of small (<3cm in length) and the number of large (>3cm in length) gastropods on each tile was recorded at approximately 11pm for four consecutive nights (until the plants had begun to degrade).

The following morning at approximately 7:30am, the number of small and large gastropods encountered on each tile was again recorded (to ascertain if gastropods were commuting to and fro the area), along with an estimation of the number of damaged leaves. Each individual leaf was deemed as damaged if any part of the lamina, petiole or stem was missing. If a whole plant was consumed, this was recorded as four damaged leaves.

Data were analysed using generalised linear mixed models (GLMMS) using R (R Development Core Team 2008). GLMMs allow for the control of repeated measures and both random and fixed factors are modelled (Schall 1991). The significance of linear mixed models was recorded as a χ^2 for each term during standard model simplification (Crawley 2014). 'Crop damage', 'total gastropods encountered', 'total gastropods encountered during the day' and 'total gastropods encountered during the night', were all analysed employing the 'presence' or 'absence' of a spiked barrier, as a fixed factor. Spatial heterogeneity in gastropod abundance was accounted for by including 'tile pair' as a random factor in the analysis.

Results

The use of a spiked barrier significantly reduced the total number of gastropods encountered on or in the vicinity of study lettuces, compared to those left unprotected ($\chi^2 = 72.6$, $df = 1$, $p < 0.001$) and the total number of gastropods encountered at night ($\chi^2 = 72.26$, $df = 1$, $p < 0.001$) but did not significantly affect the number of gastropods encountered during the day ($\chi^2 = 0.177$, $df = 1$, $p = 0.673$).

A mean of 2.6 gastropods per tile was encountered when using a spiked barrier (see Fig 2) and a mean of 4.46 per tile without a barrier (see Fig 2) representing a 41.7% decrease in mean gastropods per tile when using a spiked barrier compared to control tiles.

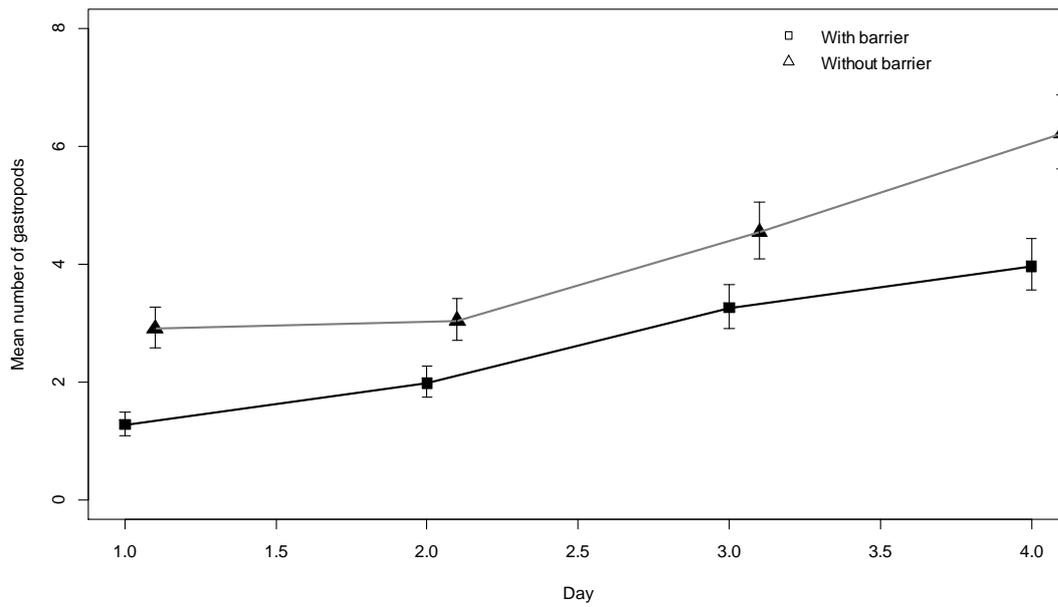


Figure 2 - Mean number of gastropods per tile per night with standard deviation, indicating significantly fewer gastropods encountered within the vicinity of lettuces when a barrier is employed.

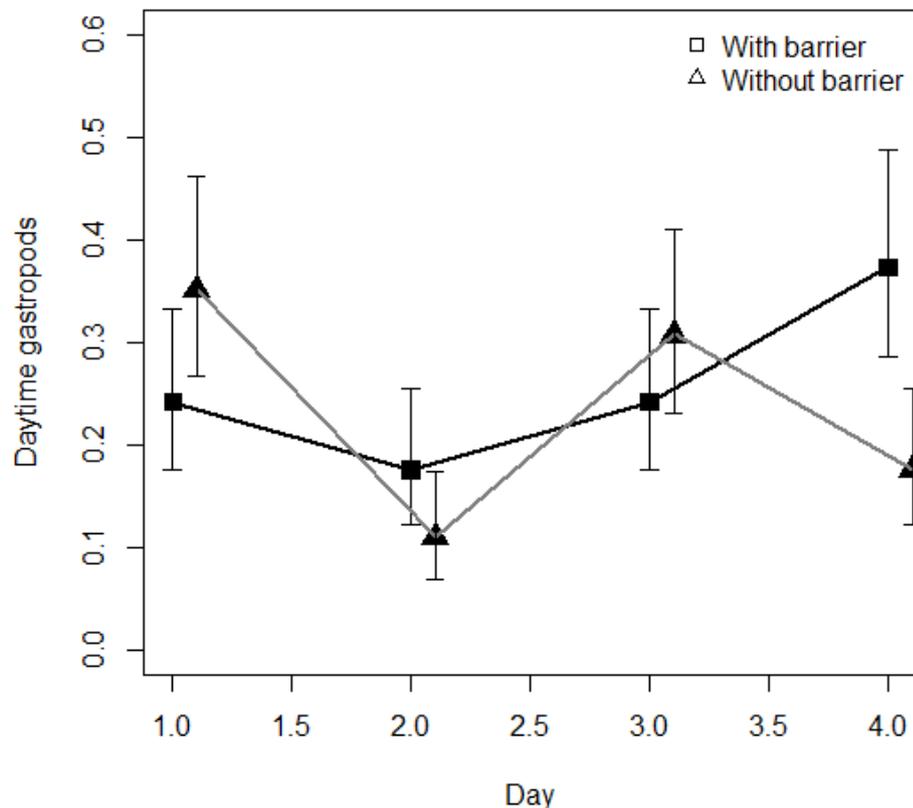


Figure 3 - Mean number of gastropods encountered per condition per day count, showing no effect of a barrier on the number remaining in the morning.

The use of a spiked barrier was found to significantly reduce crop damage (the number of damaged leaves) ($\chi^2 = 77.59$, $df = 1$, $p < 0.001$).

A mean of 7.96 leaves were damaged per tile when using a spiked barrier (see Fig 4) and a mean of 10.98 leaves without a barrier (See Fig 4), representing 31.14% fewer damaged leaves when a spiked barrier was used.

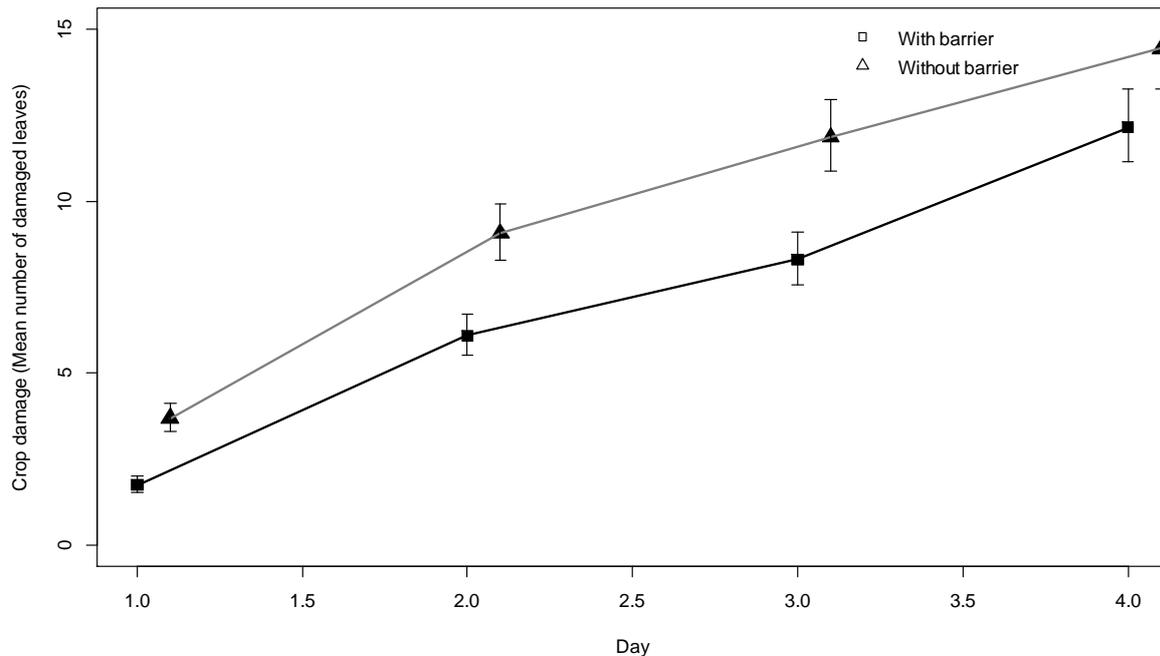


Figure 4 - Mean crop damage (number of damaged leaves per lettuce) per day, with standard error of the mean, showing a significant reduction in leaf damage when using a barrier. The degree of significance of this effect was lessened with time as shown by the overlapping standard error at day 4.

