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The application of a functional group approach to algal-grazer interactions

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Abstract

Algal morphology is considered to be influenced by physiological and environmental factors such as stress and disturbance; one key disturbance exerted on algae is grazing pressure from herbivores. The factors upon which herbivores base their food preferences include algal attractiveness and edibility. Steneck and Watling's (1982) functional group model advocates the combination of algal species into seven groups based upon morphology and ecological function; the basic premise being that algal attractiveness and edibility will decrease hierarchically from functional group one to functional group seven, and that species within a group will be similarly attractive and edible. Two grazers commonly found in the intertidal area are the gastropod *Littorina littorea* and the isopod *Idotea granulosa.* Attractiveness and edibility experiments were conducted in which both grazers were presented with algae representing functional groups two to seven in single- and two-way choice experiments, in order to determine whether their food preferences could be predicted using a functional group approach. Although *L. littorea* and *I. granulosa* exhibited preference for algae between functional groups with regards to attractiveness and edibility, preference for both functional group and algal species differed between grazers. When the effect of algal morphology was investigated by presenting grazers with algal homogenates in agar, both grazers displayed a preference for algal extracts in agar over whole plant material, and exhibited preference for algae both between- and within-functional groups. The fact that not all species within a functional group were consumed in similar amounts contradicts the functional group model. Further investigation of the effect of algal availability on the feeding preferences of *L. littorea* showed their food choices could not be predicted based upon the algal species dominant in their habitat of origin. Results from all experimental manipulations suggest that both the habitat and food requirements of a herbivore, along with algal characteristics such as morphological, structural and chemical defences, algal availability and nutritional composition all influence herbivore food choice. As such, the functional group model proposed by Steneck and Watling (1982) cannot be accurately used to predict the feeding preferences of *L. littorea* or *I. granulosa.* It is suggested that unless models are modified to meet the requirements of a specific question, current functional group approaches are not a useful tool for predicting algal-grazer interactions.

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"I do not know what I may appear to the world, but to myself I seem to have been only like a boy playing on the sea shore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay undiscovered before me".

Sir Isaac Newton, from Brewster, D. (1855) Memoirs of the Life, Writings, and Discoveries of Sir Isaac Newton. Vol. II, Ch. 27.

"Many zoologists conside algae only as fodder, and many phycologists consider grazers merely as a nuisance to their algae".

Stephen Hawkins and Richard Hartnoll, (1983) Oceanography and Marine Biology: An Annual Review, 21: 195-282.

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Author's Declaration

The candidate confirms that the work submitted is her own work and the appropriate credit has been given where reference has been made to the work of others.

Chapter 1

General Introduction

1.1 Algal defences to grazers

Algae possess a number of traits that allow them to be defended from grazing herbivores. Examples of primary methods of defence include structural, morphological and chemical defences and the ability of a plant to escape herbivores both spatially and temporally (Atsatt and Dowd, 1976; Cronin *et al*, 2002; Rohde *et al*, 2004).

1.1.1 Structural and morphological defences

Algae display a range of morphologies, which may be related to life history. Morphological diversity has been linked to different survival strategies in terms of an alga's ability to grow and reproduce, compete for resources (*i.e.* light, space, nutrients), resist predation and tolerate environmental fluctuations (Littler and Littler, 1980). Late successional algae, such as fucoids, have tougher thalli than ephemeral, opportunistic species, such as *Ulva* sp. Linnaeus[†] where selection for structural tissue has occurred in longer lived species in established communities (Littler and Littler, 1980). It has long been believed that tougher plant tissue is avoided by herbivores as it is less palatable (Feeny, 1970; Horn *et al*, 1982; Huston, 1994). Other morphologies considered to be resistant to grazers include crustose growth forms (Lubchenco and Cubit, 1980; Bertness *et al*, 1983; Dethier, 1994). Fleshy algal crusts such as *Hildenbrandia rubra* (Sommerfelt) Meneghini and *Ralfsia verrucosa* Areschoug are more resistant to grazing by intertidal grazers such as *Littorina littorea* Linnaeus than the erect algae *Chondrus crispus* Stackhouse and *Ulva lactuca* Linnaeus (Bertness *et al*, 1983) (see section 3.4.1 of chapter 3 for further discussion). Some species can change their growth form in response to grazers (Lewis *et al*, 1987). Many algal species have heteromorphic life stages, during which they display alternating morphological generations that differ in their susceptibility to grazing pressure (Lubchenco and Cubit, 1980; Slocum, 1980; Littler and Littler, 1983). *Padina* sp. Adanson displays morphological plasticity as it assumes a prostrate, turf growth form in the presence of grazers but a foliose growth

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[†] Throughout this thesis algal nomenclature follows Hardy and Guiry (2003), with the exception of *Ulva intestinalis* synonym *Enteromorpha intestinalis* (Linnaeus) Nees (Hayden *et al*, 2003).

form when grazers are absent (Lewis *et al*, 1987) (see section 5.1.1 of chapter 5 for further discussion). Algal susceptibility encompasses how well defended a species is against factors such as herbivory. In experiments where differences in algal morphology have been removed by presenting grazers with algae in 'artificial' forms, such as in agar, feeding preferences are often altered (*e.g.* Geiselman and McConnell, 1981; Hunter, 1981; Galan Jimenez *et al*, 1996; Heckscher *et al*, 1996; Cronin *et al*, 2002; Jormalainen *et al*, 2005) (see section 5.4.2 of chapter 5 for further discussion).

Structural defences provide plants with an important front-line defence against herbivores (Padilla, 1989). Herbivores identify potential food items through handling, taste and plant nutritional quality (Cronin *et al*, 2002). If a herbivore is unable to remove plant tissue because of structural defences, any other deterrents present, such as chemical defences or low nutritional quality will be irrelevant (Padilla, 1989). Algae can be structurally resistant to herbivores through calcification, for example *Corallina officinalis* Linnaeus. Calcification occurs in a range of forms and to varying degrees (Padilla, 1989), and is thought to act primarily by increasing an alga's mechanical resistance to tissue loss by grazing herbivores (Steneck, 1982). Algal calcium carbonate ($CaCO_3$) occurs in two forms; aragonite and calcite, though they are never both present in the same algal species (Borowitzka, 1977). Other carbonates found in algae include magnesium carbonate ($MgCO_3$) and strontium carbonate ($SrCO_3$) (Borowitzka, 1977). The level of calcification is influenced by factors ranging from water motion, competition, and acquisition of light and nutrients and through herbivores themselves (Littler and Littler, 1980; Steneck and Watling, 1982; Steneck, 1986). Calcified algae are generally constrained to the lower shore, with the exceptions that they can survive in pools and under canopy algae (Hawkins and Hartnoll, 1983). They appear to have a more restricted distribution upon shores than non-calcified plants due to their level of desiccation tolerance (Steneck and Dethier, 1994), which may reduce their contact with grazers, providing them with a further degree of protection as they are physically remote from many herbivores.

Structural $CaCO_3$ reduces algal palatability and calorific values, thus making plants less susceptible to herbivores (Paine and Vadas, 1969; Raffaelli, 1979; Horn *et al*, 1982; Steneck, 1982; Breitburg, 1984; Hay, 1991; Benedetti-Cecchi *et al*, 2000; Belliveau and Paul, 2002). There is conflicting evidence in the literature regarding the effectiveness of calcification as a defence. Pennings and Paul (1992) showed that the sea hare *Dolabella auricularia* Lightfoot was deterred by plant calcification as feeding preferences were negatively correlated with plant toughness. In an experiment with a number of tropical limpet species, Padilla (1989) found that increased levels of algal calcification decreased the plants resistance to herbivory. More tissue was lost from crustose and calcareous algae than from species with sheet or branching morphologies (Padilla, 1989). Calcareous plants were thought to be more brittle than fleshy algae and so more likely to break when a force was applied. Fleshy algae yield under applied force by absorbing energy transferred from the grazing herbivore (Padilla, 1989). However, as docoglossan limpets have a different feeding mechanism to both grazers investigated in this study (see section 1.4 below for description of experimental grazers), the degree to which calcification influences algal susceptibility to grazing may differ between herbivores (Padilla, 1989). Schupp and Paul (1994) observed that some species of herbivorous fish preferentially consumed artificial food containing powered aragonite, while other fish species avoided food containing $CaCO_3$. Hily *et al* (2004) found that the gastropod *Jujubinus striatus* Linnaeus consumed the calcareous crustose algae *Pneophyllum* sp. Kuetzing that dominated during winter months when non-calcareous species were sparse. Kennish *et al* (1996) also found seasonal differences in the consumption of both encrusting coralline and encrusting fleshy algae. The herbivorous crab *Grapsus albolineatus* Lamarck preferentially consumed *Corallina* sp., *Ralfsia* sp. and *Hildenbrandia* sp. during the summer, but encrusting algae were only a minor component of their diet during the winter when filamentous algae were dominant (Kennish *et al*, 1996). Other authors have shown that although herbivores reduce crust biomass, grazing may have an overall positive effect on crustose algae through the removal of epiphytes and the stimulation of new growth (Steneck, 1982, 1997; Breitburg, 1984; Stoner, 1989; Neckles *et al*, 1993; Littler *et al*, 1995; de O. Figueiredo *et al*, 1996; Belliveau and Paul, 2002; Wai and Williams, 2005; Underwood, 2006).

An alternative strategy exhibited by some algal species is to employ $CaCO_3$ as a chemical defence (Hay *et al*, 1994). In a series of feeding experiments, the urchin *Diadema antillarium* Philippi, the isopod *Cymadusa filosa* Savigny and a mixed-species group of parrotfish were presented with an artificial food containing powdered $CaCO_3$. Both *D. antillarium* and *C. filosa* reduced their feeding on the artificial food. This led Hay *et al* (1994) to suggest that $CaCO_3$ can act as a chemical defence by altering a herbivore's gut pH, which in turn increases the effectiveness of other chemical defences.

1.1.2 Chemical defences

Many of the algae used in this investigation are known to produce a wide range of secondary compounds: *Polysiphonia* Greville and *Rhodomela* Agardh contain halophenolics (Hay and Fenical, 1988); fucoids, such as *Ascophyllum nodosum* (Linnaeus) Le Jolis and *Fucus serratus* Linnaeus produce significant amounts of phlorotannins (Hay and Fenical, 1988) and *Osmundea* Stackhouse is known to produce around 500 different secondary metabolites (Hay, 1996) including acetogenins, haloacetogenins, haloterpenes, indoles (Hay and Fenical, 1988), halogenated sesquiterpenes and diterpenes (de Nys *et al*, 1998). The terpenoid secondary metabolites produced by *Osmundea* occur in intracellular vesicles termed *corps en cerise*, which only release their contents when the plant cells are damaged (Dworjanyn *et al*, 1999), for example when being consumed by herbivores. However, other algal species, such as *Cladophora rupestris* (Linnaeus) Kützing and *U. lactuca* are more vulnerable to grazing as they have limited investment in chemical or structural defences (Littler and Littler, 1980). Later successional, slow growing (k-selected) plants invest a lot of energy in chemical defences (Herms and Mattson, 1992), whereas early successional, fast growing ephemeral (r-selected) plants protect themselves from grazing by investing less energy in anti-herbivore chemical defences in individual plants, and more in ensuring that their population is sustained within the environment (Nicotri, 1980). As ephemeral species persist through maintaining their population rather than concentrating their energy on the growth of individual plants, their growth patterns are often spatially and temporally unpredictable, which may act as a defence against grazing by herbivores.

However, studies have shown that structurally tough, chemically-defended plants are often consumed by herbivores (Malm *et al*, 1999; Jormalainen *et al*, 2001a), and *Idotea baltica* Pallas is one herbivore that is known to exert a detrimental effect on *Fucus vesiculosus* Linnaeus (Kotta *et al*, 2000; Jormalainen *et al*, 2001a) (see sections 3.4.1 and 3.4.2.2 of chapter 3 for further discussion).

For those algae with defensive chemicals, the distribution of secondary metabolites varies within a plant (Hammerstrom *et al*, 1998). In kelp species such as *Laminaria digitata* (Hudson) J.V. Lamouroux, holdfasts and stipes often have higher concentrations of phlorotannins than the blade tissue; also for those species with midribs they contain one-half to one-third more of the phlorotannins of blade tissue (Van Alstyne *et al*, 2001). Similarly in fucoids, the concentration of phlorotannins in the blade tissue is around 10% less than in the midrib (Van Alstyne *et al*, 2001) and apical parts contain significantly less phlorotannins than basal parts (Hemmi *et al*, 2005). By allocating defensive compounds differentially within a plant, algae may overcome the costs associated with producing secondary metabolites. Crawley (1983) suggested that there are three main costs to a plant producing chemical defences. The first is the cost of manufacturing, translocation and storing the chemical compounds. The second involves the cost of maintaining and running the defences and the cost of repair. The third cost is described as the opportunity cost, which is associated with the loss in productivity and competitive ability compared to plants that do not posses chemical defences (Crawley, 1983). Using the differential allocation of secondary metabolites as a trade-off against these costs could increase overall plant fitness (Cronin and Hay, 1996). Alternatively, algae could reduce costs through inducible defences.

1.1.2.1 Inducible defences

Inducible defences can be defined as 'responses by the plant to herbivore injury that decrease the negative fitness consequences of attacks on the plant' (Karban and Myers, 1989). Therefore, defences can be regarded as inducible if they are always present at low levels but activated to be produced at higher levels via environmental stimulation, for example in response to a disturbance such as herbivory. Inducible defences require new structures and chemical constituents to be produced, and also bring about changes in the structures or concentrations of existing chemical compounds (Harvell, 1990). To be effective against herbivores, inducible defences should be immediate and all parts of a plant should be able to induce defences, which then should decrease if no further damage from herbivores occurs (Hammerstrom *et al*, 1998). An advantage of inducible defences to a plant is the concept of 'memory'. 'Memory' in this context is presumed to allow successive responses to disturbance to be significantly quicker than the initial response (Harvell, 1990). This is beneficial in environments where a plant is dependent upon the nature of the disturbance and where the probability of disturbance occurring is high. However, there are also costs associated with the production of inducible defences. The cost to a plant of manufacturing inducible defences has been difficult to quantify, but has been suggested to affect plant fitness, for example growth rates and reproduction (Harvell, 1990). Nevertheless, inducible defences seem to be less costly than constitutive defences that are always present in high enough concentrations to reduce disturbance. The benefits to a plant of inducible defences compensate for the associated costs (Harvell and Tollrian, 1999) and so inducible defences provide a viable means of protection against disturbance by herbivores.

1.2 The components of food preference

Food preference may be related to factors that do not directly relate to algal nutritional quality (Nicotri, 1980) (see section 3.1 of chapter 3 for a full definition). Mesoherbivores are benthic herbivores that utilise algae both as a source of food and as a habitat (Rogers *et al*, 1998). Mesoherbivores that live among plants may utilise algal species as a habitat as well as a food source as the algae offer protection and shelter from predators and wave action. Such characteristics may have an effect on herbivore preference as much as the food value of the plant (Nicotri, 1980).

Food preference is considered to have two components: edibility and attractiveness. Edibility reflects the speed at which a given food item meets the physiological needs of a grazer, and also the ease with which the item can be manipulated and ingested (Nicotri, 1980). Edibility can be measured by quantifying the amount of an alga consumed during active feeding, and assuming that all grazers have experienced equivalent hunger conditions, the preferred foods should be consumed in greater quantities than the least preferred foods. Attractiveness incorporates a number of additional factors regarding selection, including the capacity of a herbivore to detect plant odours, and also non-nutritive characteristics that are used to determine the value of a plant as a habitat (Watson and Norton, 1985a). Attractiveness can be measured by offering grazers a choice between two or more potential food items (Nicotri, 1980; Watson and Norton, 1985a). As edibility and attractiveness measure different aspects of choice, both components of food preference were investigated in the current study.

1.3 Algal functional group models

1.3.1 The history of the functional group approach

Algal functional groups are considered by many authors to be the biological units which can be satisfactorily used to describe or assess the status of algal communities without recourse to species level identification (Littler, 1980; Littler and Littler, 1980; Littler and Arnold, 1982; Steneck and Watling, 1982; Steneck and Dethier, 1994; Philips *et al*, 1997; McClanahan *et al*, 2002; Wilson, 2002; Buonopane *et al*, 2005; Micheli and Halpern, 2005). Early classifications of algal species into groups were generally based on phylogeny and life history traits (*e.g.* Chapman and Chapman, 1976). However, because such classifications were not based on ecological interactions and physiological characteristics, they are often regarded as having limited real world value (Littler and Arnold, 1982). Littler and Littler (1980) proposed a functional-form model that provided evidence that factors such as successional status, photosynthetic ability, calorific value and structural toughness showed predictable trends based upon the morphology of algal species that were independent of their phylogenetic affinity. Subsequent studies (*e.g.* Littler, 1980; Hay, 1981) generated further evidence for the relationship between algal morphology and ecological characteristics. In a study using the previously published data of a number of authors, Steneck and Watling (1982) examined functional group interactions between algae and molluscan grazers, with both algae and grazers arranged into one of seven or four functional groups respectively (*e.g.* Foster, 1964; Haven, 1973; Creese and Underwood, 1976; Kitting, 1980; but see Appendix I in Steneck and Watling (1982) for a comprehensive list). Based upon their analysis Steneck and Watling (1982) suggested that molluscs exhibited distinct feeding patterns in relation to algae from different functional groups. Most herbivorous molluscan species consumed algal forms that were either very minute (for example microalgae and filamentous algae) or very large and expansive (for example kelps and crustose algae), with intermediate size algae consumed to a lesser extent (Steneck and Watling, 1982). Their functional group model was expanded by Steneck and Dethier (1994), who proposed a refined version. In Steneck and Dethier's (1994) model algal species were arranged into one of nine functional groups similar to those of Steneck and Watling (1982) and used to describe the effects of stress and disturbance upon algal communities. Stress can be defined as a factor that influences the productivity potential of a plant, including light, nutrients and desiccation (Steneck and Dethier, 1994).

Disturbance includes factors that limit plant biomass by causing partial or total destruction, for example herbivory or scouring (Steneck and Dethier, 1994). Steneck and Watling's (1982) approach was based upon similarities among unrelated algal species and concluded that herbivore-induced disturbances had functionally similar effects on morphologically and anatomically similar algae regardless of their taxonomic or geographic affinities (Steneck and Dethier, 1994). Subsequent studies, including those of Lavorel *et al* (1997), Zamora (2000) and Boaventura *et al* (2002) have also maintained that algal species that are phylogenetically quite distinct may be considered to be so similar at some levels that they serve a similar function within a community. For functional groups to be regarded as good indicators of environmental variables, all species within a functional group must respond in a similar way and to a similar degree to each variable (Carney, 1996).

1.3.2 Steneck and Watling's (1982) functional group model

One of the aims of the current study is to determine if the functional group model proposed by Steneck and Watling (1982) can be used to predict the feeding preferences of herbivores. This model was chosen in preference to those by Littler and Littler (1980) and Steneck and Dethier (1994) because algae are arranged into functional groups based not only upon their morphological and anatomical characteristics but also the size and toughness of the algae relative to the feeding abilities of molluscan grazers. This is an advantage over other models as it demonstrates how the functional group approach can help to understand the mechanisms of plant-animal interactions between algae and molluscan grazers.

According to the model, algal functional groups are designated a number from one to seven and ranked according to toughness, which Steneck and Watling (1982) defined as the ability to resist being scratched. Table 1.1 presents and overview of the functional group approach suggested by Steneck and Watling (1982) and provides examples of algal species within each group. Functional group one (the least 'tough') comprises the microalgae, for example unicellular and filamentous algal forms that have no holdfasts for attachment, such as diatoms. This group includes spores and zygotes of algae from other groups. Functional group two are the filamentous algae, and are represented by larger algal filaments that are attached by holdfasts, and usually have little or no cortication. Sporelings of many other species are also included in this group.

Functional group three, the foliose algae, is represented by algae with thin sheet and tube morphologies that are only one or two cells thick. Functional group four includes the corticated macrophytes. These algae represent morphologically complex species that usually have wiry, tough and ramifying forms, which are capable of growing erect and filling three-dimensional space. The thalli of these algae have an outer corticated layer surrounding the medulla. Functional group five includes the leathery macrophytes, and is represented by extensively corticated species with complex morphologies, that provide them with the structural strength required to grow very large (often over 100 m long). This group also includes non-calcified crusts, which although are not as morphologically complex as some of the species within this group, they are equally tough to scrape due to the dense packing of cells in the outer tissue. Functional group six incorporates articulated calcareous algae, which include algae that contain calcium carbonate in their cell walls. Species within this group are of similar size and shape to species of functional group four, but differ morphologically in that they are composed of a series of short calcareous segments connected by flexible joints. Functional group seven includes the crustose coralline algae. Species comprising this group also contain calcium carbonate in their cell walls and they are heavily calcified. They differ from algae in functional group six in that they grow prostrate over substrata. *Verrucaria maura* Wahlenberg, a lichen rather than alga, is structurally similar to the coralline species of functional group seven and so may play as important a function within the ecosystem as other members of the group. *Verrucaria maura* was included in this investigation because it is widely distributed in the intertidal region of temperate rocky shores. As can be seen from Table 1.1, Steneck and Watling (1982) hypothesised that as algal structural complexity increases from functional group one through to functional group seven, algal susceptibility to grazing by herbivores decreases.

Table 1.1. Algal functional groups (adapted from Steneck and Watling, 1982). The species investigated were organised into the functional groups suggested by Steneck and Watling (1982) to test their prediction that as algal structural complexity increases, susceptibility to grazing by herbivores will decrease.

Functional Group	**Representatives**	**Susceptibility to Grazing**
FG1. Microalgae	Diatoms	High
FG2. Filamentous algae	*Cladophora rupestris*$^{2, 2, 3, 3, 5, 5}$ *Ceramium* sp.$^{2, 2, 3, 3, 5, 5}$ *Polysiphonia* sp.$^{3, 4}$	↑
FG3. Foliose algae	*Ulva lactuca*$^{2, 2, 3, 3, 5, 5}$ *Ulva intestinalis*$^{3, 4}$ *Dumontia contorta*$^{2, 3}$ *Palmaria palmata*$^{3, 5}$ *Porphyra* sp.$^{2, 3, 5}$	
FG4. Corticated macrophytes	*Mastocarpus stellatus*$^{2, 2, 3, 3, 5, 5}$ *Osmundea pinnatifida*$^{2, 2, 3, 3, 5, 5}$ *Rhodomela confervoides*^{3}	
FG5. Leathery macrophytes	*Fucus serratus*$^{2, 2, 3, 3, 5, 5}$ *Ascophyllum nodosum*^{3} *Laminaria digitata*$^{2, 2, 3, 3, 5, 5}$	
FG6. Articulated calcareous algae	*Corallina officinalis*$^{2, 2, 3, 3, 5, 5}$	
FG7. Crustose coralline algae (including a lichen)	*Lithophyllum incrustans*^{3} *Verrucaria maura*^{3}	Low

[2] Used in Chapter Two experiments (blue = *L. littorea*; green = *I. granulosa*).

[3] Used in Chapter Three experiments (blue = *L. littorea*; green = *I. granulosa*).

[4] Used in Chapter Four experiments (blue = *L. littorea*; green = *I. granulosa*).

[5] Used in Chapter Five experiments (blue = *L. littorea*; green = *I. granulosa*).

1.3.3 The experimental algae

The intertidal zone along the east coast of England is dominated by many species of algae that together exhibit a diverse range of morphologies representing all seven of the functional groups proposed by Steneck and Watling (1982). Algae representing functional group one were not included in this study because (as described in section 1.3.2 above and 6.2.1 of chapter 6) this group comprises sporelings and zygotes of algae from other functional groups. This may have led to difficultly in the interpretation of results, as two life history stages of the same alga could be presented to grazers. Additionally, problems with the identification and grouping of microscopic algae could have caused unreliable results to be collected. Algae found along the rocky shores of the east coast from functional group two include the green alga *C. rupestris*; and the red algae *Ceramium* sp. Roth and *Polysiphonia* sp. Within functional group three, the green algae *Ulva intestinalis* Linnaeus; and *U. lactuca*, and the red algae *Dumontia contorta* (S.G. Gmelin) Ruprecht; *Palmaria palmata* (Linnaeus) Kuntze and *Porphyra* sp. Agardh were collected for use in this study. Algae representing functional group four were the red algae *Mastocarpus stellatus* (Stackhouse) Guiry; *Osmundea pinnatifida* (Hudson) Stackhouse and *Rhodomela confervoides* (Hudson) P.C. Silva. Species comprising functional group five used in this study were the brown algae *F. serratus*; *A. nodosum*; and *L. digitata*. There was only one species of algae representing functional group six found within the collection area and that was the red alga *C. officinalis*. For functional group seven, the red encrusting alga *Lithophyllum incrustans* Philippi was collected along with the lichen *V. maura*, which is also common on many shores. Algae are important components of intertidal ecosystems as they provide both food and shelter for a range of intertidal grazers.

One of the factors that the presence and growth of macroalgae on a shore is considered to be dependent upon is grazing pressure, which is a function of grazer density and behaviour (McQuaid, 1996). Grazer density is influenced by many factors, including the presence of predators, the availability of suitable habitats such as crevices or algal canopy, and also life history patterns and recruitment success (McQuaid, 1996). Grazer pressure is also influenced by several factors, including the position of the grazers on the shore in relation to tidal height, as most will only feed when submerged or in a damp environment (Alexander, 1960; Lubchenco, 1978). Grazing by herbivores has been shown in numerous studies to have a negative impact on algal populations

(Lubchenco, 1978, 1980, 1983; Lubchenco and Menge, 1978; Sze, 1980; Lubchenco and Gaines, 1981; Petraitis, 1983, 1987; Underwood and Jernakoff, 1984; Salemaa, 1987; Janke, 1990; Davies and Beckwith, 1999; Kotta *et al*, 2000; Nilsson *et al*, 2004; Hemmi *et al*, 2005).

1.4 The experimental grazers

Two grazers commonly found within the intertidal of the east coast are the prosobranch snail *Littorina littorea* and the isopod *Idotea granulosa* Rathke. *Littorina* and *Idotea* distribution overlap on many rocky shores, including the ones visited in this study and so they could potentially be utilising the same algal species (Nicotri, 1980). *Littorina littorea* was chosen as an experimental grazer because it has a widespread distribution and commonly occurs on rocky shores, with a range extending from the high littoral zone to the sublittoral (Gardner and Thomas, 1987). The species tolerates wide ranges of temperature, salinity, and exposure and adapts well to laboratory conditions (McQuaid, 1996). *Littorina littorea* have also been shown to be important in structuring algal communities on many shores (Lubchenco, 1978; Sze, 1980; Underwood and Jernakoff, 1984; Petraitis, 1987; Janke, 1990; Davies and Beckwith, 1999).

Littorina is an opportunistic generalist grazer and in temperate areas, mid- and low-shore species such as *L. littorea* occur in areas dominated by dense macroalgal communities and tend to graze either macroalgae or algal epiphytes (McQuaid, 1996). *Littorina littorea* has been shown to exhibit a clear order of species preference among macroalgae, favouring ephemeral species such as *U. intestinalis* above algae such as *F. vesiculosus*, while *A. nodosum*, *C. crispus* and encrusting algae are not consumed at all (Lubchenco, 1978, 1980, 1982; Bertness *et al*, 1983; Watson and Norton, 1985a).

Densities and distributions of *Idotea* populations are determined by several factors, mainly the physiological adaptability of animals, habitat selection and biotic interactions of communities (Salemaa, 1979). Idoteids are generally considered to be omnivorous, as they are able to consume red, green and brown algae (Nicotri, 1980; Robertson and Mann, 1980; Hawkins and Hartnoll, 1983; Kotta *et al*, 2000) but have also been shown to be cannibalistic (Naylor, 1955; Franke and Janke, 1998). They are considered to be less important grazers than *L. littorea* on the lower shore (Nicotri, 1980), however numerous studies have been conducted on the effect *Idotea* spp., specifically *I. baltica* have had in recent years on populations of *F. vesiculosus* in the Baltic Sea (Salemaa, 1979, 1987; Schaffelke *et al*, 1995; Engkvist *et al*, 2000; Kotta *et al*, 2000; Goecker and Kall, 2003; Engkvist *et al*, 2004; Nilsson *et al*, 2004; Rohde *et al*, 2004). *Idotea* was chosen as the second experimental grazer as less work has been conducted on their food preferences compared with *L. littorea*, possibly due to labour-intensive collection, taxonomic difficulties, the fact that they are more difficult to see on plants and the misconception that mesoherbivores only eat diatoms and small filamentous algae (Hay *et al*, 1988). However, *Idotea* spp. thrive in laboratory conditions as they are able to develop and maintain stable populations (Franke and Janke, 1998) and it has been suggested that the feeding preferences of mesoherbivores play a crucial role in structuring algal communities (Pavia *et al*, 1999a). Mesoherbivores are thought to have evolved different food preferences to macroherbivores because they utilise algae as both a food source and a habitat (Duffy and Hay, 1994). *Idotea* have been shown to prefer large, robust plants to those with softer filaments and blades (Nicotri, 1980; Cervin and Aberg, 1997; Franke and Janke, 1998; Engkvist *et al*, 2000; Jormalainen *et al*, 2001a; Engkvist *et al*, 2004), though they also consume foliose algae (Kammermans *et al*, 2002; Goecker and Kall, 2003) and filamentous algae (Jormalainen *et al*, 2001a; Goecker and Kall, 2003), especially algal epiphytes occurring on fucoids (Karez *et al*, 2000; Kotta *et al*, 2000; Svensson *et al*, 2004). *Idotea baltica* exhibit sexual dimorphism in habitat choice but have been found to display no sexual differences in food preference (Merilaita and Jormalainen, 2000; Jormalainen *et al*, 2001b). It has generally been suggested that food preferences may be determined by algal morphology, nutritional quality or colour of algae (Kotta *et al*, 2000).

1.5 General Aims

This study aims to assess the utility of the functional group model of Steneck and Watling (1982) in predicting the feeding preferences of the intertidal grazers *L. littorea* and *I. granulosa*. Both components of food preference (attractiveness and edibility) will be considered in line with the functional group model.

Both grazers will be presented with algal species in two-way attractiveness experiments to determine if algal attractiveness decreases from functional group two to functional group six, and also if all species within a functional group will be similarly attractive to grazers (chapter two).

To determine if algal species and functional groups are similarly susceptible to grazing by both *L. littorea* and *I. granulosa*, grazers will be presented with whole plants of algae in single-choice edibility experiments. The aim will be to test whether algal susceptibility to grazing decreases as functional group number, and thus algal complexity increases and also to assess the susceptibility of algal species within a functional group (chapter three). The relationship between algal attractiveness and edibility for *I. granulosa* will also be studied by testing if *I. granulosa* consume more of the algal species they found the most attractive (chapter three).

To further explore the results collected from the attractiveness and edibility experiments in relation to grazers' behaviour in the natural environment, *L. littorea* from two habitats within a shore dominated by two different algal species will be presented with the two algae. Data will be used to determine if grazers will be more attracted to the algal species dominant in their habitat of origin; and if they consume more of the algal species dominant in their habitat of origin when presented with algae in single-choice experiments (chapter four). To put the results collected from these experiments into context with the functional group model, *L. littorea* from both habitats will be presented with the two algae simultaneously to determine if they preferentially consume algae from functional group two over algae from functional group three (chapter four).

To further investigate the impact of algal morphology on feeding preferences, experiments will be conducted in which algal morphology as a controlling factor is eliminated. Both *L. littorea* and *I. granulosa* will be presented with algae as whole plants and as homogenates in agar to test whether both morphological types are similarly susceptible to grazing or whether algal susceptibility to grazing will decrease from functional group two to functional group six (chapter five). The susceptibility of algae within a functional group will also be investigated for both whole plant material and algal homogenates (chapter five). The results collected from all experimental work will then be discussed in relation to the utility of Steneck and Watling's (1982) functional group model, and in the wider context of the functional group approach proposed by other authors (chapter six).

Published papers related to aspects of this work are presented in appendices II and III. The published research was conducted alongside the work presented in chapter's two to five and is included separately as although the papers are not central to the main thesis, the findings are relevant to the study and compliment the work undertaken in the experimental chapters.

Chapter 2

Attractiveness of algal functional groups to intertidal grazers

2.1 Introduction

Macroalgal fronds offer refuge for mesoherbivores from both biological and physical factors (see section 2.1.1 below) and also provide food for mesoherbivores (see section 2.1.2 below) (Duffy and Hay, 1991; Brawley, 1992; Rogers *et al*, 1998). A herbivore's selection between two or more potential food items can be attributed to the attractiveness of each item. Herbivores are able to make choices both at close range via tactile and chemical sampling, and over larger distances by means of chemoreception (Watson and Norton, 1985a).

2.1.1 Algae as a habitat

Generally, habitat structure is composed of two major factors: complexity and heterogeneity (McCoy and Bell, 1991). Complexity incorporates variation in habitat structure characteristic of the absolute abundance of individual structural components, which are the distinct physical elements of a habitat *e.g.* rocks, pools and crevices (McCoy and Bell, 1991). Heterogeneity incorporates variation in habitat structure attributable to variation in the relative abundance of different structural components (McCoy and Bell, 1991). The structural shelter provided by algal fronds to mesoherbivores protect against wave action (Wieser, 1952), desiccation (Wieser, 1952) and predation (Duffy, 1989; Drake *et al*, 1995). *Littorina littorea* are often found in dense clusters upon algae (Newell, 1958), and it has been suggested that aggregation could offer protection from environmental factors such as predation, desiccation and wave action. It is considered that aggregation is aided by trail following (Davies and Beckwith, 1999).

2.1.1.1 Trail following

During movement the pedal gland of *L. littorea* secretes mucus, which is deposited on the substratum as a trail, over which the animal glides or crawls (Bretz and Dimock, 1983; Davies and Beckwith, 1999). It is known that *L. littorea* follow mucus trails (see Davies and Beckwith (1999) and references therein), though the purpose of this behaviour is still unclear. As well as offering protection from environmental factors such as wave action, aggregation may also increase humidity within the microenvironment (Lalli and Parsons, 1997) thus reducing desiccation rates. Aggregation may also provide protection against predators by reducing an individual's chances of being preyed upon (Davies and Beckwith, 1999). Another theory suggesting why trail following may occur is that mucus trails may be a potential food source to other *L. littorea*, as they are thought to contain a supply of nutrients (Davies *et al*, 1992), as proteins and carbohydrates constitute the principle components of pedal mucus (Bretz and Dimock, 1983). If *L. littorea* follow mucus trails to acquire a proportion of their nutritional requirement, variation in behaviour may occur as a result (Davies and Beckwith, 1999). *Littorina littorea* prefer to follow the trails of conspecifics rather than their own and are more likely to follow a trail with microalgae in it (Davies and Beckwith, 1999). Therefore, it can be concluded that *L. littorea* use trail following for grazing purposes. As trail following may be an important factor affecting the behaviour of *L. littorea* it may have an impact on preference behaviour. Individual snails may not be demonstrating a preference for an alga because they consider it to be the most attractive; rather they may be following a trail previously set by themselves or a conspecific. To account for this potential complication, the methodology outline below (see section 2.2.2.1 below) was designed to eliminate trail following behaviour. There is no evidence in the literature to suggest that *I. granulosa* use cues produced by themselves or conspecifics to aid their foraging behaviour, therefore it was not required to be considered during the experimental design.

The level of structural organisation of algae has important effects on the distribution and abundance of mesoherbivores (Nelson, 1979; Edgar, 1983c; Dean and Connell, 1987a; Johns and Mann, 1987; Gibbons, 1988; Hacker and Steneck, 1990; Martin-Smith, 1993; Jarvis and Seed, 1996; Beck, 2000; Ruitton *et al*, 2000; Schreider *et al*, 2003; Kraufvelin and Salovius, 2004; McAbendroth *et al*, 2005). Studies have shown that species richness, total animal density and population densities increase with habitat complexity (Dean and Connell, 1987a; Jarvis and Seed, 1996; Beck, 2000; Kraufvelin and Salovius, 2004). However, other studies have found the opposite relationship to exist, with more complex habitats having lower species richness, abundance, and diversity (Edgar, 1983c; Schreider *et al*, 2003).

2.1.1.2 Protection from predators

As introduced in section 2.1.1 above, many algae provide herbivores with protection from predators. Holomuzki and Short (1988) reported that densities of the stream-dwelling isopod *Lirceus fontinalis* Rafinesque were higher on *Cladophora* sp. than on the bare substratum. *Lirceus fontinalis* do not feed upon algae, instead they consume decaying leaf tissue (Holomuzki and Short, 1988) indicating that they use *Cladophora* sp. predominately has a habitat. The use of *Cladophora* sp. by *L. fontinalis* was not dependent on the presence of predatory fish; apart from in microhabitats such as small pools (Holomuzki and Short, 1988). Under experimental conditions in the laboratory, the emergence of grazers from algae in pools was reduced in the presence of fish, and entrances into algae and movements between open areas decreased (Holomuzki and Short, 1988). For some organisms, predation risk decreases with increasing algal biomass and structural complexity (Nelson, 1979; Stoner, 1982), therefore mesoherbivores can reduce their risk of detection by visually searching predators (such as fish) by preferring microhabitats that provide the most protection (Merilaita and Jormalainen, 1997). *Idotea* are at risk from predation by shrimp, crabs and fishes (Bruno and O'Connor, 2005). Steingrund and Ferno (1997) recorded high predation on *I. baltica* by *Gadus morhua* Linnaeus. Smelt *Osmerus eperlanus* Linnaeus, several species of pipefish in the genus *Syngnathus* spp., the rocklings *Rhononemus cimbrius* Linnaeus and *Ciliata mustela* Linnaeus, bass *Dicentrarchus labrax* Linnaeus, corkwing *Crenilabrus melops* Linnaeus, weever fish *Trachinus vipera* Cuvier, the gobies *Pomatoschistus minutus* Pallas, *Gobius niger* Linnaeus and *Gobius paganellus* Linnaeus, shanny *Blennius pholis* Linnaeus, bull rout *Myoxecephalus scorpius* Linnaeus and lumpsucker *Cyclopterus lumpus* Linnaeus are also known to consume isopods, particularly *Idotea* spp. (Wheeler, 1969).

Mesoherbivores can also escape predation from visually searching predators through camouflage (Hacker and Madin, 1991). If an organism is of similar colour to its environment, predators will be less likely to spot individuals, thus increasing the chance of individual survival. *Idotea* use camouflage as they display genetically determined colour polymorphism. In colour polymorphic organisms, the crypsis (how concealed an organism is within its habitat) of different colour morphs can vary between visually different microhabitats (Merilaita and Jormalainen, 1997). Individuals with dark, uniform colouration are easily concealed on brown algae (Merilaita and

Jormalainen, 1997, 2000) and some darker red algae, but would be visible to predators when on green algae. Individuals that are lighter and greener in colour are more camouflaged on green algae, and some of the lighter-coloured brown algae. However, in an experiment with *albafusca* (white-spotted) and *uniformis* (unicoloured) individuals of *I. baltica*, Merilaita and Jormalainen (1997) found no colour morphic dependence on microhabitat choice upon *Fucus* with and without white epizoites. Although their evidence suggests that different colour morphs of *I. baltica* displayed no preference in habitat, there is no information on whether *I. granulosa* may choose habitats in relation to colour morph. Additionally, the colour perceived may alter not only between herbivores, but also with depth and differing water conditions.

