An investigation of gardening in the sedentary caddisfly *Tinodes waeneri* across a nutrient gradient

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I certify that this thesis, and the research to which it refers, are the product of my own work, and that any ideas or quotations from the work of other people, published or otherwise, are fully acknowledged in accordance with the standard referencing practices of the discipline. I acknowledge the helpful guidance and support of my supervisors, Dr Jonathan Grey and Professor Alan Hildrew.

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This thesis is dedicated to Tom.

Abstract

Sedentary species face a trade-off between the advantages of exploiting food close to their homes and the cost of defending it. Consequently, the net benefit of this lifestyle may be greatest at intermediate productivity. In aquatic systems, it has been suggested that some sedentary grazers can increase the range of circumstances under which they are able to compete with mobile grazers by enhancing food resources within their feeding territories through 'gardening'. This was examined for the retreat-building sedentary larvae of the caddis *Tinodes waeneri*, which are often dominant in the littoral of lakes. The hypotheses tested were 1) *T. waeneri* gardens by fertilising its retreat (a fixed 'gallery' on which periphyton grows), and 2) gardening will be more important in lower productivity lakes. Detailed field sampling across a lake productivity gradient was coupled with a laboratory mesocosm study. A natural abundance stable isotope technique was developed to identify gardening. A survey of six populations in the English Lake District indicated that larvae garden as they fertilise gallery biofilm with excreted nitrogen and feed on their galleries. Galleries also contained more food than the epilithon and larval assimilation of galleries was related to food availability. Galleries contained a higher proportion of diatoms than the epilithon, and gallery diatom communities were associated with higher nutrient levels, especially in the lower productivity lakes. Gardening also occurred in the experimental mesocosms. Furthermore, the amount of gardening was related to nutrient levels; more gardening occurred at low nutrients than at high nutrients. Thus, 'gardening' is widespread in *T. waeneri* populations and may allow this species to be successful in low resource environments. It may also substantially affect ecosystem processes within the littoral of lakes by influencing patterns of nitrogen retention and enhancing overall productivity.

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Chapter 2

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Chapter 1 - Introduction

1.1 General introduction

Traditional ecological theory primarily focuses on the importance of abiotic factors and negative biotic interactions, such as predation, competition and parasitism in influencing the structure of communities. However, positive interactions have increasingly been shown to be important in shaping community organisation, as well as in influencing the success of individuals and the distribution and growth of populations (Bruno *et al.*, 2003; Brooker *et al.*, 2008). Facilitative interactions occur where at least one organism benefits from the interaction and no organisms are harmed (Bruno *et al.*, 2003). Mutualisms are facilitative interactions where both of the interacting species benefit more than they are harmed (Hay *et al.*, 2004). Ecosystem engineers, which generate state changes in biotic or abiotic materials and thus modify the environment and create or maintain habitats for other species (Jones *et al.*, 1994), can also be classed as facilitators where their effects are positive.

Herbivores can have important impacts on ecosystem function, due to their position within networks, sandwiched between the plants they consume and their own consumers (Schmitz, 2008), and can therefore mediate effects passing up or down food chains (Schmitz, 2008). Thus, the way in which herbivores respond to resource limitation, the stoichiometric mismatch between plants and themselves, the trade-off between resource capture and predation risk, as well as their feeding mode, will all have the potential to influence key ecosystem processes (Schmitz, 2008). Herbivores are generally perceived to have negative trophic interactions with plants. However, both trophic and ecosystem engineering interactions can result from a single action by a herbivore (Wilby *et al.*, 2001). Moreover, whilst plant-herbivore interactions are negative when viewed from a pairwise perspective, they can actually be mutualistic in a community setting (Hay *et al.*, 2004). For example, territorial herbivorous damselfish maintain algal mats, on which they graze (Hay *et al.*, 2004; Irving & Witman, 2009). The presence of the damselfish allows a richer and more diverse community of filamentous algae to exist, than occurs elsewhere (Ceccarelli *et al.*, 2001). Although the resident consumes these algae, defence of the algal turf ensures that the filamentous algae are not grazed to local extinction by other herbivorous reef inhabitants (Irving & Witman, 2009). In addition, algal productivity is higher within the algal turf (Ceccarelli *et al.*, 2001), further benefiting the filamentous algae (Hay *et al.*, 2004). Thus, in this type of mutualism,

although plants are consumed by the herbivore, this is offset from the plants perspective by the fact that the presence of the herbivore reduces the total amount of consumption of the plant community and/or increases the productivity of the community (Hay *et al.*, 2004). The herbivore benefits from having exclusive rights to the food resource.

The range of environmental conditions under which a species is able to maintain viable populations (fundamental niche) is usually reduced through the impacts of inter-specific interactions, in particular competition (and this is the realised niche). However positive interactions occur between species (as in the case of facilitation) and may counteract the reduction in niche space. Under some circumstances the realised niche has the potential to be larger than the fundamental niche (Bruno *et al.*, 2003). Thus, facilitation may increase the range of conditions over which a species can exist, compared to when it lives alone (Bruno *et al.*, 2003).

Species which are facilitators under one set of conditions have the potential to be negative interactors (such as competitors or predators) under a different set of conditions (Daleo & Iribarne, 2009) and their ecological importance is also likely to change depending on environmental conditions (Crain & Bertness, 2006). Bruno and Bertness (2001) predicted that both the strength and importance (relative to other factors under consideration) of positive interactions should increase with increasing background environmental stress. Thus, where environmental stress is low, the facilitator is of no benefit to the species in question, and negative competitive interactions will be more important (Bruno & Bertness, 2001). In contrast, where environmental stress is high, it is predicted that the impacts of facilitation will be more important, although the magnitude of this will depend on whether competition between the two species remains constant over the environmental stress gradient (smaller effect of facilitation), or whether competition also decreases with increasing environmental stress (larger effect of facilitation; Bruno & Bertness, 2001). Where the two species interacting occupy different trophic levels, competition between the two species is likely to be negligible (Bruno & Bertness, 2001). The environmental stress model of Menge and Sutherland, (1987), which predicts that as one moves from benign to stressful environments predation, competition and then abiotic stress will be the most important variables structuring the community, has been modified to predict the importance of facilitation relative to other interaction types (Bruno & Bertness, 2001; Bruno *et al.*, 2003). Predictions suggest that positive interactions should be most important in

reducing environmental stress at medium high to high stress levels (Bruno *et al.*, 2003; Brooker *et al.*, 2008) and also be the most important factor (as compared to competition, predation and abiotic factors) influencing community structuring at these stress levels. However, as environmental stress increases there will come a point where the facilitator will no longer be able to shield the associated species from the stress and at this point abiotic factors will have the greatest effect on community structure (Bruno & Bertness, 2001). Similarly, ecosystem engineers that are predicted to have the greatest positive impacts on a community are those which alleviate resource or other limitations through their presence (Crain & Bertness, 2006).

Environmental stresses can include physical and biological stresses as well as resource limitation (Bruno & Bertness, 2001). In the littoral of lakes, benthic organisms, including grazers, will experience gradients of environmental stress. These include physical stresses such as turbulence, wave-washing, water level changes (Scheifhacken *et al.*, 2007) and water chemistry (e.g. pH, Schartau *et al.*, 2008), and biological stresses including food availability (Harrison & Hildrew, 2001), which may be limiting to grazers under some circumstances (Lamberti, 1996). Lakes vary widely in the amount of nutrients contained within the water column and are classified on this basis (Lampert & Sommer, 2007). Primary producer biomass and production, including that of the periphyton (a key resource for littoral grazers), is positively related to nutrient levels in the water column (Tolonen *et al.*, 2005). Therefore lake productivity can be viewed as an environmental stress gradient.

Here I investigate the possibility that the grazer *Tinodes waeneri* (L.) is able to maintain dominance over a large range of lake productivity due to a positive gardening interaction. A nutrient gradient is employed to characterise the interaction and assess the effectiveness of the interaction at low resource levels.

In the next sections, periphyton and the key factors that affect it are introduced and the ideas behind consumer-mediated fertilisation of resources are discussed. Further, the constraints of a sedentary lifestyle are outlined and gardening is described. Information on the ecology of *T. waeneri* is presented and preliminary indications of gardening in this species are summarised. As one of the main tools used to study gardening was stable isotope analysis, a brief outline of this technique is also included. Finally, the main aims and structure of the thesis are outlined.

1.2 Periphyton

Periphyton is a natural assemblage, predominantly consisting of algae, fungi and bacteria (Lowe, 1996) that can be found attached (by adhesive, carbohydrate-rich exopolymers (EPS); Wotton, 2004) to most surfaces in aquatic habitats. Where periphyton is attached to hard substrata that are larger than algal cells, such as gravel, cobbles and boulders, it is classed as epilithon (Stevenson, 1996). Photosynthetic benthic assemblages in lake littoral habitats are similar to those in streams and contain mainly cyanobacteria and a wide variety of algae; green algae, diatoms and sometimes red algae (Lowe, 1996). Furthermore, they are extremely species rich and diverse, and will therefore contain species with a range of traits and tolerances (Darcy-Hall, 2006). Periphyton has a three dimensional structure, (Boston & Hill, 1991), with the vertical component becoming more pronounced during community development. Algal succession on suitable surfaces generally starts with the colonization of prostrate (adnate) algae, which are tightly attached to the substratum. Apically attached erect forms are the next colonizers and they shade the adnate algae. Erect forms are then overgrown with filamentous algae and stalked diatoms, which have better access to water column nutrients and lights. Motile species are able to navigate their way around the filaments and survive in these communities (Stevenson, 1996).

Periphyton often dominates primary production in littoral habitats (Hillebrand, 2002), and can make a substantial contribution to whole lake productivity (Vadeboncoeur *et al.*, 2008). Furthermore, periphyton is important in lake nutrient cycles (e.g. Axler & Reuter, 1996) and as a carbon source to lake food webs (e.g. Hecky & Hesslein, 1995; Hadwen & Bunn, 2005). Additionally, periphyton communities can stabilise sediment and provide invertebrates, such as meiofauna and chironomids, with shelter (Stevenson, 1996).

The biomass, quality (e.g. C:N:P ratio) and composition of periphyton can be influenced by a range of factors. These include the availability of crucial resources such as nutrients (Borchardt, 1996), light (Hill, 1996; Qin *et al.*, 2007) and space (Lowe, 1996), as well as by abiotic factors such as temperature (DeNicola, 1996), pH and other water characteristics, substratum type (e.g. Wetzel, 1983a; Burkholder, 1996; Vadeboncoeur *et al.*, 2006), water currents or wave-washing (Cattaneo, 1990), and other physical disturbances (Steinman & McIntire, 1990). Furthermore, biotic factors such as grazing and algal pathogens can also influence periphyton biomass, stoichiometry and

composition (Steinman, 1996). Nutrient availability and grazing are two of the most commonly studied factors (e.g. Rosemond *et al.*, 1993; Hillebrand & Kahlert, 2001; Darcy-Hall & Hall, 2008) and important in the Lake District (King, 1999) where the fieldwork for this thesis was undertaken. In particular, nutrient (bottom-up) versus grazing (top-down) control of benthic algae has been investigated in detail, and meta-analyses suggest that both may be important in limiting periphyton (Hillebrand, 2002; Gruner *et al.*, 2008). However, other combinations of factors have also been investigated (e.g. Lamberti *et al.*, 1989; Marks & Lowe, 1993; Liess *et al.*, 2009).

1.3 Nutrients and periphyton

Epilithic algae either take up nutrients from the water column (for which they may have to compete with phytoplankton: Hansson, 1990; Axler & Reuter, 1996) or nutrients recycled internally within the biofilm (King *et al.*, 2006). Nitrogen and phosphorus are both commonly limiting to primary producers in freshwater systems (Elser *et al.*, 2007), and these are the most studied nutrients. Impacts of nutrients and nutrient limitation on the biomass and production of lake periphyton have been studied extensively using nutrient enriched substrata (e.g. Fairchild *et al.*, 1989; Marks & Lowe, 1993; King, 1999; Maberly *et al.*, 2002), fertilisation of the water (e.g. Pringle, 1990; Hillebrand & Kahlert, 2001) and by comparisons across gradients of productivity and nutrient availability (e.g. Cattaneo, 1987; Liboriussen & Jeppesen, 2006; Vadeboncoeur *et al.*, 2006).

The growth rate can be controlled by the rate at which the limiting nutrient is supplied, although potential yield will be determined by total nutrient availability (Borchardt, 1996). The nutrient status of benthic algae can be determined through comparison of their C:N:P ratio with an optimal C:N:P ratio (Kahlert, 2001) calculated as 158:18:1 by Kahlert (1998) and 119:17:1 by Hillebrand & Sommer (1999) for benthic algae. Very high C:N:P ratios are associated with nutrient limitation (Kahlert, 1998), and in addition, the identity of the limiting nutrient can also be derived from C:N:P ratios. For example, C:P ratios of over 369 and/or N:P ratios of over 32 indicate P limitation, whilst C:N ratios of over 11 may indicate N limitation (Kahlert, 1998).

Lake littoral periphyton C:N:P ratios can be very variable both temporally and spatially (Kahlert, 2002; Fink *et al.*, 2006). However, Darcy-Hall (2006) in her cross lake study (14 lakes in Michigan TP 13.5–77.5µg l^{-1}; TN 208.9-1869.0µg l^{-1}) reported the greatest

benthic algal nutrient limitation in the low nutrient lakes. Moreover, benthic algal limitation declined significantly along the nutrient gradient; algae in high productivity systems were less nutrient limited than benthic algae growing in low productivity lakes (Darcy-Hall, 2006).

Algal communities have the ability to respond quickly to changes in nutrient availability since algae can reproduce rapidly (Darcy-Hall, 2006). Furthermore, different species within the periphyton probably have different nutrient requirements. Some species are also likely to be better competitors for nutrients than others (Borchardt, 1996), and therefore nutrients levels may influence community structure. For example, there may be distinct algal guilds depending on the nutrient that is limiting (Carrick *et al.*, 1988). It is possible to define precisely the optimum nutrient concentrations for each taxon (Hall & Smol, 1999), for example through the use of 'transfer functions' (e.g. King *et al.*, 2000; Miettinen, 2003; DeNicola *et al.*, 2004). This has been exploited (especially in the case of diatoms) to examine eutrophication and recovery in palaeolimnological studies (e.g. Bennion *et al.*, 1996; Barker *et al.*, 2005) and in the ecological assessment and monitoring of water bodies (e.g. Kelly *et al.*, 2008).

1.4 Impacts of grazers on periphyton

Periphyton is the main food source available to freshwater herbivores (although grazing on macrophytes does also occur, Lodge, 1991) and the interaction is characterised by the relatively small size of the individual algae in relation to the body size of the grazers.

Consumption of periphyton by grazers generally results in a reduction in algal biomass (e.g. in ~70% of studies reviewed by Feminella & Hawkins, 1995 and Steinman, 1996). Although, biomass-specific production often increases in response to grazing, the effect is usually insufficiently large to compensate for losses of biomass due to grazing (Feminella & Hawkins, 1995). Grazers can also have an impact on algal community composition and structure, with the most pervasive effect being a reduction in percentage of over-storey algae and an increase in the contribution of under-storey algae (Steinman, 1996). Over-storey algae may be more easily dislodged and consumed by grazers, although this will depend on the grazer in question; caddisflies and snails, that respectively scrape and rasp, had the biggest impact in the review by Steinman (1996). Under-storey algae are tightly attached to the substratum and may therefore be more

resistant to grazing. Some algal species may also be unpalatable and thus favoured by grazing. Examples of unpalatable species include filamentous algae such as *Oedogonium* spp. (Steinman *et al.*, 1992) and algae which produce large amounts of mucilage such as *Chaetophora* spp. (Liess & Kahlert, 2009). There is very little suggestion that grazers are able to selectively consume algal species (Steinman, 1996), although feeding efficiencies may vary for different species (Peterson, 1987).

Any impacts of grazing on periphyton that are not directly related to consumption can be classed as indirect impacts. The importance of these indirect effects is often positively related to the amount of biomass removal by the grazer (Liess & Hillebrand, 2004). Three categories of indirect effects have been identified (Liess & Hillebrand, 2004). Firstly, grazers can indirectly increase the abundance of an algal taxon through grazing on its competitors. This could occur through selective grazing (but see above), passive selection as a function of grazer mouthpart morphology, or grazer avoidance of unpalatable species. Secondly, predators, either through direct impacts on grazer density or indirect impacts on grazer behaviour (Peckarsky *et al.*, 2008), may result in an increase in periphyton abundance (although this will depend on whether periphyton is limited by top-down or bottom-up mechanisms). Lastly, grazers may increase periphyton abundance through alleviating other constraints on periphyton production. For example, grazers may be able to increase habitat availability for periphyton or increase availability of nutrients limiting periphyton production. Grazers have been found significantly to lower periphyton C:N and C:P ratios across studies (Hillebrand *et al.*, 2008) and there are three main pathways through which they are able to do this. Firstly, grazers may reduce the proportion of detritus in the periphyton (detritus is C rich and nutrient poor; Frost *et al.*, 2005). Secondly, grazers may increase nutrient supplies by changing the structure of the periphyton, reducing cell numbers and disrupting boundary layers (Lamberti & Moore, 1984). All these changes will mean that each remaining algal cell will have increased access to water column nutrients or to nutrients recycled internally within the periphyton mat. Lastly, excretion or egestion of nutrients by grazers may increase the supply to the periphyton (Hillebrand *et al.*, 2008). This last mechanism has been the focus of much recent research and is discussed in more detail in the next section.

1.5 Resource fertilisation and consumer-mediated nutrient recycling

Nutrients are released by consumers either as faeces or as excretions. Excretions are the most direct way in which consumers can release nutrients, as they are usually produced in an inorganic form and are thus readily assimilated by algae and bacteria (Vanni, 2002). Consumer excretions are recognised as playing an important role in nutrient dynamics within benthic communities (e.g. Devine & Vanni, 2002; Vanni, 2002) as well as in pelagic systems (e.g. Urabe *et al.*, 2002). For example, excretions from the benthic invertebrate assemblage in a desert stream were found to supply up to 70% of N required by the algal community (Grimm, 1988), and the fish assemblage in a neotropical stream was calculated to provide up to 49 and 126% of algal N and P demand, respectively (Vanni, 2002).

Individual species have increasingly been recognised as having significant impacts on nutrient recycling. The New Zealand mud snail (*Potamopygrus antipodarum* (Gray)) excretes sufficient ammonium to fulfil an estimated 65% of the demand of biofilm in a productive stream (Hall, 2003) and P recycling by zebra mussels (*Dreissena polymorpha* (Pallas)) had a major influence on P fluxes in Lake Erie (Arnott & Vanni, 1996). In an experimental stream study, excretion by the snail *Elimia clavaeformis* (I. Lea) was estimated to provide approximately 14% of the P taken up by the periphyton (Mulholland *et al.*, 1991).

The impacts of consumers on nutrient dynamics will depend on the abundance of animals as well as their body size (Vanni, 2002; Hall *et al.*, 2007) and identity (Evans-White & Lamberti, 2005). Thus, spatial and temporal variation in species distribution and abundance can influence nutrient recycling. Where organisms are both patchily distributed and play important roles in mediating biogeochemical cycling, as is often the case in streams (McIntyre *et al.*, 2008) and lake littorals, biogeochemical hotspots (*sensu* McClain *et al.*, 2003) may occur. For example, spatial variation in fish density and community composition gave rise to nutrient recycling hotspots in a neotropical stream with a diverse fish community (McIntyre *et al.*, 2008). Furthermore, consumer-mediated nutrient recycling is predicted to be most important to nutrient dynamics in systems where background nutrient concentrations are low (Cross *et al.*, 2005) and be less important where they are high (Evans-White & Lamberti, 2006), although little is known as yet about how the impacts of this process vary along gradients in productivity (Vanni *et al.*, 2006).

Grazers in freshwater systems are often limited by the quantity and quality of their food. In contrast to periphyton, which can be highly variable in terms of stoichiometry, consumers are much more constrained in their body C:N:P ratios across systems and time (e.g. Frost *et al.*, 2003; Liess & Hillebrand, 2005; Fink *et al.*, 2006). Moreover, grazers often have much lower C:N:P ratios than the periphyton they are consuming (e.g. Fink *et al.*, 2006). Thus, there is commonly an elemental imbalance between the grazer and its food source. Grazer growth rates are reduced where food is of poor quality, although food quantity modifies this relationship (Frost & Elser, 2002; Stelzer & Lamberti, 2002; Fink & Von Elert, 2006). A further consequence of elemental imbalances between grazers and the periphyton is that they will lead to differences in the relative release of nutrients (N and P) in grazer excretions (Sterner & Elser, 2002), although other factors such as periphyton community composition may also play a role (Rothlisberger *et al.*, 2008).

Thus, nutrient excretions provide a potentially important feedback mechanism (Elser & Urabe, 1999) through which grazers have the potential to alter the quantity and/or quality of their food supply (Hillebrand *et al.*, 2008). In benthic habitats, resource fertilisation through grazer excretions has largely been studied in mobile grazers (especially gastropods, e.g. Liess & Haglund, 2007). Excretions from small grazers that live in close association with periphyton (e.g. some chironomids) may have greater impacts as excreted nutrients are likely to be more efficiently retained and taken up by the biofilm community. Conversely, excretions from large grazers are more likely to be washed away (Frost *et al.*, 2002b). Similarly, consumer-mediated nutrient recycling may be much more important in sedentary grazers, where excretions can be better targeted at feeding sites, and thus increase the quantity or quality of food available to individual grazers.

1.6 Sedentary grazers

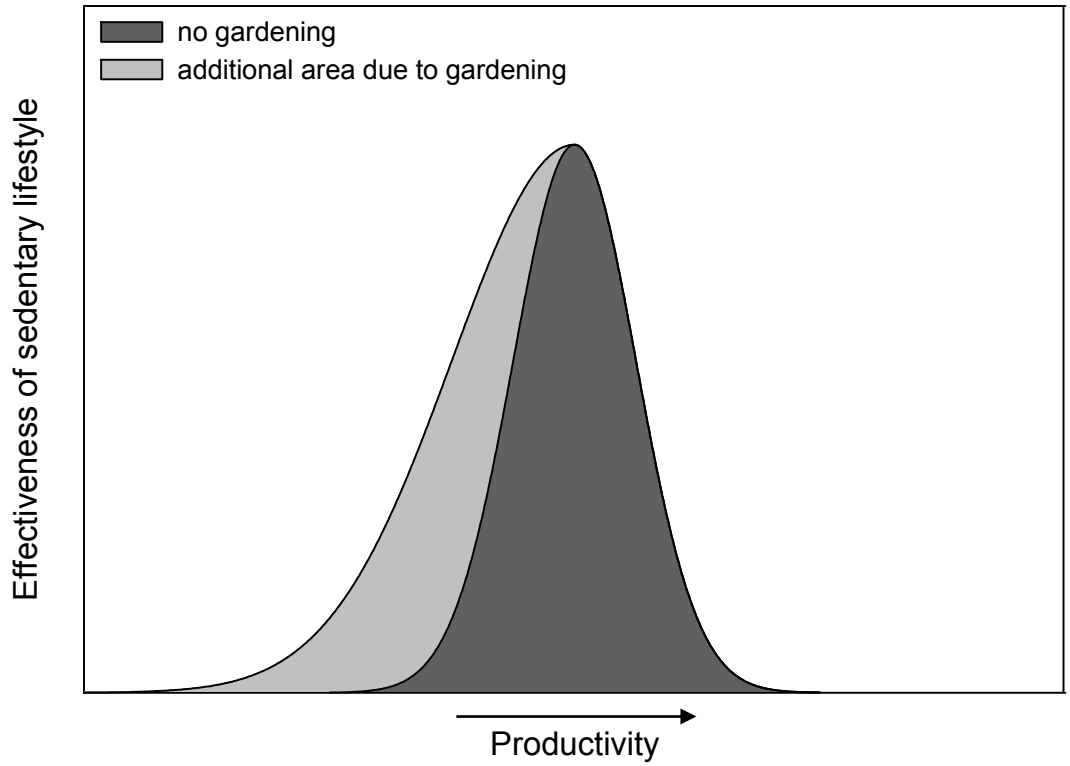
Sedentary animals are common in the benthic communities of both streams (McAuliffe, 1984b) and lake littorals (Harrison & Hildrew, 1998b) and can often be locally abundant (Bergey, 1995) and dominant (Harrison & Hildrew, 2001). Whilst mobile grazers can easily locate and switch between good feeding patches, sedentary grazers need to obtain all necessary resources from the area surrounding their retreats and sometimes also from the retreat itself (Bergey, 1995). This can, therefore, potentially limit their distribution

and abundance. Sedentary grazers often defend these feeding patches (McAuliffe, 1984b) against intruders (presumably to maximise food availability) and they can thus be viewed as being territorial (in line with the definition of Brown and Orians, 1970).

Territoriality has been postulated to occur only where the resource in question is both limiting and defendable (Brown, 1964). Defensibility in this instance includes the energetic and physical costs of defence to the individual as well as whether it is actually possible physically to defend the resource (Brown, 1964). Models developed and tested using nectar feeding birds indicate that territoriality should only be a viable strategy where territories are economically defensible. In other words, territories should only be maintained where the benefits gained through the increased food supply outweigh the costs of defence (Gill & Wolf, 1975; Carpenter & MacMillen, 1976). Therefore, where food is generally plentiful there is no advantage to occupying and defending a territory. Conversely, there will be a point at which the availability of food is so low that costs of defending a space large enough to satisfy food requirements are too great to be met by the additional amount of resources obtained (Gill & Wolf, 1975; Carpenter & MacMillen, 1976). Furthermore, it has been argued that the renewal rate of a resource, as well as its standing crop, may be important in determining the conditions under which territoriality should occur (Hart, 1987). Thus, along a gradient of background resource availability, territoriality is only viable somewhere in the middle of the gradient (at intermediate resource levels and/or renewal rates, Fig. 1.1), although the exact region will be dependent on the species as well as other biotic and abiotic factors.

These predictions will also be relevant to territorial grazer species in freshwater systems as algal patches often form discrete entities that can be physically defended (Wiley & Warren, 1992). Further, grazers are often considered to be food limited ((McAuliffe, 1984b; Lamberti, 1996), with high levels of inter- and intra-specific competition often being apparent (e.g. McAuliffe, 1984a; Feminella & Resh, 1990; Kohler, 1992). Data for the sedentary hydroptilid caddisfly *Leucotrichia pictipes* (Banks) confirm that these models are applicable to freshwater benthic systems. Hart (1985a) reported that in fifth instar *Leucotrichia* larvae, which are sessile in fixed cases, territory size was related to both density of a limiting resource (food) and larval resource requirements (territory size increased when food was removed from within the territory). These territories were also defended from con-specifics and other grazers.

Figure 1.1 The impact of gardening on the effectiveness of a sedentary lifestyle across a productivity gradient.



1.7 Gardening

Sedentary species may be able to alter the balance between resource availability and the cost of defence of a territory through gardening. Gardening has been defined as 'interactions in which plant assemblages within a fixed site are modified through the activities of a grazer that selectively enhances the food value of the grazed plants for the grazer' (Plagányi & Branch, 2000). This definition can be extended to include microbes as well as plants and food value should be viewed both in terms of quality and quantity. In terrestrial systems, short-lived invertebrate grazers are only likely to influence food quality for later generations (e.g. Frost & Hunter, 2007). However, in freshwaters, grazers may be able to have more immediate impacts on periphyton communities as the latter contain very small organisms with short generation times which rapidly respond to changes in their environment. Thus, gardening should be a particularly useful strategy for sedentary species in aquatic systems.

The simplest form of gardening (and least active) is based on the provision of a substratum, creating more space for algal or microbial growth (Woodin, 1977; Liess & Hillebrand, 2004; Stief *et al*., 2005). A second method of gardening is weeding (e.g. in damselfish; Hata & Kato, 2002). Weeding has been shown to occur in a caddisfly (*Leucotrichia*) which feeds on epilithic algae just outside its case. The algal community in its feeding area is dominated by diatoms rather than the cyanobacteria which dominate elsewhere. Gut contents analysis and displacement experiments have shown that the caddisfly larvae remove (but do not ingest) the cyanobacteria from their territories and thus reduce competition for their preferred food source (Hart, 1985b).

However, fertilisation of the algal or microbial community through the excretion and egestion of nutrients is the most commonly reported form of gardening (e.g. Connor & Quinn, 1984; Pringle, 1985; Hershey *et al.*, 1988; Plagányi & Branch, 2000; Stief & Becker, 2005; Deines *et al.*, 2007), and will lead to nutrient cycling occurring within a more or less closely coupled system. The effectiveness of this gardening method is likely to depend on a number of factors, including:

1) the nutrient status of the system as a whole; benthic algae in a eutrophic system would be expected to be able to meet nutrient demands through uptake from the surrounding water, and therefore grazer nutrient excretions should make little difference to algal nutrient status (Kahlert, 2001).

2) the stoichiometry (C:N:P ratio) at which the nutrients are re-supplied and the stoichiometry of all the components of the system (Liess & Hillebrand, 2004).
3) the physical environment; in streams excreted nutrients are likely to be washed away more quickly than in lakes, making them less available for algal and microbial assimilation.

As well as enhancing food value, other benefits of gardening to a grazing consumer may include: survival during periods of food stress such as floods (Cox & Wagner, 1989), provision of a more predictable food supply (Woodin, 1977), extension of the conditions under which a grazer can live (especially in relation to nutrients) (Fig. 1.1), an increase in the density of consumers which can occupy a suitable habitat patch, and a reduction in competition between them for limiting food.

However, gardening may also have important feedbacks on the whole system as it may influence the cycling of nutrients and enhance primary productivity, as well as aiding local nutrient retention. Gardening may thus have an impact on overall ecosystem processes within freshwater systems. Furthermore, it may affect community diversity and composition by altering the competitive balance between species, and may provide a habitat for a greater range of algal and microbial species than would otherwise be present.

1.8 The study organism - *Tinodes waeneri*

1.8.1 General ecology

Tinodes waeneri (Trichoptera: Psychomyiidae) inhabits stony substrata in the littoral zone of lakes, but is also present in rivers (Edington & Hildrew, 1995). It is common and often dominant in mesotrophic and moderately eutrophic lakes (Edington & Hildrew, 1995). For example, it occurred in all 36 Danish lakes of this type examined by Bordersen *et al*., (1998) and accounted for 20% of all animals collected from the stony littoral of the 39 lakes examined.

Larvae build galleries made of sediment bound by silk on the surfaces of rocks, and periphytic algae account for about 90% of all the biomass associated with the gallery silk (Kahlert & Baunsgaard, 1999). Food particles and nutrients may adhere to the silk: blackfly silk has been found to have high sorption coefficients for a range of pesticides (Brereton *et al*., 1999) and silken nets of the hydropsychid *Cheumatopsyche* Wallengren

form sites of calcium carbonate precipitation in a karst stream (Drysdale, 1999). However, studies of net spinning caddis, suggest that non-sieving mechanisms (particle adhesion to the net structure) play only a minor role in food capture (e.g. Edler & Georgian, 2004; Loudon, 1990).

Gut contents analysis has shown that the diet of the larvae generally consists of algae, detritus and inorganic material (Dall *et al.*, 1984), although Dunn (1954) also reported the presence of some animal remains.

Tinodes waeneri was found to have three distinct life-cycles in Lake Esrom, Denmark (Dall *et al.*, 1984). Populations from sheltered sites were univoltine (one generation a year) whilst those from exposed sites were bivoltine (two generations a year). Another population studied in this lake also had two flight periods but only a proportion of the cohort that was laid as eggs in early summer emerged later in the summer, with the rest emerging the following year (Dall *et al.*, 1984).

1.8.2 Preliminary evidence for gardening

There are several lines of evidence that point to a potential role of gardening in the nutrition of *T. waeneri. Tinodes waeneri* larvae can occur at extremely high densities within the stony littoral zone of lakes, with values of 11500 individuals m^{-2} recorded at one site in Lake Esrom (Dall *et al.*, 1984) and densities of 1000-2000 individuals m^{-2} commonly reported elsewhere (e.g. Hasselrot, 1993a). Traditionally it has been suggested that the larvae feed by scraping algae from rocks immediately outside their galleries (Dall *et al.*, 1984), but at such high larval densities food may become extremely limiting if epilithic algae are the sole food source.

Silk production in *Tinodes* is costly (calculated as ~20% of total annual production for two cased caddis species; Iversen, 1980; Huryn & Wallace, 1985), as it contains high levels of amino acids (Alecke *et al.*, 2005), yet galleries are several times longer than the resident larva (Alderson, 1969). The galleries are therefore probably larger than they need to be simply to provide protection from predation and displacement by waves and currents. Furthermore, laboratory-derived time budgets of *T. waeneri* larvae suggest that they spend 35% of their time feeding, but only approximately 1% of their time extruding from the gallery (Hasselrot, 1993a). Similar observations have also been made for *T. rostocki* McLachlan (Stief & Becker, 2005).

Hasselrot (1993a) found that ammonia concentrations were higher (by 35-40μg l^{-1}) inside the gallery than just outside the wall. He went on to suggest that larval egestion and excretion of nutrients could be responsible for this difference. These egested/excreted nutrients could be taken up and assimilated by the algae on the gallery wall, which are also the main food source for the resident. In other words *T. waeneri* may be gardening. Although this requires confirmation, Hasselrot (1993a) also suggested that diatoms were the main component of this system as their motility gave them the unique opportunity to make use of both the high light outside the gallery and the high nutrient concentrations within. Further circumstantial evidence of nutrient recycling between the larva and the algae living on the gallery wall has been obtained using ^{15}N addition. Galleries were found to assimilate ^{15}N faster and to a greater extent during the incubation period, and retained it a week longer than the epilithic community (Kahlert & Baunsgaard, 1999).

However, the above work has only been carried out in a single lake (Lake Erken in Sweden) and no rigorous test of gardening in *T. waeneri* has yet been carried out. Further work is required to test whether *T. waeneri* does garden, and whether gardening is a general phenomenon in this species. In particular, study of this interaction over a nutrient gradient, will provide an understanding of its importance as a facilitative mechanism that may allow *T. waeneri* to live under lower nutrient conditions (i.e. occupy a wider niche) than would otherwise be possible. Natural abundance stable isotope analysis is a good method for investigating gardening.

1.9 Use of natural abundance stable isotopes (C and N) to investigate trophic relationships and nitrogen cycling

Isotopes are atoms of the same element which differ in the number of neutrons they contain (Sulzman, 2007). Ninety five percent of all nitrogen and carbon isotopes occur in the lighter form (^{14}N and ^{12}C). A 'δ value' is a difference measurement of the abundance of the stable isotope relative to a standard, and it provides a direct indication of the percentage of heavy isotope (e.g. ^{15}N and ^{13}C in the sample; Fry, 2006): samples with higher δ values contain a greater amount of the heavy isotope compared to samples with lower δ values. Such samples are thus 'enriched' in the heavy isotope. Conversely, samples with lower δ values are 'depleted' in the heavy isotope and contain more of the lighter isotope. Changes in the relative proportions of the heavy and light isotopes occur

during mixing and fractionation (an increase in the isotopic differences between a source and a product; Fry, 2006). Fractionation occurs during chemical reactions because heavy and light isotopes have different bond strengths and vibration frequencies (Sulzman, 2007), and therefore are differentially favoured during reactions.

A common use of stable isotopes is to examine trophic relationships and trace the transfer of energy or nutrients through food webs. The use of stable isotopes in trophic studies relies on the fact that different food sources have distinct isotopic values that are conservatively and predictably represented in consumers. Stable isotopes have the advantage that they can be used in systems where it is not possible to observe feeding links directly (e.g. in the soil or in many aquatic systems), or where gut contents analysis does not provide useful information (Grey, 2006: e.g. in animals with a gastric mill such as atyiid shrimps; Yam & Dudgeon, 2005). In addition, they are able to integrate information on dietary contributions over longer time periods than gut contents, although rate of turnover of different food web components (Grey, 2006) and variability in isotopic signatures of dietary sources need to be considered (Peterson, 1999).

Carbon is particularly useful in dietary studies as fractionation between dietary sources and consumers is generally small (mean +0.5±0.13‰ in meta-analysis, McCutchan *et al.*, 2003), making it easy to trace through the food web. Furthermore, sources are often quite distinct (especially in freshwaters; Fry, 2006).

The fraction of the heavy nitrogen isotope is less conserved and thus is often used to estimate trophic position rather than food source *per se*, but can also be used to examine dietary contribution. Thus, ^{15}N-enrichment between trophic levels is typically about +3.4‰ (Minagawa & Wada, 1984), although the degree of enrichment can be highly variable between (e.g. -0.8‰ and +5.9‰ in a meta-analysis by McCutchan *et al.*, 2003). The form of nitrogenous waste excreted by organisms (Vanderklift & Ponsard, 2003), resource quality (e.g. C:N ratio, Adams & Sterner, 2000) and related nutritional stress (e.g. starvation; Gannes *et al.*, 1997), have all been shown to influence the magnitude of ^{15}N-enrichment of consumers relative to their food resources.

Mixing models are used to estimate the dietary contributions of several possible food sources to a consumer. However, inaccuracies commonly arise through 1) insufficient

knowledge of the actual fractionation factors between consumers and their diet (McCutchan *et al.*, 2003), 2) isotopic variability within food sources and the consumer not being incorporated (Phillips & Gregg, 2001), and 3) differences in the concentration of carbon and nitrogen between food sources not being taken into account (Phillips & Koch, 2002). This variation can be included in the new generation of mixing models based on Bayesian statistics that have been developed recently (e.g. Moore & Semmens, 2008; Jackson *et al.*, 2009).

Carbon stable isotopes have been very useful in understanding the importance of different subsidies to food webs, especially in aquatic systems (e.g. discrimination between autochthonous and allochthonous contributions to lakes, Grey *et al.*, 2000, 2001). As large differences between sources are rarer for nitrogen isotopes, they are used more frequently as an integrator of the nitrogen cycle (Robinson, 2001; but see Bartz & Naiman, 2005 as an example of use as a tracer). Processes that produce nitrogen fractionation, mixing of nitrogen pools, and nitrogen loss or gain will all be reflected in the $\delta^{15}N$ value of the system under consideration, making it ideal as an integrator of the nitrogen cycle, especially when interpreted in combination with other information (Robinson, 2001).

1.10 Aims of the research

The main aim of this study was to investigate gardening as a means through which a grazer can extend its range and maintain dominance under low resource conditions. *Tinodes waeneri* makes an ideal study organism as it is naturally dominant over a wide productivity gradient (Edington & Hildrew, 1995) in the stony littoral of lakes and aggressively defends its retreats and their immediate surroundings against intruders (Hasselrot, 1993a). This thesis examines in detail the '*Tinodes* system' which comprises the larvae, the galleries in which they reside, and the epilithon surrounding those galleries (Fig. 1.2).

The main objectives were to assess whether the sedentary caddisfly *Tinodes waeneri* gardens through fertilisation of its gallery and, if so, to examine how this interaction changes along a resource gradient. In this case the resource gradient used was a natural gradient in lake productivity across six lakes in the English Lake District. I also manipulated nutrient concentration, epilithon availability and larval density in the laboratory, as a test of field patterns under controlled conditions. Initial predictions as to

how specific elements of gardening should change along a nutrient gradient were derived from the literature and are illustrated in Fig. 1.3.

Specifically, at low productivity, excreted nutrients are expected to contribute a greater amount to the total nutrient budget of the gallery biofilm than where background nutrient levels are high (as discussed in sections 1.3 & 1.5; Fig. 1.3a). Thus, fertilisation should have a greater positive impact on gallery biofilm stoichiometry and gallery biofilm biomass relative to the epilithon in systems of low productivity. As productivity increases, and excreted nutrients have less of an impact on the gallery biofilm, gallery biofilm and epilithon should become more similar in terms of quality and quantity (Fig. 1.3b). The greater quantity and quality of resources on the gallery relative to the epilithon in low productivity systems should translate into greater consumption and assimilation of gallery relative to epilithic material (Fig. 1.3c). As productivity increases and the difference between the two food sources is reduced, epilithon should increasingly become a more important dietary source. These predictions were tested and are described in the following chapters.

Figure 1.2 The key components of the *Tinodes waeneri* system studied. Note that the gallery is several times longer than the larva and the faecal pellets expelled from the back end of the gallery. This photograph was taken in the laboratory.

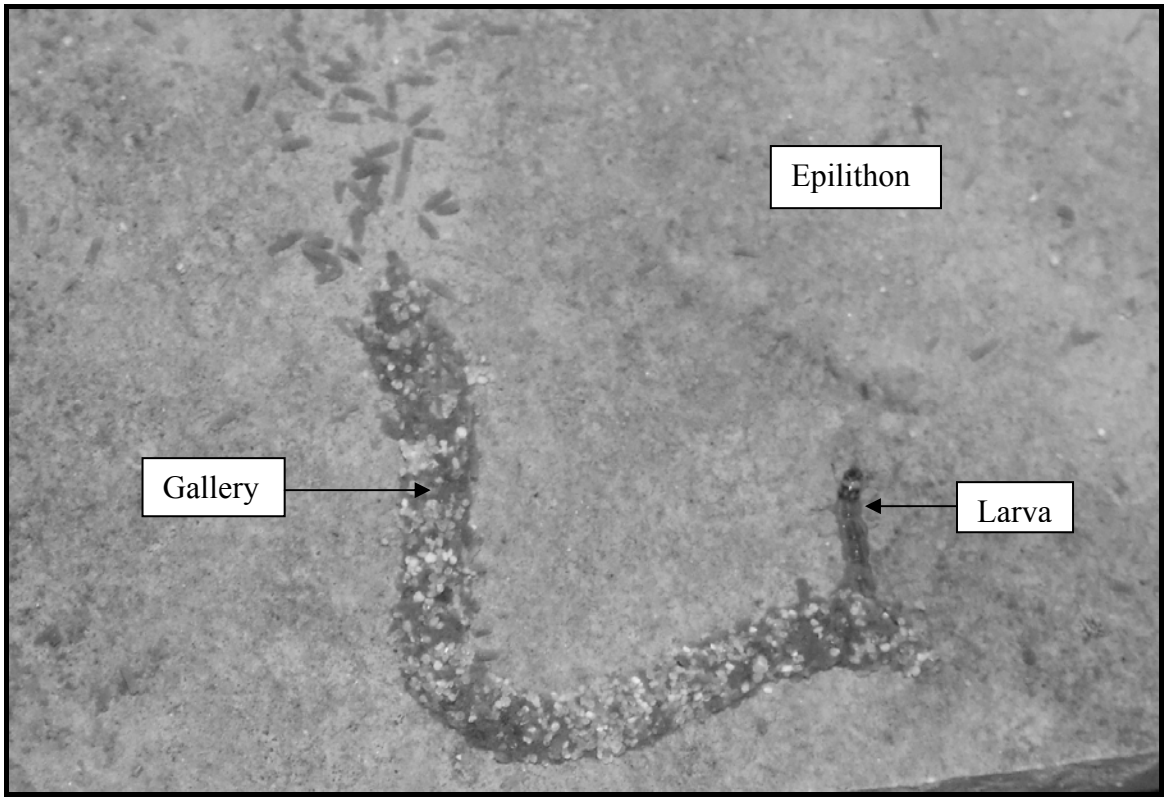
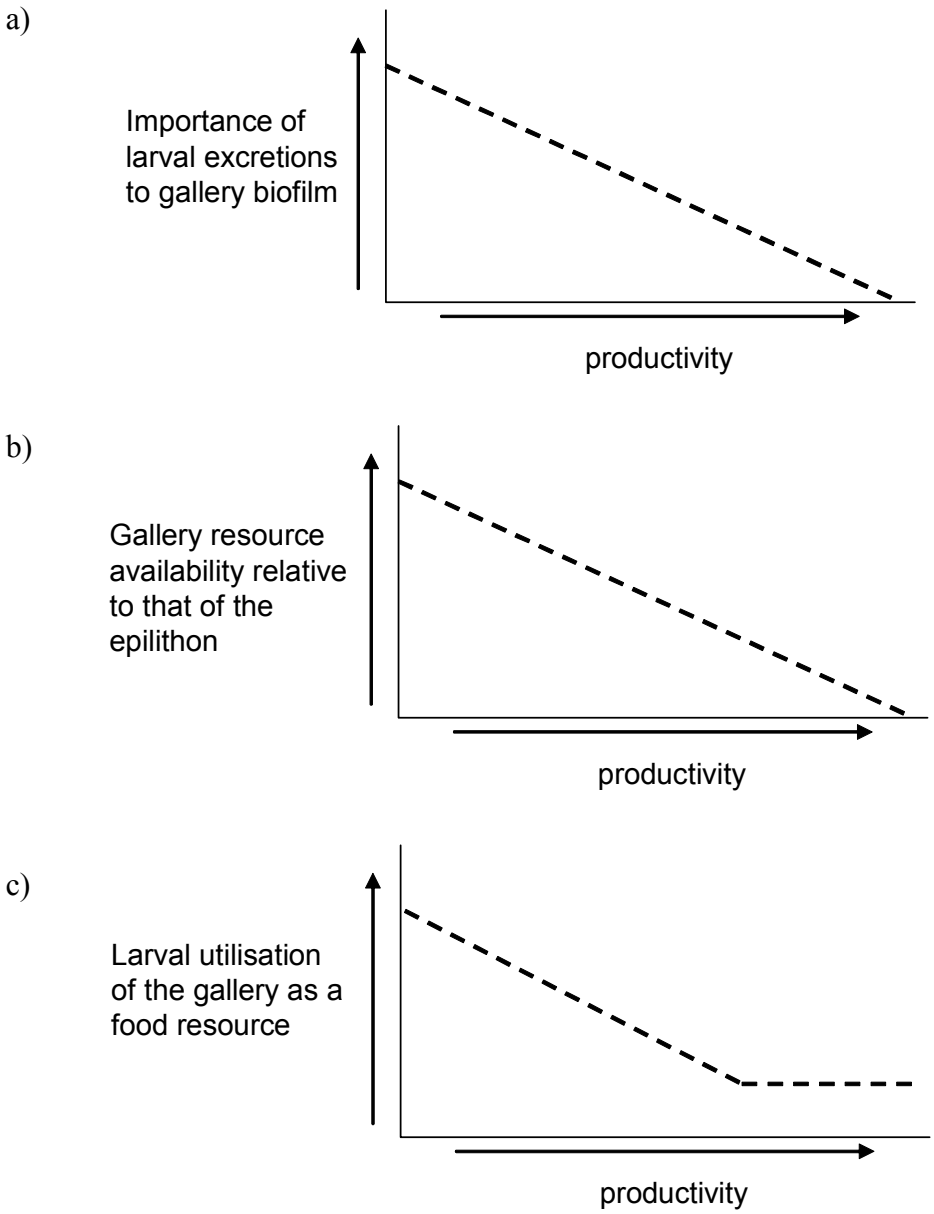


Figure 1.3 Conceptual illustration of how the different components of gardening might vary across a productivity gradient.



1.11 Structure of the thesis

This thesis is divided into four data chapters, which all contain Introduction, Methods, Results and Discussion sections, followed by a final discussion chapter. In addition there are three short appendices containing additional data relevant to some of the points made in the discussions.

Chapter 2 – The occurrence of gardening in *T. waeneri* is assessed using six natural populations inhabiting a productivity gradient in the English Lake District. Natural abundance stable isotope analysis was employed. The following two criteria needed to be met for gardening to be confirmed: 1) excreted nutrients are assimilated within the gallery and 2) the larvae are assimilating gallery material. Temporal patterns were also investigated.

Chapter 3 – The quantity and quality of food available to the larvae on the gallery is compared to that in the epilithon across the six lakes studied in Chapter 1. As *T. waeneri* is thought to garden through fertilisation, it was predicted that galleries should contain greater and/or better food. In addition, other possible factors (including disturbance and competition for limiting space) that may influence the amount of gallery versus epilithon assimilated by the larvae were assessed.

Chapter 4 – Algal communities on galleries and within the epilithon were compared in five lakes. Some gardening species are able to maintain monocultures, or distinct algal communities, within their gardens. Therefore, one aim was to assess whether there was a specific algal consortium associated with the galleries. Alternatively, it was predicted that fertilisation of the gallery should result in a different algal community to that on the epilithon, and that this community would be more distinct from that of the epilithon where background productivity was low. To test these hypotheses, the relative abundance of algal classes was assessed using pigment analysis and diatoms were identified to species.

Chapter 5 – Gardening was investigated under controlled experimental conditions using a single population of *T. waeneri*. Three nutrient treatments and two density levels were arranged in a fully factorial design. Confounding influences such as predation pressure and exposure to wave energy were thus removed and larval behaviour and gallery movement could be studied precisely. In addition, the impacts of the larvae on

the wider epilithon could be assessed through comparison of the epilithon in the larval treatments to ungrazed epilithon grown under the same conditions. A second experiment investigated the effects of removal of epilithon from in front of larval galleries on larval growth and gallery movement.

Chapter 6 – This chapter draws together the findings of all the preceding chapters to assess whether *Tinodes waeneri* is gardening through fertilising the gallery biofilm community and how the gardening interaction relates to productivity. In particular, the hypotheses presented in section 1.10 (Fig. 1.3) are evaluated in light of the data collected. Suggestions for further research are also included.

Chapter 2 - Gardening by the psychomyiid caddisfly *Tinodes waeneri*: evidence from stable isotopes

2.1 Introduction

Sedentary benthic animals are common and can often be dominant in freshwater systems (e.g. in the stony littoral of lakes; Brodersen *et al.*, 1998; Harrison & Hildrew, 2001). Yet, this lifestyle is expected to impose severe restrictions on the range of conditions under which they are likely to be successful (Hart, 1987), and thus on their distribution and abundance. Organisms that are sedentary must be able to obtain all of their resources to sustain growth and development from the immediate vicinity of their retreats. Sedentary grazers will experience a trade-off between maintaining a grazing patch that is large enough to provide them with the necessary resource, whilst minimizing the costs of defending that feeding patch against intruders (Hart, 1987). Thus, this life-style should be most successful at intermediate resource levels (Fig. 1.1). At high food abundances sedentary organisms may be out-competed by more mobile species, whereas when food resources are low, large areas would need to be defended, making this a costly strategy (Hart, 1987).