Discussion

The use of non-lethal methods of gastropod control is certainly an inviting prospect for the conscientious gardener, particularly for those wishing to limit environmental damage. However, the efficacy of such methods must be such that gastropod numbers are reduced to damage levels that the plant can tolerate.

These results indicate that the use of a spiked barrier significantly reduced both the total number of gastropods and leaf damage by 41.7% and 31.14% respectively. Comparison of the mean gastropod number over the duration of the experiment

between both conditions indicates a similar upward trend in gastropod number. This similarity suggests the use of a barrier served to effectively reduce the number of gastropods without affecting the behaviour of those that were able to cross it.

A similar trend was noted in the mean number of damaged leaves between both conditions, with an overall reduction in leaf damage when employing a spiked barrier. However, the overlap of the standard error on day four, suggests the importance of this effect may have decreased through time.

Foraging individuals were commuting to and from the study area on a nightly basis, as only a small number of gastropods were encountered within the vicinity of the lettuces in both conditions during the day. Such a 'homing' behaviour has been noted in *C. aspersum* and as such, it is feasible that other gastropod species may show a similar affinity for returning to specific foraging locations (Dunstan & Hodgson 2014). This 'homing' ability may result in individuals that crossed the barrier developing an affinity for a particular foraging site. It was noted that smaller gastropods, particularly *D. reticulatum*, were observed to follow larger individuals. Such behaviour may potentially result in larger gastropods inadvertently aiding other individuals to gain access to the resources.

Far fewer gastropods were found on the tiles during the day than at night, however, there appeared to be a steady increase in gastropods on the tiles with a barrier than without. This suggests a barrier may prevent some of the gastropods that crossed the barrier from leaving; therefore its efficacy may be improved if measures are implemented to physically remove and relocate such individuals.

There is potential for mucus trails to act as a source of social information for individuals to locate the lettuce within the protected area. Should one individual

discover a means of circumventing the barrier, they may leave a mucus message that aids their own navigation back through the barrier. Such a message may be ripe for exploitation by conspecifics who may use the foraging success of such individuals to navigate through the barrier. Such behaviour is common in eusocial insects, such as ants (Wolf & Wehner 2000), who leave progressively stronger pheromone trails for kin. While kin selection is an unlikely explanation for these findings, there is potential for dilution effects to favour information sharing (Lima 1995).

A growing number of individuals successfully crossed the barrier, suggesting it served to limit the rate at which the resources were discovered rather than preventing access. In attempting to prevent gastropods from accessing plants, the efficacy of a spiked barrier may be limited to plant species that can tolerate a degree of destruction or that are vulnerable for a limited period of time. Combining its use with the physical removal and relocation of gastropods may improve its viability as a means of control due to the reduced gastropod burden relocation offers (Dunstan & Hodgson 2014).

These results warrant further investigation into the potential for information sharing in gastropods. The mucus trail gastropods leave in their wake has metabolic benefits if followed by conspecifics (Tankersley 1989; Davies & Blackwell 2007), however, these data suggest there is potential for their use as a means of social information sharing.

Conclusion

Gastropod damage can be rapid and devastating to gardeners and attempts at limiting their consumption may be improved by means of physical exclusion. These data suggest gastropods commute regularly to feeding sites and conspecifics may eavesdrop on the foraging success of others. Therefore, reducing the number of individuals that successfully discover a patch of food may serve to slow the inevitable increase in foraging individuals. While a spiked exclusion device reduced the number of individuals foraging on a patch, the barrier was regularly crossed, therefore such methods may only be successful if used in conjunction with other gastropod reduction protocols.

Chapter 2 – An investigation into the use of conspecific trails as a form of social information

Abstract

Managing uncertainty is important for organisms to survive in a world in which resources are not always predictable. To reduce uncertainty, individuals may gather information from their own personal experiences or from the experience of others. The common garden snail (*Cornu aspersum*) provides a unique model to investigate the use of information transfer through its habit of leaving a mucus trail as it moves. I investigate the phenomenon of trail following in this species to determine whether unfed individuals use the trails left by individuals that have successfully foraged to guide their own behaviour. I show that the probability of trail following depends on the nutritional status of both the leading snail and the following snail. Unfed individuals were significantly more likely than fed individuals to follow the trail left by a fed individual than any other combination.

Introduction

Organisms exist in an uncertain world. Whether uncertainty pervades on a landscape-wide scale or simply through the changing behaviour of conspecifics, managing uncertainty is key to an organism's fitness. Gathering information in its many forms is crucial if an organism is to manage uncertainty and modify its own behaviour accordingly (Dall & Johnstone 2002).

Deciding where to forage is a key concern for many organisms and represents an important source of uncertainty. When an individual has prior knowledge of the landscape, such as the location or quality of resources, it may choose to use this information to direct its foraging behaviour. However, gregarious species may benefit from using the knowledge of others to improve their own foraging efficiency. When individuals are separated in either space or time, the ability to communicate useful information to conspecifics can prove beneficial for all.

If an organism's foraging trips are separated in time and resources are unpredictable or patchy, it may benefit an individual to communicate information that will increase the foraging efficiency of its future self and kin. Many ant species lay pheromone trails between a food source and the nest that become progressively stronger as more individuals follow the trail and deposit their own pheromone trail (Wolf & Wehner 2000), however because messages must remain long enough to be read at a later time, this information may be open to exploitation.

In contrast to social information, organisms may inadvertently produce information on the location and quality of resources which may allow others to benefit from the experience of others. Such inadvertent social information, or ISI, may act as a social attractant and might allow patchy resources to be optimally exploited (Dall *et al.* 2005). For example, the bumblebee, *Bombus terrestris* has been found to locate productive patches of flowers more efficiently when a demonstrator bee was seen to be foraging there (Baude *et al.* 2008).

If individuals are closely related, sharing information with kin may improve fitness (Sober 1992). However, this benefit may be reduced if unrelated individuals are able to eavesdrop on these messages (Waibel *et al.* 2011). In these circumstances, an

individual may offset the cost of sharing resources with unrelated individuals via indirect benefits, such as the use of many eyes to spot danger, or through simple dilution effects (Lima 1995).

The production of mucus is well documented in many species of gastropods and serves a range of functions, from aiding locomotion (Tankersley 1989) and digestion (Davies *et al.* 1990), to adhesion and protection from desiccation (Denny 1983).

Many species of gastropod possess a supropedal gland on the anterior third of the foot which consists of various mucus producing cells (Shirbhate & Cook 1987). The substances produced by these cells include proteins and muco-polysaccharides and combine with both acid and base substances to form the mucus that gastropods commonly leave behind them (Shirbhate & Cook 1987).

Analysis of the mucus secretions left by the marine limpet, *Patella vulgata* indicated seasonal variation in its protein, carbohydrate and ash content (Davies *et al.* 1990). Such variation was thought to be a product of differential nutrient partitioning as more resources were required for gonadal maintenance (Davies *et al.* 1990). A difference was also found in the carbohydrate content of small and large individuals suggesting mucus production may have a differing function depending on an individual's size.

Mucus production is key to gastrod locomotion, therefore, it can be hypothesised that its additional use as a means of communication, may have evolved, provided such messages provided a net benefit to the producer.. The carnivorous snail *Euglandina rosea* consumes other snails by following the trails they leave behind. Similarly, in a behavioural crossover, during courtship, *Euglandina rosea* pursues its potential mate

by following the mucus trail it leaves in its wake, until the two meet and copulate (Cook 1985).

It is clear that following mucus trails is a key component of gastropod ecology with potentially multitudinous underlying roles. In this paper I present a simple experiment designed to unravel the communicative role of trail following in the common garden snail (*Cornu aspersum*) in which the following questions are addressed; 1) Do snails show a preference for following the trails left by individuals from their own colony? 2) Does the prior feeding success of the follower snail influence its tendency to follow a trail? 3) Does the prior feeding success of the snail that left a trail influence the likelihood that it will be followed?

Material and methods

Thirty three snails were collected from four separate, geographically isolated colonies located in Penryn, Cornwall, UK.

Snails were housed in pairs, consisting of two individuals collected from the same colony in Perspex boxes, lined with capillary matting. Boxes were housed in an incubator at 24 degrees Celsius.

Each box was misted with warm water an hour prior to the start of the trials, in order to encourage activity in the snails and the boxes were returned to the incubator.

Half of the snail pairs were randomly selected and assigned to one of two conditions, fed or unfed. The fed boxes were provided with food ad libitum, consisting of spring greens and carrots, while the unfed condition was denied food for 14 days.

A trial arena was created consisting of a 10cm diameter circle printed on plain white paper.