2.1.2 Algae as a food source

Mesoherbivore habitat preferences are often closely related to food selection, as these organisms live on their source of food. However, the algae that offer the best habitat and protection may not be the algae with the highest food values (Nicotri, 1980; Salemaa, 1987; Buschmann, 1990; Duffy and Hay, 1994; Toth and Pavia, 2002). The feeding preferences of mesoherbivores occupying algae may have fundamental effects on algal communities (Pavia *et al*, 1999a) as mesoherbivores often inhabit algae covered with epiphytes, and in some cases the epiphytic macroalgae are preferred over the host algae (Nicotri, 1980; Gunnill, 1982; Shacklock and Doyle, 1983; D'Antonio, 1985; Brawley and Fei, 1987; Arrontes, 1990; Duffy, 1990; Anderson *et al*, 1998; Bostrom and Matilla, 1999; Karez *et al*, 2000; Ruesink, 2000). Branched, filamentous epiphytes usually have a high level of structural complexity, which can affect the suitability of a host plant as a habitat for mesoherbivores (Pavia *et al*, 1999a), both by making the host plant more attractive to herbivores, and by providing a suitable habitat themselves. When *I. baltica* were presented with a choice between living and artificial seagrass as shelter, they preferred live epiphytes on the artificial plants (Bostrom and Mattila, 1999). When choosing between food and shelter they again preferred the live epiphytic food over shelter provided by both artificial and natural plants (Bostrom and Mattila, 1999). However, when choosing in the presence of a predatory fish *I. baltica* showed no preference for shelter (Bostrom and Mattila, 1999). In an investigation by Pavia *et al* (1999a) densities of *I. granulosa* were significantly higher on *A. nodosum* plants with epiphytes than those plants without epiphytes, suggesting that *I. granulosa* may utilise algal epiphytes as a habitat. However, this interaction effect was only significant at some sites (Pavia *et al*, 1999a). In feeding preference experiments, large individuals of *I. granulosa* significantly consumed young, meristematic parts of *A. nodosum*, whereas small individuals significantly consumed the epiphytes *Ceramium nodulosum* (Lightfoot) Ducluzeau and *Pilayella littoralis* (Linnaeus) Kjellman (Pavia *et al*, 1999a), implying that grazers utilise epiphytes as a source of food. However, some mesoherbivores have been shown to preferentially consume the host plant (Arrontes, 1990; Duffy, 1990; Poore, 1994).

Inter-specific plant differences between habitat and food choice have also been observed. Toth and Pavia (2002) investigated the habitat choice and feeding preferences of the specialist limpet *Ansates pellucida* Linnaeus and the generalist littorinid *Lacuna vincta* Montagu upon *Laminaria hyperborea* (Gunnerus) Foslie. Regarding habitat choice, a significantly higher number of *A. pellucida* were found on old fronds of *L. hyperborea* than new fronds in both the natural environment and laboratory choice experiments (Toth and Pavia, 2002). There was no significant difference in the natural distribution for *L. vincta* (Toth and Pavia, 2002). However, when presented with a choice in the laboratory they significantly preferred new frond material to old (Toth and Pavia, 2002). Regarding food choice, *L. vincta* significantly preferred new fronds of *L. hyperborea*, whereas no significance in consumption was observed for *A. pellucida* (Toth and Pavia, 2002). The results suggest that the habitat choice for *L. vincta* was in accordance with food preference, whereas the habitat choice of *A. pellucida* may possibly be due to some other factor. Toth and Pavia (2002) compared both the tissue nitrogen and phlorotannin content between old and new *L. hyperborea* fronds and found no significant differences between them. They therefore suggested that habitat choice in *A. pellucida* may be determined by plant toughness (Toth and Pavia, 2002). The older fronds of *L. hyperborea* will be tougher than new growth, and so may provide a more protective and stable habitat.

2.1.3 Between-group variation in the attractiveness of algal functional groups

In order to assess the utility of the functional group approach, the work of other authors (presented below) will be discussed in the context of Steneck and Watling's (1982) functional group model (see section 1.3.2 of chapter 1 for characteristics of functional groups). Nicotri (1980) investigated the attractiveness of a range of algae to *I. baltica* in paired choice experiments. Algae were assigned a preference ranking (high, medium and low) based upon their attractiveness to *I. baltica*. With the exception of two algal species (*Spongomorpha* sp. Kutzing from functional group two and *Codium* sp. Stackhouse), all of the algae assigned a 'high' preference ranking were algae from functional groups five and six (Nicotri, 1980). *Codium* sp. was not assigned a functional group as it does not clearly fit any of the functional groups described by Steneck and Watling (1982) (Phillips *et al*, 1997). The algae considered in the 'medium' preference category (such as *Petalonia* sp. Derbes and Solier and *Chondrus* sp.) were from functional groups three and four, with algae comprising the 'low' preference category from functional groups two and three, for example *Ceramium* sp. and *U. lactuca* (Nicotri, 1980). Nicotri (1980) compared the results observed for *I. baltica* with those collected for *L. littorea* by Lubchenco (1978). This was possible due to similar methodologies being used for both investigations, though not all of the same algal species were tested. For *L. littorea*, all of the algae with a 'high' preference ranking were algae from functional groups two (*e.g. Spongomorpha* sp.) and three (*e.g. U. intestinalis*). The one alga assigned a 'medium' preference ranking was from functional group three, while all of the algae from the 'low' preference category were those from functional groups four and five (such as *Chondrus* sp. and *Fucus* sp.) (Nicotri, 1980).

Watson and Norton (1985a) also tested the attractiveness of a range of algae using paired choice experiments. Again, evaluating their findings in the context of functional groups showed that algae from functional group three were the most attractive to *L. littorea*, with algae from functional groups four and five comprising a second preference category and *C. officinalis* from functional group six the least attractive alga (Watson and Norton, 1985a). In another experiment, both *Littorina obtusata* Linnaeus and *Littorina mariae* Sacchi et Rastelli were attracted to fucoids, while *C. rupestris* and *U. lactuca* (functional groups two and three respectively) were avoided by both grazers (Watson and Norton, 1987). The preference among fucoids differed between the grazers, with *L. obtusata* preferring *F. vesiculosus* whereas *L. mariae* found *F. spiralis* more attractive (Watson and Norton, 1987).

2.1.4 Aims

In the following laboratory experiments, the aims were: i) to determine whether algal attractiveness would decrease from functional group two to functional group six for both *L. littorea* and *I. granulosa*; and ii) to determine for both grazers if all algae within a functional group were similarly attractive.

2.2 Methods

2.2.1 Study Sites

Samples were collected from two study sites: Holbeck, North Yorkshire, UK (54°16'N, 0°25'W) and Filey Brigg, North Yorkshire, UK (54°13'N, 0°16'W). Holbeck lies to the south of Scarborough's sandy South Bay. The shore is moderately exposed and is comprised of a large wavecut limestone platform. The algal community is largely dominated by a canopy of fucoid algae throughout the year and ephemeral green algae in the spring and summer months. There are moderately high abundances of understorey, turf, and encrusting algal species. The intertidal fauna is dominated by littorinids, patellids and mussels. Filey Brigg lies to the north of the sandy Filey Bay. The Brigg is comprised of hard limestone that projects approximately 1 km into the North Sea in an east/south-east direction. The north side of the Brigg is exposed to wave action which has formed rock platforms combined with large boulders. The south side is more sheltered and is comprised mainly of rock platforms with some large pools. Changes in abundance of both flora and fauna occur along the Brigg. Fucoid cover is patchy, mainly due to high abundances of mussels and barnacles. There are also high numbers of littorinids and patellids. The more sheltered areas of the Brigg are high in abundances of ephemeral green and filamentous red algae.

2.2.2 Between- and within-group variation in the attractiveness of algal functional groups

Attractiveness experiments were conducted in which grazers were presented with algae in two-way choice experiments. Preliminary experiments were performed

(Appendix I), after which the experimental design was modified as outlined below (see section 2.2.2.1)

2.2.2.1 *Littorina littorea*

Mature *L. littorea* (mean shell height 2.02 ± 0.01 cm S.E.) and 5 species of algae representing functional groups two to six (Table 1.1, chapter 1) were collected between October 2003 and May 2004 from Holbeck (see section 2.2.1 above for site description). Following collection *L. littorea* were food deprived for four days in filtered seawater to ensure that they were not satiated prior to the start of experiments (see section 3.4.4.1 of chapter 3 for discussion on the effects of food deprivation on attractiveness preferences). Algae were cleaned to remove epifauna and epiphytes by washing in running seawater and examination under a dissecting microscope. Epifauna were removed so that any changes observed were due to the experimental grazers and not other fauna attached to the algae. Removing epiphytes ensured that any changes observed were as a result of preference for the experimental algae, rather than the epiphytes. All algae used in experiments were mature plants with no reproductive tissue. Twenty *L. littorea* were placed in experimental tanks (18 x 12.5 x 5 cm) with 600 ml of filtered seawater and allowed to explore their surroundings for 30 minutes, and thereby acclimate to the experimental conditions. After 30 minutes all snails were returned to the centre of the experimental tank and equal weights of two algal species were introduced in paired choice experiments with one species at each end of the tank. If *L. littorea* employ trail following during foraging (as discussed in section 2.1.1.1 above), it is assumed the volume of mucus laid down during the exploratory period may have been so great and so prevent individual trails from being utilised. Experiments were left to run for a further 30 minutes, during which the snails were allowed freedom of movement throughout the experimental tank. After this period the number of *L. littorea* on each alga was counted. To identify any difference in attractiveness between functional groups, one alga from each functional group (two to six) was presented in combination with algae from every other functional group (resulting in a total of 10 combinations). The experiment was repeated six times for each combination, using new snails for each replicate. An attractiveness hierarchy was determined by summing the total number of *L. littorea* choosing each alga over all combinations involving that alga and dividing by the total number of *L. littorea* present in each set of experiments (adapted from Nicotri, 1980):

$$\text{Number of grazers choosing algae from each functional group} = \frac{\sum n}{n_r rc} \qquad \text{(Equation 1)}$$

Where $\sum n$ = total number of grazers choosing each alga

n_r = number of grazers in each replicate

r = number of replicates of each combination involving alga

c = number of combinations involving alga.

2.2.2.2 *Idotea granulosa*

Mature *I. granulosa* (mean body length 1.68 ± 0.01 cm S.E.) and algae representing functional groups two to six (Table 1.1, chapter 1) were collected during November 2004 from Holbeck (see section 2.2.1 above for site description). Following collection *I. granulosa* were stored until use in filtered seawater with *U. lactuca* providing food and shelter and algae were cleaned to remove epifauna and epiphytes by washing in running seawater and examination under a dissecting microscope. Epifauna were removed so that any changes observed were due to the experimental grazers and not other fauna attached to the algae. Removing epiphytes ensured that any changes observed were as a result of preference for the experimental algae, rather than the epiphytes. All algae used in experiments were mature plants with no reproductive tissue. Six *I. granulosa* of similar colouration were placed in experimental tanks (17 x 11.5 x 4 cm) with 200 ml of filtered seawater containing equal weights of two algal species in paired choice experiments with one species at each end of the tank. Experiments were left to run for 7 days, during which the grazers were allowed freedom of movement throughout the experimental tank. The number of *I. granulosa* on each alga was counted at the same time every day for the duration of the experiment. Experiments ran for longer than those with *L. littorea* as it is considered that idoteids utilise algae more as a habitat than littorinids, so leaving trials to run for only 30 minutes may not have accurately reflected the amount of time *I. granulosa* spend upon algae. To identify any difference in attractiveness between functional groups, one alga from each functional group (two to six) was presented in combination with algae from every other functional group (resulting in a total of 10 combinations). The experiment was repeated six times for each combination, using new *Idotea* for each replicate. An attractiveness hierarchy was determined using equation 1 (see section 2.2.2.1 above).

The data collected were used to test the null hypotheses that for both grazers there would be no significant difference in algal attractiveness from functional group two to functional group six, and that all species within a functional group would be similarly attractive to *L. littorea* and *I. granulosa.*

2.2.3 Statistical Analyses

Data analyses were conducted using the SPSS version 11.0 for Windows package. Data were screened using the Kolmogorov-Smirnov normality test and Levene's test to test for normality of distribution and homogeneity of variances respectively (Sokal and Rohlf, 1995). As the attractiveness hierarchy data calculated were proportional and the data had heterogeneous variances due to the upper and lower constraints imposed upon proportional data (Underwood, 1981), *a priori* arc-sine transformation was carried out (Fowler *et al*, 1998). Data not meeting the assumptions of homogeneity were analysed at P = 0.01 to reduce the risk of Type I error (Underwood, 1997).

2.2.3.1 Between-group variation in the attractiveness of algal functional groups

For both grazers one-way analysis of variance (ANOVA) was used to test the null hypothesis that there would be no significant difference in mean algal attractiveness from functional group two to functional group six. *Post hoc* Tukey pairwise comparisons were determined where appropriate to identify differences between the number of grazers choosing each functional group.

To determine whether the results collected supported Steneck and Watling's (1982) prediction that algae would be less attractive to grazers as functional group number increased, the data recording the number of grazers choosing each group for functional groups two to six were ranked from the most chosen group to the least chosen group, to indicate an order of preference shown by *L. littorea* and *I. granulosa.* The rank order of functional group preference recorded was compared to the rank order of functional group preference predicted by Steneck and Watling's (1982) model. The null hypothesis that both *L. littorea* and *I. granulosa* would show no significant preference for algae from functional group two to functional group six was tested using the test for non-parametric data, Spearman Rank Correlation Coefficient.

2.2.3.2 Within-group variation in the attractiveness of algal functional groups

For both grazers Chi-square tests (Fowler *et al*, 1998) were conducted using pooled replicate data to test the null hypothesis that species within a functional group would be similarly attractive for species from functional groups two to five. Data for functional group six were not included in the analysis as only one algal species (*C. officinalis*) was available from this group, and so no comparison could be made. During the experiments, some individual grazers did not make a 'choice' between the two algal species (though this was always less than half of the number of grazers). These data were omitted from the statistical tests as the number of grazers not making a choice may have skewed the data so that any differences in preference between the two algae were masked. As there were only two categories for each functional group, and thus only one degree of freedom, data were corrected using Yates' Correction for Continuity (Fowler *et al*, 1998). Data were analysed using Chi-square tests rather than Mann-Whitney *U*-tests in order to determine any difference in the frequency of grazers choosing each alga.

2.3 Results

The effect of algal attractiveness on the food preferences of *L. littorea* and *I. granulosa* were investigated by presenting grazers with a choice of two algae simultaneously and counting the number of grazers on each species, which was assumed to indicate a choice preference.

2.3.1 Data Screening

The results of the Kolmogorov-Smirnov normality test showed that for both grazers the data at both functional group and species level were normally distributed (Kolmogorov-Smirnov, $P > 0.15$). After arc-sine transformation, data for *L. littorea* showed no significant departure from homogeneity (Levene's, $L_{4,115} = 0.847$, $P > 0.15$); therefore, it can be assumed that all samples were drawn from populations with equal or similar variances. Transforming the data for *I. granulosa* was not sufficient to achieve homogeneity of variances (Levene's, $L_{4,835} = 39.290$, $P < 0.05$), however as ANOVA is robust to departures from the assumption (Underwood, 1997) it was appropriate to proceed with the analysis. To minimise the increased risk of Type I error, data were analysed at significance level $P = 0.01$ (Underwood, 1997).

2.3.2 Between-group variation in the attractiveness of algal functional groups

2.3.2.1 *Littorina littorea*

The number of *L. littorea* choosing algae from each functional group varied, with the grazers showing an obvious preference for functional group two with an average of 45.25 ± 2.99 *L. littorea* (mean ± standard error, n = 24) and the least preferred being functional group six with an average of 9.00 ± 1.47 (mean ± standard error, n = 24) grazers choosing algae from this group (Fig. 2.1).

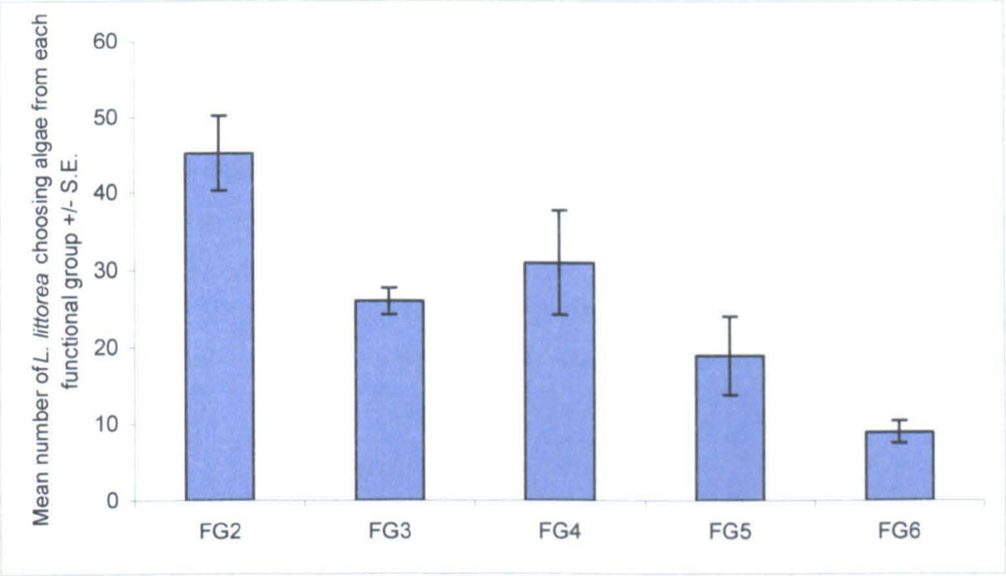

Figure 2.1 – Mean number of *Littorina littorea* choosing algae from each functional group (± standard error) in two-way choice experiments (n = 120).

The difference in choice exhibited by *L. littorea* showed a general decrease from functional group two to functional group six (Fig. 2.1). The one-way ANOVA comparing the mean number of grazers choosing algae from each functional group showed a significant difference in choice between functional groups (Table 2.1).

Table 2.1 – One-way analysis of variance to investigate the attractiveness of algal functional groups to *Littorina littorea* using arc-sine transformed data (level of significance *** $P < 0.001$).

Source of Variation	df	SS	MS	F	*P*
Between Groups	4	286.981	71.745	17.982	< 0.001***
Within Groups	115	458.837	3.900		
Total	119	745.818			

Post hoc Tukey pairwise comparison ($P = 0.05$) revealed that *L. littorea* found algae from functional group two significantly more attractive than algae from functional groups three, five and six; functional group three was significantly more attractive than functional group 6; and algae from functional group four was significantly more attractive than those from functional groups five and six, but these groups were not significantly different from each other (Fig. 2.1, Table 2.2).

Table 2.2 – *Post hoc* Tukey pairwise comparison revealing differences in the attractiveness of algal functional groups to *Littorina littorea* (functional groups listed down the left hand side were significantly more attractive than functional groups listed across the top where marked by *).

	FG2	FG3	FG4	FG5	FG6
FG2		*		*	*
FG3					*
FG4				*	*
FG5					
FG6					

2.3.2.2 *Idotea granulosa*

The number of *I. granulosa* choosing algae from each functional group also varied, with the grazers again showing an obvious preference for algae from functional group two with an average of 33.21 ± 1.08 *I. granulosa* (mean ± standard error, n = 24) choosing algae from this group. The least preferred algae were those from functional group five with an average of 4.25 ± 0.58 (mean ± standard error, n = 24) grazers choosing algae from this group (Fig. 2.2).

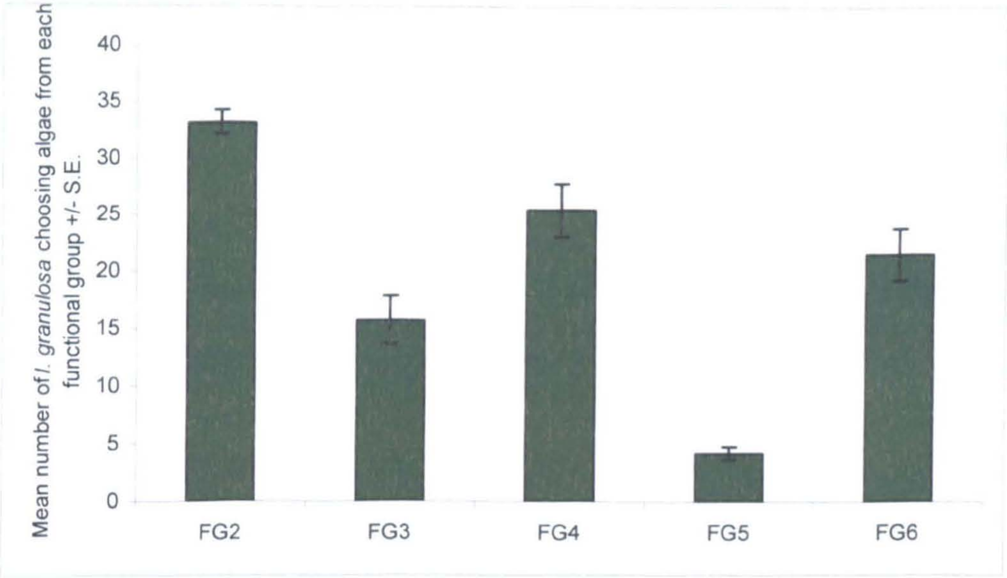

Figure 2.2 – Mean number of *Idotea granulosa* choosing algae from each functional group (± standard error) in two-way choice experiments (n = 120).

The difference in choice exhibited by *I. granulosa* showed no general pattern in choice from functional group two to functional group six (Fig. 2.2), however when the mean number of grazers choosing algae from each group were compared using one-way ANOVA, the difference in choice between the groups was significant (Table 2.3).

Table 2.3 – One-way analysis of variance to investigate the attractiveness of algal functional groups to *Idotea granulosa* using arc-sine transformed data (level of significance *** $P < 0.001$).

Source of Variation	df	SS	MS	F	*P*
Between Groups	4	6173.650	1543.412	147.854	< 0.001***
Within Groups	835	8710.547	10.432		
Total	839	14884.107			

Post hoc Tukey pairwise comparison ($P = 0.05$) revealed that algae from functional group two was significantly more attractive to *I. granulosa* than algae from functional groups three, four, five and six; functional group three was significantly more attractive than functional group five; and algae from functional groups three and five were significantly less attractive than those from functional groups four and six, but these groups were not significantly different from each other (Fig. 2.2, Table 2.4).

Table 2.4 – *Post hoc* Tukey pairwise comparison revealing differences in the attractiveness of algal functional groups to *Idotea granulosa* (functional groups listed down the left hand side were significantly more attractive than functional groups listed across the top).

	FG2	FG3	FG4	FG5	FG6
FG2		*	*	*	*
FG3				*	
FG4		*		*	
FG5					
FG6		*		*	

The data collected suggest that algal attractiveness to *L. littorea* generally matches the functional group model predicted by Steneck and Watling (1982) as the number of grazers choosing algae from each group showed a decrease from functional group two to functional group six (Fig. 2.1). However, the rank order of algal edibility recorded was not significantly related to that predicted by Steneck and Watling (1982) (Spearman Rank Correlation Coefficient, $r_s = 0.90$, $P > 0.1$) (Fig. 2.3). Algal attractiveness to *I. granulosa* did not follow the pattern predicted by Steneck and Watling's (1982) model as although they found algae from functional group two the most attractive, the number of grazers choosing algal from groups three to six fluctuated (Fig. 2.2). As such, the rank order of algal edibility recorded was not significantly related to that predicted by Steneck and Watling (1982) (Spearman Rank Correlation Coefficient, $r_s = 0.71$, $P > 0.05$) (Fig. 2.3).

Both correlation coefficients showed a strong positive relationship. Data were analysed using Spearman Rank Correlation Coefficient as they were non-parametric and thus did not meet the assumptions required for Pearson's Correlation Coefficient. Had Pearson's Correlation been computed, it is more likely that the correlation coefficients would have been significant, given the strength of the relationships and the small sample sizes tested.

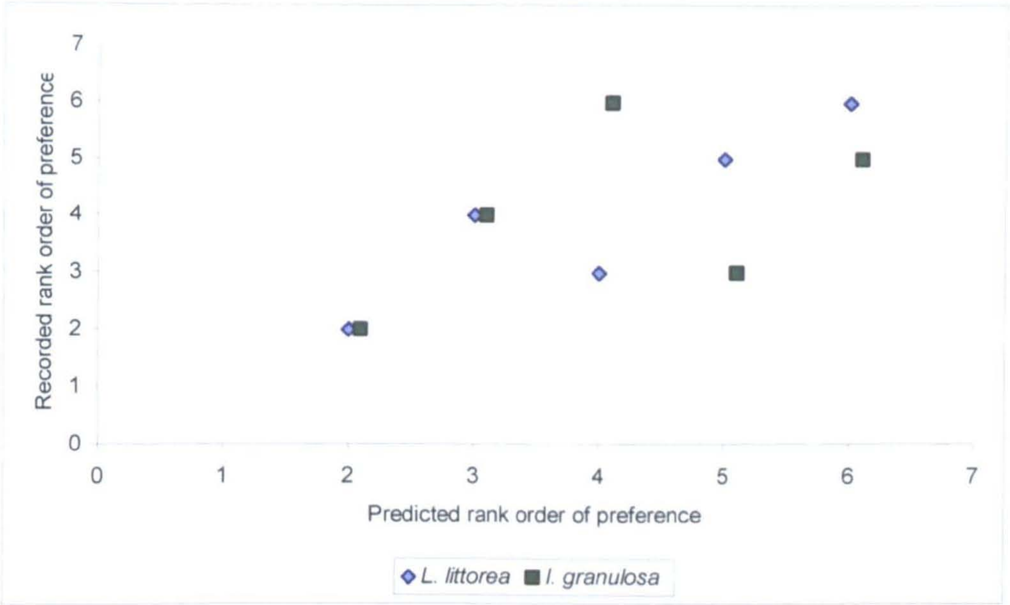

Figure 2.3 - Relationship between the rank order of functional group preference as predicted by Steneck and Watling (1982) and the rank order of functional group preference recorded for *Littorina littorea* (n = 5) and *Idotea granulosa* (n = 5). NB: data points for *I. granulosa* are off set by 0.1 to increase clarity of the figure.

2.3.3 Within-group variation in the attractiveness of algal functional groups

2.3.3.1 *Littorina littorea*

Differences were exhibited in the number of *L. littorea* selecting each algal species from the same functional group, when these algae were offered in combination with each other (Fig. 2.4).

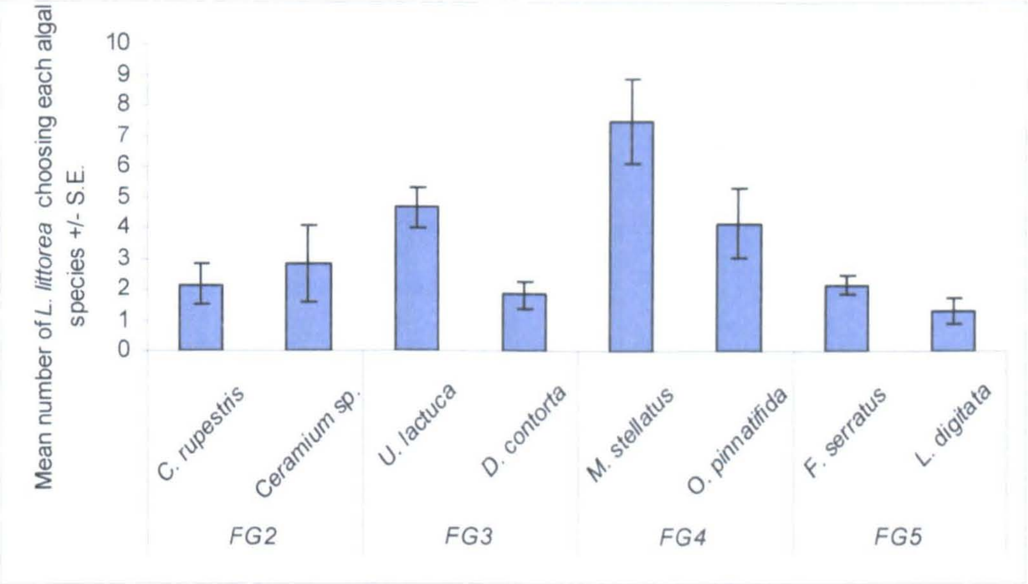

Figure 2.4 – Mean number of *Littorina littorea* choosing each algal species (± standard error) when presented in combination with algae from the same functional group in two-way choice experiments (n = 48).

The results of the Chi-square tests suggest that species' within functional groups three ($\chi^2_1 = 7.44$, $P < 0.01$) and four ($\chi^2_1 = 5.73$, $P < 0.05$) were significantly different in attractiveness to *L. littorea*. Within functional group three more grazers chose *U. lactuca* (mean = 4.67 ± 0.67 standard error, n = 6) than *D. contorta* (mean = 1.83 ± 0.48 standard error, n = 6) (Fig. 2.4), while *M. stellatus* (mean = 7.50 ± 1.38 standard error, n = 6) was chosen more than *O. pinnatifida* (mean = 4.17 ± 1.14 standard error, n = 6) within functional group four (Fig. 2.4). There was no significant difference in the number of *L. littorea* choosing each algal species within functional group two ($\chi^2_1 = 0.57$, $P > 0.05$) or functional group five ($\chi^2_1 = 1.24$, $P > 0.05$) (Fig. 2.4), which suggests that the species presented within these groups were similarly attractive to *L. littorea*.

2.3.3.2 *Idotea granulosa*

Algal species within functional groups two and three were chosen in similar numbers by *I. granulosa*, with more of a preference shown between algae within functional groups four and five (Fig. 2.5).

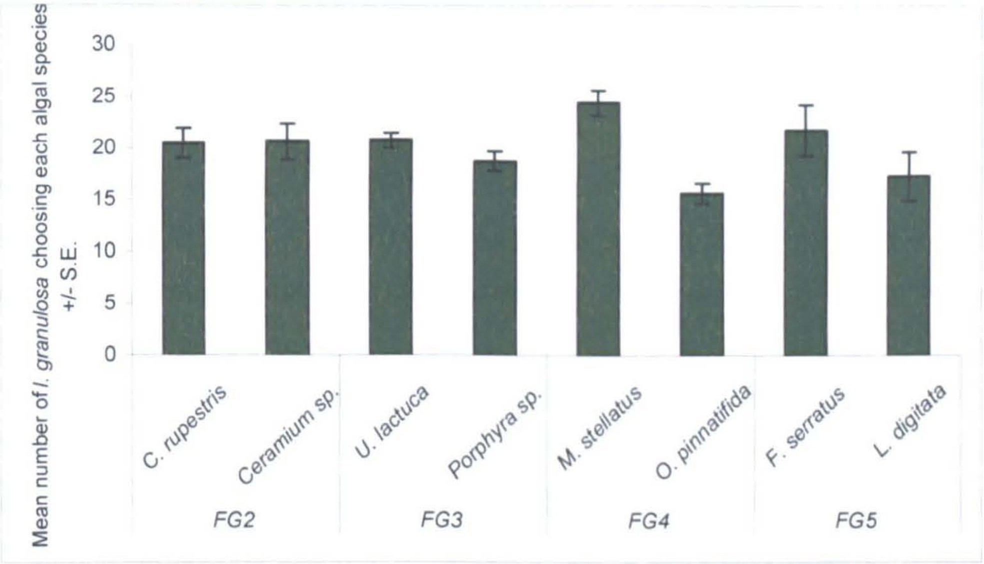

Figure 2.5 – Mean number of *Idotea granulosa* choosing each algal species (± standard error) when presented in combination with algae from the same functional group in two-way choice experiments (n = 48).

However, the results of the Chi-square tests suggest that only species' within functional group four (χ^2_1 = 11.66, $P < 0.01$) were significantly different in attractiveness to *I. granulosa* with more grazers choosing *M. stellatus* (mean = 24.5 ± 1.18 standard error, n = 6) than *O. pinnatifida* (mean = 15.67 ± 1.00 standard error, n = 6) (Fig. 2.5). There was no significant difference in the number of *I. granulosa* choosing each algal species within functional group two (χ^2_1 = 0.01, $P > 0.05$), functional group three (χ^2_1 = 0.61, $P > 0.05$), or functional group five (χ^2_1 = 3.11, $P > 0.05$) (Fig. 2.5), which suggests that the species presented within these groups were similarly attractive to *I. granulosa*.

2.4 Discussion

Using ideas from the functional group model proposed by Steneck and Watling (1982), it was suggested that algal species varied in their attractiveness to grazing herbivores based upon their morphological and anatomical features (Table 1.1, chapter 1). The hypothesis proposed was that as algal structural complexity increases through functional groups, the attractiveness of each species to grazers is decreased. This hypothesis was supported by data collected for *L. littorea*, but not for *I. granulosa*.

2.4.1 Between-group variation in the attractiveness of algal functional groups

The preferences of *L. littorea* with regard to algal attractiveness differed between functional groups (Fig. 2.1, Table 2.1); with an overall preference for algae from functional group two, the filamentous algae (Fig. 2.1). When Nicotri (1980) assigned the attractiveness data collected by Lubchenco (1978) for *L. littorea* into preference categories, all of the algae with a 'high' preference ranking were algae from functional groups two and three (see section 2.1.3 above). In the current study, algae from functional group four were the second most attractive to *L. littorea.* In another attractiveness investigation, Watson and Norton (1985a) reported that algae from functional groups four and five comprised a second preference category to algae from the 'preferred' functional group three. It is perhaps surprising that algae from functional group five were not preferred over algae from functional groups three and four by *L. littorea*, given that it dominates on the shore from which grazers were collected (see section 2.2.1 above) and so is widely available as a habitat. Watson and Norton (1987) attributed *L. obtusata*'s preference for *F. vesiculosus* to that fact that the grazers occupy an area of the shore dominated by canopy fucoid algae and so use fucoids as a habitat. When *L. obtusata* were presented with algae in the laboratory they were more attracted to the fucoid algae, rather than *C. rupestris* and *U. lactuca*, which are usually characterised as understorey species (Watson and Norton, 1987). But as Williamson and Creese (1996) recognize, the interactions between littorinids and habitat structure are not straightforward. In all investigations, *C. officinalis* (functional group six) was the least preferred algae to *L. littorea* with regard to algal attractiveness (Fig. 2.1; Watson and Norton, 1985a); which supports the functional group model as it is considered to be a structurally complex algal species (Steneck and Watling, 1982). The attractiveness of an alga incorporates both its suitability as a habitat and its availability as a food source (see section 1.2 of chapter 1 for full discussion). For *L. littorea*, less structurally complex habitats may be preferred as the grazers do not require as much protection due to their protective shell (Williamson and Creese, 1996). Therefore the habitat requirements of *L. littorea* may be influenced more by their requirement for food than refuge.

Idotea granulosa also found algae from functional group two to be the most attractive (Fig. 2.2, Table 2.3), possibly because the dense growth form of filamentous algae not only provide mesoherbivores with a habitat comparatively inaccessible to external predators, they also provide an abundant food resource (Edgar, 1983b). However, these results contradict those collected by Nicotri (1980) for *I. baltica*. Relating Nicotri's (1980) results to functional groups, *I. baltica* displayed a preference for algae from functional groups five and six, with algae from functional groups two and three the least preferred (Nicotri, 1980). In the current study, algae representing functional group three had simple, flat, membranaceous thalli (Edgar, 1983a) and so were not very structurally complex. While they may have provided *I. granulosa* with a good source of food (Fig. 3.4, chapter 3), they did not offer much protection as a habitat. Algae from functional groups four and six were also chosen in high numbers by *I. granulosa* (Fig. 2.2). As predation risk decreases with increasing algal biomass (Nelson, 1979; Stoner, 1982) *I. granulosa* utilising *C. officinalis* as a habitat may be better protected from predators (Akioka *et al*, 1999). Algae from functional group five were the least attractive to *I. granulosa* (Fig. 2.2). In an experiment using the amphipod *Gammarellus angulosus* Rathke, Hacker and Steneck (1990) found significantly higher densities of amphipods on algae and algal mimics with branched (functional groups two and six) and filamentous (functional group two) morphologies (such as *Ceramium* sp. and *C. officinalis*) than those with foliose (functional group three) and leathery macrophyte (functional group five) morphologies (such as *U. lactuca* and *L. digitata*). The authors attributed their results to differences in the thallus and interstitial volumes of algae. Foliose algae (functional group three) have a low thallus-to-interstitial volume ratio, whereas leathery macrophytes (functional group five) have a high thallus-to-interstitial volume (Hacker and Steneck, 1990). The thalli of both types of algae have large, undissected sheet-like fronds surrounded by interstitial space (Hacker and Steneck, 1990), which provides little available space for mesograzers to live within. Leathery algae have the higher volume of the two types due to greater cortication providing more structural support (Hacker and Steneck, 1990). Algae with filamentous (functional group two) and corticated (functional groups four and six) morphologies have intermediate thallus-to-interstitial volumes due to them having many, small, dissected fronds with more interstitial space surrounding the fronds (Hacker and Steneck, 1990). Algae with these morphologies provide mesoherbivores with more living space, which given the results collected in this study may provide evidence to support the theory that they are more attractive to mesograzers.

As with *L. littorea*, *I. granulosa* living on algae may separate the use of them as a habitat and a source of food (Buschmann, 1990). Kraufvelin *et al* (2006) showed that the habitat preference hierarchy for *Gammarus locusta* Linnaeus was red algae > green algae > brown algae > periphyton, which was the opposite of the food that provided the grazers with the highest growth rate in laboratory feeding preference experiments. This suggests that *G. locusta* value algae more as a habitat than a source of food.

As *I. granulosa* are mobile invertebrates, they may be able to partition their behaviour between habitat and food requirements by spending time on algae favoured as a habitat but moving to feed on more nutritious algae and then returning (Buschmann, 1990). Buschmann (1990) observed that three species of amphipod in the genus *Hyale* separated their use of algae as a habitat and a source of food by preferentially consuming *Iridaea laminarioides* Bory even though this alga had the lowest amphipod density. This suggests that the amphipods were utilising other algae species (such as *Centroceras clavulatum* (C. Agardh) Montagne, *Ahnfeltia durvillaei* (Bory) J. Agardh and *Laurencia chilensis* De Toni, Forte & Howe (Buschmann, 1990)) as habitats and moving to feed upon *I. laminarioides* as the number of amphipods utilising *I. laminarioides* increased during the night (Buschmann, 1990). Rogers *et al* (1998) investigated the diel movements of a range of mesograzers (the sea hare *Aplysia parvula* Guilding in Moerch; the sea urchin *Holopneustes purpurascens* Agassiz; and the prosobranch mollusc *Phasianotrochus eximius* Perry) using macrophytes as both a habitat and food source. All mesograzers studied exhibited diel movement on host algae (that differed in their morphologies) by occupying significantly higher positions at night than during the day. One of the factors they attributed this to was predation pressure (Rogers *et al*, 1998). Predation can strongly affect plant use by mesograzers as the avoidance of diurnally active, visually feeding predators causes them to shelter among basal fronds during the day and move up to the canopy at night (Rogers *et al*, 1998).

Although there has been no recorded incidence of diel movement by *Idotea* it may be possible that their movement behaviour between plants may change as a result of predation pressure, as many fish have been shown to be more active during the night (Jadot, *et al*, 2002; Cartamil *et al*, 2003; Pessanha and Araújo, 2003; Cartamil and Lowe, 2004; Daugherty and Sutton, 2005; Yamamoto and Tominaga, 2007). Holomuzki and Hoyle (1990) showed that in the laboratory, the amphipod *Gammarus minus* Say significantly reduced swimming activity at night in response to water conditioned with green sunfish *Lepomis cyanellus* Rafinesque. In the field, however, amphipods were only slightly less active at night when exposed to chemical cues of *L. cyanellus*. Holomuzki and Hoyle (1990) concluded that the distribution of amphipods in streams was influenced by the presence of fish, but chemical cues alone may not be important in triggering predator avoidance behaviours in nature.

Kotta *et al* (2000) suggested that habitat selection by *Idotea* is driven both by algal morphology and food quality. They presented gazers with two algae: *Pilayella littoralis* (functional group two) and *Furcellaria lumbricalis* (Hudson) Lamouroux (functional group four) and concluded that *F. lumbricalis* offered a better refuge from predators whereas *P. littoralis* provided a better quality of food. Considering algae from the three functional groups preferred by *I. granulosa* (*C. rupestris*, *M. stellatus* and *C. officinalis*) all have branched structures providing more available living space and increased food levels (Dean and Connell, 1987b). The greater habitat complexity offered by the branching may provide the grazers with protection from predators, as they can conceal themselves within the structure of the algae.