In fresh waters, periphyton can be a limiting resource for grazers (Lamberti, 1996), both temporally (e.g. during the summer) and spatially (e.g. in low productivity systems). Nevertheless, some sedentary grazers are very abundant, even during periods of resource (food or space) limitation. One possible explanation is that, via gardening, such grazers provide themselves with a better food resource within their local patch (Plagányi & Branch, 2000). An active form of gardening is the fertilisation of photosynthetic and other microbial assemblages through the egestion of nutrients (as in chironomids; Pringle, 1985; Hershey *et al.*, 1988; Hirabayashi & Wotton, 1999; Deines *et al.*, 2007, and in marine limpets; Plagányi & Branch, 2000).

Tinodes waeneri (Trichoptera: Psychomyiidae) is an ideal study organism for studying gardening. It is naturally dominant over a wide productivity gradient (Edington & Hildrew, 1995) in the stony littoral of lakes and aggressively defends its retreats (galleries made from sediment and silk) and their immediate surroundings against intruders (Hasselrot, 1993a). Furthermore, it lives at high densities (regularly 1000 to 2000 individuals per m^2 Hasselrot, 1993a) although densities of 11500 individuals per

m^2 have been recorded (Dall *et al.*, 1984). The recognition that galleries are much longer than would be required if their sole purpose was larval protection, as well as measurement of high concentrations of ammonium and phosphate within the galleries, and high chlorophyll concentrations on the gallery walls, first led Hasselrot (1993b) to postulate that *T. waeneri* gardens by fertilising algae in its own patch. However, this hypothesis remains untested.

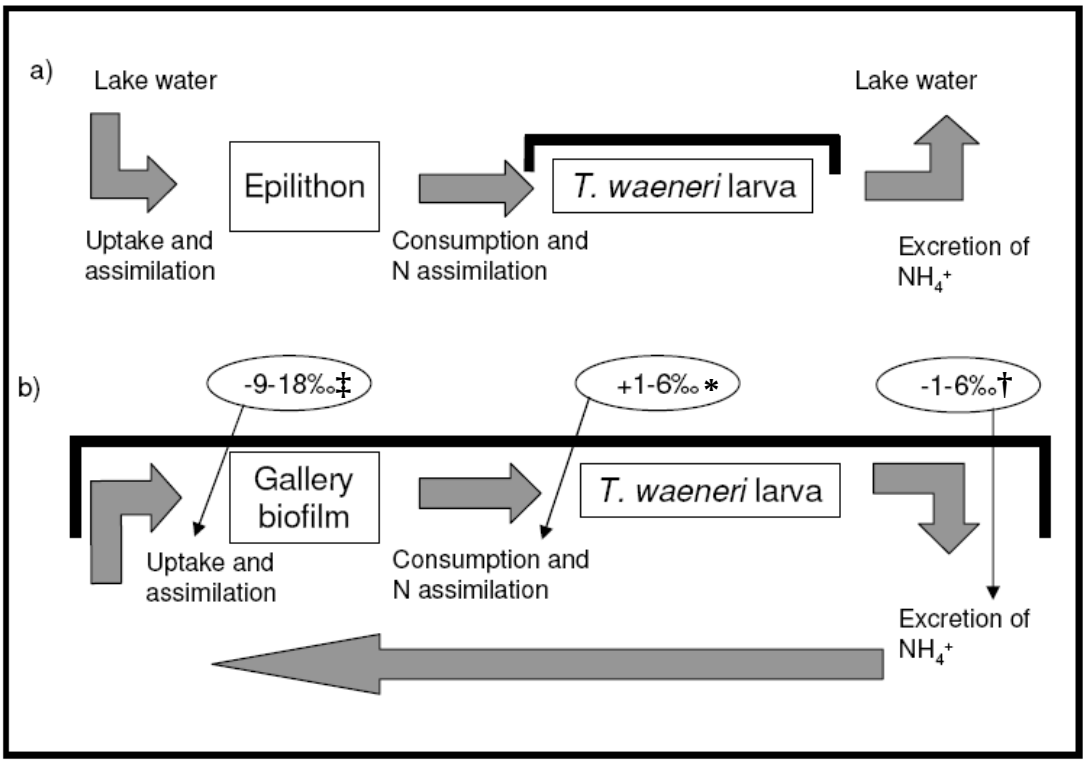
My aim was to investigate gardening in *Tinodes waeneri,* using natural abundance stable isotope analysis, across a natural nutrient gradient (six lakes). I propose that, for the process to be accepted as 'gardening', *T. waeneri* larvae must, firstly, enhance the quality and/or quantity of their food resources in a fixed location and, secondly, consume these fertilised resources. Therefore, I tested two main hypotheses that: 1) *T. waeneri* enhances its food resources by fertilising the gallery biofilm (Fig. 2.1) and 2) that the gallery is a significant food source for the larvae. I also expected that the gallery should be a more important food source in low productivity systems (where nutrient recycling should have more of an impact) and during the summer in all lakes (when food is potentially most limiting, lake water nitrogen concentrations are low, and *T. waeneri* achieves its highest density).

2.2 Methods

2.2.1 Rationale for detecting gardening through fertilisation

To infer whether nitrogen recycling is taking place, and thus whether the first criterion for gardening is fulfilled, the fact that discrimination against the heavier ^{15}N isotope occurs both during nitrogen excretion by the larvae (Peterson & Fry, 1987) and during uptake of inorganic nitrogen by algae (Evans, 2001, Fig. 2.1) was exploited. This rationale has also been successfully applied in other systems (e.g. Grey *et al.*, 2004; Muscatine *et al.*, 2005). If nitrogen recycling is occurring within the gallery community I would expect the gallery biofilm to be ^{15}N-depleted relative to the adjacent epilithon. To determine the dietary importance of galleries to the larvae, and thus assess whether the second criterion of gardening is fulfilled, both the carbon and nitrogen stable isotope ratios of the general background epilithon and the gallery were used as potential food resources in dietary mixing models.

Figure 2.1 A diagrammatic representation of nitrogen flow through the *Tinodes waeneri* community when a) no gardening (nutrient recycling) is taking place (i.e. it is a completely open system) and b) nutrient recycling within a closed gallery system is taking place. Figures in b) indicate typical fractionation values derived from Robinson (2001). Larvae will typically be ^{15}N-enriched by between 1 and 6‰ (*) and most likely ~2‰ relative to their food source (e.g. McCutchan *et al.*, 2003; Vanderklift & Ponsard, 2003). Within the gallery, larvae will continuously excrete nitrogenous waste in the form of ammonium (Vanderklift & Ponsard, 2003), which by mass balance, should be ^{15}N -depleted by a similar amount (†). The biofilm community within the gallery architecture should exploit this ^{15}N -depleted source of ammonium, resulting in the gallery biofilm becoming more ^{15}N -depleted than the epilithon on the rock (which does not have access to larval excretions). During uptake and assimilation by algae further discrimination against the heavier ^{15}N isotope will occur (Evans, 2001) (‡). If the larva then grazes from the ^{15}N -depleted biofilm, its excretions will theoretically be further ^{15}N -depleted relative to the gallery biofilm it was feeding from. Uptake of the excretions by the gallery biofilm will lead to biofilm with an even lower δ^{15}N value. Thus, continued cycling of nitrogen between the larva and its gallery should lead to further ^{15}N -depletion of the gallery biofilm relative to the epilithon.



2.2.2 Study sites

Six lakes in the English Lake District were selected (Figs. 2.2 & 2.3), ranging from moderately oligotrophic to eutrophic (Table 2.1), representing the range of conditions under which *T. waeneri* occurs in the locality. All sites were sampled in April, August and October 2006 and in January 2007. In addition, the populations at Rydal Water, Ullswater and Bassenthwaite Lake were also sampled in July 2006, when sites were visited whilst carrying out other fieldwork. Sampling of all lakes within a given sample period took less than one week.

Figure 2.2 A map of the Lake District with sampled lakes illustrated in black. Arrows point to the approximate locations of sampling sites. The map is modified from Knudson (1954).

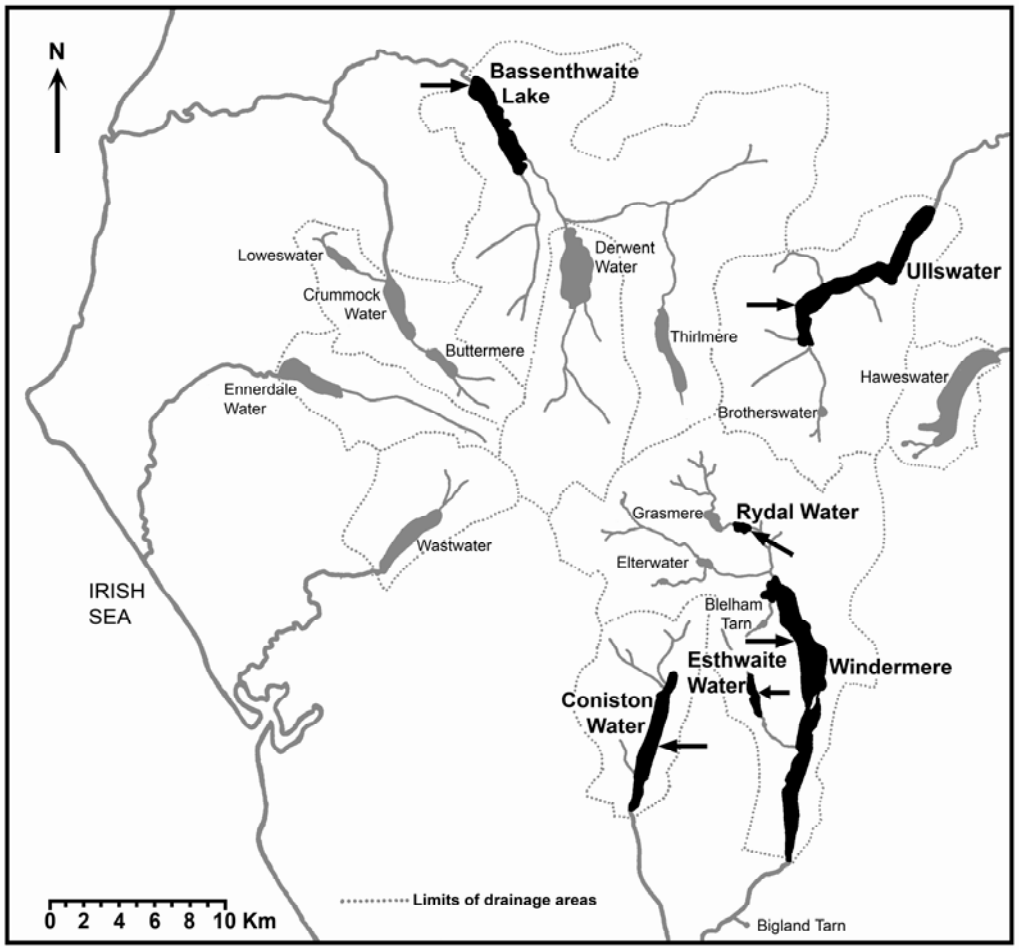


Figure 2.3 Photographs of the six sample sites.

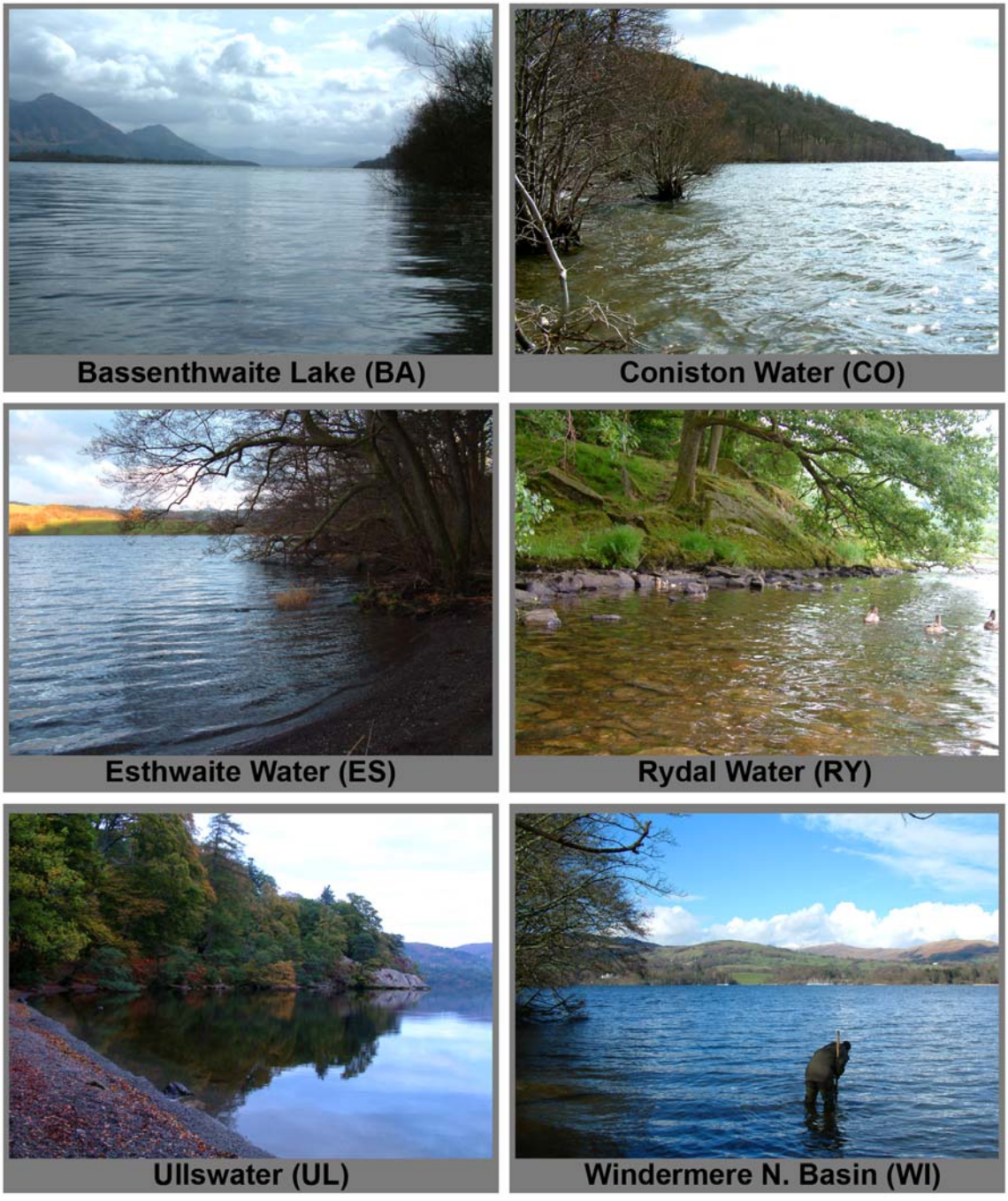


Table 2.1 Sample site locations and lake characteristics from Maberly *et al.* (2006). Annual mean total phosphorus, nitrate-nitrogen and phytoplankton chlorophyll *a* are values from a 2005 survey of the English Lake District (Maberly *et al*., 2006).

Lake	Grid reference	Lake area (km^2)	Mean depth (m)	Volume (m^3x10^6)	Mean retention time (days)	Mean total phosphorus (mg m^{-3})	Mean nitrate-nitrogen (mg m^{-3})	Mean phytoplankton chlorophyll *a* (mg m^{-3})
Bassenthwaite	NY201317	5.3	5.3	27.9	30	20.4	279	14.4
Coniston	SD309952	4.9	24.1	113.3	340	10.5	328	5.3
Esthwaite	SD363967	1.0	6.4	6.4	100	31.0	456	15.0
Rydal Water	NY361062	0.3	4.4	1.5	9	15.5	268	14.7
Ullswater	NY387181	8.9	25.3	223.0	350	9.8	216	4.5
Windermere (North Basin)	SD386994	8.1	25.1	201.8	180	14.2	332	8.6

2.2.3 Sampling methods

At each site, 10 rocks were chosen at random for the sampling of galleries, epilithic algae and *T. waeneri* larvae for stable isotope analysis. To reduce inter-site variability, rocks were taken from similar depths (30 to 60cm) in all lakes. Four galleries were labelled on each rock and photographed with an appropriate scale bar (for area and length calculations) before the resident larvae were carefully removed. The individual galleries were stored separately and their occupants were kept alive in individual vials filled with lake water until their guts had been cleared. Keeping larvae separately avoided fights between larvae and ensured that individual larvae could be associated with the galleries they had occupied in the field. Scrapings of epilithon were taken from the same rocks as the galleries and larvae, from areas not beneath or adjacent to galleries (i.e. so as to represent the general 'background' epilithic biofilm), using a clean scalpel. Galleries and algal scrapings were kept on ice in the field and frozen on return to the laboratory.

Particulate organic matter (POM) was also collected from the water column, as this could constitue an alternative larval food source. Approximately 20 litres of water were collected from each lake and concentrated using a Minitan II ultra filtration system (pore size 0.2μm). The concentrate was then filtered through a pre-ashed Whatman GF/F filter (pore size 0.7μm) and this filter was then prepared for stable isotope analysis.

2.2.4 Baseline

To allow comparisons between lakes, the $\delta^{15}N$ of total dissolved nitrogen (TDN) in the water was used as a baseline. Samples were collected in Febraury 2007, a time at which water nitrogen concentrations are high and biological activity is low. Three 5L water samples were collected from each site, after wading out to a depth of approximately 1.3m. Water samples were filtered through Whatman GF/C filters (pore size 1.2μm) on site, kept cold (less than 8 °C) in the dark and frozen on return to the laboratory. Defrosted samples were filtered through Whatman GF/F filters (pore size 0.7μm) before the filtrate was freeze-dried. All solid material remaining once the sample was dry was carefully collected, ground and analysed for $\delta^{15}N$. Only two samples were available for analysis for Esthwaite (one sample having unfortunately defrosted during storage).

2.2.5 Silk

On each sampling occasion additional galleries were photographed with an appropriate scale bar and the galleries and their resident larvae collected. Galleries were frozen after collection and were later used to determine their total (silk and biofilm) organic content. Twenty larvae collected from each of the lakes during the October sample period were allowed to clear their guts (maintained individually in lake water for 24 hours, with faecal material removed at regular intervals), rinsed in deionised water and then placed into laboratory aquaria (one per lake) containing deionised water, a tile and pre-ashed sediment from their respective lakes and maintained at 10°C. Once larvae had built retreats (after 24 to 48 hours) their galleries were photographed and both galleries and larvae were harvested and dried. Only galleries containing a resident larva were used as these represented functioning galleries rather than aborted building attempts. These galleries contained only inorganic sediment and larval silk and were also ashed to obtain a measure of the silk content. Gallery lengths and areas were measured from the photographs using Image J and the quantities of organic matter in both types of gallery (i.e. built in the laboratory and containing only silk and ashed sand, and those taken from the field) were calculated as mg cm^{-2}. The proportion of gallery biofilm within natural galleries was taken to be the difference in organic matter (mean mg cm^{-2}) between the galleries collected from the field from each of the six lakes over the four main sampling occasions and that of the experimental galleries containing only silk.

It was not possible to measure the nitrogen and carbon stable isotope values of the silk from these laboratory galleries because of the low mass of organic matter present. As an alternative, 120 *T. waeneri* larvae were collected from each of the sampling sites at Bassenthwaite, Esthwaite, Rydal Water, Windermere and Ullswater in October 2007. Each larva was placed in a separate labelled vial containing lake water and allowed to clear its gut fully, before being rinsed and inspected for any attached silk and transferred to a clean vial containing deionised water. In such circumstances, larvae readily built and occupied sheet-like silk shelters which were attached to both the vial wall and floor. Once a high proportion of larvae had built silken shelters (after approximately 48 hours), silk was carefully removed from the vials and placed into small pre-weighed tin capsules. Silk from 25 larvae from each lake was pooled to provide one stable isotope sample. Tin capsules were dried, crimped and reweighed. Larvae were grouped identically and prepared for stable isotope analysis.

2.2.6 Stable isotope analysis

Gut-cleared *T. waeneri* larvae were inspected for attached silk and rinsed in deionised water before being frozen. All samples for stable isotope analysis (larvae, galleries, epilithon and POM) were dried (at 60°C in an oven for a minimum of 24 hours or freeze-dried for 72 hours) and ground with an agate pestle and mortar. Ground samples were weighed into tin cups and stored in a desiccator prior to analysis. Tin-cups were combusted in an elemental analyser (Flash EA, 1112 series, Thermo-Finnigan) coupled to a continuous flow mass spectrometer (Finnigan MAT Delta^{Plus}, Thermo-Finnigan).

Reduced columns (for dimensions and details refer to Houghton *et al.*, 2002; Tod, 2007) were used to analyse small larvae (<0.6mg) and silk samples. To reduce the background carbon values, gloves were worn during sample processing and smaller, ultra light weight tin cups (4x3.2mm) were used (Carman & Fry, 2002).

All isotope ratios are expressed in parts per mille (‰). The secondary standards ammonium sulphate (RM 8547) and sucrose (RM 8542), of known isotopic composition in relation to atmospheric nitrogen in air (N) and Pee Dee Belemnite (C), respectively, were used to calibrate stable isotope ratios. A urea dilution series (0 to 0.6 mg urea for standard columns) was included in every run and used to calculate the elemental mass of carbon and nitrogen in the samples. For the repeated analysis of a standard (Cyclohexanone-2,4-Dinitrophenylhydrazone) typical accuracy (i.e. ±1SD) for N was ±0.08 when using standard columns and ±0.25 for reduced columns and for carbon was ±0.19 and ±0.12 for standard and reduced columns, respectively.

2.2.7 Data analysis

All data for galleries, larvae and epilithic algae were calculated using mean values per rock. Data for galleries and larvae were included only when both the gallery and its resident had been analysed. Data analysis was performed using SPSS version 13.

2.2.7.1 Corrections due to silk

Mean gallery (defined here as silk and other organic matter) $\delta^{15}N$ values for each lake and sample period were corrected for silk to provide a $\delta^{15}N$ value of the gallery biofilm (defined here as all organic matter other than silk). Known variables included the proportion of non-silk organic matter, the total gallery $\delta^{15}N$ values, and the $\delta^{15}N$-value of the silk (calculated as the mean value of the larvae +1.7‰, see Results for

information on silk $\delta^{15}N$ values) and these were used in a re-arranged standard two-source mixing model to calculate the $\delta^{15}N$ value of the gallery biofilm.

2.2.7.2 Mixing models

To provide an estimate of the amount of gallery and epilithon assimilated by the *T. waeneri* larvae, the Bayesian mixing model Stable Isotope Analysis in R (SIAR; Parnell *et al.*, 2008; Jackson *et al.*, 2009) was used. The advantage of using this type of model over standard mixing models (e.g. Phillips & Gregg, 2001) is that it is able to integrate sources of variability (e.g. associated with fractionation or among samples) and can be informed by the careful inclusion of prior information (e.g. gut contents data) which can increase its ability to distinguish between possible sources (Moore & Semmens, 2008). Model outputs thus reflect the uncertainty associated with the estimates of source contributions, but still rely on the assumption that carbon and nitrogen route together, both ecologically and physiologically.

Separate mixing models were run for each lake at each sampling date and variation among rock means in the $\delta^{15}N$ and $\delta^{13}C$ values of the two end-members (gallery and epilithon) and the mixture (*T. waeneri* larvae) were included. Gallery $\delta^{15}N$-values that were not corrected for silk (and thus represent the gallery as a whole) were used as a potential food source. Fractionation factors between the end-members (gallery and epilithon) and the larvae were assumed to be 2.2±1.47 for $\delta^{15}N$ and 0.5±1.31 for $\delta^{13}C$ and were held constant in all models. These values represent the fractionation factors for nitrogen of consumers fed on plant or algal diets and the fractionation factors for carbon for all animals in the meta-analysis performed by McCutchan *et al*. (2003).

2.3 Results

2.3.1 Nitrogen fertilisation of the gallery community

Tinodes waeneri larvae were ^{15}N-enriched relative to both potential dietary sources (background epilithon and galleries) in all samples except Rydal Water in July, where larvae were ^{15}N-depleted relative to the epilithon (Fig. 2.4). Larvae generally had higher $\delta^{15}N$ values in April (other than at Coniston and Windermere) and in January than in the summer/autumn sampling periods. The difference in larval and gallery $\delta^{15}N$ was consistent within lakes after April; ^{15}N-enrichment of larvae relative to galleries (mean ±1SE) was 2.28±0.15‰ across lakes in the August to January samples, but 4.49±0.61‰ in the April sample. Galleries were consistently ^{15}N-depleted relative to the epilithon in

Esthwaite and Rydal Water, whereas gallery and epilithon $\delta^{15}N$ values were very similar to each other at Ullswater and Coniston (Fig. 2.4). In the January sample there was less gallery ^{15}N-depletion relative to the epilithon in all lakes and, with the exception of Esthwaite and Rydal Water, galleries were ^{15}N-enriched (Fig. 2.4). Low variability in the $\delta^{15}N$ values of the individual components measured was found within sample periods within individual lakes.

The organic material in *T. waeneri* galleries is a combination of both biofilm and larval-derived silk, which holds the sediment particles in place to form the gallery structure. In this study, silk accounted for 11.5±0.6% of gallery mass in all lakes excluding Esthwaite (21.5±2.5%). Silk made up the smallest proportion of gallery organic matter in April (10.1±1.6%) in all lakes and its contribution increased over the sampling periods (to 15.3±1.3% in January). Stable isotope analysis indicated that there was no difference in $\delta^{13}C$ values between the larvae and the silk they produced ($t=0.3$, $df=19$, $P=0.76$, Table 2.2). In contrast, silk was significantly ^{15}N-enriched relative to the larvae that produced it by an average of 1.7‰ ($t=11.7$, $df=19$, $P<0.0001$), and therefore has the potential to mask ^{15}N-depletion of the gallery biofilm relative to the epilithon. However, correction of galleries for silk only reduced $\delta^{15}N$ values by 0.65±0.6 ‰.

Table 2.2 Nitrogen and carbon stable isotope values of *Tinodes waeneri* larvae and their silk. Values are means (1SE).

Lake		$\delta^{15}N$(‰)		$\delta^{13}C$(‰)	
	N	Larvae	Silk	Larvae	Silk
Bassenthwaite	4	7.96 (0.06)	8.85 (0.10)	-17.23 (0.12)	-17.18 (0.09)
Esthwaite	5	10.75 (0.17)	12.46 (0.07)	-18.66 (0.63)	-17.80 (0.45)
Rydal Water	4	6.83 (0.13)	9.11 (0.35)	-19.62 (0.09)	-19.53 (0.18)
Ullswater	4	5.12 (0.12)	7.29 (0.16)	-20.51 (0.27)	-20.45 (0.41)
Windermere	3	5.86 (0.03)	7.34 (0.33)	-14.57 (0.22)	-15.87(0.53)

The ^{15}N-depletion of the gallery biofilm relative to the epilithon ranged from 4.26 (Rydal Water, April) to -0.86 (Coniston Water, January), once the influence of silk on

$\delta^{15}N$ had been accounted for (Fig. 2.5 – positive values indicate gallery biofilm ^{15}N-depletion relative to the epilithon). The magnitude of this difference varied significantly among lakes (two-way ANOVA without replication: $F_{5,15}=10.506$, $P<0.0001$), and was generally lower in the less productive group of lakes (Fig. 2.5) which comprised Windermere, Coniston and Ullswater (chlorophyll *a* concentrations ranging from 4.5 to 8.6 mg m^{-3}, Table 2.1). Furthermore, these three lakes all displayed similar temporal patterns, with the ^{15}N-depletion of gallery biofilm (relative to the epilithon) peaking in the October sample (Fig. 2.5). In contrast, in the three more productive lakes (chlorophyll *a* concentrations ranging from 14.4 to 15.0 mg m^{-3}, Table 2.1), this peak occurred in April, and values steadily decreased thereafter. Esthwaite and Rydal Water displayed the greatest difference (always greater than 1.1‰) between the $\delta^{15}N$ values of the epilithon and gallery biofilm.

Galleries were more ^{13}C-depleted than the epilithon in all cases (Table 2.3), whereas the larvae and the epilithon were much more similar in terms of $\delta^{13}C$ values. High epilithon $\delta^{13}C$ values were measured for Coniston in August (-8.61±0.48). POM values, measured in August, October and January, were always ^{13}C-depleted in comparison to the other components measured (Table 2.3).

2.3.2 Galleries as a carbon and nitrogen source

Concentration dependence was not built into the SIAR mixing models used to determine gallery consumption as *post-hoc* tests indicated no significant differences between galleries and algae in terms of C:N ratio (Tukey HSD $P>0.1$). Mean silk C:N ratios (atomic) ranged from 4.3 to 5.1 and thus did not substantially reduce the C:N ratio of the gallery.

The SIAR mixing models clearly indicated that galleries formed an important source of carbon and nitrogen to the larvae (Fig. 2.6). This was true for all samples other than for Bassenthwaite in April and August and Windermere for August, October and January, where the probability intervals spanned zero and it is therefore possible that gallery nitrogen and carbon were not assimilated by the larvae. Similarly, epilithon carbon and nitrogen may not have been assimilated at Rydal Water in July or in Ullswater in April, suggesting that galleries may have been the main source of carbon and nitrogen.

Figure 2.4 Mean δ15N (±1SE) values of galleries, background epilithon and *Tinodes waeneri* larvae across the sampling periods for each of the lakes. The samples for the TDN baseline (dotted line) were collected in February 2007.

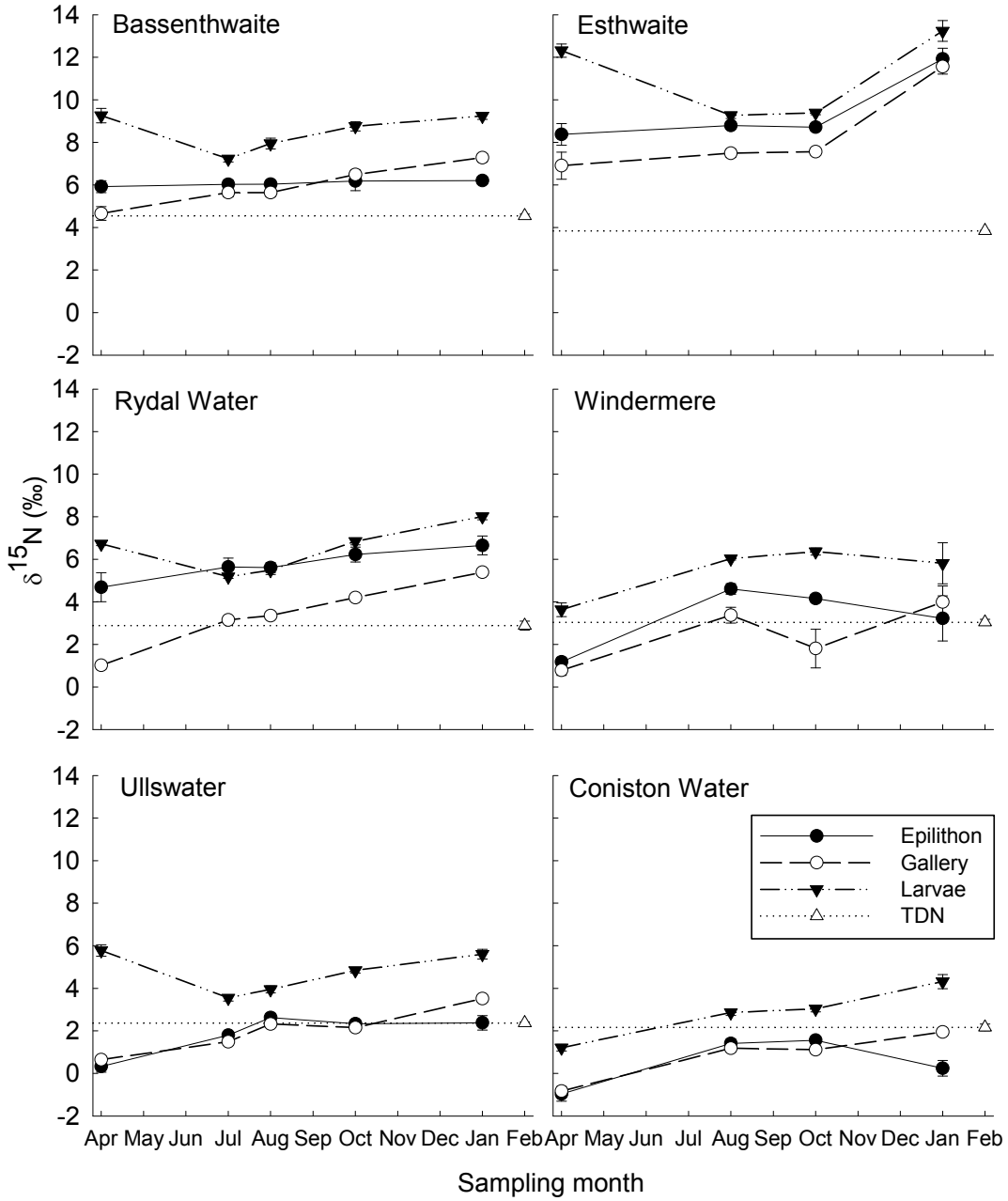


Figure 2.5 The mean difference in $\delta^{15}N$ values between the epilithon and the gallery biofilm (i.e. raw gallery values have been corrected for silk) for the six lakes across the four main sample periods. Positive values suggest nitrogen recycling within the gallery community. Error bars represent ±1SE and give an indication of the among rock variability in the difference in $\delta^{15}N$ between epilithon and galleries. They do not represent error associated with the correction of the mean gallery values for silk.

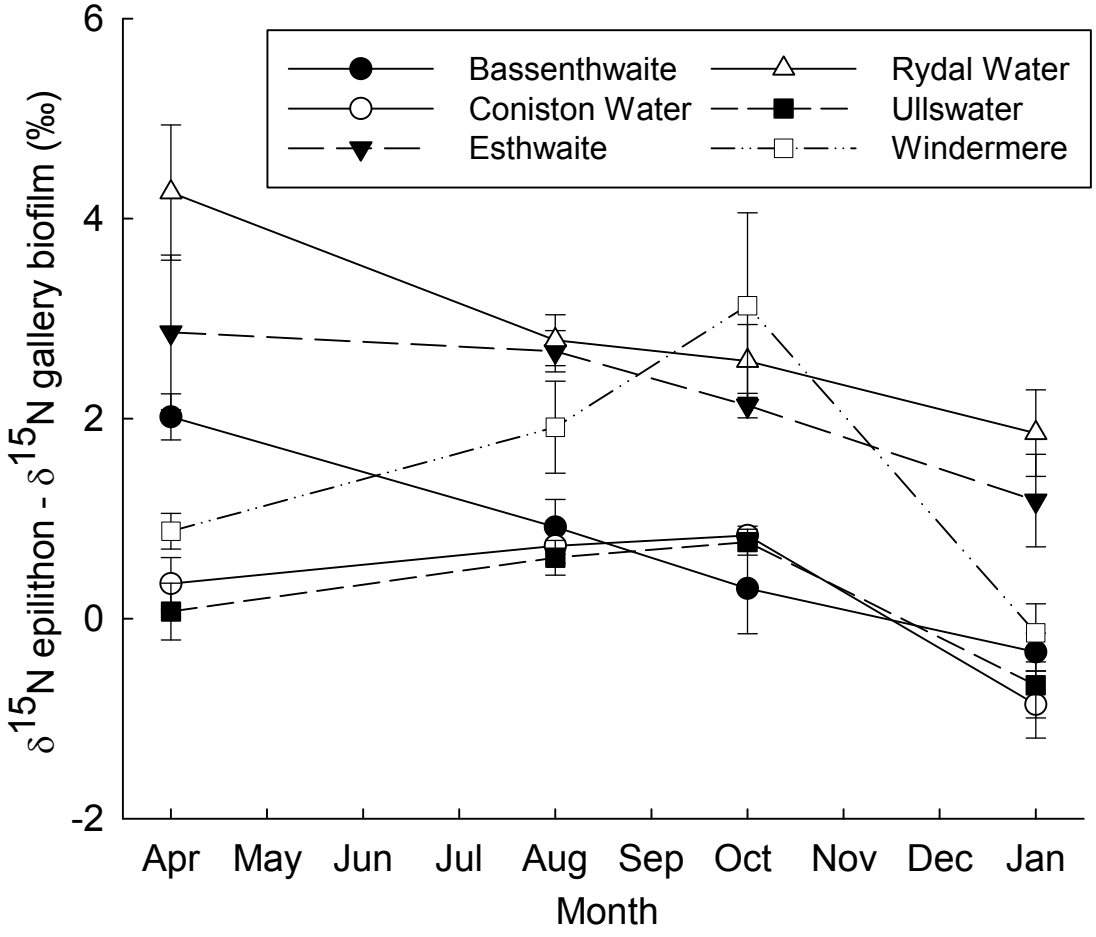
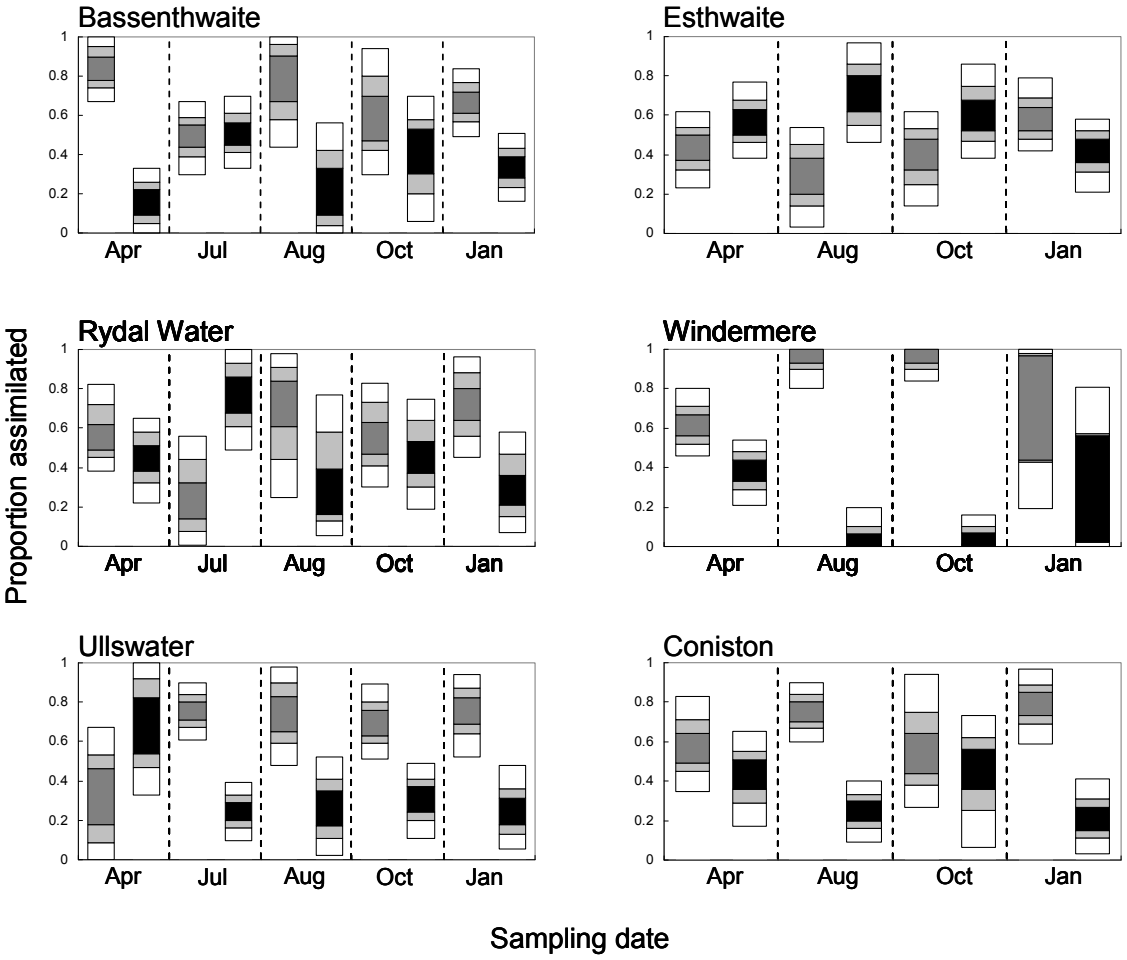


Table 2.3 $\delta^{13}C$ values for *Tinodes waeneri* larvae, galleries, epilithon and water column POM for all lakes and sampling periods Values are means (1SE).

Date	Item	Site Bassenthwaite		Coniston		Esthwaite		Rydal Water		Ullswater		Windermere	
		Mean (S.E)	N	Mean (S.E)	N	Mean (S.E)	N	Mean (S.E)	N	Mean (S.E)	N	Mean (S.E)	N
Apr	Larvae	-17.42 (0.26)	7	-14.25 (0.47)	7	-20.45 (0.37)	7	-20.68 (0.37)	7	-18.88 (0.79)	6	-16.64 (0.29)	5
	Galleries	-21.74 (0.28)	7	-17.95 (0.65)	7	-23.18 (0.63)	7	-23.06 (0.23)	7	-19.94 (0.52)	6	-22.16 (0.32)	5
	Epilithon	-17.76 (0.17)	7	-14.14 (1.29)	7	-17.04 (0.66)	7	-18.74 (0.71)	7	-17.03 (0.44)	6	-14.52 (0.67)	5
Jul	Larvae	-18.22 (0.24)	7					-19.65 (0.89)	8	-15.12 (0.58)	10		
	Galleries	-21.11 (0.48)	7					-22.82 (0.58)	8	-22.75 (0.49)	10		
	Epilithon	-16.27 (0.40)	7					-20.43 (0.79)	8	-13.98 (0.64)	10		
Aug	Larvae	-19.02 (0.38)	7	-9.69 (0.44)	8	-18.51 (1.35)	9	-18.15 (0.37)	10	-14.36 (0.71)	9	-13.22 (0.34)	10
	Galleries	-22.75 (0.32)	7	-17.00 (1.02)	8	-20.50 (0.29)	9	-23.82 (0.19)	10	-21.06 (0.52)	9	-21.57 (0.45)	10
	Epilithon	-20.04 (0.36)	7	-8.61 (0.48)	8	-16.73 (0.86)	9	-18.86 (0.72)	10	-15.90 (1.19)	9	-14.65 (0.48)	10
	POM	-29.6	1	-28.45	1	-30.40	1	-27.01	1	-28.07	1	-26.74	1
Oct	Larvae	-21.08 (0.43)	10	-10.40 (0.34)	12	-17.97 (0.66)	8	-20.84 (0.67)	8	-20.01 (0.34)	10	-14.90 (0.47)	10
	Galleries	-24.59 (0.32)	10	-17.59 (0.68)	12	-20.66 (1.02)	8	-25.27 (0.28)	8	-24.65 (0.44)	10	-24.14 (0.57)	10
	Epilithon	-22.72 (0.63)	10	-15.38 (0.81)	12	-17.13 (1.23)	8	-21.00 (0.96)	8	-19.96 (0.66)	10	-16.31 (0.51)	10
	POM	-31.03	1	-28.08	1	-33.32	1	-31.82	1	-31.23	1	-30.06	1
Jan	Larvae	-21.33 (0.52)	9	-15.96 (0.51)	9	-21.38 (0.60)	10	-22.06 (0.30)	11	-20.48 (0.74)	9	-15.06 (0.59)	2
	Galleries	-26.62 (0.31)	9	-22.27 (0.39)	9	-26.60 (0.54)	10	-26.77 (0.20)	11	-27.27 (0.30)	9	-25.56 (0.63)	2
	Epilithon	-20.09 (0.59)	9	-15.91 (0.3)	9	-19.54 (1.07)	10	-23.09 (0.62)	11	-19.89 (0.74)	9	-18.53 (2.07)	2
	POM	-28.61	1	-29.68	1	-30.66	1	-28.48	1	-29.04	1	-29.14	1

Figure 2.6 Posterior estimates of the contributions of background epilithon (grey central bar) and gallery (black central bar) to the larvae for each of the sampling periods based on the SIAR mixing model. Results for the six lakes are shown on separate graphs. 50% probability intervals are illustrated by the central dark grey or black areas of the bars, 75% probability intervals also include the light grey areas of the bars and extend to the boundary between the light grey and white areas and 95% probability intervals also include the white areas and are thus represented by the entire bars.



Larvae from the different lakes showed varying temporal assimilation patterns. Larvae in Windermere and Ullswater assimilated considerably higher proportions of C and N from the epilithon than from the gallery after April (Fig. 2.6). Bassenthwaite larvae showed a similar pattern, although gallery contributions were higher (Fig. 2.6). At Rydal Water, mean gallery contributions were at least 30%, although epilithon was still the main contributor. Finally, at Esthwaite, mean gallery contribution was above 57% at all times other than January. In all cases the mixing models for the January sample suggested that carbon and nitrogen had mainly been assimilated from the epilithon (Fig. 2.6).

There was no relationship between the amount of gallery N and C assimilated by the larvae and the amount of ^{15}N-depletion of the gallery biofilm as compared to the adjacent epilithon. Indeed, opposing patterns were found in different lakes: trends were positive (i.e. in line with predictions) for Esthwaite (r=0.808, n=4, P=0.192), Rydal Water (r=0.654, n=4, P=0.346) and Coniston (r=0.572, n=4, P=0.428) and negative for Bassenthwaite (r=-0.869, n=4, p=0.131) and Windermere (r=-0.894, n=4, p=0.106). There was also no significant relationship between productivity (mean annual TP; Table 2.1) and the proportion of material assimilated by the larvae derived from the gallery in any sampling period (April: r=-0.055, n=6, P=0.918; August: r=0.802, n=6, P=0.055; October: r=0.607, n=6, P=0.202; January: r=0.776, n=6, P=0.070).

2.4 Discussion

2.4.1 Nitrogen fertilisation of the gallery community

Using nitrogen isotopes, this study demonstrates that nitrogen recycling occurs within the gallery community in natural populations of *T. waeneri*. The lower δ^{15}N values of galleries relative to the epilithon indicates that nitrogen was being recycled between the *T. waeneri* larvae and the gallery biofilm, and thus the first criterion necessary to show gardening has been met.

Gallery organic matter consists of silk produced by the larva and a biofilm community. To investigate nitrogen recycling within the gallery community it was crucial to focus only on the biofilm component, as this contains the algae and bacteria that are likely to assimilate any larval excretions. It was necessary to measure the δ^{15}N of *T. waeneri* silk and the proportion of gallery organic matter that it represents in order to calculate the δ^{15}N of the biofilm itself. The silk values measured in this study (^{15}N-enriched by a

mean of 1.7‰ relative to the larvae that produced it) are similar to the values measured by Tibbets *et al.* (2008) for the silk cocoons of the silkworms, *Bombyx mori* (L.) (silk ^{15}N-enriched by 1.1‰ relative to the silkworms). The amino acids, serine, glycine and arginine are the predominant components of caddisfly silk (Engster, 1976, Rudall & Kenchington, 1971), and as they are essential amino acids they will have undergone little change in isotopic composition (Pinnegar & Poulin, 1999). The silk values obtained here should reflect natural values; the method used in this study was not found to affect the N and C stable isotope signatures of the larvae and a single removal of the silken nets produced by the predatory caddis *Plectrocnemia conspersa* (Curtis) larvae had no significant effect on larval body mass, although the nets built were smaller (Beveridge & Lancaster, 2007).

Despite silk being taken into consideration, ^{15}N-depletion of the gallery biofilm compared to the epilithon was relatively low throughout this study, given the potential fractionation factors involved (Fig. 2.1). This could in part be a result of sampling entire galleries. There is an age gradient along the galleries of *T. waeneri* as they move their galleries by demolishing one end and incorporating new material onto the other. Hence, if nitrogen recycling is taking place, I would expect that older gallery parts would be more ^{15}N-depleted than newly constructed sections. Some evidence for this has been shown in *Tinodes rostocki*. Stief & Becker (2005) found that the oldest sections of *T. rostocki* galleries contained higher chlorophyll content and respiratory/ photosynthetic activity than younger gallery sections (which were similar to the background epilithon). Therefore, as it was not possible to distinguish and separate old and new gallery sections for *T. waeneri* in the field, the magnitude of ^{15}N-depletion measured for whole galleries is likely to underestimate the amount of nitrogen recycling occurring in older gallery sections. Also, the level of ^{15}N-depletion will be dependent on mean gallery age and the speed at which the *T. waeneri* moves its gallery, both factors that are difficult to measure in the field.

A second explanation for the small amount of ^{15}N-depletion of the gallery compared to the epilithon could be incorporation of faecal pellets into the gallery structure (Edington & Hildrew, 1995), which could mask gallery biofilm ^{15}N-depletion, as faecal material is usually ^{15}N-enriched compared to the diet (Peterson & Fry, 1987). However, I found no evidence of faecal pellets being incorporated into galleries in *T. waeneri* and sediment grains were the principal building material used. Moreover, in laboratory studies (Fig.

1.2 & Chapter 5) *T. waeneri* larvae evicted faecal pellets from the back end of their galleries, and in wave washed lakes these would be washed away.

Low levels of ^{15}N-depletion of the gallery compared to the epilithon may suggest that the gallery/resident community was not a tightly-coupled system. This may be as a result of two distinct mechanisms. Firstly, as galleries are usually open-ended, abdominal undulations by *T. waeneri* larvae promote water exchange between the interior of the gallery and the external environment. Consequently, epilithon immediately adjacent to galleries, especially where galleries are short or at high densities, may also assimilate larval excretions resulting in isotopic homogenisation. Alternatively, the larvae may graze on the epilithon as well as on the gallery (as suggested by the mixing models). Thus, the total diet of the larvae would be less ^{15}N-depleted than if larvae were consuming only gallery material, resulting in larval excretions that are also less ^{15}N-depleted. In turn, this would reduce levels of ^{15}N-depletion of the gallery biofilm relative to the epilithon. Ingesting epilithon from outside the gallery and then excreting epilithon-derived nitrogen into the gallery interior may allow larvae to retain epilithic nitrogen within the larval territory, replacing any that is lost.

In addition to benefiting from larval N excretion the gallery biofilm may also be using larval-derived carbon. Galleries were consistently ^{13}C-depleted relative to the epilithon and this could be the result of uptake of ^{13}C-depleted carbon dioxide (Finlay & Kendall, 2007) respired by the *T. waeneri* larvae within their galleries.

2.4.2 Galleries as a larval carbon and nitrogen source

The outputs of the mixing models clearly indicate that galleries form an important source (e.g. mean gallery-derived dietary contribution of 25% or greater in 21 out 0f 27 of the mixing models run) of carbon and nitrogen for the resident larvae across the lakes studied here. *Tinodes waeneri* larvae not only modify their food resources, but also consume them. Thus, the second criterion has also been fulfilled and the data show that *T. waeneri* larvae do garden by fertilising their galleries.