A 'blazer' snail was randomly selected from the pool of available snails and was used to produce a 10cm mucus trail on the circular arena, starting at one edge.

A 'follower' snail was randomly selected from the pool of available snails, the arena was turned and the follower snail was placed at the starting point of the trail created by the blazer snail on the arena. A stopwatch was set for 15 minutes. After 15 minutes, if the snail stopped moving or if the snail reached the edge of the arena, the trial was stopped. Both the length of the follower snail's mucus trail and the length of the overlap between its trail and the blazer trail were measured.

The procedure was repeated twice with each follower snail for the following blazer snails: 1) An unfed snail from the same colony, 2) A fed snail from the same colony 3) An unfed snail from a different colony, 3) A fed snail from a different colony

At the end of each trial, the follower snail was weighed (g), the circumference of its aperture was measured (cm) and a mass index was calculated, by dividing the mass (g) by the aperture (cm) to account for size differences in mass.

Statistical analysis

Due to a bimodal distribution, data were transformed into those that follow and those that did not follow. Any individual with a trail following index of 0.4 or greater was classed as following while those less than 0.4 were classed as not following.

The logit probability of an individual choosing to follow a conspecific trail was calculated in order to analyse this binary response variable (Bewick *et al.* 2005) and further analysed via generalised linear mixed modelling. GLMM analysis controls for repeated measures and allows both fixed and random factors to be modelled (Schall 1991). The probability of following was analysed using GLMM, employing the

'feeding status of the follower snail', 'the feeding status of the blazer snail' and the 'colony' from which they came, as fixed effects. Individual variance was accounted for by including the 'identity of the blazer' and the 'identity of the follower' snail as random factors in the analysis. All data was analysed using R (R Development Core Team 2008).

Ethics

All work was conducted following ethical approval by the University of Exeter ethical approval board and a full risk assessment was conducted.

Results

Unfed follower snails showed a greater probability of following a trail left by a fed blazer, and were less likely to follow a trail left by an unfed blazer, as shown in Fig 5 (GLMM: $\chi^2 = 12.34$, $df=1$, $p < 0.001$). Fed followers showed no significant preference for following conspecific trails.

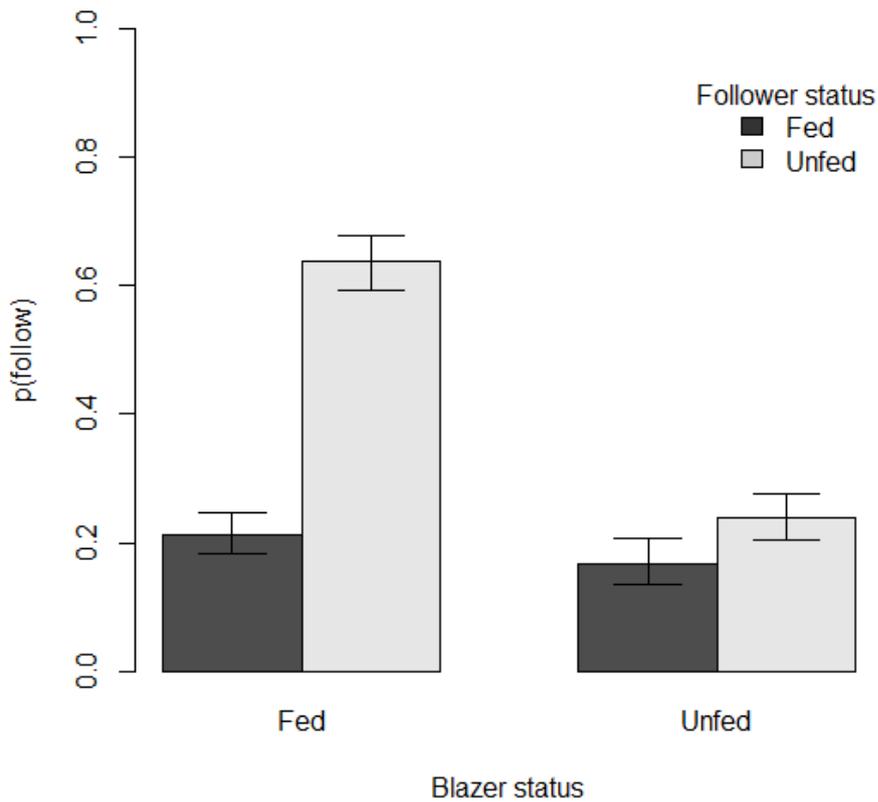


Figure 5 - Histogram of the probability of following a trail showing a significant interaction between the feeding status of the blazer and follower.

Although non-significant, a trend was found for the probability of following a trail decreasing with increasing mass index as shown in Figure 6 ($\chi^2 = 3.7206$, $df=1$, $p = 0.157$).

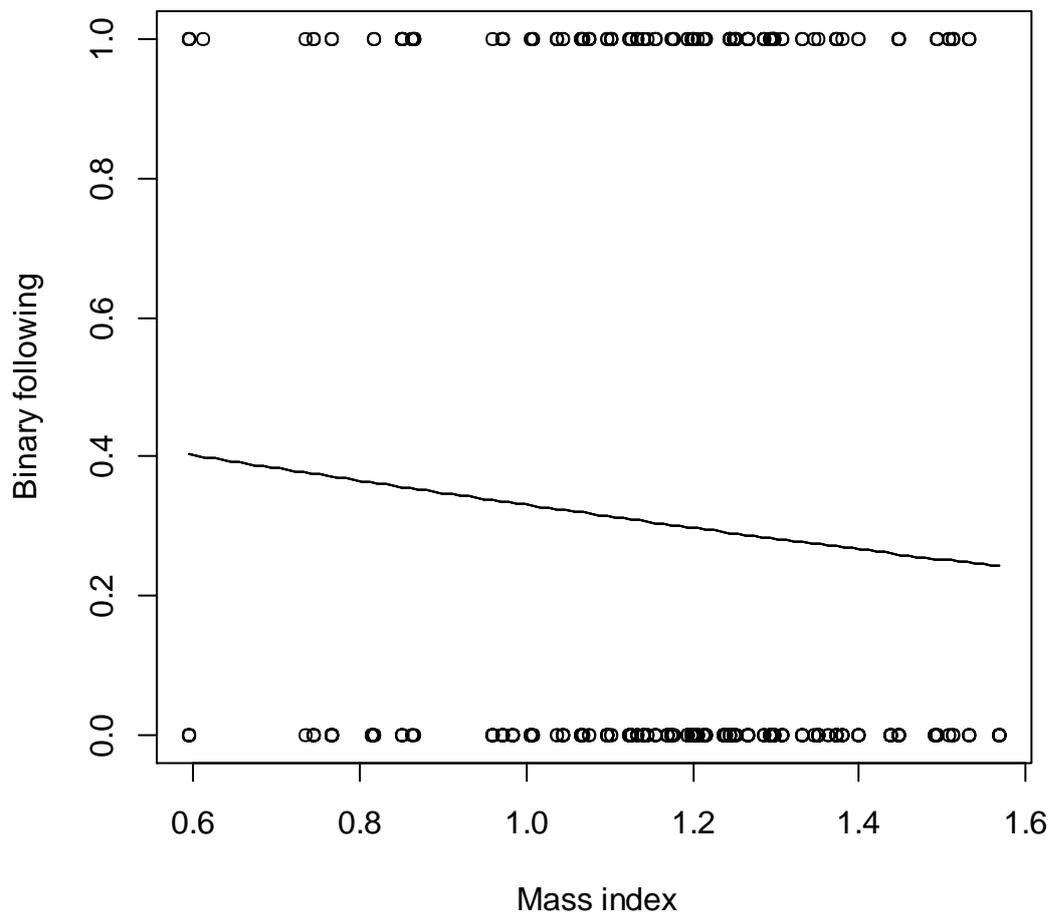


Figure 6 - The effect of mass on deciding to follow or not follow, showing an overall (non-significant) reduction in following with increasing mass.

The colony status (coming from the same or different colony) of the blazer snail did not significantly affect the probability of following, as shown in Figure 7 ($\chi^2 = 1.00$, $df=1$, $p = 0.315$).