2.4.2 Within-group variation in the attractiveness of algal functional groups

As algae were arranged into functional groups based upon their morphologies, species within a functional group should be similarly attractive to grazers if they base their habitat choices upon algal form. For *L. littorea*, within group variation occurred between algae from functional groups three and four with regards to attractiveness. Within functional group three, *U. lactuca* was significantly more attractive to *L. littorea* than *D. contorta* (Fig. 2.4). Although *U. lactuca* and *D. contorta* occur within the same functional group due to the fact that they are foliose algae that are only one or two cells thick, they differ in their morphologies as *U. lactuca* has a thin, sheet-like thallus, while *D. contorta* has a tube growth form. The sheet-like thallus of *U. lactuca* may be more attractive to *L. littorea* as it may be better able to support the weight of the grazer due to its larger surface area (see section 4.4.1 of chapter 4 for further discussion). These results suggest that morphological functional groups could be split into two subgroups. This was first proposed by Steneck and Dethier (1994), whose functional group model was based upon nine functional groups rather than the seven groups of Steneck and Watling (1982) (see section 1.3.1 of chapter 1). They recommended that algae within functional group three be divided into two groups: foliose algae (functional group three) and corticated foliose algae (functional group 3.5); with *U. lactuca* representing functional group three and *D. contorta* representing the relatively more complex group 3.5. The results collected support the theory that functional groups can be divided into sub groups, as *L. littorea* found the less complex *U. lactuca* more attractive than *D. contorta.*

Both *L. littorea* and *I. granulosa* displayed a difference in attractiveness within functional group four by being significantly more attracted to *M. stellatus* than *O. pinnatifida* (Figs. 2.4, 2.5). As *M. stellatus* is a larger plant with a more leafy structure than the turf species *O. pinnatifida*, it should be able to accommodate more individual grazers, due to their being more space available within the fronds. As results for *I. granulosa* were recorded every day over a period of seven days, rather than just after 30 minutes as for *L. littorea*, the attractiveness of algae may be more related to its value as a food source. Comparison of the nutritive value of the two species within functional group four shows that *M. stellatus* has a slightly higher calorific value than *O. pinnatifida* (Marsham *et al*, 2007, Appendix II). It also contained less fat, fibre and calcium than *O. pinnatifida* and had a lower ash content, which may make *M. stellatus* a more suitable food for *I. granulosa* than *O. pinnatifida* (Marsham *et al*, 2007, Appendix II).

2.5 Summary

- For *L. littorea* algal attractiveness decreased in general from functional group two to functional group six.

- *Idotea granulosa* also found algae from functional group two significantly more attractive than algae from the other functional groups; however fewer chose algae from functional group five suggesting it was the least attractive.

- Although both grazers displayed a strong positive relationship between the rank order of algal attractiveness of functional groups and the rank order predicted by Steneck and Watling (1982), the Spearman Rank Correlation Coefficients showed no significant relationship.

- Within a functional group, *L. littorea* found algae within functional groups two and five similarly attractive, but significantly preferred *U. lactuca* over *D. contorta* within functional group three, and *M. stellatus* over *O. pinnatifida* within functional group four.

- Algae within functional groups two, three and five were found to be similarly attractive to *I. granulosa*; within functional group four *M. stellatus* was significantly preferred over *O. pinnatifida.*

- Differences observed between the two grazers may be due to variation in their requirements relating to habitat choice and food preference.

Chapter 3

Edibility of algal functional groups by intertidal grazers

3.1 Introduction

Factors determining the food choice of a herbivore are divided into a number of categories that influence grazing preference once a food is encountered: the edibility or palatability of a food; and its value in comparison with other foods (Wakefield and Murray, 1998). Herbivores differ over a range of variables, including phyla, size, mobility, feeding modes, digestive physiology, and habitat requirements. In 'response' algae will differ in their edibility, palatability, and nutritional value (Wakefield and Murray, 1998). Edibility relates to the capacity a given food item has to being consumed, whereas palatability is more dependent on how acceptable the food 'tastes' to the consumer. The nutritional value of a food item incorporates the ease with which the consumer assimilates the food, after which it uses the energy gained for growth and replacement of tissues. Key determinants of algal edibility that may control the capacity of a grazer to consume a plant include size, shape, and material composition (Steneck and Dethier, 1994). In contrast, chemical deterrents that may affect algal palatability usually involve taste and so could be important in modifying a herbivore's subsequent foraging behaviour, if a grazer is capable of memory and of visually or chemically recognising a previously encountered plant species (Steneck and Dethier, 1994). If a food requires the consumer to utilise a lot of energy during digestion, absorption and assimilation, its nutritional value will be low.

Factors determining the feeding behaviour of prosobranch molluscs such as *L. littorea* are yet to be fully understood, but are thought to involve both the ability of a herbivore to recognise potential food items and the specific digestive capabilities of that herbivore (Imrie *et al*, 1990). Imrie *et al* (1990) have also suggested that most studies have concentrated on the ability herbivores have to process food items, whilst assuming factors determining preference at pre-ingestive stages of feeding were less relevant on account of the apparent excess of food in natural communities. The contribution that an alga makes to the nutrition of a grazer is dependent on both the total value of the component being considered (*i.e.* organic matter, calories, protein etc) and on the rate at which the alga is ingested (Nicotri, 1980). The nutritive value of an alga is considered to be important in determining food choice and may also affect the algal species consumed by herbivores, especially at within-functional group levels (Steneck and Dethier, 1994).

It has been suggested that herbivores may be nitrogen-limited (Mattson, 1980; Edwards *et al*, 2006) and may therefore prefer foods with higher nitrogen concentrations (Van Alstyne *et al*, 2001). Vegetative algal thalli low in both nitrogen and dietary availability of nitrogenous compounds may thus act as a limiting resource to herbivores (Mattson, 1980; White, 1985). Herbivores might therefore be expected to preferentially consume algae rich in nitrogen in order to meet their nitrogen requirements (Wakefield and Murray, 1998). However, preferred foods of herbivores are not always those of greatest nutritional quality. Previous research (Carefoot, 1967, 1970; Himmelman and Carefoot, 1975; Steneck, 1982; Watanabe, 1984) failed to detect a consistently strong relationship between nutritional content and algal position in preference rankings (Wakefield and Murray, 1998).

The nutritional state of the grazer may affect its food choices. Grazers are often food deprived for time periods between 24 hours to four days prior to feeding preference experiments in order to ensure they consume a measurable amount of an alga before it grows or deteriorates (*e.g.* Schaffelke *et al*, 1995; Iken, 1999; Van Alstyne *et al*, 1999, 2001; Hanley *et al*, 2003; Gauslaa, 2005; Sorensen *et al*, 2005). However, the effect of food deprivation is unclear as it is not known whether herbivores respond to food deprivation by being less selective or if food deprivation affects preferences for intact algae (Van Alstyne *et al*, 1999) (see section 3.4.4.1 below for further discussion). Watson and Norton (1985a) suggest that edibility is not influenced by the nutritional state of the grazer, and any increases in the edibility of a species may result from higher nutritional value or from a reduction in anti-herbivore defences.

3.1.1 Algal grazing by *Littorina* and *Idotea*

Differences in food choice exhibited by grazers may be due to differences in feeding apparatus between species (Van Alstyne *et al*, 2001). All grazing molluscs use radulae in feeding (Hawkins and Hartnoll, 1983). The form of radula and feeding movements are adapted to the types of food consumed (Hawkins and Hartnoll, 1983). The radulae of mesogastropods such as *Littorina* are taenioglossate, *i.e.* have reduced numbers of teeth (Brusca and Brusca, 2002), allowing them to consume a variety of algae, including microalgae, small encrusting forms and fleshy macroalgae (Hawkins and Hartnoll, 1983; Wilhelmsen and Reise, 1994; Alyakrinskaya, 2005). Other adaptations include larger rachidian and lateral teeth, which along with the strengthening of the muscular jaws allow grazers such as *Littorina* to rasp the substratum and surface cell layers of algae (Hawkins and Hartnoll, 1983; Brusca and Brusca, 2002; Alyakrinskaya, 2005). The greater flexibility and thus stronger forces exerted in feeding are facilitated by more mobile odontophore cartilages, which are used in both the retraction process and the erection of radula teeth against substratum (Hawkins and Hartnoll, 1983).

Raffaelli (1985) investigated the relationship between the diets of herbivorous molluscs and feeding morphology. Gut content analysis of a range of molluscan herbivores revealed that herbivores with mouthparts of the same morphology did not consume the same species of algae (Raffaelli, 1985). Other factors such as algal availability, consumption of non-plant material and the way the radula are utilised may be more important in determining herbivore feeding choices (Raffaelli, 1985).

A variety of crustaceans graze upon algae but many of them are omnivorous (Hawkins and Hartnoll, 1983). Isopods such as *Idotea* have a feeding mechanism which is similar in the major herbivore genre but shows no obvious adaptations to an algal diet (Hawkins and Hartnoll, 1983). Macrophagous, herbivorous, and scavenging crustaceans feed by holding on to a food source and biting off small portions with the mandibles whilst the other mouthparts assist by holding food and pushing it into the mouth (Hickman, 1967; Hawkins and Hartnoll, 1983; Brusca and Brusca, 2002).

Both *Littorina* and *Idotea* have feeding structures that enable them to feed on morphologically defended algal species, *i.e.* those with thalli composed of cells which have structural fibres in their cell walls (Padilla, 1989). However, algal toughness can cause severe radula damage to *L. littorea* feeding on crustose algae (Bertness *et al*, 1983). *Littorina littorea* grazing on the crustose species *Hildenbrandia rubra* and *Ralfsia verrucosa* all had damaged radula, caused by extreme rasping upon tough surfaces (Bertness *et al*, 1983). The authors suggested that the toughness of an alga in relation to radula penetration could be explained by both the size and arrangement of cells, and the presence of an outer cuticle. Small cells enclosed by a protective cuticle appeared to act as an effective feeding deterrent by increasing the density of structural cell material that *L. littorea* had to physically manipulate in order to consume tough algae (Bertness *et al*, 1983). Encrusting algae composed of small cells offer extensive structural resistance throughout their tissue (Bertness *et al*, 1983; Phillips *et al*, 1997). The physical toughness of structural compounds is known to reduce grazing (Cronin *et al*, 2002); and the presence of a cuticle seems to be effective in deterring grazing by *Idotea*. *Idotea wosnesenskii* Brandt presented with portions of mature *Iridaea cordata* (Turner) Bory with the cuticle removed from half of the blade surface fed at much higher rates on cuticle-free plants, although they did consume plants with the cuticle still intact (Gaines, 1985). As *I. wosnesneskii* feed by biting the margin of algal fronds, rather than scraping the surface like *L. littorea*, Gaines (1985) suggested that the presence of a cuticle may be useful as a grazing deterrent to *Idotea* due to its feeding mode. The increased toughness provided by the cuticle to the plant may act as barrier to biting herbivores such as *Idotea* (Gaines, 1985).

Differences in grazer feeding modes can influence plant communities (Carpenter, 1986). Ceccarelli *et al* (2005) investigated the effect of 'foragers' (relatively mobile grazing fishes that feed in loose single or multi-species schools) and 'farmers' (highly site-attached fish species that defend small territories from conspecifics and all other herbivores, including foragers) on benthic communities. When 'foragers' were excluded, there were substantial increases in both the cover and biomass of macroalgae and a decline in some corals, regardless of the presence of 'farmers' (Ceccarelli *et al*, 2005). When 'farmers' were excluded, some of the algal food species showed a moderate decline, regardless of whether "foragers" had access (Ceccarelli *et al*, 2005). Relating this to intertidal communities, *Littorina* primarily forage on both rocky substrata and whole algal plants (Watson and Norton, 1985a). They are known to have dramatic effects on macroalgal communities (Lubchenco, 1978; Sze, 1980; Underwood and Jernakoff, 1984; Petraitis, 1983, 1987; Janke, 1990; Davies and Beckwith, 1999). Excluding them from the shore for even short periods of time leads to an increase in algal cover (Bertness *et al*, 1983; Cervin *et al*, 2004). In comparison, *Idotea* are considered to have little effect on the structuring of algal communities (as discussed in section 1.4 of chapter 1).

3.1.2 Feeding preference experiments

Knowledge of herbivore food preferences is useful in predicting which algal species are expected to be consumed in the field, and therefore which species are likely to persist in natural algal communities (Van Alstyne *et al*, 1999). Preference for a particular algal species is an indication of the probability of that species being chosen if offered on an equal basis with other algae (Johnson, 1980). Feeding preference experiments are considered to be the most appropriate method for analysing food selection because food types are presented under controlled conditions and known availability (Cueto *et al*, 2001). There are two contrasting methodologies in use to determine food preferences: single- or multiple-choice feeding experiments.

In single-choice experiments, food items are presented to the grazer individually and the recorded consumption is later compared with that for other food options (Cueto *et al*, 2001) (*e.g.* Hay *et al*, 1986; Barker and Chapman, 1990; Schaffelke *et al*, 1995; Stachowicz and Hay, 1996; Anderson *et al*, 1998; Sotka and Hay, 2002; Macaya *et al*, 2005; Sorensen *et al*, 2005; Taylor and Brown, 2006; Wessels *et al*, 2006). In multiple-choice experiments, different potential food items are presented simultaneously to consumers within a single experimental arena (Cueto *et al*, 2001) (*e.g.* Carefoot, 1967; Hunter, 1981; Horn and Neighbors, 1984; Gaines, 1985; Hay *et al*, 1986, 1990, 1994; Barker and Chapman, 1990; Renaud *et al*, 1990; Schaffelke *et al*, 1995; Stachowicz and Hay, 1996; Merilaita and Jormalainen, 1997; Anderson *et al*, 1998; Wakefield and Murray, 1998; Pavia *et al*, 1999a; Karez *et al*, 2000; Van Alstyne *et al*, 2001; Sotka and Hay, 2002; Borell *et al*, 2004; Heaven and Scrosati, 2004; Hemmi *et al*, 2004; Rohde *et al*, 2004; Svenssen *et al*, 2004; Gassmann, 2005; Gauslaa, 2005; Macaya *et al*, 2005; Poore and Hill, 2005; Agrell *et al*, 2006; Taylor and Brown, 2006; Wessels *et al*, 2006). There is some discussion as to which approach yields the most reliable results. Some researchers recommend the use of multiple-choice experiments because during the course of the trial, the grazer has the opportunity to express a dietary choice (Peterson and Renaud, 1989). Multiple-choice experiments are a measure of edibility, rather than palatability (Watson and Norton, 1987), and are thought to have biological relevance as herbivores in natural conditions are presented with multiple choices among food species (Barker and Chapman, 1990) and normally encounter more than one alga simultaneously. This leads them to assess the food available and then choose the preferred item (Cueto *et al*, 2001). Multiple-choice experiments provide information on the level of edibility of a range of offered algal species (Wessels *et al*, 2006). However, in natural conditions grazers could encounter food items consecutively and examine each individually to determine whether to consume or reject it (Smallwood and Peters, 1986). For mesoherbivores it is considered unlikely that grazers in their natural habitat will choose among several algae when starting to feed as it is expected that they will feed on their host alga (Macaya *et al*, 2005).

Multiple-choice experiments may increase differences in preference by masking the consumption of some less preferred algal species (Cueto *et al*, 2001; Lippert and Iken, 2003). The consumption of less preferred species may depend on the abundance of preferred algae, as they may only be chosen when the preferred species are absent, as is the case in single-choice experiments (Cueto *et al*, 2001). These less preferred algae could be essential for the survival of grazers in the field when preferred species are scarce (Cueto *et al*, 2001). Multiple-choice experiments may underestimate the consumption of algae whose preference depends upon the presence of preferred species (Cueto *et al*, 2001). In contrast, single-choice experiments could produce deceptive results because a tested food may be consumed at higher rates than expected simply because there is nothing else to eat (Cueto *et al*, 2001) *i.e.* the consumption of food items that are not usually preferred may be overestimated (Roa, 1992). Single-choice experiments may determine the maximum feeding rate for each algal species, but provide no information regarding herbivore preferences in natural conditions (Wessels *et al*, 2006). One potential shortcoming of multiple-choice experiments is that traditional parametric statistics cannot be used to test for significant preferences among species due to lack of independence (Barker and Chapman, 1990; Karez *et al*, 2000; Goecker and Kall, 2003). However there are non-parametric alternatives to these analyses which enable meaningful analysis.

Research comparing single- and multiple-choice experiments often report apparently conflicting results. Watson and Norton (1985a) showed that the preference rankings observed by grazing *L. littorea* on the shore confirmed the macroalgal preference rankings recorded in multiple-choice laboratory experiments. This suggests that multiple-choice experiments are a potentially useful indicator of natural interactions upon the shore. However, Barker and Chapman (1990) conducted preference experiments in the field by presenting *Fucus* sp. to a mixed guild of herbivores (gastropods, amphipods and isopods). In the field *Fucus evanescens* C. Agardh was the most consumed species with *Fucus distichus* Linnaeus the least consumed (Barker and Chapman, 1990). These results were not in agreement with laboratory multiple-choice tests as the preference rankings showed that *Littorina* preferred *F. vesiculosus* over *F. evanescens*, which in turn was preferred over *F. distichus*, and *F. spiralis* was the least consumed species (Barker and Chapman, 1990). However, the results from preference tests in the field matched the results of the single-choice laboratory experiments as again *F. evanescens* was the most consumed fucoid and *F. distichus* the least (Barker and Chapman, 1990). Cueto *et al* (2001) reported that the Rufous-collared sparrow *Zonotrichia capensis* Swainson showed preferences in seed consumption but when their data were analysed to determine the preferred foods the authors arrived at different conclusions. Seeds preferentially consumed in multiple-choice experiments were always heavily consumed in single-choice experiments, suggesting that the consumption of less preferred foods was dependent upon the relative abundance of preferred food items (Cueto *et al*, 2001). Wessels *et al* (2006) showed consumption rates for the amphipod *Gammarellus homari* Fabricius and the urchin *Strongylocentrotus droebachiensis* Muller determined in single-choice experiments corresponded well with the feeding preferences determined in multiple-choice experiments.

3.1.2.1 Between-group variation in the edibility of algal functional groups

Watson and Norton (1985a) tested the edibility of a range of algae to *L. littorea* by presenting grazers with a 'test' alga and a 'reference material' (*U. lactuca*) in paired choice experiments. A ratio of the amount of test alga consumed (mg) to the amount of reference material consumed (mg) was calculated to determine a palatability index. Translating their results into the context of the functional group approach shows that algae from functional group three with foliose morphologies and some algal species from functional groups four (*O. pinnatifida*) and five (*F. serratus*) were preferentially consumed. Algae from functional group two and other algal species from functional group four (*M. stellatus*) were not consumed during the feeding experiment, but *L. littorea* did begin to feed upon them up to 20 days after the start of the experiment. Other algae from functional group five (*A. nodosum*) and algae from functional group six were not consumed by *L. littorea* (Watson and Norton, 1985a).

Nicotri (1980) investigated the edibility of a range of algae to *I. baltica* in a series of single-choice feeding experiments. Algae were ranked based upon the amount (mg) of each species ingested by five large isopods per day (Nicotri, 1980). Assigning the algal species tested into functional groups suggests that algae from functional groups two and three were preferentially consumed over algae from functional groups four and five, while algae from functional group six were the least consumed (Nicotri, 1980). The alga with the highest preference rank for *I. baltica* was *Codium* (Nicotri, 1980) (see section 2.1.3 of chapter 2 for information relating to the position of *Codium* in the functional group model).

3.1.3 Aims

In the following experiments, the aims were: i) to determine whether algal species were similarly edible to both *L. littorea* and *I. granulosa*; ii) to determine for both *L. littorea* and *I. granulosa* whether algal edibility decreases as functional group number increases; iii) to determine for both grazers whether all algae within a functional group were similarly edible; and iv) to determine whether algal edibility is related to algal attractiveness by testing if *I. granulosa* consume more of the algae they found the most attractive.

3.2 Methods

3.2.1 Between- and within-group variation in the edibility of algal functional groups

Edibility experiments were conducted in which the grazers *L. littorea* and *I. granulosa* were presented with algae from functional groups two to six, in both single and two-way choice experiments.

3.2.1.1 *Littorina littorea*

Mature *L. littorea* (mean shell height 2.02 ± 0.01 cm S.E.) and algae representing functional groups two to six (Table 1.1, chapter 1) were collected between February 2003 and May 2003 from Holbeck (see section 2.2.1 of chapter 2 for site description). Following collection *L. littorea* were food deprived for four days in filtered seawater to ensure that they were not satiated prior to the start of experiments (see section 3.4.4.1 below for discussion on the effects of food deprivation on feeding preferences). Algae were cleaned to remove epifauna and epiphytes by washing in running seawater and examination under a dissecting microscope. Epifauna were removed so that any changes observed were due to the experimental grazers and not other fauna attached to the algae. Removing epiphytes ensured that any changes observed were as a result of preference for the experimental algae, rather than the epiphytes. All algae used in experiments were mature plants with no reproductive tissue, and were used within 48 hours of collection. Three *L. littorea* were placed in experimental tanks (17 x 11.5 x 4 cm) with 200 ml of filtered seawater. The algae were blotted dry using a standard procedure (algae were placed on paper towels and blotted six times) and weighed (error of balance ± 0.001 g). A known weight of algae ranging from 1 g to 5 g (depending on the species) was added to the tanks in single-choice experiments. Experiments were conducted in an aquarium with a constant temperature of 12 °C and a 12 hour dark: light cycle. After five days the grazers were removed and the algae reweighed. Alongside the experimental tanks, grazer free controls were set up to allow for autogenic changes (defined as all changes in the potential prey that occur independent of the action of the grazers, including reproduction, mortality, growth, photosynthesis, water loss or gain, or any other process that may affect the response variable (Peterson and Renaud, 1989)). The water in both the experimental and control tanks was changed every second day. The experiment was repeated six times for each species, using new snails for each replicate. Once the algae had been reweighed, any change in weight was calculated using the formulae:

$$\Delta W = G_0 - G_f \qquad \text{(Equation 2)}$$

Where G_0 = initial weight of algae exposed to grazers

G_f = final weight of algae exposed to grazers

$$\Delta W = C_0 - C_f \qquad \text{(Equation 3)}$$

Where C_0 = initial weight of algae in control tanks

C_f = final weight of algae in control tanks.

It can be assumed that a decrease in weight of algal material recorded in the experimental tanks equates to the amount of the plant material consumed by the grazing *L. littorea*, and that the total amount consumed is a measure of edibility (Cronin *et al*, 2002).

For species from functional group seven, *L. incrustans* and *V. maura*, this methodology was modified as follows: *L. littorea* were collected from Holbeck (see section 2.2.1 of chapter 2 for site description) and kept in filtered seawater without food for four days (see section 3.4.4.1 below for discussion on the affects of food deprivation). The two algal species from functional group seven were collected from Filey Brigg (see section 2.2.1 of chapter 2 for site description) by chiselling them off the rock. Portions of crust were cleaned to remove epifauna and epiphytes by running under filtered seawater and then examination under a microscope. As these are crustose species, a 1 cm^2 section was placed under a dissecting microscope and the percentage of grazed surface area was calculated. Experiments were conducted in an aquarium with a constant temperature of 12 °C and a 12 hour dark: light cycle. The sections of crust were then offered to three *L. littorea* for five days, after which the grazers were removed and the crusts re-examined to determine any change in grazed surface area. Controls without grazers were set up to allow for autogenic changes, and each species was replicated six times, using new grazers for each replicate. Any change in the percentage of surface area grazed was calculated using the formulae:

$$\Delta\,\% = GP_{0\%} - GP_{f\%} \qquad \text{(Equation 4)}$$

Where $GP_{0\%}$ = initial percentage of surface area grazed exposed to grazers

$GP_{f\%}$ = final percentage of surface area grazed exposed to grazers

$$\Delta\,\% = CP_{0\%} - CP_{f\%} \qquad \text{(Equation 5)}$$

Where $CP_{0\%}$ = initial percentage of surface area grazed in control tanks

$CP_{f\%}$ = final percentage of surface area grazed in control tanks.

Once again it can be assumed that any increase in the percentage of the surface area grazed equalled the amount consumed by *L. littorea* and that the total amount consumed was a measure of edibility (Cronin *et al*, 2002).

3.2.1.2 *Idotea granulosa*

Mature *I. granulosa* (mean body length 1.68 ± 0.01 cm S.E.) and algae representing functional groups two to six (Table 1.1, chapter 1) were collected during November 2004 from Holbeck (see section 2.2.1 of chapter 2 for site description). Following collection *I. granulosa* were stored until use in filtered seawater with *U. lactuca* providing food and shelter; and algae were cleaned to remove epifauna and epiphytes by washing in running seawater and examination under a dissecting microscope. Epifauna were removed so that any changes observed were due to the experimental grazers and not other fauna attached to the algae. Removing epiphytes ensured that any changes observed were as a result of preference for the experimental algae, rather than the epiphytes. All algae used in experiments were mature plants with no reproductive tissue, and were used within 48 hours of collection. Six *I. granulosa* were placed in experimental tanks (17 x 11.5 x 4 cm) with 200 ml of filtered seawater. The algae were blotted dry using a standard procedure (algae were placed on paper towels and blotted six times) and weighed (error of balance ± 0.001 g) and a known weight of algae (approximately 2 g) was added to the tanks in single-choice experiments. Experiments were conducted in an aquarium with a constant temperature of 12 °C and a 12 hour dark: light cycle. After seven days the grazers were removed and the algae reweighed. Alongside the experimental tanks, grazer free controls were set up to allow for autogenic changes. The water in both the experimental and control tanks was changed every second day. The experiment was repeated six times for each species, using new grazers for each replicate. Once the algae had been reweighed, any change in weight was calculated using equations 2 and 3 (see section 3.2.1.1 above). It can be assumed that a decrease in weight of algal material recorded in the experimental tanks equates to the amount of the plant material consumed by the grazing *I. granulosa*, and that the total amount consumed is a measure of edibility (Cronin *et al*, 2002).

The data collected were used to test the null hypothesis that for both grazers there would be no significant differences in algal edibility from functional group two to functional group six. As a different methodology was used to record the consumption by *L. littorea* of species representing functional group seven, these data were not combined with the data from the other functional groups for analysis. The second null hypothesis, that all species within a functional group would be similarly edible, was tested using data from functional groups two to seven for *L. littorea*, and data from functional groups two to six for *I. granulosa*.

3.2.2 Between-group variation in the edibility and attractiveness of algal functional groups

3.2.2.1 *Idotea granulosa*

During the attractiveness experiment (see section 2.2.2.2 of chapter 2) the amount consumed of each species by *I. granulosa* presented in a two-way choice experiment was recorded. Prior to the algae being added to the tanks, it was blotted dry using a standard procedure (algae were placed on paper towels and blotted six times) and weighed (error of balance ± 0.001 g). After 7 days the grazers were removed and the algae reweighed and any change in weight was calculated using equations 2 and 3 (see section 3.2.1.1 above). The data were analysed to test the null hypothesis that *I.*

granulosa would not significantly consume algae from the functional groups that were recorded as the most attractive.

3.2.3 Statistical Analyses

Data were screened using the Kolmogorov-Smirnov normality test and Levene's test to test for normality of distribution and homogeneity of variances respectively (Sokal and Rohlf, 1995). Data not meeting the assumptions of homogeneity were log transformed prior to analysis.

3.2.3.1 Between-species variation in the edibility of algal species

To determine whether both *L. littorea* and *I. granulosa* exhibited any food preferences, data were analysed using two-factor ANOVA at the species level. A two-factor ANOVA was chosen with GRAZER PRESENCE/ABSENCE and FUNCTIONAL GROUP as factors as this was considered to be the most appropriate analysis. Previous work has suggested random pairing of control foods (without grazers present) with experimental foods (with grazers present) to correct for autogenic changes (Roa, 1992). The main problem with this technique is that random pairing could give rise to numerous configurations of the data (Prince *et al*, 2004). Therefore, the significance of the results collected could depend on the random allocation of the control data with the experimental data (Manly, 1993). Applying a two-factor ANOVA to the data with GRAZER PRESENCE/ABSENCE as two separate variables should allow the interactions among experimental factors to be seen.

For both grazers, the null hypothesis that algal edibility would be the same for each algal species was tested with GRAZER PRESENCE/ABSENCE was a fixed factor with two levels. The second factor in the analysis was SPECIES, a fixed factor with fourteen levels for *L. littorea* and nine levels for *I. granulosa*, as the species used in the investigation were chosen from a fixed available range for each functional group (Underwood, 1997). GRAZER PRESENCE/ABSENCE and SPECIES were the independent variables and the weight of each species consumed was the dependent variable. *Post hoc* Tukey pairwise comparisons were determined where appropriate to identify differences between the mean amounts consumed of each species.

3.2.3.2 Between-group variation in the edibility of algal functional groups

To test the null hypothesis that algal functional groups were similarly edible to both *L. littorea* and *I. granulosa* data were analysed using two-factor ANOVA. GRAZER PRESENCE/ABSENCE a fixed factor with two levels and FUNCTIONAL GROUP a fixed factor with five levels (Underwood, 1997). GRAZER PRESENCE/ABSENCE and FUNCTIONAL GROUP were independent variables, and the weight of each functional group consumed was the dependent variable. *Post hoc* Tukey pairwise comparisons were determined where appropriate to identify differences between the mean amounts consumed of each functional group.

To determine whether the results collected supported Steneck and Watling's (1982) prediction that algae would be less susceptible to grazing as functional group number increased, the data recording the weight of algae consumed for functional groups two to six were ranked from the most consumed group to the least consumed group, to indicate an order of preference shown by *L. littorea* and *I. granulosa*. Steneck and Watling (1982) predicted that a herbivore's preference for algae from a functional group would decrease from functional group two to functional group six as algal susceptibility to grazing decreased. The rank order of functional group preference recorded was compared to the rank order of functional group preference predicted by Steneck and Watling's (1982) model. The null hypothesis that both *L. littorea* and *I. granulosa* would show no significant preference for algae from functional group two to functional group six was tested using the test for non-parametric data, Spearman Rank Correlation Coefficient.

3.2.3.3 Within-group variation in the edibility of algal functional groups

One-way ANOVAs were conducted on the species data to compare the weight of each species consumed within functional groups two to five for *L. littorea*. *Post hoc* Tukey pairwise comparisons were determined where appropriate to identify differences between the mean amounts consumed of each species within a functional group. As percentage cover grazed by *L. littorea* was measured for species within functional group seven, a non-parametric test was used to determine if the species within the group were similarly edible to grazers. Examination of the data prior to analysis revealed that there was no change in the percentage cover of grazed algae in control tanks, so it was appropriate to use a Mann-Whitney *U*-test as data were non-parametric and did not meet the assumptions required for a parametric test. Student t-tests assuming equal variances were conducted on the species data for *I. granulosa*, to determine whether species within a functional group were similarly edible to grazers. Data for functional group six were not analysed as only one species (*C. officinalis*) was available for this group and so no comparison could be made.

3.2.3.4 Between-group variation in the edibility and attractiveness of algal functional groups

The null hypothesis that *I. granulosa* would not significantly consume algae that they found the most attractive was tested by comparing the attractiveness rank order of functional group preference to the edibility rank order of functional group preference using the test for non-parametric data, Spearman Rank Correlation Coefficient.

3.3 Results

The food preferences of *L. littorea* and *I. granulosa* were investigated by offering grazers samples of up to fourteen species of algae (singularly or in paired choice experiments) and recording changes in weight of the plants over the experimental time period. Any change in weight was assumed to equate to the amount of algae consumed by the grazers.

3.3.1 Data Screening

The results of the Kolmogorov-Smirnov normality test showed that the data at both functional group and species level for both grazers were normally distributed (Kolmogorov-Smirnov, $P > 0.15$). For *L. littorea* Levene's test of homogeneity of variances showed no significant differences in variance in the amount of each functional group consumed (Levene's, $L_{4,79} = 1.514$, $P > 0.15$) and the amount of each species consumed (Levene's, $L_{13,70} = 1.801$, $P > 0.05$). Therefore, it can be assumed that all samples were drawn from populations with equal or similar variances. As Levene's test of homogeneity of variances showed significant differences in the amount of each functional group (Levene's, $L_{9,98} = 2.582$, $P < 0.05$) and species (Levene's, $L_{17,90} =$

3.278, $P < 0.001$) consumed by *I. granulosa*, data were log transformed. After transformation, data for the number of each functional group showed no significant departure from homogeneity (Levene's, $L_{9, 98} = 1.895$, $P > 0.05$); therefore it can be assumed that all samples were drawn from populations with equal or similar variances. Transforming the species data was not sufficient to achieve homogeneity of variances, however as ANOVA is robust to departures from the assumption (Underwood, 1997) it was appropriate to proceed with the analysis. To minimise the increased risk of Type I error, data were analysed at significance level $P = 0.01$ (Underwood, 1997).

3.3.2 Between-species variation in the edibility of algal species

3.3.2.1 *Littorina littorea*

When comparing all species together the weight of each algal species consumed by *L. littorea* varied, with *Ceramium* sp. being the most consumed species (mean = 1.615 ± 0.130 g S.E., n = 6) and *D. contorta* being the least consumed species (mean = 0.135 ± 0.013 g S.E., n = 6) (Fig. 3.1).

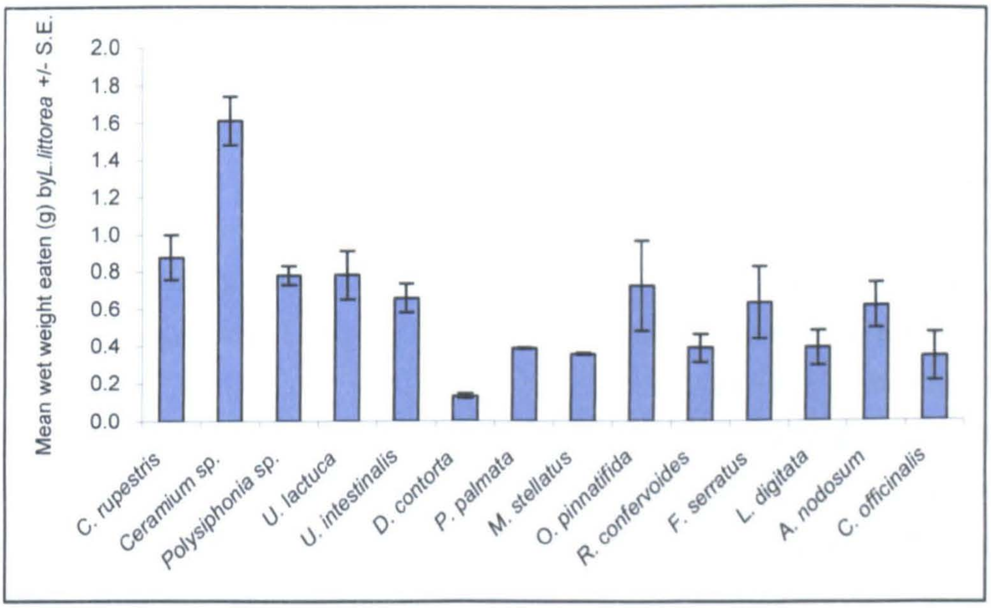

Figure 3.1 – Mean wet weight of each algal species consumed (± standard error) by *Littorina littorea* (n = 84).

When the mean weight of each algal species consumed by *L. littorea* was compared using two-factor ANOVA, there was a significant difference in mean consumption weights (Table 3.1).

Table 3.1 – Two-factor analysis of variance of the effects of GRAZER PRESENCE/ABSENCE and SPECIES on the weight of each species consumed by *Littorina littorea* (level of significance ** $P < 0.01$, *** $P < 0.001$).

Source of Variation	df	SS	MS	F	*P*
GRAZER +/-	1	7.243	7.243	117.800	<0.001***
SPECIES	13	9.535	0.733	11.929	<0.001***
GRAZER +/- * SPECIES	13	2.081	0.160	2.603	0.003**
Error	140	8.608	0.061		
Total	168	56.247			

GRAZER PRESENCE/ABSENCE and SPECIES were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the weight of each species consumed). The change in weight (g) of each species differed significantly between GRAZER PRESENCE/ABSENCE and also between SPECIES (Table 3.1). As the interaction effect was also significant there was always greater weight loss when grazers were present, irrespective of the algal species (Table 3.1). Although it was not possible to carry out *post hoc* comparisons on GRAZER PRESENCE/ABSENCE due to there being only two categories, the data showed that algae presented to grazers displayed greater weight loss (mean = 0.662 ± 0.027 g S.E., n = 84) than those in control tanks (mean = 0.206 ± 0.027 g S.E., n = 84). *Post hoc* Tukey pairwise comparison ($P = 0.05$) comparing the weight of each species consumed revealed that *Ceramium* sp. was consumed significantly more than every other species, and these species differed in that *C. rupestris* was consumed significantly more than *M. stellatus*, *R. confervoides*, *L. digitata* and *A. nodosum*; *Polysiphonia* sp. was consumed significantly more than *C. officinalis* and *D. contorta* was consumed significantly less than *C. rupestris*, *Polysiphonia* sp., *U. lactuca* and *O. pinnatifida* (Fig. 3.1, Table 3.2).

Table 3.2 - *Post hoc* Tukey pairwise comparison revealing differences in the edibility of algal species to *Littorina littorea* (species listed down the left hand side were consumed significantly more than species listed across the top where marked by *).

	C. rup	*Cer* sp.	*Poly*	*U. lac*	*U. int*	*D. con*	*P. pal*	*M. ste*	*O. pin*	*R. con*	*F. ser*	*L. dig*	*A. nod*	*C. off*
C. rup						*		*		*		*	*	
Cer sp.		*	*	*	*	*	*	*	*	*	*	*	*	*
Poly sp.						*								*
U. lac						*								
U. int														
D. con														
P. pal														
M. ste														
O. pin						*								
R. con														
F. ser														
L. dig														
A. nod														
C. off														

3.3.2.2 *Idotea granulosa*

The weight of each algal species consumed by *I. granulosa* differed, with *Ceramium* sp. being the most consumed species (mean = 0.448 ± 0.077 g S.E., n = 6) and *L. digitata* being the least consumed species (mean = 0.044 ± 0.010 g S.E., n = 6) (Fig. 3.2).

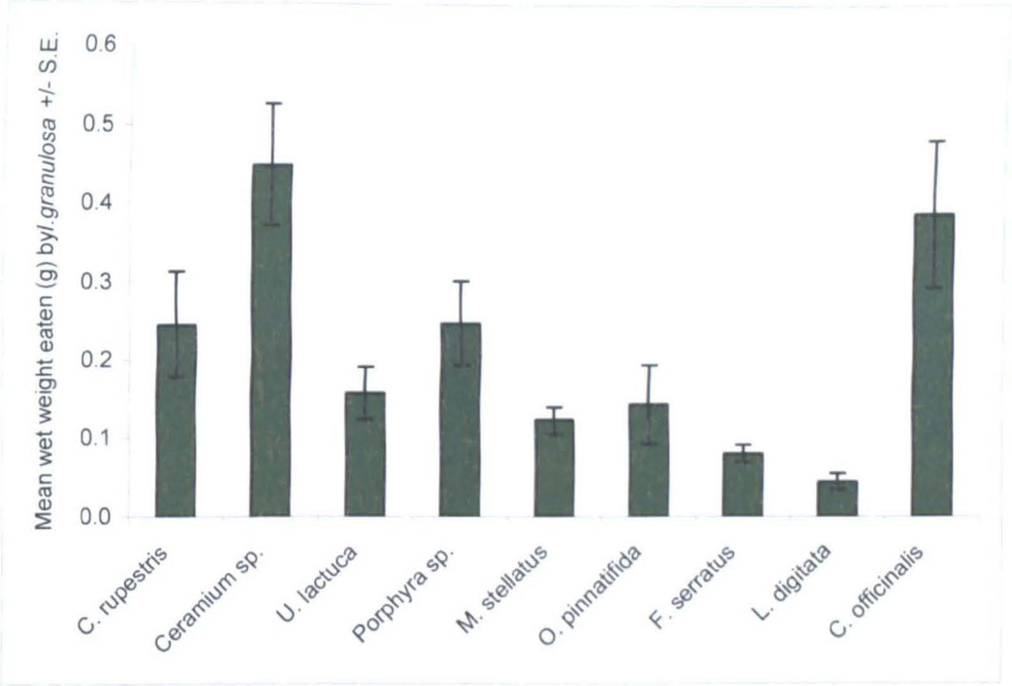

Figure 3.2 – Mean wet weight of each algal species consumed (± standard error) by *Idotea granulosa* (n = 54).