The difference between larvae and their galleries in terms of δ^{15}N values are consistent across lakes and time periods other than April (Fig. 2.4). Moreover, the difference (2.28 ‰) is close to the value (2.2‰) suggested by McCutchan *et al*. (2003) for the trophic

shift between consumers and their algal or plant diet, suggesting that galleries are an important nitrogen source for the larvae. This may be partly because they contain silk.

Silk is costly for invertebrates to produce because it contains large amounts of amino acids (Rudall & Kenchington, 1971; Engster, 1976). Forcing the caddisfly *Odontocerum albicorne* (Scopoli) to build new silk-bound cases reduced pupation duration and adult flight ability (Stevens *et al.*, 1999) and repeated removal of silken nets resulted in lower body masses in the larval polycentropodids *Polycentropus flavomaculatus* (Pictet) and *Plectrocnemia conspersa* (Dudgeon, 1987; Beveridge & Lancaster, 2007). It has also been calculated that recycling silk in spiders reduces the cost of spinning an orb web by 34% (Opell, 1998). Therefore, feeding on galleries may be important in the recycling of silk in *T. waeneri*. However, as galleries have been found to be rich in chlorophyll and support diatom dominated algal assemblages (Hasselrot, 1993a) the gallery biofilm should itself be a valuable larval food source. Moreover, if diatoms are more abundant on galleries than in the epilithon, then galleries could represent a significant source of lipids (Becker, 1990) and, in particular, polyunsaturated fatty acids which larvae must obtain from their diet (Desvilettes *et al*., 1997). Thus, gardening may allow this species to maintain dominance in low productivity systems and enable them to live in lakes of lower productivity than they would otherwise be able to inhabit.

2.4.3 Gardening in relation to resource availability

Unexpectedly, the mixing models showed that galleries contributed a greater proportion of assimilated C and N to the larvae in the more productive lakes (Bassenthwaite, Esthwaite and Rydal Water) than in Windermere, Ullswater and Coniston. Furthermore, on a temporal within lake scale, the findings were similar; galleries made up the greatest proportion of larval N and C supply in the summer only in the three higher productivity lakes, with peaks in April in the other lakes. Thus larvae in the lower productivity lakes were not more reliant on galleries than larvae in higher productivity lakes.

The strongest evidence of gardening was for the gallery community in Esthwaite, the most productive lake in this study. The lakes were chosen to reflect the range in lake productivity associated with the distribution of *T. waeneri* in the English Lake District. However, on a wider scale, these lakes fall in the lower to intermediate section of the productivity gradient, with many lakes (for example in the UK the Shropshire Meres, Grey *et al.*, 2000) containing substantially higher concentrations of nutrients and

chlorophyll *a*. Thus, on this larger scale, Esthwaite could be classed as a lake of intermediate trophic status and sedentary grazers are expected to be most successful at intermediate resource supply (Hart, 1987; Fig. 1.1). However, the amount of gallery relative to epilithon assimilated was not significantly related to productivity (mean annual TP) across the six lakes sampled in any sample period. It may be that water column nutrient levels are a poor measure of benthic productivity and/or that nutrient status at a much finer scale may also show a better relationship with gardening. Furthermore, it is also predicted that other biotic factors, such as *T. waeneri* larval density and life-stage, will also play a role. This warrants further investigation.

Other factors, such as predation risk and palatability of food may affect food selection by *T. waeneri* larvae. For example, at Esthwaite, the background epilithic algal community is dominated by filamentous green algae and these may be a less favourable food source for the larvae than galleries, which are often diatom-dominated (Hasselrot, 1993a). Predation by fishes, leeches and other invertebrate predators (Jones, 1967) may also influence *T. waeneri* feeding behaviour, as grazing on epilithic algae requires the larva to protrude from the relative safety of its gallery.

In terms of nitrogen recycling, greater amounts of gallery ^{15}N-depletion relative to epilithon were also recorded in the three more productive lakes (Esthwaite, Rydal Water and Bassenthwaite). In contrast, the amount of gallery ^{15}N-depletion relative to the biofilm peaked over the summer period in the three less productive lakes (Coniston, Windermere and Ullswater) but in April in the other lakes. In all lakes, differences between epilithon and gallery $\delta^{15}N$ were lowest in the January sampling period. At this time, water column nutrient levels are relatively high, algal growth is slow and *T. waeneri* nutrient excretions reduced, as larvae are not actively growing (Jones, 1967; Dall *et al.*, 1984). This probably leads to less of a distinction between the galleries and epilithon in terms of nutrient availability and the available nutrient source.

However, the magnitude of the gallery ^{15}N-depletion relative to the biofilm may not be an accurate measure of the amount of nitrogen recycling occurring in the gallery community between sampling dates and across lakes. I have assumed that the differences in $\delta^{15}N$ value between the epilithon and the gallery are mainly a consequence of the two biofilm communities using nitrogen with different $\delta^{15}N$ signatures, due to the gallery biofilm having access to ^{15}N-depleted ammonium from

larval excretions (see Section 2.1). However, I must also consider other factors that could explain differences between $\delta^{15}N$ values of galleries and adjacent epilithon.

The ^{15}N-depletion of galleries relative to the surrounding epilithon could be the result of differences in algal species composition (Needoba *et al.*, 2003; Vuorio *et al.*, 2006) or environmental conditions (Vuorio *et al.*, 2006) between the gallery and the epilithon. However, I consider this to be unlikely for several reasons. Firstly, only $\delta^{15}N$ values from epilithon and galleries taken from the same rock (i.e. they were subject to similar environmental conditions) were compared. In addition, little variation in isotope signatures was measured across rocks within sampling periods. Secondly, although different species of algae growing under identical conditions (including the same nitrogen source) can have different $\delta^{15}N$ values (Needoba *et al.*, 2003; Vuorio *et al.*, 2006), there is as yet no indication that fractionation during nitrogen incorporation varies in any systematic way among groups of algae (Needoba *et al.*, 2003). The only exception are nitrogen fixing cyanobacteria (Goericke *et al.*, 1994) which are characterised by low $\delta^{15}N$ values, often close to 0‰ (Goericke *et al.*, 1994). However, cyanobacteria were not an appreciable component of either periphyton community in October in any of the lakes studied (Chapter 4), nor did they make up a substantial proportion (by biovolume) of epilithic samples from Esthwaite and Coniston, over a two year period (King *et al.*, 2002). Moreover, nitrogen fixation in cyanobacteria is usually reduced under high nitrogen conditions (Wetzel, 2001) such as those occurring within galleries (Hasselrot, 1993a). Finally, other more subtle differences in algal community composition are also unlikely to have caused the observed patterns in $\delta^{15}N$ values. In contrast to phytoplankton blooms, where one or a few species make up the majority of the biomass, epilithic and gallery biofilms contain a much more diverse mix of species and thus any inter-specific differences in fractionation should be averaged out across the community.

The source of nitrogen incorporated into gallery and epilithic biofilm could influence the measured $\delta^{15}N$ values. More specifically, the low $\delta^{15}N$ values of the gallery relative to the epilithon could be the result of assimilation of different nitrogen sources by the epilithon and the gallery. Algae are able to use ammonium, nitrite and nitrate as sources of inorganic nitrogen (Goericke *et al.*, 1994), but incorporation of nitrate is accompanied by less discrimination against the ^{15}N isotope than that of ammonium (York *et al.*, 2007). This difference is important because, in the lakes studied, inorganic

nitrogen is predominantly present in the form of nitrate, whereas ammonium usually occurs at very low (undetectable) concentrations within the water column (Maberly *et al.*, 2006). Thus, the main source of nitrogen available to the epilithon is probably nitrate. In contrast, galleries contain high concentrations of ammonium (Hasselrot, 1993a), which is probably the main inorganic nitrogen source available to the gallery biofilm.

Nevertheless, both the gallery and the epilithon may be mainly incorporating ammonium. Phytoplankters often preferentially incorporate ammonium rather than nitrate, even where ammonium makes up only a small fraction of the dissolved inorganic nitrogen pool (York *et al.*, 2007 and references therein). The gallery biofilm would be exposed to much higher ammonium concentrations than the epilithon and would therefore discriminate against the heavier isotope (^{15}N) more; nitrogen starved cells and low ammonium concentrations both promote lower discrimination factors (Waser *et al.*, 1999; York *et al.*, 2007). This would result in lower gallery than epilithic $\delta^{15}N$ values. Nitrogen available to the epilithon may have been especially limited if substantial boundary layers built up or if epilithic biofilms were thick. The high $\delta^{13}C$ value for the epilithon in Coniston (-8.61) in August, at the upper limit of values (-47 to -8‰) quoted for periphyton by Finlay & Kendall (2007), suggests that boundary layers may have limited the exchange of at least some substances (e.g. carbon dioxide). Thus, it is possible that the patterns observed are a result of discrimination against the ^{15}N isotope during excretion by the larvae (as suggested in the Introduction, section 2.1) as well as decreased fractionation on uptake of ammonium by the more nitrogen limited epilithon. Importantly, and even if this was the case, the patterns obtained in this study still reveal the occurrence of nitrogen recycling within the gallery community. Thus, the dominance and high abundance of *T. waeneri* within the littoral communities of these lakes means that nitrogen recycling within the gallery system could have important impacts on the functioning of the littoral community as a whole. *Tinodes waeneri* communities may contain greater quantities of nitrogen and be able to support greater production than surrounding rock surfaces, and algal community composition may also be affected.

2.4.4 Conclusions

This large scale stable isotope investigation (across six lakes and spanning a year) suggests that gardening in *T. waeneri* may be a common occurrence. Significant

nitrogen recycling within the gallery community was observed in four of the six lakes studied and nitrogen and carbon stable isotopes also suggested that gallery nitrogen and carbon was being assimilated into the larvae in all lakes and across all time periods. However gallery-derived carbon and nitrogen was assimilated by larvae in greater proportions, relative to epilithon, in the more productive lakes. The next challenge is to explain patterns of gallery assimilation among lakes and time periods. It will be important to assess how food quality and quantity varies between the gallery and the epilithic algae across nutrient gradients and how this influences feeding and growth of *T. waeneri.*

Chapter 3 – Why do *Tinodes waeneri* larvae consume their galleries?

3.1 Introduction

Sedentary grazers are common in freshwaters (Hart, 1987) and are often a dominant component of the community. They commonly construct fixed retreats (e.g. galleries, cases), or live within mines in aquatic macrophytes (Bergey, 1995). However, a sedentary lifestyle places restrictions on the resources (including food, shelter and building materials), such organisms can secure. Competition from mobile grazers has the potential to reduce further available resource levels. In addition, sedentary animals could be particularly vulnerable to predation by mobile predators and to environmental disturbance (e.g. scouring or falling water levels).

Psychomyiid larvae (Trichoptera), including *Tinodes waeneri*, are sedentary and reside in galleries that contain organic and/or sediment particles bound by larval silk, attached to hard surfaces (Edington & Hildrew, 1995). The location of these galleries is not entirely fixed in relation to the substratum, as the larvae generally add gallery material to one end and demolish the other end (e.g. Alderson, 1969; Spänhoff *et al.*, 2003; Alecke *et al.*, 2005), thereby gradually shifting the gallery position. Such movement of the gallery has been assumed to be a mechanism by which these caddis larvae are able to reach new food patches, which they can then consume by feeding from the front of their galleries, by protruding their armoured head and prothorax (e.g. Jones, 1967). Galleries would thus have a protective function, shielding the larvae from wave washing and predation. However, an alternative role for larval galleries has been proposed more recently by Hasselrot (1993a). He postulated that the gallery and its larval resident function as a closely coupled, mutualistic system (similar to a coral reef) in which larvae fertilise the gallery biofilm with their excretions and subsequently consume the fertilised biofilm from the gallery wall (Hasselrot, 1993a). Thus, under this hypothesis, the primary purpose of the gallery would be as a ‘garden’.

Gardening has been defined as ‘interactions in which plant assemblages within a fixed site are modified through the activities of a grazer that selectively enhances the food value of the grazed plants for the grazer’ (Plagányi & Branch, 2000). Therefore, if *T. waeneri* larvae are using their galleries as a garden, the galleries should be a better food

resource (in terms of quality and/or quantity) than the general background (unfertilised) epilithon surrounding them (the alternative resource available). Apart from simply providing more and/or a better quality food, however, there could be further advantages to gardening. It could firstly provide grazers with a more predictable continuous food supply (Woodin, 1977), thus reducing the impacts of fluctuations in availability. Secondly, a related benefit may be that the gallery acts as a supplementary or backup food supply (i.e. a larder), that is used intermittently during periods of reduced food availability or increased demand. Experiments on the biofilm grazing cased caddis *Agapetus fuscipes* Curtis indicated that the chlorophyll (a surrogate for algae) in 'dirty looking' cases enabled the larvae to survive for an average of ten days longer than larvae with clean cases, when starved under laboratory conditions (Cox & Wagner, 1989). Cases that looked dirty initially were clean by the end of the experiment, suggesting that the larvae were using material on the cases as a food source. The experimental manipulations mimicked times of food shortage (such as might be associated with periods of spate), and the algae residing on the cases may thus function as an important backup option for these caddisflies (Cox & Wagner, 1989).

The use of galleries as a food source could also be to reduce competition for living space. *T. waeneri* larvae live in dense aggregations on stones within the littoral of lakes and have been recorded at densities of up to 11,500 individuals per m^2 (Dall *et al.*, 1984), although densities of around 1000 to 2000 individuals per m^2 are more usual (e.g. Hasselrot, 1993a). The females lay several egg masses, containing 100 to 300 eggs, on partly submerged stones (Jones, 1967). Sometimes, several females exploit suitable laying sites (Jones, 1967) resulting in dense aggregations of larvae. Furthermore, larvae have restricted dispersal capabilities (Harrison & Hildrew, 2001), leading to slow colonization of other suitable habitat.

Another possibility is that galleries could provide a less risky food source in circumstances when emerging from the gallery to feed on epilithon is associated with a high predation risk; for instance, predator cues (kairomones) result in changes in feeding behaviour of *Chironomus riparius* Meigen (Hölker & Stief, 2005). Emerging from the gallery to feed on epilithon could also increase the risk of larval detachment from the substratum due to water movements and wave washing (Dall *et al.*, 1984).

Thus, gardening is only one possible explanation for larval feeding from the gallery. An assessment of nitrogen recycling using stable isotopes demonstrated that gallery biofilm communities were using larval excretions as a nitrogen source. Moreover, galleries were indeed a dietary source for these larvae (Chapter 2), suggesting that the galleries have the potential to act as a garden, at least in the six lakes studied. However, the primary reason (reduced predation *versus* a better food source *versus* reducing competition for space) for assimilating gallery material remains unclear. The aim of the study described in this chapter, therefore, was to evaluate whether galleries do provide the larvae with a higher value food resource and, secondly, to assess whether resource availability explained the variation in the proportion of gallery material assimilated (as revealed by stable isotopes). If the provision of more/better food, i.e. gardening, explains larval consumption of gallery biofilm, I would expect galleries to contain a greater quantity and/or better quality of resources and also that assimilation of gallery material would be related to resource availability.

3.2 Methods

Epilithic and gallery food resources available to *T. waeneri* larvae, epilithic macroinvertebrates and environmental variables were assessed in August and October 2006 and in January 2007 at sampling sites in six lakes in the English Lake District. Lakes were chosen to represent a productivity gradient and ranged from moderately oligotrophic to eutrophic (based on total phosphorus (TP) and mean phytoplankton chlorophyll *a*; Maberly *et al.*, 2006 Table 2.1). Samples were taken from the same location and, in the case of macroinvertebrates and C:N ratios, from the same rocks from which the samples for stable isotopes were collected (details of sampling sites are given in Chapter 2).

3.2.1 Are galleries a better larval food resource than the background epilithon?

3.2.1.1 Resource quality

C:N was used as a measure of resource quality. The molar C:N ratio of the whole gallery (silk plus other organic components) was compared to that of the surrounding epilithon, and larval and silk C:N ratios were also measured. The C:N data derives from the same samples as the stable isotope data; samples were therefore processed using the same methods (see Chapter 2 for methods) and the April data are also included. A urea dilution series (0 to 0.6mg urea for standard columns) was included at the start of every

run on the mass spectrometer and was used to calculate the elemental mass of carbon and nitrogen in the samples. For the repeated analysis of a standard (Cyclohexanone-2,4-Dinitrophenylhydrazone), typical accuracy (i.e. ±1SD) was ± 0.12.

3.2.1.2 Resource quantity

Resource quantity was measured as chlorophyll *a* concentration and ash free dry mass (AFDM). AFDM was measured in October 2006 and January 2007 only. No measurement was made in August due to low epilithic biomass. On each sampling occasion, epilithon was sampled from five rocks using a periphyton sampler modified from Stockner and Armstrong (1971). The sampler was made from a 20ml syringe from which the tip had been removed. Half a toothbrush head was glued onto the plunger and the plunger was rotated for four complete turns in each direction whilst the syringe was pressed against the rock. Rocks were sampled after removal from the water. The material collected in the toothbrush bristles was washed into a clean beaker using deionised water. Six such samples were amalgamated into one epilithon sample and washed into a container. Samples were kept cool (on ice) and dark in the field and frozen immediately on return to the laboratory. The syringe had an internal diameter of 19mm and sampled an area of 2.84cm^2. Therefore the total area of epilithon sampled per rock was 17.04cm^2. The periphyton sampler used in this study is smaller than those widely used by other workers (e.g. area sampled 5.3cm^2 in study by Loeb, 1981), to enable deployment in areas away from feeding territories. The syringe sampler effectively removed biofilm attached to tiles during laboratory tests and is recognized as being efficient at removing algal biofilms from substrata (e.g. Aloi, 1990). Four randomly selected galleries were labelled, and photographed with a small section of ruler adjacent to them to provide a scale bar. These galleries were then carefully removed, to avoid contamination from the surrounding epilithon, and placed into labelled Eppendorf tubes, kept cool and dark and frozen on return to the laboratory.

Epilithon samples were defrosted and carefully divided into two equal volumes, using the methods described by Biggs & Kilroy (2000). Samples were filtered onto pre-weighed and pre-ashed GF/C filter papers. For chlorophyll analysis these epilithic samples and two randomly selected galleries from each rock were freeze-dried in the dark. Freeze-drying prior to extraction promotes higher extraction efficiencies for both epilithon (Hagerthey *et al.*, 2006) and sediment (Hansson, 1988). Samples were extracted on ice, overnight, in 90% acetone, before spectrophotometric measurement of

chlorophyll *a*. Galleries for AFDM determination were placed in pre-weighed clean aluminium capsules (7.5x6mm). Galleries and filters were oven dried, reweighed and then ashed in a muffle furnace (500°C for 5 hours).

3.2.1.3 Gallery and territory size

Gallery attributes were measured from digital photographs (with scale bars) taken in the field, using Image J. The area of rock surface covered by the gallery (gallery footprint) was measured for all samples and this was also used to calculate a maximum gallery surface area (based on the height of the gallery equalling a third of the width of the gallery).

In the October sample the feeding territories of fifth instar larvae were also measured. A feeding territory is defined as the gallery area (gallery footprint) plus the area of epilithon surrounding the gallery that the larva is able to reach without fully leaving its gallery. Therefore, feeding territories were the area of the gallery plus a strip the length of the larva, extending out from the perimeter of the gallery. Although, larvae usually protrude from the ends of their galleries, they were also commonly observed extending out of the sides of their galleries, presumably having bitten an exit hole.

3.2.1.4 Nutrient concentrations

Sample sites were chosen to lie along a productivity gradient (moderately oligotrophic to eutrophic). Water column nutrient concentrations (phosphate, nitrate and nitrite) were measured using a segmented flow autoanalyser (Skalar) and standard colorimetric techniques (Grasshoff *et al.*, 1999) for five water samples from each sampling site, for the October and January samples. Water temperature was also measured.

3.2.2 Gallery function - Habitat availability

3.2.2.1 The epilithic invertebrate community

Once samples for stable isotope analysis (see Chapter 2) had been collected from rocks, all remaining sedentary invertebrates were scraped off the top surface of the rock using a toothbrush. Upon removal from the water, rocks were immediately placed into individual white trays, and therefore any sedentary invertebrates that left the rock during sampling were retained within the tray. Scrapings were placed in sealed plastic bags and kept cool until return to the laboratory. The surface area sampled was calculated by tracing the scraped area of the rock onto acetate, which was later excised and weighed.

The rock scrapings were sorted and all invertebrates were retained and frozen for later identification and measurement. Invertebrates other than *T. waeneri* were grouped into orders and counted. Head widths and larval lengths were measured for all *T. waeneri* larvae (i.e. those collected for stable isotope analysis and those subsequently scraped from the rock surface) from each rock sampled. Larvae were sometimes damaged and, in these cases, the minimum number of larvae on the rock was assessed and the instars were determined with the additional use of bristle number on the anal prolegs as described by Jones (1967). Instar distribution and density of *T. waeneri* larvae per rock was determined. Head widths were used to calculate individual larval dry mass using the equation developed specifically for lake dwelling *T. waeneri* by Baumgärtner & Rothhaupt (2003). In the few cases where head widths could not be measured, the mean head width for the appropriate instar was used in the calculations.

3.2.2.2 Substratum characteristics

The particle size distribution of the substratum at each site was assessed in August using a 0.5 by 0.5m quadrat, divided into 0.1 by 0.1m squares resulting in 36 cross over points. The substratum directly below each cross over point in 10 randomly located quadrats was assigned to one of the following categories: rocks <2cm, rocks 3-5cm, rocks 6-10cm, rocks 11-25cm and rocks >25cm on their longest axis, sediment and bedrock.

3.2.3 Gallery function - Larval protection

Due to lake dimensions and aspect, the sampling sites also potentially varied in the levels of wave-washing they received. Attempts to use plaster of Paris blocks to measure relative water motion (Thompson & Glenn, 1994) were unsuccessful as the blocks were too visible in the water (despite being dyed) and were disturbed or relocated by the public. As an alternative, mean weighted fetch and exposure was calculated using the method described in Brodersen (1995) and wind data for Keswick obtained from www.windfinder.com. The annual distribution of wind directions and the wind speed averaged across the 12 months was used. Sampling depth was held constant at 0.4m.

3.2.4 Gallery function - Proportion of gallery-derived C and N assimilated by the larvae

The proportion of larval assimilated material derived from the gallery was calculated for each lake and sample period using the SIAR mixing model, as described in Chapter 2. Mean values derived from the model are used here.

3.2.5 Data analysis

All data are presented as means (±1SE). Two way ANOVAs and Tukey HSD *post hoc* tests were used to test for differences between the epilithon and galleries in terms of resource quality or quantity. Spearman's rank correlations were run to look for correlations between the various resource level (chlorophyll *a* concentrations, AFDM, C:N ratios, gallery length and area) competition (number of *T. waeneri* larvae and chironomids per m^2, biomass of *T. waeneri* per m^2 and substratum characteristics) and protection (mean weighted wind fetch and wind exposure) parameters and the mean proportion of assimilated larval diet (C and N) derived from the gallery. All analyses were performed using SPSS version 13.

3.3 Results

3.3.1 Are galleries a better larval food resource than the background epilithon?

3.3.1.1 Resource quality

The mean larval C:N value was 5.30±0.55 with individuals ranging from 4.47 to 6.61. In comparison, mean gallery and epilithic C:N ratios were 7.71±0.29 and 7.89±0.29, respectively. There was no significant difference in the C:N ratio of these two possible larval food sources (ANOVA, $F_{1,3}$=0.132, P=0.741) and there was no consistency in the direction of differences among lakes or across dates (Fig. 3.1). Coefficients of variation had similar ranges among lakes for epilithon (13-24%) and galleries (11-22%), but were lower in galleries than in the epilithon in all lakes other than Coniston and Rydal Water (Table 3.1). However, there was a significant item (i.e. epilithon, gallery) x lake x date interaction (ANOVA $F_{15,382}$= 3.703, P<0.001). There was also significant among lake variation in resource (gallery and epilithon) C:N ratios (ANOVA $F_{5,15.1}$=7.065, P=0.001; Fig. 3.1). C:N ratios of both these potential larval food sources also varied temporally and were generally lower (better quality) in April and January than in the August and October sampling periods (Fig. 3.1). In April, there was a greater difference in C:N ratios between the larvae and their food sources in the less productive lakes (Coniston Water, Ullswater and Windermere) compared to the other lakes (Fig. 3.1). There was no

relationship between epilithic C:N ratios and the nutrient concentrations measured in the October and January sample periods.

Figure 3.1 Mean (±1 SE) molar C:N ratios of the epilithon, galleries and *Tinodes waeneri* larvae for the four sample periods. Lakes (UL = Ullswater, CO = Coniston Water, WI = Windermere, RY = Rydal Water, BA = Bassenthwaite and ES = Esthwaite) are arranged in order of increasing productivity.

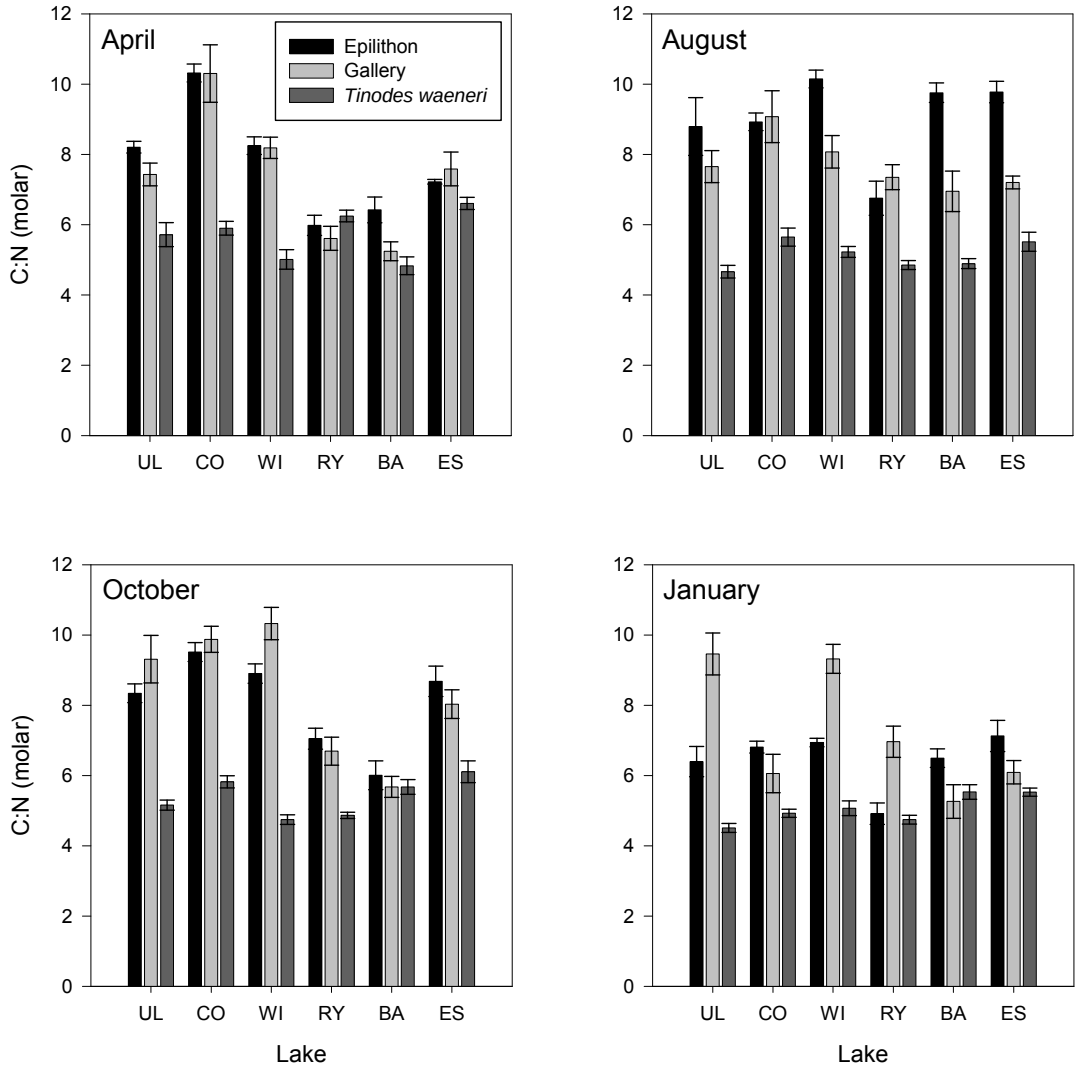


Table 3.1 Coefficients of variation for C:N ratios and chlorophyll *a* values within gallery and epilithon samples from the different lakes.

Lake	C:N ratio		Chlorophyll *a*	
	Epilithon	Gallery	Epilithon	Gallery
Bassenthwaite	23.9	12.1	46.5	55.1
Coniston	16.9	21.7	68.3	49.4
Esthwaite	15.5	11.5	77.1	38.2
Rydal Water	13.4	16.3	60.5	12.1
Ullswater	17.0	10.9	67.6	17.2
Windermere	15.6	11.8	51.6	38.5

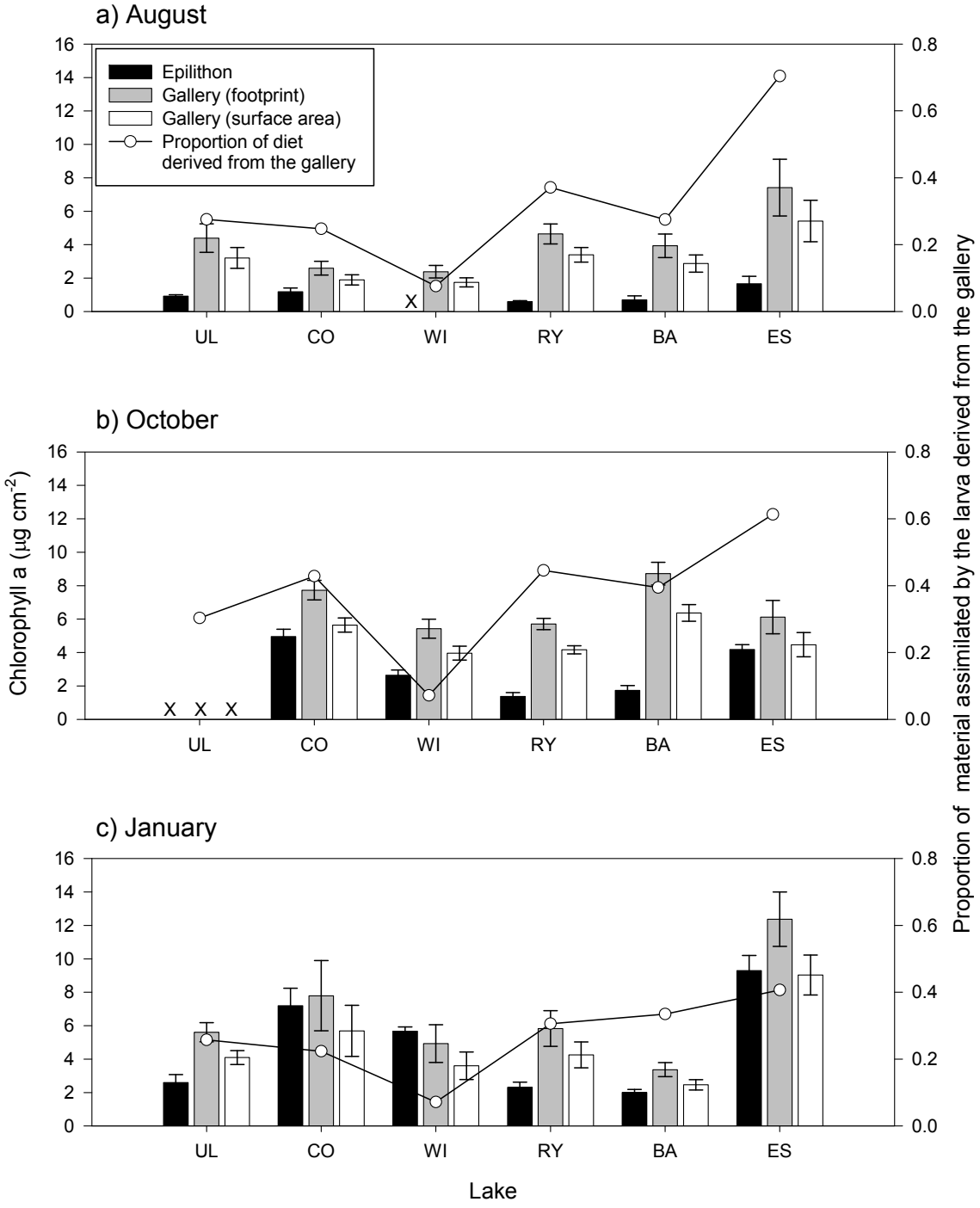
Galleries contain silk as well as the organic matter associated with the biofilm (including algae) and the C:N ratio of silk was 4.75±0.08 compared to 5.05±0.09 for the larvae that produced it (larvae collected in October 2007). Silk should therefore reduce the gallery C:N ratio relative to the epilithon that lacks silk, and thus the gallery biofilm portion itself would probably have been of equal or worse quality relative to the epilithon.

3.3.1.2 Resource quantity

Chlorophyll *a* content was significantly higher on the gallery than in the epilithon in all sampling periods (August 2006: item (i.e. gallery and epilithon) x lake interaction $F_{4,54}$=2.598, P=0.046; October 2006: item x lake interaction $F_{4,65}$=4.087, P=0.05; January 2006: item $F_{1,70}$=9.065, P=0.004; Fig. 3.2a-c), with the magnitude of difference in chlorophyll concentrations between the gallery and epilithon varying among lakes. Epilithic chlorophyll *a* concentrations were uniformly low during the August sample period (Fig. 3.2a), although chlorophyll *a* concentrations at Esthwaite were significantly higher than those at Coniston and Ullswater (Tukey HSD *post hoc* tests). In contrast, in January differences in chlorophyll *a* concentrations between galleries and epilithon were generally much smaller (and non significant when maximum gallery surface area is used) than in the earlier sampling periods, and this occurred against higher background epilithic chlorophyll concentrations. Coefficients of variation for gallery and epilithic chlorophyll *a* values ranged from 12 to 55% and 46 to 77%, respectively, among lakes, with lower values measured for the gallery in all lakes other than Bassenthwaite (Table 3.1). There was no correlation between gallery or epilithic chlorophyll *a* content and

any of the water column nutrient concentrations measured across lakes in either October or January.

Figure 3.2 Mean (±1 SE) chlorophyll *a* content (µg cm-2) of the epilithon (black bars) and *Tinodes waeneri* galleries (grey bars (footprint) and white bars (surface area)) in the six lakes in a) August, b) October and c) January. Lakes are arranged in order of increasing productivity and X indicates missing data. The mean proportion of assimilated larval carbon and nitrogen derived from the gallery (calculated using SIAR mixing models) is shown by the open symbols on each graph. The line joining the points has been plotted for clarity and is not intended to indicate any causal relationship between the variables.



AFDM measurements also indicated that galleries contained a higher quantity of potential food resources (organic matter content) per cm^2 than the epilithon among all lakes, in October and January (Fig. 3.3). However, unlike for chlorophyll *a*, the magnitude of the difference in AFDM content between the two possible larval food sources was similar in the October (1.13±0.1mg cm^{-2}) and January (0.94±0.1mg cm^{-2}) samples (based on gallery footprints). As galleries consist of both silk (about 11.5% by weight, chapter 2) and biofilm they are expected to have higher AFDM. However, even when silk was accounted for, galleries contained over three times the amount of AFDM as the surrounding epilithon.

The gallery only accounted for between 13.8 and 18.8% of total territory area (Fig. 3.4a) for fifth instar larvae in October 2006. Potential feeding territory area was positively related to gallery length (regression, r^2=0.815, $F_{1,5}$=27.447, P=0.003), as there was little difference in the mean length of a fifth instar larva among lakes (7.74 ± 0.38mm), and galleries were straight rather than sinuous. On average, galleries were 5.0±0.2 times longer than their larval occupants (Fig. 3.4b). The largest territories and longest galleries were found in Rydal Water and Coniston. The galleries in these lakes were significantly longer than galleries in Windermere, Ullswater and Bassenthwaite (where larvae had the shortest galleries and smallest territories, Tukey HSD <0.05).

Using mean territory and gallery areas and mean chlorophyll content of gallery and epilithon it was possible to examine the relative importance of the gallery as a chlorophyll source (Fig. 3.4c). Larval territories at Coniston contained the highest total amounts of chlorophyll *a* (61.5µg) followed by Esthwaite (44.2µg). The majority (79.2 and 80.3%, respectively) of this chlorophyll *a* was attributable to the epilithon. In contrast, Rydal Water larvae had slightly larger territories than those in Coniston and Esthwaite, yet these territories only contained 25.2µg of chlorophyll, of which 42.9% was found in the gallery. This was despite the gallery representing only 15.3% of the total feeding territory area. Bassenthwaite and Windermere, also displayed low total amounts of chlorophyll in the feeding territory, with galleries in Bassenthwaite containing over half (52.8%) of the chlorophyll potentially available to the larvae (Fig. 3.4c). Similarly, among all lakes, galleries contained an average of 40% of the AFDM available to the larvae (silk was excluded from these calculations).

Figure 3.3 A comparison of the mean (±1 SE) ash free dry mass of epilithon (black bars) and gallery footprint (grey bars) and surface area (white bars) in October 2006 and January 2007. The X indicates missing data.

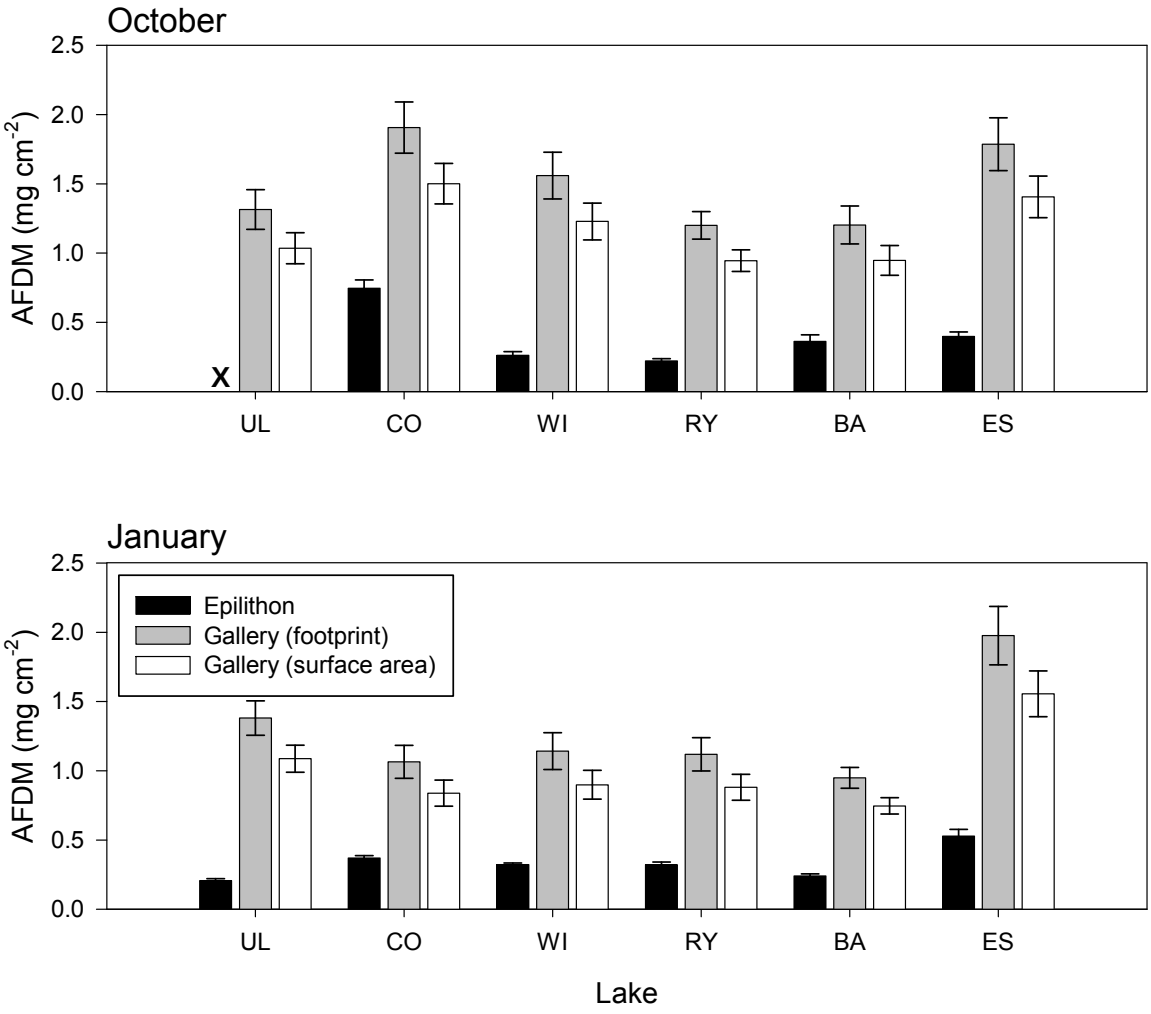
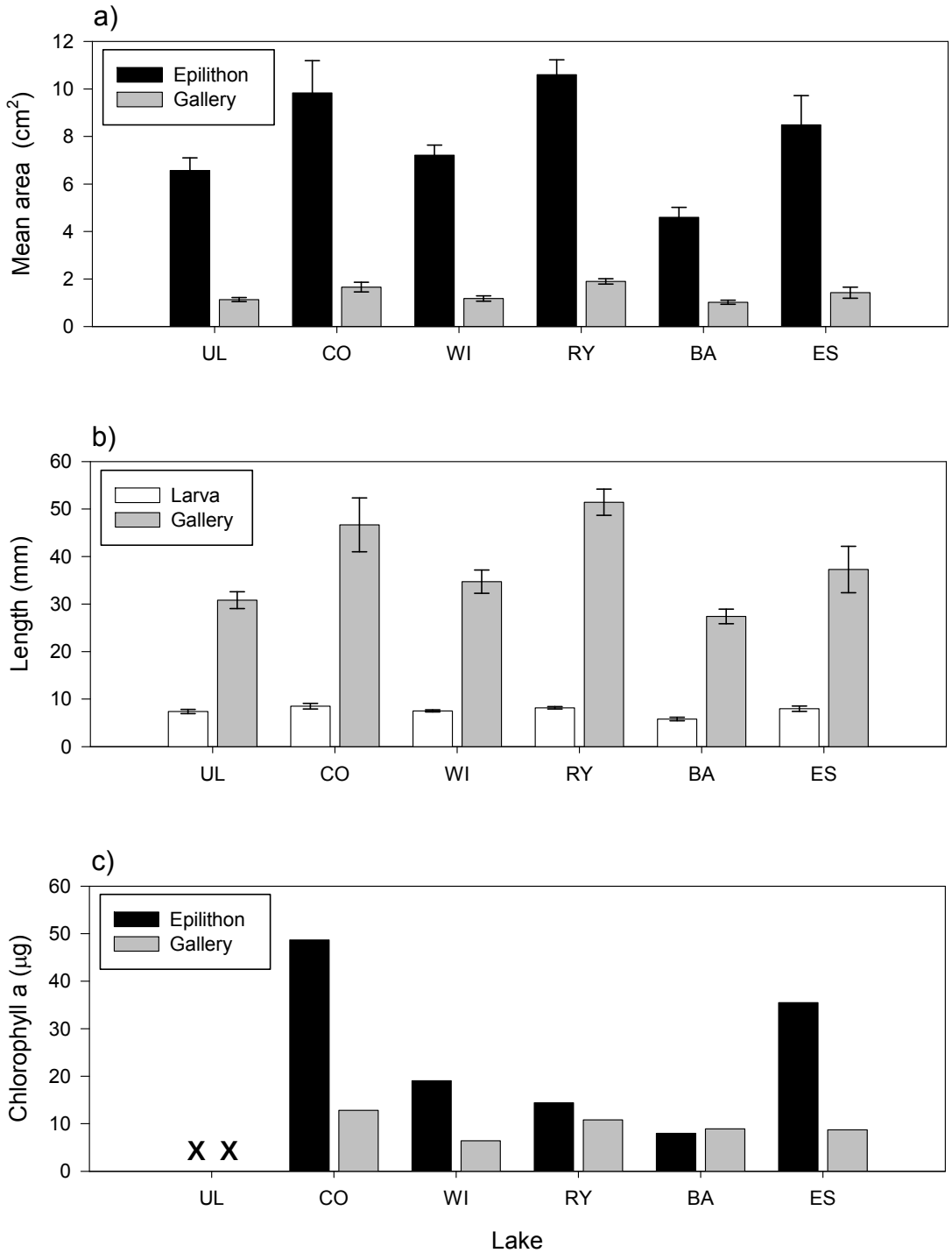


Figure 3.4 Territory characteristics of fifth instar larvae collected during the October sampling period across the six lakes; a) the mean (±1 SE) area of epilithon (black bars) and gallery (grey bars) contained within the territory area, b) the mean (±1 SE) length of larvae (white bars) and galleries (grey bars) and c) total amount of chlorophyll *a* in the epilithon (black bars) and gallery (grey bars) of an average territory. Calculations were based on mean gallery and epilithon chlorophyll concentrations and the mean area of epilithon and gallery within territories. These calculations could not be undertaken for Ullswater as chlorophyll a data were not available.



3.3.2 The function of the gallery

The mean proportion of larval carbon and nitrogen derived from the gallery (relative to the epilithon) were calculated using SIAR mixing models (Chapter 2) and the results are briefly described here. There was substantial variation in the proportionate contribution of gallery-derived nutrients to the biomass of the larvae (7 to 70% across the August to January sample periods) both spatially and temporally (Fig. 3.2). Esthwaite larvae contained the highest proportion of gallery derived material, with a peak of 70% occurring in August. Contributions peaked in October in Rydal Water (37.1%), Bassenthwaite (39.4%) and Coniston (42.8%), whereas in Ullswater and Windermere contributions were more constant at 27.0±1.3% and 7.4±0.2%, respectively. Contributions were also more similar across lakes in January (Fig. 3.2c) than in the other two sample periods (30.5% ± 0.3 for lakes other than Windermere). This variation provides the opportunity for examining the alternative hypotheses concerning the primary function of *T. waeneri* galleries (gardening, enabling larvae to reside at higher densities or larval protection). Data relating to these hypotheses are examined in turn.

3.3.2.1 Gardening

In August there was a significant positive correlation (r_s=1, n=6, P<0.001) between the mean proportion of dietary material derived from gallery and gallery chlorophyll *a* levels (Figs. 3.5 & 3.2a). However, there was no relationship between resource quantity and the relative importance of gallery material to larval biomass in October (r_s=0.1, n= 5 P=0.873; Fig. 3.2b) or in January (r_s=0.1, n=5, P=0.873; Fig. 3.2c).

In October, there was a positive correlation between the contribution of the gallery to larval biomass and the discrepancy in C:N ratio between the epilithon and the gallery (r_s=0.943, n=6 P=0.05, Fig. 3.6a). Thus, where galleries had lower C:N ratios (i.e. were of better quality) than the epilithon, gallery formed the major contribution to the larvae. There was no significant correlation between these two variables during the August 2006 (r_s=-0.29, n=6, P=0.957) and January 2007 (r_s=0.6, n=5, P=0.285) sample periods. However, there was also variation in the C:N ratio of the epilithon across sampling periods (April to January) within individual lakes. In Esthwaite, Rydal Water and Coniston there was a clear trend towards greater dietary importance of galleries as the epilithic C:N ratio increased (i.e. had reduced food quality, Fig. 3.6b), although this was only significant for Esthwaite (r_s=1, n=4, P<0.001).

Figure 3.5 The relationship between the proportion of assimilated larval material derived from the gallery and gallery chlorophyll *a* for the August 2006 sample.