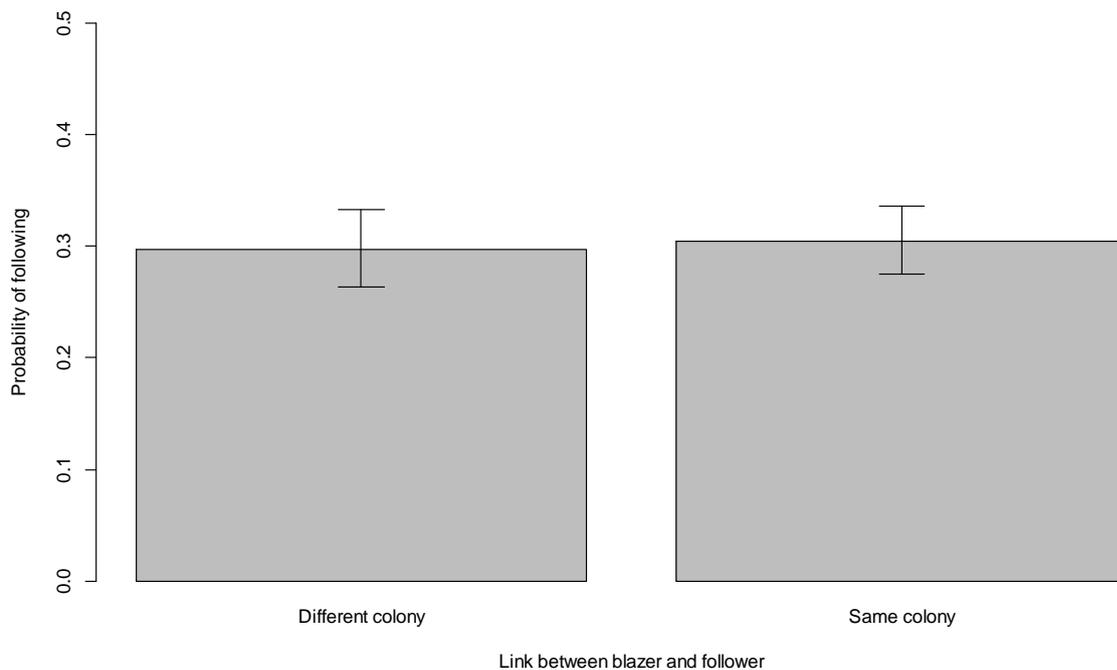


Figure 7 - Histogram of the probability of following depending on the similarity in the colony status of the blazer and follower snail, showing no significant effect of colony status.

Discussion

The feeding status of both the following individual and of the snail that produced the trail, significantly affected the probability of a snail following a trail or being followed. As the follower snail's somatic condition improved, so a trend toward not following was noted. These results indicate that *C. aspersum* uses information contained within the mucus trail along with its own nutrient profile to guide its foraging decisions.

By nature, snails are prone to desiccation and therefore, they must find food, consume it and return to safety within a limited period. Snails have strong olfactory abilities that enable them to locate food (Chase & Croll 1981) however, knowing the prior location of food may mean the difference between foraging success or failure. Should an individual reveal its foraging success in its mucus trail, it may allow it to find the food source efficiently the following evening, by following the trail it left, especially if the food is beyond its olfactory capabilities or downwind. Such a trail may be ripe for exploitation by conspecifics that may attempt to eavesdrop on the message left.

Trails left by individuals that had not recently fed were less likely to be followed, even by unfed individuals, suggesting that the trail maker's available resources have an impact on the composition of its mucus. Despite this difference it is not possible to say if fed individuals secrete substances to indicate their feeding success or if food compromised individuals are forced to modify their mucus composition in order to allocate scarce resources for somatic maintenance, revealing their foraging failure. The seasonal variation in mucus composition noted in *Patella vulgata* by Davies *et al.* (1990) correlated with gonadal changes, however, it was not clear how nutrient intake varied seasonally in this study.

Individuals with greater body condition showed a trend toward not following conspecific trails, indicating that an individual's available resources may influence its behaviour. As this research was conducted during daylight hours it can be suggested that individuals may have been motivated to seek cover to prevent desiccation. Such self preserving behaviour may help explain why unfed individuals of greater body condition were less likely to follow conspecific trails; having a greater reserve of resources may mean desiccation poses a greater risk than starvation.

Given the key role of mucus in the locomotion of many gastropods, it is likely that its composition in well-fed individuals conveys a greater benefit than attempting to change its composition to deceive others. Despite the potentially patchy distribution of food available to *C. aspersum*, the volume of food within those patches may mean direct competition for food is generally limited; therefore, the benefit of dilution effects may be great enough to encourage information sharing.

Solitary foraging organisms can assess the quality of a patch using two sources of information: their own experience or 'patch sample' information (Valone 1991), and 'pre-harvest' information, such as the memory of a patch location or olfactory information (Valone 1991). Whilst snails forage independently, their gregarious nature allows them access to a third information class, coined 'public information' with which they can assess the foraging success of conspecifics and opt to forage or not, in the same patch (Baude *et al.* 2008).

Being privy to such public information may help explain the way in which gastropods appear to consume newly planted seedlings within a few days of planting, as opposed to consuming them slowly. Potentially only a few pioneer individuals will be required to locate a new food patch for the rest of the population to follow suit. If these findings are replicated in free living individuals, then it may benefit gardeners to simply sweep or wash away the messages left the night before to limit the number of individuals locating a vulnerable patch.

Considering the evolution of information use in this context, one must consider any increase in fitness as a result of using social information, in comparison to its fitness had it ignored the information it was presented with (Seppänen *et al.* 2007). Under natural conditions when exposed to patch sample, pre-harvest and public information

along with an individual's condition and resource requirement, it may pay an individual to combine these data to guide its foraging decisions. As such, the importance of social information use in *C. aspersum* in a natural setting is a key area for future research.

Whether left as a direct signal to itself or others, or as a form of inadvertent social information due to resource reallocation, it is evident that *C. aspersum* can detect the difference between trails left by fed and unfed individuals and that they may use this public information to direct their own foraging behaviour. Future work to investigate the composition of the mucus left by fed and unfed individuals may help to ascertain if mucus messages are left for the benefit of others or simply represent a form of inadvertent social information.

Conclusion

Trail following is evidently key to the ecology of *C. aspersum* under controlled conditions, but its role in directing foraging behaviour in the wild may be less readily explained. Individuals show behavioural plasticity, by varying in their tendency to follow trails as a consequence of their own foraging success. This plasticity may have evolved to allow individuals to use public information only when absolutely necessary.

While these results reveal that the foraging success of others can be used by naive individuals to guide their own foraging behaviour, the underlying cause of these differences is less clear cut. It may be safe to say that mucus production plays such a pivotal role in the success of this species that its trails may convey multitudinous messages.

Chapter 3 – The metabolic implication of gastropod locomotion using conspecific trails

Abstract

Organisms must strive to conserve energy in order to allocate finite resources to reproduction to maximise fitness. Gastropod locomotion is costly due to the energy required for mucus production, such that mucus production greatly outweighs the cost of locomotion. I investigate the metabolic implications of the phenomenon of following conspecific trails in the common garden snail (*Cornu aspersum*) using a Field Metabolic System to measure CO₂ production. An indirect increase in locomotor efficiency was found, as individuals travelled faster on conspecific slime for no added metabolic effort.

Introduction

Organisms allocate a finite set of resources to key processes such as somatic maintenance, locomotion and reproduction. By considering this allocation of energy in terms of Darwinian fitness, it can be suggested that any energy an organism can save may be directed toward greater reproduction, increasing its overall fitness (Kozłowski 1996).

Measuring the cost of animal locomotion is comparable to measuring motor vehicle fuel economy; in essence the distance travelled per unit of energy. Any vehicle's fuel efficiency may be measured in miles per gallon of fuel; however, the reciprocal is

true for biological measurement which concentrates on the energy expenditure per unit travelled (Tucker 1975).

Various factors influence the amount of energy an organism uses to travel a given distance, namely speed of locomotion and mass. Any increase in locomotor speed requires greater energy input to travel a given distance. Organisms vary greatly in their mass both among species, and within species due to age or nutritive status. As a rule, a larger mass requires a greater effort to move; as such, an organism's mass must also be accounted for when calculating its locomotor efficiency (Tucker 1975).

Through the production of the energy required for locomotion, organisms release carbon dioxide (CO_2) as a metabolic waste product. The CO_2 released over a given time period can be used as a comparative measure of the organism's energy expenditure (Walsberg & Hoffman 2005). Therefore, to incorporate all of these variables into one numerical measure of locomotor efficiency, the organism's rate of CO_2 production must be divided by its mass, the distance it travelled and the time it took to travel that distance. This will provide the rate of CO_2 (ml/min) produced per unit of mass (g), per unit of distance (cm) per unit of time (sec) (Tucker 1975).

By incorporating these factors, species with very different physiologies can be compared, such that if species 'a' and species 'b' share the same body mass and CO_2 production but species 'a' travels twice the distance in a given time than species 'b', the efficiency of species 'a' will be greater than species 'b'. The unique form of locomotion employed by gastropods is both slow and metabolically costly, so we might ask how they optimise this unique adaptation for greatest efficiency.

Gastropods, such as snails, are well known for their habit of laying a mucus trail in their wake during locomotion. This thin layer (10-20 μm) of mucus is deposited and

pressure is placed upon it by its singular foot, providing propulsion (Denny & Gosline 1980). However, while this form of locomotion is common, it comes at a cost. The metabolic cost of the production of mucus has been shown to be 35 times more costly than that of locomotion (Denny 1980a). Despite these costs, the adhesive property of gastropod mucus lends has allowed gastropod habitat to expand into a three dimensional range (Denny & Gosline 1980), which may have been of particular benefit to herbivorous individuals.

Gastropods move by way of unidirectional, monotaxic, direct wave-like movements of the large foot, the speed of which is greater than the gastropod's propulsion (Lai *et al.* 2010). As the wave passes over a layer of non-Newtonian fluid, pressure differentials are generated due to the simultaneous compression and shearing of the fluid, generating propulsion through the forces acting on the foot (Chan *et al.* 2007). It is clear that one of the key functions of pedal mucus in gastropod locomotion is its adhesive viscosity that provides the friction and forces that are essential for the gastropod's foot to propel itself forward (Denny 1980b). As a non-Newtonian fluid, the mucus has been mathematically demonstrated to provide a substrate for this propulsive friction (Lauga & Hosoi 2006).