The difference in the mean weight of each algal species consumed by *I. granulosa* was compared using two-factor ANOVA and a significant difference was revealed (Table 3.3).

Table 3.3 – Two-factor analysis of variance of the effects of GRAZER PRESENCE/ABSENCE and SPECIES on the weight of each species consumed by *Idotea granulosa* (level of significance * $P < 0.05$, ** $P < 0.01$, NS not significant). † tested at significance level $P = 0.01$.

Source of Variation	df	SS	MS	F	*P*
GRAZER +/-	1	0.209	0.209	6.873	0.01*
SPECIES	8	0.926	0.116	3.809	0.001**
GRAZER +/- * SPECIES	8	0.571	0.071	2.351	0.024NS†
Error	90	2.734	0.030		
Total	107	4.440			

The PRESENCE/ABSENCE OF GRAZERS and SPECIES were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the weight of each species consumed). The change in weight of each species differed significantly between GRAZER PRESENCE/ABSENCE and also between SPECIES (Table 3.3). However, as the interaction effect was not significant the change in weight of algae presented to grazers was always greater than the change in weight of algae in control tanks irrespective of algal species (Table 3.3). Although it was not possible to carry out *post hoc* comparisons on GRAZER PRESENCE/ABSENCE due to there being only two categories, the data showed that algae presented to grazers displayed greater changes in weight (mean = 0.208 ± 0.024 g S.E., n = 54) than those in control tanks (mean = 0.120 ± 0.024 g S.E., n = 54). *Post hoc* Tukey pairwise comparison ($P = 0.05$) comparing the weight of each species consumed revealed that *Ceramium* sp. was consumed significantly more than *F. serratus* and *L. digitata*, but these species were not significantly different from each other (Fig. 3.2).

3.3.3 Between-group variation in the edibility of algal functional groups

3.3.3.1 *Littorina littorea*

The weight of algae consumed by *L. littorea* differed between functional groups. Functional group two was the most consumed (mean = 1.090 ± 0.108 g S.E., n = 18), and functional group six the least consumed (mean = 0.345 ± 0.130 g S.E., n = 6) (Fig. 3.3).

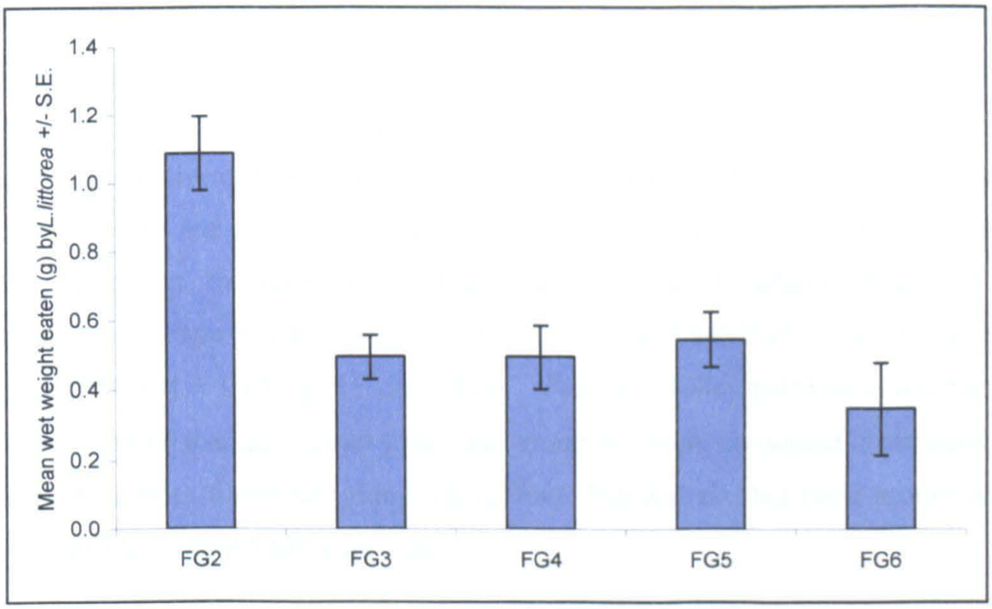

Figure 3.3 – Mean wet weight of each functional group consumed (± standard error) by *Littorina littorea* (n = 84).

The difference in the weight of each functional group consumed by *L. littorea* showed a general decrease from functional group two to functional group three, with very little difference between groups three to six (Fig. 3.3). When the mean consumption weights for each functional group were compared using two-factor ANOVA, there was a significant difference in the amounts consumed (Table 3.4).

Table 3.4 – Two-factor analysis of variance of the effects of GRAZER PRESENCE/ABSENCE and FUNCTIONAL GROUP on the weight of each functional group consumed by *Littorina littorea* (level of significance *** $P < 0.001$, NS not significant).

Source of Variation	df	SS	MS	F	*P*
GRAZER +/-	1	5.392	5.392	61.552	<0.001***
FUNCTIONAL GROUP	4	5.637	1.409	16.087	<0.001***
GRAZER +/- * FUNCTIONAL GROUP	4	0.746	0.186	2.129	0.080NS
Error	158	13.840	0.087		
Total	168	56.247			

GRAZER PRESENCE/ABSENCE and FUNCTIONAL GROUP were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the weight of each functional group consumed). The change in weight of each functional group differed significantly between GRAZER PRESENCE/ABSENCE and also between FUNCTIONAL GROUP (Table 3.4). However, as the interaction effect was not significant the change in weight of algae presented to grazers was always greater than the change in weight of algae in control tanks irrespective of their functional group (Table 3.4). Although it was not possible to carry out *post hoc* comparisons on GRAZER PRESENCE/ABSENCE due to there being only two categories, the data showed that algae presented to grazers displayed greater changes in weight (mean = 0.593 ± 0.036 g S.E., n = 84) than those in control tanks (mean = 0.191 ± 0.036 g S.E., n = 84). *Post hoc* Tukey pairwise comparison (P = 0.05) revealed that algae from functional group two were consumed significantly more than algae from functional groups three, four, five and six, but these groups were not significantly different from each other.

3.3.3.2 *Idotea granulosa*

The weight of algae in each functional group consumed by *I. granulosa* also differed. Functional group six was the most consumed (mean = 0.387 ± 0.095 g S.E., n = 6) and functional five the least consumed (mean = 0.062 ± 0.009 g S.E., n = 12) (Fig. 3.4).

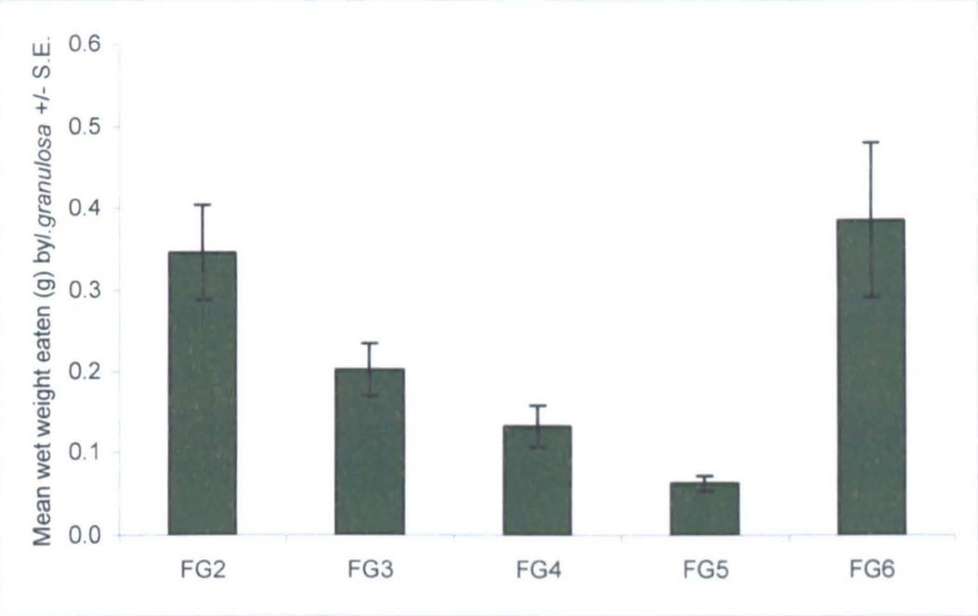

Figure 3.4 – Mean wet weight of each functional group consumed (± standard error) by *Idotea granulosa* (n = 54).

The difference in the weight of each functional group consumed by *I. granulosa* showed a general decrease from functional group two to functional group five (Fig. 3.4), with a large increase in the amount of functional group six consumed. When compared using two-factor ANOVA the difference in the change in weight was significant (Table 3.5).

Table 3.5 – Two-factor analysis of variance of the effects of GRAZER PRESENCE/ABSENCE and FUNCTIONAL GROUP on the weight of each functional group consumed by *Idotea granulosa* using log transformed data (level of significance ** $P < 0.01$ *** $P < 0.001$, NS not significant).

Source of Variation	df	SS	MS	F	*P*
GRAZER +/-	1	7.826	7.826	23.893	<0.001***
FUNCTIONAL GROUP	4	5.413	1.353	4.132	<0.004**
GRAZER +/- * FUNCTIONAL GROUP	4	2.760	0.690	2.107	0.080NS
Error	98	32.100	0.328		
Total	108	183.605			

GRAZER PRESENCE/ABSENCE and FUNCTIONAL GROUP were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the weight of each functional group consumed). The weight of each functional group consumed differed significantly between GRAZER PRESENCE/ABSENCE and also between FUNCTIONAL GROUP (Table 3.5). However, as the interaction effect was not significant the change in weight of algae presented to grazers was always greater than the change in weight of algae in control tanks irrespective of their functional group (Table 3.5). Although it was not possible to carry out *post hoc* comparisons on GRAZER PRESENCE/ABSENCE due to there being only two categories, the data showed that algae presented to grazers lost more weight (mean = 0.226 ± 0.025 g S.E., n = 54) than those in control tanks (mean = 0.116 ± 0.025 g S.E., n = 54). *Post hoc* Tukey pairwise comparison ($P = 0.05$) revealed that algae from functional group two was consumed significantly more than algae from functional group five, which in turn was consumed significantly less than algae from functional group six.

The data collected suggest that algal edibility by *L. littorea* generally matches the functional group model predicted by Steneck and Watling (1982) as the amount of each group consumed showed a decrease from functional group two to functional group three, with no significant differences between groups three to six (Fig. 3.3). However, the rank order of algal edibility recorded was not significantly related to that predicted by Steneck and Watling (1982) (Spearman Rank Correlation Coefficient, $r_s = 0.77$, $P > 0.05$) (Fig. 3.5). Algal edibility by *I. granulosa* followed Steneck and Watling's (1982) model in that the amount of each functional group consumed decreased from functional group two to functional group five (Fig. 3.4). However, as functional group six was consumed the most, the rank order of algal edibility recorded was not significantly related to that predicted by Steneck and Watling (1982) (Spearman Rank Correlation Coefficient, $r_s = 0.43$, $P > 0.05$) (Fig. 3.5).

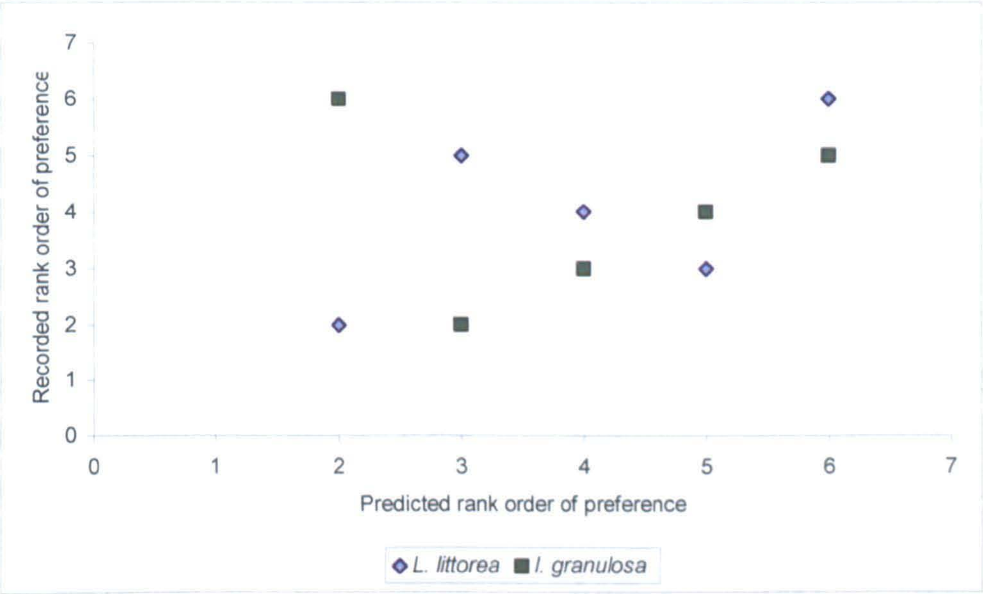

Figure 3.5 - Relationship between the rank order of functional group preference as predicted by Steneck and Watling (1982) and the rank order of functional group preference recorded for *Littorina littorea* (n = 5) and *Idotea granulosa* (n = 5).

3.3.4 Within-group variation in the edibility of algal functional groups

The results of the one-way ANOVAs conducted to determine if species within a functional group were similarly susceptible to grazing by *L. littorea* showed that the individual species of functional groups two and three were not consumed in similar amounts (Table 3.6). For functional group two, the *post hoc* Tukey pairwise comparison ($P = 0.05$) revealed that *Ceramium* sp. was consumed significantly more than *Polysiphonia* sp., but neither species were consumed in significantly different amounts than *C. rupestris* (Fig. 3.1). For functional group three, the *post hoc* Tukey pairwise comparison ($P = 0.05$) revealed that *U. intestinalis* was consumed significantly more than *D. contorta* and *P. palmata*, which in turn were consumed significantly less than *U. lactuca* (Fig. 3.1). The results also suggest that species within functional groups four and five were consumed in similar amounts, as differences in the amount of each species consumed were not significant (Table 3.6). Within functional group seven, *V. maura* (median = 0.50 ± 24 range, n = 6) and *L. incrustans* (median = 1.50 ± 7 range, n = 6) were consumed in similar amounts (Mann-Whitney *U*-test, $U_{6,6} = 15.5$, $P > 0.05$).

Table 3.6 – One-way analyses of variance to show the comparison of algal edibility by *Littorina littorea* of algal species within a functional group (level of significance * $P < 0.05$ ** $P < 0.01$, NS not significant).

Source of Variation	df	SS	MS	F	*P*
Functional Group 2					
Between Groups	2	1.457	0.729	4.774	0.025*
Within Groups	15	2.289	0.253		
Total	17	3.746			
Functional Group 3					
Between Groups	3	0.971	0.324	7.165	0.002**
Within Groups	20	0.903	0.045		
Total	23	1.874			
Functional Group 4					
Between Groups	2	0.144	0.072	0.687	0.518NS
Within Groups	15	1.574	0.105		
Total	17	1.718			
Functional Group 5					
Between Groups	2	0.097	0.049	0.331	0.724NS
Within Groups	15	2.210	0.147		
Total	17	2.308			

The results of the Student t-tests conducted to determine if species within a functional group were similarly susceptible to grazing by *I. granulosa* showed that the individual species of functional groups two to four were consumed in similar amounts. Within functional group two, there was no significant difference in the amount of *Ceramium* sp. consumed compared to *C. rupestris* (Student t-test, $t_{10} = -1.970$, $P > 0.05$) (Fig. 3.2); within functional group three, *Porphyra* sp. was consumed similarly to *U. lactuca* (Student t-test, $t_{10} = -1.410$, $P > 0.05$) (Fig. 3.2); and within functional group four *I. granulosa* consumed *O. pinnatifida* and *M. stellatus* in similar amounts (Student t-test, $t_{10} = -0.382$, $P > 0.05$) (Fig. 3.2). Within functional group five *F. serratus* was consumed significantly more than *L. digitata* (Fig. 3.2) (Student t-test, $t_{10} = 2.428$, $P < 0.05$).

3.3.5 Between-group variation in the edibility and attractiveness of algal functional groups

Algae from functional groups two, four and five were similarly edible and attractive to *I. granulosa*, though algae from functional group three were not as high in the attractiveness preference rank as would be expected and algae from functional group six were more attractive to *I. granulosa* than expected (Fig. 3.6). However, the data collected suggest that there was no significant relationship between the preferred edible algae and the algae found most attractive by *I. granulosa*. The rank order of functional group preference for attractiveness was not significantly similar to the rank order of functional group preference for edibility (Spearman Rank Correlation Coefficient, $r_s = 0.1$, $P > 0.10$) (Fig. 3.6).

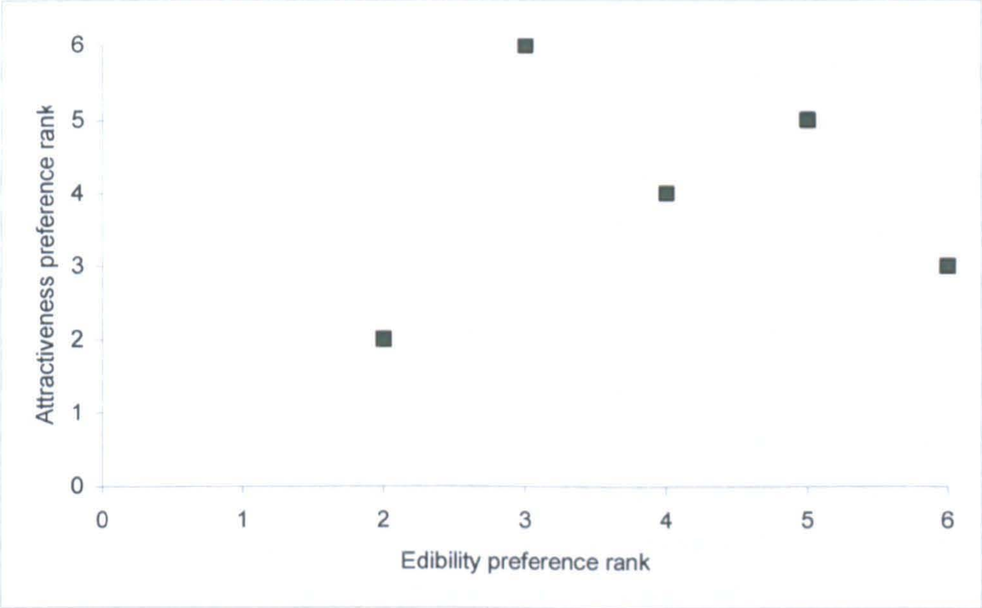

Figure 3.6 – Relationship between the rank of edibility preference and rank of attractiveness preference for *Idotea granulosa* (n = 5).

3.4 Discussion

The functional group model proposed by Steneck and Watling (1982) predicted that algal species varied in their susceptibility to grazing by herbivores based upon their morphological and anatomical features (Table 1.1, chapter 1). Their hypothesis being that as algal structural complexity increases through functional groups, the ability of each species to resist grazing by herbivores is increased.

3.4.1 Between-group variation in the edibility of algal functional groups

The results obtained from the current study on the grazing habits of *L. littorea* and *I. granulosa* go some way to support Steneck and Watling's (1982) predictions in that *L. littorea* consumed algae from functional group two significantly more than algae from functional groups three, four, five and six (Fig. 3.3; Table 3.4), while *I. granulosa* significantly preferred algae from functional groups two and six over algae from group five (Fig. 3.4; Table 3.5). This is as a result of the significantly high amount of *Ceramium* sp. consumed by both grazers (Figs. 3.1, 3.2) compared to all other algal species. *Idotea granulosa* have been shown to prefer *Ceramium nodulosum* (Lightfoot) Ducluzeau and the structurally similar *Pilayella littoralis* (Linnaeus) Kjellman (Pavia *et al*, 1999a); both of which are composite algae of functional group two. It is surprising that *L. littorea* did not consume algae from functional group three significantly more than algae from groups four, five and six (Fig. 3.3) as it has been previously shown that *L. littorea* prefer small, ephemeral algae (Littler and Littler, 1980; Lubchenco, 1978; Wilhelmsen and Reise, 1994). Ephemeral algae such as *U. intestinalis* and *U. lactuca* are thought to lack structural or chemical means of defence against herbivores (Littler and Littler, 1980; Bertness *et al*, 1983). They also display sporadic and patchy distribution, which may cause them to be too unpredictable as a source of food, particularly for specialist grazers (Wilhelmsen and Reise, 1994). As both *L. littorea* and *I. granulosa* are generalist herbivores they can efficiently use the periodic availability of these species (Lubchenco, 1978). *Idotea granulosa* have been shown to consume *U. lactuca* (Karez *et al*, 2000) and Goecker and Kall (2003) reported that *I. baltica* significantly preferred *U. intestinalis* over *Cladophora* sp., which in turn was preferred over *F. vesiculosus*. In this experiment, *I. granulosa* preferred algae from functional group two (*C. rupestris* and *Ceramium* sp.) over algae from functional group three (*U. lactuca* and *Porphyra* sp.), which in turn were preferred over algae from the least consumed functional group five (*F. serratus* and *L. digitata*). The functional group model proposed by Steneck and Watling (1982) was based upon the concept that algae in different functional groups would have different levels of structural defences and therefore exhibit different levels of 'toughness'. Results collected by Bertness *et al* (1983) suggested that the toughness of an alga in reference to the radula penetration of grazers could be explained by algal cell size and the presence or absence of an outer cuticle. Their study investigated four algal species: *U. lactuca* (functional group three), *C. crispus* (functional group four), *H. rubra* (functional group seven) and *R. verrucosa* (functional group seven). Three of these species caused radula damage to *L. littorea*. *C. crispus* has a tough outer cuticle and *H. rubra* and *R. verrucosa* are composed of small cells, which make them tough to penetrate. The most consumed species was *U. lactuca*, which was also the only species to cause no damage to the grazer's radula (Bertness *et al*, 1983). *Littorina littorea* are taenioglossan grazers whose radula has modifications that are characteristic of this group (Steneck and Watling, 1982). Taenioglossan grazers have radula with twenty functional points touching the substratum during feeding, and have more cusps than other littorinids (Steneck and Watling, 1982). The sharp cusps on the radula teeth allow *L. littorea* to efficiently tear the thallus of *U. lactuca* and so they can ingest more food per unit time compared to other algae (Watson and Norton, 1985a). These characteristics suggest that *L. littorea* are more likely to graze algae from the lower functional groups (Steneck and Watling, 1982).

Algae from functional group five were consumed slightly more than algae from functional groups four and six by *L. littorea* (Fig. 3.3), but comprised the least consumed group by *I. granulosa* (Fig. 3.4). Steneck and Watling (1982) suggested that algae from the most commonly grazed functional groups were either very small (functional groups one and two) or had very large, expansive forms (functional groups five and seven). However, although many herbivore species prefer to consume kelps (Wakefield and Murray, 1998), few benthic invertebrates such as *L. littorea* and *I. granulosa* appear to specialise on laminarian species (Hay and Steinberg, 1992). Van Alstyne *et al* (1999) found that the urchin *Strongylocentrotus purpuratus* Stimpson and the turban snail *Tegula funebralis* Adams generally preferred adult kelps to adult fucoid algae; however this was not the case for the isopod *I. wosnesenski*. In the Baltic Sea, *I. baltica* heavily consume *F. vesiculosus* to the point that they have played a role in the destruction of the fucoid belt (Kotta *et al*, 2000). In some areas, the *Fucus* population had not returned to the initial level prior to destruction yet the density of *I. baltica* remained high, which suggests they switch to an alternative diet at a low *F. vesiculosus* biomass (Kotta *et al*, 2000). Kotta *et al* (2000) showed that *I. baltica* consumed both *P. littoralis* (functional group two) and *F. lumbricalis* (functional group four), but showed a significant preference for *P. littoralis*. Goecker and Kall (2003) suggested that *Idotea* would preferentially consume *F. vesiculosus* only when no alternative food source was available, whereas, Jormalainen *et al* (2001a) showed that *I. baltica* preferred *F. vesiculosus* over *U. intestinalis* and *Ceramium* sp. when offered algae simultaneously. However, *I. baltica* feeding on *F. vesiculosus* showed reduced growth rates, leading Jormalainen *et al* (2001a) to conclude that food value may not be an important determinant of food choice.

Steneck and Watling (1982) considered algae from functional groups three, four and six (intermediate-size) to be relatively ungrazed. It has been suggested that the surface area of the thallus of intermediate-sized algae may be too small to support relatively large herbivores such as *L. littorea* (Steneck and Watling, 1982); therefore preferences may be affected not only by the palatability of an alga but more by the structural strength. An example of this is *U. intestinalis*, which has thin, tubular fronds. The morphology of the fronds may cause them to be too narrow to carry the weight of the muscular foot of *L. littorea* (Watson and Norton, 1985a). This was demonstrated by Wilhelmsen and Reise (1994) who reported that *L. littorea* fed with fragmented pieces of *U. intestinalis* and *U. lactuca* consumed more *U. intestinalis*, whereas snails fed with whole thalli consumed twice as much *U. lactuca* than *U. intestinalis*. Fragmented pieces of *U. intestinalis* may have been preferentially consumed because the tissue of *U. intestinalis* is significantly softer than that of *U. lactuca*, and also contains aqueous extracts with strong phagostimulant properties (Watson and Norton, 1985b). For whole thalli, the broad ulvoid thallus of *U. lactuca* provides a relatively stable substratum when pinned down by the snail's foot whereas a single tubular frond of *U. intestinalis* is too narrow to carry the entire foot (Watson and Norton, 1985b). Tubular fronds could also be too buoyant when inflated with gas making them difficult for grazers to hold down (Watson and Norton, 1985a; Wilhelmsen and Reise, 1994), though this was not relevant to the algae in this investigation. These factors could account for the very small amount of *D. contorta* consumed by *L. littorea* (Fig. 3.1). *D. contorta* was the least consumed of the fourteen species and has tubular fronds filled with a watery mucilage, which could make it very difficult for *L. littorea* to physically process.

Cronin *et al* (2002) reported that the freshwater crayfish *Procambarus clarkii* Giard fed more on plants with finely branched or filamentous morphologies when offered as whole tissue as they were easier to handle and consume. These factors could limit littorinid food choice, as they are restricted to feeding on algae that they can grasp and attach to (Wakefield and Murray, 1998).

3.4.2 Algal defences

3.4.2.1 Calcification as a structural defence

In a study using limpets as the experimental grazer, Padilla (1989) considered calcification as a structural defence against herbivores, with the hypothesis that there was a relationship between the force required to remove algal tissue and the degree of calcification and thallus form (as introduced in section 1.1.1 of chapter 1). Three groups of algae were investigated: non-calcified algae, lightly calcified algae and heavily calcified algae. The minimum normal force required by limpets to remove tissue decreased with increasing calcification (Padilla, 1989). The mean amount of normal force needed to remove tissue from algae in functional groups two and four (filamentous algae and corticated macrophytes respectively) was greater than the mean amount of force needed to remove tissue from algae in functional groups five, six and seven (leathery macrophytes, articulated calcareous algae and crustose coralline algae respectively) (Padilla, 1989). This can be explained by the fact that calcified algae, such as those from functional groups six and seven, are brittle and so break when a force is applied to them. Algae with 'flexible' morphologies are able to absorb the force applied to them by grazers; therefore they require a greater total force to break (Padilla, 1985). This could explain why *L. littorea* consumed algae from functional group five more than algae from functional group four in the current study.

Regarding the difference in consumption between functional groups five and six for *L. littorea*, Padilla (1989) classified algae from these two functional groups into the same group based upon calcification (NB: the leathery algal species investigated ranged in their levels of calcification from none to heavy). *Corallina officinalis* was the only alga from functional group six presented to both *L. littorea* and *I. granulosa* in this investigation. The morphological structure of *C. officinalis* causes it to be virtually impossible to consumption by gastropod grazers. The articulated joints between calcareous segments are the only points where the alga can be consumed (Watson and Norton, 1985a). However, the radula of *L. littorea* is wider than the uncalcified joints; therefore the alga's defences are effectively impenetrable. Consequently, *C. officinalis* was consumed less than *F. serratus*, *A. nodosum*, and *L. digitata* by *L. littorea* (the composite species of functional group five) (Fig. 3.1). The decrease in weight of *C. officinalis* could be due to *L. littorea* grazing upon microalgae coating the calcified fronds (Hawkins and Hartnoll, 1983). As *L. littorea* have been suggested to preferentially consume microalgae (Steneck and Watling, 1982), they could have been rasping the surface of *C. officinalis* to remove the microalgal film. This would elevate the palatability of *C. officinalis* because although algae were cleaned to remove algal epiphytes it is possible that some of the microalgal film remained.

The amount of each functional group consumed by *I. granulosa* followed the predictions of Steneck and Watling (1982) as preference decreased from functional group two through to functional group five (Fig. 3.4). However, *I. granulosa* showed a strong preference for algae from functional group six (Fig. 3.4). Preparation of algal material for feeding preference experiments involved washing the algae to remove epifauna and epiphytes. However, most algae are covered with a microalgal film, which is difficult to see and thus remove. *Idotea* are known to consume algal epiphytes (Karez *et al*, 2000; Kotta *et al*, 2000; Svensson *et al*, 2004), and so the weight lost by *C. officinalis* may be due to *I. granulosa* grazing on the microalgal film as opposed to the alga itself (Hawkins and Hartnoll, 1983). The process used to clean the algae may not have removed all algal epiphytes, which again could have been consumed by *I. granulosa*, although a large amount of epiphyte and microalgal material would have needed to be consumed to result in the weight loss observed (Fig. 3.2). The shredding action of *I. granulosa* feeding apparatus suggests that it would be difficult for *I. granulosa* to process the heavily calcified thallus of *C. officinalis*. Given the heavy weight of *C. officinalis* compared to other algal species (due to its calcified form), any grazer consuming the alga as opposed to the microalgal film would be expected to result in large changes in weight between grazed and ungrazed plants. Although the high consumption of *C. officinalis* by *I. granulosa* does not match the predictions of the functional group model, the results collected were shown to be repeatable using the same methodology (see Fig. 5.3 of chapter 5).

3.4.2.2 Algal chemical defences

Littorina littorea are considered to be deterred from consuming *A. nodosum* (Watson and Norton, 1985a; Geiselman and McConnell, 1981) by the alga's chemical defences. *A. nodosum* contains polyphenolic plant secondary metabolites called tannins (Toth, 2002). The least complex group of tannins are the phlorotannins, which are found only in brown algae, and are acetatemalonate derived polymers of phloroglucinol (1,3,5-trihydroxybenzene) (Targett and Arnold, 2001). *Ascophyllum nodosum* contains a high concentration of phlorotannins, usually between 4-15 % dry weight (Pavia *et al*, 1999b). Phlorotannin content can show intraspecific variation due to extrinsic factors such as nutrient availability, light levels, tidal height, life history stage (Hammerstrom *et al*, 1998), solar radiation, UV-B radiation and physical damage (Pavia and Brock, 2000). If extrinsic factors cause changes to occur in the production of phlorotannins, the susceptibility of brown algae to herbivory could subsequently be altered (Pavia and Brock, 2000). A number of studies have shown that phlorotannins can deter herbivore feeding by acting as defensive chemicals (Rosenthal and Janzen, 1979; Geiselman and McConnell, 1981; Steinberg, 1985, 1988; Hay *et al*, 1988; Hay and Fenical, 1988; Van Alstyne, 1988; Winter and Estes, 1992; Targett and Arnold, 1998). However, *I. baltica* preferred foods containing phlorotannins (Jormalainen *et al*, 2005). In addition the assimilation efficiency of *I. baltica* was better when fed with *F. vesiculosus* rather than *U. lactuca* (Jormalainen *et al*, 2005). These results may be surprising given the evidence of Koivikko *et al* (2005) who recorded that *I. baltica* grazing on *F. vesiculosus* caused the alga to exude more phlorotannins, probably as an induction response. Scott and Marsham (2007, Appendix III) found no evidence of *L. littorea* being deterred by the presence of chemicals, either because feeding on *Fucus* sp. did not induce a chemical response or *L. littorea* is able to consume *Fucus* sp. regardless of any defensive mechanisms employed by the alga. Phlorotannins and other chemicals present in algae are known to act primarily as anti-bacterial and anti-larval fouling agents to protect plants against epiphytes and epifauna (Hammerstrom *et al*, 1998; Wikstrom and Pavia, 2004). Any chemicals present within algae may not be palatable to grazers simply as a side effect, rather than their chemistry being geared to deterring herbivores. If herbivores do feed upon chemically defended plants encountered in their natural environment, any effect the chemicals may have could be diluted by the consumption of a range of different algae. Generalist herbivores can improve the performance of a single food item by mixing their diet (Bernays *et al*, 1994; Cruz-

Rivera and Hay, 2000, 2001), which may also improve the balance of nutrients gained from algae (Westoby, 1978; Clark, 1982).

3.4.3 Within-group variation in the edibility of algal functional groups

Within-group variation occurred between algae from functional groups two and three for *L. littorea* and between algae from functional group five for *I. granulosa*, which disagrees with the predictions of Steneck and Watling (1982). *Idotea granulosa* consumed *F. serratus* significantly more than *L. digitata* (Fig. 3.2), which is possibly due to *Idotea* ranking habitat requirements over food requirements. However, there was no significant relationship between attractiveness and edibility (Fig. 3.6). *Ceramium* sp. was consumed significantly more by *L. littorea* than *C. rupestris* and *Polysiphonia* sp. (Fig. 3.1), which is probably due to the levels of cortication in the three species. Although *C. rupestris* is ecorticate; *Ceramium* sp. is corticated only at the nodes whereas as *Polysiphonia* sp. is relatively extensively corticated along main axes, the thickness of the fronds of *C. rupestris* compared to the fronds of *Ceramium* sp. may have allowed *L. littorea* to consume *Ceramium* sp. more easily. Within functional group three, *L. littorea* preferred *U. intestinalis* and *U. lactuca* over *D. contorta* and *P. palmata*. The morphologies of all of these species would cause them to be relatively difficult for *L. littorea* to manipulate, however compared to the *Ulva* sp., *D. contorta* is more slippery and so would be harder to grasp due to its mucus-filled fronds. *Palmaria palmata* has thicker, membranous fronds, making them more difficult to penetrate during grazing. These results further support Steneck and Dethier's (1994) theory proposing that some functional groups be spilt into morphological subgroups (see section 2.4.2 of chapter 2 for introductory discussion). Functional group two could be divided into filamentous algae (functional group two) and corticated filamentous algae (functional group 2.5), with *C. rupestris* representing group two and *Ceramium* sp. and *Polysiphonia* sp. representing functional group 2.5. Although *C. rupestris* was consumed more than *Polysiphonia* sp. by *L. littorea*, the difference in consumption was not significant so the results of this study do not fully support the theory of subgroups.

The results collected for *L. littorea* showed no significant within-group variation between algae in functional groups four and five (Table 3.1). Of the algae comprising functional group four, neither *M. stellatus* (Dudgeon *et al*, 1999) nor *O. pinnatifida* were readily consumed by grazers, due to either algal morphology or chemical defences.

However, within group variation has been shown to occur between species from functional group four (Dudgeon *et al*, 1995), possibly because algae representing this functional group exhibit a very wide range of morphologies. A lack of variation between the algae in functional group five could be explained by the similarities in both morphology and chemical defences. As previously mentioned, *A. nodosum* contains phlorotannins, as do fucoids (Peckol *et al*, 1996) and kelps (Hammerstrom *et al*, 1998), making all three species similarly edible to *L. littorea.* However, a recent study by Deal *et al* (2003) suggested that phlorotannins did not deter herbivore feeding, rather it was galactolipids produced by *F. vesiculosus* that acted as a chemical defence. This supports the theory presented by Steinberg and van Altena (1992) that smaller, non-polar secondary metabolites may be more effective at deterring herbivores than phlorotannins. Their study found that generally, the least palatable algae contained non-polar metabolites such as terpenes and lactones as well as phlorotannins (Steinberg and van Altena, 1992). No significant variation was observed for *L. littorea* between *L. incrustans* and *V. maura*, the composite species of functional group seven, even though these two species do have different morphologies. Dethier (1994) identified a clear variation between crustose coralline algae with regard to the degree of calcification of cell walls, cell size, the degree of cell-cell connection, crust thickness, the rigidity and texture of the crusts' surface, and the adherence of filaments to each other and the substratum. This study was one of the first to examine differences among species within a functional group, and the results presented contradicted the predictions of the functional group model.

3.4.4 Factors affecting herbivore feeding preferences

Littorina littorea and *I. granulosa* are both generalist herbivores and as such would be expected to base their feeding preferences on multiple plant traits (Cronin *et al*, 2002), for example morphology (Littler and Littler, 1980; Steneck and Watling, 1982), structure (Bertness *et al*, 1983; Padilla, 1985, 1989), chemical defences (Targett and Arnold, 1998), nutritive value (Salemaa, 1979; Wakefield and Murray, 1998), cover offered from predators (Duffy and Hay, 1994), grazers' state of hunger and past experience (Watson and Norton, 1985a; Cronin and Hay, 1996). Algal morphology, structure and chemical defences have previously been discussed. Regarding nutritive value, foraging efficiency may be improved by grazers exhibiting a preference for nutritious food sources (Imrie *et al*, 1990). It has been suggested that herbivores should prefer nitrogen-rich plants and feed to meet a nitrogen requirement (Wakefield and Murray, 1998). However, algal thalli often have low nitrogen content so the availability of nitrogen may act as a limiting factor (Wakefield and Murray, 1998). Previous work has shown that the food preferences of herbivores are not always related to nutritional quality. Paine and Vadas (1969) found no linear relationship between ranked food preference and calorific content. The most preferred algae had intermediate calorific values and the study concluded that food preference was due to algal availability rather than absolute food value (Paine and Vadas, 1969). Similar results were collected by Carefoot (1973), who suggested that feeding preferences were not related to algal energy content or ash free dry weight. Watson and Norton (1985a) discovered that *L. littorea* preferred *U. lactuca* to *U. intestinalis*, even though *U. intestinalis* was considered to be the more attractive alga. The algae preferred by *L. littorea* display a range of nutritive chemistries. Marsham *et al* (2007) found *Ceramium* sp. to have a low calorific value, high ash content and high protein content, whereas *C. rupestris* and *U. lactuca* both had mid-range calorific values, low ash contents and high protein contents (Appendix II). Of the least preferred species *D. contorta* had a mid-range calorific value, low ash content and high protein content; *M. stellatus* showed a mid-range calorific value, low ash content and low protein content; and *L. digitata* and *C. officinalis* both had low calorific values, high ash contents and low protein contents (Marsham *et al*, 2007, Appendix II). These studies support the theory that nutritive content of algae may not be as significant a factor in determining feeding preferences as other factors. Wakefield and Murray (1998) claimed that as long as an alga meets the morphological and structural constraints of a herbivore, and contains insufficient levels of defensive chemicals that may reduce or deter feeding, it is a potential edible and palatable food source.