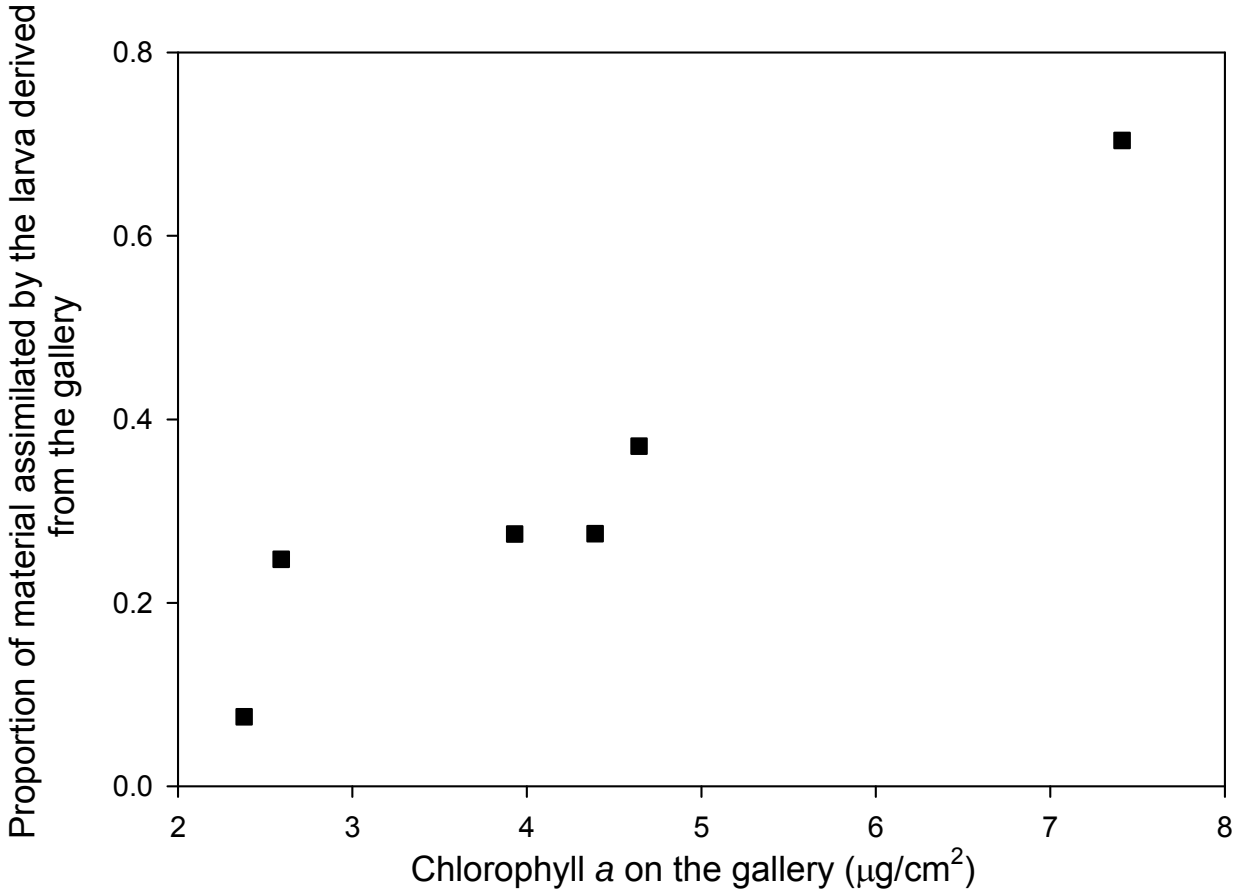
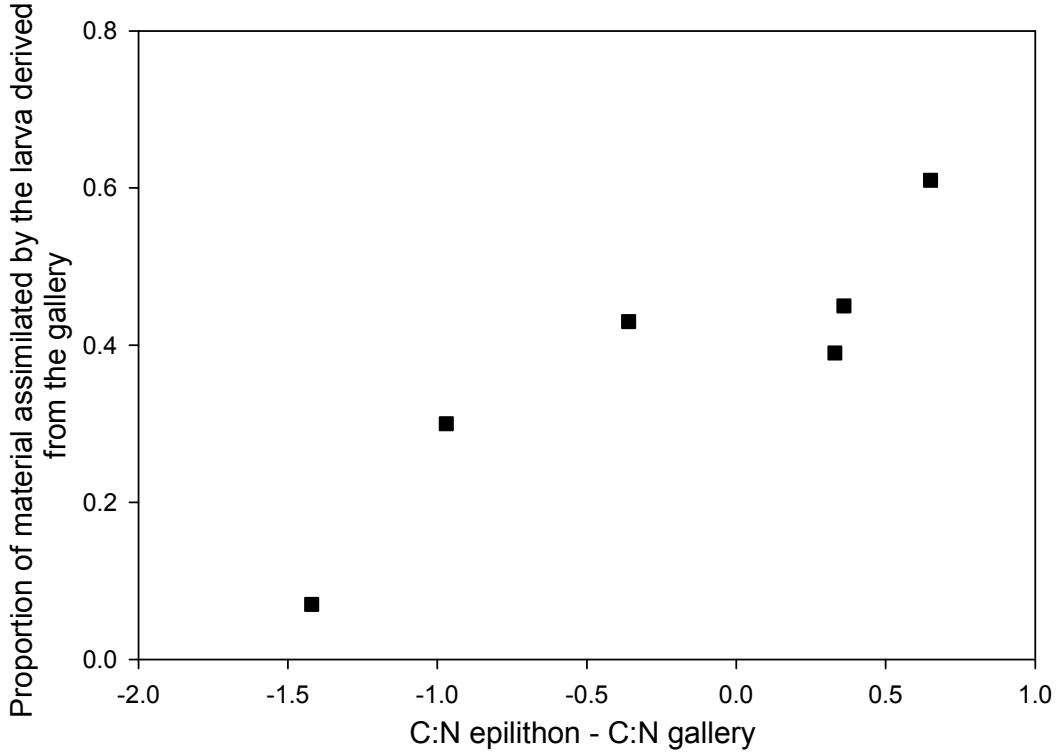
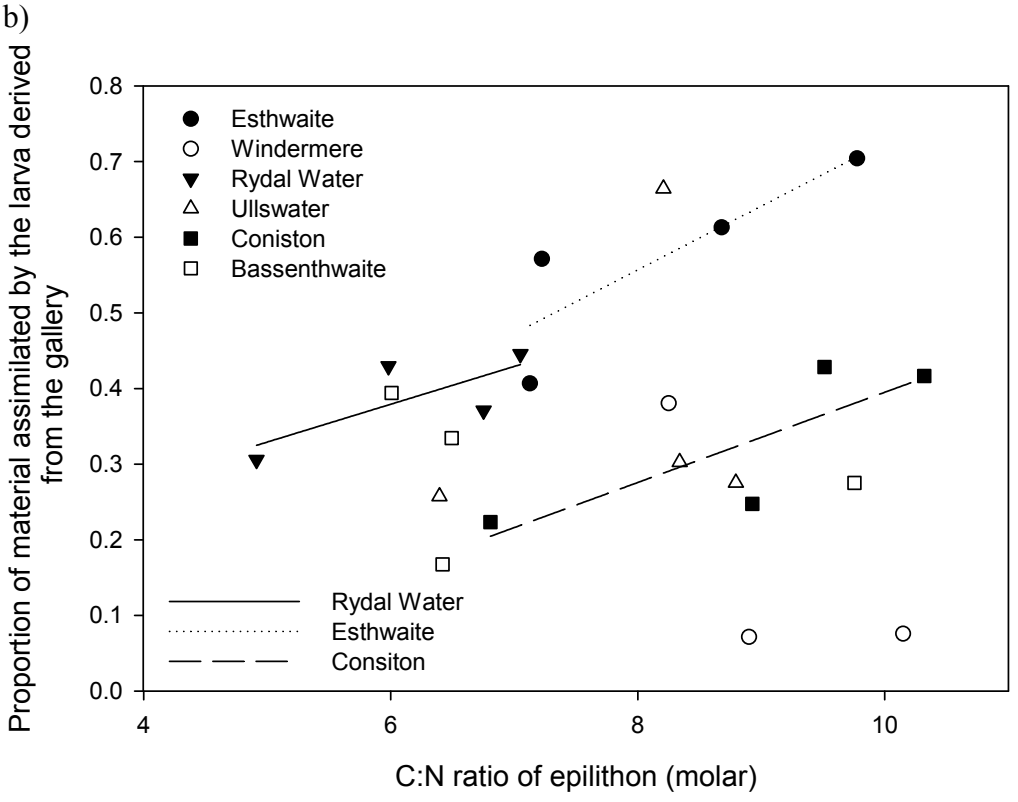


Figure 3.6 The relationship between the proportion of assimilated larval material derived from the gallery and a) the difference in C:N ratio between the epilithon and gallery across lakes in the October sample and b) the epilithic C:N ratio for each lake across all the sampling periods.

a)



b)



3.3.2.2 Habitat availability

The main sedentary invertebrates on the rocks in all lakes studied were *T. waeneri* and chironomid larvae. The few other sedentary organisms sampled included cased and caseless (Polycentropodidae) caddis larvae and freshwater limpets. The densities of *T. waeneri* (larvae per m^2 of suitable rock surface) were highly variable temporally within lakes and spatially among lakes, with high densities associated with young larvae. The highest measured density of *T. waeneri* was 2622 ± 483 individuals per m^2 at Ullswater in August, when over 50% of the larvae were either first or second instars. The lowest densities throughout the study were recorded at Coniston and Esthwaite. The biomass of *T. waeneri* in Ullswater (0.53g dry weight per m^2) was at least double that found in any of the other lakes (mean: 0.13g dry weight per m^2). Total dry weight per m^2 changed little between October and January in Bassenthwaite, Coniston, Esthwaite and Rydal Water. Similarly, the instar distribution in January showed little change from that recorded in October. Chironomids were present at higher densities than *T. waeneri* at Coniston in all three time periods examined, and densities were also higher in Bassenthwaite and Esthwaite in January and in Esthwaite in August (Fig. 3.7). Proportion of gallery incorporated into *T. waeneri* larvae was not correlated with *Tinodes* density and biomass or chironomid density in any of the sampling periods.

The substratum particle size distribution was also variable between sampling sites (Table 3.2). All sampling sites other than at Esthwaite and Rydal Water had over 90% coverage of rocks suitable for *T. waeneri* larval colonization (e.g. long axis 3cm and above). Coverage at Esthwaite was 57% with only 11% of these falling into the 6cm and greater category. At Rydal Water, 59% of the lake bed (within the portion of the sampling site inhabited by *T. waeneri*) was covered in rocks with a long axis greater than 6cm, with 70% of sampling points hitting rocks larger than 3cm. The mean contribution of gallery C and N to larval biomass was negatively correlated with the availability of rocks larger than 3cm in August (r_s=-1, n=5, P<0.001), a period of high *T. waeneri* density.

Figure 3.7 Mean (±1 SE) densities of *Tinodes waeneri* (black bars) and chironomid larvae (grey bars) across the six lakes in each of the three sampling periods.

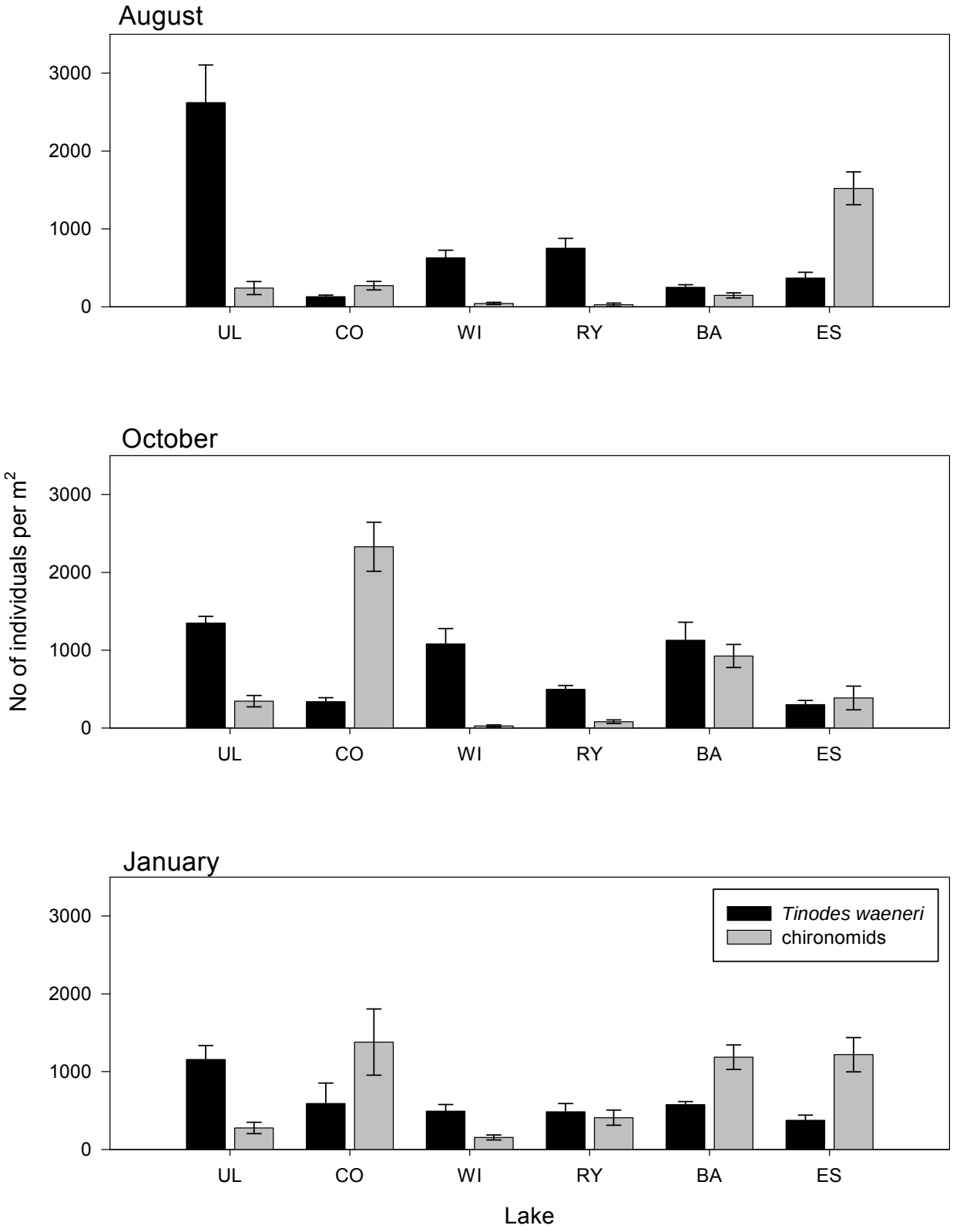


Table 3.2 Substratum characteristics (for the area of the littoral inhabited by *Tinodes waeneri* larvae) and mean weighted wind fetch and wind exposure (higher values indicate greater wind exposure) for the six sampling sites.

Sampling site	Sediment and rocks < 3cm long (% of substrate)	Rocks >3cm long (% of substrate)	Rocks >10cm (% of substrate)	Mean weighted wind fetch (km)	Wind exposure
Bassenthwaite	6.39	93.61	9.17	0.46	0.59
Coniston	3.89	96.11	27.78	1.19	1.25
Esthwaite	43.06	56.94	4.86	0.48	0.89
Rydal Water	29.44	70.56	49.72	0.15	0.26
Ullswater	2.78	97.22	43.33	0.68	0.75
Windermere	9.44	90.56	26.11	0.93	0.73

3.3.2.3 Larval protection

There was variation in how exposed, and therefore wave-washed, the sites were. The mean weighted fetch ranged from 150m at Rydal Water to 1190m at Coniston (Table 3.2) and these two sites were also the least and most exposed sites. Esthwaite was the second most exposed site, despite having a relatively short mean weighted fetch. The mean weighted wind fetch and the degree of wind exposure were not correlated with the proportion of larval biomass derived from gallery material for any of the sample periods under consideration. However wind exposure (and mean weighed fetch) was positively correlated to various measures of resource availability, particularly in the October sample period. These variables included epilithon chlorophyll *a* concentrations (r_s=1, n=5, P<0.001) and epilithon and gallery AFDM (r_s=0.9, n=5, P=0.037 and r_s=0.94, n=6, P=0.005 respectively). Epilithon chlorophyll *a* concentrations were also significantly positively correlated to wind exposures in August (r_s=0.9, n=5, P=0.037) and January (r_s=0.82, n=6, P=0.04).

3.4 Discussion

3.4.1 Are galleries a better larval food resource?

Data from the six lakes studied here clearly demonstrate that galleries contain higher quantities of food than the adjacent epilithon (the alternative food source for *T. waeneri* larvae). This is true of both chlorophyll *a* and ash free dry mass, even when silk was

taken into account. High gallery chlorophyll concentrations are likely to be the result of gardening through fertilisation by the larvae (Chapter 2). This view is supported by studies using nutrient diffusing substrata, which emulate natural point sources of nutrients (Pringle & Triska, 2007), like *T. waeneri* galleries. Increases in periphytic algal biomass in response to fertilisation, underpins the use of nutrient diffusing substrata to assess nutrient limitation (e.g. Fairchild *et al.*, 1985).

However, other factors may also contribute to high gallery chlorophyll concentrations and need to be considered. Firstly, a higher chlorophyll content of galleries than epilithon may reflect retreat defence against other grazers coupled with grazing of the surrounding epilithon by the retreat inhabitant and possibly intruders. This is unlikely to be the case in the current study as the stable isotope results suggest that galleries form an important dietary carbon and nitrogen source for the larvae. Secondly, in this study, chlorophyll concentration was reported per unit area and measured differences may be due to substratum type and structure. Whereas rock faces are relatively smooth and provide a two dimensional surface for algal colonisation, galleries are made up of sediment particles that have a three dimensional structure. They will therefore provide an increased surface area for algal attachment. Sediments are often found to have much a higher chlorophyll content than epilithon (e.g. Vadeboncoeur *et al.*, 2006) and sediments and sands harbour a specialised algal community (epipelon and epipsammon), often dominated by small diatom species (Hickman & Round, 1970), that differs from the epilithic communities. Therefore, it is possible that a *T. waeneri* larva could enhance the quantity of resources available to it simply by anchoring sediment particles to the rock and thereby creating a three dimensional gallery structure with high surface area. This would still be a simple form of gardening. However, studies on *T. rostocki* living in streams suggest that new gallery sections (those recently built) did not contain more chlorophyll than the epilithon (Stief & Becker, 2005). In addition, the stable isotope analysis has suggested that the gallery biofilm community benefits from nutrients excreted by the larvae. Moreover, a high algal biomass on sediment is at least partially attributable to high nutrient concentrations in pore water and high organic matter contents (Hansson, 1992), factors that would be directly influenced by *T. waeneri* larvae in galleries. Thus, fertilisation of galleries with larval excretions (gardening by fertilisation) is the most likely cause of the high gallery chlorophyll *a* content. A greater chlorophyll content of galleries has also been measured for *T. waeneri* in Lake Erken (Hasselrot, 1993a; Kahlert & Baunsgaard, 1999) and in the

galleries of congeneric *T. rostocki* (Stief & Becker, 2005). Therefore, this appears to be a common feature of *Tinodes* galleries. High chlorophyll concentrations or algal biomass on retreats have also commonly been measured in other taxa that may garden, including chironomids (Pringle, 1985; Hershey *et al.*, 1988), caddisflies (Cox & Wagner, 1989) and in the territories of some damselfish (e.g. Klumpp *et al.*, 1987).

Although chlorophyll *a* and AFDM were higher in galleries than in the epilithon (indicating an increased algal standing crop), differences in productivity and turnover rates between these two larval resources may also be important. If, the epilithon has a high turnover rate (e.g. due to reduced biofilm thickness and less nutrient and light limitation; Lamberti & Moore, 1984) epilithic resources may be sufficient to meet larval requirements. However, available data suggests the reverse: greater productivity in the gallery than the epilithon. Higher photosynthetic/respiratory activity has been reported for both the galleries of *T. waeneri* (Hasselrot, 1993a) and *T. rostocki* (especially in the older sections; Stief & Becker, 2005) as compared to the surrounding epilithon.

In contrast to resource quantity, food quality, as measured by C:N ratios, did not vary in a systematic manner between the epilithon and the gallery in this study. However, C:N ratios were comparatively low (<11, indicating high resource quality) throughout, compared to epilithon values measured elsewhere (e.g. benthic algal C:N ratios ranged from 4 to 51 in a review by Kahlert, 1998) and more importantly in relation to the epilithon available to a *T. waeneri* population in Lake Constance (mean C:N ratio of 26.7; Fink *et al.*, 2006). Although, a mismatch in elemental composition between a relatively elementally homeostatic grazer and a highly variable food resource can negatively influence consumer growth (e.g. Frost & Elser, 2002; Fink & Von Elert, 2006) and limit community secondary production (Sterner & Hessen, 1994), this was unlikely to have been a significant constraint on *T. waeneri* populations in this study (at least in terms of nitrogen). Larval C:N ratios averaged 5.3 (range 4.5 to 6.6) and were not much lower than those of the available food sources. The larval C:N ratios measured were in line with other studies; 5.6 in Lake Erken (Kahlert & Baunsgaard, 1999), 6.14 in Lake Constance (Fink *et al.*, 2006) and 6.69 for all psychomyiids in Lake Erken (Liess & Hillebrand, 2005).

3.4.2 The function of galleries

3.4.2.1 Gardening

Neither epilithon or gallery chlorophyll *a* concentrations, AFDM or C:N ratios were significantly correlated with water column nutrient conditions. Furthermore these resource variables did not vary in any predictable way over the productivity gradient examined in this study (mean annual TP ranged from 9.8 to 31 mg m^{-3}, Maberly *et al.*, 2006). A lack of correspondence between external nutrient concentrations and attached algal biomass or nutrient status has also been reported elsewhere (e.g. Cattaneo, 1987; Kahlert & Pettersson, 2002; Liboriussen & Jeppesen, 2006). This is commonly attributed to the negative impacts of boundary layers on the diffusion rate of water column nutrients to algal cells residing within the epilithon. Under such conditions, processes affecting nutrient availability and recycling within the biofilm of individual rocks plays a more important role in determining biofilm characteristics (Kahlert & Pettersson, 2002). Epilithon and galleries were not nutrient limited. C:N ratios provide information on the nutrient limitation status of the epilithic community: optimal ratios range between 5 and 10 and higher ratios indicate nutrient limitation (be it N or P; Hillebrand & Sommer, 1999). The lack of nutrient limitation within the epilithon during the summer months and in the lower productivity lakes may be because of rapid turnover in nutrients within the epilithic biofilm coupled with low algal densities as discussed above. Alternatively, it may be that the impacts of *T. waeneri* (and other grazer) excretions are spread over an area that extends beyond the gallery or that the larval activities such as grazing and gallery construction are also affecting nutrient cycling in the epilithon.

A significant positive correlation between the C:N ratio of the epilithon and the proportion of assimilated material derived from the gallery was found at Esthwaite. However, this relationship may be due to differences in palatability of the two food sources (which also happened to have different C:N ratios), rather that to C:N ratios *per se*. The highest epilithon C:N ratios occurred in August and October, and the presence of filamentous green algae, was an unmistakable feature of the epilithon at Esthwaite during these sample periods. Filamentous green algae generally have higher C:N ratios than diatom-dominated communities (Kahlert, 1998) and are considered to be a less palatable food source to invertebrate grazers (Brodersen, 1995). Although *T. waeneri* larvae can, and do eat green filamentous algae (Brodersen, 1995; Harrison, 1996) they may not be a preferred food source where alternatives are available.

The clear positive correlation in the difference in C:N ratios between epilithon and galleries against the relative contribution of galleries *versus* epilithon to *T. waeneri* larval biomass in October may be surprising, given the small overall differences in C:N ratios measured. Food may have been less limiting during this sample period (i.e. more food available and/or lower densities of *T. waeneri* in most lakes), making quality a more important criterion for food selection. In lakes, there is often an autumn peak in periphyton biomass and chlorophyll *a* concentrations, although, this is usually less pronounced than the spring peak (Cattaneo, 1987; Harrison & Hildrew, 1998b and also reported for Esthwaite and Coniston by King *et al.*, 2002). In contrast, the summer period is characterised by low algal biomass and chlorophyll *a* concentrations (Cattaneo, 1987). These patterns were apparent in this study in terms of both gallery chlorophyll *a* content and the amount by which galleries were enriched in chlorophyll *a* relative to the epilithon. Low chlorophyll *a* concentrations were measured in August and these coincided with high densities and rapid growth of *T. waeneri* larvae. It was the differences in food quantity between epilithon and gallery that was positively related to the relative contribution of galleries to larval biomass in August, when larvae were more food limited, in comparison to October, where food quality was important. Thus, at low resource availability, quantity is important in determining food selection by *T. waeneri* larvae (although there were no large discrepancies in C:N ratios either). The patterns reported here, correspond well with other studies. Food quantity rather than quality was found to influence the growth rate of both mayflies and gastropods provided with low quantities of food (Frost & Elser, 2002; Fink & Von Elert, 2006). However, as in our October sample, where food was plentiful food quality was more important; increased quality resulted in higher growth rates (Frost & Elser, 2002; Fink & Von Elert, 2006).

In January, resource quantity and quality may have been less important. Water temperature was between 4 and 5°C across all sites and larvae were lethargic. Although, there was some evidence of larval feeding (larvae had full guts), studies on other populations demonstrated that larval growth ceases during the winter months (Llyn Hendref, Anglesey: Jones, 1967; Lake Esrom, Denmark: Dall *et al.*, 1984), and larvae may even lose weight (Dall *et al.*, 1984) during the winter. Thus the cessation of larval growth may explain the lack of significant correlations between resource variables and gallery assimilation in January.

Although this study has shown that the proportion of gallery versus epilithon assimilated by the larvae is correlated with the magnitude of the difference in resource levels between epilithon and galleries, the epilithon still forms a substantial dietary contribution (30% or more). Several factors may explain this. Firstly, the results of the mixing models relate to assimilation rather than consumption of food resources (Grey, 2006). What an organism consumes is not necessarily directly related to what it assimilates (e.g. Aberle *et al.*, 2005): selection of food resources may be due to post-consumption differences in digestibility and ease of assimilation (Peterson, 1987), rather than selection at the time of consumption. This would only be of major relevance where food resources were markedly different in terms of quality or algal composition. Secondly, organisms are not always successful at removing algal species from the substrata to which they are attached and algal species may vary in their susceptibility to grazing. However, scrapers such as *Tinodes waeneri*, are generally fairly efficient at consuming algae, especially low profile, tightly attached forms (Steinman, 1996). Thirdly, it may be that algal distribution within the epilithon was very patchy, and this may not have been adequately reflected in the sampling regime. Larvae may have been actively selecting epilithic patches containing high quantities of food. Finally, it is possible that whilst galleries make a good supplementary food supply, the preferred larval food source may actually be the epilithon.

3.4.2.2 Habitat availability

There was no evidence in this study to suggest that assimilation of gallery versus epilithic material by *T. waeneri* larvae was related to chironomid density or *T. waeneri* density and biomass. Although chironomids were numerically more abundant at some sites, *T. waeneri* biomass was probably higher, as most of the chironomid individuals sampled were substantially smaller than *T. waeneri* larvae. In addition, although chironomids will undoubtedly compete with *T. waeneri* larvae for space, they were probably not all competing for algal resources. For example, in the stony littoral of Crosemere, only one out of the four regularly sampled chironomid species was an algal grazer (Harrison & Hildrew, 1998b). Chironomid grazers often only occupy territories for brief time periods (e.g. mean of 3h in the stream dwelling *Cricotopus bicinctus* (Meigen), Wiley & Warren, 1992), and have short lifecycles, when compared to *T. waeneri*. This may result in substantial fluctuations in the population sizes of epilithic chironomids, over relatively short time scales.

The density of *T. waeneri* was highest in August and coincided with rapid larval growth and low resource availability. Although, larval densities in this study were moderate (e.g. compared to11500 individuals m^{-2} in Lake Esrom, Dall *et al.*, 1984), intra-specific competition would probably have been most intense in August. Indeed, the data suggests that the availability of space was negatively correlated with the relative contribution of gallery C and N to larval biomass. In addition, the lakes with the greatest gallery assimilation throughout the study (Esthwaite and Rydal Water), were also those in which the supply of suitable rocks was least. A shortage of suitable substrata could place restrictions on larval territory size and influence the ease with which larvae are able to move to new grazing patches.

3.4.2.3 Protection

Assessing the potential importance of galleries for protection from predation and wave washing is more challenging, as both variables are difficult to measure in the field. Detachment and abrasion due to wave washing and ice was the chief cause of mortality in Lake Esrom (Dall *et al.*, 1984). If wave washing were an important determinant of larval food choice, it would be expected that galleries would be preferentially consumed, as under exposed conditions it would be riskier for larvae to protrude fully from their galleries. However, in this study, wind exposure was not correlated with the proportion of gallery (as compared to epilithon) assimilated by larvae, and is unlikely to have been the main driver in the use of galleries as a food source. There is evidence however that exposure may indirectly impact *T. waeneri* larval fitness, mediated by impacts on food supply; exposure was positively correlated to food quantity variables in all sampling periods. Although, severe wave washing can lead to the scouring of epilithic communities and biomass reduction (Cattaneo, 1990), at intermediate levels, wave washing can aid nutrient exchange between the epilithic mat and the overlying water and enhance algal growth (Cattaneo, 1990).

Measurements of wind exposure are likely to be underestimates, especially for Windermere, Coniston and Ullswater, as these lakes all have regular boat traffic. For example, Lake Windermere was home to over 6500 motor boats in 2001, including passenger steamers (Pickering, 2001) and Ullswater and Coniston also have regular steamer services. The wash from boats is mainly diurnal and will occur much more regularly than wind induced waves during the main tourist season (Hofmann *et al.*,

2008). Furthermore, waves from boats cause greater hydrodynamic disturbances than wind induced waves of similar amplitude (Hofmann *et al.*, 2008), and may have a greater influence on *T. waeneri* behaviour.

The effectiveness of galleries in protecting larvae from predation will depend on the identity of the main predators - galleries will provide little protection against predators that can enter galleries. References to *T. waeneri* predators are scarce, but fish (including perch, *Perca fluviatilis* L., brown trout, *Salmo trutta* L. and ruffe, *Gymnocephalus cernuus* (L.)) and leeches (*Erpobdella* spp.), appear to be the main predators (Jones, 1967; Dall *et al.*, 1984; Scheifhacken, 2006). It is not known how the abundance of these predators varies across the lakes studied, although the biomass of *Erpobdella octoculata* (L.) declines with decreasing ion content across lakes, but this is primarily as a consequence of changes in food availability (Young, 2002). Ten to fifteen percent of the diet of *Erpobdella* spp. in Lake Esrom consisted of *T. waeneri* larvae (Dall *et al.*, 1984) suggesting that they may be an important predator. However, these leeches prey upon larvae by entering galleries (Jones, 1967) and were observed in larval galleries during this study. Galleries may thus offer little protection against this predator and therefore, grazing on the gallery rather than the epilithon may not lead to increased larval survival.

3.4.3 Conclusions

Galleries contained higher quantities of algae than the epilithon across all lakes and time periods and were a less variable food supply than the epilithon. This is probably the result of the fertilisation of the gallery community by the inhabiting larva. Furthermore, this study strongly indicates that provision of a food supply is an important function of the gallery, as food quantity in August and quality in October were significantly related to the outputs of the mixing models. In addition, wave washing affects may have been meditated through the impacts of this disturbance on resource attributes and correlations between substratum availability and mixing model outputs again point to the role of food availability in determining the relative assimilation of gallery and epilithon material by the larvae. However, the impact of predation pressure on larval feeding preferences could not be assessed and it is likely that galleries also provide protection. This study demonstrates that retreats can serve as valuable grazing patches, as well as fulfilling the more obvious role of providing protection to the inhabitant.

Chapter 4 – Modification of littoral algal assemblages by *Tinodes waeneri* larvae

4.1 Introduction

Periphyton often forms an important basal resource for lake food webs (Hecky & Hesslein, 1995). Additionally, it has recently been recognised that periphyton makes a significant contribution to whole lake primary productivity (Vadeboncoeur *et al.*, 2003), and nutrient retention and recycling (Axler & Reuter, 1996) and therefore to overall ecosystem processes. Thus, grazer-mediated changes in algal species composition and structure could impact on these processes. Sedentary grazers also affect algal communities via processes beyond simple consumption (e.g. through gardening by the caseless caddisfly, *Tinodes waeneri*, Chapter 3) and these could be of particular importance in an ecosystem context.

Gardening involves a grazer modifying its environment, so as to provide itself with a better food resource. Whilst a 'better food resource' may mean one that is more plentiful, or that contains more nutrients (better food quality, see Chapter 3), it could also be one that is more palatable or easier to digest. Palatability and digestibility of algal groups varies. For example, filamentous green algae are often thought to be of a poor food quality as they contain low concentrations of lipids and proteins (Lamberti, 1996) and the cellulose cells walls are difficult to break down (see Becker, 1990 for an example with caddisflies). In contrast, diatoms are often considered to be a good food source, for grazers including Trichoptera (Becker, 1990; Lamberti, 1996), as they can contain high levels of lipids (Becker, 1990) and are highly digestible (Lamberti & Moore, 1984). At a finer scale, various growth forms or life stages may be easier to remove from the substratum and to ingest. For example, the larval caddis *Neophylax autumnus* Vorhies, classified as a scraper, was more successful at harvesting large, 'high-profile' diatoms (Peterson, 1987).

Grazers that garden can directly influence the composition of their food resources: the caddisfly *Leucotrichia pictipes* removes, but does not consume, unpalatable cyanobacteria from its feeding territories and thus maintains an algal lawn containing solely preferred food items (diatoms and the blue-green *Schizothrix*; Hart, 1985b). Some gardeners have even more distinct communities associated with their feeding territories.

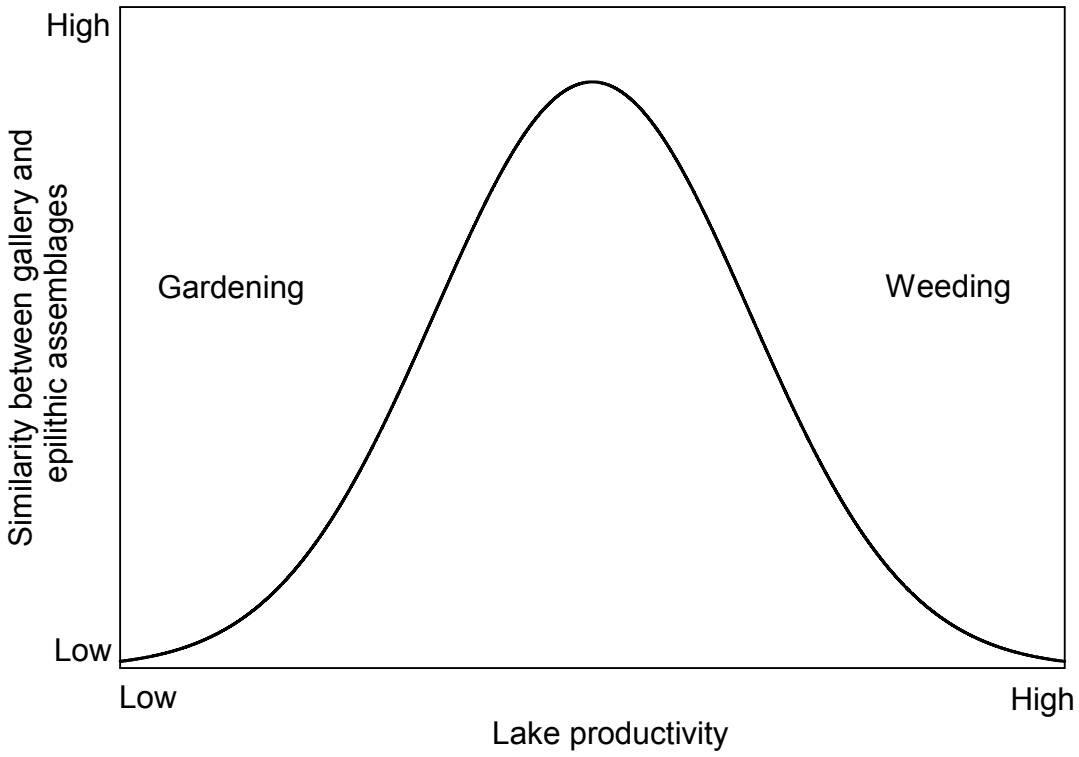
For example the silken tubes of the chironomid *Orthocladius rivulorum* Kieffer support monocultures of the diatom *Hannaea arcus* (Ehrenb.) R. M. Patrick (Hershey *et al.*, 1988). The erect growth form of this diatom species meant that the tubes could harbour a dense population. Although *H. arcus* was also present within the background epilithon, it never formed monocultures, and was rarely dominant. The distinct algal community on the tubes was found to be due to larval activity (including possible fertilisation), as pupae were not associated with the same algae. Furthermore, this consortium of alga and larva remained constant over a range of nutrient conditions (Hershey *et al.*, 1988).

I have demonstrated, using stable isotope analysis, that nitrogen fertilisation of *T. waeneri* gallery communities occurs across a range of lakes. Furthermore, gallery biofilms contain a higher standing biomass of algae compared to the adjacent epilithon. These observations suggest that *T. waeneri* has the potential to affect algal communities, within the littoral of lakes, through its gardening activities.

The aim of the research described in this chapter was to assess whether gallery biofilms contained an algal assemblage distinct from the surrounding epilithon. If this is the case the gallery algal assemblage should remain constant over a gradient of lake productivity and/or it should be more similar among lakes than it is to the background epilithon within lakes. However, even if this is not the case, larvae may still have some ability to manipulate their food. The assemblages on galleries and in the general epilithon would be expected to diverge in progressively more oligotrophic lakes (as the effect of fertilisation by the resident becomes more important; Fig. 4.1) and/or towards the more eutrophic end of the spectrum (as the effect of 'weeding' by residents of unpalatable, but competitive, algae becomes more important; Fig. 4.1).

To investigate these hypotheses, pigment analysis (Jeffrey *et al.*, 1997; Wright, 2005) was used to characterise the algal classes that were present on galleries and in the epilithon. In addition, diatoms were identified to species. They were chosen for more detailed investigation as they are often the most diverse algal assemblage in freshwaters (King *et al.*, 2006) and good ecological information about the species is available to aid interpretation. Moreover, diatoms are a common food source of *T. waeneri* larvae (Jones, 1967).

Figure 4.1 The predicted change in the degree of similarity between gallery and epilithic assemblages along a productivity gradient. The similarity between the two assemblages is expected to be lower in oligotrophic lakes (due to gardening) and in eutrophic lakes (due to weeding) than in lakes of intermediate productivity.



4.2 Methods

4.2.1 Sample collection

Samples were collected from five lakes in the English Lake District in October 2006. Lakes were chosen to represent a productivity gradient ranging from moderately oligotrophic (Coniston) to eutrophic (Esthwaite) (for mean TP and chlorophyll *a* values please refer to Chapter 2). The sites selected were the same as those used for the stable isotope study, although Ullswater was not included (see Chapter 2 for more details).

Ten randomly selected rocks, inhabited by *T. waeneri*, were collected from the littoral zone at a water depth of 30-60cm. Sampling at this depth ensured that the rocks collected contained representative *T. waeneri* populations in each lake. Also, the algae were unlikely to have been subjected to excessive disturbance via wave washing or to

photoinhibition from excessive light (King *et al.*, 2006). The epilithon was sampled using a periphyton syringe sampler, the design of which was modified from Stockner and Armstrong (1971), and is described in detail in Chapter 3. Briefly, the sampler was constructed from a 20ml syringe, with a toothbrush head attached to the plunger. An epilithic sample was derived from the internal area of the syringe held firmly in contact with the rock (=2.84cm^2) while the plunger was rotated four full turns in each direction. This was repeated in six different locations so that each epilithon sample retained originated from 17.04cm^2 of rock. Care was taken not to sample potential *T. waeneri* feeding territories (i.e. the area immediately surrounding galleries). Four galleries from each rock that was sampled were also photographed with a scale bar, carefully scraped off with forceps and preserved. Epilithic samples and the accompanying gallery samples for five of the rocks were placed in the dark on ice and frozen immediately on return to the laboratory. Epilithic samples from the other five rocks were made up to a fixed volume with deionised water, and were carefully subdivided into two samples of equal volume. Both the epilithic samples from these rocks and the matching gallery samples were immediately preserved using Lugol's solution (Biggs & Kilroy, 2000). At Coniston, the epilithon from three rocks not inhabited by *T. waeneri* was also sampled, subdivided, and preserved using Lugol's solution. Samples were stored in the dark at 5°C.

4.2.2 Pigment analysis

Epilithic samples were defrosted and filtered through Whatman GF/C filters, and then refrozen for storage and transport. Samples (the epilithon sample and one randomly chosen gallery from each rock) were freeze-dried prior to pigment extraction using an 80:15:5 acetone, methanol and water solution for 12h at -20°C. The resulting pigment solution was filtered through a 0.22µm syringe filter, evaporated to dryness under a stream of nitrogen and then kept frozen. Samples were re-dissolved in a 70:25:5 acetone: ion pairing solution: methanol solution before injection into a high-performance liquid chromatograph (HPLC). Pigment analysis was performed following standard HPLC protocols developed by Wright *et al.* (1991) using an Agilent HPLC separation system and on-line photo-diode array detector.

4.2.3 Diatom preparation and identification

Samples for diatom identification were prepared using the methods of Battarbee (1986). Briefly, one epilithon sample and one gallery sample from each rock (n=5 per lake)

were digested in hydrogen peroxide solution whilst being heated in a water bath (~50°C) and, once digestion was complete, all samples were washed in deionised water three times (with 24 hours of settling time between washes). Samples were then dried onto cover-slips and mounted using Naphrax (refractive index of 1.73, Brunel Microscopes Ltd.). Three hundred frustules from each sample were identified to species at 1000x magnification (Nikon Eclipse 50i microscope) using the keys of Krammer & Lange-Bertalot (2004b; 2004a; 2007a; 2007b), Kelly (2000), Kelly *et al*. (2005) and Hartley (1996). Broken frustules were counted only if the central area and more than half of the frustule was present. Taxonomic names follow Whitton *et al*. (2003) and authorities for all diatom species named in the text are provided in Appendix 1 .

4.2.4 Data analysis

4.2.4.1 Pigment data

The pigments responsible for the chromatogram peaks were identified by reference to their retention times and through comparison of their spectral characteristics with absorbance spectra of commercial standards and those illustrated in Jeffery *et al*. (1997). Individual pigments were quantified using calibration curves constructed by analysing a range of known concentrations of each pigment. Pigment concentrations were expressed as nanomoles of pigment per centimetre square of substratum. ANOVAs were performed using SPSS version 13, to test for differences in pigment concentrations between gallery and epilithic samples, across lakes.

4.2.4.2 Diatom community data

Diatom numbers for each sample were expressed as relative abundances. Species usually considered to be planktonic (Kelly *et al.*, 2001) were retained within the data set in line with King *et al*. (2002). Diversity indices (Shannon, Simpsons and Evenness (E)) were calculated for each sample (Magurran, 1988). Values of Simpsons index (D) are presented as 1-D, and thus the higher the value (between 0 and 1) the greater the sample diversity. The degree of similarity (SIMI) between each pair of gallery and epilithon samples was calculated using the equation of McIntire & Moore (1977). SIMI values can range from 0 to 1; if the two samples being compared have no species in common then the index would take a value of 0, whereas a value of 1 would indicate that the two samples contained exactly the same species in exactly the same proportions (Hoagland *et al.*, 1982).

To explore patterns in the diatom species data, multivariate analyses were performed using CANOCO version 4.5. Prior to analysis, species relative abundances were square-root transformed to reduce the weight of the most abundant species. An exploratory Detrended Correspondence Analysis (DCA) indicated that gradient lengths were short (less than 3) and this, together with an inspection of species responses, indicated that linear methods were most suitable (Leps & Smilauer, 2002). Relationships between species and environmental data (lake of origin, gallery and epilithon) were investigated using Redundancy Analysis (RDA). The significance of environmental variables was tested using Monte Carlo permutations and only significant variables were included in the analysis. The three epilithon samples derived from rocks lacking *T. waeneri* at Coniston were included as supplementary variables. An RDA, in which only the first axis was constrained (gallery *versus* epilithon) was also run for each lake separately.

Ordination results are represented as tri-plots with individual samples plotted using sample scores and are thus based on species composition rather than on the nominal environmental variables. The tri-plots focus on inter-sample distances and, therefore, samples (or nominal variables) that lie close together are more similar in terms of species composition, than those which are separated by a greater distance. Arrows indicate the direction of increase in species abundance, while species with the least influence were removed for clarity.

Communities were also characterised by inspection of the distribution of growth forms and diatom sizes within each sample. Diatom species were classified according to their growth form using Kelly *et al*. (2005) and Kelly *et al*. (2001). Categories were based on DeNicola *et al*., (2006) and were as follows: 1) unicellular prostrate species; 2) erect diatom species (either unicellular or colonial); 3) stalked species (e.g. *Gomphonema* spp.); 4) *Achnanthidium minutissimum*; 5) motile diatoms (e.g. *Navicula* spp.); 6) unattached non motile species (which includes planktonic species); and 7) species for which the growth form is unknown. Biovolume measurements were taken from the literature (Charles, 2001; Mize & Deacon, 2001; LaTour *et al.*, 2006; Song, 2007; Waite *et al.*, 2008). In the few cases where a biovolume measurement for a species was unavailable, the biovolume of a similar sized and shaped cell (information on size and shape taken from Krammer & Lange-Bertalot 2004b; 2004a; 2007a; 2007b) was used as an approximation.

Finally, to investigate whether any differences in the assemblage between galleries and epilithon were nutrient-related, the Trophic Diatom Index (TDI), an index that uses diatom species assemblages to assess trophic status (Kelly & Whitton, 1995), was calculated for each sample. The methodology and nutrient sensitivity (categories 1 to 5, 1= species favoured by very low nutrient concentrations, 5 = species favoured by very high nutrient conditions) and indicator values given in Kelly *et al*. (2001) were used. Possible TDI values can range from 0 (very low nutrients) to 100 (very high nutrients). Paired t-tests were used to compare TDI values between galleries and the epilithon, within each lake.

4.3 Results

4.3.1 Algal pigment composition

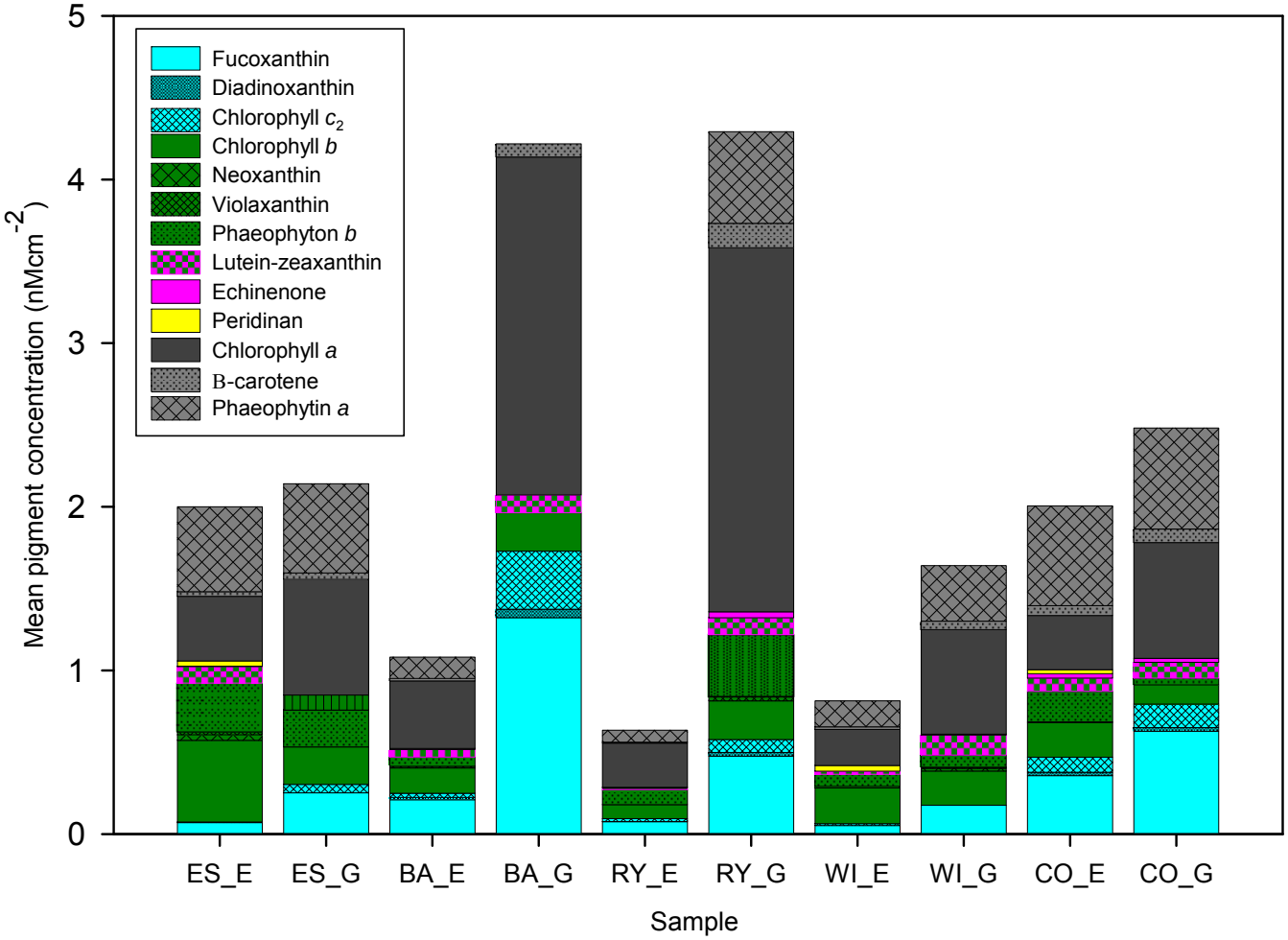
In all the lakes, higher concentrations of total pigments, and chlorophyll *a* per cm^2 of rock surface, were recorded in the galleries as compared to the epilithon, with greatest differences at Bassenthwaite and Rydal Water (Fig. 4.2). A total of 14 chlorophyll and carotenoid pigments were identified (Table 4.1). The most common and abundant pigments were the general algal pigments β-carotene, chlorophyll *a* and its derivative phaoephytin *a*, the siliceous algal pigments, chlorophyll c_2 and fucoxanthin, and the green algal pigments chlorophyll *b* and phaoephytin *b* (Fig. 4.2). The green algal pigment lutein could not be separated from the cyanobacterial pigment zeaxanthin on the HPLC set-up used. However, other cyanobacterial pigments were only present at low concentrations. Echinenone was present in some gallery and epilithon samples at Coniston, some epilithon samples at Bassenthwaite and Esthwaite, and some galleries at Windermere. However, its relative abundance was always low (<7% in all samples in which it was present other than in one gallery from Rydal Water where it accounted for 15% of total pigment concentration). Moreover, canthaxanthin, another cyanobacterial pigment, was recorded only in one sample from the epilithon at Coniston.

Table 4.1 The distribution of the pigments across algal classes (information derived from: Jeffery *et al.*, 1997; Jeffrey & Vesk, 1997; Wright, 2005; McGowan, 2007).