Given the great variation in habitat that gastropods now inhabit, there is potential for mucus deposition to have evolved secondary functions. Where the landscape is heterogeneous, hampering visual information, trails may aid navigation (Denny 1988) and following one's own trail may aid 'homing' behaviour (Santina 1994). Yet, given the high cost of mucus production, it is prudent to consider the direct metabolic benefit that trail following may impart.

Travelling on the mucus trail left by a conspecific has been shown to reduce the metabolic costs to the following individual. For example, the Marsh periwinkle, *Littorina irrorata* has been shown to require less pedal pressure to drive locomotion when crawling on a conspecific trail (Tankersley 1989). Trail following may also reduce metabolic costs by reducing the volume of mucus an individual needs to produce (Davies & Blackwell 2007). Given the large cost of mucus production and the prevalence of trail following, any metabolic benefits to following the trails left by others may help offset these costs.

Here I present an experiment in which the common garden snail, *Cornu aspersum* is used as a model to investigate the metabolic benefit to trail following using a field metabolic system, in which the following questions are addressed: 1) Does crawling on mucus affect the metabolic costs of locomotion?, 2) Does crawling on mucus affect the speed of locomotion?

I predicted that when following a trail, metabolic costs will be lower due to a potential reduced need for mucus production and due to the trail's smoother surface over which to crawl.

Methods

A sample of 26 *C. aspersum* were wild caught from 4 colonies in Penryn, Cornwall and housed in pairs in Perspex containers. Each was fed *ad lib* on a diet of carrots and spring greens and kept at a constant 21 degrees Celsius.

To measure changes in CO₂ and O₂ levels, a respirometry chamber was created. Two holes were drilled into a 10cm square plastic container, onto the lip of the container (without the lid) a rubber strip was glued using plastic adhesive; this

allowed as airtight a seal as possible to be made when this was placed on a glass arena. Clips were placed through the drilled holes and attached to rubber tubing that was used to attach to a Field Metabolic System (Field Metabolic System - Sable Systems) and data were acquired using Expedata software (version 1.3.3, Sable Systems).

A glass arena was created using a 60cm glass tank onto the underside of which a 1cm grid was stuck using adhesive tack. The area was divided in two: one half was clean and free of mucus, and the other used as a mucus arena. To create the mucus arena, a snail was gently wiped across the arena lengthways in one direction 30 times to ensure the whole arena was covered in mucus and was left for 20 minutes to dry.

The Expedata software was setup to record CO₂, O₂, water vapour and temperature which would allow the eventual calculation of the volume (ml) of CO₂ produced per minute (VCO₂).

Air was drawn through at a rate of 136ml min⁻¹ by an internal pump and gasses were analyzed using a Sable Systems FoxBox. The gas analyzers were calibrated before each use using O₂ free N₂, 1% CO₂ and dry outside air dried with drierite (Hammond) (Hawkes *et al.* 2014).

Each snail was chosen at random for testing and its mass was recorded (g). It was placed on the no-slime arena (with the FMS at baseline). When the snail began to move, the chamber was placed on top of it and a marker was placed on the Sable recording screen. The snail was observed and the length of locomotion was measured by visually counting the number of grid squares covered. Recording was stopped by placing a marker on the Sable screen and removing the chamber once

the snail had either 1) stopped moving, 2) reached the edge of the arena or 3) crawled 20cm.

The snail was placed on the slime arena and the procedure was repeated. The non-slime arena was cleaned with warm water and dried, and the procedure was repeated three times.

Following each trial, the entire arena was cleaned and a new slime arena was created for each snail.

Analysis

VCO_2 was calculated with the following equation:

$$\frac{FR \times (CO_{2e} - CO_{2i})}{(1 - CO_{2e})}$$

In which, FR= flow rate (ml/min), CO_{2e} = Fractionated maximum CO_2 , CO_{2i} = Fractionated minimum CO_2

The volume of CO_2 produced per trial (ml/trial) was calculated by dividing the VCO_2 per trial by 60 to calculate ml CO_2 per second and multiplying this figure by the length of the trial (sec).

The volume of CO_2 produced per cm (ml/cm) was calculated by dividing the volume of CO_2 per trial (ml) by the distance travelled (cm).

The volume of CO_2 produced per unit speed (ml/cm/sec) was calculated by dividing the volume of CO_2 per trial (ml) by the speed of locomotion (cm/sec).

The speed of locomotion (cm/sec) was calculated by dividing the distance (cm) travelled by the time taken (sec).

Following VCO_2 calculation, mass distance and speed was accounted for by calculating a measure of efficiency using the following equation, hereby referred to as 'efficiency':

$CO_2 / \text{mass} / \text{distance} / \text{time}$ (Tucker 1975).

Paired T-Tests were conducted comparing the volume of CO_2 produced per trial, the volume of CO_2 produced per cm, the volume of CO_2 produced per unit speed, the speed and the efficiency of snails crawling on slime and not on slime and a generalised linear mixed model was employed to measure any interactions between the slime conditions and mass (Schall 1991). The speed of travel was analysed via GLMM, with 'mass' and the 'presence' or 'absence' of a slime trail as fixed factors. Individual variance in speed was accounted for by including 'snail ID' as a random factor in the analysis.

The volume of CO_2 produced, per unit speed was also analysed via glmm, with 'mass' and the 'presence' or 'absence' of a slime trail as fixed factors. Individual variance in the volume of CO_2 produced per unit speed, was accounted for by including 'snail ID' as a random factor in the analysis. All data were analyzed using R (R Development Core Team 2008).

Results

Snails produced significantly more CO_2 (ml) per trial when not travelling on slime compared to travelling on slime ($t=3.386$, $df=24$, $p=0.02$) but there was no significant difference in the volume (ml) of CO_2 produced per cm travelled ($t=1.635$, $df=24$,

$p=0.11$) and there was no significant difference in the volume (ml) of CO_2 produced per second ($t=-0.85$, $df=24$, $p=0.399$).

There was significantly less CO_2 produced per unit speed (per second, per cm) when snails were travelling on slime than when not travelling on slime ($t=4.272$, $df=24$, $p<0.001$) (see Fig 8) and snails were significantly more efficient (ml of CO_2 per gram, per cm, per second) when travelling on slime than when not travelling on slime ($t=3.914$, $df=75$, $p<0.001$) (see Fig 9).

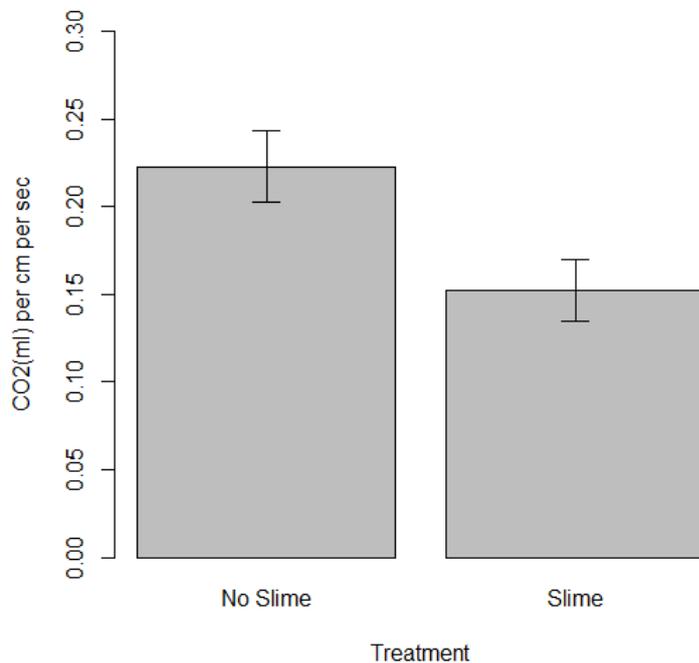


Figure 8 - Mean volume of CO_2 per cm per second, showing snails produced less CO_2 per unit of time when crawling on slime

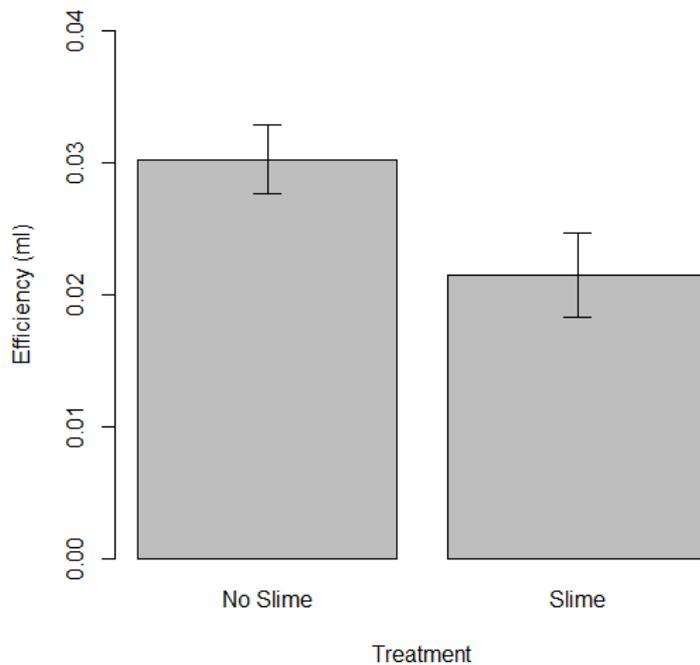


Figure 9 Mean efficiency (ml CO₂ per gram, per cm, per second) showing greater efficiency when crawling on slime

Crawling on slime significantly affected the speed of locomotion as shown in figure 10 (GLMM: $\chi^2 = 12.028$, $df = 1$, $p < 0.001$) with snails crawling on slime travelling significantly faster than those not crawling on slime ($t = -3.85$, $d.f. = 24$, $p < 0.001$), (see Fig 10), but there was no significant affect of mass on speed (GLMM: $\chi^2 = 2.994$, $df = 1$, $p = 0.08$).