Salemaa (1987) suggested that the food preferences of *Idotea* were not directly determined by the nutritional quality of algae but were significantly influenced by the morphological characteristics of algae, while Jormalainen *et al* (2001b) proposed that habitat selection dominates *Idotea* feeding preferences.

3.4.4.1 Effect of food deprivation on herbivore food preference

Effects of past feeding experience and food deprivation/starvation may be important in determining algal attractiveness preferences. All of the *L. littorea* used in the current study were food deprived for four days prior to the experiments, whereas *I. granulosa* were stored with *U. lactuca* providing food and shelter. The difference was because preliminary investigations in which *I. granulosa* were left without food prior to the start of the experiment resulted in mortality and cannibalism between animals.

It has been suggested in theory (Emlen, 1966; MacArthur and Pianka, 1966 referenced in Watson and Norton, 1985a) that herbivores may be less discriminating during feeding after a period of food deprivation; however Watson and Norton (1985a) emphasise the importance of distinguishing between edibility and attractiveness when discussing this subject. This suggests that edibility is not influenced by the nutritional state of an herbivore, but is a function of the mechanical facilities of the herbivores feeding apparatus coupled with the nutritional value of the potential food item. As attractiveness constitutes both the nutritive and non-nutritive characteristics of food it is more likely to be affected by the nutritional state of an herbivore (Watson and Norton, 1985a). If food deprivation occurs, *L. littorea* may be more likely to shift their preference priorities away from considering algae as a habitat towards algae as a food source. Watson and Norton (1985a) discovered that foraging became significantly less discriminate following food deprivation, with more herbivores choosing the less edible species when presented with the choice of two algae.

The results collected in this investigation show that *L. littorea* significantly preferred algae from functional group two over algae from groups three, five and six; they also displayed a preference for algae from functional group three over group six (Fig. 2.1, chapter 2), with *U. lactuca* and *M. stellatus* considered the most attractive species (Fig. 2.4, chapter 2). As *L. littorea* are slow moving herbivores they are likely to be unable to deal separately with both food and habitat requirements, and so must balance these two, often conflicting sets of pressures (Watson and Norton, 1985a). For *L. littorea*, feeding requirements appear to characterise their choices when determining the balance between food and habitat requirements (Watson and Norton, 1985a). *Littorina littorea* preferences could be explained by the theory that thin, foliose, sheet-like algae have higher surface area to volume ratios and so contain more energy than algae with low surface area to volume ratios, such as *M. stellatus* and *C. officinalis* (Littler and Littler, 1980). Although, once the surface of thicker algae are penetrated they may provide a high energy food supply. The least chosen species were *D. contorta* and *L. digitata* (Fig. 2.4, chapter 2), which were also two of the least edible species

(Fig. 3.1). *Littorina littorea* must regard these species as particularly inedible, as foraging herbivores are inclined to feed on the first alga encountered (regardless of the position of that alga in normal preference rankings) (Watson and Norton, 1985a). This tendency will probably increase as the herbivores hunger increases, and result in the indiscriminate grazing predicted by Optimal Foraging Theories (*e.g.* Hughes, 1980).

Optimal Foraging Theories suggest that past feeding experience and memory may influence a herbivore's response to a prey item. Watson and Norton (1985a) concluded that previous feeding experience, like nutritional status, would affect a herbivores perception of attractiveness rather than edibility. Separate individuals of *L. littorea* were used in both the edibility and attractiveness experiments, so none of the grazers encountered an alga more than once (although they could have all had prior (field) experience of all algal species). Therefore, for *D. contorta* and *L. digitata* to be the least preferred species in both sets of experiments, *L. littorea* must be familiar with these algae from foraging on the shore and may have purposefully avoided them in the attractiveness experiments.

3.5 Summary

- Algal species were not similarly edible to either *L. littorea* or *I. granulosa.* Both grazers significantly preferred *Ceramium* sp. over all other algal species, while *L. littorea* found *D. contorta* the least edible alga, and *I. granulosa* found *L. digitata* the least edible algal species.

- For *L. littorea,* algal edibility decreased from functional group two to functional group six as *L. littorea* displayed a significance preference for algae from functional group two while algae from functional group six were consumed the least.

- *Idotea granulosa* consumed algae from functional group six significantly more than algae from the other functional groups. There was a decrease in algal edibility from functional groups two to five; with algae from functional group five consumed the least.

- For both grazers there was no significant relationship between the rank order of algal edibility of functional groups and the rank order predicted by Steneck and Watling (1982).

- Within functional groups, *L. littorea* found algae within functional groups four, five, and seven similarly edible, but significantly preferred to consume *Ceramium* sp. over *Polysiphonia* sp. within functional group two. Within functional group three, *U. lactuca* and *U. intestinalis* were preferred over *D. contorta* and *P. palmata*.

- Algae within functional groups two, three and four were consumed in similar amounts by *I. granulosa*; within functional group five *F. serratus* was consumed significantly more than *L. digitata*.

- For *I. granulosa* the rank order of functional group preference for attractiveness was not significantly related to the rank order of functional group preference for edibility.

- Feeding preferences observed by grazers may be caused not only by differences in algal morphology, but also differences between the level of algal defences and algal nutritive content.

Chapter 4

Does food availability dictate *Littorina littorea* grazing habits?

4.1 Introduction

Results from previous chapters have indicated that *L. littorea* found filamentous algae representing functional two to be both more attractive than algae from other functional groups (see Fig. 2.1 and section 2.3.2.1 of chapter 2) and the most edible compared to algae from any other functional group (see Fig. 3.3 and section 3.3.3.1 of chapter 3). According to the functional group model proposed by Steneck and Watling (1982), algae representing functional group two comprise some of the least complex species, thus making them easier for grazers to consume. Although algae within this group may not provide a structurally complex habitat compared with algae in other functional groups, as a result of *L. littorea* having a protective shell, their feeding preferences may be determined more by food requirements rather than habitat requirements (as discussed in section 2.4.1 of chapter 2). However, as filamentous algae rarely dominate in the natural environment (personal observation) and algal availability may be an important factor determining feeding preference (Paine and Vadas, 1969) (see section 3.4.4 of chapter 3), the following experiment has been designed to further investigate the effect of algal availability on the feeding preferences of *L. littorea*, specifically to determine whether *L. littorea* exhibit a preference for algae dominant in their habitat or other algal species.

4.1.1 Food availability

Johnson (1980) defined the availability of a component (such as food type) as 'its accessibility to the consumer'. As *L. littorea* is a relatively mobile herbivore it has the potential to come into contact with a range of algal species within a tidal range. As discussed in section 1.2 of chapter 1, food preference is comprised of two factors: edibility and attractiveness. It may be the attractiveness of an alga that initiates a herbivores choice of food (as has been previously discussed in section 2.1 of chapter 2), but 'preconditioning' or 'habituation' can also influence food preference (Dorn *et al*, 2001). Preconditioning causes an organism to become accustomed to a situation in advance of experimental manipulation, while habituation is the process by which preconditioning occurs. Watson and Norton (1985a) suggested that previous feeding experience affected the attractiveness of algal species to grazers. They tested the effect upon *L. littorea* of prolonged contact with *F. serratus*, which they considered to be of medium to low attractiveness following their earlier experiments (Watson and Norton, 1985a). *Littorina littorea* were left to habituate for 100 days with *F. serratus*; preconditioned snails were then presented with two pairs of algae (*F. serratus*/*Pelvetia canaliculata* (Linnaeus) Decaisne and Thuret and *F. serratus*/*U. intestinalis*) (Watson and Norton, 1985a). For both pairings more snails chose *F. serratus* after habituation than before (Watson and Norton, 1985a), suggesting that they had 'learned' to prefer the alga. During foraging, herbivores use tactile and chemical sampling (Watson and Norton, 1985a) to determine the suitability of a food. Once an alga is considered suitable with regards to its attractiveness, herbivores will then 'taste' the food to confirm whether it will be beneficial to consume (Steneck and Dethier, 1994). Although species may appear similarly attractive to a herbivore, not all algal species encountered will provide the same level of nutritional quality (Marsham *et al*, 2007, Appendix II). Generally, low quality species are those that are low in nitrogen, protein, and total organic carbon (Chapman, 1950; Martinez *et al*, 1995; Galan Jimenez *et al*, 1996; Cruz-Rivera and Hay, 2000). Horn and Neighbors (1984) suggested a range of approaches grazers could utilise in order to overcome potential problems of consuming low quality dietary species, including i) consuming the most nitrogen-rich foods or parts of foods; ii) switching among food parts and species and iii) increasing consumption rates or prolonging time spent feeding. They conducted an experiment in which the herbivorous fish, the monkeyface prickleback *Cebidichthys violaceus* Girard was presented with eight species of algae ranging in nutritional quality. The algal species with the highest amounts of nitrogen and protein (*Porphyra perforate* Agardh, *Microcladia* Greville and *Smithora* Hollenberg) ranked highest in laboratory preference experiments (Horn and Neighbors, 1984). However, the authors observed that other algal species with lower nitrogen and protein contents were frequently more abundant in the diets of wild populations, which led them to conclude that additional factors, such as algal availability must be considered when predicting dietary choice (Horn and Neighbors, 1984). While considering the work of previous authors regarding herbivore food preferences in relation to their work on algal calorific values, Paine and Vadas (1969) also concluded that food availability may have been an important factor in the evolution of food preference, as algae preferentially consumed by a range of herbivores were not always those with the greatest food value, but were species abundant in the grazers' habitat.

Carefoot (1967) observed that the sea slug *Aplysia punctata* Cuvier consumed a number of different algae in its natural environment. Juvenile *A. punctata* preferred red algae such as *Delessaria sanguinea* (Hudson) Lamouroux, whereas mature sea slugs consumed green and brown algae such as *Ulva*, *Fucus* and *Laminaria* (Carefoot, 1967). Another species, *Aplysia californica* Cooper was limited to a diet of red algae in its natural environment; however they preferentially consumed *U. lactuca* and *U. intestinalis* in laboratory experiments (Carefoot, 1967). Carefoot (1967) presented sublittoral *A. punctata* with a choice of algae normally encountered in their habitat; the grazers consumed the algal species relative to the value each provided for growth, as *Plocamium coccineum* Lamouroux (considered to be the most nutritional valuable) was preferred over *Heterosiphonia plumosa* (Ellis) Batt, *Cryptopleura ramosa* (Hudson) Kylin ex Newton and *D. sanguinea* (Carefoot, 1967). When species not normally present in their habitat were included in choice experiments, the order of preference changed as *U. intestinalis* was preferentially consumed over all species, while *U. lactuca* was the second choice, even over *P. coccineum* (Carefoot, 1967). Additionally, *L. digitata* was refused when presented in choice experiments even though it was abundant in *A. punctata*'s subtidal habitat (Carefoot, 1967). The absorption of food was highest for algal species consumed in the natural habitat and those providing the best growth; and lowest for algae not usually consumed or those giving the poorest growth *e.g. D. sanguinea* and *L. digitata* (Carefoot, 1967). These results suggest that algal species which appear to be preferentially consumed may not normally be encountered in a herbivores natural habitat (see section 4.1.1 above for information relating to factors determining food quality).

In conjunction with availability, plants that are susceptible to grazing can gain protection from herbivores via association with other plant species (Pfister and Hay, 1988). Associational refuges occur when neighbouring plants reduce the apparency of the target species, thus reducing the likelihood of it being found by herbivores (Feeny, 1976). The susceptibility of target plant species may be affected by the palatability, abundance and size of neighbouring plant species due to the influence of their relative palatability, availability, accessibility and apparency (Miller *et al*, 2007). Juenger *et al* (2005) observed strong effects of plant apparency on the scarlet gilia *Ipomopsis* *aggregata* (Pursh) V. Grant ssp. *candida* (Rydb.) V. & A. Grant. Tall, early flowering plants experienced a high level of tissue damage by the herbivorous seed fly *Delia* sp. France, though after controlling for the effect of apparency, plants experienced reduced seed fly damage (Juenger *et al*, 2005).

4.1.2 The experimental algae

Polysiphonia lanosa (Linnaeus) Tandy is a filamentous alga representing functional group two. It is an epiphyte that is found growing on fucoid algae (from functional group five), specifically *A. nodosum* (Hawkins and Hartnoll, 1983), *F. serratus* and *F. vesiculosus* (Rindi and Guiry, 2004). Although it lacks cortication and is not morphologically complex, it has a very tough rigid texture (Maggs and Hommersand, 1993). *Polysiphonia lanosa* contains halophenolics (Hay and Fenical, 1988), which are considered to act as chemical defences. Fucoids are also known to produce significant quantities of chemicals (see section 3.4.2.2 of chapter 3) that act as feeding deterrents to grazers. It has been shown that carbon exchange occurs between *P. lanosa* and its host *A. nodosum* (Ciciotte and Thomas, 1997), though there is no evidence of chemical defences being exchanged between the two algae. It has been suggested that phlorotannins produced by fucoids may prevent the settlement of epiphytes, though this has only been proven to inhibit the settlement of invertebrate larvae (Wikstrom and Pavia, 2004). *Polysiphonia* sp. have a relatively high protein content and calorific value (Marsham *et al*, 2007 and references therein, Appendix II), which may make it a high quality food.

Ulva intestinalis is a foliose alga representing functional group three (Table 1.1, chapter 1) that has thin, soft, tubular fronds (see section 3.4.1 of chapter 3). It is an ephemeral alga that invests energy into fast growth rates and reproduction, rather than structural or chemical defences (see section 1.1.2 of chapter 1). Like *Polysiphonia* sp., *Ulva* sp. have high protein contents and calorific values (Carefoot, 1967, 1973; Paine and Vadas, 1969; Indergaard and Minsaas, 1991; Fris and Horn, 1993; Crossman *et al*, 2000), though contain less crude fibre and neutral detergent fibre than *Polysiphonia* sp. (Marsham *et al*, 2007 and references therein, Appendix II).

4.1.3 Aims

This part of the investigation aims to determine if *L. littorea* exhibit a preference between algal species that dominate in their habitat of origin or other algal species, specifically with the proposed theories that if algal availability affects feeding preferences i) *L. littorea* will be more attracted to the algal species dominant in their habitat of origin; and ii) *L. littorea* will consume more of the algal species dominant in their habitat of origin when presented with algae in single-choice experiments; and when presented with two algae simultaneously, under the premise of Steneck and Watling's (1984) functional group model that iii) *L. littorea* will prefer algae from functional group two over algae from functional group three.

4.2 Methods

Attractiveness and edibility experiments were conducted in which *L. littorea* from two areas of shore dominated by two different algae were presented with the two algal species in both single- and two-way choice experiments.

4.2.1 Study Site

Samples were collected from Fintry Bay, Millport, Isle of Cumbrae, UK (55°46′N, 4°56′W). Fintry Bay is a predominantly bedrock shore with several boulder beds. *Ascophyllum nodosum* is located on the mid and upper shore attached to boulders and rocks, where it can form dense stands. *Ulva intestinalis* is distributed throughout the shore from the upper shore to the sublittoral and can grow on a range of substrates from small boulders to bedrock to sediment. It forms dense mats across the surface of the substrate. On Fintry Bay, the two algal species form distinct, adjacent bands on the mid shore. *Littorina littorea* is found on *A. nodosum* plants, the surrounding boulders and in crevices and on overhangs in the boulder field. They are also widely distributed on the bedrock covered by *U. intestinalis*.

4.2.2 Availability-related attractiveness of algal functional groups

During July 2003, mature *L. littorea* (mean shell height 2.02 ± 0.01 cm S.E.) were collected from the two habitats at Fintry Bay (see section 4.2.1 above for site description): one dominated by *P. lanosa* (as an epiphyte on *A. nodosum*) and the other dominated by *U. intestinalis*. Samples of *P. lanosa* (representing functional group two) and *U. intestinalis* (representing functional group three) were also collected from Fintry Bay. Following collection *L. littorea* were food deprived for 48 hours in filtered seawater (see section 3.4.4.1 of chapter 3 for discussion on the effects of food deprivation on feeding preferences). Algae were cleaned to remove epifauna and epiphytes by washing in running seawater and examination under a dissecting microscope. Epifauna were removed so that any changes observed were due to the experimental grazers and not other fauna attached to the algae. Removing epiphytes ensured that any changes observed were as a result of preference for the experimental algae, rather than the epiphytes. All algae used in experiments were mature plants with no reproductive tissue. Twelve *L. littorea* from each habitat were placed in separate experimental tanks (18 x 12.5 x 5 cm) with 600 ml of filtered seawater and allowed to explore their surroundings for 30 minutes, and thereby acclimate to the experimental conditions. After 30 minutes all snails were returned to the centre of the experimental tank and 4 g of the two algal species were introduced in two-way choice experiments with one species at each end of the tank. Experiments were left to run for a further 30 minutes, during which the snails were allowed freedom of movement throughout the experimental tank. After this period the number of *L. littorea* on each alga was counted. The experiment was repeated six times, using new snails for each replicate. Those snails found on plant material were presumed to have demonstrated behavioural preference for that species.

The resultant data were used to investigate the possibility that *L. littorea* would be most strongly attracted to the alga that dominated their habitat upon the shore.

4.2.3 Availability-related edibility of algal functional groups presented in single-choice experiments

Littorina littorea, *P. lanosa* and *U. intestinalis* were collected from Fintry Bay (see section 4.2.1 above for site description) using the methodology outlined in section 4.2.2 above. Three *L. littorea* from each habitat were placed in separate experimental tanks (18 x 12.5 x 5 cm) with 200 ml of filtered seawater. The algae were blotted dry using a standard procedure (algae were placed on paper towels and blotted six times) and weighed (error of balance ± 0.001 g) and 5 g of each alga was added to separate tanks in single-choice experiments. After five days the grazers were removed and the algae reweighed. Alongside the experimental tanks, grazer free controls were set up to allow for autogenic changes. The water in both the experimental and control tanks was changed every second day. The experiment was repeated six times for each of the two algal species, using new snails for each replicate. Once the algae had been reweighed, any change in weight was calculated using equations 2 and 3 (see section 3.2.1.1 of chapter 3). It was assumed that a decrease in weight equalled the amount consumed by *L. littorea*, and that the total amount consumed was a measure of edibility (Cronin *et al*, 2002).

The data were used to test the hypothesis that *L. littorea* would consume more of the alga that was dominant in the area of the shore from which they were collected *i.e.* that with which it was presumed they were most familiar.

4.2.4 Availability-related edibility of algal functional groups presented in two-way choice experiments

Following on from section 4.2.3 above, this experiment was conducted to see whether the food preferences of *L. littorea* would be the same when offered two algae simultaneously. Again samples of *L. littorea*, *P. lanosa* and *U. intestinalis* were collected from Fintry Bay (see section 4.2.1 above for site description) using the methodology outlined in section 4.2.2 above. Twelve *L. littorea* from each habitat were placed in separate experimental tanks (18 x 12.5 x 5 cm) with 600 ml of filtered seawater and 5 g of both algae were presented in two-way choice experiments.

Experiments ran for four days, after which the grazers were removed and the algae reweighed. The water in the tanks was changed every second day. Three replicates were set up for each two-way choice experiment, using new grazers in each replicate. Due to time constraints, not enough algal material was collected and so no controls ran for this experiment. However, all other experiments (see sections 3.3.2.1, 4.3.2.2, 5.3.2, 5.3.3.1, 5.3.4.1) showed no significant changes in weight of algae in control tanks. Therefore, it can be assumed that any changes in weight observed in this experiment were due to grazer feeding activity. Once the algae had been reweighed, any change in weight was calculated using equation 2 (see section 3.2.1.1 of chapter 3). It was assumed that a decrease in weight equalled the amount consumed by *L. littorea*, and that the total amount consumed was a measure of edibility (Cronin *et al*, 2002).

The data were used to test the hypothesis that when presented with a choice of food, *L. littorea* would prefer algae from functional group two to algae from functional group three, and so consume more *P. lanosa* than *U. intestinalis*, in line with the functional group model predicted by Steneck and Watling (1982).

4.2.5 Statistical Analyses

4.2.5.1 Availability-related attractiveness of algal functional groups

For grazers from areas of a shore dominated by two different algae, Chi-square tests (Fowler *et al*, 1998) were conducted using pooled replicate data to test the null hypothesis that *L. littorea* from two habitats dominated by different algae would show no significant preference in attractiveness for either algal species. During the experiments, a number of individuals did not make a 'choice' between the two algal species, although this was always less than half of the total number of grazers. These data were omitted from the statistical tests as the number of grazers not making a choice may have skewed the data so that any differences in preference between the two algae were masked. As there were only two categories for each functional group, and thus only one degree of freedom, data were corrected using Yates' Correction for Continuity (Fowler *et al*, 1998).

4.2.5.2 Edibility experiments

Data for experiments outlined in sections 4.2.3 and 4.2.4 above were screened using the Kolmogorov-Smirnov normality test and Levene's test to test for normality of distribution and homogeneity of variances respectively (Sokal and Rohlf, 1995). Data not meeting the assumptions of homogeneity were log transformed prior to analysis.

4.2.5.2.1 Availability-related edibility of algal functional groups presented in single-choice experiments

To determine whether *L. littorea* exhibited any food preference between the two algal species, results were analysed using three-factor ANOVA. The null hypothesis that *L. littorea* would show no significant preference for algae from either habitat of origin was examined with GRAZER PRESENCE/ABSENCE a fixed factor with two levels, algae from their HABITAT OF ORIGIN a fixed factor with two levels and algae as a FOOD CHOICE a fixed factor with two levels (Underwood, 1997). GRAZER PRESENCE/ABSENCE, HABITAT OF ORIGIN and FOOD CHOICE were independent variables, and the weight of algae consumed was the dependent variable (Pallant, 2001). *Post hoc* Tukey pairwise comparisons could not be performed for GRAZER PRESENCE/ABSENCE, HABITAT OF ORIGIN or FOOD CHOICE, as there were fewer than three categories for each variable. Alternatively, descriptive statistics (mean ± standard error) were compared in order to determine any differences between the mean amounts of each species consumed in single-choice experiments.

4.2.5.2.2 Availability-related edibility of algal functional groups presented in two-way choice experiments

Two-factor ANOVA was used to test the null hypothesis that there would be no significant difference in food preferences of *L. littorea* from the two habitats of origin when offered the two algae simultaneously. Data were tested with HABITAT OF ORIGIN as a fixed factor with two levels and FOOD CHOICE as a fixed factor with two levels (Underwood, 1997). HABITAT OF ORIGIN and FOOD CHOICE were independent variables, and the weight of each species of algae consumed was a dependent variable (Pallant, 2001). *Post hoc* Tukey pairwise comparisons could not be performed for HABITAT OF

ORIGIN or FOOD CHOICE, as there were fewer than three categories for each variable.

ORIGIN or FOOD CHOICE, as there were fewer than three categories for each variable.

Alternatively, descriptive statistics (mean ± standard error) were compared in order to determine any differences between the mean amounts of each species consumed in two-way choice experiments.

4.3 Results

4.3.1 Availability-related attractiveness of algal functional groups

In order to determine whether *L. littorea* exhibited a preference between algae that dominated their habitat of origin or other algal species, grazers were presented with a choice of two algae simultaneously and the number of grazers on each species was counted, which was assumed to indicate a choice preference. There was no significant difference in the number of *L. littorea* from the habitat dominated by *P. lanosa* choosing either algal species ($\chi^2_1 = 0.43$, $P > 0.05$) with 1.67 ± 0.56 snails (mean ± standard error, n = 6) choosing *P. lanosa* and 2.17 ± 0.65 snails (mean ± range, n = 6) choosing *U. intestinalis* (Fig. 4.1). However, *L. littorea* from the area of shore dominated by *U. intestinalis* showed a significant preference for *U. intestinalis* (mean = 3.83 ± 1.01 standard error, n = 6) over *P. lanosa* (mean = 1.50 ± 0.56 standard error, n = 6) ($\chi^2_1 = 6.16$, $P < 0.05$) (Fig. 4.1).

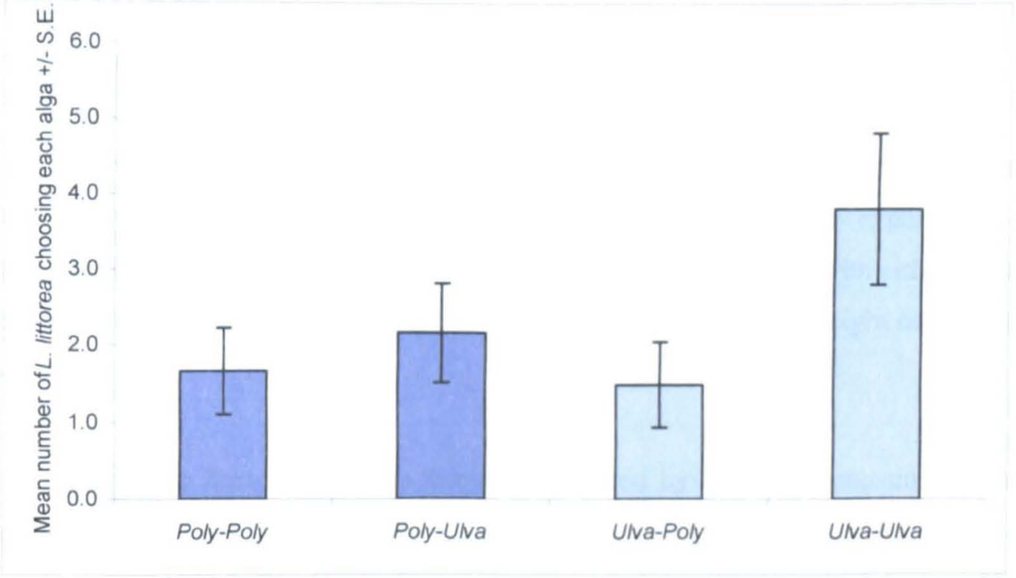

Figure 4.1 – Mean number of *Littorina littorea* from two habitats choosing alga from each of the two habitats in two-way choice experiments (± standard error) (n = 24). NB: the first algal species reported on the x-axis relates to the habitat from which the grazers originated; the second species relates to the algal species presented as food.

4.3.2 Edibility experiments

4.3.2.1 Data Screening

The results of the Kolmogorov-Smirnov normality test showed that for both the single- and two-way choice experiments all data were normally distributed (Kolmogorov-Smirnov, $P > 0.15$). Levene's test of homogeneity of variances showed a significant difference in the variance of each algal species consumed during single-choice experiments (Levene's, $L_{7, 40} = 2.425$, $P < 0.05$). However, after logarithmic transformation data were shown to be homogenous (Levene's, $L_{7, 40} = 1.624$, $P > 0.15$), therefore, it can be assumed that all samples were drawn from populations with equal or similar variances. For the two-way choice experiment, Levene's test of homogeneity of variances showed no significant difference in the variance of each algal species consumed (Levene's, $L_{3, 8} = 1.236$, $P > 0.15$). Therefore, it can be assumed that all samples were drawn from populations with equal or similar variances.

4.3.2.2 Availability-related edibility of algal functional groups presented in single-choice experiments

To determine whether *L. littorea* displayed a grazing preference for the alga that was dominant in their habitat of origin or other algal species, edibility experiments were conducted in which three grazers from each habitat were presented with either *P. lanosa* or *U. intestinalis* in single-choice experiments and any change in weight of plant tissue was calculated.

Littorina littorea from the habitat dominated by *P. lanosa* consumed more *U. intestinalis* (mean = 1.57 ± 0.38 g S.E., n = 6) than *P. lanosa* (mean = 1.19 ± 0.23 g S.E., n = 6), whereas grazers from the area of shore dominated by *U. intestinalis* consumed more *P. lanosa* (mean = 1.24 ± 0.24 g S.E., n = 6) than *U. intestinalis* (mean = 0.39 ± 0.11 g S.E., n = 6) (Fig. 4.2).

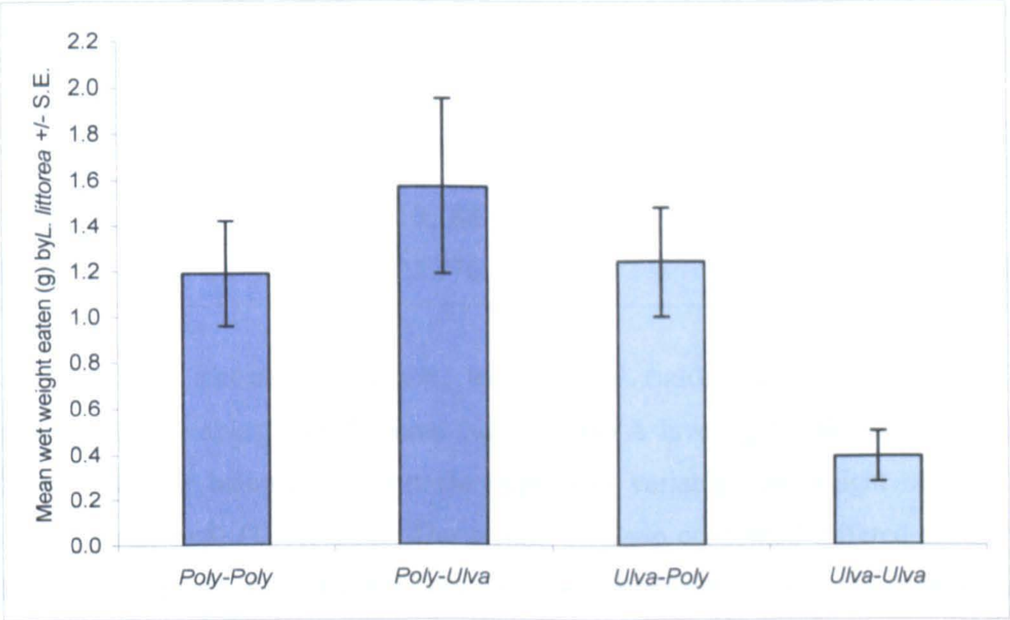

Figure 4.2 – Mean wet weight of each alga consumed in single-choice experiments by *Littorina littorea* (± standard error) (n = 24). NB: the first algal species reported on the x-axis relates to the habitat from which the grazers originated; the second species relates to the algal species presented as food.

Littorina littorea from each habitat consumed more of the algal species that was not dominant in their habitat of origin than the alga that was dominant. The difference in the mean weights of *P. lanosa* and *U. intestinalis* consumed by *L. littorea* from the *two different habitats were significant (Table 4.1).*

Table 4.1 - Three-factor analysis of variance to investigate the effects of GRAZER PRESENCE/ABSENCE, HABITAT OF ORIGIN and FOOD CHOICE on the food preferences of *Littorina littorea* (level of significance ** $P < 0.01$, NS not significant).

Source of Variation	df	SS	MS	F	*P*
GRAZER +/-	1	2.170	2.170	8.523	0.006**
HABITAT OF ORIGIN	1	0.573	0.573	2.252	0.141NS
FOOD CHOICE	1	1.913	1.913	7.513	0.009**
GRAZER +/- * HABITAT	1	0.175	0.175	0.689	0.411NS
GRAZER +/- * FOOD CHOICE	1	0.270	0.270	1.062	0.309NS
HABITAT * FOOD CHOICE	1	0.947	0.947	3.719	0.061NS
GRAZER +/- * HABITAT * FOOD CHOICE	1	0.071	0.071	0.280	0.280NS
Error	40	10.184	0.255		
Total	47	21.776			

GRAZER PRESENCE/ABSENCE, HABITAT OF ORIGIN and FOOD CHOICE were independent variables, and the three-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the weight of each algal species consumed) (Table 4.1). The weight of algae consumed differed significantly depending on GRAZER PRESENCE/ABSENCE. Algae presented as food showed greater weight loss in experimental tanks with grazers present (mean = 1.095 ± 0.116 g S.E., n = 24) than in the control tanks (mean = 0.541 ± 0.116 g S.E., n = 24), which shows that *L. littorea* were actively feeding during the experiment. There was also a significant difference in consumption between the algal species offered as FOOD CHOICE (Table 4.1). *Littorina littorea* consumed more *P. lanosa* (mean = 1.010 ± 0.116 g S.E., n = 12) than *U. intestinalis* (mean = 0.627 ± 0.116 g S.E., n = 12) regardless of the grazers habitat of origin. *Littorina littorea* from the area of shore dominated by *P. lanosa* consumed more algae overall (mean = 0.955 ± 0.116 g S.E., n = 12) than *L. littorea* from the habitat dominated by *U. intestinalis* (mean = 0.682 ± 0.166 g S.E., n = 12) regardless of the food offered, though this difference was not statistically significant (Table 4.1). As the interaction effect between GRAZER PRESENCE/ABSENCE and HABITAT OF ORIGIN was not significant (Table 4.1), there was the same pattern of consumption between habitats in that algae presented to grazers always lost more weight than algae with no grazers. The interaction effect between GRAZER PRESENCE/ABSENCE and FOOD CHOICE was also not significant (Table 4.1). These results suggest that grazers consumed the same amount of food in each habitat. As the interaction effect between HABITAT OF ORIGIN and FOOD CHOICE was not significant (Table 4.1), the grazers' HABITAT OF ORIGIN did not have a significant effect on the amount (g) of each algal species consumed. Similar amounts of algae were consumed irrespective of the habitat. Finally, the interaction between GRAZER PRESENCE/ABSENCE, HABITAT OF ORIGIN and FOOD CHOICE was not significant (Table 4.1). Regardless of GRAZER PRESENCE/ABSENCE, there was no significant difference between the changes in weight of each FOOD CHOICE consumed by *L. littorea* from either HABITAT OF ORIGIN.

4.3.2.3 Availability-related edibility of algal functional groups presented in two-way choice experiments

In order to determine whether the food preferences of *L. littorea* would be the same when offered a choice of algae, experiments were conducted in which grazers were offered two algae simultaneously.

Littorina littorea from the area of shore dominated by *P. lanosa* consumed more *U. intestinalis* (mean = 1.410 ± 0.240 g S.E., n = 3) than *P. lanosa* (mean = 0.367 ± 0.190 g S.E., n = 3); *L. littorea* from the habitat dominated by *U. intestinalis* also consumed more *U. intestinalis* (mean = 0.700 ± 0.070 g S.E., n = 3) than *P. lanosa* (mean = 0.530 ± 0.280 g S.E., n = 3) (Fig. 4.3).

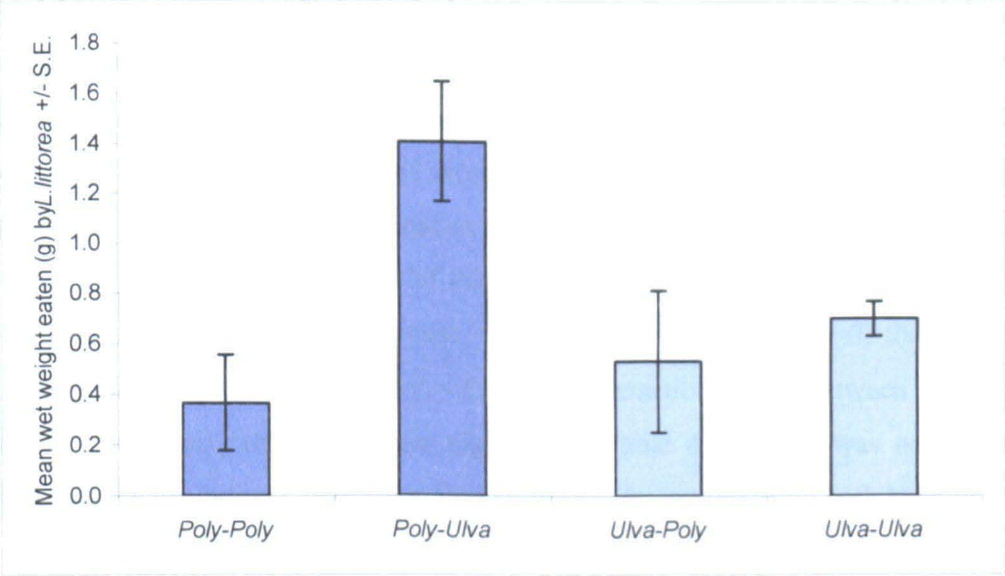

Figure 4.3 – Mean wet weight of each alga consumed in two-way choice experiments by *Littorina littorea* (± standard error) (n = 12). NB: the first algal species reported on the x-axis relates to the habitat from which the grazers originated; the second species relates to the algal species presented as food.

Littorina littorea consumed more *U. intestinalis* than *P. lanosa* regardless of the algal species that was dominant in their habitat of origin, and this difference in the change in weight was significant (Table 4.2)

Table 4.2 - Two-factor analysis of variance to investigate the effects of HABITAT OF ORIGIN and FOOD CHOICE on the food preferences of *Littorina littorea* (level of significance * $P < 0.05$, NS not significant).

Source of Variation	df	SS	MS	F	*P*
HABITAT OF ORIGIN	1	0.224	0.224	1.710	0.227NS
FOOD CHOICE	1	1.104	1.104	8.423	0.020*
HABITAT * FOOD CHOICE	1	0.572	0.572	4.364	0.070NS
Error	8	1.049	0.131		
Total	12	9.729			

HABITAT OF ORIGIN and FOOD CHOICE were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the weight of each algal species consumed). The amount (g) of food consumed by grazers from the two HABITATS OF ORIGIN, regardless of the FOOD CHOICE, was not significantly different (Table 4.2) though *L. littorea* from the habitat dominated by *P. lanosa* consumed more algae overall (mean = 0.888 ± 0.148 g S.E., n = 6) than *L. littorea* from the area of shore dominated by *U. intestinalis* (mean = 0.615 ± 0.148 g S.E., n = 6). The difference in the weight of each algal species consumed was significant (Table 4.2), regardless of the grazers' HABITAT OF ORIGIN. *Littorina littorea* consumed more *U. intestinalis* (mean = 1.055 ± 0.148 g S.E., n = 6) than *P. lanosa* (mean = 0.448 ± 0.148 g S.E., n = 6). The interaction effect between HABITAT OF ORIGIN and FOOD CHOICE was not significant (Table 4.2); there was no significant difference in the proportion of each food consumed by grazers from both habitats.

4.4 Discussion

It has been previously shown that *L. littorea* found algae from functional two more attractive than algae from other functional groups (Fig. 2.1 and section 2.3.2.1 of chapter 2) and consumed more algae from functional group two than any other functional group (Fig. 3.3 and section 3.3.3.1 of chapter 3).

4.4.1 Availability-related attractiveness of algal functional groups

When presented with a choice between *P. lanosa* and *U. intestinalis*, *L. littorea* collected from two areas within a shore (one dominated by *P. lanosa* as an epiphyte on *A. nodosum* and one dominated by *U. intestinalis*) found *U. intestinalis* slightly more attractive, though this difference was not significant. There was no difference in attractiveness between the two algal species presented to *L. littorea* from the area of shore dominated by *P. lanosa*, however, grazers from the area of shore dominated by *U. intestinalis* found *U. intestinalis* to be significantly more attractive than *P. lanosa* (Fig. 4.1 and section 4.3.1 above). According to the functional group model predicted by Steneck and Watling (1982) algae from functional group two should be more attractive to grazers than algae from functional group three, therefore the results collected do not support the models predictions. This could possibly be due to the morphology of the two algal species, as the size, shape and structure of an alga may affect the capability of a grazer to utilise a plant (Steneck and Dethier, 1994) (see section 1.1. of chapter 1).

The tubular, foliose fronds of *U. intestinalis* are wider than the wiry, tough filamentous fronds of *P .lanosa*, which may make them more attractive to *L. littorea* as they provide a greater surface area to act as a habitat. Although a single tubular frond of *U. intestinalis* may be too narrow to carry the entire foot of *L. littorea* (Watson and Norton, 1985b) (as discussed in section 3.4.1 of chapter 3); the fact that they are wider than the fronds of *P. lanosa* suggests their morphology is better suited to bearing a grazer's weight (see section 2.4.2 of chapter 2 for introductory discussion). Also, as *P. lanosa* is an epiphyte on *A. nodosum* it may not be as physically accessible to grazers as *U. intestinalis*, which forms dense mats on the rocky shore.