Pigment	Main benthic groups				Usually in phytoplankton (also in some benthic habitats)			
	Cyanophyta Cyanobacteria	Chlorophyta Green algae	Bacillariophyta Diatoms	Rhodophyta Red algae	Chrysophyta Chrysophytes	Xanthophyta Xanthophytes	Cryptophyta Cryptomonads	Pyrrophyta Dinoflagellates
Chlorophyll *a*	X	X	X	X	X	X	X	X
Phaeophytin *a*	X	X	X	X	X	X	X	X
Chlorophyll *b*		X						
Phaeophytin *b*		X						
Chlorophyll c_2			X		X			X
ß-carotene	X	X	X	X	X	X	X	X
Fucoxanthin			X		X			X
Diadinoxanthin			X		X		X	X
Canthaxanthin*	X							
Peridinin								X
Echinenone*	X							
Lutein- Zeaxanthin	X	X						
Neoxanthin		X						
Violaxanthin		X						

*= colonial cyanobacteria

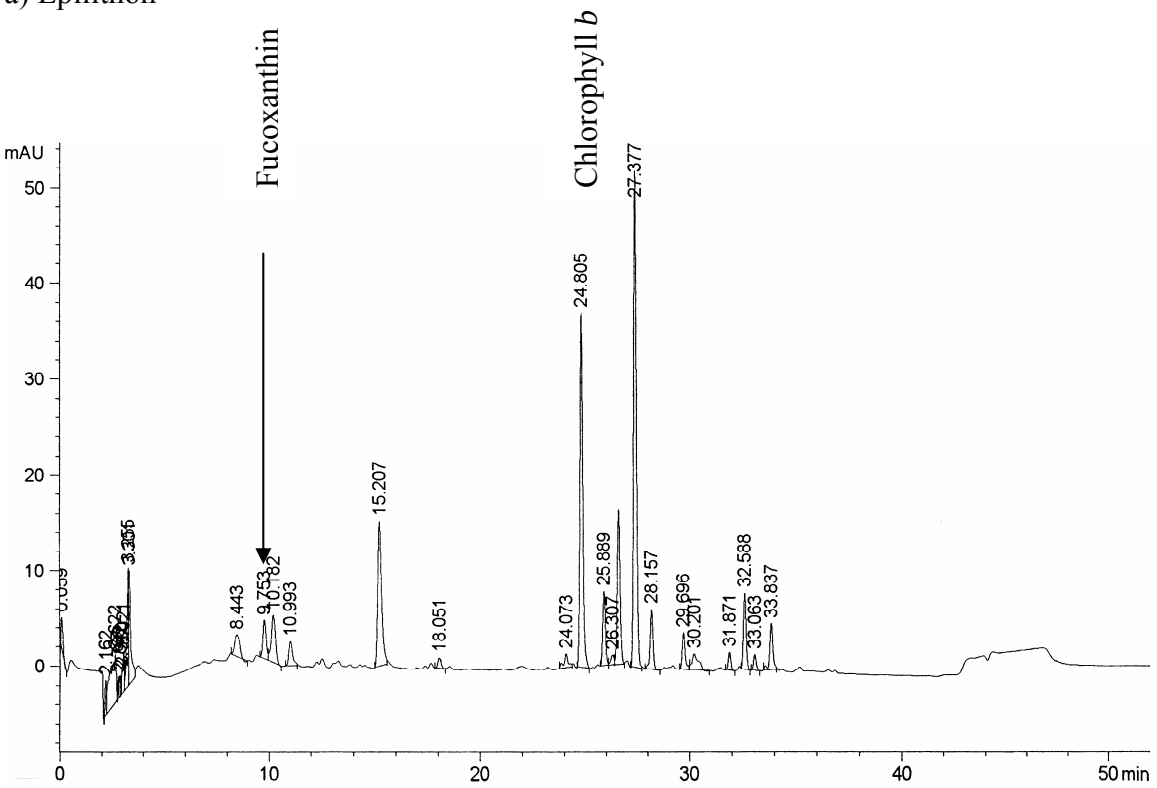
Figure 4.2 Mean densities of each of the algal pigments (nMcm^{-2} of rock surface) recorded in the epilithon (E) and gallery (G) samples in each lake. Lake codes are: ES = Esthwaite, BA = Bassenthwaite, RY = Rydal Water, WI = Windermere and CO=Coniston. Pigments are colour coded, such that blue indicates pigments associated with siliceous algae, green indicates green algal pigments, pink indicates cyanobacterial pigments, yellow indicates dinoflagellate pigments and grey denotes general algal pigments.



The principal pattern across all lakes was that galleries contained higher levels of fucoxanthin than chlorophyll *b*, whilst the opposite was true of epilithic samples (Figs. 4.2 & 4.3). In addition, chlorophyll *b* contributed a significantly greater proportion of total pigment content per cm^2 of substratum in epilithic samples than in gallery samples (lake x item (i.e. gallery and epilithon) interaction $F_{4,40}$=3.262, P=0.21, Fig. 4.4a). Chlorophyll *b* accounted for most epilithic pigment at Esthwaite and Windermere. In contrast, the relative proportion of fucoxanthin in relation to total pigment density was significantly higher in the gallery than in the epilithon (item $F_{1,49}$=25.879, P<0.001, Fig. 4.4). This difference was significantly greater at Coniston and Bassenthwaite than in the other lakes (Tukey HSD <0.05, Fig. 4.4b), although fucoxanthin concentrations were also high in the epilithon than in the other three lakes studied. A second siliceous algal pigment, chlorophyll c_2 (present in diatoms, dinoflagellates and chrysophytes; Table 4.1), also showed a similar pattern (Fig. 4.4c). In contrast, Peridinin, a pigment unique to dinoflagellates, made up only a small proportion of total pigment density (Fig. 4.4d). Furthermore, it was almost totally restricted to epilithic communities and was not recorded at Bassenthwaite. Alloxanthin, found only in chrysophytes, was not observed.

Figure 4.3 A typical chromatogram of a) an epilithon and b) a gallery sample, showing higher chlorophyll *b* compared to fucoxanthin levels in the epilithon, and the reverse pattern for the gallery. Fucoxanthin and chlorophyll *b* peaks are marked on the chromatogram. Both samples were collected from the same rock at Esthwaite.

a) Epilithon



b) Gallery

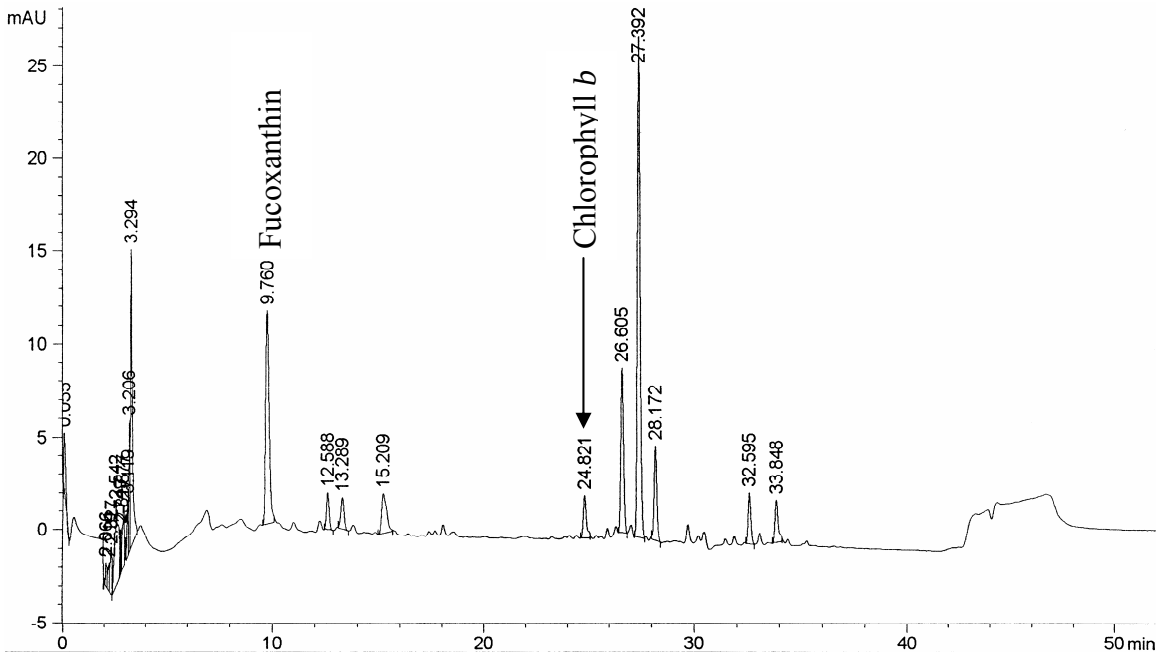
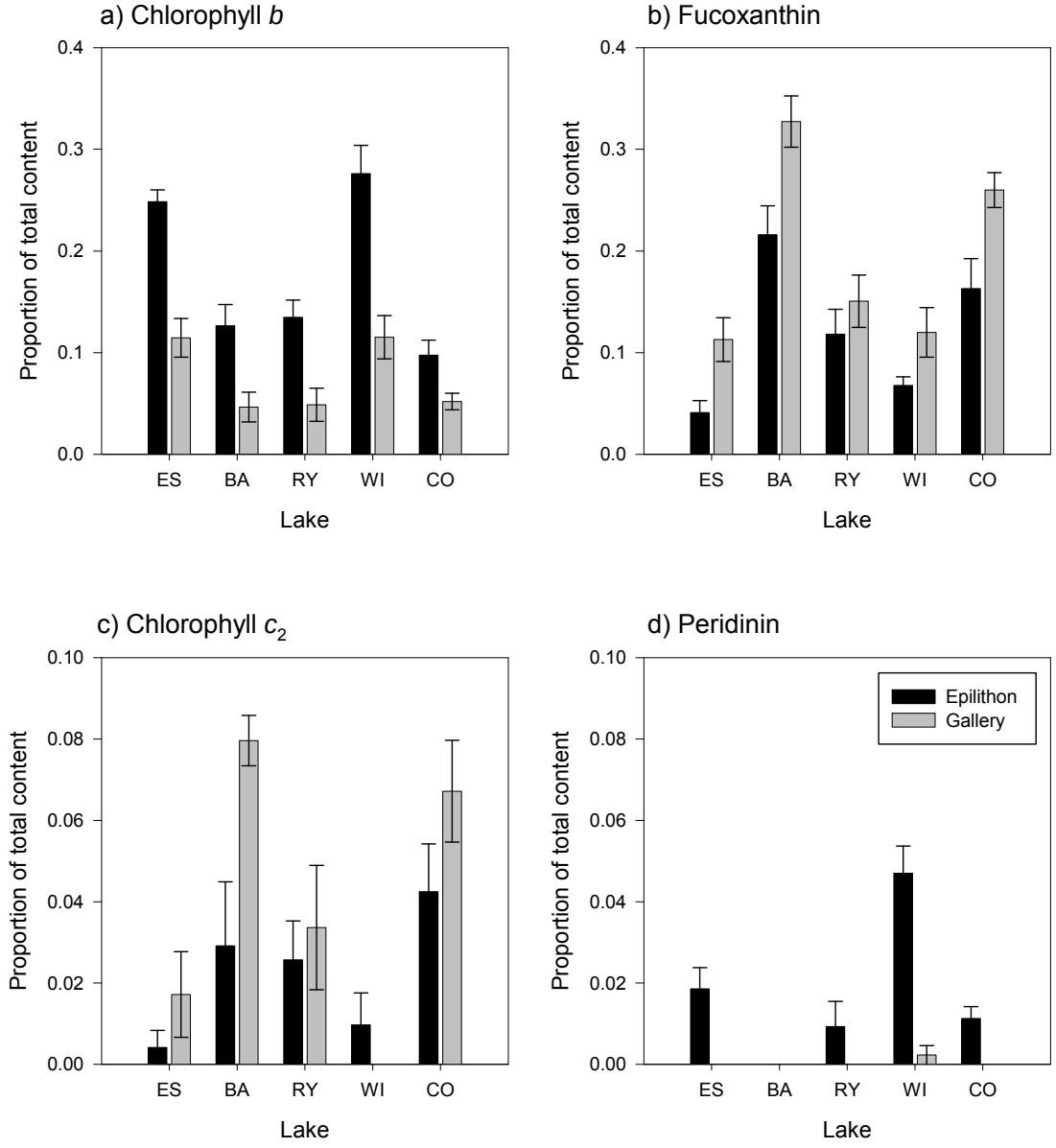


Figure 4.4 The mean (± 1 SE) relative proportion of a) chlorophyll *b* b) fucoxanthin c) chlorophyll c_2 and d) peridinin in the epilithon (E) and galleries (G) across the five lakes. Lake codes are: ES = Esthwaite, BA = Bassenthwaite, RY = Rydal Water, WI = Windermere and CO = Coniston.



4.3.2 Diatom community composition

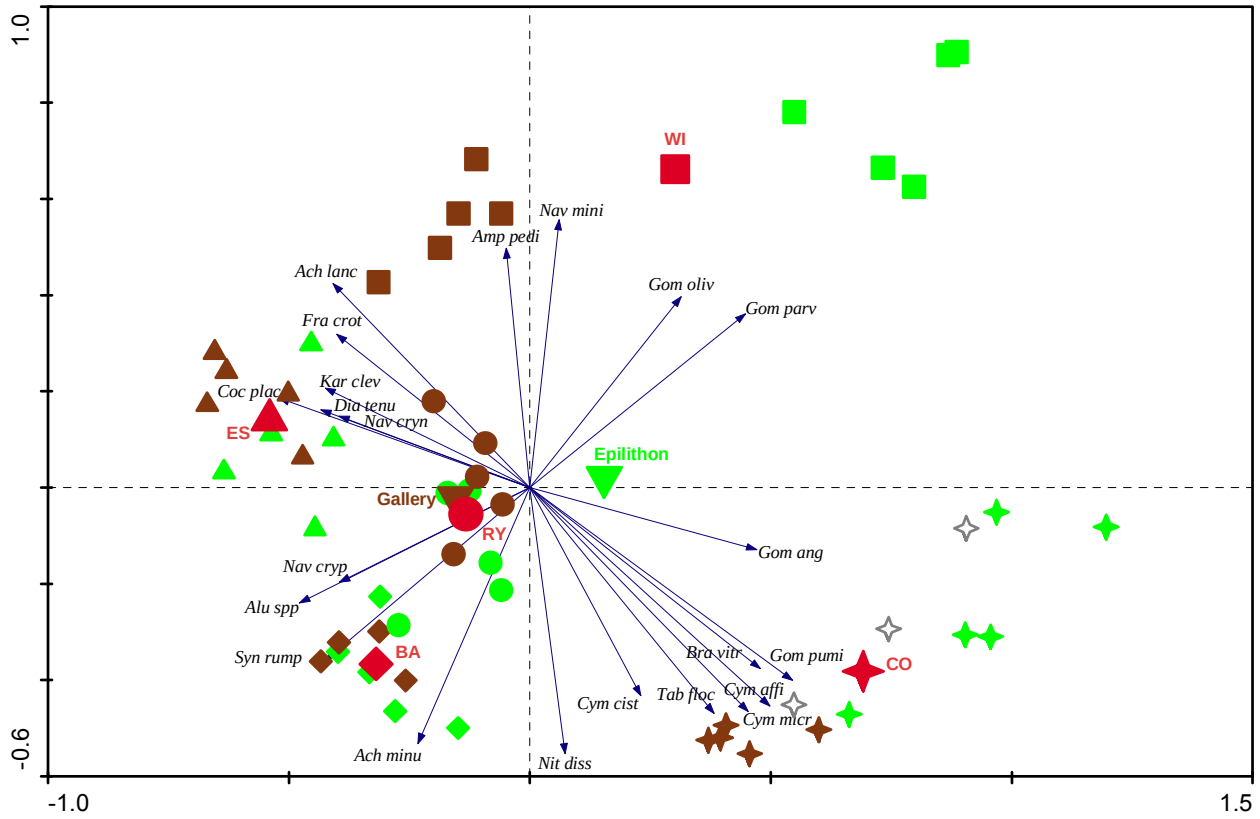
4.3.2.1 All lakes

A total of 124 species from 34 genera were recorded, with nine species recorded solely from the epilithon and another 26 species found only on galleries. The RDA for all lakes together (Fig. 4.5) indicated that all nominal environmental variables (lake of origin, gallery, epilithon) were significant (Monte Carlo permutations, P=0.002) and the first two axes explained 39.6% of the variation in species data. Gallery and epilithic assemblages were significantly different. However, the lake of origin had a larger influence on the diatom assemblage present (lake centroids were further apart on the tri-plot than the gallery and epilithon centroids). Lakes fell from right to left in order of increasing productivity, with Coniston (least productive) on the right and Esthwaite (most productive) on the left. The gallery centroid also fell to the left of the epilithon centroid. There was less variation between samples at Esthwaite, Rydal Water and Bassenthwaite, than at Coniston and at Windermere. Windermere also displayed the greatest difference between gallery and epilithon samples. The SIMI index supported this pattern (Table 4.2), with Windermere galleries and epilithon having low similarity to each other. Diatom assemblages at Bassenthwaite contained a substantially higher abundance of *Nitzschia* than elsewhere, while Coniston contained relatively high numbers of *Cymbella* species.

Table 4.2 The mean degree of similarity between the epilithic and gallery diatom assemblage within each lake calculated using the similarity index of McIntire & Moore (1977). Values of 0 indicate no similarity and values of 1 indicate high similarity and the grades of similarity assigned are based on Rohr (1977) and cited in King *et al.* (2002).

Lake	SIMI	Grade of similarity
Bassenthwaite	0.991	High
Coniston	0.873	Similar
Esthwaite	0.960	High
Rydal Water	0.992	High
Windermere	0.485	Little

Figure 4.5 A Redundancy Analysis (RDA) tri-plot with axes constrained by lake (red symbols: diamonds = Bassenthwaite (BA), stars = Coniston (CO), triangles = Esthwaite (ES), circles = Rydal Water (RY) and squares = Windermere (WI)) and sample type (down- triangles: galleries = brown and epilithon = green). Individual samples are plotted using Samp scores, thus their positions reflect variation within lakes and sample types. Grey stars indicate the projected location (not used in analysis) of epilithon samples from rocks not inhabited by *Tinodes waeneri*. Arrows show the direction of increased abundance for the species with the most influence. (**Ach lanc** = *Achnanthes lanceolata*, **Ach minu** = *Achnanthidium minutissimum*, **Alu spp.** = *Aulcosiera* spp., **Amp pedi** = *Amphora pediculus*, **Bra vitr** = *Brachysira vitrea*, **Coc plac** = *Cocconeis placentula*, **Cym affi**, **Cym cist** = *Cymbella cistula*, **Cym micr** = *Cymbella microcephala*, **Dia tenu** = *Diatoma tenue*, **Fra crot** = *Fragilaria crotonensis*, **Gom ang** = *Gomphonema angustum*, **Gom oliv** = *Gomphonema olivaceum*, **Gom parv** = *Gomphonema parvulum*, **Gom pumi** = *Gomphonema pumilum*, **Kar clev** = *Karayevia clevei*, **Nav cryp** = *Navicula cryptocephala*, **Nav cryn** = *Navicula cryptotenella*, **Nav mini** = *Navicula minima*, **Nit diss** = *Nitzschia dissipata*, **Syn rump** = *Synedra rumpens* and **Tab floc** = *Tabellaria flocculosa)*



4.3.2.2 Esthwaite

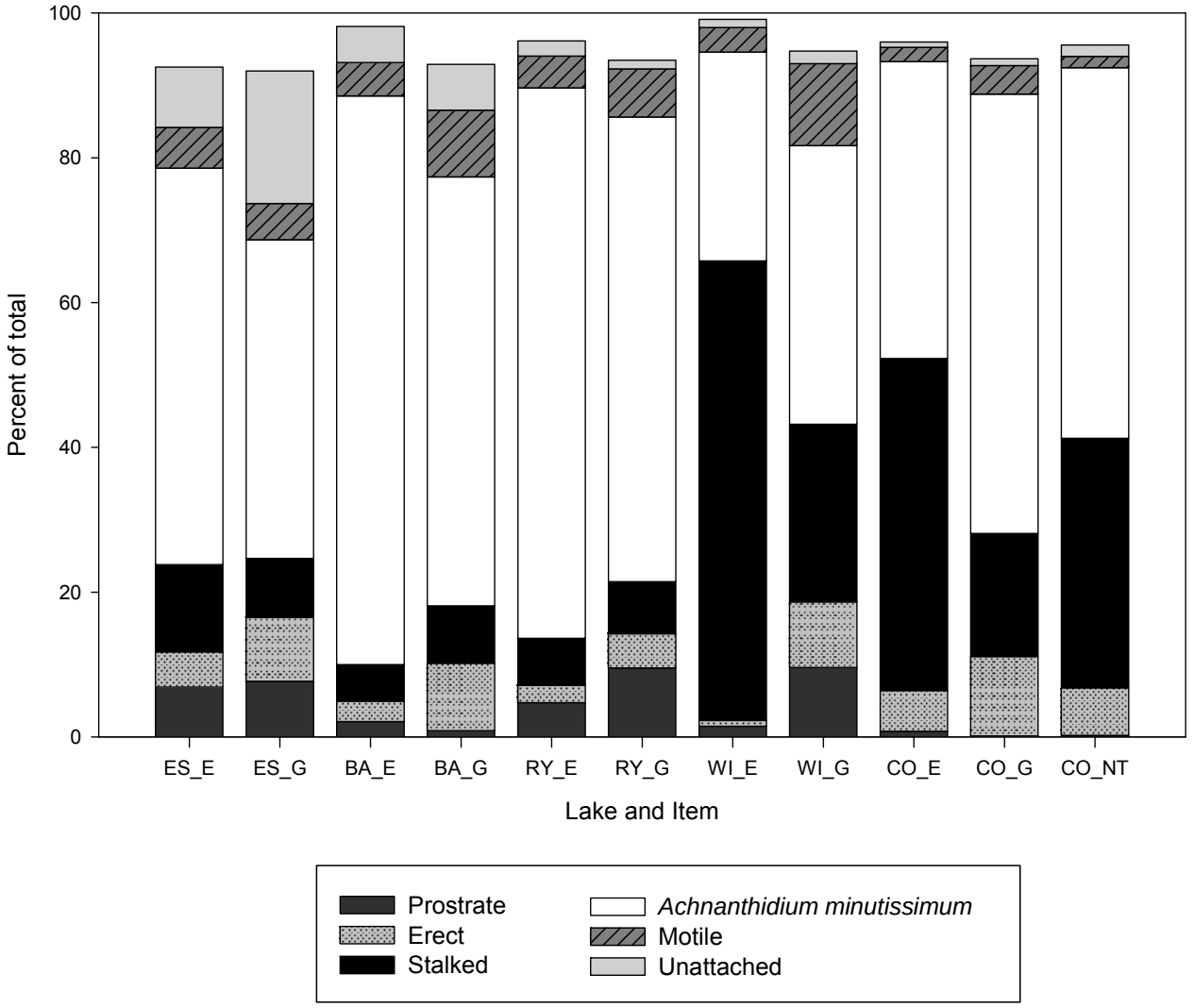
All diatom growth forms were well represented in both the galleries and the epilithon at Esthwaite (Fig. 4.6), and species diversity was comparatively high in samples from this lake (Table 4.3). Samples also contained species that are usually associated with the phytoplankton, including *Fragilaria crotonensis* and *Diatoma tenue* (Fig. 4.7) and the epilithon contained *Rhoicosphenia abbreviata*, a species typically epiphytic on green algae (Fig.4.7). *Achnanthidium minutissimum* was dominant and was more abundant in the epilithic biofilm, where it accounted for approximately 50% of all diatoms present, than in gallery biofilms (Fig. 4.6). This greater dominance of *A. minutissimum* within the epilithon was also reflected in the size distribution data: a higher proportion of small (less than 100μm^3) individuals were recorded in the epilithon than in the gallery (Fig. 4.8). However, larger diatoms (400-900μm^3) also made up a substantial portion of the community in both sample types (Fig. 4.8).

Although the mean similarity between samples was high (Table 4.2), the RDA indicated that 22.8% of the variance in the diatom species data was explained by whether a sample was derived from the gallery or from the epilithon. The TDI index was almost identical (and low) for galleries and the epilithon, although the value for galleries was slightly higher (Table 4.4).

Table 4.3 A comparison of the diatom diversity of galleries and the epilithon (mean ±1SE) within each lake using the Shannon index, Simpson's Index and evenness (E) measures.

Lake	Shannon Index		Simpson's Index (1-D)		Evenness	
	Gallery	Epilithon	Gallery	Epilithon	Gallery	Epilithon
Bassenthwaite	1.79±0.04	1.08±0.11	0.61±0.01	0.36±0.04	0.53±0.01	0.34±0.03
Coniston	1.57±0.13	1.88±0.11	0.57±0.05	0.74±0.04	0.51±0.04	0.62±0.03
Esthwaite	2.17±0.10	1.88±0.03	0.76±0.03	0.64±0.01	0.65±0.03	0.56±0.01
Rydal Water	1.62±0.07	1.02±0.17	0.53±0.03	0.34±0.06	0.47±0.02	0.32±0.04
Windermere	2.31±0.09	1.48±0.08	0.79±0.02	0.64±0.03	0.67±0.02	0.50±0.02

Figure 4.6 The relative abundance of the diatom growth form categories in the gallery (G) and epilithon (E) in the five lakes (ES = Esthwaite, BA = Bassenthwaite, RY = Rydal Water, WI = Windermere and CO = Coniston). Samples from rocks without *T. waeneri* (NT) are included for comparison. Bars do not reach 100% as the growth form of some of the diatom species in unknown.



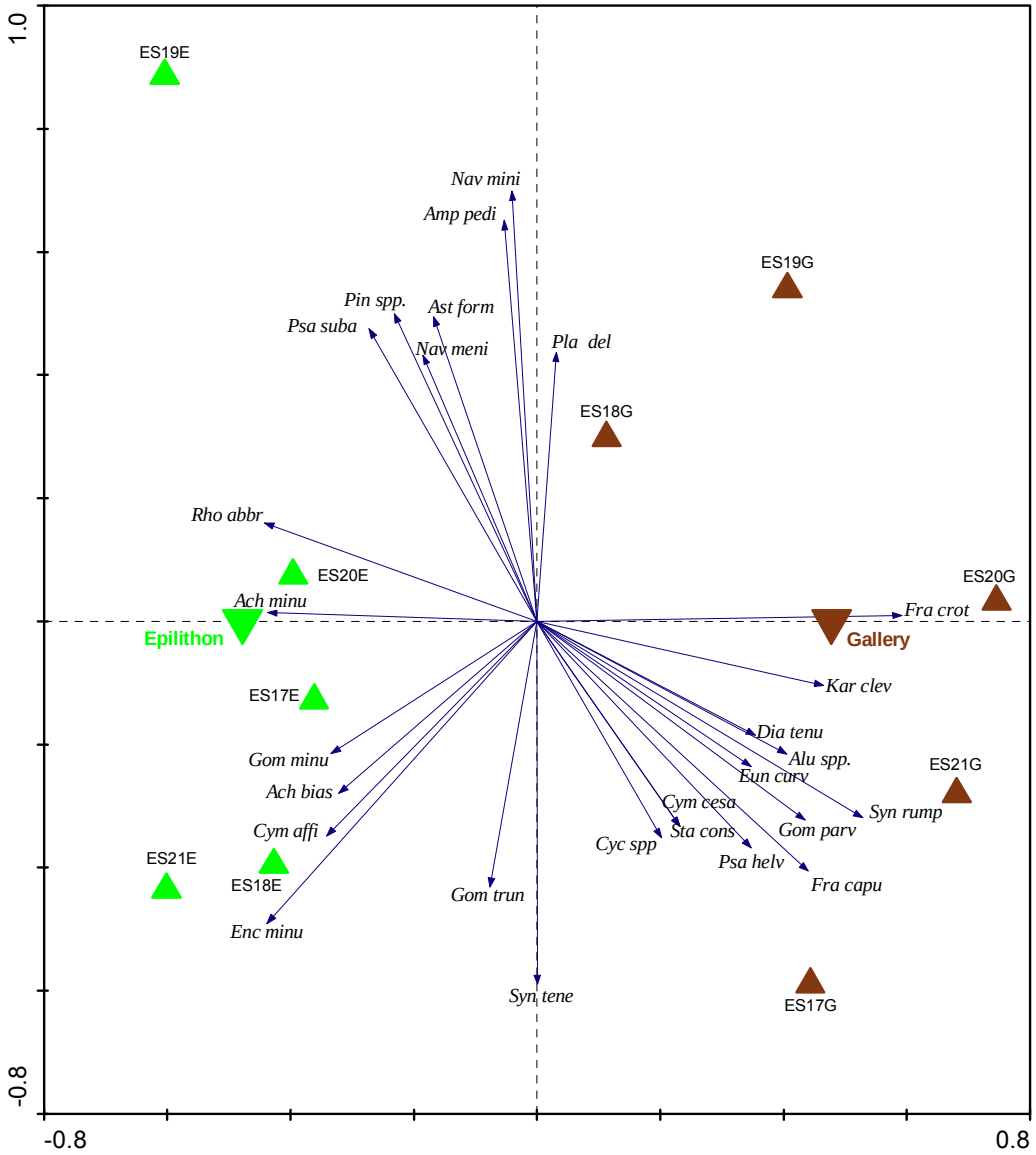


Figure 4.7 The RDA tri-plot for Esthwaite, constrained by *sample type* (galleries = brown and epilithon = green). Individual samples are plotted using Samp scores, thus their positions reflect variation within sample type. Down-triangles indicate the position of the gallery and epilithon centroids. Arrows show the direction of increased abundance for the species with the most influence. (**Ach bias** = *Achnanthidium biasolettiana*, **Ach minu** = *Achnanthidium minutissimum* **Alu spp.** = *Aulcosiera* spp., **Amp pedi** = *Amphora pediculus*, **Ast form** = *Asterionella formosa*, **Cyc spp** = *Cyclotella* spp., **Cym affi** = *Cymbella affinis*, **Cym cesa** = *Cymbella cesatii*, **Dia tenu** = *Diatoma tenue*, **Enc minu** = *Encyonema minutum*, **Eun curv** = *Eunotia curvata*, **Fra capu** = *Fragilaria capucina*, **Fra crot** = *Fragilaria crotonensis*, **Gom minu** = *Gomphonema minutum*, **Gom parv** = *Gomphonema parvulum,* **Gom trun** = *Gomphonema truncatum,* **Kar clev** = *Karayevia clevei,* **Nav meni** = *Navicula menisculus***, Nav mini** = *Navicula minima,* ***Pin spp*** = *Pinnularia* spp., **Pla del** = *Planothidium delicatulum*, **Psa helv** = *Psammothidium helveticum*, **Psa suba** = *Psammothidium subatomoides*, **Rho abbr** = *Rhoicosphenia abbreviata*, **Sta cons** = *Staurosira construens*, **Syn rump** = *Synedra rumpens* and **Syn tene** = *Synedra tenera*).

Figure 4.8 The relative abundance of frustules in the different size classes (μm^3) in the gallery (G) and the epilithon (E) for each of the lakes (ES = Esthwaite, BA = Bassenthwaite, RY = Rydal Water, WI = Windermere and CO = Coniston).

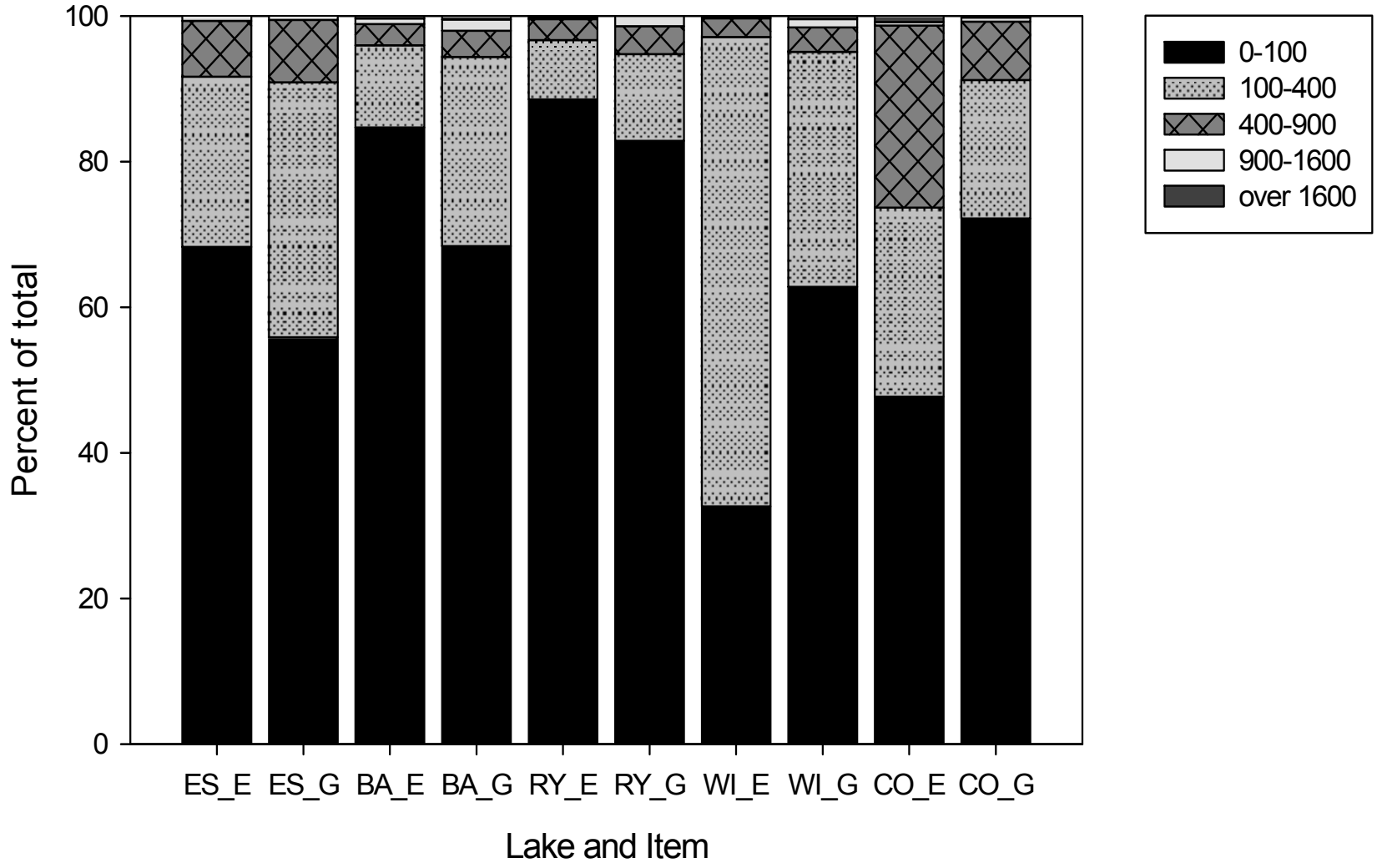


Table 4.4 Mean (± 1 SE) values of the trophic diatom index (TDI).

Lake	Gallery	Epilithon
Bassenthwaite	34.96 ± 1.09	30.69 ± 1.09
Coniston	30.65 ± 1.80	45.88 ± 4.12
Esthwaite	37.11 ± 0.63	35.93 ± 0.97
Rydal Water	33.62 ± 1.23	31.17 ± 1.08
Windermere	49.33 ± 2.18	78.97 ± 2.93

4.3.2.3 Bassenthwaite

Diatom communities at Bassenthwaite were dominated by *A. minutissimum* (Fig. 4.6), which was relatively more abundant within the epilithon than on the gallery (Figs. 4.6 & 4.9). This was also reflected in the biovolume distribution, with the greatest proportion of diatoms, including *A. minutissimum*, falling into the smallest size class (Fig. 4.8). Galleries were more taxonomically diverse (Table 4.3), and also contained a higher relative abundance of motile and erect species (Fig. 4.6) than the epilithon. Motile species were more abundant on galleries than in the epilithon, and included a number of *Nitzschia* species and *Navicula cryptocephala* (Fig. 4.9). Despite these differences in species abundances, the degree of similarity between gallery and epilithic samples was high (SIMI index, Table 4.2) and, in the RDA, 27.2% of variance in species data was due to samples being derived from galleries or epilithon. There was however, a significant difference in mean TDI values of galleries and epilithon (paired t-test, t=3.329 df=4 P=0.029, Table 4.4), with gallery communities representing higher nutrient conditions.

4.3.2.4 Rydal Water

Similarity between gallery and epilithic samples was highest in this lake with a SIMI value of 0.992 (Table 4.2). This was also reflected in the RDA, where the first axis explained only 18.2% of the variance in species data (the lowest value in this study). As in Bassenthwaite, both the epilithon and the galleries were dominated by *A. minutissimum* (Fig. 4.6), although this species was more abundant in the epilithon than on galleries (Figs. 4.6 & 4.10). Due mainly to the high abundance of *A. minutissimum,*

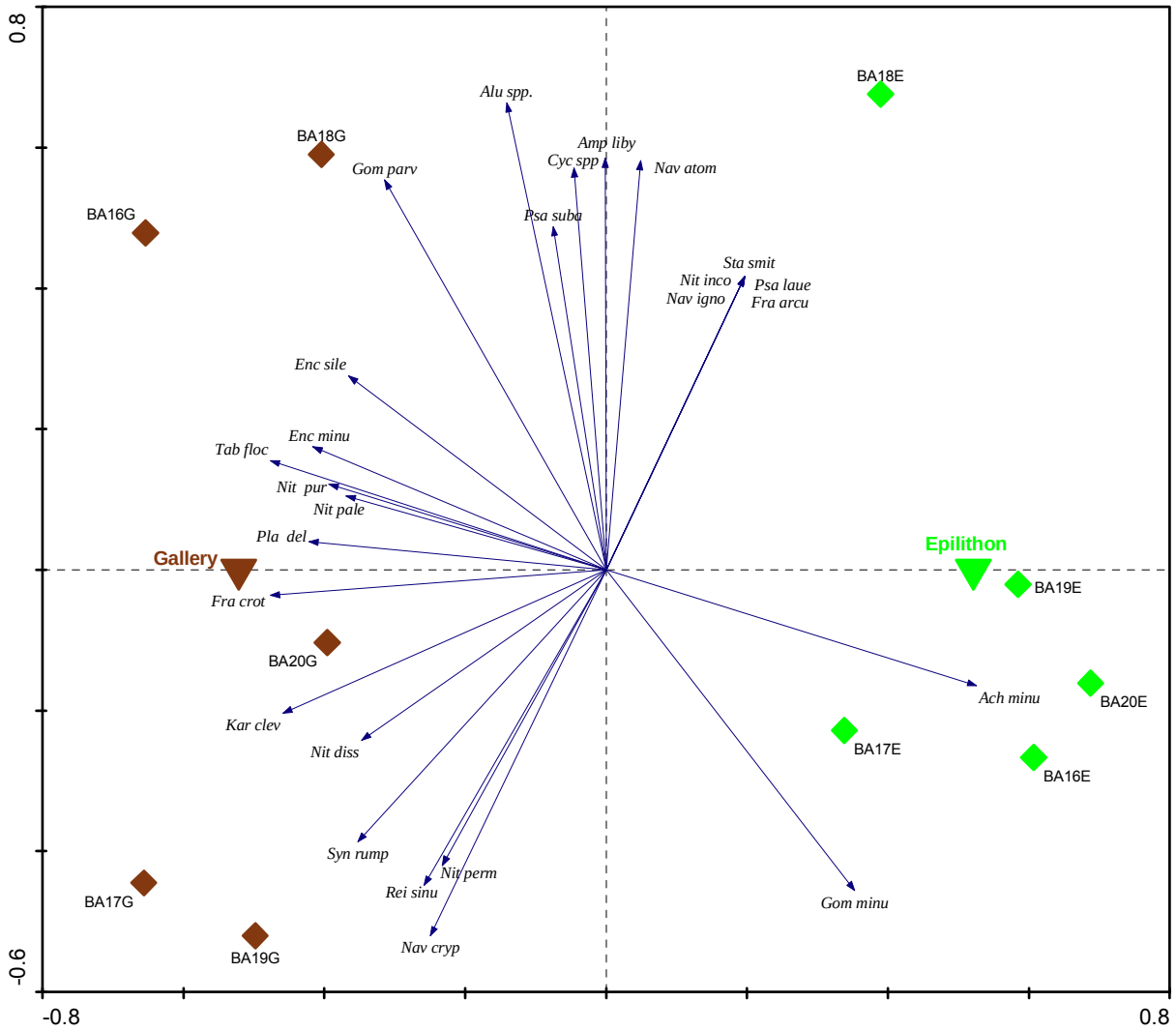
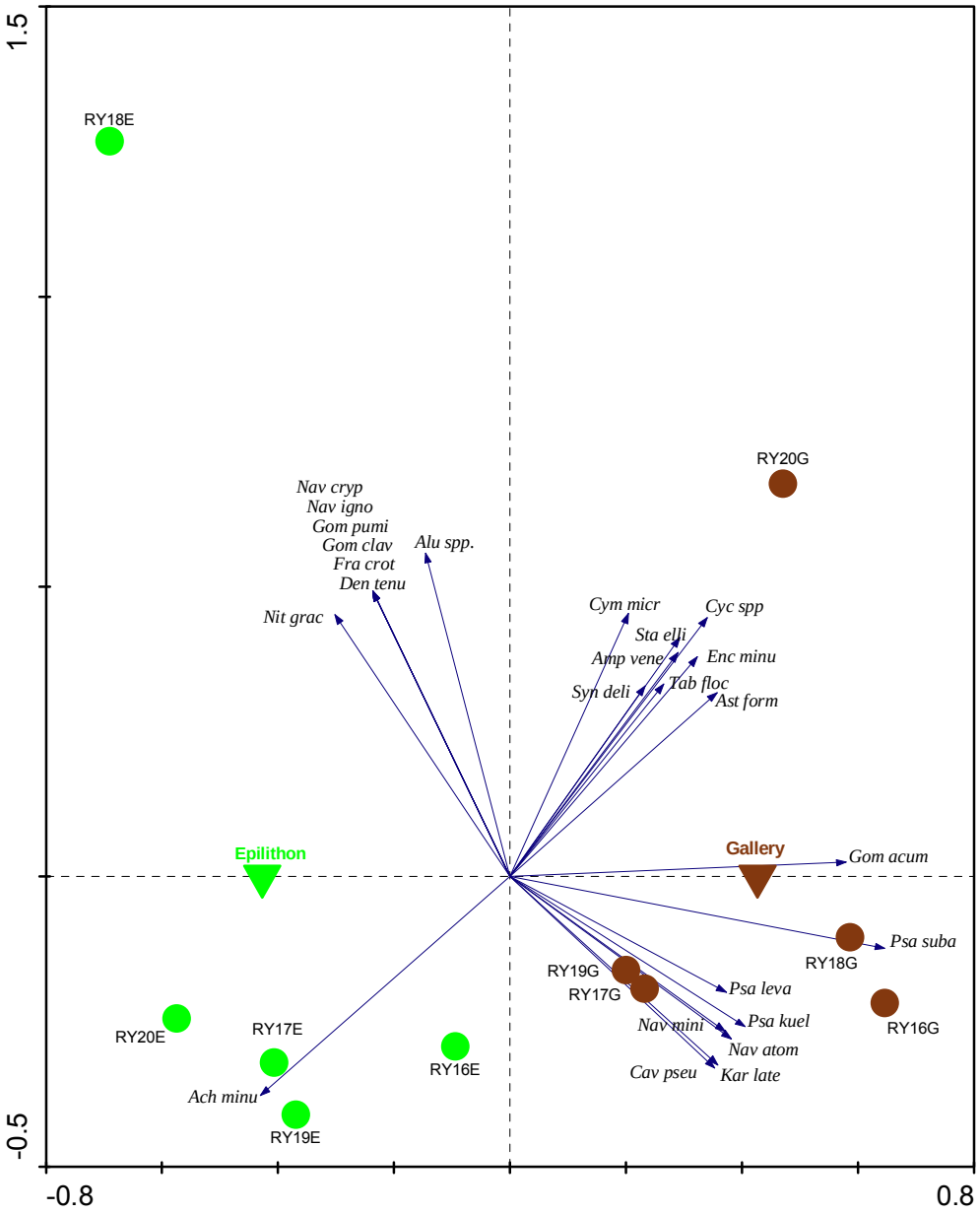


Figure 4.9 The RDA tri-plot for Bassenthwaite Lake, constrained by sample type (galleries = brown and epilithon = green). Individual samples are plotted using Samp scores, thus their positions reflect variation within sample type. Down-triangles indicate the position of the gallery and epilithon centroids. Arrows show the direction of increased abundance for the species with the most influence. (**Ach minu** = *Achnanthidium minutissimum*, **Alu spp**. = *Aulcosiera* spp., **Amp liby** = *Amphora libyca*, **Cyc spp** = *Cyclotella* spp., **Enc minu** = *Encyonema minutum*, **Enc sile** = *Encyonema silesiacum*, **Fra arcu** = *Fragilaria arcus*, **Fra crot** = *Fragilaria crotonensis*, **Gom minu** = *Gomphonema minutum*, **Gom parv** = *Gomphonema parvulum*, **Kar clev** = *Karayevia clevei*, **Nav atom** = *Navicula atomus*, **Nav cryp** = *Navicula cryptocephala*, **Nav igno** = *Navicula ignota*, **Nit diss** = *Nitzschia dissipata*, **Nit inco** = *Nitzschia inconspicua*, **Nit pale** = *Nitzschia palea*, **Nit perm** = *Nitzschia perminuta*, **Nit pur** = *Nitzschia pura*, **Pla del** = *Planothidium delicatulum*, **Psa laue** = *Psammothidium lauenburgianum*, **Psa suba** = *Psammothidium subatomoides*, **Rei sinu =** *Reimeria sinuata,* **Sta smit =** *Stauroneis smithii,* **Syn rump** = *Synedra rumpens* and **Tab floc** = *Tabellaria flocculosa).*

Figure 4.10 The RDA tri-plot for Rydal Water, constrained by *sample type* (galleries = brown and epilithon = green). Individual samples are plotted using Samp scores, thus their positions reflect variation within sample type. Down-triangles indicate the position of the gallery and epilithon centroids. Arrows show the direction of increased abundance for the species with the most influence. **(Ach minu** = *Achnanthidium minutissimum,* **Alu spp**. = *Alucosiera* spp., **Amp ven** = *Amphora veneta*, **Ast form** = *Asterionella formosa,* **Cav pseu** = *Cavinula pseudoscutiformis*, **Cyc spp** = *Cyclotella* spp., **Cym micr** = *Cymbella microcephala*, **Den tenu** = *Denticula tenuis*, **Enc minu** = *Encyonema minutum*, **Fra crot** = *Fragilaria crotonensis*, **Gom acum** = *Gomphonema acuminatum*, **Gom clav** = *Gomphonema clavatum*, **Gom pumi** = *Gomphonema pumilum*, **Kar late** = *Karayevia laterostrata*, **Nav atom** = *Navicula atomus*, **Nav cryp** = *Navicula cryptocephala*, **Nav igno** = *Navicula ignota*, **Nav mini** = *Navicula minima*, **Nit grac** = *Nitzschia gracilis*, **Psa kuel** = *Psammothidium kuelbsii*, **Psa lev** = *Psammothidium levanderi*, **Psa suba** = *Psammothidium subatomoides*, **Sta elli** = *Staurosira elliptica*, **Syn deli** = *Synedra delicatissima* and **Tab floc** = *Tabellaria flocculosa*).



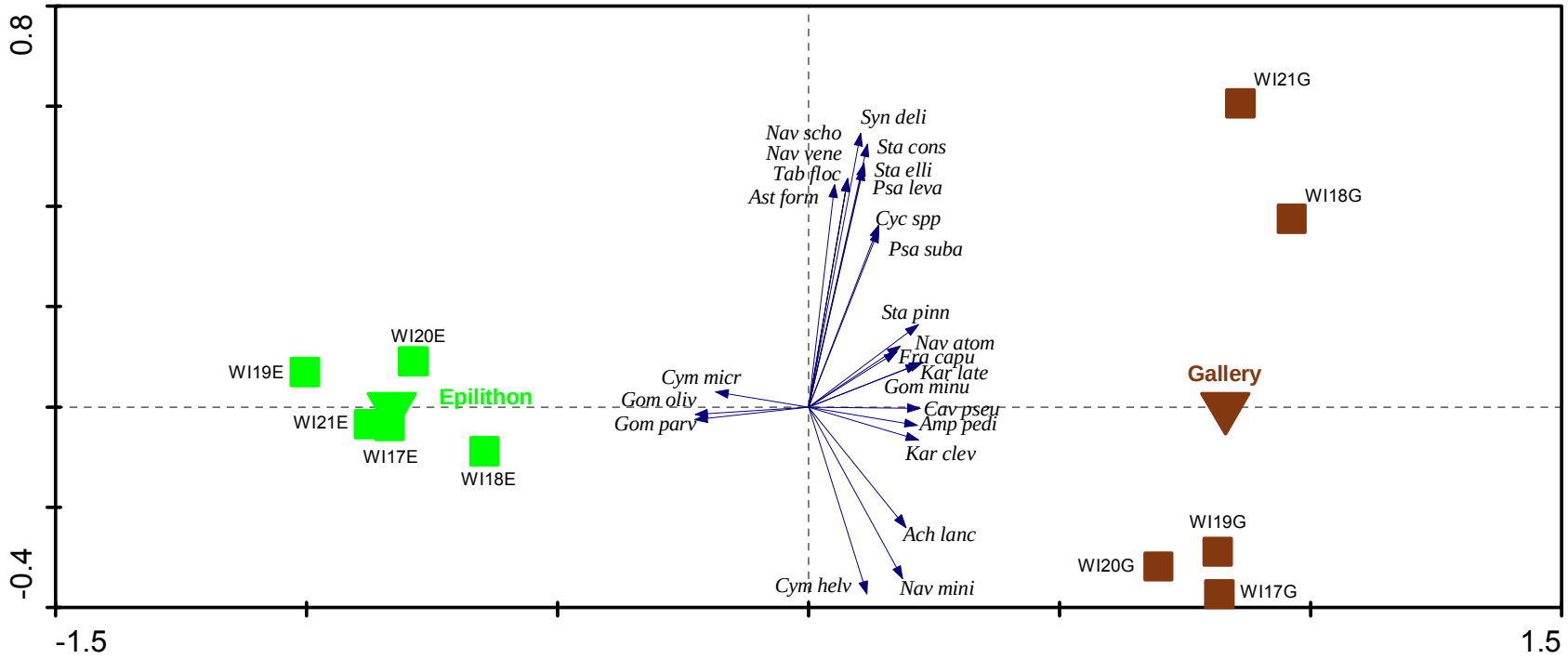
over 80% of the community consisted of small diatoms (biovolumes of less than 100μm^3, Fig. 4.8). Other small diatoms occurring at higher abundances in galleries as compared to in the epilithon included *Psammothidium subatomoides*, *Psammothidium levanderi*, *Navicula atomus* and *Navicula minima* (Fig. 4.10) and galleries had higher mean diversity and evenness scores (Table 4.3). However, although the mean TDI value was slightly higher for galleries than epilithon (Table 4.4), it was not significantly so (paired t-test, t=2.65 df=4 P=0.057).