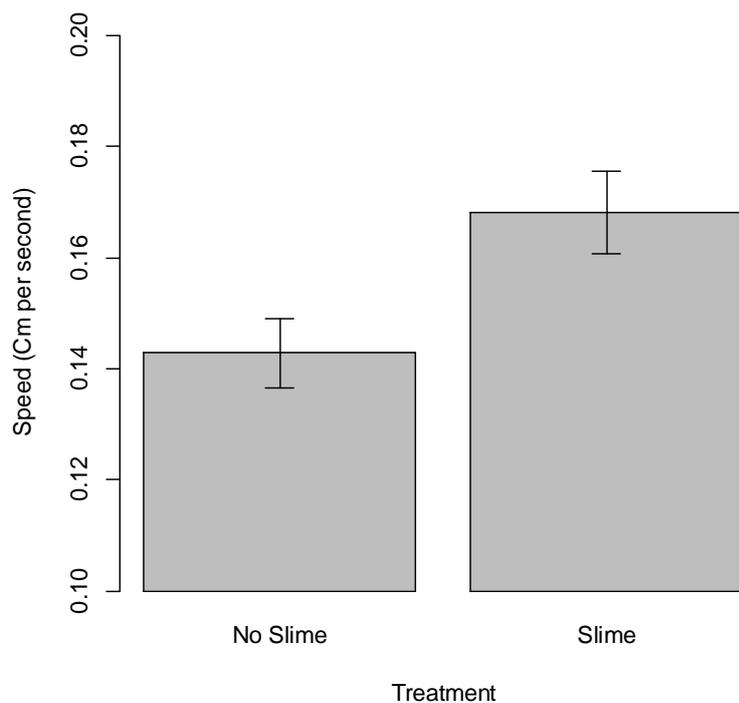


Figure 10 - Mean speed of locomotion (cm per second) showing snails travelled faster on slime

The volume of CO₂ produced per unit speed increased with mass when not crawling on slime, but this effect was reduced when crawling on slime (see Fig 11), as shown by a significant interaction between slime treatment and mass on (GLMM: $\chi^2=4.084$, $df=1$, $p<0.001$).

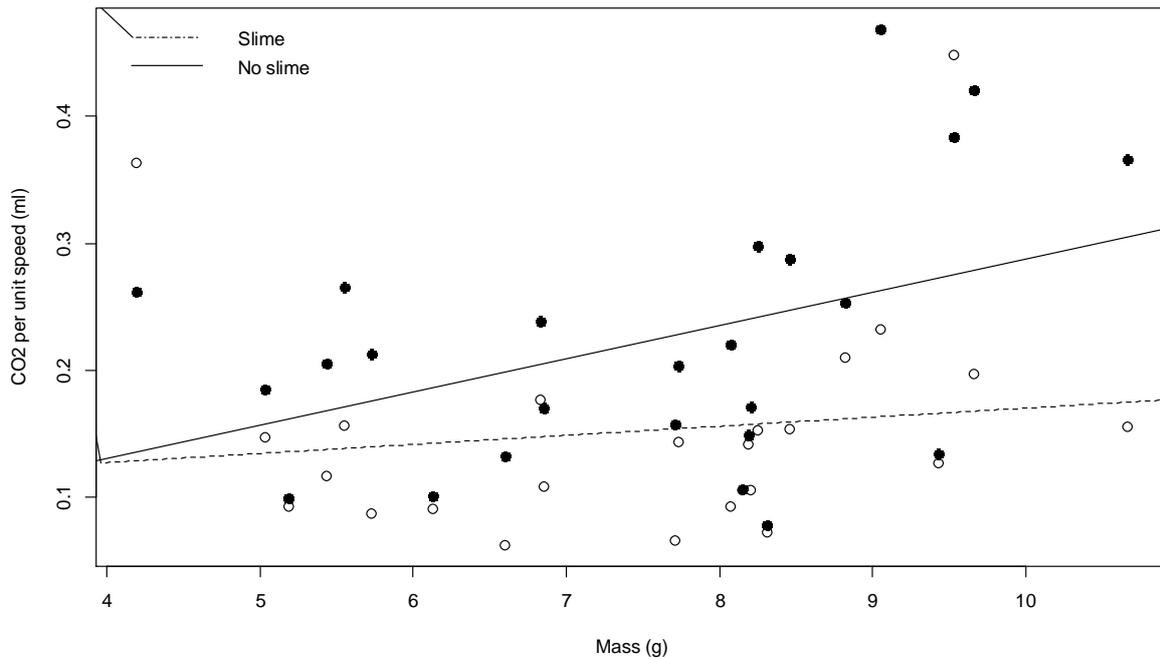


Figure 11 - Volume of CO₂ (ml) per unit speed by mass showing CO₂ per unit speed increased with mass when not crawling on slime, but this was reduced when crawling on slime

Discussion

Crawling on conspecific mucus allowed snails to crawl faster for the same energetic expenditure, thus effectively saving energy. As a snail's mass increased, its efficiency decreased, but this trend was reduced when snails were crawling on slime.

Snails saved energy through increasing the speed of locomotion when crawling on slime as shown by finding no difference in both the volume of CO₂ (ml) produced per cm travelled and the volume (ml) of CO₂ produced per second. This was further supported by finding less CO₂ (ml) was produced per unit speed (per second, per cm) and a greater efficiency when snails were travelling on slime.

The complex interaction between an individual's mass, speed and locomotor behaviour are likely indicative of the high metabolic cost of mucus production and the benefit snails receive from this unique form of locomotion.

These results suggest it may benefit larger snails to produce a greater volume of mucus to reduce the metabolic cost of locomotion, however, mucus production has a far greater cost than the actual act of locomotion (Denny 1980a) therefore, any cost saving would likely be negated by the added cost of mucus production.

As a snail increases in mass it can be hypothesised that while the size of its foot increases with mass, the relationship between mass and foot size may not be isometric. This is supported by earlier work that found a lower level of foot loading (foot to mass ratio) in larger individuals than would be expected under isometric allometry (Tattersfield 1989).

The propulsive friction required for forward motion relies upon changes in the non-Newtonian mucus in response to pressure from the ventral foot providing a suitable substrate (Denny 1980b), therefore, these results demand further investigation into the relationship between foot loading and the propulsive friction force involved in locomotion and the adhesive force required to prevent dislodgment.

That trail following allows energy to be saved is clear, however, the overall indirect saving through an increased speed of locomotion is compelling. Previous research has shown that individuals with average or lower than average standard metabolic rate were favoured by selection (Artacho & Nespolo 2009), which lends support to the energetic definition of fitness in which reproduction can be considered in terms of units of energy (Kozlowski 1996).

Any energy saved through the act of trail following may allow greater energy to be directed toward reproduction, increasing lifetime reproductive success, or fitness. In travelling faster for the same energetic cost when travelling on slime, it is likely that this strategy not only saves energy as the overall duration of locomotion is shorter for a given distance, but it may allow individuals to forage more efficiently and for a longer duration.

The risk of desiccation and predation restricts *C. aspersum* to foraging in the cooler, damp, dark evenings during which there are fewer avian predators and the risk of desiccation is lessened. In order to survive they must return to a safe day time roost where they can seal themselves inside their shell, preventing desiccation (Bailey 1981). This restriction on foraging activity means snails must forage and return to safety within a specific window, with the cost of not doing so being desiccation and ultimately mortality.

It is easy to see how increasing speed through following conspecific trails is ultimately of great benefit and may allow more resources to be consumed in a given period, with a reduction in the length of time needed to commute to and from the roost, allowing a longer foraging period and potentially producing a net positive energy budget.

The extreme cost of using mucus for locomotion, particularly the cost of mucus production, suggests the benefit lies in it allowing snails to forage in a three dimensional environment (Denny & Gosline 1980). Therefore, saving energy through following the mucus trails of conspecifics may help recoup some of these high costs. The gastropod foot is complex and there are many forces involved in its use in locomotion. It is therefore necessary to gather further data to elucidate the exact

means with which the use of conspecific mucus trails generates these metabolic savings.