It may be important to note that experimental conditions in the laboratory did not replicate the natural conditions occurring upon the shore. Upon the shore intertidal organisms are subjected to changes and disturbances caused by incoming and outgoing tides. Incoming tides cause algae lying flat upon the substrata to stand upright, thus altering the community structure. In the laboratory the water in experimental tanks was motionless and so the algae presented to grazers did not change in its position and shape throughout the experimental period. Herbivores' perceptions of algae presented under artificial conditions with reduced levels of disturbance may be altered, as algae may differ in their susceptibility to be manipulated by herbivores.

4.4.2 Availability-related edibility of algal functional groups presented in single- and two-way choice experiments

In single-choice edibility experiments *L. littorea* consumed more of the algal species that did not dominate in their habitat of origin (Fig. 4.2), with grazers collected from the area of shore dominated by *P. lanosa* consuming more algae overall regardless of the food offered. These results suggest that *L. littorea* may have switched their diet to adapt to the food presented to them.

4.4.2.1 Diet Switching

Diet switching occurs when the abundance of a previously avoided food changes relative to the abundance of other food (Crawley, 1983). As grazers were only presented with one alga at a time, the abundance of the alga that did not dominate in their habitat of origin was over-represented in relation to the amount of the alga available. Overall, *L. littorea* consumed more *P. lanosa* than *U. intestinalis* regardless of their habitat of origin. However, when presented with *P. lanosa* and *U. intestinalis* simultaneously, *L. littorea* consumed more *U. intestinalis* than *P. lanosa*, regardless of which algal species dominated on their shore of origin (Fig. 4.3). While investigating the effect of plant chemicals on feeding behaviour, Imrie *et al* (1990) presented *L. littorea* with extracts of *U. lactuca* and *Porphyra* sp. simultaneously to determine feeding preference. They suggested that the preference *L. littorea* displayed for each of the two algal species was enhanced when grazers experienced the two algal species simultaneously (Imrie *et al*, 1990). By examining other authors work relating to the chemically-mediated basis of food preference, Imrie *et al* (1990) concluded that juvenile *L. littorea* that had been removed from a site where both *U. lactuca* and *Porphyra* sp. occurred displayed a strong preference for extracts of *Porphyra* sp.. Juveniles preconditioned with either *U. lactuca* extract or *Porphyra* sp. extract consumed similar amounts of both algal species, although those habituated to *Porphyra* sp. consumed more algae overall (Imrie *et al*, 1990). Juveniles previously maintained on a mixed diet of extracts of both *U. lactuca* and *Porphyra* sp. consumed more *Porphyra* sp. than *U. lactuca* (Imrie *et al*, 1990). The authors attributed this to the fact that the individual grazers used in the investigation had prior experience of encountering either one or both of the algal species in their natural environment; though suggest the preference for *Porphyra* sp. was due to a higher concentration of a phagostimulant chemical found within its tissues that was present in both algal species (Imrie *et al*, 1990). This implies that the presence of a phagostimulant chemical increases a herbivores' preference to an alga, though there is no suggestion of whether this is by increasing the nutritional value of the alga or increasing the palatability of the alga.

4.4.2.2 Compensatory feeding

As *U. intestinalis* may be the more favourable alga, when *L. littorea* from the area of shore dominated by *U. intestinalis* were presented with *P. lanosa* they may have exhibited compensatory feeding. Grazers may compensate for lower prey quality by consuming more, rather than less, prey tissue (Cruz-Rivera and Hay, 2000). Higher quality algae may improve a grazers' fitness and so it would be advantageous for it to be selected when available. However, a grazer may not always have access to high quality foods due to predation pressure, competition between grazers or abiotic stresses limiting distribution (for example upper or lower limits upon a shore) (Cruz-Rivera and

Hay, 2000). Grazers consuming lower quality foods may still acquire sufficient nutrients by increasing their consumption rates or assimilation efficiency (Cruz-Rivera and Hay, 2000). Cruz-Rivera and Hay (2000) examined the relationship between compensatory feeding and food quality. Three species of amphipod, the non-tube dwelling species *Gammarus mucronatus* Say and *Elasmopus levis* Smith and the tube-building *Ampithoe longimana* Smith were offered manipulated high- and low-quality foods made by mixing *U. intestinalis*, *U. lactuca*, *Hypnea* Lamouroux, *Graciliaria* Greville and fish flakes. Both *G. mucronatus* and *E. levis* preferred the high quality diet (composed of fish food high in protein, nitrogen, and total organic carbon) when offered simultaneously, whereas *A. longimana* did not differentiate (Cruz-Rivera and Hay, 2000). When presented with only the low quality diet (low in protein, nitrogen, and total organic carbon created using all four algal species), all three amphipods exhibited compensatory feeding.

Littorina littorea residing on a shore dominated by *P. lanosa* may not have access to higher quality algae, though they still obtain sufficient nutrients from *P. lanosa* by increasing the amount of algae consumed. When *L. littorea* from the habitat dominated by *U. intestinalis* were presented singularly with *P. lanosa* they may have increased their consumption rate in order to obtain similar levels of nutrients as normally provided by *U. intestinalis*. It is possible that *L. littorea* from the habitat dominated by *P. lanosa* consumed more *U. intestinalis*, as it is a higher quality food due to it containing a lower proportion of fibre (Marsham *et al*, 2007 and references therein, Appendix II). Grazers may have consumed more algae on average to gain nutrients while they were available. When presented with the two algae simultaneously, *L. littorea* from both habitats consumed more *U. intestinalis*. When the preferred and less preferred algae were offered together, it is likely that the more attractive, more beneficial *U. intestinalis* was rapidly consumed, especially by *L. littorea* from the area of shore dominated by *P. lanosa*. The increased consumption of *U. intestinalis* probably resulted in reduced consumption of *P. lanosa* (Barker and Chapman, 1990). According to their theory, had all of the *U. intestinalis* in this experiment been consumed, *P. lanosa* would have become sufficiently attractive for grazers to consume it in greater quantities (Barker and Chapman, 1990).

4.4.2.3 Factors affecting herbivore feeding preference

The fact that *L. littorea* found *U. intestinalis* more attractive than *P. lanosa* and consumed more of it when presented with a choice of the two algae raises the question of why do they live in a habitat dominated by *P. lanosa*. The shore from which samples were taken during the investigation had an area dominated by *P. lanosa* as an epiphyte on *A. nodosum* and an area dominated by a large mat of *U. intestinalis* (see section 4.2.1 above for site description). The two areas of algae were not so close as to overlap but were not so far apart that *L. littorea* could not move between the two. *Littorina littorea* have been shown to migrate up and down a shore (Williams and Ellis, 1975), although Lubchenco (1978) observed that immigration and emigration of adult *L. littorea* in tide pools was rare. However, it has also been suggested that individuals are located within a specific region and if displaced they will move back into their zone of normal distribution (Alexander, 1960). *Ulva intestinalis* is a relatively small alga, and so provides grazers with little protection, for example from predators, wave exposure and desiccation. As *P. lanosa* is an epiphyte on *A. nodosum*, *L. littorea* gains a protective habitat as well as a food source by choosing to feed on *P. lanosa*. The large fronds of *A. nodosum* protect *L. littorea* from predators, wave exposure and provide a damp habitat during low tide. Additionally, *U. intestinalis* is an ephemeral species and so will change in quantity throughout the year. During the winter months when the amount of *U. intestinalis* present on the shore is depleted, *L. littorea* may need to utilise other available algal species as sources of food. However, the grazers metabolic rate may also decrease during winter, thus reducing the amount of algal consumed. Owing to competition for resources between grazers on a shore, and the fact that the benefits provided by *P. lanosa* and *U. intestinalis* meet different requirements of *L. littorea*; the feeding preferences of *L. littorea* are far from being fully understood.

4.5 Summary

- *Littorina littorea* found *U. intestinalis* more attractive than *P. lanosa* when presented with the two algae, regardless of the alga that was dominant in their habitat of origin.

- When offered in single-choice experiments, *L. littorea* preferentially consumed the alga that was not dominant in their habitat of origin.

- *Littorina littorea* from the area of shore dominated by *P. lanosa* consumed more *U. intestinalis*, whereas grazers from the habitat dominated by *U. intestinalis* consumed more *P. lanosa.*

- Overall, *L. littorea* consumed more *P. lanosa* than *U. intestinalis*, regardless of the grazer's habitat of origin.

- When offered the two algae simultaneously, *L. littorea* preferred *U. intestinalis* to *P. lanosa* regardless of which alga dominated in their habitat of origin.

- In both single- and two-way choice experiments, *L. littorea* from the area of shore dominated by *P. lanosa* consumed more food on average than *L. littorea* from the habitat dominated by *U. intestinalis*, regardless of the algal species offered.

- These results further support the observation that *L. littorea* base their food choices on both feeding and habitat requirements.

Chapter 5

Does algal morphology influence the feeding preferences of intertidal grazers?

5.1 Introduction

A main premise of Steneck and Watling's (1982) functional group model is that algal structural morphology may affect herbivore feeding preference. The two main types of morphological defences are growth form and plant toughness. Algae differ considerably in their size, shape, toughness and degree of calcification, which in turn may affect their susceptibility to being grazed by herbivores (Hay, 1997). As such, morphology is considered as one of a plant's primary means of defence (Mulkern, 1967; Feeny, 1970; Atsatt and Dowd, 1976; Littler and Littler, 1980, 1983; Bertness *et al*, 1983; Littler *et al*, 1983; Gaines, 1985; Lewis, 1985; Terbough and Robinson, 1986; Salemaa, 1987; Bernays and Janzen, 1988; Hay *et al*, 1988; Padilla, 1989; Paul *et al*, 1990; Hay, 1991, 1997; Schupp and Paul, 1994; Heckscher *et al*, 1996; Kennish *et al*, 1996; Van Alstyne *et al*, 2001; Cronin *et al*, 2002; McClanahan *et al*, 2002; Mayeli *et al*, 2004; Salovius and Bonsdorff, 2004), as most herbivores are unable to graze tough plant material.

5.1.1 Algal growth forms

Many algal species are heteromorphic in that they display two (or more) distinct growth forms that allow them to persist in habitats where herbivory is intense (Lewis *et al*, 1987). For example, the red alga *Gigartina papillata* (C. Agardh) J. Agardh has a heteromorphic life cycle that alternates between a fleshy, non-calcified crust and an upright blade growth phase, which respond differently to grazing by herbivores (Slocum, 1980). Removing a range of grazers from an area of shore inhabited by both growth forms resulted in a decrease in crustose plants, but an increase in the number of blade plants (Slocum, 1980). Slocum (1980) concluded that although the crust growth form was more persistent in the environment, it was susceptible to overgrowth by other algal species, which were regularly removed in the presence of grazers. In contrast, the blade growth form was more susceptible to grazing and so flourished in the absence of grazers (Slocum, 1980). As briefly outlined in section 1.1.1 of chapter 1, Lewis *et al* (1987) showed that the brown alga *Padina jamaicensis* (Collins) Papenfuss exhibited two different morphologies in response to the intensity of fish grazing. Reducing the number of grazers present in the experimental area resulted in a shift in morphology from a prostrate, highly branched turf growth form to an erect foliose growth form, which was known to be preferred by herbivorous fishes (Lewis *et al*, 1987).

5.1.2 Algal toughness

The toughness of a plant can be defined as its "resistance to penetration" (Feeny, 1970). Steneck and Watling (1982) quantified toughness using a measure of mechanical force as proposed by Littler and Littler (1980). As discussed in section 1.3.2 of chapter 1, Steneck and Watling (1982) predicted that algal susceptibility to grazing should decrease as algal structural complexity increases from functional group one to seven, so that micro-, filamentous and foliose algae are the most susceptible to herbivores with tougher leathery, calcareous and crustose algae the least susceptible. Littler *et al* (1983) suggested that herbivore feeding preference was a function of both the level of plant toughness and calorific value. The order of susceptibility proposed by Steneck and Watling (1982) may partly be determined by the decrease in food value that could occur as more structurally complex algae allocate a greater proportion of their mass to indigestible structural materials, thus making them tougher for herbivores to consume (Hay, 1997).

Results reported in section 3.3 of chapter 3 present some evidence to support the ideas discussed above, however the results of the edibility experiments for *L. littorea* and *I. granulosa* do not fully examine the possibility that algal morphology could be acting as a feeding deterrent. As the work of other authors show that herbivore feeding preferences may change in response to changes in algal morphology (Geiselman and McConnell, 1981; Hunter, 1981; Galan Jimenez *et al*, 1996; Heckscher *et al*, 1996; Jormalainen *et al*, 2001a, 2005; Cronin *et al*, 2002; Rohde *et al*, 2004; Wessels *et al*, 2006), removing algal morphology as a factor affecting herbivore feeding preferences may result in changes in algal susceptibility to grazing. The most common method of manipulating algae to determine controlling factors is to add homogenised algal material to an agar solution (Geiselman and McConnell, 1981; Hunter, 1981; Targett *et al*, 1986; Steinberg, 1988; Heckscher *et al*, 1996; Stachowicz and Hay, 1996; Schnitzler *et al*, 2000; Jormalainen *et al*, 2001a, 2005; Cronin *et al*, 2002; Hemmi and Jormalainen, 2002; Sotka and Hay, 2002; Kubanek *et al*, 2004; Wessels *et al*, 2006). If algal susceptibility to grazing is controlled by morphology, the functional group model predicted by Steneck and Watling (1982) may not show the same results if herbivores were presented with homogenised algal material, as the morphological differences between functional groups would be removed. By utilising the methods of the above authors, the impact algal morphology has on the application of the functional group approach may be better understood.

5.1.3 Aims

This set of experiments aim to examine the effect algal morphology has on herbivore food preference by eliminating algal morphology as a controlling factor, and specifically to test whether i) algal species as whole plants were similarly edible to *L. littorea* and *I. granulosa*; ii) algal edibility for both *L. littorea* and *I. granulosa* would decrease for whole plants from functional group two to functional group six; iii) algal homogenates (for algal species and functional groups) would be similarly edible to both grazers; and iv) species within a functional group would be similarly edible to *L. littorea* and *I. granulosa* for both whole plant material and algal homogenates.

5.2 Methods

Edibility experiments were conducted in which grazers were presented with algae in two morphological forms: algae as whole plants and algae as homogenates in agar.

5.2.1 Comparison of agar and algae as a source of food

To firstly determine whether grazers would utilise agar as a source of food, experiments were conducted in which *L. littorea* and *I. granulosa* were presented with three types of agar discs in single-choice experiments: i) agar only; ii) agar containing *Ceramium* sp. and; iii) agar containing *F. serratus*. *Ceramium* sp. and *F. serratus* were chosen as comparison species as they represent opposite ends of the range of algae consumed by both grazers. Both *L. littorea* and *I. granulosa* readily consumed *Ceramium* sp., but found *F. serratus* less edible (Figs. 3.1, 3.2, chapter 3).

Mature *L. littorea* (mean shell height 2.02 ± 0.01 cm S.E.) and *I. granulosa* (mean body length 1.68 ± 0.01 cm S.E.) and the two algal species were collected from Holbeck (see section 2.2.1 of chapter 2 for site description) during August 2004. Upon return to the laboratory, the gastropods were kept for two days in filtered seawater, whereas the isopods were stored until use in filtered seawater with *U. lactuca* providing food and shelter. Algae were cleaned to remove epifauna and epiphytes by washing under running seawater and examination under a dissecting microscope, and used within 48 hours of collection. Agar discs were prepared using homogenised algae. Algae were blotted dry using a standard procedure (algae were placed on paper towels and blotted six times) and macerated in a household blender until pieces were approximately 0.5 cm^2. 50 ml of homogenised algal material was mixed with 25 ml of distilled water and the whole algal solution was added to agar solution (6 g $litre^{-1}$) that had been boiled to 90 °C and cooled to 50 °C. For the plain agar discs, agar was prepared as outlined above omitting the addition of homogenised algal material. The agar was then poured in to Petri dishes (5.4 cm diameter) and allowed to cool. Once cool the agar discs were removed from the Petri dishes, weighed, and placed in experimental tanks (17 x 11.5 x 4 cm) containing 200 ml of filtered seawater and either three *L. littorea* or six *I. granulosa* in single-choice experiments. Experiments were conducted in an aquarium with a constant temperature of 12 °C and a 12 hour dark: light cycle. After seven days the grazers were removed and the agar discs reweighed. Alongside the experimental tanks, grazer free controls were set up to allow for autogenic changes. The water in both the experimental and control tanks was changed every second day. The experiment was repeated six times for each type of agar disc, using new gastropods or isopods for each replicate. Once the agar discs had been reweighed, any change in weight was calculated using equations 2 and 3 (see section 3.2.1.1 of chapter 3).

5.2.2 Between- and within-group variation in the edibility of algal functional groups presented as whole plants

Although the food preferences of both grazers in relation to whole plant material had previously been investigated (chapter 3), experiments using whole plants were repeated in order to further reliably compare results for feeding experiments conducted using agar containing algal extracts (see section 5.2.3 below). *Littorina littorea* and *I. granulosa* were presented with a known weight of whole plants of algae from functional groups two to six (Table 1.1, chapter 1), ranging in weight from 1 g to 5 g (depending on the species) in single-choice experiments as described in section 5.2.1 above.

5.2.3 Between- and within-group variation in the edibility of algal functional groups presented as algal homogenates

Simultaneously with experiment 5.2.2 described above, *L. littorea* and *I. granulosa* were presented with algal homogenates of the same algal species as used in section 5.2.2 solidified in agar in single-choice experiments (as described in 5.2.1 above).

The data collected were used to test the null hypotheses that i) for both grazers there would be no significant difference in consumption of algal material presented as whole plants or homogenates in agar; ii) for both whole plant material and algal homogenates algal edibility would not be significantly different between algal species or functional groups; and iii) there would be no significant difference in edibility within a functional group for both whole plant material and algal homogenates.

5.2.4 Statistical Analyses

Data were screened using the Kolmogorov-Smirnov normality test and Levene's test to test for normality of distribution and homogeneity of variances respectively (Sokal and Rohlf, 1995). Data not meeting the assumptions of homogeneity were log transformed prior to analysis.

5.2.4.1 Comparison of agar and algae as a source of food

To ascertain whether both *L. littorea* and *I. granulosa* would utilise agar as a source of food data were analysed using two-factor ANOVA. GRAZER PRESENCE/ABSENCE and AGAR DISC TYPE were fixed factors with two and three levels respectively (Underwood, 1997). GRAZER PRESENCE/ABSENCE and AGAR DISC TYPE were the independent variables and weight of each disc consumed was the dependent variable. *Post hoc* Tukey pairwise comparisons were determined where appropriate to identify differences between the mean amounts consumed of each type of agar disc.

5.2.4.2 Between-species variation in the edibility of algal species in relation to algal morphology

To determine whether algal species presented as whole plants or algal extracts in agar were similarly susceptible to grazing by both *L. littorea* and *I. granulosa*, data were analysed using two-factor ANOVA. The first factor in the analysis was GRAZER PRESENCE/ABSENCE (a fixed factor with two levels). The second factor was algal SPECIES MORPHOLOGY, which was a fixed factor with eighteen levels for both grazers, as the species used in the investigation were chosen from the fixed range of composite species of a functional group available (Underwood, 1997). GRAZER PRESENCE/ABSENCE and SPECIES MORPHOLOGY were the independent variables and the weight of each species consumed was the dependent variable. *Post hoc* Tukey pairwise comparisons were determined where appropriate to identify differences between the mean amounts consumed of each species.

5.2.4.3 Between-group variation in the edibility of algal functional groups in relation to algal morphology

To determine whether both *L. littorea* and *I. granulosa* exhibited any preference between whole plants and artificial food, data were analysed using two-factor ANOVA at the functional group level.

The null hypotheses that grazers would show no significant preference for algal homogenates in agar or whole plant material between the five functional groups, and that algal edibility would not be significantly different between functional groups were tested with GRAZER PRESENCE/ABSENCE a fixed factor with two levels (Underwood, 1997) and FUNCTIONAL GROUP MORPHOLOGY as a fixed factor with ten levels for both grazers. GRAZER PRESENCE/ABSENCE and FUNCTIONAL GROUP MORPHOLOGY were independent variables, and the weight of each functional group morphology type consumed was the dependent variable. *Post hoc* Tukey pairwise comparisons were determined where appropriate to identify differences between the mean amounts consumed of each functional group.

5.2.4.4 Within-group variation in the edibility of algal functional groups in relation to algal morphology

The null hypothesis that species within a functional group would not be consumed in significantly different amounts regardless of algal morphology was tested with one-way ANOVAs. *Post hoc* Tukey pairwise comparisons were determined where appropriate. Data for functional group six were not analysed as only one species was used for this group and so no comparison could be made.

5.3 Results

The effect of algal morphology on the food preferences of *L. littorea* and *I. granulosa* were investigated by offering both grazers samples of nine species of algae as whole plant material and algal homogenates in agar, and recording changes in weight of the food over the experimental time period. Any change in weight was assumed to equate to the amount of algae consumed by the grazers.

5.3.1 Data Screening

5.3.1.1 Comparison of agar and algae as a source of food

The results of the Kolmogorov-Smirnov normality test showed that data for all three agar types for both grazers were normally distributed (Kolmogorov-Smirnov, $P > 0.01$). As Levene's test of homogeneity of variances showed significant differences in the weight of each agar type consumed by *L. littorea* (Levene's, $L_{5, 30} = 5.452$, $P < 0.05$) and *I. granulosa* (Levene's, $L_{5, 30} = 7.511$, $P < 0.001$), data were log transformed. After transformation, data for both grazers showed no significant departure from homogeneity (*L. littorea*: Levene's, $L_{5, 30} = 1.477$, $P > 0.15$; *I. granulosa*: Levene's, $L_{5, 30} = 2.503$, $P > 0.05$); therefore it can be assumed that all samples were drawn from populations with equal or similar variances.

5.3.1.2 Between- and within-group variation in the edibility of algal species and functional groups

The results of the Kolmogorov-Smirnov normality test showed that data at both the functional group and species level for both grazers were normal (Kolmogorov-Smirnov, $P > 0.01$). Homogeneity of variances at the functional group level were heterogeneous for *L. littorea* (Levene's, $L_{19, 196} = 7.528$, $P < 0.001$) and *I. granulosa* (Levene's, $L_{19, 196} = 6.901$, $P < 0.001$), even after logarithmic transformation. At the species level Levene's test of homogeneity of variances showed significant differences in the amount consumed by *L. littorea* (Levene's, $L_{35, 180} = 4.251$, $P < 0.001$) and *I. granulosa* (Levene's, $L_{35, 180} = 4.557$, $P < 0.001$), again even after logarithmic transformation. However, where the data are balanced and relatively large samples are used, ANOVA is robust to deviations from the assumption of homogeneity of variances (Underwood, 1997). To further enhance the reliability of the results α was set at $P = 0.01$.

5.3.2 Comparison of agar and algae as a source of food

The amount (g) of the three types of agar consumed by both *L. littorea* and *I. granulosa* differed, with *L. littorea* consuming more agar containing *Ceramium* sp. (mean = 2.849 ± 0.714 g S.E., n = 6), while *I. granulosa* preferred to consume agar containing extracts of *F. serratus* (mean = 3.079 ± 0.613 g S.E., n = 6) (Fig. 5.1).

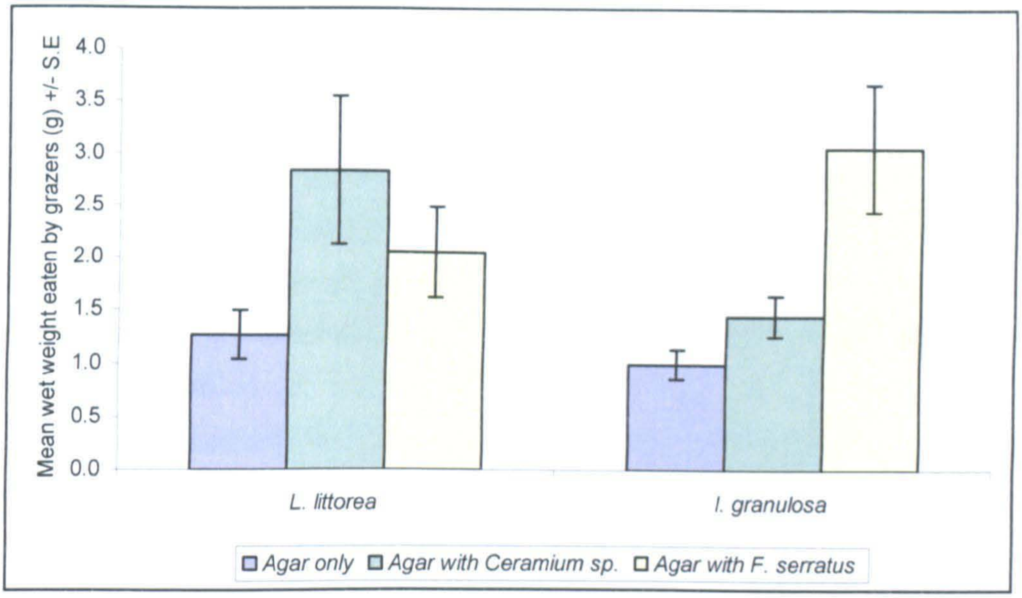

Figure 5.1 – Mean wet weight of each type of agar consumed by *Littorina littorea* and *Idotea granulosa* (± standard error) (n = 24).

Both grazers consumed more of the agar discs containing algal extracts than the plain agar. There were significant differences in the mean weight of agar consumed by both grazers across the three disc types (Tables 5.1, 5.2).

Table 5.1 - Two-factor analysis of variance to investigate the effects of GRAZER PRESENCE/ABSENCE and AGAR DISC TYPE on the weight of each agar disc type consumed by *Littorina littorea* using log transformed data (level of significance ** $P < 0.01$, *** $P < 0.001$, NS not significant).

Source of Variation	df	SS	MS	F	*P*
GRAZER +/-	1	9.248	9.248	43.418	<0.005**
AGAR DISC TYPE	2	2.690	1.345	6.316	<0.001***
GRAZER +/- * AGAR DISC TYPE	2	0.832	0.416	1.952	0.160NS
Error	30	6.390	0.213		
Total	35	19.159			

GRAZER PRESENCE/ABSENCE and AGAR DISC TYPE were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the amount (g) of each agar disc type consumed). The weight of each disc type consumed by *L. littorea* differed significantly between GRAZER PRESENCE/ABSENCE and also between AGAR DISC TYPE (Table 5.1). The interaction effect was not significant which suggests that discs presented to grazers always lost more weight irrespective of the type of agar disc offered (Table 5.1). Although it was not possible to carry out *post hoc* comparisons on GRAZER PRESENCE/ABSENCE due to there being only two categories, the data showed that agar discs presented to grazers displayed greater weight loss (mean = 2.066 ± 0.219 g S.E., n = 18) than those in control tanks (mean = 0.362 ± 0.219 g S.E., n = 18). *Post hoc* Tukey pairwise comparison ($P = 0.05$) comparing the weight of each type of agar disc consumed revealed that for *L. littorea*, agar discs containing extracts of *Ceramium* sp. and *F. serratus* were consumed significantly more than plain agar discs, though were not significantly different from each other (Fig. 5.1).

Table 5.2 - Two-factor analysis of variance to investigate the effects of GRAZER PRESENCE/ABSENCE and AGAR DISC TYPE on the weight of each agar disc type consumed by *Idotea granulosa* using log transformed data (level of significance ** $P < 0.01$, *** $P < 0.001$, NS not significant).

Source of Variation	df	SS	MS	F	*P*
GRAZER +/-	1	8.649	8.649	46.260	<0.001***
AGAR DISC TYPE	2	2.998	1.499	8.019	<0.002**
GRAZER +/- * AGAR DISC TYPE	2	0.853	0.426	2.280	0.120NS
Error	30	5.609	0.187		
Total	35	18.108			

GRAZER PRESENCE/ABSENCE and AGAR DISC TYPE were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the amount (g) of each agar disc type consumed). The weight of each type of disc consumed by *I. granulosa* differed significantly between GRAZER PRESENCE/ABSENCE and also between AGAR DISC TYPE (Table 5.2). As the interaction effect was not significant discs presented to grazers always lost more weight irrespective of the type of agar disc offered (Table 5.2). Although it was not possible to carry out *post hoc* comparisons on GRAZER PRESENCE/ABSENCE due to there being only two categories, the data showed that agar discs presented to grazers displayed greater weight loss (mean = 1.854 ± 0.173 g S.E., n = 18) than those in control tanks (mean = 0.362 ± 0.173 g S.E., n = 18). *Post hoc* Tukey pairwise comparison ($P = 0.05$) comparing the type of each agar disc consumed revealed that for *I. granulosa*, agar discs containing extracts of *Ceramium* sp. and *F. serratus* were consumed significantly more than plain agar discs, though were not significantly different from each other (Fig. 5.1).

5.3.3 Between-species variation in the edibility of algal species in relation to algal morphology

5.3.3.1 *Littorina littorea*

The weight of each algal species presented as different morphologies consumed by *L. littorea* differed from one another, with *C. rupestris* in agar being the most consumed food (mean = 0.686 ± 0.311 g S.E., n = 6) and whole plants of *C. officinalis* the least consumed (mean = 0.071 ± 0.023 g S.E., n = 6) (Fig. 5.2).

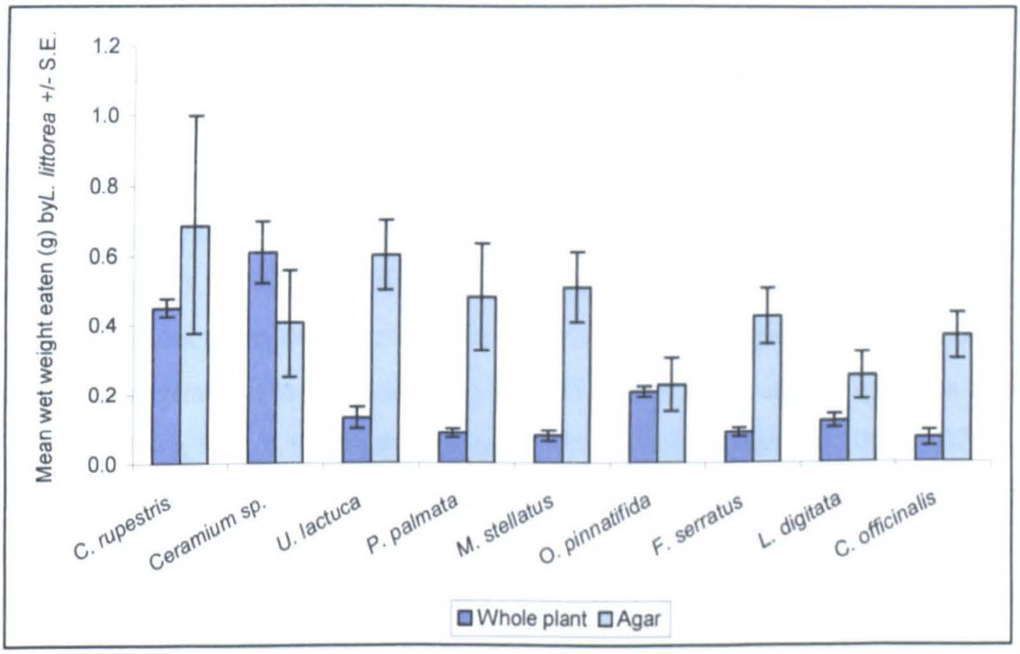

Figure 5.2 – Mean wet weight of each morphological type of each algal species consumed (± standard error) by *Littorina littorea* in single-choice experiments (n = 108).

With the exception of *Ceramium* sp., *L. littorea* consumed more of each species in agar than whole plant material (Fig. 5.2), and this difference between the change in weight of algal material was statistically significant (Table 5.3).

Table 5.3 - Two-factor analysis of variance to investigate the effects of GRAZER PRESENCE/ABSENCE and SPECIES MORPHOLOGY on the weight of each algal species consumed by *Littorina littorea* (level of significance *** $P < 0.001$, NS not significant). † tested at significance level $P = 0.01$.

Source of Variation	df	SS	MS	F	*P*
GRAZER +/-	1	2.649	2.649	62.441	<0.001***
SPECIES MORPHOLOGY	17	3.652	0.215	5.064	<0.001***
GRAZER +/- * SPECIES MORPHOLOGY	917	1.391	0.082	1.929	0.018NS†
Error	180	7.636	0.042		
Total	216	24.963			

GRAZER PRESENCE/ABSENCE and SPECIES MORPHOLOGY were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the amount (g) of each species morphology consumed). For *L. littorea*, the change in weight of each species differed significantly between GRAZER PRESENCE/ABSENCE and also between SPECIES MORPHOLOGY (Table 5.3). At $\alpha = 0.01$ the interaction effect was not significant which shows that discs presented to grazers always lost more weight irrespective of the type of agar disc offered (Table 5.3). Although it was not possible to carry out *post hoc* comparisons on GRAZER PRESENCE/ABSENCE due to there being only two categories, the data showed that algae in experimental tanks containing grazers displayed greater weight loss (mean = 0.322 ± 0.020 g S.E., n = 108) than algae in control tanks (mean = 0.100 ± 0.020 g S.E., n = 108). *Post hoc* Tukey pairwise comparison ($P = 0.05$) comparing the weight of each species consumed revealed that for *L. littorea, C. rupestris* in agar was consumed significantly more than whole plants of *U. lactuca, P. palmata, M. stellatus, F. serratus, L. digitata* and *C. officinalis*, which along with whole plants of *O. pinnatifida* were consumed significantly less than *U. lactuca* in agar (Fig. 5.2, Table 5.4). *Mastocarpus stellatus* in agar was also consumed significantly more than whole plants of *P. palmata, M. stellatus, F. serratus, L. digitata* and *C. officinalis* (Fig. 5.2, Table 5.4). There were no significant differences in the amount of agar discs consumed.

Table 5.4 - *Post hoc* Tukey pairwise comparison revealing differences in the edibility of algal species of differing morphologies to *Littorina littorea* (species listed down the left hand side were significantly more edible than species listed across the top where marked by *).

	C. rup wp	*C. rup* agar	*Cer* sp. wp	*Cer* sp. agar	*U. lac* wp	*U. lac* agar	*P. pal* wp	*P. pal* agar	*M. ste* wp	*M. ste* agar	*O. pin* wp	*O. pin* agar	*F. ser* wp	*F. ser* agar	*L. dig* wp	*L. dig* agar	*C. off* wp	*C. off* agar
C. rup wp																		
C. rup agar					*		*		*				*		*		*	
Cer sp. wp																		
Cer sp. agar																		
U. lac wp																		
U. lac agar					*		*		*		*		*		*		*	
P. pal wp																		
P. pal agar																		
M. ste wp																		
M. ste agar							*		*				*		*		*	
O. pin wp																		
O. pin agar																		
F. ser wp																		
F. ser agar																		
L. dig wp																		
L. dig agar																		
C. off wp																		
C. off agar																		

5.3.3.2 *Idotea granulosa*

The weights of each algal species presented as different morphologies consumed by *I. granulosa* also differed from one another, with *O. pinnatifida* in agar being the most consumed food (mean = 1.846 ± 0.579 g S.E., n = 6) and whole plants of *L. digitata* the least consumed (mean = 0.044 ± 0.010 g S.E., n = 6) (Fig. 5.3).

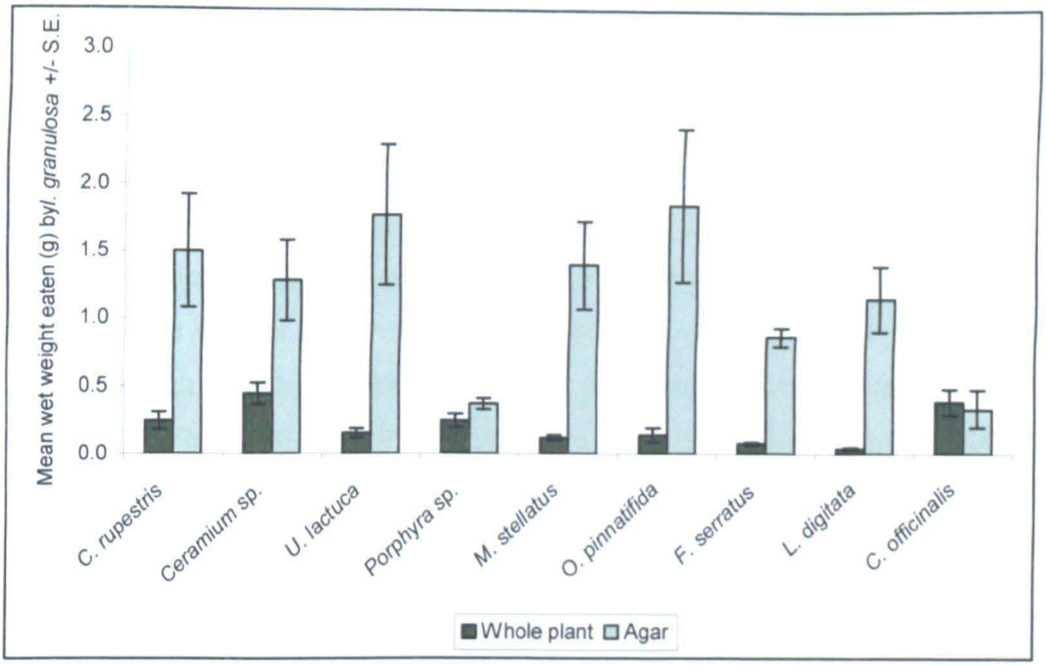

Figure 5.3 – Mean wet weight of each morphological type of each algal species consumed (± standard error) by *Idotea granulosa* in single-choice experiments (n = 108).

With the exception of *C. officinalis*, *I. granulosa* consumed more of each species in agar than whole plant material (Fig. 5.3) and this difference in the mean change in weight was significant (Table 5.5). There appeared to be no trend in either the amount of whole plant material consumed or algal homogenates in agar consumed between algal species (Fig. 5.3).

Table 5.5 - Two-factor analysis of variance to investigate the effects of GRAZER PRESENCE/ABSENCE and SPECIES MORPHOLOGY on the weight of each algal species consumed by *Idotea granulosa* (level of significance *** $P < 0.001$).

Source of Variation	df	SS	MS	F	*P*
GRAZER +/-	1	14.485	14.485	67.239	<0.001***
SPECIES MORPHOLOGY	17	26.039	1.532	7.110	<0.001***
GRAZER +/- * SPECIES MORPHOLOGY	917	16.064	0.945	4.386	<0.001***
Error	180	38.778	0.215		
Total	216	135.056			

GRAZER PRESENCE/ABSENCE and SPECIES MORPHOLOGY were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the amount (g) of each species morphology consumed). The change in weight of each algal species differed significantly between GRAZER PRESENCE/ABSENCE and also between SPECIES MORPHOLOGY (Table 5.5). The interaction effect was significant indicating that weight lost in experimental tanks with grazers present did not follow the same pattern for each species morphology (Table 5.5). Although it was not possible to carry out *post hoc* comparisons on GRAZER PRESENCE/ABSENCE due to there being only two categories, the data showed that algae in experimental tanks containing grazers displayed greater weight loss (mean = 0.688 ± 0.045 g S.E., n = 108) than algae in control tanks (mean = 0.170 ± 0.045 g S.E., n = 108). *Post hoc* Tukey pairwise comparison (P = 0.05) of the weight of each species consumed revealed that for *I. granulosa*, *C. rupestris* in agar was consumed significantly more than whole plants of *C. rupestris*, *U. lactuca*, *Porphyra* sp., *M. stellatus*, *F. serratus* and *L. digitata*, but these were not significantly different to each other (Fig. 5.3, Table 5.6). In addition to those species, *Porphyra* sp. and *C. officinalis* in agar and whole plants of *O. pinnatifida* and *C. officinalis* were consumed significantly less than *U. lactuca* in agar, which along with whole plants of *Ceramium* sp. were also consumed significantly less than *O. pinnatifida* in agar (Fig. 5.3, Table 5.6), but there were no significant difference between these species. *M. stellatus* in agar was also consumed significantly more than whole plants of *C. rupestris*, *U. lactuca*, *Porphyra* sp., *M. stellatus*, *F. serratus* and *C. officinalis* in agar (Fig. 5.3, Table 5.6). Finally, *L. digitata* in agar was consumed significantly more than whole plants of *F. serratus* and *L. digitata* (Fig. 5.3, Table 5.6). There were no significant differences in the amount of agar discs consumed.