4.3.2.5 Windermere

The difference between galleries and epilithon was most pronounced in this lake, with 68.9% of variance of the species data being explained by the first axis (gallery *versus* epilithon) of the RDA (Fig. 4.11). The epilithic community was more tightly clustered than the gallery community, which showed greater variation on the second (unconstrained) axis (Fig. 4.11). The epilithic community was dominated by *Gomphonema* species (61.2±4.3% of total community) and especially *G. parvulum* (49.2±45.3% of total community). In contrast, *Gomphonema* species only made up an average of 16.6±2.9% of the total community in galleries collected from the same rocks. The most abundant species on galleries was *A. minutissimum,* which was the second most abundant species within the epilithon. Other species characteristic of galleries (arrows pointing towards gallery centroid in Fig. 4.11) included *Amphora pediculus, Cavinula cocconeiformis*, and *Karayevia clevei.*

There was a greater abundance of stalked diatoms (Fig. 4.6) and diatoms in the 100-400μm^3 size class (Fig. 4.8) in the epilithon as compared to the galleries, and indeed as compared to all other samples in this study. This was attributable to the dominance of the stalked diatom *G. parvulum* within the epilithon. This species was at least partly responsible for the extremely high mean TDI value calculated for the epilithon (Table 4.4) as it has a value of 4 (as compared to 2 for *A. minutissimum*). Galleries were more taxonomically diverse than the epilithon (Table 4.3) and contained a more even distribution of growth forms (Fig. 4.6). In particular, a greater proportion of the community consisted of motile species on the Windermere galleries, than in the Windermere epilithon, or in any other sample (Fig. 4.6).

Figure 4.11 The RDA tri-plot for Windermere, constrained by *sample type* (galleries = brown and epilithon = green). Individual samples are plotted using Samp scores, thus their positions reflect variation within sample type. Down-triangles indicate the position of the gallery and epilithon centroids. Arrows show the direction of increased abundance for the species with the most influence. (**Ach lanc** = *Achnanthes lanceolata,* **Amp pedi** = *Amphora pediculus*, **Ast form** = *Asterionella formosa,* **Cav pseu** = *Cavinula pseudoscutiformis*, **Cym micr** = *Cymbella microcephala*, **Cym helv =** *Cymbella helvetica,* **Cyc spp** = *Cyclotella* spp., **Fra capu** = *Fragilaria capucina,* **Gom minu** = *Gomphonema minutum*, **Gom oliv** = *Gomphonema olivaceum*, **Gom parv** = *Gomphonema parvulum,* **Kar clev** = *Karayevia clevei*, **Kar late** = *Karayevia laterostrata*, **Nav atom** = *Navicula atomus*, **Nav mini** = *Navicula minima*, **Nav scho** = *Navicula schoenfeldii*, **Nav vene** = *Navicula veneta*, **Psa lev** = *Psammothidium levanderi*, **Psa suba** = *Psammothidium subatomoides*, **Sta cons** = *Staurosira construens*, **Sta elli** = *Staurosira elliptica*, **Sta pinn** = *Staurosirella pinnata*, **Syn deli** = *Synedra delicatissima* and **Tab floc** = *Tabellaria flocculosa*).

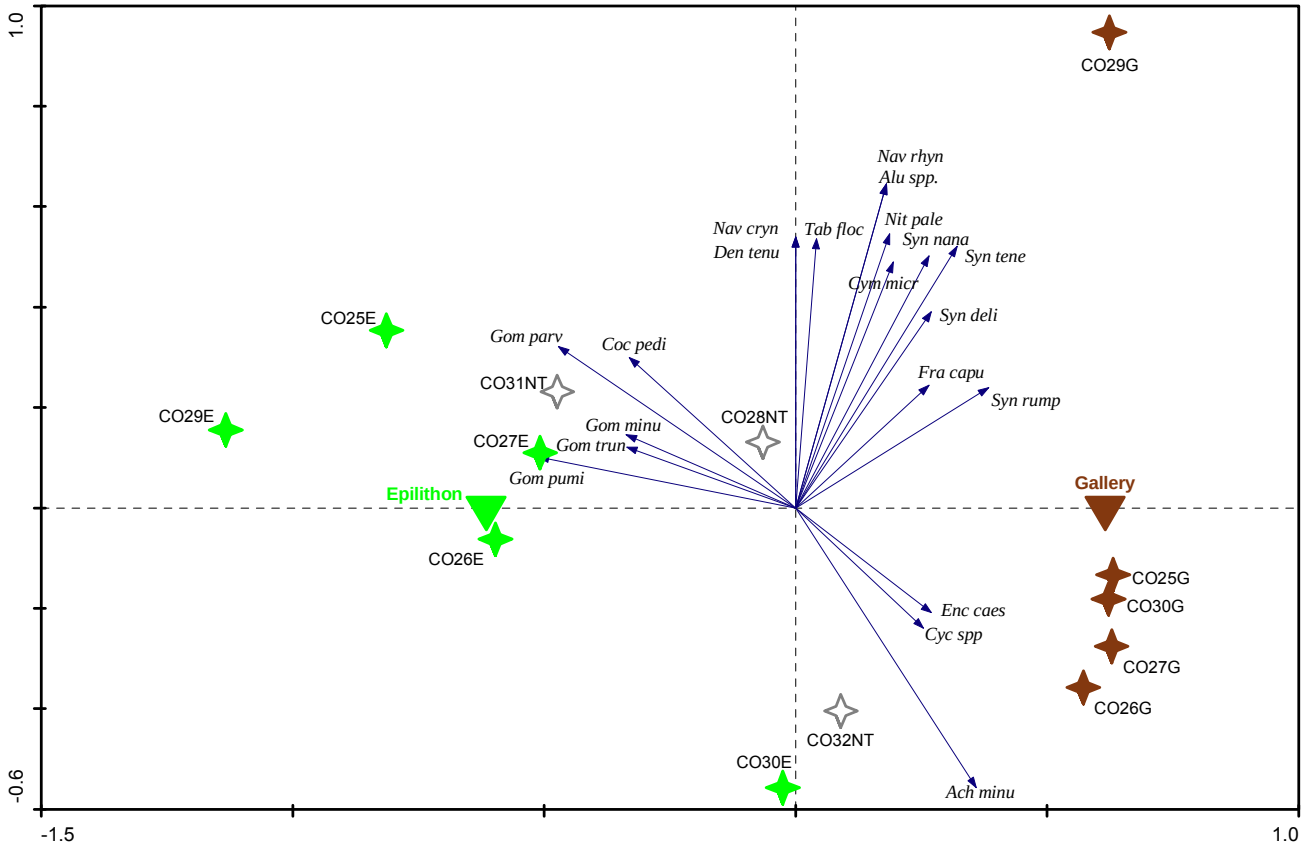


4.3.2 6 Coniston

In contrast to any other lake studied, diversity (Shannon and Simpson indices) and evenness were higher for the epilithon than for the galleries (Table 4.3) at Coniston. The difference in species composition between galleries and the epilithon was substantial, and this explained 37.9% of the variance in species data in the RDA analysis. The SIMI value (Table 4.2) fell into the 'similar' category, and was substantially lower than at Bassenthwaite, Rydal Water and Esthwaite. The most abundant species on both galleries and in the epilithon was *A. minutissimum,* although it was more common in galleries (60.7±5.4% of community) than in the epilithon (41.0±5.4% of community, Fig. 4.6). Epilithon contained high abundances of *Gomphonema* species (36.6±6.2% in the epilithon compared to only 6.9±0.7% in galleries). *G. parvulum* and *G. pumilum* were the main *Gomphonema* species present. These species contributed to the higher mean TDI value of the epilithon as compared to the gallery (Table 4.4).

Samples of epilithon from rocks devoid of *Tinodes waeneri* galleries were also collected, and these fell between gallery and epilithon samples in the RDA tri-plot (Fig. 4.12), although they aligned more closely with the epilithic samples. These samples were also intermediate in terms of growth form distribution (Fig. 4.6), especially in relation to contributions by *A. minutissimum* (51.2±6.1% of total) and *Gomphonema* species (24.1±5.9% of total) to the community.

Figure 4.12 The RDA tri-plot for Coniston, constrained by *sample type* (galleries = brown and epilithon = green). Individual samples are plotted using Samp scores, thus their positions reflect variation within sample type. Down-triangles indicate the position of the gallery and epilithon centroids. Grey stars indicate the projected location of epilithon samples taken from rocks not inhabited by *Tinodes waeneri*. Arrows show the direction of increased abundance for the species with the most influence. (**Ach minu** = *Achnanthidium minutissimum*, **Alu spp**. = *Aulcosiera* spp., **Coc pedi =** *Cocconeis pediculus*, **Cym micr** = *Cymbella microcephala*, **Cyc spp** = *Cyclotella* spp. **Den tenu =** *Denticula tenuis*, **Enc ceas =** *Encyonema caespitosum*, **Fra capu** = *Fragilaria capucina*, **Gom minu** = *Gomphonema minutum*, **Gom oliv** = *Gomphonema olivaceum*, **Gom parv** = *Gomphonema parvulum*, **Gom trun** = *Gomphonema truncatum*, **Nav cryn** = *Navicula cryptotenella*, **Nav rhyn =** *Navicula rhynchocephala*, **Nit pale =** *Nitzschia palea*, **Syn deli** = *Synedra delicatissima*, **Syn nana =** *Synedra nana*, **Syn rump** = *Synedra rumpens*, **Syn tene** = *Synedra tenera* and **Tab floc** = *Tabellaria flocculosa).*



4.4 Discussion

Using a combination of pigment analysis and community composition, it is clear that *T. waeneri* galleries contained a different assemblage of photosynthetic microorganisms, characterised by a greater content of diatom pigments, including fucoxanthin, as well as a distinctly different diatom community when compared to the neighbouring epilithon. Furthermore, this pattern was a general one, repeated at all five lakes studied. Within the diatom assemblage, there was no discrete consortium of species specifically associated with galleries among lakes. Thus, whilst there was no specific algal consortium associated with *T. waeneri* galleries, there was a general trend towards an assemblage of diatoms within the gallery, and this assemblage could itself be classed as a consortium. There was also a greater divergence in the diatom community on galleries from that in the epilithon in the less productive lakes, which lends support to my second hypothesis, that the influence of fertilisation of *T. waeneri* galleries would have the greatest impact on algal assemblages in lakes of lower productivity (Fig. 4.1).

As the lakes in this study were specifically selected to cover a substantial nutrient gradient, it is perhaps not surprising that the lake of origin had a greater impact on the diatom assemblages than the substratum from which samples originated (gallery *versus* epilithon). This difference in background nutrient availability (for instance, dissolved in the water column) between an oligotrophic and a eutrophic lake had a rather greater effect than the differences between fertilised galleries and unfertilised epilithon from the same rocks within a single lake. There were very few diatom species that were present only on one substratum type and none of these was common. Nevertheless, the magnitude of the difference between galleries and the epilithon was remarkable, particularly in some lakes.

A comparison of values of the similarity index obtained here with values calculated in other studies provides a framework to evaluate the differences in diatom assemblage between galleries and epilithon. Galleries were similar at Bassenthwaite and Rydal Water (greater than 0.99), and in Esthwaite (0.96). However the value for Esthwaite was only marginally greater than the similarity (0.95) calculated for autumn epilithon collected from 12 locations around the shores of Bassenthwaite (although pooled stone samples were used, thereby potentially reducing variation; King, 1999; King *et al.*, 2006). Similarity indices for Coniston, and particularly Windermere, were much lower in the current study (0.873 and 0.485 respectively). In comparison, average SIMI values

from monthly samples collected from single sites over a two year period were 0.74 for Coniston and 0.61 for Esthwaite (King *et al.*, 2002), suggesting marked distinction between the gallery and epilithic communities. The results of the similarity indices were corroborated by results from the RDA which indicated that over 18% of the variation in species data was explained by sample origin (gallery or epilithon) in all the lakes, with 37% and 68% of the variance explained in Coniston and Windermere, respectively. If this divergence in diatom assemblage between the epilithon and the gallery is due to larval gardening by fertilisation, one would expect gallery assemblages to reflect the higher nutrient availability in the galleries. Trophic state (measured as TP of the water column) is a significant factor in controlling Lake District epilithic assemblages across lakes (King *et al.*, 2000). It is therefore likely that diatom communities would also respond to fertilisation from larval excretions at the smaller among-patch scale.

Although it is difficult to infer the underlying mechanisms influencing assemblages from diatom assemblage data, there are a number of lines of evidence that suggest that gallery assemblages were shaped by higher nutrient availability. Firstly, the arrangement of samples in the RDA tri-plot (Fig. 4.5) suggested that the galleries contained diatom communities characteristic of higher nutrient levels; the lakes were arranged in order of increasing productivity from right to left and, for each lake, the gallery samples fell to the left (more productive side) of the epilithic samples. Secondly, at Esthwaite, Rydal Water, and Bassenthwaite, galleries harboured a greater proportion of diatoms larger than 100μm^3 than did the epilithon. A positive relationship between average size of diatoms within periphytic communities and the trophic status of the system has commonly been reported in both among lake comparisons (e.g. Cattaneo, 1987) and, more importantly, from within lake enrichment studies (DeNicola *et al.*, 2006), which provide a good comparison to the impacts being investigated here. However, the reverse pattern was apparent at Windermere and Coniston, mainly due to the high abundances of the relatively large *G. parvalum* in the epilithon of these two lakes. Size distributions were also heavily influenced by *A. minutissimum*, the most abundant species found during the study, which has a biovolume of less than 100μm^3. This benthic diatom is ubiquitous and was identified in all 51 samples taken from 17 lakes (including the five studied here) in the Lake District (King *et al.*, 2000) and *Achnanthes* species have also been found to dominate galleries of the stream dwelling *T. rostocki* (Hasselrot *et al.*, 1996). In itself, the distribution of this dominant species also suggests that gallery communities were at least partially shaped by nutrient availability.

Achnanthidium minutissimum abundance was greatest in Bassenthwaite and Rydal Water. It also had a lower relative abundance on the galleries as compared to the epilithon in the more productive lakes (Esthwaite, Bassenthwaite and Rydal Water), whereas at Coniston and Windermere it was more abundant on the galleries than in the epilithon. This pattern may be due to the fact that *A. minutissimum* has a TDI score of 2 (favoured by low nutrient concentrations: Kelly *et al*., 2001), and its optimal nutrient conditions (10.94µgL^{-1} TP King *et al.*, 2000 or 21.38µgL^{-1} TP DeNicola *et al.*, 2004) probably fall in between Windermere and the more productive lakes. Experimental manipulations using nutrient diffusing substrata under low ambient nutrient conditions resulted in an increased abundance of *A. minutissimum* with nutrient enrichment (e.g. Fairchild *et al.*, 1985; Carrick & Lowe, 1989). This response can be more pronounced where the additional nutrients are diffusing into the biofilm from the substratum (as in the case of galleries) rather than from the water column (Pringle, 1990). In contrast, in more productive lakes, a lower relative abundance of this species has been reported (DeNicola *et al.*, 2006), with abundances decreasing still further with nutrient enrichment (nutrient diffusing substrata).

Galleries were also more taxonomically diverse (Shannon index) than epilithic samples collected from the same rocks in all lakes except Coniston. In freshwater ecosystems, meta-analyses (Worm *et al.*, 2002; Hillebrand *et al.*, 2007), and a more recent controlled experiment (Liess *et al.*, 2009), have indicated that species diversity increases with fertilisation, and thus larval nutrient enrichment of the gallery community may have contributed to the observed patterns in species richness. However, only diatoms were identified to species level and it is possible that the inclusion of all algal groups would have altered the patterns in richness observed, especially in the light of the higher abundance of pigments associated with green algae in the epilithon. Furthermore, high diversity may be linked to other factors, an obvious one being the difference in substratum type between the galleries and the epilithon. Pringle (1990) hypothesized that the high diversities she measured on sand compared to adjacent glass slides was due to the physical structure of the sand grains, which produced a greater range of microhabitats. Additionally, the diatom communities associated with sediments are often distinct from epilithon. Thus, sediment is often associated with a specific suite of species, such as *Navicula* and *Nitzschia* species (Stevenson, 1996; Kelly *et al.*, 2001), or small *Fragilaria* species (King *et al.*, 2006). However, such diatom species did not

make up an appreciable proportion of gallery communities, and were also present in epilithic samples. Sediment diatoms assemblages are impacted by the unique environmental conditions found in benthic sediments, especially their instability and their potentially high organic content and readily available nutrients in the pore water (Hansson, 1992). In contrast, sediment particles within galleries are fastened into position by larval silk, and nutrients surrounding the sediment grains will either be those generally available in the water column/epilithon or those excreted by the gallery occupant. Alternatively, higher gallery diatom diversity may reflect reduced grazing pressure on the gallery community relative to the epilithon, as herbivory generally reduces species richness (Hillebrand *et al.*, 2007).

Increased grazing pressure on the epilithon relative to the gallery may be either due to larval grazing on the epilithon or larval defence of galleries against other grazers. The position in the RDA of the epilithic samples from rocks lacking *T. waeneri* at Coniston suggested that these communities were more similar to the gallery than was the epilithon on the rocks from which the galleries were collected. Alternatively, this arrangement of samples may simply reflect the spatial variability in epilithic communities from a highly unstable and wave-washed shore. Incorporation of epilithic samples from *Tinodes*-free rocks was attempted in all lakes, but proved unsuccessful due to human interference and the ability of *T. waeneri* to colonise vacant substrata efficiently. However, epilithic samples were collected in areas away from galleries (at least 1cm), so that samples would not reflect potential larval feeding territories, thus minimising the influence of grazing by *T. waeneri* on the patterns reported. Even so, if intense grazing of the entire epilithon was occurring it could have influenced the observed algal community differences between galleries and epilithon. Stable isotope analysis suggested that epilithon was the main source of C and N for Windermere larvae in the October sampling period (Chapter 2) and, thus, larval grazing or some other disturbance may have led to the dominance of *G. parvalum* within the epilithic community in Windermere. Although, *G. parvalum* is sometimes associated with high nutrient conditions (Kelly *et al.*, 2001; DeNicola *et al.*, 2006), it is an extremely variable species that can colonise hard surfaces over a large range of nutrient conditions (Hoagland *et al.*, 1982; Krammer & Lange-Bertalot, 2007b), in much the same way as *A. minutissimum*. However, its low growth form and larger size makes it susceptible to shading by other algae and to grazing (King *et al.*, 2005).

It has been hypothesised that motile diatoms should be abundant on *Tinodes* galleries as: 1) they will be attracted to and be able to move towards the higher nutrient conditions within the gallery (Hasselrot, 1993a); and 2) that once living within the gallery structure they may be able to move between the high light conditions on the outside surface of the gallery and the high nutrient conditions within the gallery interior (Stief & Becker, 2005). Furthermore, as well as being motile, *Navicula* and *Nitzschia* species have been demonstrated to be facultatively heterotrophic (King *et al.*, 2006) and may thus be able to deal with low light within the galleries (Tuchmann, 1996). Gallery communities did not contain species typically associated with light limited conditions (King *et al.*, 2006). In contrast to the tubes of the gardening chironomid species *Pseudodiamesa* cf. *pertinax* (Garrett), which contained significantly greater abundances of both *Navicula* and *Nitzschia* species than unmodified substrata (Pringle, 1985), motile diatoms were only slightly more common on the gallery than in the epilithon. Furthermore, even on the galleries they only formed a small proportion of the diatoms present. The relative lack of motile species in the assemblage may also be related to the length of time over which the gallery biofilm had developed, as they are usually at their most abundant in relatively mature assemblages (Stevenson, 1996).

Community data from the five lakes suggests that the galleries were in an early successional stage. The pigment data indicated that galleries were dominated by diatoms in all five lakes and supports results obtained for galleries in Lake Erken, using spectrophotometric techniques (Hasselrot, 1993a). Pigments associated with siliceous algae (especially fucoxanthin) attained higher densities in the galleries than on the epilithon within the individual lakes. As fucoxanthin is involved in light harvesting, rather than photo-protection (Wright, 2005), it can be used as a proxy for biomass in a similar way to chlorophyll *a* (Goericke & Montoya, 1998). Diatoms were the main group of siliceous algae contributing to fucoxanthin, particularly within gallery biofilms. No alloxanthin was recorded (a marker pigment for chrysophytes), and the marker pigment for dinoflagellates (peridinin) was scarce and mainly associated with the epilithon. In comparison, pigments associated with green algae (e.g. chlorophyll *b*) were more common in the epilithon than within galleries. Furthermore, at least in Esthwaite, high levels of chlorophyll *b* were probably related to the abundance of green filamentous algae (a late successional stage, Stevenson, 1996) within the epilithon (King *et al.*, 2002). In addition, *A. minutissimum,* the dominant species, is also often an early coloniser (Fairchild *et al.*, 1985) and usually grows directly attached to the

substratum, or attached through a short stalk (Kelly *et al.*, 2005), and thus typifies an early successional stage (Hoagland *et al.*, 1982; Stevenson, 1996).

Younger community stages are often more productive (Abe *et al.*, 2007) and the rate of biomass accrual declines as communities reach maturity (McCormick & Stevenson, 1991). For example, *A. minutissimum* - the dominant species on galleries – grows fast and, in a study in a Kentucky stream, its ability to outgrow other species enabled it to remain dominant for several weeks (McCormick, 1996). Diatom-dominated communities, such as those uncovered on galleries here, are also commonly observed on retreats and cases (e.g. Pringle, 1985; Hershey *et al.*, 1988), and may form a high quality food source for grazers (Lamberti & Moore, 1984) such as *T. waeneri*. Stable isotope results suggest that galleries are an important food resource for the larvae (Chapter 2) and they are therefore likely to be consuming diatoms. Gut contents analysis of *T. waeneri* larvae from Windermere suggested that diatoms were their main food source (Jones, 1967), although they will also consume green algae (Brodersen, 1995; Harrison, 1996). Similarly, diatoms formed a large proportion of the diet of *T. rostocki* in the German Breitenbach (Becker, 1990), despite an epilithon dominated by cyanobacteria. Thus, it is likely that the diatoms in *T. rostocki* guts are also derived from their galleries, which had high fucoxanthin contents (Stief & Becker, 2005).

The mechanism by which larvae maintain the galleries at an early successional stage is not known, but it may be related to gallery movement. Galleries are built from sediment bound together by silk, and larvae will extend one end of the gallery whilst demolishing, and presumably feeding upon, the other end, resulting in the unidirectional, slow migration of galleries across the substratum. Algal communities will therefore only have time to progress some way along the succession, depending on the rate of gallery movement and the productivity of the lake system, before they are consumed by the resident larva. Consequently, there should be a gradient in successional stage along the galleries. Indeed there is evidence to suggest this is the case for *T. rostocki* where higher standing stocks of algal biomass (Stief & Becker, 2005) and greater diatom abundance (Hasselrot *et al.*, 1996) have been measured on older gallery sections. Gallery communities are also likely to be affected by other aspects of larval behaviour; the composition of communities already existing on the sediment incorporated into galleries will depend on whether the sediment is being re-used or whether new sediment is being included (see Appendix 2). Furthermore, gallery communities may be more similar to

the epilithon if the larvae insert sections of biofilm into the gallery during the building process. A further possibility is that larvae are able to exclude green algae, and especially filamentous forms, from their galleries. Thus, *T. waeneri* grazing of *Cladophora* propagules was responsible for the spatial structuring of *Cladophora* at Crosemere (Harrison & Hildrew, 2001), although this occurred at a greater scale than that being examined here.

This study clearly illustrates that *T. waeneri* gallery algal communities are distinct from epilithic communities among the five lakes studied. Galleries contained more fucoxanthin and less chlorophyll *b* than the adjacent epilithon, indicating that they are more diatom-dominated. Furthermore, gallery and epilithic samples from within individual lakes are also different, with the greatest divergence being in the low nutrient lakes, and this will result in increased patchiness within the stony littoral (Pringle *et al.*, 1988). As *T. waeneri* occurs at such high density in these lakes (up to 22% of rock surfaces covered by galleries; chapter 3), the influence of larval activities on the algal communities of the littoral of these lakes is likely to be important to their function; in particular, productivity and nitrogen cycling (Ishida *et al.*, 2008) could be altered.

Chapter 5 – Bottom-up effects: the influence of resource availability on gardening

5.1 Introduction

Excretion by consumers is recognised as playing an important role in nutrient dynamics in both benthic (e.g. Devine & Vanni, 2002; Vanni, 2002) and pelagic systems (e.g. Urabe *et al.*, 2002). Excretions from benthic invertebrate assemblages as a whole can meet significant proportions of algal nutrient demand (Grimm, 1988). Furthermore, there is increasing evidence that a high biomass of dominant species can also have substantial impacts on nutrient recycling (Arnott & Vanni, 1996; Hall, 2003; Cross *et al.*, 2005).

In addition to species' abundances and characteristics, the background nutrient status of the system will also determine the impact of excretions by animals on nutrient dynamics. It has been predicted that nutrients excreted by grazers will be of greater significance to producers in circumstances where nutrients limit production than when nutrients are apparently already available (Liess & Hillebrand, 2004; Cross *et al.*, 2005). However, this prediction has rarely been assessed over nutrient gradients (Vanni, 2002).

Understanding the mechanisms which enable grazers to remain dominant, even when resources are limiting (Cavanaugh *et al.*, 2004) and/or productivity is low is a major goal in ecology (Power *et al.*, 1988). One such mechanism is gardening, and the most common form of gardening involves the grazer fertilising its food resource with its own excretions (e.g. Connor & Quinn, 1984; Pringle, 1985; Hershey *et al.*, 1988). Thus the effectiveness of gardening by fertilisation will also be dependent on the nutrient status of the system; under high nutrient conditions grazer excretions are expected to have little effect on algal nutrient status or biomass. Algal nutrient demand is likely to be met through uptake from the surrounding water and therefore there will be little benefit to the grazer in feeding on the fertilised resource. Conversely, in low nutrient environments, excreted nutrients may be an important algal resource and fertilised algae may provide grazers with a superior food resource.

A grazer that could exert a powerful influence on nutrient dynamics within the stony littoral of lakes is the sedentary caseless caddisfly *Tinodes waeneri.* This species is often

highly dominant (Dall *et al.*, 1984; Brodersen *et al.*, 1998; Harrison & Hildrew, 2001) and recent stable isotope analysis has provided evidence of gardening through fertilisation in this species (Chapter 2). Moreover, the gallery (the site of gardening) contained more chlorophyll than the surrounding epilithon (the alternative food source), suggesting fertilisation may be enhancing resource supply. The assimilation of C and N also varied with food availability, further indicating that the galleries were an important larval food source (Chapter 3).

Although, *T. waeneri* is classified as a sedentary grazer (e.g. in Harrison & Hildrew, 1998b), its gallery does not occupy a fixed position in relation to the substratum (see Fig. 5.1 for an example). Gallery movement occurs because the larvae will slowly demolish one end of the gallery and extend the other end of the gallery. In addition, gallery lengths can also vary. For example, they ranged from being only slightly longer than the larva (~1cm) to being 4 to 7cm long in a study by Jones (1967). Gallery length, rate of movement and therefore also gallery age, will be under control of the larva and could influence the importance of gardening, but are difficult to measure in the field.

Furthermore, although gardening in *T. waeneri* was studied across a natural gradient in lake productivity, there were no consistent relationships between gardening and background productivity, as measured in the field. Other differences between lakes (e.g. in predation pressure and algal community structure) may have overshadowed any potential relationship. Thus, an important next step is to study gardening in *T. waeneri* in relation to resource limitation under more controlled conditions. Therefore, the importance of gardening across a nutrient gradient and at two larval densities (resources should be more limiting under higher densities) was examined under controlled laboratory conditions. In a second experiment, the amount of epilithon within the larva's main grazing patch was manipulated.

The main hypotheses were: a) nutrient recycling and feeding on galleries would be more important under low than high background resource availability; and b) that gallery movement and larval grazing behaviour would reflect this. Under these hypotheses it is predicted that in the first experiment gardening should result in: i) a greater importance (i.e. absolute or relative amount) of excreted larval N in the gallery biofilm and a greater larval dependence on galleries in the low nutrient treatment than the high nutrient treatment; ii) a greater proportion of chlorophyll and/or lower C:N in galleries (relative

to in the ungrazed epilithon), as well as longer larval galleries, in the low as compared to high nutrient treatment; and iii) less time spent grazing on epilithon by the larvae in the low nutrient treatment as compared to the high nutrient treatment. In addition, if gardening is effective there should be no difference in growth between the high and low density treatments within nutrient treatments. In the second experiment it is predicted that galleries will be moved more rapidly where epilithon is removed from in front of the galleries if epilithon is the primary food source.

5.2 Methods

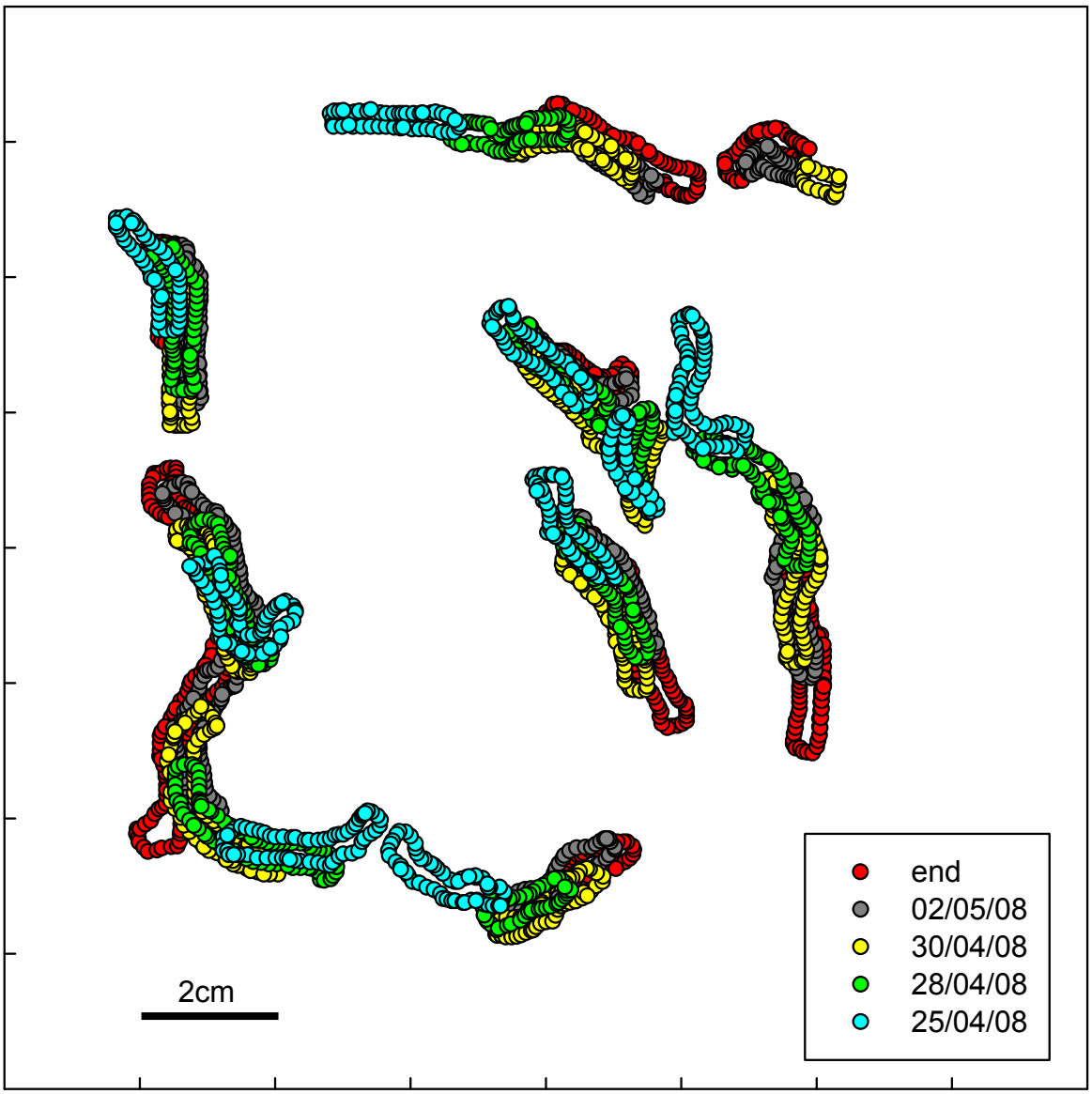
5.2.1 Experiment 1

5.2.1.1 Experimental design

In this experiment all larvae were grown on unglazed quarry tiles in the laboratory. The experiment contained three nutrient levels and two *T. waeneri* density levels arranged in a fully factorial design. Five replicates of each treatment were run through to the end of the experiment. There were also an additional three replicates of each of the six individual treatments, but without larvae (it was only possible to include three such replicates due to space limitations). These served as a comparison for the larval tile, thus enabling larval impacts on the epilithon to be quantified. In addition, one entire set of replicates of the experiment (six *Tinodes* tiles and six tiles without *Tinodes*) was sampled for epilithic algae at the beginning of the experiment (at the time when larvae were added to the mesocosms).

Nutrients were added to GF/F filtered lake water collected from Coniston, to provide the high and medium nutrient levels. Nitrogen and phosphorus were added as ammonium nitrate and potassium phosphate, respectively. Final concentrations of N were 860.65±1.9, 454.44±1.9 and 260.13±9.51μgL^{-1} in the water added at the beginning of the experiment in the high, medium and low nutrient levels. Initial phosphate concentrations were 123.43±2.65 (High), 66.93±3.66 (Medium) and 28.53±7.01 (Low) μgL^{-1}.

Figure 5.1 The position of galleries on a tile at two day intervals, showing the movement of galleries across the tile.



To achieve the two densities (numbers per unit area), the area of epilithon available (tile area) rather than larval number per experimental mesocosm was manipulated. This was achieved by using tiles of two sizes. Therefore, as larval number remained constant, the nutrients excreted and consumed were similar in the two density treatments. The area (mean ± SE) of the small tiles was 119.5±1.7cm^2 (~10x12cm) whereas the larger tiles had an area of 225cm^2 (15x15cm). Ten larvae were added to each tile, resulting in larval densities of 839.4±12.2 on small tiles and 444.4 individuals m^{-2} on large tiles.

5.2.1.2 Experimental set-up

Epilithic material taken from the littoral of Windermere and Coniston was sieved to remove larger particles and metazoan animals, mixed and used to colonise the quarry tiles. These tiles had previously been acid washed and thoroughly rinsed in deionised water for 1 month. Tiles were arranged on the bottom of large containers placed under artificial daylight (Sylvania Activa 172 Fluorescent tubes, illuminance 1517.8±97.6 Lux) on a 12h light:dark cycle at 11.49±0.05°C and left to colonise for one month. Tiles for each block of the experiment were arranged together in the same container.

At the start of the experiment, tiles were placed in experimental containers (2 litres, approximate internal dimensions 16.7x16.7x9.5cm), on a bed of washed gravel and placed under the same light environment used during colonisation. Equal quantities (~1ml) of pre-ashed sand were added to the tiles in all containers to serve as a gallery building material for the larvae. These containers were pre-filled with 1.5L of nutrient amended GF/F (Whatman; pore size 0.7μm) filtered lake water and the water level was marked on the side of the container. Water levels were kept constant through the addition of deionised water where needed. One third (500ml) of the water was replaced from each container once every week to maintain the difference in nutrient levels between treatments (Liess & Hillebrand, 2006). All experimental containers were connected to an air supply.

Fourth instar *T. waeneri* larvae were collected from Red Nab on the north basin of Windermere (SD386994) on 29 and 30 March 2008, and kept cold in individual glass vials until use in the experiment (for a maximum of 3 days). Fourth instar larvae were used as these would moult into fifth instars during the experiment and feeding and growth rates are highest in fifth instar larvae (Bergey, 1995). Ten *T. waeneri* larvae were added to each tile one week after the epilithon was exposed to the nutrient

treatments. Only healthy larvae were selected and these were randomly allocated to each treatment. Larvae for each treatment were placed in a white tray with a small amount of water and a scale bar and photographed before being carefully released onto the tile. All larvae initially settled onto the tiles, although a small proportion of larvae moved to the gravel during the experiment (see results). Experimental treatments were maintained for one month under the same conditions used during colonisation of the tiles.

At the end of the experiment each tile was carefully removed from the container and all galleries were labelled (by placing plastic labels next to the gallery) before the tile was photographed. A sample (17.04cm^2 total area sampled, taken from six equally sized locations) of the epilithon was taken using a periphyton toothbrush sampler (see Chapter 3 for further details) and additional (three, where possible) epilithon samples were taken for stable isotope analysis. Galleries were then carefully removed from the tile and placed into individual Eppendorf tubes. Larvae inhabiting the galleries were placed in individual vials containing filtered lake water and labelled to enable them to be associated with their particular galleries. Gallery and epilithic samples were frozen immediately after collection. Larvae were left to clear their guts for 24 hours before being rinsed in deionised water, inspected under the microscope for attached silk or other material (any found was removed), and then frozen.

5.2.1.3 Measurements

i) Larval growth

A mean larval growth rate was calculated for each mesocosm (as an increase in total larval weight/number of larvae) as larvae were not housed individually during the experiment and therefore could not be distinguished from one another. At the beginning of the experiment, 50 fourth instar larvae were used to obtain a regression equation for calculating dry mass from photographs. Each larva was photographed in a Petri dish, resting on 1mm graph paper, and was then placed into a pre-weighed aluminium capsule. These capsules were oven-dried and re-weighed to provide a measure of dry mass. Image J was used to obtain key larval measurements from the photographs and these were used to develop an equation for calculating dry weight of the *Tinodes* larvae from photographs (Dry weight (mg) = (0.0899 x total area) - 0.1279, r^2=0.7545, n=50). The total area of each of the larvae placed into each mesocosm was measured from the photographs and the regression equation was used to convert these measurements into

dry mass. Dry mass was also measured for each individual larva that was recovered at the end of the experiment, and thus the total dry mass of larvae in each mesocosm at the end of the experiment could also be estimated. The number of living larvae collected from each mesocosm also provided a measure of survival.

ii) Larval behaviour

Emergence from galleries - During the experiments, digital video recordings, using a video camera (Sony DCR-SR190E Camcorder) with an infrared light (night shot mode), were made of individual tiles to quantify the amount of time that larvae spent protruding from their galleries. Pairs of high density (small) tiles were recorded from the high (n=5) and the low nutrient treatments (n=5), with one tile from each pair recorded on consecutive days. Recording was started towards the end of the light period each day and a one hour video segment, beginning 30 minutes after the lights were turned off (at 18:00), was analysed. Videos were recorded in the dark as previous recordings (over 24 hours) had shown no obvious differences in activity patterns between daylight and darkness, larval movements were easier to analyse in night shot mode and evening recordings ensured that disturbance from other researchers using the aquariums was minimal. The 30 minute delay was included to allow the larvae to return to their normal nocturnal behaviour after installation of the camera and the change from light to dark.

During analysis of the videos, larval protrusion from their galleries was scored by inspecting one frame every minute (using Adobe Premiere Elements, version 4) over the one hour section of interest, giving a number of emergences out of sixty for each gallery present. Where no larva was observed protruding from a particular gallery during the section of video analysed, the remainder of the thirteen hour video recording was checked to assess whether the gallery was occupied or not. If no larva was seen protruding from the gallery within thirteen hours the gallery was considered to be unoccupied. Only occupied galleries were used in the calculations.

Gallery movement - To assess rates of gallery movement across the tiles, digital photographs of each tile were taken at two day intervals. Co-ordinates of multiple points around the outline of each gallery were obtained to provide information on the location of the gallery perimeter, relative to the tile edges. The co-ordinates were obtained using Image Pro-Plus (version 4.1) for four dates spanning eight days, with the last date falling two days before the experiment ended, The co-ordinates of occupied galleries,

from the photographs of individual tiles taken at the end of the experiment, were also calculated. Co-ordinates for each of the dates were plotted (e.g. Fig 5.1) and the distance that the front and back end of the gallery had moved over the eight days was measured for all galleries that were still occupied at the end of the experiment. Time stamps from the photographs were used to calculate the time interval between the first and the last photograph and, thereby, the rate of gallery movement.

5.2.1.4 Stable isotope analysis

Stable isotopes were used in the same way as in Chapter 2. The $\delta^{15}N$ values of the gallery and the epilithon were used to assess whether gallery biofilm was assimilating nitrogen derived from larval excretions. Where this is the case, the gallery should be ^{15}N-depleted relative to the epilithon (see Fig. 2.1 for rationale behind this). The $\delta^{15}N$ value of the ungrazed epilithon was measured, and comparison with the $\delta^{15}N$ values for the grazed biofilm provided information on uptake of excreted N by the epilithon. Additionally, $\delta^{13}C$ and $\delta^{15}N$ values of the gallery, epilithon and larvae were used in SIAR mixing models (Jackson *et al.*, 2009) to assess gallery contributions to larval biomass in each of the experimental treatments. Larvae and epilithon collected at the start of the experiment were also analysed to provide information on the way in which $\delta^{13}C$ and $\delta^{15}N$ changed during the experiment.

Samples of epilithon from all tiles at the end of the experiment, five gallery samples and matching larval samples from each tile, and larval and epilithic samples from the beginning of the experiment, were all prepared for stable isotope analysis.

Samples were oven-dried and then ground using an agate pestle and mortar. Ground samples were weighed into tin capsules and combusted in an elemental analyser (Flash EA, 1112 series, Thermo-Finnigan) coupled to a continuous flow mass spectrometer (Finnigan MAT Delta^{Plus}, Thermo-Finnigan). Larval samples (n=13) from the start of the experiment (fourth instar larvae) were of insufficient mass to run through the normal column and were therefore run using a reduced column (Chapter 2).

All isotope ratios are expressed in parts per mille (‰) and were calibrated using the secondary standards ammonium sulphate (RM 8547) and sucrose (RM 8542), which are of known isotopic composition in relation to the international standards, atmospheric nitrogen in air (N) and Pee Dee Belemnite (C).

Gallery $\delta^{15}N$ values were corrected for silk. Larvae from high density replicates run concurrently with the main experiment were collected at the end of the experiment. They were then allowed to clear their guts before being introduced to clean tiles in pure water and supplied with inorganic sand. Once galleries had been constructed, they were photographed with a scale bar and harvested and the resident larvae were frozen. Galleries were placed in pre-weighed aluminium capsules and ashed to obtain a measure of the amount of silk built into galleries by the larvae. These were compared to galleries harvested from the experiment (see below) to calculate the proportion of organic matter attributable to silk for each nutrient level.

5.2.1.5 Resource availability

Resource quality was measured as the C:N ratio of the epilithon and the whole gallery, and was calculated from samples run for stable isotope analysis. Elemental mass of carbon and nitrogen in each sample was calculated using a urea dilution series (0 to 0.6mg urea for standard sized columns), which was included at the beginning of every run on the mass spectrometer.

Chlorophyll *a* was the main measure of resource quantity and was measured for the epilithon samples (at the start and at the end for both grazed and ungrazed) and a gallery sample composed of two randomly selected galleries (occupied at the end of the experiment) from each tile. Epilithon samples were defrosted and filtered onto Whatman GF/C filters, after which all samples were freeze-dried before overnight extraction in 90% acetone in a freezer. Chlorophyll *a* was measured using standard spectrophotometric techniques (Biggs & Kilroy, 2000). Gallery lengths and areas were measured for all galleries that were occupied at the end of the experiment using Image J. This provided a measure of the area of rock surface covered by the gallery (gallery footprint). The maximum gallery surface area was calculated (i.e. minimum chlorophyll a concentration) by multiplying the foot print area by 1.27. This conversion factor is based on the height of the gallery equalling a third of the width of the gallery.

5.2.2 Experiment 2

This experiment was designed to examine the impacts of a reduction in epilithon availability directly in front of the gallery on gallery size, gallery movement and larval growth. The basic set-up of this experiment was similar to the one described above:

fourth instar larvae, collected from Windermere, were grown on unglazed quarry tiles (15x15cm) that had been pre-colonised with epilithon collected from Coniston. Ten tiles were placed individually on a bed of washed gravel on the bottom of 2L containers, filled with 1.5L of GF/F filtered lake water, collected from Coniston. Equal quantities of pre-ashed sand were added to each of the tiles, for the larvae to use in gallery construction. Water levels were kept constant through the addition of deionised water as needed and one third of the water was substituted with fresh GF/F filtered lake water once a week.

Nine larvae were photographed on a white tray with a scale bar and then added to each of the 10 tiles at the start of the experiment. Larvae were allowed to settle for one week before experimental manipulations were started. Tiles were randomly allocated to two treatment groups, which contained five tiles each. In one treatment (epilithon removal treatment) a 1cm wide strip of epilithon, extending 1cm to either side of the gallery was removed from directly in front of the gallery entrance, using a stiff paint brush, whereas in the second treatment (control treatment) an equivalent area of epilithon was removed, but from areas away from the galleries. This epilithon removal was carried out every other day and the experiment was run for one month.

To measure gallery movement, tiles were photographed daily. Gallery movement was measured using the same methodology as for experiment 1 (see Section 5.2.1.3 for details) and gallery movement rates were calculated for the period from the 22/08/08 to the 28/08/08 for all galleries that contained resident larvae when the experiment was ended on the 31/08/08.

At the end of the experiment, each tile was carefully removed from the water. All the galleries were labelled using plastic labels and a small section of ruler was placed on the gallery to act as a scale bar. A digital photograph was taken of each tile, and larvae were then carefully removed from their galleries and placed into vials containing lake water, together with the plastic label. Larval lengths were measured for all larvae, and these were used in the regression equation of Baumgärtner and Rothhaupt (2003). Gallery lengths were measured using exactly the same methodology as in experiment 1 (Section *5.2.1.5*).

5.2.3 Data analysis

All data analysis was preformed using SPSS (version 13) and R (version 2.8.1) was used for the mixing models. Data was log(x+1) transformed prior to analysis where variances were not homogenous. Larval proportions of gallery- and epilithon-derived N and C were calculated using Stable Isotope Analysis in R (SIAR) mixing models (Parnell *et al.*, 2008; Jackson *et al.*, 2009, for more details see Chapter 2). The amount of gallery ^{15}N-depletion per day was calculated for each tile as the total amount of gallery ^{15}N-depletion relative to the epilithon over the mean age of the galleries.

5.3 Results

5.3.1 Experiment 1

5.3.1.1 Resource availability

The overall chlorophyll content of the epilithon and galleries was strongly influenced by nutrient levels (using gallery surface area; $F_{2,83}$=7.494 P<0.001), with the high nutrient treatments containing more chlorophyll *a* than the medium and low nutrient treatments (Tukey HSD, P<0.05, Fig. 5.2). At the end of the experiment, epilithon samples collected from tiles inhabited by *Tinodes* larvae contained significantly lower chlorophyll concentrations than samples taken from tiles without larvae. Chlorophyll *a* concentrations on galleries were between 2.2 and 4.7 times higher than in the matching epilithon samples (calculated using maximum gallery surface area; Fig. 5.2) and also significantly higher than in ungrazed epilithon (Tukey HSD, P<0.05). Within galleries, there was a trend towards lower chlorophyll in the low density treatments and also towards decreasing chlorophyll densities with decreasing nutrient level (Fig. 5.2).

Nutrient level significantly affected the amount of organic matter present in galleries in the high density treatments ($F_{2,9}$=5.585, P=0.026), with galleries built at high nutrient availability containing significantly more organic matter per mg of sediment than galleries in the other two nutrient levels (Tukey HSD, P=0.026, Fig. 5.3). Thus, galleries from the high nutrient level contained just over twice as much organic material per mg of sediment than galleries from the low nutrient concentration (Fig. 5.3).

Figure 5.2 Mean (±1SE) chlorophyll *a* content (µg cm^{-2}) in each of the experimental treatments. Epilithic chlorophyll content was measured at the start of the experiment (2 tiles per treatment) and at the end of the experiment for tiles not occupied (n=5 per treatment) and tiles occupied by *Tinodes waeneri* (n=5). Chlorophyll *a* was also measured for larval galleries from the five occupied tiles in each treatment and values for both the gallery footprint and surface area are presented.