Conclusion

The following of conspecific trails allows individuals to save energy through increased speed at no added metabolic cost. This was supported by finding crawling on slime increased efficiency (CO_2 per gram, per cm, per second) and reduced the volume of CO_2 per unit speed (cm per second) but finding no significant effect of crawling slime on CO_2 per trial, or per cm, or per second.

Efficiency decreased with mass when not crawling on slime, but this lessened when crawling on slime, suggesting this behaviour may benefit some individuals more than others.

Gastropod locomotion is complex and costly and the relationship between trail following and energy saving is equally multifaceted. In order to understand the role saving energy plays in the evolution of trail following behaviour, it is essential to consider the many other potential benefits such as mate finding, navigation and acquisition of information on habitat quality.

Chapter 4 – The prevalence and behavioural implication of infection of the snail *Cornu aspersum* with the tropically transmitted nematode, canine lungworm (*Angiostrongylus vasorum*)

Abstract

Parasites have a complex ecology and many must utilise multiple hosts through trophic transmission in order to reproduce. The canine lung worm *Angiostrongylus vasorum* is a parasite that affects both wild and domestic canids such as the red fox (*Vulpes vulpes*) and domestic dog (*Canis lupus*) and causes infection via a gastropod intermediate host. I investigate the prevalence of parasite infection in the common garden snail (*Cornu aspersum*) in a park in Swansea, Wales and the effect infection has on intermediate host defensive behaviour. No infection was found in all individuals tested (N=68) therefore no conclusion could be drawn on the effect of infection. Further research is needed to ascertain if *C. aspersum* is a suitable host for *A. vasorum* or if these results are due to increased preventative medication of domestic dogs with commercial anthelmintics.

Introduction

Parasites are broadly considered to be organisms that live in or on the body of another species in order to obtain food and/or shelter (O'Connor 1985). Whilst many species complete a whole life cycle within a single organism, a great number require an intermediate host, within which its larval form may develop, before eventually reproducing within its end host.

While the evolution of trophically transmitted species with such a complex multi-host life cycle may appear hard to explain, it has been suggested that the addition of an intermediate host can improve transmission to the intended host (Choisy *et al.* 2003). Initial evolutionary pressure to survive the death of a host following its predation, may have encouraged trophic transmission, as the most straightforward means of surviving the predation of a host, is to simply parasitise the host's predator (Lafferty 1999).

In areas in which the density of the intended host is low, the addition of an intermediate host facilitates transmission of the parasite; for example the tapeworm *Echinococcus multilocularis*, that infects red foxes (*Vulpes vulpes*) has no critical intended host density at which the parasite would die out (Vervaeke *et al.* 2006).

In order for a trophically transmitted parasite to be transmitted to its intended host, its intermediate host must be predated by its intended host. Given the importance of this transmission, it may be beneficial for the parasite to increase the likelihood that its intermediate host will be consumed by changing its behaviour, such as increasing its vulnerability. Such parasite increased trophic transmission, or more specifically, 'parasite increased susceptibility to predation' (Beckage 1997), may increase the parasite's fitness through the simple act of increased transmission (Lafferty 1999).

Should the behaviour of a parasitised host differ from that of unparasitised conspecifics, it might be considered that the behaviour has been modified by its parasite. However, while it was once considered that host pathology was unaffected by coevolved parasitism, in fact the cost of parasitism can be high (Minchella 1985). The host needs only to survive for long enough for it to be predated by the intended

host for the parasite to complete its lifecycle; therefore, the cost of parasitism cannot be discounted if behavioural phenotypic differences are evident.

It has been suggested if both direct and indirect costs of parasitism to an intermediate host's reproductive success are high, then host resistance may develop. Such resistance itself may also incur a fitness cost compared to those without the resistant genotype therefore, a high likelihood of infection is required to drive the evolution of host resistance (Minchella 1985).

Such resistance may be either passive, in which the somatic environment is simply unfavourable for the parasite, or active in nature in which an immune response is elicited, encysting and destroying any parasites (Lie *et al.* 1977). It might be assumed that both forms may incur a cost, with the latter being the most costly (Minchella 1985). Laboratory experiments with the aquatic snail *Biomphalaria glabrata*, a polymorphic species that may be resistant or susceptible to the parasite, *Schistosoma mansoni* found the resistant phenotype to be at a selective disadvantage compared to susceptible individuals, even in the presence of the parasite (Minchella & Loverde 1983).

This interesting paradox suggests that the cost to reproductive success to resistant individuals may be so great, that in mixed populations containing both phenotypes, there is no selective advantage to being resistant. The factors underpinning this cost are unknown, although a reduction in competitive ability of resistant individuals has been proposed.

Whatever the mode of action, the selective disadvantage to resistance may explain the continued prevalence of both the susceptible host and the parasite in this

instance and may suggest that the costs of susceptibility to parasitism are not great enough to drive selection for resistance.

The potential variability in host susceptibility to infection is key when ascertaining the risk of parasitism in domestic species, such as commercial livestock and pets. Of these parasites, canine lungworm, *Angiostrongylus vasorum* is an emerging disease in the UK, having first been noted in imported greyhounds (Jacobs & Prole 1975). It has been confined to areas of the South West England and South Wales but is thought to be spreading throughout the UK.

The canine lungworm is a metastrongylid nematode of canids which, as adults, resides in the heart and pulmonary arteries, causing a range of disease symptoms, from coughing and dyspnoea to heart failure and death (Morgan *et al.* 2005).

The parasite requires an intermediate gastropod host within which its larvae develop into L3 larvae, before being consumed by a canine host such as the domestic dog (*Canis lupus*) (Chapman *et al.* 2004), or *V. vulpes* (Morgan *et al.* 2008).

While *A. vasorum* has a number of intermediate and intended hosts, the common garden snail (*Cornu aspersum*) represents one of the most readily recognisable for laypeople. A simple online image search for the terms 'lungworm' and 'snail' generate numerous images of *C. aspersum* with 50% of the first 10 images showing this species.

The familiarity this species has gained may be due in part to its habit of living in parks and gardens and its conspicuous nature. Living in close proximity to people *C. aspersum* may represent a key intermediate host for *A. vasorum* and a source of infection for *C. lupus* and urban *V. vulpes*.

Should infection with *A. vasorum* change the behaviour of *C. aspersum*, especially if such changes result in increased trophic transmission, then this may play a role in its potentially increasing prevalence in the UK. Equally, such parasite increased trophic transmission may suggest that only a low population of unprotected *C. lupus* or *V. vulpes* may be required to maintain the risk of infection.

Here I present a study into the prevalence and behavioural implication of *A. vasorum* infection in *C. aspersum* from an urban population, in which the following questions are addressed: 1) Is *C. aspersum* a host to *A. vasorum*? 2) Does parasite status influence *C. aspersum* defensive behaviour?

Material and methods

1) Behaviour experiment

Sixty eight *C. aspersum* were collected from an urban park in Swansea, Wales. This area was selected due to its documented high prevalence of *A. vasorum* in canine hosts. These were separated into 2 groups (n=34) and housed in an incubator at 21 degrees Celsius.

One group was fed *ad libitum* with carrots and spring greens and the other was denied food for 2 weeks.

Each *C. aspersum* was individually numbered with correction fluid and pen.

Prior to the start of the trials, individuals were misted with warm water to encourage activity and returned to the incubator. Each was removed in turn and placed on a clean piece of paper. Each was tapped gently using a finger on the shell and a stopwatch was started. The time at which it exuded a) its foot and b) both of its antennae, was noted.

This procedure was repeated 10 times for each individual.

The feeding condition of both groups of *C. aspersum* was then alternated so that the fed group was denied food and the food deprived group was fed and both were left for a two week period. The experimental procedure was repeated as previously.

2) Ascertaining parasite status

Each *C. aspersum* was euthanised by crushing between two plastic boards which had been covered with cling wrap. Using forceps, each was masticated and placed into a plastic test tube containing a 50ml solution of 0.7% HCL and 0.5% pepsin and incubated at 37 degrees Celsius for 2 hours (Jarvi *et al.* 2012).

After incubation, any *A. vasorum* larvae were collected via a modified Baermann technique (Baermann 1917) in which each digested sample was poured into a small piece of muslin cloth and tied with an elastic band. This was then suspended inside a lukewarm water filled funnel with a clamped piece of 10m rubber tubing attached and left for 24 hours.

After 24 hours one ml of water was drawn off the bottom of the sample and observed via light and phase contrast microscopy for the presence of *A. vasorum* larvae.

All data was analysed using R (R Development Core Team 2008).

Ethics

All work was conducted following ethical approval by the University of Exeter ethical approval board and a full risk assessment was conducted.

Results

The mean difference (sec) in the time taken for antennae to be exuded between the first and last trial was 60 seconds (± 254.7).

The distribution of the time taken for antennae to be exuded indicated most individuals showed little difference apart from a small number of individuals at either extreme (as shown in Fig 12).