Table 5.6 - *Post hoc* Tukey pairwise comparison revealing differences in the edibility of algal species of differing morphologies to *Idotea granulosa* (species listed down the left hand side were significantly more edible than species listed across the top where marked by *).

	C. rup wp	*C. rup* agar	*Cer* sp. wp	*Cer* sp. agar	*U. lac* wp	*U. lac* agar	*Por* sp. wp	*Por* sp. agar	*M. ste* wp	*M. ste* agar	*O. pin* wp	*O. pin* agar	*F. ser* wp	*F. ser* agar	*L. dig* wp	*L. dig* agar	*C. off* wp	*C. off* agar
C. rup wp																		
C. rup agar	*				*		*		*				*		*			
Cer sp. wp																		
Cer sp. agar																		
U. lac wp																		
U. lac agar								*			*						*	*
Por sp. wp																		
Por sp. agar																		
M. ste wp																		
M. ste agar	*				*		*		*				*				*	
O. pin wp																		
O. pin agar																		
F. ser wp																		
F. ser agar																		
L. dig wp																		
L. dig agar													*		*			
C. off wp																		
C. off agar																		

5.3.3.3 Comparison of between-species data

Comparing the whole plant data collected in chapter 5 (see sections 5.3.3.1 and 5.3.3.2 above) with previous results from chapter 3 (see sections 3.3.2.1 and 3.3.2.2 of chapter 3) revealed that the pattern of edibility of algal species were similar for both *L. littorea* and *I. granulosa* (Table 5.7).

Table 5.7 – Comparison of the rank order of whole plant species preference observed in chapters 3 and 5 for *Littorina littorea* (n = 9) and *Idotea granulosa* (n = 9).

Littorina littorea	Rank of species data from chapter 3	Rank of species data from chapter 5
C. rupestris	2	2
Ceramium sp.	1	1
U. lactuca	3	4
P. palmata	7	7
M. stellatus	8	8
O. pinnatifida	4	3
F. serratus	5	6
L. digitata	6	5
C. officinalis	9	9
Idotea granulosa		
C. rupestris	4	4
Ceramium sp.	1	1
U. lactuca	5	5
Porphyra sp.	3	3
M. stellatus	7	7
O. pinnatifida	6	6
F. serratus	8	8
L. digitata	9	9
C. officinalis	2	2

The rank order of algal species preference displayed by *L. littorea* differed in that *U. lactuca* (functional group three) was once place higher up the preference ranking than *O. pinnatifida* (functional group four) in chapter 3, though this preference was reversed in chapter 5 (Table 5.7). Within functional group five, *L. littorea* displayed a preference for *F. serratus* over *L. digitata* in chapter 3, but this was reversed in chapter 5 (Table 5.7). Although the differences observed between species were not the same for both chapters, the rank order of preference was similar. The results collected for *I. granulosa* in both chapters 3 and 5 were identical, suggesting that its preference for algal species was repeatable.

5.3.4 Between-group variation in the edibility of algal functional groups in relation to algal morphology

5.3.4.1 *Littorina littorea*

The amount (g) of food material consumed by *L. littorea* differed between algal morphology for functional groups. Algae from functional group two in agar was the most consumed food offered (mean = 0.545 ± 0.171 g S.E., n = 6), and whole plants from functional group six the least consumed (mean = 0.071 ± 0.023 g S.E., n = 6) (Fig. 5.4).

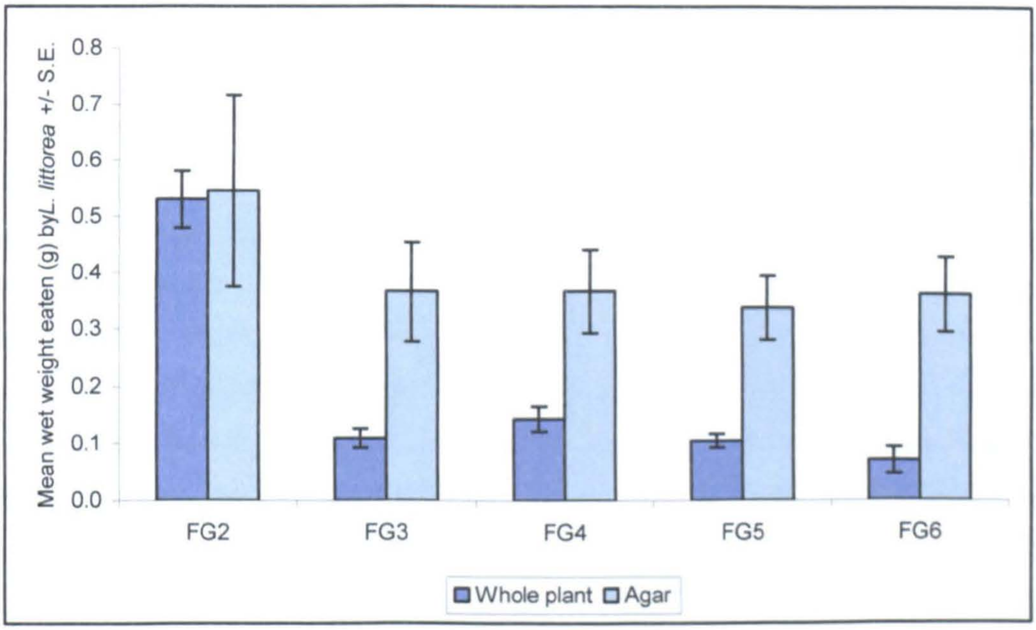

Figure 5.4 – Mean wet weight of each morphological type from each functional group consumed (± standard error) by *Littorina littorea* in single-choice experiments (n = 108).

For all functional groups *L. littorea* consumed more agar than whole plant material (Fig. 5.4). There was also a difference in the weight of whole plant material consumed between functional groups (Fig. 5.4), and these differences were significant (Table 5.8).

Table 5.8 - Two-factor analysis of variance to investigate the effects of GRAZER PRESENCE/ABSENCE and FUNCTIONAL GROUP MORPHOLOGY on the weight of each functional group consumed by *Littorina littorea* (level of significance ** $P < 0.01$, *** $P < 0.001$).

Source of Variation	df	SS	MS	F	*P*
GRAZER +/-	1	2.228	2.228	50.486	<0.001***
FG MORPHOLOGY	9	2.911	0.323	7.330	<0.001***
GRAZER +/- * FG MORPHOLOGY	9	1.119	0.124	2.818	0.004**
Error	196	8.649	0.044		
Total	216	24.963			

GRAZER PRESENCE/ABSENCE and FUNCTIONAL GROUP MORPHOLOGY were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the amount of each functional group consumed). The change in weight of each functional group differed significantly between GRAZER PRESENCE/ABSENCE and also between FUNCTIONAL GROUP MORPHOLOGY (Table 5.8). Even at $\alpha = 0.01$ the interaction effect was significant indicating that weight lost in experimental tanks with grazers present did not follow the same pattern for each functional group morphology (Table 5.8). Although it was not possible to carry out *post hoc* comparisons on GRAZER PRESENCE/ABSENCE due to there being only two categories, the data showed that algae in experimental tanks containing grazers displayed greater weight loss (mean = 0.311 ± 0.021 g S.E., n = 108) than algae in control tanks (mean = 0.100 ± 0.021 g S.E., n = 108). *Post hoc* Tukey pairwise comparison ($P = 0.05$) revealed that both whole plants and algae in agar from functional group two, and algae in agar from functional group three were consumed significantly more than whole plants from functional groups three, four, five and six, but these morphologies were not significantly different from each other (Fig. 5.4, Table 5.9). Functional group four in agar was also consumed significantly more than whole plants from functional groups three and five (Fig. 5.4, Table 5.9).

Table 5.9 - *Post hoc* Tukey pairwise comparison revealing differences in the edibility of algal functional groups of differing morphologies to *Littorina littorea* (functional groups listed down the left hand side were significantly more edible than functional groups listed across the top where marked by *).

	FG2 wp	FG2 agar	FG3 wp	FG3 agar	FG4 wp	FG4 agar	FG5 wp	FG5 agar	FG6 wp	Fg6 agar
FG2 wp			*		*		*		*	
FG2 agar			*		*		*		*	
FG3 wp										
FG3 agar			*		*		*		*	
FG4 wp										
FG4 agar			*				*			
FG5 wp										
FG5 agar										
FG6 wp										
FG6 agar										

5.3.4.2 *Idotea granulosa*

The weight of food material consumed by *I. granulosa* also differed between algal morphology for functional groups. Algae in agar from functional group four was consumed the most (mean = 1.620 ± 0.324 g S.E., n = 6), and whole plants from functional group five were the least consumed (mean = 0.062 ± 0.009 g S.E., n = 6) (Fig. 5.5).

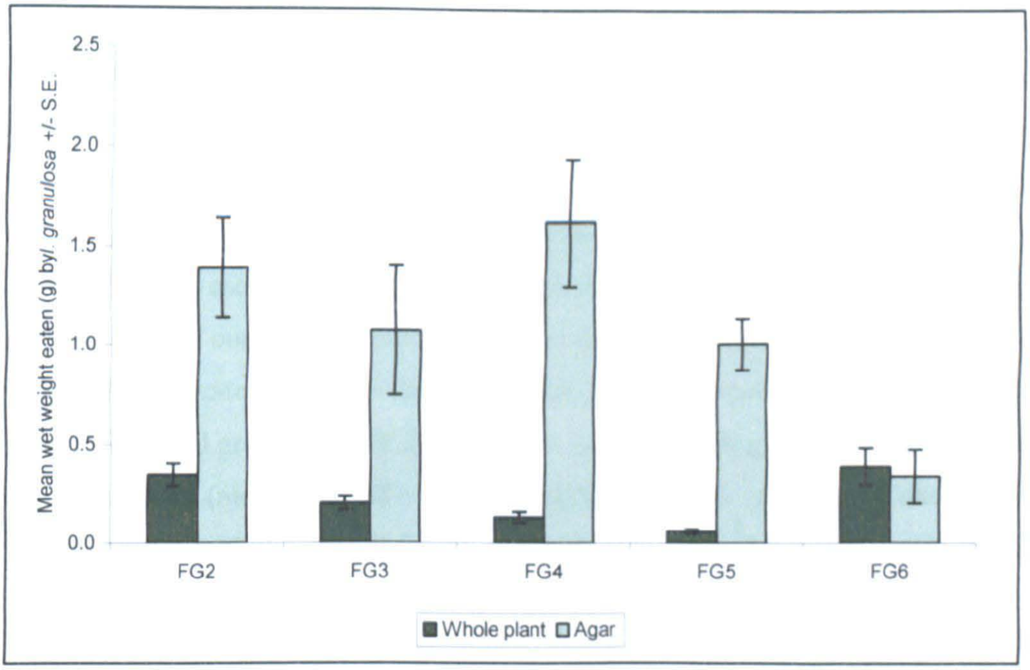

Figure 5.5 – Mean wet weight of each morphological type from each functional group consumed (± standard error) by *Idotea granulosa* in single-choice experiments (n = 108).

With the exception of functional group six, *I. granulosa* consumed more algae in agar than whole plant material (Fig. 5.5). There was also a declining trend from functional group two to functional group five in the weight of whole plant material consumed (Fig. 5.5), and these differences were significant (Table 5.10).

Table 5.10 - Two-factor analysis of variance to investigate the effects of GRAZER PRESENCE/ABSENCE and FUNCTIONAL GROUP MORPHOLOGY on the weight of each algal functional group consumed by *Idotea granulosa* (level of significance *** $P < 0.001$).

Source of Variation	df	SS	MS	F	*P*
GRAZER +/-	1	12.340	12.340	52.340	<0.001***
FG MORPHOLOGY	9	21.653	2.406	10.205	<0.001***
GRAZER +/- * FG MORPHOLOGY	9	13.018	1.446	6.135	<0.001***
Error	196	46.211	0.236		
Total	216	135.056			

GRAZER PRESENCE/ABSENCE and FUNCTIONAL GROUP MORPHOLOGY were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the amount of each functional group consumed). The change in weight of each functional group differed significantly between GRAZER PRESENCE/ABSENCE and also between FUNCTIONAL GROUP MORPHOLOGY (Table 5.10). Even at $\alpha = 0.01$ the interaction effect was significant indicating that weight lost in experimental tanks with grazers present did not follow the same pattern for each functional group morphology (Table 5.10). Although it was not possible to carry out *post hoc* comparisons on GRAZER PRESENCE/ABSENCE due to there being only two categories, the data show that algae in experimental tanks containing grazers displayed greater weight loss (mean = 0.655 ± 0.049 g S.E., n = 108) than algae in control tanks (mean = 0.158 ± 0.049 g S.E., n = 108). *Post hoc* Tukey pairwise comparison ($P = 0.05$) revealed that algae in agar from functional group two was consumed significantly more than whole plant material from functional groups two, three, four and five and algae in agar from functional group six, which along with whole plants from functional group six, were consumed significantly less than algae in agar from functional group four, but these morphologies were not significantly different from each other (Fig. 5.5, Table 5.11). Algae in agar from functional group three were consumed significantly more than whole plants from functional groups three and five, which along with whole plant material from functional group four, were consumed significantly less that algae in agar from functional group five, though these morphologies were not significantly different from each other (Fig. 5.5, Table 5.11).

Table 5.11 - *Post hoc* Tukey pairwise comparison revealing differences in the edibility of algal functional groups of differing morphologies to *Idotea granulosa* (functional groups listed down the left hand side were significantly more edible than functional groups listed across the top where marked by *).

	FG2 wp	FG2 agar	FG3 wp	FG3 agar	FG4 wp	FG4 agar	FG5 wp	FG5 agar	FG6 wp	Fg6 agar
FG2 wp										
FG2 agar	*		*		*		*			*
FG3 wp										
FG3 agar			*				*			
FG4 wp										
FG4 agar	*		*		*		*		*	*
FG5 wp										
FG5 agar			*		*		*			
FG6 wp										
FG6 agar										

5.3.4.3 Comparison of between-functional group data

Comparing the whole plant data collected in chapter 5 (see sections 5.3.4.1 and 5.3.4.2 above) with previous results from chapter 3 (see sections 3.3.3.1 and 3.3.3.2 of chapter 3) revealed that the pattern of edibility of algal species were similar for both *L. littorea* and *I. granulosa* (Table 5.12).

Table 5.12 – Comparison of the rank order of whole plant functional group preference observed in chapters 3 and 5 for *Littorina littorea* (n = 9) and *Idotea granulosa* (n = 9).

Littorina littorea	Rank of functional group data from chapter 3	Rank of functional group data from chapter 5
Functional Group 2	1	1
Functional Group 3	3	4
Functional Group 4	2	3
Functional Group 5	4	2
Functional Group 6	5	5
Idotea granulosa		
Functional Group 2	2	2
Functional Group 3	3	3
Functional Group 4	4	4
Functional Group 5	5	5
Functional Group 6	1	1

The rank order of algal functional group preference displayed by *L. littorea* was the same in that algae from functional group were the most preferred in both chapters, and algae from functional group six were the least preferred in both chapters (Table 5.12). The results differed between the two chapters as algae from functional groups three, four and five occupied different positions in the edibility rank (Table 5.12). The results collected for *I. granulosa* in both chapters 3 and 5 were again identical, suggesting that its preference for algal functional groups was repeatable (Table 5.12).

5.3.5 Within-group variation in the edibility of algal functional groups in relation to algal morphology

5.3.5.1 *Littorina littorea*

The results of the one-way ANOVAs conducted to determine if species with differing morphologies within a functional group were similarly susceptible to grazing by *L. littorea* showed that functional group two was the only group in which the composite species were consumed in similar amounts (Table 5.13). Species comprising functional groups three, four and five were not consumed in similar amounts (Table

5.13). For functional group three, the *post hoc* Tukey pairwise comparison ($P = 0.05$) revealed that *U. lactuca* in agar was consumed significantly more than whole plants of *U. lactuca* and *P. palmata*, which in turn were consumed significantly less than *P. palmata* in agar (Fig. 5.2). Within functional group four, *M. stellatus* in agar was consumed significantly more than *M. stellatus* whole plants, which in turn were consumed significantly less than whole plants of *O. pinnatifida* (Fig. 5.2). Within functional group five, *F. serratus* in agar was consumed significantly more than whole plants of *F. serratus* and *L. digitata* (Fig. 5.2).

Table 5.13 – One-way analyses of variance to investigate the levels of edibility by *Littorina littorea* of algal species presented as different morphologies within a functional group (level of significance ** $P < 0.01$, *** $P < 0.001$, NS not significant).

Source of Variation	df	SS	MS	F	*P*
Functional Group 2					
Between Groups	3	0.598	0.199	0.708	0.558NS
Within Groups	20	5.630	0.282		
Total	23	6.229			
Functional Group 3					
Between Groups	3	2.814	0.938	13.530	<0.001***
Within Groups	20	1.387	0.069		
Total	23	4.201			
Functional Group 4					
Between Groups	3	1.895	0.632	9.068	0.001**
Within Groups	20	1.393	0.070		
Total	23	3.288			
Functional Group 5					
Between Groups	3	1.500	0.500	5.271	0.008**
Within Groups	20	1.897	0.095		
Total	23	3.397			

5.3.5.2 *Idotea granulosa*

For *I. granulosa* species within each functional group were not consumed in similar amounts (Table 5.14). Within functional group two the *post hoc* Tukey pairwise comparison ($P = 0.05$) revealed that *C. rupestris* in agar was consumed significantly more than whole plants of *C. rupestris* and *Ceramium* sp. (Fig. 5.3). For functional group three, *U. lactuca* in agar was consumed significantly more than whole plants of *U. lactuca* and *Porphyra* sp., and *Porphyra* sp. in agar (Fig. 5.3). Within functional group four, *O. pinnatifida* in agar was consumed significantly more than whole plants of both *M. stellatus* and *O. pinnatifida* (Fig. 5.3). Within functional group five, *F. serratus* in agar was consumed significantly more than whole plants of *F. serratus* and *L. digitata*, which in turn were consumed significantly less than *L. digitata* in agar (Fig. 5.3).

Table 5.14 – One-way analyses of variance to investigate the levels of edibility by *Idotea granulosa* of algal species presented as different morphologies within a functional group (level of significance ** $P < 0.01$, *** $P < 0.001$).

Source of Variation	df	SS	MS	F	*P*
Functional Group 2					
Between Groups	3	6.786	2.262	5.366	0.007**
Within Groups	20	8.430	0.421		
Total	23	15.215			
Functional Group 3					
Between Groups	3	10.424	3.475	8.221	0.001**
Within Groups	20	8.452	0.423		
Total	23	18.876			
Functional Group 4					
Between Groups	3	13.893	4.631	6.941	0.002**
Within Groups	20	13.343	0.667		
Total	23	27.235			
Functional Group 5					
Between Groups	3	5.548	1.849	19.157	<0.001***
Within Groups	20	1.931	0.097		
Total	23	7.478			

5.4 Discussion

5.4.1 Comparison of agar and algae as a source of food

Both *L. littorea* and *I. granulosa* utilised agar as a source of food and readily consumed algal extracts presented in agar. Significant differences were observed in the amount consumed between plain agar discs and discs containing algal extracts, with both grazers preferring discs containing *Ceramium* sp. and *F. serratus* over discs containing no extracts (Fig. 5.1; Tables 5.1, 5.2). Although there were no significant preferences observed between discs containing the two algal species, the results suggested that *L. littorea* and *I. granulosa* would consume homogenised algae in agar, rather than just plain agar and there was the potential for preferences to be exhibited as *L. littorea* preferentially consumed discs containing *Ceramium* sp. (Fig. 5.1), while discs containing *F. serratus* were favoured by *I. granulosa* (Fig. 5.1).

5.4.2 Between-species variation in the edibility of algal species in relation to algal morphology

Combined analysis of the single-choice experiment food preference data for both whole plant material and artificial food (see section 5.2.4.2 above for full details) showed that neither *L. littorea* nor *I. granulosa* displayed any preferences for algal species presented as whole plants. No significant differences were observed in the consumption of whole plant material across the range of species offered (see section 5.3.3 above), suggesting that all species were similarly susceptible to grazing by *L. littorea* and *I. granulosa*. This is surprising given that in previous experiments both grazers displayed a significant preference for *Ceramium* sp. (see sections 3.3.2.1 and 3.3.2.2 of chapter 3). However, there were major similarities in the data as the rank order of preference for algal species displayed by *L. littorea* was similar in both chapters, while the results collected for *I. granulosa* found were identical in chapters 3 and 5 (see sections 5.3.3.3 and 5.3.4.3 above). Both *L. littorea* and *I. granulosa* preferentially consumed algal extracts in agar over whole plant material, though their preferences differed slightly. *Littorina littorea* consumed agar discs containing *C. rupestris*, *U. lactuca* and *M. stellatus* significantly more than a range of algal species presented as whole plants (see section 5.3.3.1 above), while *I. granulosa* preferentially consumed *O. pinnatifida* and *L. digitata* in agar in addition to those artificial foods consumed by *L. littorea* (see section 5.3.3.2 above).

Grazers would be expected to consume algal homogenates in agar more then whole plant material as the morphological defences provided in the structure of whole plants cease to exist and so no longer act as grazing deterrents (Cronin *et al*, 2002). Wessels *et al* (2006) observed that both the amphipod *Gammarellus homari* Fabricius and the sea urchin *Strongylocentrotus droebachiensis* Muller significantly consumed homogenised algae in agar over whole plant material; however, Jormalainen *et al* (2001a) showed that *I. baltica* consumed more natural food than artificial food.

Littorina littorea displayed no preference for extracts of any algal species in agar, suggesting that all agar discs were similarly edible (see section 5.3.3.1 above). Heckscher *et al* (1996) recorded similar results for the amphipod *Microdeutopus gryllotalpa* Costa. They offered grazers a range of algal species as agar discs in pair-wise feeding experiments and found all species were consumed in equal amounts by *M. gryllotalpa* (Heckscher *et al*, 1996). *Idotea granulosa* showed significant differences in its feeding preferences between agar discs (see section 5.3.3.2 above). Discs containing extracts of *C. officinalis* were consumed in significantly lower quantities than discs containing *U. lactuca*, *M. stellatus* and *O. pinnatifida* (Fig. 5.3). Agar discs containing extracts of *U. lactuca* and *O. pinnatifida* were also preferentially consumed over discs containing *Porphyra* sp. (Fig. 5.3). These findings indicate that factors other than algal morphology may be affecting the food choices of *I. granulosa*.

Jormalainen *et al* (2001a) recorded a change in feeding preferences between natural and artificial foods for *I. baltica*. Grazers were presented with several species of algae, in either their natural form or as agar pellets, in multiple-choice feeding experiments (Jormalainen *et al*, 2001a). The order of preference when presented with whole plant material was *F. vesiculosus* (functional group five) > *Dictyosiphon foeniculaceus* (Hudson) Greville (functional group two) > *Elachista fucicola* (Velley) Areschoug (functional group two) > *P. littoralis* (functional group two) > *U. intestinalis* (functional group three) > *Ceramium tenuicorne* (Kützing) Waern (functional group three) (Jormalainen *et al*, 2001a). However, when *I. baltica* were presented with artificial food, *P. littoralis* was the most preferred species and *F. vesiculosus* the least (Jormalainen *et al*, 2001a). Changes in feeding preferences have also been recorded for other herbivores. Cronin *et al* (2002) showed that the freshwater crayfish *P. clarkii* consumed plants with finely branched or filamentous morphologies, that were also low in nitrogen and protein, when presented with whole plant material, but altered their preferences to tough plants that were high in nitrogen and protein when presented with plants in agar (Cronin *et al*, 2002). These results suggest that the physical morphology of plants is an important factor in reducing grazing on whole plant material. However, Cronin *et al* (2002) also found differences between plants once morphology had been removed as a factor affecting feeding preference. *Procambarus clarkii* exhibited preferences between plants presented in agar, leading the authors to conclude that other factors such as defensive chemicals and nutritive quality may be important in determining feeding preferences (Cronin *et al*, 2002).

Wessels *et al* (2006) observed that the amphipod *G. homari* preferentially consumed the filamentous green alga *Acrosiphonia penicilliformis* (Fosl.) Kjellman, the foliose red algae *P. palmata*, *Devaleraea ramentacea* (Linnaeus) Guiry and *Phycodrys rubens* (Linnaeus) Batters, and showed some preference for the blades of the leathery species *L. digitata* and *Laminaria solidungula* Agardh in single-choice experiments. Compared to the amphipods, the sea urchin *S. droebachiensis* showed a clear preference for both the blades and stipes of *L. digitata*, *L. solidungula* and *Laminaria saccharina* Lamouroux; they also consumed *P. palmata* (Wessels *et al*, 2006). When presented with homogenised algae in agar the preferences of *G. homari* showed a shift towards preferential consumption of the filamentous brown alga *Desmarestia viridis* (Muller) Lamouroux, the blade of the leathery kelp *Alaria esculenta* (Linnaeus) Greville and the stipes of *L. digitata* and *L. solidungula* (Wessels *et al*, 2006). The increased consumption of the tough, leathery species when morphological defences were removed suggests that algal physical toughness was an effective deterrent for *G. homari*. *Strongylocentrotus droebachiensis* continued to prefer kelp species when presented as homogenates in agar, indicating that factors other than algal morphology may account for their feeding preferences. They also displayed a change in preference by increasing their consumption of the filamentous algae *Polysiphonia arctica* Agardh and *D. viridis*, and the foliose alga *P. rubens* (Wessels *et al*, 2006).

The findings of Jormalainen *et al* (2001a) (discussed above) contradict the results of this experiment, as *I. granulosa* found whole plants of both *F. serratus* and *L. digitata* to be the least edible, but consumed both species when presented as artificial food (Fig. 5.3). This suggests that it may be the structure of *F. serratus* and *L. digitata* that prevent *I. granulosa* from grazing them in their natural form. As with other algae from functional group five, *Fucus* and *Laminaria* are known to produce secondary metabolites that may act as grazing deterrents (Geiselman and McConnell, 1981; Irelan and Horn, 1991; Targett *et al*, 1992; Kubanek *et al*, 2004). If such compounds were preventing *I. granulosa* from consuming these algal species, it is unlikely that they would be consumed when presented in artificial form. In an experiment investigating the effect of the presence of herbivores on the production of anti-herbivore defences in *F. vesiculosus*, Rohde *et al* (2004) found that both *L. littorea* and *I. baltica* displayed no significant preferences between *F. vesiculosus* presented as both whole plants and reconstituted material in agar, leading them to conclude that chemical factors, rather than morphological factors were controlling the feeding preferences of the two grazers. It may be possible that secondary metabolites are reactive and only produced when intact plants are grazed; thus they will be absent from artificially macerated plants as any damage caused would be through human manipulation rather than contact with grazers. Artificially damaging plants during homogenisation may damage plants in a way that is different to the actions of herbivores, thus chemicals may not be released. Jormalainen *et al* (2001a, 2005) found that *I. baltica* were not deterred by the presence of chemicals within algae as they preferentially consumed those species with the highest concentrations of phlorotannins; it is therefore possible that *I. granulosa* were not deterred by secondary metabolites. Alternatively, it is possible that any chemical deterrents present in the algae may have leached out of the agar into the surrounding water; current evidence as to the likelihood of this occurring is conflicting in the literature as Kubanek *et al* (2004) concluded that phlorotannins present in *F. vesiculosus* did not leach out from agar, whereas Jormalainen *et al* (2005) suggested that phlorotannins leach from agar into the surrounding water within four hours.

5.4.3 Between-group variation in the edibility of algal functional groups in relation to algal morphology

Steneck and Watling's (1982) functional group model was again partially supported by the results from this experiment as *L. littorea* consumed significantly more algae as whole plants from functional group two than any other functional group (Fig. 5.4). Algae within functional group two were the least structurally complex and so should be the most susceptible to grazing. There were no differences in consumption between the remaining groups, confirming the results collected in section 3.3.3.1 of chapter 3 (Table 5.7). In both experiments, algae from functional group six were consumed the least (Figs. 3.3, 5.4). Again this supports the functional group model as algae comprising this group are considered structurally complex and thus more difficult to consume. In previous edibility experiments (see section 3.3.3.2 of chapter 3) *I. granulosa* showed significant preferences for algae from functional groups two and six, however they displayed no significant preferences for algae between functional groups during this experiment, suggesting they found all groups presented as whole plants similarly susceptible to grazing. Although there were no significant differences in the preference for functional groups in chapter 5, the rank order of functional group preference displayed by *I. granulosa* was identical to the rank order of preference displayed in chapter 3 (Table 5.12). As results collected from two experiments (chapters 3 and 5) displayed comparable patterns for both grazers, reliable evidence has been provided that is repeatable over time.

Again, both *L. littorea* and *I. granulosa* preferentially consumed algal extracts in agar over whole plant material (Figs. 5.4, 5.5; Tables 5.8, 5.10), though their preferences differed slightly. As for algal species, *L. littorea* displayed no preference for extracts of algae from any functional group in agar, suggesting that all agar discs were similarly edible (see section 5.3.4.1 above). By removing structural defence as a factor affecting feeding preferences, it may be expected that algae from all functional groups would be similarly susceptible to grazing. However, Geiselman and McConnell (1981) revealed that *L. littorea* consumed agar containing extracts of *Ceramium* sp. (functional group two), *U. lactuca*, *U. intestinalis* and *Porphyra* sp. (all functional group three) but avoided artificial food containing *C. crispus* (functional group four), *F. vesiculosus* and *A. nodosum* (both functional group five), leading them to conclude that factors other than algal toughness act as feeding deterrents.

Idotea granulosa showed significant differences in its feeding preferences between agar discs (see section 5.3.4.2 above). Discs containing extracts of algae from functional groups two and four were consumed significantly more than discs containing algae from functional group six (Fig. 5.5), which again suggests that factors other than algal morphology were influencing the feeding preferences of *I. granulosa*. As discussed in section 1.1.1 of chapter 1, the calcium carbonate found in the structure of

C. officinalis may act as a chemical defence (Hay *et al*, 1994), thus making it less edible to herbivores.

Algae presented as homogenates in agar provide a different nutritive composition to herbivores than algae presented as whole plants (Jormalainen *et al*, 2005). Apart from the fact that the agar discs had little structural defences and so were easier for the grazers to consume, both grazers possibly consumed more algae in agar overall than whole plant material in order to compensate for the agar's lower nutritional quality and gain the same level of nutrients provided by whole plant tissue.

5.4.4 Within-group variation in the edibility of algal functional groups in relation to algal morphology

Within-group variation occurred between algae from functional groups three, four and five for *L. littorea* and between algae from functional groups two, three, four and five for *I. granulosa*, which disagrees with the predictions of Steneck and Watling (1982). The differences observed within groups two and five for *L. littorea* and groups two, four and five for *I. granulosa* were due to the fact that algal extracts presented in agar were consumed more than the whole plant equivalents of the same species (Figs. 5.2, 5.3). Within functional group four, *L. littorea* preferentially consumed whole plants of *O. pinnatifida* over whole plants of *M. stellatus* (Fig. 5.2). As discussed in section 3.4.3 of chapter 3, species within functional group four display a wide range of morphologies; therefore they may be differentially susceptible to grazing. There were no significant differences in consumption between agar discs, which further suggest that the feeding preferences of *L. littorea* may be determined by algal morphological characteristics rather than the presence of chemical deterrents. *Idotea granulosa* however, significantly consumed extracts of *U. lactuca* in agar, not only over whole plants of *U. lactuca* and *Porphyra* sp., but also over extracts of *Porphyra* sp. in agar (Fig. 5.3). As discussed in section 4.4.2.1 of chapter 4, Imrie *et al* (1990) suggested that the preference juvenile *L. littorea* exhibited for *Porphyra* sp. over *U. lactuca* was due to higher concentrations of a phagostimulant chemical within the tissues of *Porphyra* sp.. Grazers often have different reactions to chemicals found within food material, and although *I. granulosa* seem to show little avoidance for algal species containing chemical compounds, the phagostimulant present in *Porphyra* sp. that may attract *L. littorea* to feed upon this alga may have a different effect upon *I. granulosa*. The preference observed for extracts of *U. lactuca* in agar may be due to differences in nutritional value between the two species, or the presence of unknown chemicals.

5.5 Summary

- Both *L. littorea* and *I. granulosa* readily consumed algal homogenates in agar when presented with agar discs as a source of food.

- In general, both grazers consumed more algae in agar than whole plant material, and significantly preferred extracts of algae in agar than some algal species presented as whole plants.

- Neither *L. littorea* nor *I. granulosa* showed any significant preference for algal species presented as whole plant material, suggesting all species were edible.

- *Littorina littorea* displayed no preference for extracts of any algal species in agar, while *I. granulosa* significantly preferred agar discs containing *U. lactuca*, *M. stellatus* and *O. pinnatifida*.

- *Littorina littorea* consumed significantly more algae as whole plants from functional group two, though displayed no preferences between algal presented as homogenates in agar. However, the opposite was found for *I. granulosa*. They displayed no preferences for algae between functional groups when presented with whole plant material, but preferentially consumed agar discs containing extracts of algae from functional groups two and four.

- Within functional group four, *L. littorea* consumed whole plants of *O. pinnatifida* significantly more than whole plants of *M. stellatus*, suggesting that plants within this group were not similarly edible. They displayed no preferences when presented with extracts of algae in agar.

- However, within functional group three, *I. granulosa* significantly consumed extracts of *U. lactuca* in agar over extracts of *Porphyra* sp. in agar.

- For both grazers, the rank order of preference for both algal species and functional groups was comparable to the rank order of preference observed in chapter 3.

- The feeding preferences of *L. littorea* seem to be affected by algal morphology as functional group differences were observed between whole plants with differing morphologies but not agar discs with similar morphologies. *Idotea granulosa* displayed no differences in consumption between whole plant material, but significantly consumed agar discs containing extracts of some algal species, suggesting that it may be the chemical and/or nutritional composition of algae that influence their food preferences.

Chapter 6

General Discussion

6.1 Predicting herbivore feeding preferences

Algal functional groups have been considered to be a useful ecological tool for assessing intertidal communities as they may reduce the requirement for identification to species level (Littler and Littler, 1980; Littler, 1980; Steneck and Watling, 1982; Steneck and Dethier, 1994). In introducing their approach, Steneck and Watling (1982) further suggested that functional groups may be used to predict the effect of interspecific interactions and interpret patterns relating to community structure. A key interspecific interaction upon the shore is that between herbivores and algae, as distribution patterns of algae are often influenced by herbivory (Lubchenco, 1978, 1983; Sze, 1980; Underwood and Jernakoff, 1984; Petraitis, 1983, 1987; Salemaa, 1987; Janke, 1990; Davies and Beckwith, 1999; Kotta *et al*, 2000; Nilsson *et al*, 2004; Hemmi *et al*, 2005). Like most organisms, the diets of herbivores are limited to not only the species available within their habitat, but also to the sub-set of them that they are able to manipulate and consume. Herbivores can therefore be considered to assess both the attractiveness and the edibility of a potential food item when making feeding decisions (Nicotri, 1980). Factors that may influence a herbivore's perception of attractiveness and edibility include algal size, morphology and toughness. Steneck and Watling (1982) proposed an algal classification system based upon morphological characteristics in which algae were ranked from functional group one through to functional group seven according to their toughness. Steneck and Watling (1982) defined toughness as the ability of a plant to resist being scratched and related level of toughness to trends in grazing difficulty *i.e.* the tougher a plant, the more difficult herbivores find it to graze. Bertness *et al* (1983) suggested a similar definition in that the toughness of an alga is related to both the size and arrangement of algal cells, and the presence of an outer cuticle. Small cells enclosed by a protective cuticle act as an effective feeding deterrent by increasing the density of structural cell material grazers have to physically manipulate in order to consume tough algae (Bertness *et al*, 1983). An increase in the physical toughness of structural compounds may cause a reduction in grazing by herbivores (Cronin *et al*, 2002). Steneck and Watling (1982) predicted that as algal toughness increased from functional group one to seven, the susceptibility to grazing by herbivores would decrease.

6.2 The findings of this study

The work reported in this study comprises a series of investigations into the application of a functional group approach in predicting the feeding preferences of intertidal grazers. The findings of the investigations conducted are discussed in each of the relevant chapters. However, in order to discuss the utility of applying a functional group approach to algal-grazer interactions a summary of the main findings is provided below.

6.2.1 Herbivore feeding preferences

The use of Steneck and Watling's (1982) functional group model as a predictor of the feeding preferences of *Littorina littorea* and *Idotea granulosa* was investigated via attractiveness and edibility experiments.

Between-group variation in the attractiveness of algal functional groups

To test whether the attractiveness of algal species decreased as algal structural complexity increased from functional group two to functional group six, both *L. littorea* and *I. granulosa* were presented with algae from each functional group in two-way choice experiments. In line with the functional group model proposed by Steneck and Watling (1982) it would be expected that algae from functional group two would be the most attractive, with algae from functional group six the least attractive to grazers. Both *L. littorea* and *I. granulosa* displayed a preference for the filamentous algae representing functional group two. The order of preference shown by *L. littorea* generally followed the proposed model as algal attractiveness decreased as functional group number increased. For all grazers, the attractiveness of an alga is affected by both habitat and food requirements (Nicotri, 1980; Watson and Norton, 1985a). As *L. littorea* is a molluscan herbivore it may prefer less structurally complex species as they do not generally utilise algae for protection from predators and as a consequence may have reduced habitat requirements (Williamson and Creese, 1996).

The order of attractiveness preference displayed by *I. granulosa* differed slightly from the predicted order of preference as they found algae from functional group five the least attractive, rather than the coralline algae representing functional group six. The overall pattern indicated a preference for algae with branched morphologies. These tend to have greater habitat complexity which increases the level of protection offered to small herbivores such as *I. granulosa* (Edgar, 1983a, 1983b; Hacker and Steneck, 1990; Akioka *et al*, 1999). Branched algal species also provide increased food levels and so act not only as a shelter but a good source of food (Edgar, 1983b; Dean and Connell, 1987b; Kotta *et al*, 2000).

Between-group variation in the edibility of algal functional groups

The order of edibility was similar to the order of attractiveness for *I. granulosa*, as they found algae from functional group six more edible than algae from the other functional groups. They then displayed a decrease in consumption from functional groups two to five; with algae from functional group five consumed the least. Although *I. granulosa* displayed a preference for algae from functional group six, given the composition of algae within the group, *Idotea* may not have been feeding upon the algae, but rather microalgae living upon the fronds (Hawkins and Hartnoll, 1983). Alternatively, low nutritional value may not be an important factor in determining their food choice (Salemaa, 1987; Jormalainen *et al*, 2001a). Other idoteid species have been shown to consume fucoid algae from functional group five known to contain defensive chemicals (Jormalainen *et al*, 2001a, 2005), though in some cases only when no other food was available (Goecker and Kall, 2003).

Buschmann (1990) proposed that mobile invertebrates such as *I. granulosa* may be able to separate their behaviour between habitat and food requirements by feeding on more nutritious algae as necessary and moving to reside on a species preferred as a habitat. However, moving between plants presents a higher predation risk (Holomuzki and Hoyle, 1990; Rogers *et al*, 1998; Bakker *et al*, 2005). In this investigation there were no predators within the experimental system, and the use of single-choice experiments for the edibility study suggest that *I. granulosa* were making preference choices based on algal characteristics.