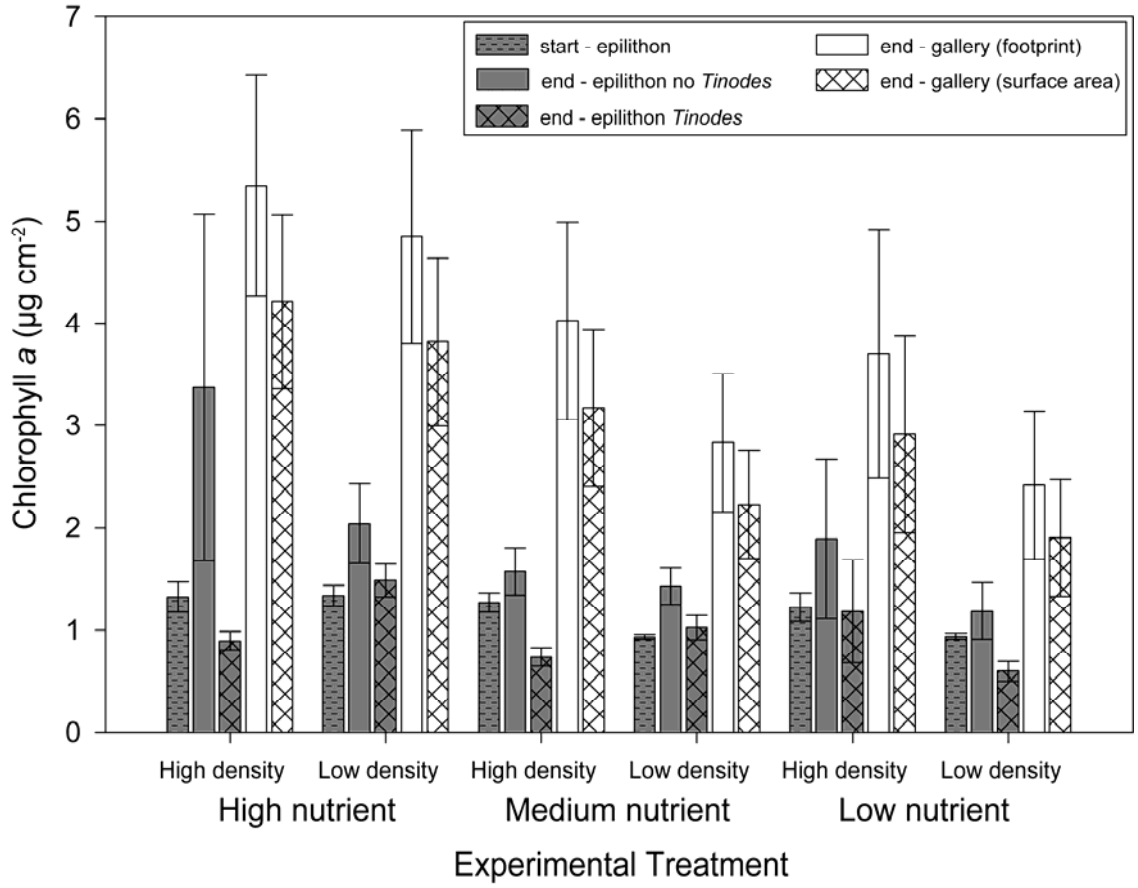
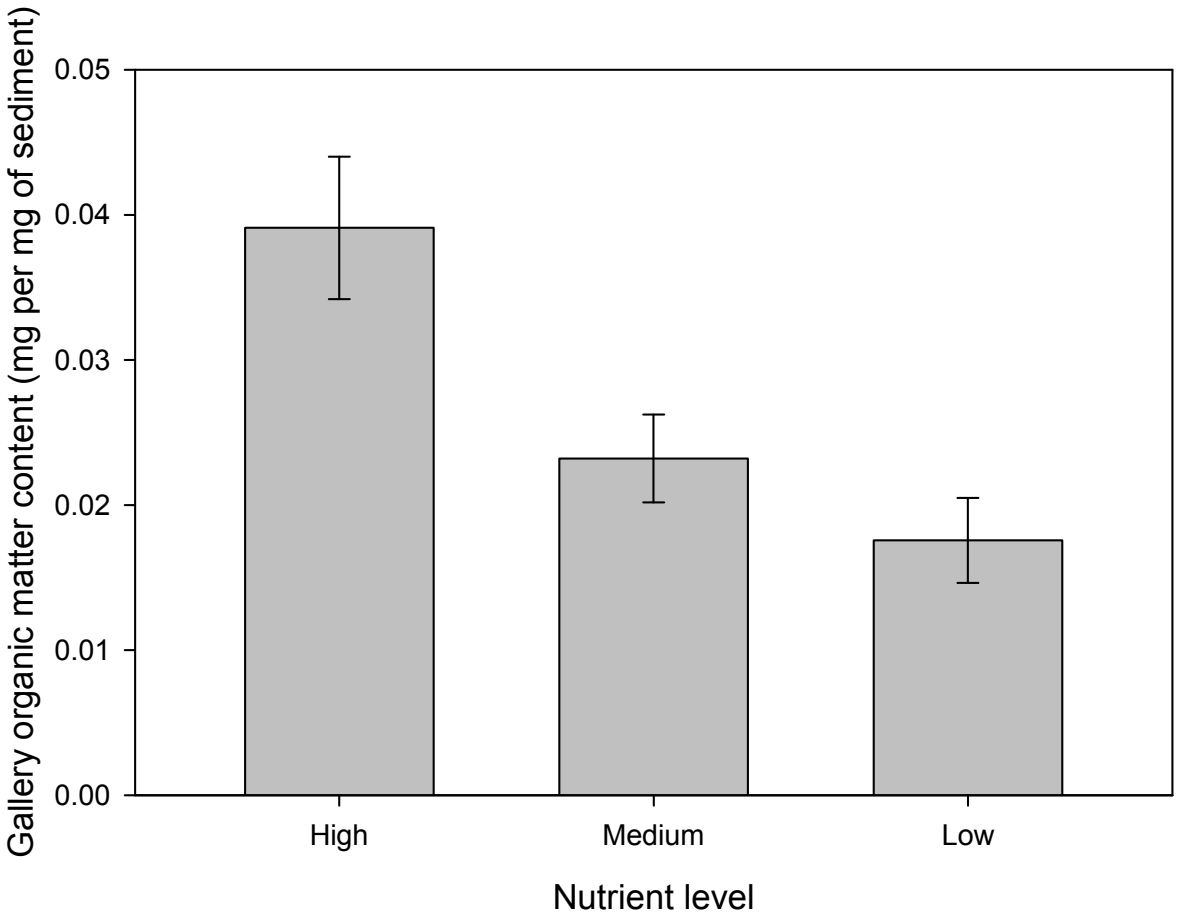


Figure 5.3 Mean (±1SE) organic matter content of galleries (amount of organic matter (mg) per mg of inorganic sediment) at high larval densities, for the three nutrient levels.



Overall, gallery length and area were not significantly affected by treatment (Table 5.1), although for both medium and high nutrient levels, the galleries were slightly longer when larval densities were high. Galleries also tended to be shortest in the low nutrient treatments.

Table 5.1 Mean gallery size and the mean length of time that gallery sections remain in place in the six treatments.

Treatment		Gallery area (cm^2)	Gallery length (mm)	Length of time a typical gallery section exists (days)
Nutrient level	Density	Mean (SE)	Mean (SE)	Mean (SE)
High	High	0.91 (0.13)	26.70 (3.16)	7.86 (0.61)
	Low	0.67 (0.06)	22.91 (1.25)	7.35 (0.43)
Medium	High	0.86 (0.08)	27.04 (2.09)	10.40 (3.76)
	Low	0.68 (0.08)	21.90 (1.70)	7.40 (1.18)
Low	High	0.69 (0.05)	20.02 (1.57)	8.02 (0.88)
	Low	0.72 (0.09)	20.46 (1.92)	4.84 (0.36)

C:N ratio was used as a measure of the quality of the resources available to larvae. Nutrient level had a significant impact on epilithic C:N ratios ($F_{2,66}$=3.599, P=0.033), with the ratio being significantly lower (i.e. higher quality) at high nutrient concentrations than at the two lower nutrient levels (Tukey HSD, P<0.017). In contrast, density had no significant impact on C:N ratio (Fig. 5.4). Epilithon present at the beginning of the experiment had significantly lower C:N ratios than epilithon and galleries sampled at the end of the experiment (Tukey HSD, P<0.001). Epilithon collected from the treatments lacking *Tinodes* also had higher C:N ratios than both the epilithon and the galleries present in mesocosms containing larvae across all nutrient and density treatments.

5.3.1.2 Larval behaviour

Video footage revealed that larvae (at high density) in both the high and low nutrient concentrations were highly active, and individual larvae were observed protruding from their galleries for between 2 and 50 of the 60 observation periods (one minute intervals during one hour). However, there was no significant difference in larval protrusion between the pairs (high and low nutrient) of tiles (paired t-test t=0.334, df=4, P=0.748)

with larvae spending a mean of 32.40% (±2.47) and 30.56% (±3.76) of the observation points extending out of their galleries at high and low nutrient levels, respectively (Fig. 5.5). Within individual pairs, there was only a significant difference between the two tiles in the first pair to be filmed (8 and 9 days after larvae were introduced into the experimental mesocosms) ($t=2.376$, $df=14.983$, $P=0.031$).

Galleries were moved across the tiles at a significantly faster rate in the low density (3.92 ± 0.23mm day^{-1}) treatments than in the high density (2.85 ± 0.24mm day^{-1}) treatments ($F_{1,24}=17.647$, $P<0.001$, Fig. 5.6). Nutrient levels had no significant impact on the rates of gallery movement (Fig. 5.6). Galleries generally remained on the top surface of the tiles, and by the end of the experiment, only 4.0% of occupied galleries were located on the gravel and 4.4% located exclusively on the side of the tiles.

Calculations using values of mean gallery lengths and mean rates of gallery movement per day indicate that the length of time a typical gallery section persisted ranged from 10.4 to only 4.8 days (Table 5.1). Galleries sections remained in position for longer in the high density treatments than in the low density treatments, as gallery movement rates were lower and galleries were longer (Table 5.1).

Figure 5.4 Mean (±1SE) C:N ratios (molar). C:N ratios of epilithon were measured at the start and end (for tiles occupied and not occupied by *Tinodes waeneri*) of the experiment. Gallery C:N ratios were also measured at the end of the experiment.

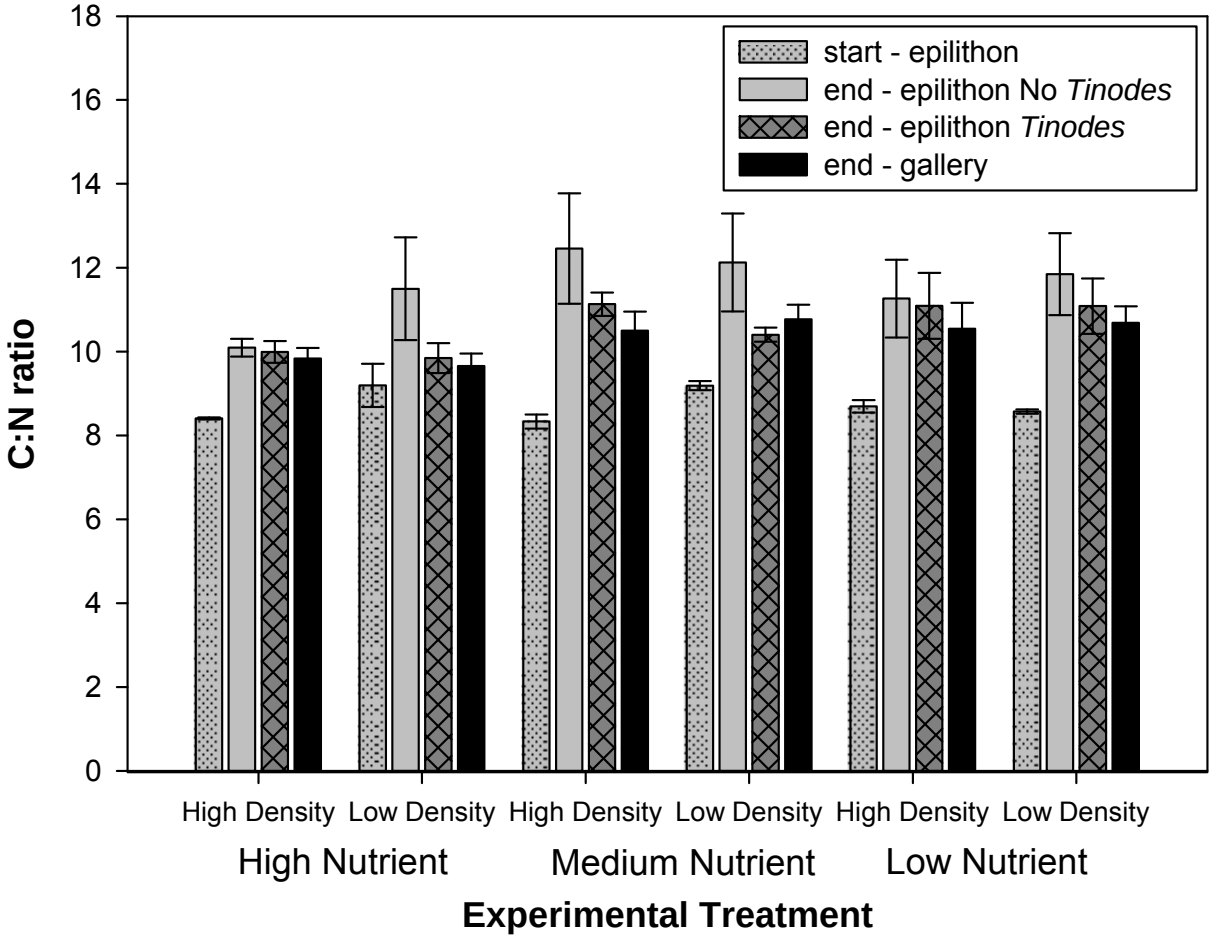


Figure 5.5 Mean percentage (±1SE) of time spent protruding from the gallery in the high (black bars) and low (light grey bars) nutrient treatments, for each of the five replicates recorded. Both nutrient level treatments recorded contained larvae living at high density and larvae were scored as either protruding from their galleries or not, at minute intervals over the course of one hour.

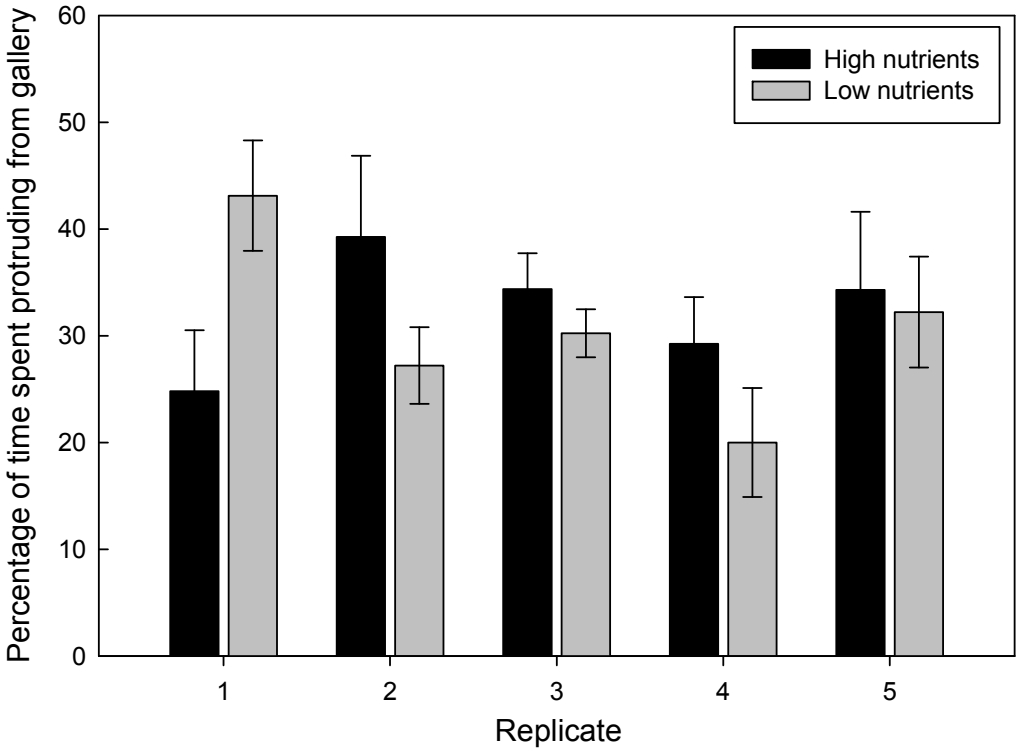
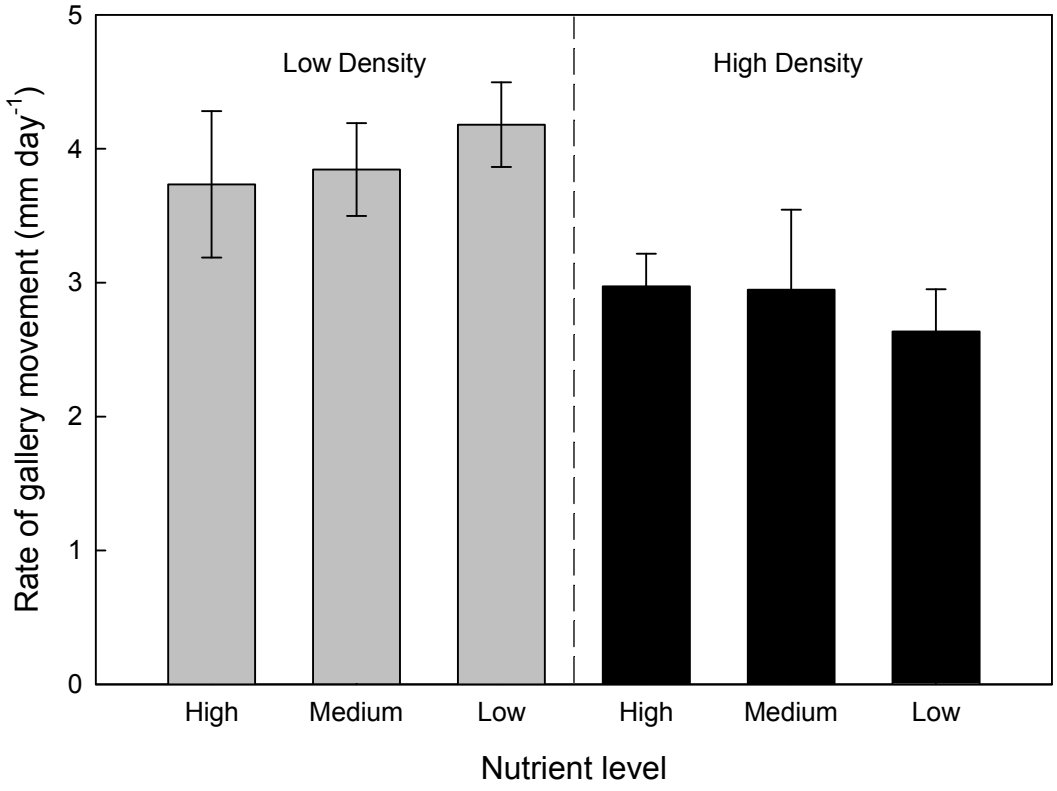


Figure 5.6 The mean (±1SE) distance (mm) that the galleries moved forward per day (24 hours) in each of the six treatments. Low density treatments are shown as light grey bars and high density treatments as black bars.



5.3.1.3 Stable isotopes

Correction of the $\delta^{15}N$ values of galleries to obtain values of gallery biofilm, had most impact in the low nutrient treatment, due to a combination of both a lower organic content and more silk in the galleries. Galleries and epilithon in treatments housing *Tinodes* larvae were ^{15}N-depleted (i.e. lower values) when compared to the epilithon sampled from the mesocosms without larvae (Fig. 5.7). Furthermore, galleries were also ^{15}N-depleted relative to the epilithon in all treatments containing larvae (Fig. 5.7), but differences in $\delta^{15}N$ values between the epilithon and the galleries did not vary significantly across treatments, although they were lower in the high density x high nutrient treatment. However, when gallery age was taken into account, and the rate of ^{15}N-depletion per unit time was calculated, the ^{15}N-depletion of gallery relative to the nearby epilithon was related to nutrients ($F_{2,24}$=4994, P=0.015) with the greatest ^{15}N-depletion of galleries (relative to the epilithon) at low nutrient levels (Fig. 5.8). These calculations of mean gallery age were based on mean rates of gallery movement and mean gallery lengths (measured towards the end of the experiment) and assumed that gallery length remained constant. There was no significant difference in gallery length between the start and end date of the gallery movement analysis (paired t-test, t=0.93, df=29, P=0.926), suggesting that this was a valid assumption.

At the beginning of the experiment, larvae had a $\delta^{15}N$ value of 4.02±0.19. Larvae at the high nutrient concentrations became more ^{15}N-depleted during the experiment, whereas at the other nutrient levels larvae became ^{15}N-enriched, and this mirrored the values of their two potential food sources (Fig. 5.7). Larvae also became more ^{13}C-depleted in the low nutrient and medium nutrient, low density treatments (Table 5.2). However, there was little difference in terms of $\delta^{13}C$ values between the epilithon and the gallery in all treatments.

SIAR mixing models provided a large range of possible outcomes due to the small differences between epilithic and gallery values (particularly in terms of $\delta^{13}C$). However, the results indicate that larvae were assimilating both gallery and epilithic material in all treatments, with it being most likely that between one third and two thirds of assimilated material originated from the gallery (Fig. 5.9). Furthermore, there was a trend toward an increasing mean (50% probability interval), and particularly minimum (lower end of 95% probability interval), larval reliance on gallery material with decreasing nutrient levels (Fig. 5.9).

Figure 5.7 Mean (±1SE) $\delta^{15}N$ values (‰) for the larvae, galleries and grazed and ungrazed epilithon sampled at the end of the experiment across the six treatments.

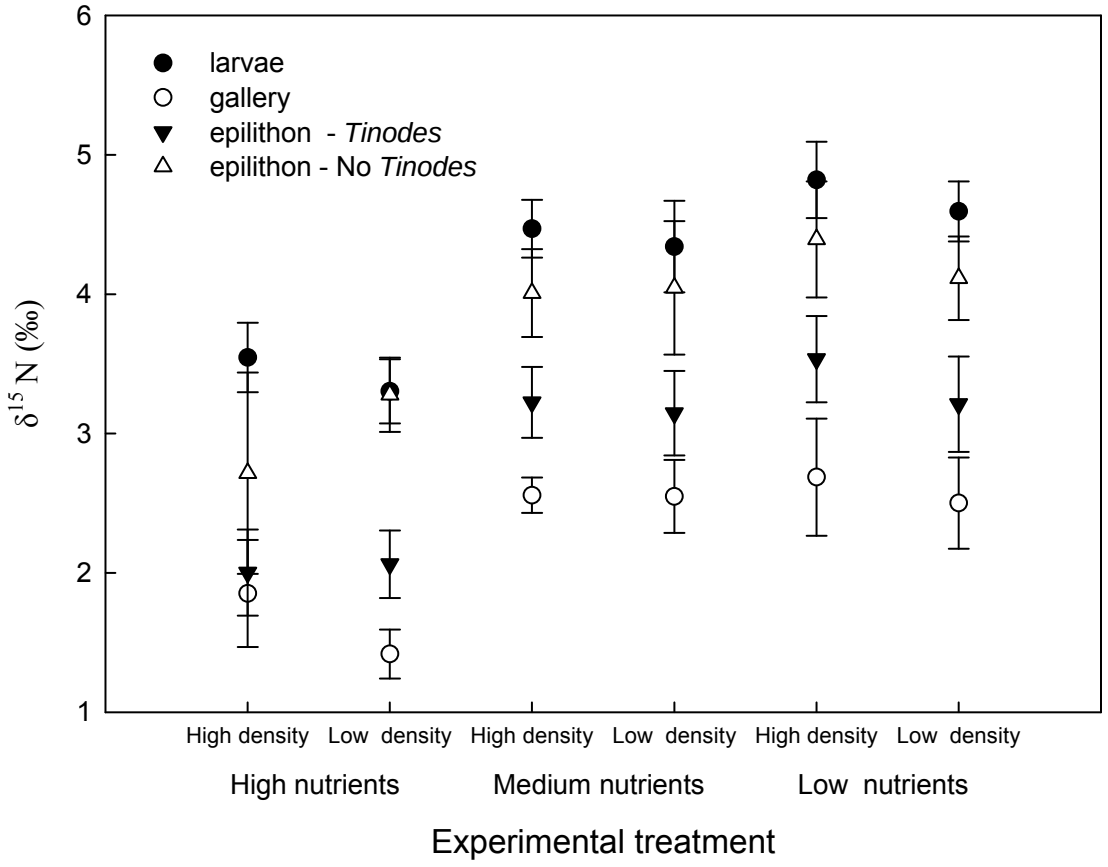


Figure 5.8 The mean difference (±1SE) in $\delta^{15}N$ values between the grazed epilithon and the galleries, corrected for the mean age of the gallery, across the three nutrient levels. Low density treatments are shown as light grey bars and high density treatments as black bars.

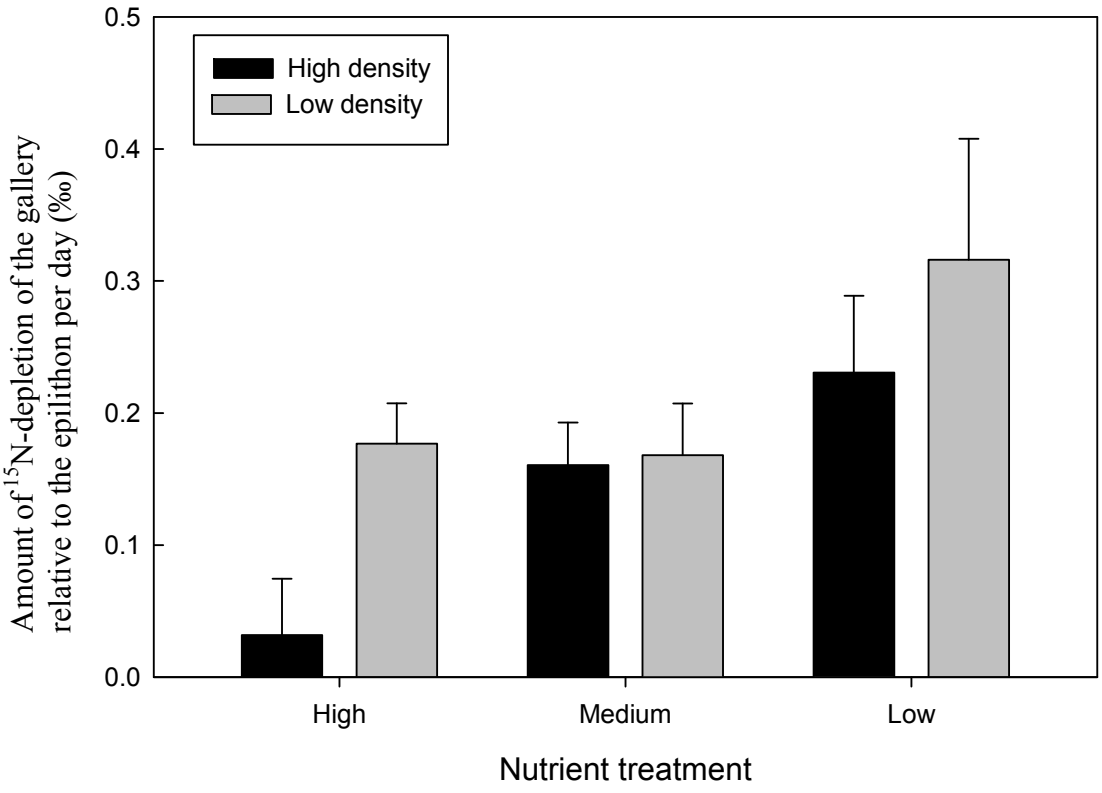
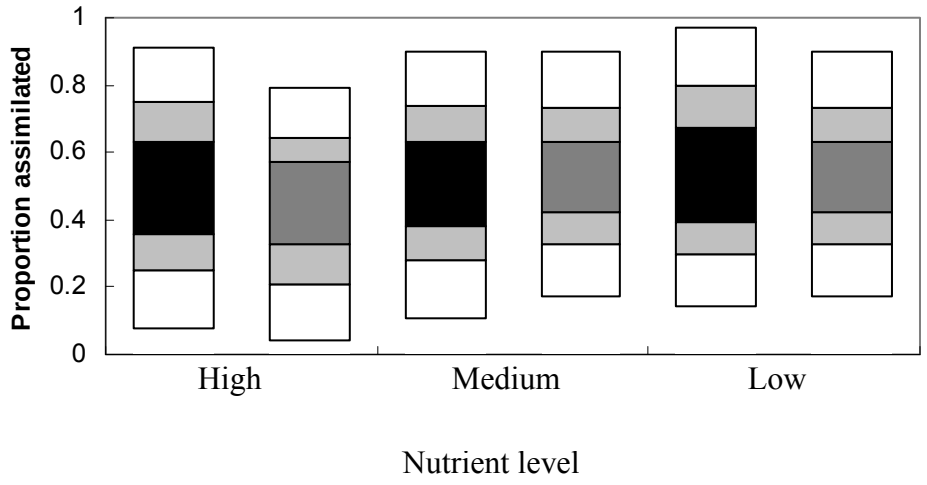


Table 5.2 Mean $\delta^{13}C$ values (‰) across treatments for the different components of the system at the beginning and end of experimental manipulations.

Nutrient Treatment	Density	Larvae		Gallery		Grazed epilithon		Ungrazed epilithon		Epilithon at the start	
		Mean (SE)	N	Mean (SE)	N	Mean (SE)	N	Mean (SE)	N	Mean (SE)	N
Start	All	-15.47 (0.23)	13	_	_	_	_	_	_	_	_
High	High	-15.52 (0.95)	5	-16.65 (1.14)	5	-15.87 (1.10)	5	-14.77 (1.58)	3	-14.40 (0.07)	2
High	Low	-15.50 (0.42)	5	-17.53 (0.12)	5	-16.23 (0.59)	5	-14.22 (0.25)	3	-14.78 (0.37)	2
Medium	High	-15.27 (0.60)	5	-16.76 (0.80)	5	-16.04 (0.92)	5	-13.96 (1.47)	3	-14.92 (0.19)	2
Medium	Low	-16.29 (0.31)	5	-17.41 (0.27)	5	-16.46 (0.54)	5	-15.91 (1.30)	3	-14.87 (0.15)	2
Low	High	-17.93 (1.46)	5	-18.28 (1.45)	5	-18.21 (1.70)	5	-16.67 (1.78)	3	-13.86 (0.15)	2
Low	Low	-17.64 (0.89)	5	-18.75 (0.88)	5	-18.82 (1.05)	5	-18.11 (1.32)	3	-15.54 (0.96)	2

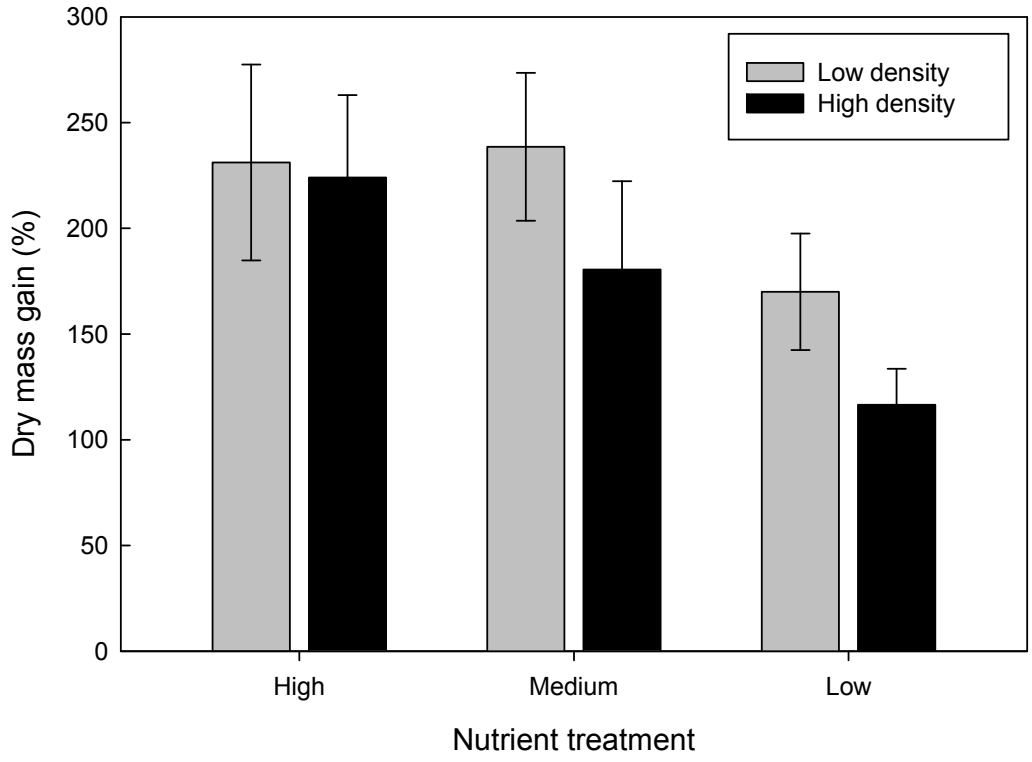
Figure 5.9 Posterior estimates of the contributions of gallery C and N to the larvae at the three nutrient levels, using the SIAR mixing model. 50% probability intervals are represented by the dark central bar (black for high density treatments and dark grey for low density treatments), with 75% confidence intervals including this area and the lighter grey area. 95% probability intervals are represented by the entire bars.



5.3.1.4 Larval growth

Larval survival was high, with 90% or greater survival in all treatments other than the low nutrient/ high density treatment (7.8±0.58 individuals recaptured out of 10). Most larvae moulted into fifth instars during the experiment and dry mass increases per larva were measured in all treatments (Fig. 5.10). Dry mass gain was greatest in the high nutrient treatments and the low density/medium nutrient treatment. Larvae gained less weight in the high density treatments, with this becoming more pronounced with further reductions in nutrient concentrations. The initial mean C:N ratio of the larvae was 5.59±0.13 but this increased marginally in all treatments, other than the low nutrient x high density treatment, and ranged between 5.97±0.18 and 5.45±0.15 at the end of the experiment.

Figure 5.10 The mean dry mass gain calculated as a percentage of original dry mass per larva for high and low larval densities across the three nutrient levels.



5.3.2 Experiment 2

Gallery lengths and movement rates (Table 5.3) were similar to those in experiment 1 (Table 5.1). However, there was no difference in gallery length or movement rate (Table 5.3) between the epilithon removal treatment (epilithon removed from directly in front of each gallery) and the control treatment (same amount of epilithon removed but away from galleries). In addition, there was no difference in the mean dry mass per larva in the two treatments at the end of the experiment (Table 5.3), although larvae survived less well in the epilithon removal treatment.

Table 5.3 Rates of gallery movement, gallery sizes and larval dry mass for the epilithon removal experiment (Experiment 2).

Treatment	Gallery length (mm)		Rate of gallery movement (mm day^{-1})		Mean dry mass per larva (mg)		Mean number of larvae per tile	
	Mean (SE)	N	Mean (SE)	N	Mean (SE)	N	Mean (SE)	N
Control	20.96 (1.27)	5	3.31 (0.45)	5	0.54 (0.03)	5	7.6 (0.68)	5
Epilithon removal	22.20 (1.68)	4	3.64 (0.50)	4	0.53 (0.03)	5	6.2 (0.66)	5

5.4 Discussion

The results from the highly controlled factorial design of experiment 1 indicate that gardening was taking place under all experimental treatments. More importantly, the results suggest that gardening was playing more of a role where nutrient levels were low. The density at which the larvae are living was also found to influence larval behaviour. Further support for galleries being an important larval food resource was gained from the second experiment.

5.4.1 Gardening in Tinodes waeneri

The stable isotope analysis (nutrient and density experiment) provided evidence that gardening through fertilisation, defined as both i) fertilisation of the gallery through larval excretions and ii) consumption of the gallery by larvae, occurred across all experimental treatments. Galleries had lower $\delta^{15}N$ values than the epilithon in all cases (although not significantly so in the high nutrient x high density treatment) and larvae did assimilate gallery material. ^{15}N-depletion relative to the epilithon (mean of 1.49‰ when ungrazed epilithon was used in the comparison) was similar to values measured for the Lake District populations studied, under natural conditions (Chapter 2).

Mixing models indicated that galleries formed an important component of the diets of larval *Tinodes* (circa 50%) under all experimental conditions. Observations during the experiment confirmed that both epilithon and gallery material was being consumed. Galleries were surrounded by a zone with a width, the approximate length of the resident, in which there was very little epilithon. Gallery consumption also occurred as galleries were disassembled down to the level of individual sand grains. Mixing models rely on the assumption that grazers are in isotopic equilibrium with their food source (Gratton & Forbers, 2006; Kaufman *et al.*, 2008). Although no information on C and N isotope turnover rates in similar organisms was available, it is likely that this assumption of mixing models was met. Larvae were grazing within the experimental treatments for one month, during which time they at least doubled in mass and in most cases had also moulted. Thus, larvae were actively growing and metabolic activity is also likely to have been reasonably high given the temperature (11.5°C) under which the experiment was performed.

Galleries were a better food resource for the larvae because they harboured significantly higher concentrations of chlorophyll *a* both per unit rock area and per unit gallery

surface area than both grazed and ungrazed epilithon in all treatments. The chlorophyll concentrations (2.4 to 5.3 μg cm^{-2}) were within the range measured for *T. waeneri* galleries in the Lake District (Chapter 3) and in Lake Erken (Hasselrot, 1993a), as well as on the cases of other caddisflies (e.g. *Glossosoma intermedium* (Klapalek); Cavanaugh *et al.*, 2004).

5.4.2 Resource availability and the importance of gardening

In these experiments, resource availability was manipulated in three ways: 1) by altering nutrient levels of the water surrounding the tiles (experiment 1); 2) by changing the amount of epilithon available for the larvae to feed upon (i.e. larval density in experiment 1); and 3) by manipulating the availability of epilithon directly in front of larval galleries (experiment 2). As expected, in experiment 1, epilithic chlorophyll content responded to nutrient addition, with the high nutrient treatment containing significantly greater quantities of chlorophyll *a* per cm^2 than treatments at the other two nutrient levels. There was however, no impact of the nutrient additions on the C:N ratio of the epilithon, nor was the epilithic C:N ratio different from that of the galleries. This suggests that any effects revealed are due to resource quantity rather than quality. Thus, compensatory feeding, a strategy used by herbivores to overcome low resource quality (Price *et al.*, 1980; Cruz-Rivera & Hay, 2000), should not have affected the results.

Importantly, in line with the initial hypothesis, recycled nitrogen from grazers contributed a greater proportion of N per unit time to the gallery biofilm in the low nutrient treatments than in high nutrient treatments, at both densities (Fig 5.8). There is also some indication that the minimum amount of larval N and C assimilated from the gallery increased with a decrease in productivity (Fig. 5.8). However, some caution is necessary when assessing such patterns because the mixing model data is difficult to interpret due to the low discrimination in $\delta^{13}C$ values between the galleries and epilithon and the variability within food sources. Despite this, both these lines of evidence support the original hypothesis that gardening is more important to the larvae under low nutrient levels than under high nutrient concentrations. Furthermore, these experiments once again indicate the fact that gardening is a common facet of this grazer's lifestyle.

The differential importance of gardening across the nutrient gradient was however, not reflected in the amount of food available in the gallery as compared to the epilithon. The

difference in chlorophyll *a* concentrations between the gallery and the ungrazed epilithon was no higher in the low nutrient level than the high nutrient level. Furthermore, the amount of gallery organic matter was twice as high in the high nutrient treatment compared to the low nutrient treatment. However, it is possible that any impacts of enhanced gallery fertilisation may have been masked by more intense gallery grazing by the resident in the low nutrient treatment than in the high nutrient treatment, or due to differences in mean gallery age.

Mean gallery age is a combination of the length of the gallery and the rate at which the gallery is moved across the substratum. Rates of gallery movement in the first experiment were primarily affected by the density at which larvae were living. At low densities, galleries were moved at a faster rate across the tile surface than in the higher density treatment. Previous studies of *Tinodes* species have suggested that gallery length and shape (number of curves) is constrained by intra-specific interactions (Hasselrot, 1993a; Alecke *et al.*, 2005). Such intra-specific interactions can result in fights, with death or gallery (and territory) loss being the costly outcome. It is therefore plausible that gallery forward movement in the high density treatments was dictated by the movement of con-specifics. Alternatively, it is possible that movement was restricted by resource availability. This seems less likely as growth was not resource limited in the high nutrient treatment, yet galleries still moved slower when larval density was high.

Gallery movement rates in this study were similar to those of galleries *in situ* at Lake Erken (2.56 to 2.91mm per day at a larval density of 587 individuals per m^2, calculated using my methodology from Figure 2 in Hasselrot, 1993b). This suggests that the experimental conditions were not causing unnatural behaviour. On the other hand, in experiment 1 larval activity was much higher (they protruded from their galleries on 30% of all occasions on which they were observed) than reported by Hasselrot (1993a). He suggested that larvae spend a median of 99% of their time inside their galleries, although a large proportion of larvae observed (~70%) did protrude from their galleries (Hasselrot, 1993a). However, his results were based on 30 minute video observations of *Tinodes* galleries on rocks transferred from the field to the laboratory. Thus, as *Tinodes* larvae (personal observation) are very sensitive to disturbance (e.g. shadowing during the set up of video cameras) Hasselrot's (1993a) observations were probably an artefact of his experimental procedures. In my study, only video footage taken 30 minutes after

switching on the camera was analysed, and activity during 60 minutes was observed. Furthermore, field based observations of *T. waeneri*, also at Lake Erken in Sweden, have revealed that larvae do frequently protrude from their galleries (Cyvaite & Dolinskaite, 2003).

It is important to note that gallery movement may not provide the full picture in terms of gallery consumption as gallery disassembly during movement is not the only point at which larval feeding can occur. Larvae have also been reported to i) bite holes into the gallery wall and consume any silk and associated biofilm before re-plugging the hole with fresh silk and ii) graze algal cells off the inside of the gallery walls (Hasselrot, 1993a). Behaviour very similar to that in ii) has previously been interpreted as larvae strengthening gallery walls through silk addition (Jones, 1967), and is thus not necessarily related to feeding. Furthermore, these feeding behaviours have been observed for larvae whose galleries were transplanted onto clean microscope slides (Hasselrot, 1993a), and therefore lacked an alternative food supply. They may not be characteristic of larval feeding in more natural situations. The rate of gallery movement also provides information on epilithon consumption, as the forward motion of galleries allows larvae to access new unexploited feeding areas. Thus, in my first experiment, larvae were able to utilise a greater area of epilithon over the course of the experiment in the low density treatments as compared to the high density treatments. However, in the second experiment, run at a slightly lower density, galleries were not moved significantly faster across the epilithon, despite the epilithon being removed from directly in front of the gallery (the main epilithon feeding area of larvae). Furthermore, final mean larval biomass was the same in this treatment as in the control. This suggests that the amount of epilithon outside the gallery did not influence larval behaviour and that galleries were likely to have been the main food source.

The slower rate of gallery movement, and associated reduction in access to epilithon in the higher density treatments, could have been counter-balanced by the fact that larvae maintained longer galleries (at least under the two higher resource levels). Galleries therefore had the potential to contribute a larger proportion of resources to the larval diet (as there is more gallery available, and less epilithon is available due to the slower gallery movement rate) under high density treatments (there is also some indication of this in the mixing model results; Fig. 5.9). Thus, gardening may also have been more important under high larval densities where resource availability (epilithon) is reduced.

Contrary to expectation, galleries were shortest at the lowest nutrient level, being only about twice the length of a fifth instar larva. The reason for this remains unclear, as it is unlikely that gallery length was constrained by the cost of silk production because larvae at the lowest nutrient level built the most silk into their galleries. Furthermore, building material, i.e. sand, was not limiting at any point during the experiments.

Although there were differences in the rate of gallery movement, no resource limitation was apparent in the highest nutrient level treatment, even at high larval density (there was no difference in dry mass gain between the two density treatments). Similarly, in the medium nutrient concentration, where galleries were also longer under high density, differences in weight gain between the two densities were not significant. In contrast, when nutrient levels were low, density had an appreciable impact on larval weight gain, and indeed survival. This was probably due in part to the fact that galleries were not lengthened in response to density. Similar impacts of resource limitation on larval growth have been reported for the psychomyiid *Psychomyia flavida* Hagen (Hart & Robinson, 1990). However, even in the lowest nutrient level, *T. waeneri* larvae still doubled in weight during the experimental period and thus, did not appear to be food limited. Galleries may have been important in maintaining growth rates, but testing this is difficult as it is not possible to isolate larvae from their galleries for extended periods.

The short time frame over which fertilisation of the biofilm is able to occur, given the high rates of gallery movement, has implications for the food supply that the gallery can provide. Biofilm biomass accrual generally follows a sigmoid curve (Stevenson, 1996), with the rate of biomass increase levelling off over time, unless further disturbances occur (Stevenson, 1996). Thus, there should be an optimum time point at which to harvest the gallery. However, the time taken to reach this optimum level will depend on the resource of interest (algae, microbes or EPS) and the nutrient status of the system (the gallery in this case); under lower nutrient levels periphyton builds up more slowly than when nutrient concentrations are higher (King *et al.*, 2006). The extent to which biofilm communities are already present on sand grains used to construct the galleries would be important in determining gallery biofilm biomass. This will be dependent on whether sand grains used are ones that have been grazed by the larva during demolition of the gallery (lower quantities of biofilm, although larval removal efficiency may be low) or new sand grains that are collected from the epilithon (higher initial quantities of biofilm; see Appendix 2). Although it is probable that both types of sand grain were

incorporated into the gallery, the relative proportions of each are unknown. Also, observations during the experiment suggested that larvae were including epilithic material as well as sediment into the gallery that they were constructing. This is expected to be beneficial to the larvae, as the gallery would serve as a temporary food store. During its period of residence in the gallery wall, the included epilithic material would be fertilised and, therefore would become a better food source to the larvae at the point of consumption. A less likely explanation is that the inclusion of epilithon into the gallery structure may provide increased camouflage of galleries against their background.

Chapter 6 – General Discussion

6.1 Does *Tinodes waeneri* garden through fertilisation of its gallery?

Tinodes waeneri clearly qualifies as an ecological 'gardener' because it fertilises and feeds from its own gallery (Table 6.1). Using a combination of carbon and nitrogen stable isotopes it has been possible for the first time to demonstrate, both in the field (Chapter 2) and in the laboratory (Chapter 5), that galleries are an important dietary source for the larvae and that the gallery community is utilising nitrogen excreted by the larvae. Further, this study has shown that galleries contained a higher chlorophyll concentration per unit area than the epilithon and that larvae manipulated their food source, to some extent, as the gallery assemblage was also more diatom-dominated. Thus, galleries represent a better food source for the larvae (Becker, 1990) and this gardening interaction can be viewed as a mutualism (Hay *et al.*, 2004). Benefits from feeding on the gallery are however difficult to quantify precisely as it is not possible to separate larvae from their galleries. Thus, I can confirm that fertilising and feeding from its own gallery is an integral part of the 'economy' of *Tinodes waeneri* and this goes some way to explaining its dominance in the stony lake littoral.

Nevertheless, epilithon does also account for a large portion of the food grazed and subsequently assimilated by the larvae. For example, the mixing models (Chapter 2) indicated that galleries contributed 50% or more of the assimilated material in 37% of the Lake District samples, and 80% or more of assimilated material in about 11% of samples (using the 50% probability intervals estimated by SIAR, Figure 2.6), with the remainder comprising epilithon. In addition, in the experimental study (Chapter 5) there were obvious grazed zones surrounding galleries, and these were also occasionally observed in the field, most notably at Windermere, where galleries contributed least to larval carbon and nitrogen.

Even so, larvae do fertilise their galleries and also actively manipulate gallery algal communities. In the experiment (Chapter 5), larvae built epilithon scraped from the tile surface into the structure of their galleries, and this epilithon presumably remained in place until the gallery section was dismantled, 5 to 10 days later. During this time, the 'ready made' algal community will have benefited from the higher nutrient availability within the gallery lumen, and this delay in consumption probably would have increased its nutritional value to the larvae. Parallels can be drawn to detrital systems, where the

build up of microbial communities (both internal and invading) during conditioning increases faecal quality (Wotton & Malmqvist, 2001). In a well studied desert stream, collector-deposit feeders frequently re-ingested their faeces at 2-3 day intervals and thus benefited from the increased quality associated with the conditioning of the faeces (Fisher & Gray, 1983).

Ingesting epilithon may also be a mechanism by which 'new' nutrients can be harvested, thus replacing those that may leak from the gallery system. Feeding on epilithon and excreting epilithon-derived nutrients into the gallery lumen introduces such 'new' nutrients into the gallery. These nutrients will be taken up by the gallery biofilm and thus conserved within the larval territory. The gallery system into which the nutrients are being released is not totally sealed, as galleries are open ended and larvae promote water currents through the gallery interior. However, studies on *T. rostocki,* which has similar galleries and larval behaviour, have reported differences in pH and oxygen concentrations between the gallery interior and the outside water (Stief & Becker, 2005). This suggests that the gallery is sufficiently separate from the water column for a high proportion of excreted nutrients to potentially be retained within the gallery. Thus, larvae are conserving nutrients within their territories, whilst also making the most of any epilithon that is available to them.

Taken together, these results indicate that larval activities, including 1) the provision of increased surface area for algal colonization through building of the gallery, 2) incorporation of epilithon into gallery walls during gallery construction, and 3) fertilisation of the gallery biofilm, will all provide this sedentary species with a supplementary food supply throughout its development. Building a retreat is the only way that a non-case bearing sedentary larva can exploit the resource-rich upper surfaces of stones in environments subject to strong hydrological disturbances such as those experienced on shallow and wave-washed shores of lakes and streams. Whilst the gallery primarily provides shelter and protection, it also crucially provides food and limiting nutrients. Thus gardening is an integral part of the suite of ecological characteristics that define the 'niche' of this species.

Table 6.1 Summary of the main hypotheses tested in each chapter and the results obtained. (BA = Bassenthwaite, CO = Coniston, ES = Esthwaite, RY = Rydal Water, UL = Ullswater and WI = Windermere)

	Hypotheses tested	**Methods**	**Does *T. waeneri* garden?**	**Are galleries a better food source?**	**How does gardening vary across a nutrient gradient?**
Chapter 2	- *T. waeneri* fertilises the gallery biofilm - Gallery is a significant dietary C and N source for the larvae - Galleries are a more important food source in low productivity systems -Recycling should have more of an impact in low productivity systems	Field survey using natural abundance stable isotope analysis to assess gallery versus epilithon assimilation and use of recycled nutrients by the gallery community across six lakes differing in productivity	- Galleries were consistently ^{15}N-depleted relative to the epilithon (indicating uptake of N from larval excretions) in ES and RY, but differences were much smaller in UL and CO - Galleries formed an important C and N source for the larvae		- Less ^{15}N-depletion of galleries in lower productivity lakes (WI, CO & UL), peaking in October. Greater ^{15}N-depletion in more productive lakes (ES, RY & BA), peaking in April. - Differences in $\delta^{15}N$ values between epilithon and gallery biofilm were greatest at ES and RY. - Galleries contributed a greater proportion of assimilated C and N to the larvae in the more productive lakes (BA, ES and RY) than in WI, UL and CO. Contributions were greatest in the summer in the three higher productivity lakes and in April in all other lakes.
Chapter 3	- Galleries contain a greater quantity and/or better quality of resources - Gallery assimilation patterns are related to resource availability	Comparison of gallery and epilithon in terms of food quality and quantity across six lakes (August to January). Investigated relationship between resource availability and larval gallery assimilation.	- Proportion of gallery versus epilithon assimilated by the larvae greater where gallery is a much better resource than the epilithon (August sample)	- Chlorophyll *a* concentrations and AFDM always significantly higher in the gallery than in the epilithon - 2 food sources not different in C:N ratio	- Food quality and quantity not related to water column nutrient concentrations or to lake productivity. - Epilithon was not nutrient limited in any lake

Chapter 4	- Gallery biofilms contain an algal assemblage distinct from the surrounding epilithon and the gallery assemblage remains constant over the lake productivity gradient - If no consortium exists, then larval fertilisation of galleries will create the greatest divergence in algal communities between galleries and epilithon at low ambient nutrient concentrations	Comparison of pigments and diatom communities from galleries and the epilithon for October samples from five Lake District study sites (BA, CO, ES, RY and WI)	- Gallery contained diatom assemblages representative of slightly higher nutrient concentrations	-*T. waeneri* galleries had higher levels of diatom pigments than epilithon -Gallery diatom community different from neighbouring epilithon, especially in WI and CO - No discrete consortium of diatom species associated with galleries	- Increased divergence in diatom communities between the epilithon and galleries with decreasing lake productivity
Chapter 5	- Nutrient recycling and gallery assimilation more common at low nutrients than at high nutrient levels - This will be reflected by gallery movement and grazing behaviour - Gardening should result in: i) Greater uptake of excreted larval N by gallery biofilm and greater larval assimilation of galleries at low nutrients. ii) Higher proportion of chlorophyll and/or lower C:N in galleries relative to ungrazed epilithon, and longer larval galleries at low nutrient levels iii) Less time spent grazing epilithon at low nutrient levels iv) No difference in gallery movement rate in experiment 2	Laboratory experimental study with 3 nutrient levels and two density treatments. Experiment also included un-grazed treatments. Measured stable isotope ratios of all system components, food quantity and quality, larval grazing behaviour and gallery movement. Second experiment removed epilithon from directly in front of the galleries	- Galleries were ^{15}N-depleted relative to the epilithon in all treatments containing larvae -Both galleries and the epilithon were assimilated, with circa 50% of dietary contribution derived from the gallery - Gallery movement rate unaffected by epilithon removal	- Gallery chlorophyll *a* concentrations were between 2.2 and 4.7 times higher than in the matching epilithon samples. Also significantly higher concentrations than in ungrazed epilithon - No significant difference in gallery lengths between treatments	- Gallery AFDM highest at high nutrients - No difference in time spent protruding from the gallery with nutrient level - Rate of gallery movement linked to density (galleries in place for longer in high density treatments) - ^{15}N-depletion of gallery relative to the epilithon related to nutrient levels (greatest at low nutrients) - Trend towards greater gallery contribution to larval assimilation of gallery N and C with decreasing nutrients - Larval dry mass gain greatest at the high nutrient level and the low density x medium nutrient treatment. Larvae gained less weight in the high density treatments, with this trend becoming more pronounced with reducing nutrient concentrations.