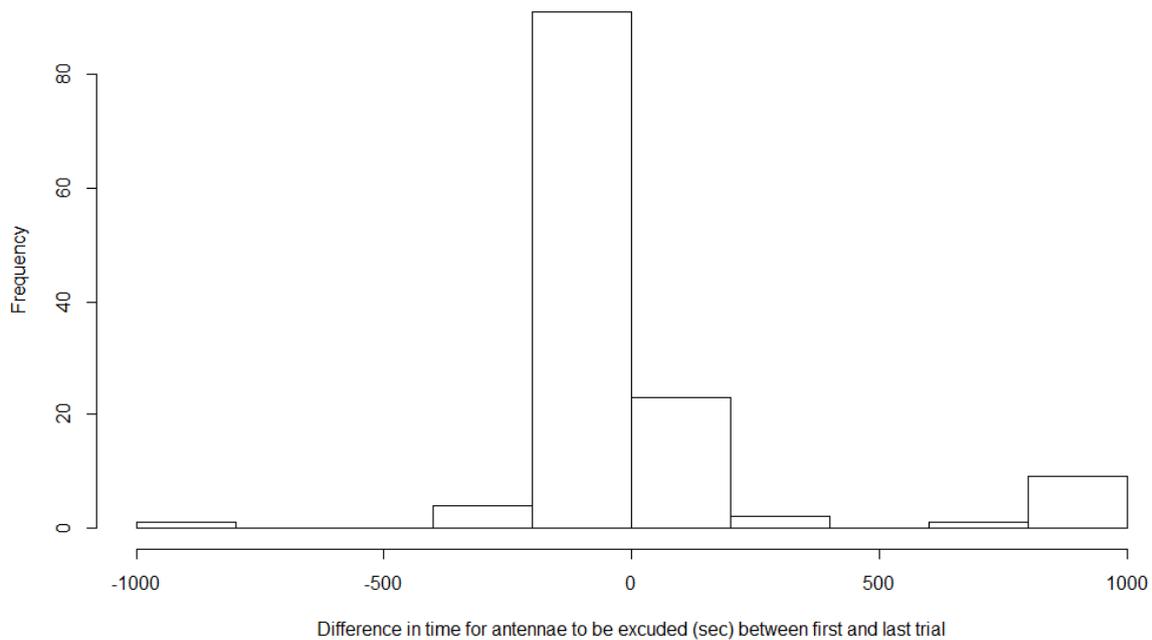


Figure 12 - Frequency histogram of the difference in the time for antennae to be exuded between the first and last trial indicating very little difference in the mean time.

No *A. vasorum* larvae were found in any of the sample snails therefore no formal statistical calculation was performed.

Discussion

No *A. vasorum* larvae were found in the sample analysed. The area from which the snails were collected has a high incidence of *A. vasorum* in dogs, therefore, these results suggest that either *C. aspersum* is not a common intermediate host to *A. vasorum* or its level of infection is so low as to not be readily identifiable.

Finding no individuals infected with *A. vasorum* is certainly compelling, given the reported high canine infection in the area from which they were collected. Should the selective disadvantage of individuals with an unsusceptible phenotype as found by Minchella & Loverde (1983), be applicable to *A. vasorum*, we might expect to find more infected than uninfected individuals.

When such unexpected results are obtained, the potential for methodological errors must be considered. While the methodology employed had been proven valid for Jarvi et al (2012), being unable to obtain a sample of known parasite status with which to validate the methodology means the possibility of concluding a false negative parasite status cannot be discounted.

The effect of seasonal variation on the lack of infected individuals found cannot be discounted. The sample snails were obtained in early November – while studies suggest *V. vulpes* has a higher incidence of infection in these months, the prepatent period of *A. Vasorum* indicates the season for infection would be greatest in spring (Morgan *et al.* 2008). Further investigation with repeated sampling over multiple seasons would address this potential confounding variable.

Alternatively, the lack of infection may be indicative of *C. aspersum* not being a preferred intermediate host of *A. vasorum* and thus attention for the control of

intermediate hosts in high risk areas may be best focussed toward other species. Previous studies have also failed to find infected *C. aspersum*, adding support to these findings. A study by Kim *et al*, found no *C. aspersum* infected with the similar rat lungworm (*Angiostrongylus cantonensis*) in 8 separate study locations (Kim *et al*. 2014). The authors suggested that this lack of infection may be due to their sample being collected from locations greater than 600m above sea level, however, the sample was collected from just 18m above sea level therefore such altitude effects can be discounted.

A further study conducted in New Caledonia found a similar lack of infection in over 100 individuals (Ash 1976). The low elevation of this collection site, along with these findings, suggest elevation effects may not be involved in the lack of infection in this species.

The lack of infection found in multiple studies, suggests physiological mechanisms common to *C. aspersum* across populations, may limit their suitability as a host; specifically mechanisms that serve to limit the maturation of larvae.

It has been found that compounds within the haemolymph of *C. aspersum* inhibit the maturation of the nematode *Rhabditis maupasi* that matures within in snail's mantle. Such an effect was found to be non-species specific, reducing both the number of the insect grub parasite *Neoplectana glaseri*, that reached maturation and the number of larvae each female produced (Ratanarat-Brockelman 1975).

Further investigation into the prevalence of *A. vasorum* in other gastropod species in the sampled area may help explain if these results are indicative of physiological mechanisms or an overall reduction in infection across species.

Given that earlier work suggested that anti-parasitic mechanisms are so costly that unsusceptible individuals are at a selective disadvantage compared to susceptible individuals, it might be proposed that the sample population was not heterogeneous for resistance, with all individuals expressing the resistant phenotype. Alternatively, the particular costs of parasitism in this sample may have been so high as to negate this selective disadvantage, or the particular resistant mechanism involves little investment of resources.

Conclusion

No snails were found to be infected with *A. vasorum* therefore it is not possible to state how this parasite might affect the behaviour of *C. aspersum*. Understanding the relationship between the parasite and both its intermediate and final host is important to ensure the health of both domestic and wild animals.

Finding no infected individuals demands further investigation as there are several potential hypotheses that may explain this. Primarily, it may be suggested that *C. aspersum* is simply not a suitable host for this parasite and further research to experimentally infect this species may allow this hypothesis to be tested.

Assuming *C. aspersum* is a suitable host, the lack of infection found may be indicative of greater vigilance by pet owners and veterinarians in the sampled area in ensuring domestic dogs are given preventative anthelmintics, therefore closing a loop in the infection cycle. Further research into infection prevalence in multiple gastropod species as well as *V. vulpes* and *C. lupus* in the study area may help to provide a clearer picture of how these findings can be interpreted.

Conclusion

The common garden snail is an extremely successful species and is well adapted to the niche it occupies. From these findings it is evident that it has a range of behaviours that aid its foraging efficiency and may be implicated in its success. Of all the behaviours studied to date, the use of conspecific trails may play many crucial roles in maximising resource acquisition while minimising energy expenditure. It is clear this trail following behaviour has many benefits depending on the individual circumstances and it may help explain why this species is able to thrive in a range of habitats.

Despite the high cost of mucus production and its use in gastropod locomotion, these results show it has multiple functions including aiding communication, increasing locomotor speed and increasing locomotor efficiency. Benefiting from the foraging information of others may maximise foraging efficiency, particularly when resources are unpredictable or patchy. In finding that unfed snails preferentially follow fed individuals, it is clear snails can determine which conspecifics have successfully foraged and in a laboratory setting were found to use this to guide their own behaviour. To further understand the relevance of this behaviour, research is required to investigate its use in a natural setting.

It is clear that the use of conspecific trails has metabolic benefits to the following individual through increased speed of locomotion; however, the relationship between a snail's mass and its use of conspecific trails is less straightforward. In finding that larger individuals benefit from the use of conspecific trails more than smaller individuals, leads one to question the plasticity of this behaviour in the wild. In

essence, larger unfed individuals face a dilemma; is the reduced cost of following the trail of another individual worth the extra competition for resources?

The energetic demands on an individual snail may depend on multitudinous factors, from the distribution of resources to the pressure from potential parasites. The lack of *A. vasorum* infection may be due in part to nematode inhibitors within the hemolymph which increase the metabolic burden on those with a resistant genotype (Minchella & Loverde 1983). Accounting for parasite status when investigating the prevalence of trail following may shed new light on both the plasticity of the behaviour and the metabolic tradeoffs involved in parasite resistance.

While these data suggest the benefits of using conspecific trails are many, it is clear that this behaviour can be used to our advantage to limit the access gastropods have to areas we wish to protect. These data suggest that foraging efficiency will be reduced by physically limiting the number of pioneering individuals that discover a new food source, by employing a physical barrier. With fewer individuals leaving a mucus message to inform others of the location of the food source the speed at which the numbers of foraging individuals build will be lower than if the food was unprotected. Conversely, in finding the barrier to not be permeable these data indicate that those individuals that passed through the barrier, will indeed reveal to others where the food source is and how to access it, therefore only delaying the inevitable consumption of the food.

It is clear that conspecific trails likely play a key role in the ecology of *C. aspersum* and their use may have multiple functions. This knowledge may guide the development of new and innovative methods of gastropod control through the removal of trails, potentially reducing foraging efficiency and subsequently breeding

success. However, before this is attempted, further research must be conducted to ascertain how these findings are mirrored in a natural setting.

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