It is likely that gardening of the type described for *T. waeneri* holds true for other psychomyiid caddisflies, and especially *Tinodes* species. They have similar lifestyles and can also reach high abundances (e.g. 2010 *Psychomyia flavida* larvae per m^2; Hart & Robinson, 1990: 3100±548 *T. rostocki* larvae per m^2; Stief & Becker, 2005: and 2000 *T. unicolor* (Pictet) individuals per m^2; Alecke *et al.*, 2005). Additionally, I have measured gallery ^{15}N-depletion relative to the epilithon (2.71±0.34‰, 13 galleries analysed), suggesting uptake of excreted N by the gallery biofilm, in a small scale stable isotope study of a *T. assimilis* McLachlan population in one North Somerset stream. *Lype* species that live on wood, and feed on the biofilm associated with wood (Spänhoff *et al.*, 2003), could also potentially benefit from gardening. Larvae build long galleries from wood particles (Spänhoff *et al.*, 2003) and fertilisation of the high surface area of these fragments could lead to a much greater and higher quality biofilm resource. This remains to be tested.

The stable isotope approach used in this study was highly successful for examining gardening and can also be applied to other systems (e.g. Appendix 3 for research on *Philopotamus montanus* (Donovan)). The stable isotope technique is useful in distinguishing dietary sources, as gardeners are likely to use similar food sources (e.g. fertilised and unfertilised epilithon) that are not easy to separate using other methods, such as gut contents and fatty acid analyses (Desvilettes *et al.*, 1997), or molecular techniques (Symondson, 2002). Furthermore, the uptake of nitrogen excretions and/or respired carbon by the 'garden' can also be confirmed concurrently. Stable isotope techniques have the additional benefit that they can be used in situations where it is difficult to observe the interaction and/or to separate the grazer from its garden experimentally. However, as with all stable isotope analyses, additional complementary ecological and behavioural data are also needed for interpretation of results (Finlay & Kendall, 2007) and other factors that may differentially affect the stable isotope signatures of the alternative food resources need be understood (for example current velocity, periphyton thickness and light intensity on periphyton $\delta^{13}C$ values in streams; Boston & Hill, 1991; MacLeod & Barton, 1998; Finlay *et al.*, 1999). Also, adequate mixing models that allow for variation in fractionation and stable isotope values of the end members and the mixture (e.g. SIAR; Jackson *et al.*, 2009) need to be used.

6.2 Is the importance of gardening linked to resource availability?

The mutualistic interaction between the larvae and the biofilm on their galleries (gardening) was studied over a range of nutrient concentrations reflecting an environmental stress gradient. The main hypothesis, in line with theory on positive interactions (Bruno & Bertness, 2001), was that gardening should play more of a role at low nutrient concentrations than at high nutrient concentrations. A summary of the main findings of this research are included in Table 6.1 and Figure 6.1 illustrates the original hypotheses (see Chapter 1) and the results from the lake survey (Chapters 2 to 4) and experimental study (Chapter 5) in a conceptual manner. These results are discussed in more detail in the following sections.

6.2.1 Importance of larval excretions to gallery biofilm

Uptake of larval nutrient excretions by the gallery biofilm community was expected to be negatively related to lake productivity, so that larval excretions supply a larger proportion of nutrient demand where external nutrient concentrations are low (Figure 6.1). This variable was measured as the amount of ^{15}N-depletion of the gallery relative to the adjacent epilithon, and the expected relationship occurred within the experimental treatments, but only after gallery age (i.e. the amount of time available for the biofilm to take up larval excretions) had been taken into account. The opposite relationship was true in the Lake District, although equivalent information on gallery age and rate of movement was not available. However it is interesting to note that a stable isotope investigation carried out during a single sample period (November 2005) at Crosemere, a more eutrophic lake in Shropshire (mean TP = 214 mg m^{-3}; Moss *et al.*, 1994, Appendix 4), failed to find any evidence of nitrogen recycling within the gallery community, suggesting that larval excretions may indeed, become less important when background nutrient levels are high.

In this thesis, nitrogen was the nutrient of interest for several reasons: 1) stable isotopes could be used to investigate nitrogen flow through the system, 2) nitrogen is a key component of larval silk, and most importantly 3) previous research had suggested that nitrogen cycling within the gallery community might be a significant process (e.g. Hasselrot, 1993a; Kahlert & Baunsgaard, 1999). However, while nitrogen is a useful indicator for confirming the presence of nutrient recycling in general within the gallery community, nitrogen will not always be the main driver of the relationship between productivity and periphyton nutrient status. Within the experimental study (Chapter 5),

water nutrient levels were amended in such a way as to ensure that nitrogen, rather than phosphorus, was the limiting nutrient. However, in the field survey, nitrogen may not have been the nutrient limiting periphyton production. In the Lake District, phosphorus can often be the limiting nutrient for both the phytoplankton (e.g. Barbosa, 1989; Talling, 1999) and periphyton (King, 1999). Thus, the uptake of non-limiting nitrogen from larval excretions by the gallery biofilm may not necessarily be expected to be related to lake productivity.

6.2.2 Resource availability in the gallery relative to the epilithon

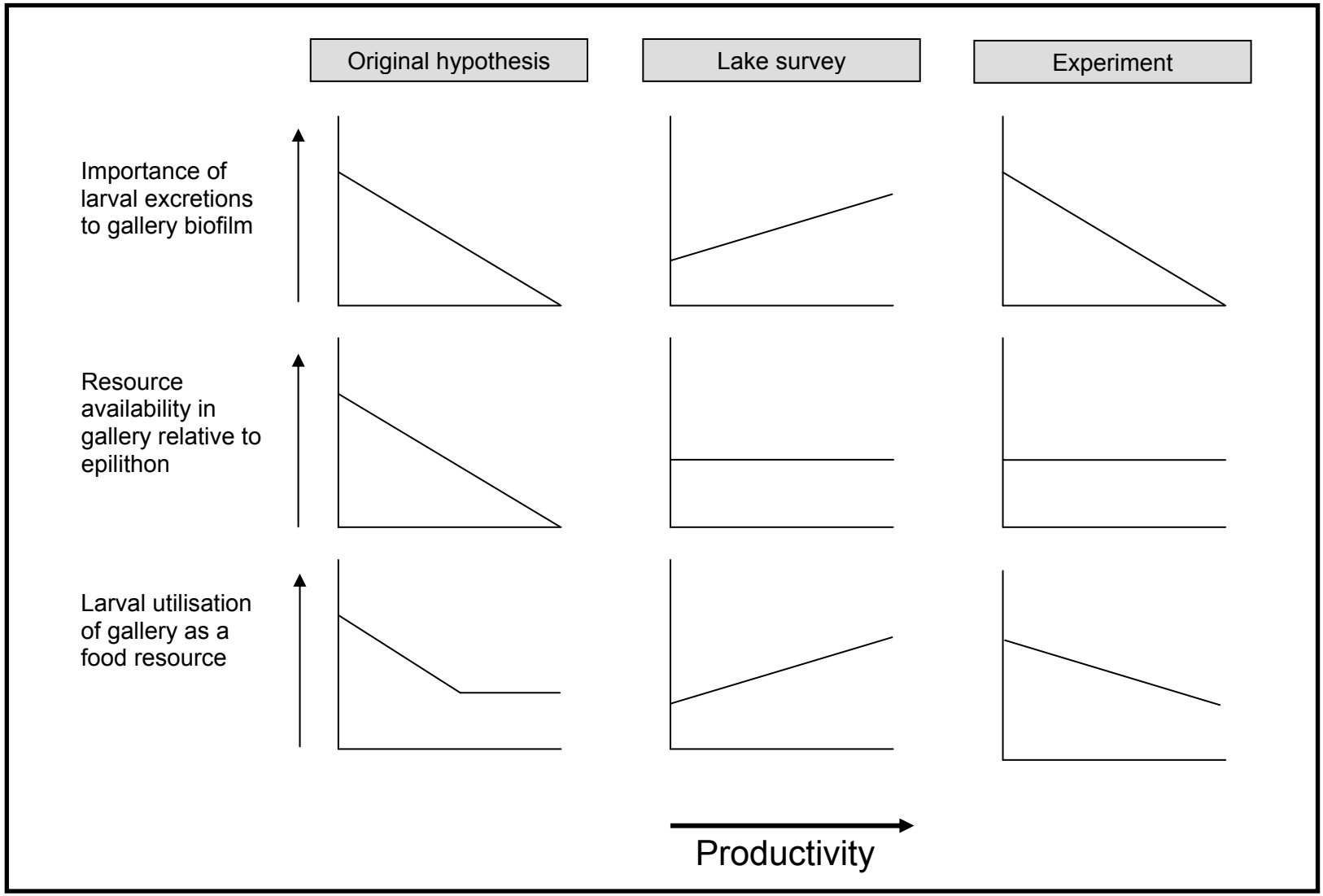
Neither the field survey nor the experiment showed an increased divergence between epilithon and gallery resource availability (chlorophyll *a* concentrations and C:N ratios) in lower productivity than in higher productivity system (Fig. 6.1). Nevertheless, as already mentioned, galleries did contain more chlorophyll than the epilithon. One reason for this discrepancy may the fact that the biomass of epilithic periphyton in lakes does not always reflect patterns in concentration in the water (e.g. Cattaneo, 1987; Hillebrand & Kahlert, 2001; DeNicola *et al.*, 2006). Furthermore, a study of Lake District epilithic communities also failed to find a significant correlation between TP and various measures of epilithon biomass (King, 1999). In contrast, where increased nutrients are supplied from below (as during deployment of nutrient enriched substrata) responses are more pronounced (e.g. Pringle, 1990). In essence, *T. waeneri* galleries are analogous to nutrient diffusing substrata.

In terms of food quality (measured here as the C:N ratio), there was little evidence that galleries were a better food resource than the epilithon and there was no pattern across the nutrient gradients studied. Although it is possible, that additional nutrients from larval excretions simply went into increased biomass production rather than being taken up by existing cells in the biofilm, this seems unlikely as C:N ratios were consistently low (less than 11) in all samples analysed. It is possible that, due to low C:N ratios of the background epilithon (e.g. due to low fraction of detritus in the biofilm, and/ or low light; Hillebrand *et al.*, 2004; Liess & Hillebrand, 2004), larval excretions failed to have any additional measurable impact. Alternatively, in this study, the consistently low C:N ratios may have been the result of carbon limitation within the biofilm. This may have resulted from high photosynthetic rates coupled with slow diffusion of carbon dioxide through boundary layers and/or from the presence of a thick periphyton mat (Frost *et al.*, 2002a).

Carbon limitation of algal growth has been reported in epilithic algae in softwater lakes (e.g. Fairchild *et al.*, 1989; Fairchild & Sherman, 1992) and, more importantly, has also been inferred from carbon stable isotope values in alkaline fast flowing streams with moderate dissolved inorganic carbon (DIC) concentrations (Hill & Middleton, 2006). ^{13}C-enrichment due to carbon limitation is the result of a switch in inorganic carbon source from carbon dioxide to bicarbonate and discrimination against the heavier ^{13}C isotope upon uptake of bicarbonate. This discrimination will create a ^{13}C-enriched bicarbonate pool (as bicarbonate is not replaced sufficiently rapidly due to constraints on diffusion) that algae will use (Hill & Middleton, 2006). Although $\delta^{13}C$ values of the epilithon will be largely dependent on the $\delta^{13}C$ values of the DIC (which was not measured here), some of the $\delta^{13}C$ values obtained in this study, notably Coniston in August, are towards the higher end of the range measured for periphyton (-47 to -8‰; Finlay & Kendall, 2007). This suggests that carbon limitation may have been occurring in the epilithon. Furthermore, galleries were consistently ^{13}C-depleted relative to the epilithon in the Lake District field study and this could be the result of uptake of ^{13}C-depleted carbon dioxide (Finlay & Kendall, 2007) respired by the larvae inhabiting the galleries. Whilst carbon does not limit potential yield of epilithic biofilm, a shortage may have a detrimental impact on algal growth rates (Borchardt, 1996). Thus, boundary layers, the complex nature of periphyton mats and internal nutrient recycling within the mats may all moderate the response of the biofilm to external nutrient concentrations, and this may have contributed to the discrepancy between the simple prediction made in this study and actual responses of the periphyton across the productivity gradient.

This prediction (increased divergence between epilithon and gallery resource availability) was however met when diatom communities were assessed, suggesting that they may be a more sensitive indicator of nutrients (at least over the nutrient range studied here), than algal biomass as a whole. Diatom community composition and relative abundance is known to be sensitive to, and respond rapidly to, changes in water nutrient conditions (e.g. within a week in an oligotrophic river, Lavoie *et al.*, 2008). Their high diversity coupled to different species tolerances and their short life-cycles allows them to respond to environmental change rapidly, and makes them particularly good indicator species (King *et al.*, 2005).

Figure 6.1 A comparison of the original predictions for gardening over a productivity gradient with patterns obtained under natural and experimental conditions.



6.2.3 Larval utilisation of the gallery as a food resource

Finally, it was expected that larval dependence on galleries as a food resource would be highest in the low productivity lakes and decline across a productivity gradient (Fig. 6.1). It was expected that even in high productivity systems larvae would continue to assimilate a small amount of gallery material, consumed during the deconstruction of galleries, as galleries need to be moved to enable new epilithic feeding patches to be reached (Fig. 6.1). This prediction was tested in both the field survey and the experimental study using the outcomes of mixing models, which provide an estimate of the proportion of gallery-derived nitrogen and carbon assimilated by the larvae. Data from the laboratory experiment appeared to support the hypothesis (although estimates of larval assimilation of galleries were accompanied with high levels of uncertainty). In contrast, data from the field experiment showed the opposite trend (Fig. 6.1), with the greatest assimilation of gallery-derived carbon and nitrogen by the larvae occurring in Esthwaite, the most eutrophic lake studied. It is thus likely that other differences between lakes (e.g. biotic factors, including inter- and intra-specific competition for space and food and predation pressure and abiotic factors such as wave-washing) overshadowed the effects of productivity on resource availability in the lake survey.

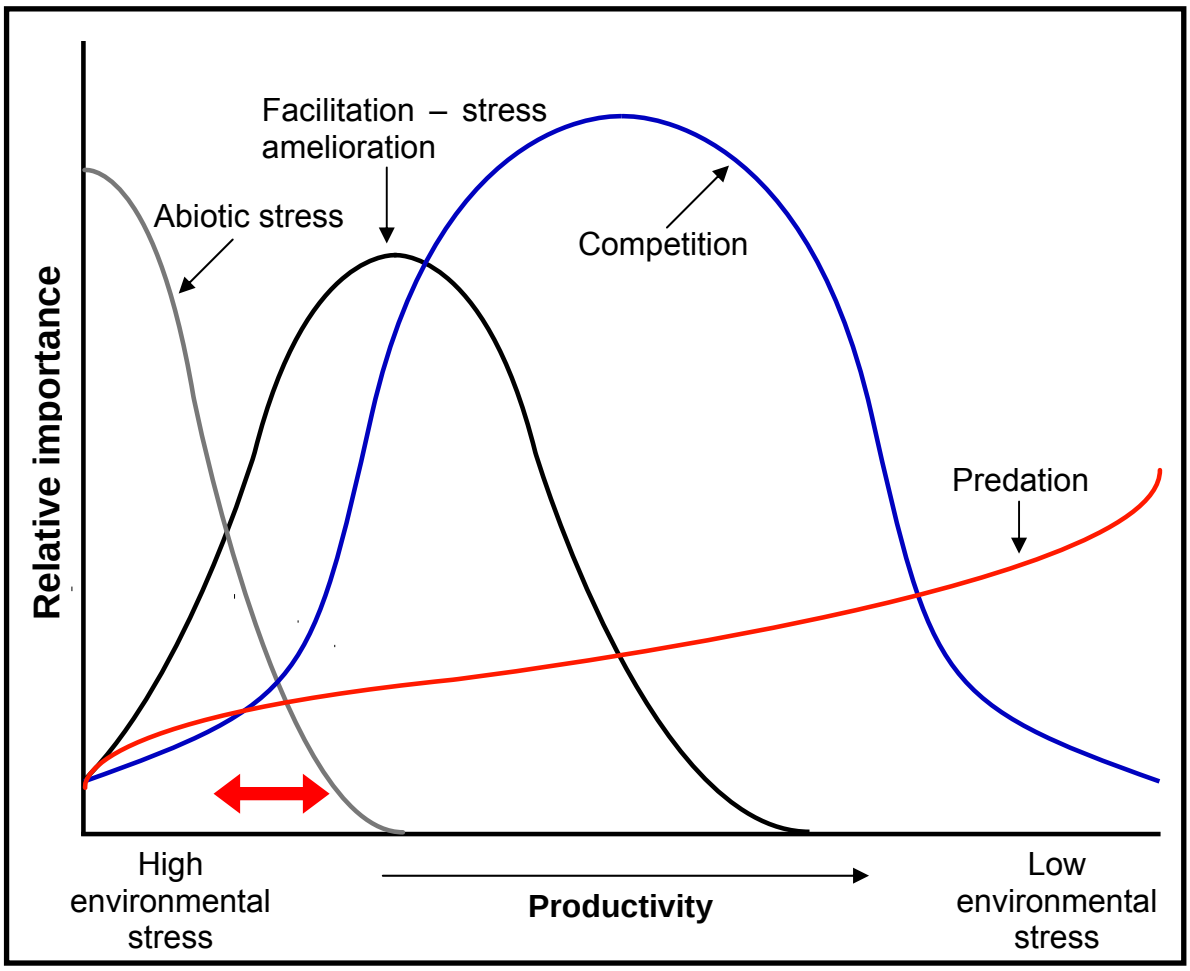
Although algal class differences between galleries and the epilithon were consistent among lakes in the field survey, species composition within algal classes was likely to have been more variable (Chapter 4). In the more productive lakes, filamentous green algae were a feature of the epilithon, which were probably less palatable than the diatom-dominated communities on the galleries. Thus, in these lakes, choice of feeding location (gallery *versus* epilithon) may have been governed by algal community structure, a factor that may have been of less importance in the lower productivity lakes. Predation pressure is more difficult to measure, but may also have been more severe in the more productive lakes.

Interestingly, in the experiment (Chapter 5), gallery movement rate was strongly influenced by larval density. Furthermore, there was a slight suggestion that density also affected the gallery-derived dietary contribution to the larvae (Chapter 5). Biotic interactions have been recognised as playing a major role in the structuring of stream communities (e.g. McAuliffe, 1984a; Feminella & Resh, 1990) and in the stony littoral of Crosemere (Harrison, 1996; Harrison & Hildrew, 2001). Strong intra-specific interactions have also previously been reported in several psychomyiid species. These

include *Psychomyia flavida,* which competes for silken tunnels (Hart, 1983), *Tinodes rostocki,* where competition for space forced larger larvae to vacate rocks and relocate (Becker, 1993), and both *T. waeneri* and *T. unicolor* change gallery direction in response to conspecifics (Hasselrot, 1993a; Alecke *et al.*, 2005). In my experiment, larval density influenced gallery movement rate, which was slower when larval density was high. At the two lower nutrient levels, the larvae living at the higher density also had lower growth rates. These experimental treatments contained natural densities of *Tinodes*, and may be applicable to lake littorals. For example, in the lake survey (Chapter 3), suitable habitat space was most limiting in the lakes with the highest levels of gallery assimilation.

An important alternative explanation for the results of the field survey may be that the gardening mutualism was not as effective in the low productivity lakes. At high environmental stress levels, facilitation is predicted to increase population densities (Bruno & Bertness, 2001; Fig. 6.2), but as environmental stresses increase, facilitation will function less well and there will come a point on the environmental stress gradient where it can no longer compensate for abiotic stresses (Bruno & Bertness, 2001, Fig. 6.2). It is possible that the portion of the environmental stress gradient (productivity) investigated here, fell within this region (Fig. 6.2) Thus, across the gradient used, I may have been seeing an increase in the importance of facilitative interactions such as gardening with greater productivity, as facilitation was increasingly able to compensate for abiotic stress. This view is supported by the fact that the mean TP concentrations in the lakes studied (9.8 to 31 mg m^{-3}; Maberly *et al.*, 2006) was at the very low end of the range of TP concentrations over which *T. waeneri* was recorded to occur in a survey of Danish lakes (14 to 450 mg m^{-3}; Brodersen *et al.*, 1998).

Figure 6.2 A conceptual model illustrating the relative importance of abiotic stress, competition, predation and facilitation through stress amelioration, across an environmental stress gradient (modified from Bruno & Bertness, 2001). The suggested potential location of the lakes in the survey on the environmental stress gradient is indicated by the red arrow.



6.3 Wider implications of this research and impacts of *Tinodes waeneri* on the lake littoral

Dominant species are usually superior competitors (Lancaster *et al.*, 2008). Previous studies have suggested several mechanisms by which *Tinodes waeneri* is able to live at high densities and maintain dominance. Firstly, the gallery provides them with good protection from predation (Jones, 1967). Secondly, their lifecycle dictates that larvae remain present within the epilithon for much of the year, allowing them to monopolise space. Moreover they can be a superior competitor for epilithic resources, as well as space (Harrison & Hildrew, 2001), especially when epilithon is limiting. In a productive

system (Crosemere), it was suggested that competition between the larvae and other sedentary invertebrates was indirect and occurred through the control of *Cladophora* by *T. waeneri* (Harrison & Hildrew, 2001).

Here, I have demonstrated another mechanism by which this species can maintain high population densities and dominance: through the fertilisation of biofilm communities on their galleries. In contrast to the previous mechanism, where they control algae at high nutrient levels, this strategy could allow them to maintain dominance in low productivity systems, and enable them to extend their 'niche' into lower productivity systems that they would otherwise be unable to inhabit. As already alluded to, the systems studied here, were at the extreme low end of the productivity gradient that this species has been reported to inhabit in Denmark (Brodersen *et al.*, 1998). Despite this, high larval densities were recorded (e.g. 1348±89 individuals per m^2 in Ullswater in October, Chapter 3), although local smaller scale variability in nutrient conditions may also have been important.

Tinodes waeneri larvae contribute to the patchiness within the stony littoral of lakes at scales ranging from tens of metres (Harrison, 1996) to less than a centimetre (this study). Patchiness at the larger scale was manifested as differences in *Cladophora* cover on rocks, between shaded (under or near trees) and open sites (away from trees). Epilithic patches devoid of *Cladophora* occurred in the shade at Crosemere, whilst *Cladophora* dominated the epilithon in open areas (Harrison, 1996; Harrison & Hildrew, 2001). Patchiness at this scale appeared to be due to a combination of factors. *T. waeneri* larvae hatch in the shade, as this is the preferred egg laying site for adult females (Harrison & Hildrew, 1998a), and therefore, larvae are concentrated in this habitat, at a time when *Cladophora* propagules are vulnerable to grazing. Although larvae disperse out of shaded areas, the delay in doing so meant that *Cladophora* had grown to a size where it was no longer vulnerable to grazing by the time the larvae had colonised the open areas (Harrison, 1996; Harrison & Hildrew, 2001).

These researchers also uncovered patchiness on the among rock scale that was also linked to *T. waeneri* presence or absence. *T. waeneri* mainly disperse by crawling which makes colonisation of tall rocks more difficult (further to climb) than for flat rocks (Harrison & Hildrew, 2001). The result is that these two rock types harbour distinct

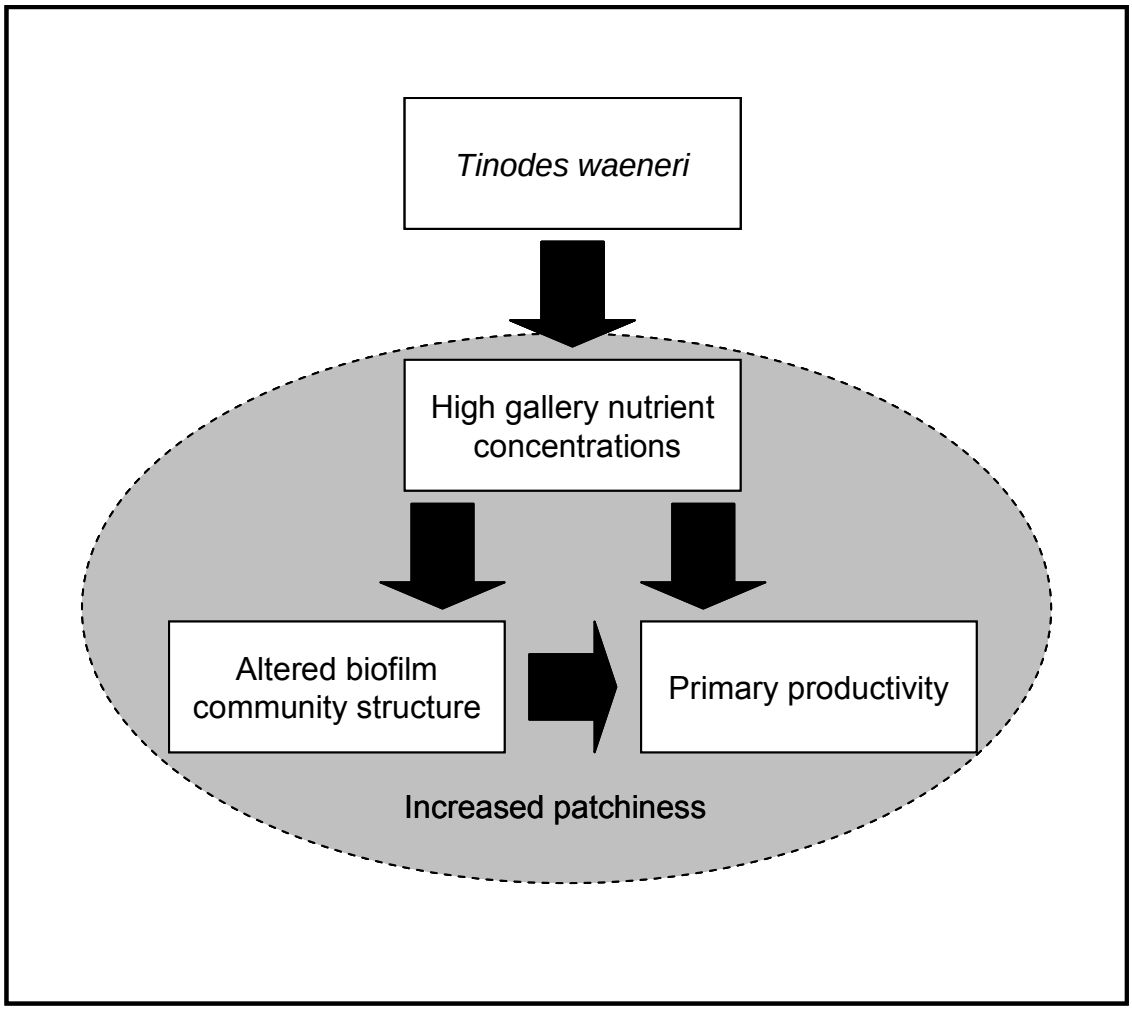
algal and invertebrate communities (Harrison & Hildrew, 2001), further adding to the patchiness of the stony littoral.

In this thesis, patchiness at an even finer scale has been revealed. In all lakes studied, the pigment analysis (Chapter 4) revealed that epilithic communities were dominated by green-algae whereas galleries were rich in diatoms. Galleries were also nutrient hotspots and made of a different substratum (sediment). Even finer scale patchiness may be occurring along the length of galleries as this represents a gradient in gallery age (Stief & Becker, 2005). Furthermore, the experimental study highlighted the fact that there were also differences in algal biomass between grazed areas within larval territories, and ungrazed areas out of larval reach.

A high diversity of resource patches may confer stability to the system and the processes that control this fine scale patchiness will be important in influencing ecosystem function (Pringle *et al.*, 1988). Algal community composition, productivity and nutrient retention and recycling all have the potential to be altered by this grazer (Fig 6.3).

Firstly, nutrient retention occurs because *T. waeneri* excretions are released into the confines of the gallery, and therefore have the potential to be taken up by the gallery biofilm. Consequently, these nutrients are not released back into the general lake water and 'lost' to the periphyton, as would be the case for more mobile grazers. Thus, these nutrients are retained within the gallery biofilm, and can then be utilised by grazers, or internally recycled within the biofilm.

Figure 6.3 The links between *Tinodes waeneri* larvae and key ecosystem and community traits.



Additionally, nutrients excreted by a grazer such as *T. waeneri* will locally fertilise the biofilm community associated with its gallery, potentially affecting algal community structure. This may occur because there are greater quantities of nutrients available, or because the ratio at which nutrients are re-supplied (dependent on the stoichiometric imbalance between the grazer and its food supply; Elser & Urabe, 1999) favours some species over others. In addition, larval grazing activities and substratum type (sediment versus rock) may also impact on algal community structure (Steinman, 1996; Wetzel, 1996). In my study, there was evidence that larvae were affecting algal community structure (Chapter 4). Gallery communities contained much higher concentrations of diatom-associated pigments and were consistently distinct from epilithic communities,

where pigment profiles suggested dominance by green algae. There were also differences in the diatom community.

Larval excretions may also influence productivity, a key ecosystem process, both directly and indirectly (e.g. through changes in biofilm community composition). Lake littorals can account for a substantial portion of whole lake primary productivity (e.g. up to 62% in a review by Wetzel, 1983b) and, thus, changes to littoral productivity due to the presence of *T. waeneri* could be significant to lakes as a whole.

Thus, *Tinodes waeneri* larvae have the potential to modify significantly the structure and processes occurring within the epilithon of stony littorals, both through their roles as ecosystem engineers and through fertilisation and consumption of galleries. They may be expected to have a particularly strong impact as they often occur at very high population densities (up to 11500 individuals per m^2; Dall *et al.*, 1984), and this may have a cumulative effect on ecosystem level properties (Pringle *et al.*, 1988). As larvae are small, they will have relatively high excretion rates per unit mass (Hall *et al.*, 2007). Moreover, organisms will have the most impact on nutrient dynamics when they are highly abundant and when they are excreting a nutrient that is limiting (Vanni *et al.*, 2006). In the case of *T. waeneri,* larval densities are at their highest during the summer when water column nutrients (N and P) are expected to be more limiting for algal growth, and thus impacts may be greatest at this time of year.

6.4 Future research directions

To gain an in-depth understanding of the impacts of gardening by *T. waeneri* on ecosystem processes, further research should examine the gardening interaction at both a much finer scale (measurement of nitrogen flow between the resident larva, the gallery and the epilithon) and a larger, within littoral scale (quantification of the impact of *T. waeneri* excretions on productivity and algal community structure).

Nitrogen flow and retention can be quantified at the scale of the gallery community using nitrogen stable isotope addition experiments (Fry, 2006). In a laboratory setting, it should be possible to label the three components (larva, gallery and epilithon) of the community individually and to measure the incorporation of the label into the two unlabelled components.

The impact of *Tinodes* on epilithic production throughout the growth period of the larvae has never been quantified. This is despite frequent speculation that *Tinodes* may have significant positive impacts on production (e.g. Hasselrot, 1993a; Kahlert & Baunsgaard, 1999; Stief & Becker, 2005). To investigate the impacts of *T. waeneri* larval fertilisation of galleries on total periphyton productivity and community structure at the much larger scale of whole lake littorals, comparisons between artificial substrata inhabited and not inhabited by *T. waeneri* larvae need to be made on a regular basis. Algal and microbial productivity would be measured (using ^{14}C incorporation; Ward, 2007, and [H^3] incorporation into proteins respectively; Bott, 2007) together with algal community composition, algal standing crops and larval densities.

The proportion of epilithic demand, for the limiting nutrient, met by *T. waeneri* excretions within the lake littoral could also be measured using a supply/demand approach (e.g. Vanni *et al.*, 2006). Larval nitrogen and phosphorus excretion rates would be measured at set intervals (e.g. using incubations such as those described in Devine & Vanni, 2002) and larval abundances and biomass would be used to scale up nutrient excretion rates to the littoral scale. These values could be compared with algal nutrient demand (based on autotrophic production rates and C:N and C:P ratios of the epilithon, to give a measurement of nutrient demand). From such data it would be possible to calculate the proportion of epilithic nutrient demand satisfied by *T. waeneri* across time.

6.5 Summary of main conclusions

- *Tinodes waeneri* larvae garden through fertilising their galleries as gallery biofilm takes up larval nitrogen excretions and the larvae feed on their galleries. Gardening is thus, a mutualistic interaction and may enable this species to maintain dominance when food is limiting.
- Larval activities result in higher gallery chlorophyll *a* concentrations and ash free dry mass as compared to the epilithon, but do not impact upon C:N ratios. Gallery community structure is also distinct from that of the epilithon, at algal class level, and there are some differences in diatom species assemblages.
- Gardening was apparently not more important in low productivity systems in the field, although under controlled laboratory experiments the opposite was true. Additional factors such as intra-specific competition, palatability and predation

pressure and, in particular, abiotic stress may have modified any patterns, at least over the nutrient gradient studied in the field.

- Gardening has the potential to impact on key ecosystem processes including nitrogen flow and productivity, especially as larvae are numerous, and may thus have far reaching impacts on littoral systems.
- The stable isotope approach used may also be useful to study gardening in other systems. Gardening is likely to be more widespread across sedentary species than previously acknowledged.

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Appendix 1 – Authority names for the diatom species referred to in Chapter 4

Species	Authority
Achnanthidium biasolettiana	(Grunow) L.Bukhtiyarova 1995
Achnanthidium minutissimum	(Kütz.) Czarnecki 1994
Amphora libyca	Ehrenb. 1840
Amphora pediculus	(Kütz.) Grunow in Schmid et al. 1875
Amphora veneta	Kütz. 1844
Asterionella formosa	Hassall 1855
Aulacoseira	G.H.K.Thwaites 1848
Brachysira vitrea	(Grunow) R.Ross in B.Hartley 1986
Cavinula pseudoscutiformis	(Hust.) D.G.Mann et A.J.Stickle in Round et al. 1990
Cocconeis pediculus	Ehrenb. 1838
Cocconeis placentula	Ehrenb.1838
Cyclotella	Kütz. ex. Bréb. 1838
Cymbella affinis	Kütz. 1844
Cymbella cesatii	(Rabh.) Grunow in A. Schmidt 1881
Cymbella cistula	(Ehrenb.) Kirchner 1878
Cymbella helvetica	Kütz. 1844
Cymbella microcephala	Grunow 1880
Denticula tenuis	Kütz. 1844
Diatoma tenue	Agardh 1812
Encyonema caespitosum	Kütz. 1849
Encyonema minutum	(Hilse in Rabenh.) D.G.Mann in Round et al. 1990
Encyonema silesiacum	(Bleisch in Rabenh.) D.G.Mann in Round et al. 1990
Eunotia curvata	(Kütz.) Lagerst. 1884
Fragilaria capucina	Desm. 1925
Fragilaria crotonensis	Kitton 1869
Gomphonema pumilum	(Grunow) Reichardt & Lange-Bert.(1991)
Gomphonema acuminatum	Ehrenb. 1836
Gomphonema angustum	Agardh 1831
Gomphonema clavatum	Ehrenb. 1832
Gomphonema minutum	(Ag.) Agardh 1831 1831
Gomphonema olivaceum	(Hornem.) Bréb. 1838
Gomphonema parvulum	(Kütz.) Kütz. 1849
Gomphonema truncatum	Ehrenb. 1832
Karayevia clevei	(Grunow in Cleve et Grunow) Round et L.Bukhtiyarova 1996
Karayevia laterostrata	(Hust.) Kingston 2000
Navicula atomus	(Kütz.) Grunow 1860
Navicula cryptocephala	Kütz. 1844

Species	Authority
Navicula cryptotenella	Lange-Bert. 1985
Navicula ignota	Krasske 1932 em. J.W.G.Lund 1946
Navicula menisculus	Schumann 1866
Navicula minima	Grunow in Van Heurck 1880
Navicula rhynchocephala	Kütz. 1844
Navicula schoenfeldii	Hust. 1930
Navicula veneta	Kütz. 1844
Nitzschia dissipata	(Kütz.) Grunow 1862
Nitzschia gracilis	Hantzsch 1860
Nitzschia inconspicua	Grunow 1862
Nitzschia palea	(Kütz.) W.Sm. 1856
Nitzschia perminuta	(Grunow) M.Perag. 1903
Nitzschia pura	Hustedt 1954
Pinnularia	Ehrenb. 1843
Planothidium delicatulum	(Kütz.) Round et L.Bukhtiyarova 1996
Planothidium lanceolatum	(Bréb.) Lange-Bert. 1999
Psammothidium helveticum	(Hust.) L.Bukhtiyarova et Round 1996
Psammothidium kuelbsii	(Lange-Bert. in Lange-Bert. et Krammer) L.Bukhtiyarova et Round 1996
Psammothidium lauenburgianum	(Hust.) L.Bukhtiyarova et Round 1996
Psammothidium levanderi	(Hust.) L.Bukhtiyarova et Round 1996
Psammothidium subatomoides	(Hust.) L.Bukhtiyarova et Round 1996
Reimeria sinuata	(Greg.) Kociolek et Stoermer 1987
Rhoicosphenia abbreviata	(C.Agardh) Lange-Bert. 1980
Stauroneis smithii	Grunow 1860
Staurosira construens	Ehrenb. 1843 F2136
Staurosira elliptica	(Schumann) D.M.Williams et Round 1987
Staurosirella pinnata	(Ehrenb.) D.M.Williams et Round 1987
Synedra acus	Kütz. 1844
Synedra delicatissima	W.Sm. 1853
Synedra nana	Meister 1912
Synedra rumpens	Kütz. 1844
Synedra tenera	W.Sm. 1856
Tabellaria flocculosa	(Roth) Kütz. 1844

Appendix 2 – Sediment use in gallery construction

This small-scale investigation was designed to examine whether *Tinodes waeneri* larvae re-used sediment from the dismantled section of their gallery, and incorporated it into the new gallery section that they were constructing.

Larvae collected from the Lake District in August 2006, were released into an aquarium containing quarry tiles that had been pre-colonised with algae. In addition, the aquarium also contained yellow sand as a gallery building material. After 1 week, the tiles were carefully removed from the water and any sand not built into a gallery was carefully rinsed off the tile. Once tiles had been placed back into the aquarium, red sand was added.

Gallery building was observed, and in all cases new gallery sections contained red sand rather than yellow sand (Fig. A.1). Thus, at least under conditions of relatively high sediment availability larvae did not re-use sediment when building their galleries.

Figure A.1 A photograph of a typical gallery, showing yellow sand in the old section (towards the left) and red sand in the more recently constructed section (towards the right).

Appendix 3 - Exploring potential gardening in the net spinning caddisfly *Philopotamus montanus* using stable isotope analysis

A3.1 Introduction

Philopotamus montanus is found in small, stony, upland streams, which tend to be unproductive. The larvae filter food from the water using a very fine meshed silken net attached to the underside of a rock, and their gut contains fine detritus, diatoms and small bits of moss (Jones, 1949; Elliott, 1981). A considerable variety of diatom species have been found in their guts, and Jones (1949) considered this to be the main food of this species.

This species has been found to be univoltine in the Lake District streams in which it has been studied (Elliott, 1981). Growth rates of *P. montanus* are comparable to those of *T. waeneri* (Dall *et al.*, 1984), with the highest growth rate (6-7.5% dry weight day^{-1}) occurring in July and August, and the larvae grow to a large size (mean dry weight in 5th instar = 5.183mg, Elliott, 1981). However, *P. montanus* is able to achieve high growth rates in what can be a very unproductive habitat. Furthermore, in streams in which tree leaf litter is the main source of detritus, only low levels will be available during the period of greatest larval growth.

In addition, it is generally suggested that the nets of suspension-feeding caddisflies are inefficient at capturing particles (capture efficiency of <0.03% to 0.4% for *Hydropsyche* species; Brown *et al.*, 2005), although, this is based on a low number of studies on one genus of caddisfly. Furthermore, the shape of the nets and the activities of the *P. montanus* larvae mean that only small particles are retained within the net (Edington & Hildrew, 1995). These considerations led to the suggestion that *P. montanus* may have access to an alternative food supply, and that it may be gardening. This was investigated using stable isotope analysis. If gardening is taking place, the larvae and their nets should be ^{15}N-depleted relative to the seston (see Fig. 2.1 for rationale).

A3.2 Methods

Approximately 20 individuals of *Philopotamus montanus* together with their nets, were sampled from each of six streams in the Lake District and the southwest of England, between November 2005 and May 2006. All streams, other than Markham Brook (which was sampled twice) were sampled on one occasion. Riparian vegetation differed between the Lake District and Somerset streams, and was dominated by grassland and woodland respectively (Fig. A.2). In some Somerset streams *P. montanus* was found to co-occur with a second philopotamid species, *Wormaldia occipitalis* (Pictet), and individuals of this species were also collected. In addition, water samples (approximately 10L) were collected, and these were filtered through GF/F filters in the laboratory to collect the seston. Filter papers and nets were subsequently oven dried (60°C, for at least 48h). The larvae were kept cool in vials containing stream water for 24 hours to allow them to clear their guts before they were also oven dried. Seston and invertebrate samples were prepared for stable isotope analysis and analysed using the methods described in Chapter 2 (section 2.2). A small number of whole nets were analysed as it was not possible to separate the contents from the silken architecture of the net.

Figure A.2 Photographs of four of the sampled streams, showing the differences in bankside vegetation.

A3.3 Results

Streams in the south west of England contained greater amounts of seston than the Lake District streams, with 1.5 litres of water providing adequate amounts of seston for the stable isotope analysis, whereas the amount filtered from Lake District streams was approximately 10 litres. Seston in the south west was mainly brown in colour and appeared to contain mainly detritus, whereas seston from the Lake District contained a greater proportion of algal material.

Differences in $\delta^{15}N$ values between the larvae and the seston were very variable among streams (ranging from -0.2‰ to 4.43‰, Table A.1). Low $\delta^{15}N$ values for *P. montanus* and small differences between N isotope ratios for seston and larvae occurred in the Lake District streams and in the Quantocks (sampling took place approximately 50-100m from the stream source). Quantock nets were slightly ^{15}N-depleted compared to the seston, but the same did not hold true for nets collected from Snow Cove Gill (Lake District).

$\delta^{13}C$ values were inconsistent across streams, with larvae having lower isotope ratios in some streams and higher ones in others when compared to the seston. In the Quantocks, and at Markham Brook, there was a clear separation in $\delta^{15}N$ but not in $\delta^{13}C$, between *P. montanus* and *W. occipitalis*. At Sidcot, the other stream in which both species co-occurred, there was a similar trend, although only a small number of *P. montanus* larvae were sampled due to low abundances.

Table A.1 Summary of $\delta^{15}N$ and $\delta^{13}C$ values (mean ± 1SE) for *Philopotamus montanus* larvae and their nets, *Wormaldia occipitalis* larvae and seston.

Site	Date	Location		Seston	*Philopotamus montanus*					*Wormaldia occipitalis*		
					Larvae	Larvae – Seston	n	Nets	n	Larvae	Larvae-Seston	n
Carrock Beck Seep	04/06	Lake District	$\delta^{15}N$	1.78	3.89 ± 0.07	2.12 ± 0.07	7			_	_	_
			$\delta^{13}C$	-30.08	-33.14 ± 0.18	-2.33 ± 0.18	7			_	_	_
Snow Cove Gill	04/06	Lake District	$\delta^{15}N$	2.27	4.87 ± 0.12	2.6 ± 0.12	9	3.58 ± 0.48	5	_	_	_
			$\delta^{13}C$	-29.42	-27.98 ± 0.16	1.45 ± 0.16	9	-30.34 ± 0.47	3	_	_	_
Hardknott Gill	05/06	Lake District	$\delta^{15}N$	3.93	3.73 ± 0.09	-0.2 ± 0.09	6			_	_	_
			$\delta^{13}C$	-28.52	-27.81± 0.12	0.71 ± 0.12	6			_	_	_
Sidcot	04/06	North Somerset	$\delta^{15}N$	2.70	6.29 ± 0.23	3.59 ± 0.23	3			6.46 ± 0.09	3.76 ± 0.09	7
			$\delta^{13}C$	-30.52	-29.57 ± 0.29	0.95 ± 0.29	3			-29.60 ± 0.08	0.92 ± 0.08	7
Markham Brook	04/06	North Somerset	$\delta^{15}N$	3.17	7.6 ± 0.10	4.43 ± 0.10	6			7.77 ± 0.08	4.6 ± 0.08	6
			$\delta^{13}C$	-29.81	-30.03 ± 0.11	-0.23 ± 0.11	6			-29.99 ± 0.16	-0.19 ± 0.16	6
Markham Brook	11/05	North Somerset	$\delta^{15}N$	2.30	6.68 ± 0.07	4.38 ± 0.07	22			_	_	_
			$\delta^{13}C$		-29.54 ± 0.08		22			_	_	_
Quantocks	11/05	Somerset	$\delta^{15}N$	0.80	2.18 ± 0.05	1.38 ± 0.05	14	0.137 ± 0.13	5	2.8 ± 0.11	2.00 ± 0.11	5
			$\delta^{13}C$	-31.93	-29.21 ± 0.07	2.72 ± 0.07	14	-32.49	1	-29.37 ± 0.13	2.56 ± 0.13	5

A3.4 Discussion

For *P. montanus* there appears to be a regional pattern in the data, with both lower $\delta^{15}N$ and a smaller amount of fractionation between larvae and seston occurring in the Lake District and also in the Quantocks. The Lake District streams flowed through rough grassland and were probably less productive than Markham Brook and Sidcot. The stream in the Quantocks was sampled near its source and although it was surrounded by some trees, the predominant vegetation type was upland heathland/grassland.

Net values were expected to be slightly ^{15}N-enriched relative to the seston as nets also contain silk (see Chapter 2) and this was the case at Snow Cove Gill. The net capture efficiency and selectivity for different size particles may explain both the differences in stable isotope values between the seston and larvae and seston and nets, as well as between the two philopotamid species (*P. montanus* and *W. occipitalis*). Silk strands in *P. montanus* nets form a rectangular structure, with longitudinal strands 10-13μm apart and fine strands running perpendicularly at a spacing of 1μm (Edington & Hildrew, 1995). In comparison, nets of a *Wormaldia* sp. consist of two overlapping layers creating pore sizes of just 0.4 by 0.4μm (Merritt & Wallace, 1981), which should allow them to capture smaller particles of seston than *P. montanus*. This perhaps explains the $\delta^{15}N$ values which suggested that the two species had a slightly different diet. However, nets do not act as simple sieves, with adhesion to silk strands also playing a role in particle capture, and furthermore, nets generate complicated flow patterns which may further influence the size of particles captured by the nets (Edler & Georgian, 2004).

The net design will mean that they will filter out only a small proportion of the bulk seston that was flowing through the streams. Similarly, the relationship between nets and seston may also be a function of how the isotope ratio of the seston fraction within the net relates to the seston as a whole. The bulk seston sample analysed here may therefore not be a realistic measure of the stable isotope ratios of the potential larval food resource within the streams, and better measures of the size range of particles captured by nets and eaten by *P. montanus* are needed. However, this data does suggest that food is not being modified through gardening.

Appendix 4 - Crosemere

Tinodes waeneri larvae were sampled from the littoral of Crosemere (a eutrophic lake) in November 2005. Table A.2 provides information on the sample location and the key characteristics of the lake for comparison with the Lake District lakes in Table 2.1. Crosemere was sampled as the *T. waeneri* population has previously been well studied (Harrison, 1996; Harrison & Hildrew, 1998b; Harrison & Hildrew, 1998a; Harrison & Hildrew, 2001). Sampling protocol, sample processing and stable isotope analysis of the samples was carried out in exactly the same way as described in section 2.2 with the exception that 7 rather than 10 rocks were sampled and 5 larvae and galleries rather than 4 were collected per rock.

Table A.2 Location of the sample site and the key characteristics of Crosemere (data from Lewis, 1990; Moss *et al.*, 1994; James *et al.*, 2003).

Measurement	Value
Grid reference	SJ307431
Lake area (km^2)	0.152
Mean depth (m)	4.8
Volume (m^3 x 10^4)	73.0
Mean retention time (years)	1.06
Mean total phosphorus (mg m^{-3})	214
Mean nitrate-nitrogen (growth season; mg m^{-3})	240
Mean phytoplankton chlorophyll *a* (mg m^{-3})	33

Organic matter made up an average of 9.59% by weight of the gallery. Larvae collected were mainly fifth instars (only 8% were fourth instar). Stable isotope results are displayed in Fig A.3 and analysis of variance indicated that there was no significant difference between the three components of the *Tinodes* system in terms of either $\delta^{15}N$ ($F_{2,17}$=4.82, P<0.05) or $\delta^{13}C$ values ($F_{2,17}$=3.24, P>0.05). Larvae were however slightly ^{15}N-enriched and slightly ^{13}C-depleted relative to their potential food sources. There was no therefore no indication of N recycling within the gallery community, and as there was no difference in $\delta^{13}C$ between the food sources, the dietary contributions of galleries and epilithon to the larvae could not be calculated.

Figure A.3 Mean (± 2SE) stable isotope values (‰) for the larvae, galleries and epilithon collected from Crosemere in November 2005. Gallery $\delta^{15}N$ values represent the entire gallery as the values have not been corrected for silk.