Littorina littorea also displayed similar results to the attractiveness preference in relation to the edibility preference order. *Littorina littorea* preferred algae from functional group two and consumed calcareous algae from functional group six the least, which may be explained by structure of their feeding apparatus. The radula teeth of *L. littorea* comprise sharp cusps that efficiently tear unstructurally defended algae (Watson and Norton, 1985a), such as those from the lower functional groups (Steneck and Watling, 1982). In comparison, the structurally tough *C. officinalis* (from functional group six) is composed of calcareous segments linked by articulated joints, which prevent it from being consumed by *L. littorea* due to their radula being wider than the uncalcified joints (Watson and Norton, 1985a). However, the recorded order of edibility preference varied from the predicted order as algae from functional group five were consumed more than algae from groups three and four.

Algal availability

Although *L. littorea* may base their feeding preferences more on food rather than habitat requirements, the availability of algae within their natural environment may be an important factor determining food preferences. *Littorina littorea* not only require protection from predators, but also from changes within their environment such as increased desiccation risk (Wieser, 1952). During emersion *L. littorea* congregate under large canopy fucoid algae as they maintain a damp environment allowing grazers to continue to feed (Alexander, 1960; Lubchenco, 1978). As algae comprising functional group five was widely available as a habitat due to it dominating on the shore from which grazers originated, it may have been consumed more due to previous feeding experience (Watson and Norton, 1985a). Although all of the individuals in this study were only used in one experimental trial, because they were collected from the natural environment they would have prior experience of encountering the algae presented. As previous feeding experience is more likely to affect attractiveness choices rather than edibility (Watson and Norton, 1985a), *L. littorea* may exhibit a preference for fucoid algae as it not only provides suitable protection during emersion, but it is potentially edible. Time spent feeding may also be an important factor controlling the feeding preferences observed. Presenting grazers with one algal species at a time provides them with the maximum amount of time available to feed upon that species. Presenting grazers with more than one algal species may cause them to partition their time to allow feeding on all available food sources.

The effect of algal availability upon the feeding preferences of *L. littorea* was further investigated by collecting grazers from two areas within a shore: one area dominated by *P. lanosa* (as an epiphyte on *A. nodosum*) representing functional group two, and one area dominated by *U. intestinalis* representing functional group three. Using methodologies outlined in chapters 2 and 3, grazers were subjected to a range of both attractiveness and edibility experiments in order to determine whether feeding preferences could be predicted in relation to algal availability. In line with the functional group model it would be expected that grazers would find *P. lanosa* more attractive than *U. intestinalis*. However, the results collected did not support the model as *L. littorea* from the area of shore dominated by *P. lanosa* displayed no preference in attractiveness between the two algal species, while *L. littorea* from the area of shore dominated by *U. intestinalis* found *U. intestinalis* significantly more attractive than *P. lanosa*. These results suggest that algal availability may affect the feeding preferences of *L. littorea*. To further test this theory, when *L. littorea* from both areas of the shore were presented with both algal species in single-choice experiments, grazers from both areas preferentially consumed the algal species that did not dominate in the area of the shore from which they originated. This may indicate that *L. littorea* has the capacity to switch their diet in response to changes in availability, as would be expected of a generalist grazer. Generalist grazers increase their survival by consuming a range of species with differing nutritional and chemical properties (Wiggins *et al*, 2006) and diet switching occurs when the relative abundances of foods of differing preference change in relation to each other (Crawley, 1983). As grazers from both areas of the shore preferred the alternative algal species, *L. littorea* may have switched their diet as an adaptation to changes in the abundance of algal species normally present in their natural environment. Diet switching is beneficial to generalist grazers as it maximises their nutritional intake (Westoby, 1978; Clark, 1982; Wiggins *et al*, 2006) and dilutes the effect of any defensive chemicals, thus improving their dietary performance (Bernays *et al*, 1994; Cruz-Rivera and Hay, 2000, 2001).

When presented with algae in two-way edibility experiments grazers from both areas of the shore preferentially consumed *U. intestinalis* (from functional group three) over *P. lanosa* (from functional group two), which again contradicts the functional group model. Although in the functional group model *U. intestinalis* is considered to be structurally tougher then *P. lanosa*, overall *L. littorea* found it more attractive and consumed it in greater quantities, especially those grazers from the area of shore dominated by *P. lanosa*. This suggests that *U. intestinalis* may be easier to manipulate than *P. lanosa*, and may be more beneficial in relation to nutritional value provided (Marsham *et al*, 2007 and references therein, Appendix II). The results collected in chapter 4 indicate that the effect of algal availability on *L. littorea* feeding preferences cannot be predicted using Steneck and Watling's (1982) functional group model.

Algal chemical defences

Algae comprising functional group five are known to contain phlorotannins (Hay and Fenical, 1988; Targett and Arnold, 2001; Van Alstyne *et al*, 2001; Hemmi *et al*, 2005) that can act defensive chemicals and deter feeding by herbivores (Rosenthal and Janzen, 1979; Steinberg, 1985, 1988; Targett and Arnold, 1998). In this investigation both grazers displayed a difference in preference for chemically defended algae. *Littorina littorea* consumed algae from functional group five suggesting that either they are able to consume species regardless of the presence of any defensive chemicals, or grazing by *L. littorea* does not induce the production of chemicals within fucoid algae (Scott and Marsham, 2007, Appendix III). In comparison *I. granulosa* least consumed algae from functional group five, suggesting that it may be deterred by defensive chemicals. This difference in consumption of chemically defended algae may be related to the feeding strategies of the grazers (Van Alstyne *et al*, 2001). *Littorina littorea* remove the surface cell layers by rasping the surface of an alga, whereas *I. granulosa* employ a shredding motion and bite off portions of an alga (Hawkins and Hartnoll, 1983; Brusca and Brusca, 2002; Alyakrinskaya, 2005). The allocation of defensive chemicals within a plant may prevent them from being released due to damage caused by *L. littorea* as their feeding method does not always cause deep excavation of the algal frond (personal observation.). Feeding by *I. granulosa* causes a greater concentration of damage in small areas of the algal frond, which may induce the production of defensive chemicals.

Algal morphology

Under the premise of the functional group model, morphologically similar species should respond in a similar way to grazing disturbance, and species within a group should be functionally equivalent as they utilise characteristics of their environment in similar ways (Steneck and Watling, 1982). The results collected in chapter 2 (attractiveness experiments), chapter 3 (edibility experiments) and chapter 5 (morphology experiments) do not support hypothesis.

Within-group variation in the attractiveness of algal functional groups

Within-group differences in attractiveness were observed for both *L. littorea* and *I. granulosa*. Within functional group three, *L. littorea* found *U. lactuca* significantly more attractive than *D. contorta*, possibly as a consequence of differences in algal morphology. Due to their physiology, *L. littorea* must physically occupy the algae they feed upon (Steneck and Watling, 1982). Unlike the tubular fronds of *D. contorta*, the sheet-like morphology of *U. lactuca* is able to accommodate the large muscular foot of *L. littorea* (Watson and Norton, 1985b; Wilhelmsen and Reise, 1994), thus making it more attractive to grazers. Both *L. littorea* and *I. granulosa* displayed differences in preference for algae within functional group four, by finding *M. stellatus* significantly more attractive than *O. pinnatifida*. As with functional group three, this could be related to algal morphology as *M. stellatus* has a larger, leafier structure enabling it to accommodate *L. littorea*. For *I. granulosa*, *M. stellatus* may have the preferential morphology as more individuals will be able to seek protection within the channelled fronds. Phillips *et al* (1997) suggested that the assumption that all species within a group will respond in a similar way could not be supported due to within-group variability. Algae within functional group four display a wide range of differing morphologies (Dudgeon *et al*, 1995) which may account for variability within the group. Phillips *et al* (1997) highlighted a number of species that were not easily classified using one of the existing functional group models (Littler and Littler, 1980; Steneck and Watling, 1982; Steneck and Dethier, 1994). In such cases the morphological features of the species are considered and plants are assigned to the group most closely resembling its morphology. As functional group four represent the broadly defined corticated macrophytes (Steneck and Watling, 1982) it is one of the largest groups as many algal species that do not meet the morphological requirements of other functional groups are assigned to group four. This problem with the functional group model may be overcome by reassigning the criteria on which algae within each functional group are characterised in order to further spilt the groups and thus allow more algal species to be correctly categorised.

Between-group variation in the edibility of algal functional groups

To further investigate the effect of algal morphology on feeding preferences, the methodology employed in chapter 3 was used for single-choice edibility experiments, with the addition that grazers were presented with algal homogenates in agar in order to remove algal morphology as a factor that may affect feeding preference.

Both grazers utilised agar as a source of food, thus allowing it to be used as a food item. When grazers were presented with algal species as whole plants neither *L. littorea* nor *I. granulosa* displayed preferential feeding, as would be expected under the premise of the functional group model. When presented with algae as homogenates in agar, *L. littorea* displayed no preference between species, suggesting that they considered all algae to be similarly edible. These results support Steneck and Watling's (1982) model as it would be expected that algae with similar morphologies would be equally edible, thus indicating that algal morphology may be an important factor in determining *L. littorea* feeding preferences.

Idotea granulosa showed significant preference for certain species as they consumed *Ceramium* sp. over *F. serratus* and *L. digitata.* They also showed preferential consumption for *U. lactuca* and *O. pinnatifida* over *Porphyra* sp.. This contradicts the underlying premise of Steneck and Watling's (1982) model as it would be expected that species with similar morphologies would be similarly edible. This indicates that algal morphology may not solely affect the feeding preferences of *Idotea*. However, removing morphology as a factor did alter the feeding preferences of *I. granulosa*. For example, they did not consume *F. serratus* and *L. digitata* (from functional group five) when presented as whole plants, but readily consumed agar discs containing extracts of each species. This suggests it may be the morphology of these two algal species that prevent *I. granulosa* from utilising them as a food source. Wessels *et al* (2006) recorded similar results for the amphipod *Gammarellus homari*, which increased its consumption of tough, leathery species once morphological defences were removed. As previously discussed, algae from functional group five are known to produce secondary metabolites that act as feeding deterrents (Geiselman and McConnell, 1981; Irelan and Horn, 1991; Targett *et al*, 1992; Kubanek *et al*, 2004). Given that *I. granulosa* consumed agar discs containing extracts of both *F. serratus* and *L. digitata*, it is possible that any secondary metabolites present in these species were not deterring *Idotea*. Rohde *et al* (2004) recorded that *I. baltica* displayed no significant preferences between *F. vesiculosus* presented as both whole plants and reconstituted material in agar, leading them to conclude that chemical factors, rather than morphological factors were controlling their feeding preferences. Alternatively, as evidence in the literature is contradictory (Kubanek *et al*, 2004; Jormaleinen *et al*, 2005) it may be possible that any chemical deterrents present may have leached out of the agar.

Differences were observed in *L. littorea*'s consumption of whole plants between functional groups. The results collected in chapter 5 generally supported those of chapter 3 as *L. littorea* displayed a significant preference for algae from functional group two but least preferred algae representing functional group six. When presented with the same algae as homogenates in agar, no preferences were recorded, suggesting that all functional groups were similarly edible to *L. littorea*. This again supports the premise of the functional group model as it demonstrates that algal morphology may be affecting the feeding choices of *L. littorea*.

However, results collected for *I. granulosa* did not support the functional group model as they did not display any preference for whole plants between functional groups. Under the functional group model, it would be expected that algae from functional group two would be consumed the most, and the order of preference would decrease through to functional group six. The results collected in chapter 5 for *I. granulosa* support those collected in chapter 3, as in both experiments they displayed a preference for algae from functional group six and least preferred algae representing functional group five. Additionally, in chapter 5 *I. granulosa* displayed preferential consumption of algal extracts in agar representing functional groups two and four over extracts of algae from functional group six. Given that in chapter 3, algae from functional group six were the most preferred it appears that the morphology of algae within this group may act as a factor controlling the feeding preferences of *I. granulosa*. As previously discussed, *I. granulosa* utilise algae as a habitat as well as a source of food. These results suggest that in chapter 3 *I. granulosa* displayed a preference for algae from functional group six as they offered the most suitable habitat due to their morphology. When morphology was removed as a factor, *I. granulosa* did not consume algae from this group as it may not have been beneficial for them to do so, given the nutritional composition of the algae (Marsham *et al*, 2007, Appendix II) and the fact that the calcium carbonate found in the structure of *C. officinalis* may act as a chemical defence (Hay *et al*, 1994).

Within-group variation in the edibility of algal functional groups

Both *L. littorea* and *I. granulosa* displayed edibility preferences for algae within a functional group. In chapter 5, within-group differences were observed in most cases due to preferential consumption of algal homogenates in agar over whole plant material. This may be accounted for by differences in the nutritional composition of algae in agar compared to whole plant material (Jormalainen *et al*, 2005). Algae presented as homogenates in agar may be less nutritious than the same species presented as whole plants due to nutritional components being lost during maceration. If this is the case it may cause grazers to consume greater quantities in order to gain the required nutrients. Additionally, as morphology would not have been a factor affecting the ability of grazers to manipulate agar discs, both grazers may have found them easier to consume (Cronin *et al*, 2002).

Littorina littorea consumed significantly more *Ceramium* sp. than either *C. rupestris* or *Polysiphonia* sp. within functional group two (chapter 3). In later work, Steneck and Dethier (1994) expanded Steneck and Watling's (1982) seven group model to a nine group model by splitting functional groups two and three into two sub-groups based upon levels of cortication within the algal thallus. According to Steneck and Dethier's (1994) model, *C. rupestris* would be classified as filamentous algae (functional group two), with *Ceramium* sp. and *Polysiphonia* sp. as corticated filamentous algae (functional group 2.5). Applying the theory of subgroups would predict *C. rupestris* to be consumed more than *Ceramium* sp., which was not supported by the results of this investigation. Although *C. rupestris* has lower levels of cortication than *Ceramium* sp., the branching structure and arrangement of fronds in *C. rupestris* may make it more difficult for *L. littorea* to manipulate, thus causing them to preferentially consume *Ceramium* sp.. *Littorina littorea* also displayed within-group preference in functional group three, by significantly consuming *U. lactuca* and *U. intestinalis* over *D. contorta* and *Porphyra* sp. (chapter 3). These results do support Steneck and Dethier's (1994) model as they split functional group three into two subgroups with *U. lactuca* and *U. intestinalis* representing foliose algae (functional group three) and *D. contorta* and *Porphyra* sp. representing corticated foliose algae (functional group 3.5). Differences were observed within functional group four for *L. littorea* as they preferentially consumed whole plants of *O. pinnatifida* over whole plants of *M. stellatus* (chapter 5). This again not only contradicts the functional group model, as all species within a group should be similarly susceptible to grazing, but also the results presented in chapter 3, as previously *L. littorea* consumed species within functional group four in similar amounts. The difference observed within this group could again be accounted for by the high level of morphological variability between composite species of the group. In their comparison of the morphology of *M. stellatus* and *C. crispus* in response to physiological stress, Dudgeon *et al* (1995) suggested that species within functional group four displayed as much within-group variation as there was variation between functional groups.

Idotea granulosa displayed within-group preferences for algae comprising functional group three (chapter 5). Within functional group three, *U. lactuca* in agar was consumed significantly more than *Porphyra* sp. presented in agar. As morphology had been removed, another factor (such as chemical or nutritional composition (Cronin *et al*, 2002)) may have affected the edibility of these two species, causing *U. lactuca* to be preferred over *Porphyra* sp.. Steneck and Dethier (1994) suggested that differences in the nutritional value of algae may have a greater effect upon within-group variation in algal consumption, than variation in the consumption of algae from different functional groups. *Idotea granulosa* also showed within-group variation within functional group five by preferentially consuming *F. serratus* over *L. digitata* (chapter 3). As with species within other groups, these species display differences in structure as although they are both corticated, leathery macrophytes, *L. digitata* has more substantial cortication than *F. serratus*, which increases frond toughness thus making it more difficult for *I. granulosa* to shred during feeding. The shore from which the grazers were collected was dominated by a fucoid belt. Although there are small patches of *L. digitata* in lower shore pools, in the natural environment *F. serratus* was more available to *I. granulosa* than the sub-tidal kelp species.

In comparing the results of chapters 3 and 5, it is evident that although some differences were recorded in the feeding preferences of both *L. littorea* and *I. granulosa*, the overall rank order of edibility preference was similar for *L. littorea* and identical for *I. granulosa*, though they were not similar to each other. This suggests that the two grazers employ different methods of feeding and may have different requirements affecting their preferences. This may affect the level of variability within their feeding regimes, thus making it difficult for predictions to be made. Results collected for *L. littorea* support Steneck and Watling's (1982) model in relation to general preference patterns. Using the model to predict progression patterns from one functional group to the next is outside of the capabilities of the model, partially because as Steneck and Watling (1982) suggested, functional groups may be useful as a reference tool but groups do not represent discrete morphological 'jumps'. Assigning an algal species to a certain group is open to discrepancy due to natural within-species variation (Phillips *et al*, 1997); a more realistic view would take into account the fact that algae progress on a continuum with the mid-point of each morphological type falling within a functional group (Steneck and Watling, 1982). Problems relating to within-species variation arise due to species crossing functional groups through different life stages (Steneck and Watling, 1982). Sporelings of a species may initially be assigned to a low functional group (*e.g.* group one or two) but reclassified as the species grows and its structural morphology changes. Additionally, the composite parts of some of the more complex species may be assigned to different functional groups based upon their morphology, for example many fucoids have leathery stipes (representing functional group five) but a crustose holdfast (representing functional group seven) (Steneck and Dethier, 1994). As all of the plant material used in this investigation was mature frond portions with no reproductive tissue, each species was assigned to a functional group based upon uniform morphological criteria.

None of the results collected for *I. granulosa* support the functional group model proposed by Steneck and Watling (1982). As their proposed model was based upon the feeding capabilities of molluscs, it is not unexpected that the model cannot be used to predict the feeding preferences of isopods, given differences in their feeding requirements. However, it has been constructive to test the model using a different type of grazer in order to propose suggestions for development of the model for future research. As for *Idotea*, there are some molluscan grazers for whom algae act as both a habitat and a food source. Although *Idotea* employ a different feeding mode to molluscan grazers (shredding as opposed to rasping), Steneck and Watling (1982) emphasize that differences in feeding preferences are a reflection of functional variation in the feeding apparatus of grazers. Their model was based not only upon functional differences between algae, but also between grazers within the same phylum (Steneck and Watling, 1982). As *L. littorea* and *I. granulosa* are both generalist grazers, they are capable of displaying adaptive feeding behaviour in relation to the presence of algae, providing that no higher preference species is available (Hawkins and Hartnoll, 1983). Although morphology is an important factor affecting the feeding preferences of both grazers, there are many other controlling factors acting upon both *L. littorea* and *I. granulosa* that cause difficulties in using a single model to predict feeding preference.

Habitat and food requirements

Given that in both attractiveness and edibility experiments, *L. littorea* displayed a preference for structurally fine, easily consumed algae from functional group two and least preferred the structurally tough algae from functional group six, food requirements may be more important than habitat requirements in determining their feeding preferences, which in turn may be controlled by a combination of algal morphology, structural toughness and algal nutritional composition.

In both attractiveness and edibility experiments, algae from functional group five were the least preferred by *I. granulosa*, suggesting that it is unsuitable as both a habitat due to it providing little protection (Hacker and Steneck, 1990); and food source due to its nutritional and/or chemical composition. Obvious preference for algae from functional group six, which provide very little nutritional value (Marsham *et al*, 2007, Appendix II) but offer a habitat well protected from predators (Nelson, 1979; Stoner, 1982; Akioka *et al*, 1999) suggests that as *Idotea* utilise algae as a habitat as well as a food source, habitat requirements may be more important in determining their feeding preferences.

6.3 Critique of study and further work

Algal availability

Although it was not covered in this study, it would be useful to investigate the effect of algal availability upon the feeding preferences of *I. granulosa*. Individuals collected from specific plant species could be offered their host plant in combination with additional algal species and data could be collected to determine if they feed more upon the alga with which they were originally associated or another algal species. This would complement the data collected which tested whether *I. granulosa* consumed more of the algae to which they were most attracted. For *I. granulosa* the rank order of attractiveness recorded was not significantly related to the rank order of edibility recorded, suggesting that *Idotea* have different habitat and food requirements. An algal species that provides a suitable habitat may not be the most nutritious food, so ideally *Idotea* should be able to separate habitat and food requirements in order to generate feeding preferences.

The effect of algal availability on the feeding preferences of both grazers could be further investigated by presenting both *L. littorea* and *I. granulosa* with algae from a range of functional groups, rather than just from two groups presented to *L. littorea* in this study. This would allow all functional groups to be compared in relation to each other and their availability. Additionally, a combination of species abundant within each grazer's environment, and algal species previously unencountered could be offered to both grazers. This would not only test whether grazers were making feeding choices based upon algal morphology, but would also examine the effect of past feeding experience.

Algal chemical defences

Although Scott and Marsham (2006) examined whether any chemicals produced by juvenile *Fucus* sp. had a deterrent effect on feeding by *L. littorea*, assumptions were made based upon their findings and no chemical analysis was conducted as part of the investigation, or this study. To fully understand the effect of any chemical defences present in the algal species used in this study, full chemical analysis is required as data are not available from the literature regarding all of the species. Additionally, experimental manipulation of the concentration of chemical defences present in algae presented to both *L. littorea* and *I. granulosa* may provide further evidence of any effect such chemicals have on the feeding preferences of these intertidal grazers.

Algal morphology

The effect of algal morphology could be further investigated by presenting grazers with artificial plants of similar morphologies to live material. Rather than homogenising algae into agar, artificial structures could be produced that mimic algae in their size, shape, thickness and branching patterns. The artificial plants would provide no nutritional value to the grazer, and would have no chemical deterrents. This would allow attractiveness preferences to be recorded that were entirely separate from the edibility requirements of the grazer.

To further determine how algal toughness influences the feeding preference of herbivores, work could also be conducted to quantify the proportion of an alga's mass utilised as indigestible structural materials. If more structurally complex algae do allocate a greater proportion of their mass to indigestible materials, making them tougher for herbivores to consume, calculating the amount of structural material within their thallus would allow comparison against their observed feeding preferences.

Within-group variation in the attractiveness and edibility of functional groups

Variation within functional group four could be investigated further by increasing the number of species within this group presented to *L. littorea* and *I. granulosa* in both attractiveness and edibility experiments. In this study both grazers were presented with *M. stellatus* and *O. pinnatifida* representing functional group four. Given the high level of variation between species within this group, the range of species offered to grazers could be extended in order to gain more comprehensive results for the group. Increasing the number of species may reduce variation in the results collected, and possibly allow redefinition of the characteristics an algal species is required to display in order to be included in functional group four.

Habitat and food requirements

It may have been valuable to investigate the attractiveness and edibility preferences of algae with epiphytes to compare with the results collected in this study for algae without epiphytes. Presenting grazers with algae covered in epiphytes may have allowed them to fulfil both their habitat and feeding requirements from one source, thus eliminating their need to move between algal species.

In order to provide further evidence of the factors controlling feeding preferences of both *L. littorea* and *I. granulosa*, all of the algal species used in this study could be measured for toughness. Feeny (1970) and Littler and Littler (1980) measured plant toughness using a penetrometer which calculated the amount of force required to pierce the plant blade, whereas Steneck and Watling (1982) linked plant toughness to grazing difficulty by suggesting that the more difficult it was for a herbivore to graze a plant, the tougher that plant was. As this study was based upon the functional group model of Steneck and Watling (1982), plant toughness was assumed under the predictions of the model. However, work by Padilla (1989) raised the possibility that it may not be an alga's toughness that deters feeding, as the more structurally tough algae required less force to remove tissue than the fleshier, less structurally defended species. Estimating the toughness of all of the algal species used in this study would allow the feeding preferences of both grazers to be discussed in relation to algal toughness.

Another factor that could be investigated is plant structural complexity. Steneck and Watling (1982) based their functional group model on the premise that as functional group number increased from one to seven, plant structural complexity increased. Although more morphologically complex species will be structurally tougher, they provide increased food levels, living space and protection from predators (Dean and Connell, 1987b), which may trade-off against increasing toughness in preference choices. Algal morphological complexity can be assessed via a variety of methods including order of branching (Steingraeber *et al*, 1979), bifurcation ratio of the first to second order branches (Steingraeber *et al*, 1979; Canham, 1988), frond density (Grahame and Hanna, 1989), interstitial volume (Hacker and Steneck, 1990) and perimeter fractal dimension (Gee and Warwick, 1994). Assigning a measure of structural complexity to the algal species used in this study may offer further explanation of the feeding preferences displayed by *L. littorea* and *I. granulosa.*

6.4 Other functional group approaches

For both grazers no significant relationship was observed between the recorded rank order of edibility and the rank order predicted by Steneck and Watling's (1982) model, suggesting that the functional group continuum from group two to six may not be a useful predictor of feeding preferences. However, as Steneck and Watling (1982) contradict themselves by stating that most herbivorous molluscs consume algae of either minute or expansive forms, it may be outside the basis of the functional group model for it to be a useful tool in predicting sequential preference. Considering their model in relation to a progression from group one to seven, it would be expected that the amount an alga within each functional group grazed would decrease in proportion to an increase in structural complexity. Given that there are many factors affecting the feeding preferences of grazers (*e.g.* algal availability, plant size in relation to the grazer, suitability as a habitat, chemical defences, and nutritive value) it is improbable that a linear relationship exists between feeding preference and functional groups.

Steneck and Watling (1982) were not unique in suggesting a functional group model. Other authors have proposed and commented upon the use of variations on the approach (Littler, 1980; Littler and Littler, 1980; Littler and Arnold, 1982; Coen, 1988; Padilla, 1985; Steneck and Dethier, 1994; Stone, 1995; Didham *et al*, 1996; Philips *et al*, 1997; Bonsdorff and Pearson, 1999; Padilla and Allen, 2000; Gibb and Hochuli, 2002; McClanahan *et al*, 2002; Wilson, 2002; Coomes and Grubb, 2003; Biber *et al*, 2004; Hooper and Dukes, 2004; Buonopane *et al*, 2005; Johnson *et al*, 2005; Micheli and Halpern, 2005). The original intention of this study was to investigate whether composite species of functional groups exhibited similar responses in relation to environmental stresses and disturbances, with a view to being able to use functional groups as an indicator of environmental conditions. The study began by investigating the effect of grazing disturbance on algae both between and within functional groups. However, as the complex relationship between the many factors affecting herbivore feeding preferences raised many more questions, the emphasis of the study changed and became focused upon whether the feeding preferences of intertidal grazers could be predicted using a functional group model. Although the use of other functional groups models has not been quantitatively studied, the results obtained using Steneck and Watling's (1982) approach can be discussed in relation to other models.

The constraints of Steneck and Watling's (1982) model may limit its use in predicting the feeding preferences of the two experimental grazers used in this study. Applying the data collected in this study to an alternative model may have yielded different preference patterns. Bremner *et al* (2003) compared the use of two different approaches (a taxon composition approach and a biological trait analysis) in assessing functional diversity in marine ecosystems and concluded that using the two different approaches generated contrasting answers. Using another model in which species were either assigned to groups based upon different criteria, or grouped using fewer or more than the seven groups of Steneck and Watling (1982) may have caused the species used in this study to be categorised into different functional groups. Recategorisation of species may provide stronger evidence that a functional group model could be used to predict feeding preferences.

Functional group models may be most useful when tailored to a specific experimental factor, organism or ecosystem. In their discussion on the use of functional groups in phytoecology, Medail *et al* (1998) concluded that functional groups would be the most useful when determined by an investigators' specific objectives. Using the ideas of Merritt and Cummins (1996), Lakly and McArthur (2000) advocated the use of 'functional feeding groups' based upon the morphological, behavioural and food gathering characteristics of macroinvertebrates. They proposed that this approach would consider how dependent on a specific resource the invertebrates were in relation to the exploitation of a habitat or food source (Lakly and McArthur, 2000). Padilla and Allen (2000) recommended that groupings should be based upon a specific function and species should be assigned to groups based upon functional criterion. Similarly, Underwood and Petraitis (1993) suggested that functional groupings would only be useful if the similarity of 'function' of all of the species within each group was known and not simply assumed. Biber *et al* (2004) proposed a model for three functional groups of macroalgae based upon extensive data sets and literature. They demonstrated that their model was a useful tool for the seagrass habitat studied, and concluded that it could be used to model similar habitats exhibiting the same conditions on which the model was based (Biber *et al*, 2004). In studying the spatiotemporal variation in abundance of algae within the same functional group, Collado-Vides *et al* (2005) concluded that the composite species could be assigned to a single functional group in order to describe one factor relating to changes in abundance, however they suggested regarding algae at genus or even species level may be more useful in illustrating shifts in algal community structure. In their study, using a functional group approach was useful in assessing one aspect of an algal community but could not be used to discuss overall patterns.

Implications for the functional group approach

Given the recent literature discussed above regarding the use of functional group approaches, and the results reported from this study, it may be unfeasible to apply a wide-ranging functional group model to ecological data. The work presented in chapters 2 to 5 have quantitatively tested the functional group model of Steneck and Watling (1982) and have shown that the model cannot be applied to the feeding preferences of intertidal grazers. Unless models are customised to meet the requirements of a specific question, natural variation within the environment will prevent general predictions being made regarding an organism's response to environmental stresses and disturbances. Extensive experimental work needs to be conducted on both biotic and abiotic factors acting upon organisms in order to increase understanding of interactions between organisms and their environment to allow predictions to be made.

The work reported in this study could be taken forward in relation to using an overall functional group approach by exploring the original aim. The responses of species within a functional group to a range of stresses and disturbances (such as desiccation, temperature, salinity, photosynthetic recovery, wave exposure, and sand scour) could be experimentally tested in both laboratory and field investigations. Assigning species to functional groups based upon their morphological responses to each stress or disturbance may allow specific questions to be answered concerning the use of algal functional groups as indictors of environmental conditions. The solution may be that a model needs to be created from answers rather than used to predict answers from a theoretical model.

6.5 Summary

In conclusion, the work reported in this study suggests that using a general functional group approach to predict the feeding preferences of intertidal grazers would not be useful, largely due to variation between algal species within a functional group. Additionally, other factors not correlated with algal morphology are important in determining the dietary choices of herbivores. The data collected do not fully support the hypotheses that both the attractiveness and edibility of algae would decrease as functional group number increased. As differences were also observed between species within a functional group, the hypotheses that all species within a functional group would be similarly attractive and edible to grazers were not supported. By experimentally testing the model, rather than undertaking a literature review, this study makes a significant contribution to the application of functional groups in understanding algal-grazer interactions. The functional group model proposed by Steneck and Watling (1982) was more useful in predicting the feeding preferences of *L. littorea* than *I. granulosa*, due to differences in the requirements of each grazer in relation to habitat and food choice. Applying a model based on a specific function or organism would allow more reliable predictions to be made.

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Appendices

Appendix I

Attractiveness of algal functional groups to intertidal grazers

I.1 Background

The following study was conducted as a preliminary investigation to the attractiveness experiment described in section 2.2.2.1 of chapter 2. The initial aim of the experiment was to determine if algal species were similarly attractive to *L. littorea* and compare the attractiveness of algal species between functional groups (with up to four species of algae representing each functional group) in order to determine if algal attractiveness decreased from functional group two to group six in line with Steneck and Watling's (1982) functional group model. Within group comparisons were also going to be conducted to ascertain whether all species within a functional group were similarly attractive to grazers. The data were also used to determine if algal attractiveness changed over time.

I.2 Methods

I.2.1 Variation in the attractiveness of algal functional groups and species

Mature *L. littorea* (mean shell height 2.02 ± 0.01 cm S.E.) and 14 species of algae representing functional groups two to six (Table 1.1, chapter 1) were collected between February 2003 and June 2003 from Holbeck and Filey Brigg (see section 2.2.1 of chapter 2 for site descriptions). Following collection *L. littorea* were food deprived for four days in filtered seawater, and algae were cleaned to remove epifauna and epiphytes. All algae used in experiments were mature plants with no reproductive tissue. Twenty *L. littorea* were placed in experimental tanks (18 x 12.5 x 5 cm) with 600 ml of filtered seawater and allowed to explore their surroundings for 30 minutes, and thereby acclimate to the experimental conditions. After 30 minutes all snails were returned to the centre of the experimental tank and equal weights of two algal species were introduced in paired choice experiments with one species at each end of the tank. Experiments were left to run for a further 30 minutes, during which the snails were allowed freedom of movement throughout the experimental tank. After this period the number of *L. littorea* on each alga was counted. Each algal species was tested in combination with every other species (resulting in a total of 78 combinations). The experiment was repeated six times for each combination, using new snails for each replicate. An attractiveness hierarchy was determined using equation 1 (see section 2.2.2 of chapter 2)

The data were used to test the null hypotheses that there would be no significant difference in algal attractiveness from functional group two to functional group six, and that all species within a functional group would be similarly attractive to *L. littorea.*

I.2.2 Statistical Analyses

Data were screened using the Kolmogorov-Smirnov normality test and Levene's test to test for normality of distribution and homogeneity of variances respectively (Sokal and Rohlf, 1995). As the attractiveness hierarchy data calculated were proportional and the data had heterogeneous variances due to the upper and lower constraints imposed upon proportional data (Underwood, 1981), *a priori* arc-sine transformation was carried out (Fowler *et al*, 1998).

I.2.2.1 Between-species variation the attractiveness of algal species

To determine whether algal species were similarly attractive to *L. littorea* data were analysed using two-factor ANOVA. SPECIES was a fixed factor with fourteen levels as the species used in the investigation were chosen from a fixed range of available species within each functional group (Underwood, 1997). The second factor in the analysis was TIME (a fixed factor with five levels). SPECIES and TIME were the independent variables and the weight of each species consumed was the dependent variable. *Post hoc* Tukey pairwise comparisons were determined where appropriate.

I.2.2.2 Between-group variation in the attractiveness of algal functional groups

Two-factor analysis of variance (ANOVA) was used to test the null hypothesis that algal attractiveness would not be significantly different from functional group two to functional group six as structural complexity increased, and that algal attractiveness would not change over time. Factors in the analysis were FUNCTIONAL GROUP (a fixed factor with five levels) and TIME (a fixed factor with five levels) (Underwood, 1997). Both FUNCTIONAL GROUP and TIME were independent variables, and the species position in the attractiveness hierarchy was a dependent variable (Pallant, 2001). *Post hoc* Tukey pairwise comparisons were determined where appropriate.

I.2.2.3 Within-group variation in the attractiveness of algal functional groups

One-way ANOVAs were conducted on the species data to compare attractiveness of each consumed within functional groups two to five for *L. littorea*. *Post hoc* Tukey pairwise comparisons were determined where appropriate. Data for functional group six were not included in the analysis as only one algal species (*C. officinalis*) was available from this group, and so no comparison could be made.

I.3 Results

The effect of algal attractiveness on the food preferences of *L .littorea* was investigated by presenting grazers with a choice of two algae simultaneously and counting the number of grazers on each species, which was assumed to indicate a choice preference.

I.3.1 Data Screening

The results of the Kolmogorov-Smirnov normality test showed that the data at both functional group and species level were normal (Kolmogorov-Smirnov, $P > 0.15$). Levene's test of homogeneity of variances showed no significant difference in the number of each functional group chosen (Levene's, $L_{18, 62} = 1.109$, $P > 0.15$) and the number of species chosen (Levene's, $L_{34, 46} = 0.950$, $P > 0.15$). Therefore, it can be assumed that all samples were drawn from populations with equal or similar variances.

I.3.2 Between-species variation in the attractiveness of algal species

Differences were exhibited in the number of *L. littorea* selecting each algal species, with the grazers showing a preference for *U. lactuca* with an average of 32 ± 57 individuals (median ± range, n = 11). The least chosen species was *C. officinalis* with an average of 5 ± 7 grazers (median ± range, n = 4) choosing this algal species (Fig. Ia).

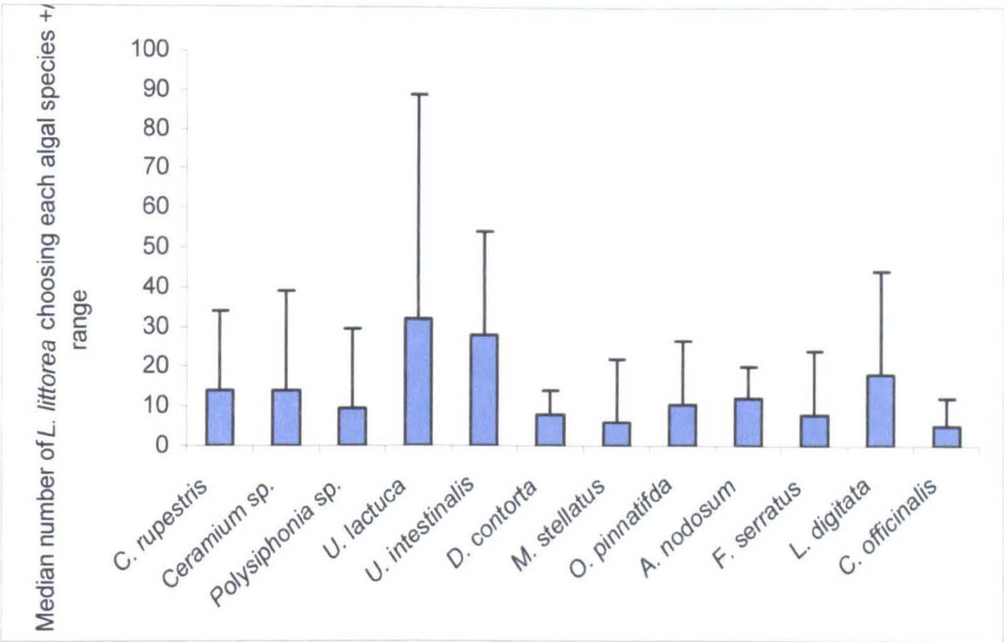

Figure Ia - Median number of *Littorina littorea* choosing each algal species (± range) (n = 80).

The results collected showed that species were not similarly attractive to grazers (Fig. Ia), and the difference in the number of *L. littorea* selecting each species when all species were compared together was significant (Table Ia).

Table Ia – Two-factor analysis of variance of the effects of SPECIES and TIME on algal attractiveness preference of *Littorina littorea*, using arc-sine transformed data (level of significance * $P < 0.05$, *** $P < 0.001$, NS not significant).

Source of Variation	df	SS	MS	F	*P*
SPECIES	11	868.669	78.970	3.284	0.002*
TIME	4	1329.200	332.300	13.819	<0.001***
SPECIES * TIME	19	376.450	19.813	0.824	0.669NS
Error	46	1106.183	24.047		
Total	81	37526.920			

SPECIES and TIME were independent variables, and the two-factor ANOVA investigated the effect of these, and their interaction, upon the dependent variable (the position of species in the attractiveness hierarchy). Attractiveness differed significantly over TIME and also between SPECIES. However, as the interaction effect was not significant, *L. littorea* displayed the same pattern of attractiveness through TIME *i.e.* the attractiveness of each algal species was consistent through time. *Post hoc* Tukey pairwise comparison ($P = 0.05$) revealed that *U. lactuca* differed significantly from *C. rupestris*, *Ceramium* sp., *Polysiphonia* sp., *D. contorta*, *M. stellatus*, *O. pinnatifida*, *A. nodosum*, *F. serratus*, *L. digitata* and *C. officinalis*, but these species were not significantly different from each other. The *post hoc* Tukey also revealed that *U. intestinalis* was significantly different from *Polysiphonia* sp., *D. contorta*, *M. stellatus*, *O. pinnatifida*, *F. serratus*, *L. digitata* and *C. officinalis*, but these species did not differ significantly from each other.

I.3.3 Between-group variation in the attractiveness of algal functional groups

The number of *L. littorea* choosing each functional group varied, with the grazers showing an obvious preference for functional group three with an average of 25 ± 57 *L. littorea* (median ± range, n = 27) and the least preferred being functional group six with an average of 5 ± 7 grazers (median ± range, n = 4) choosing algae from this group (Fig. Ib).

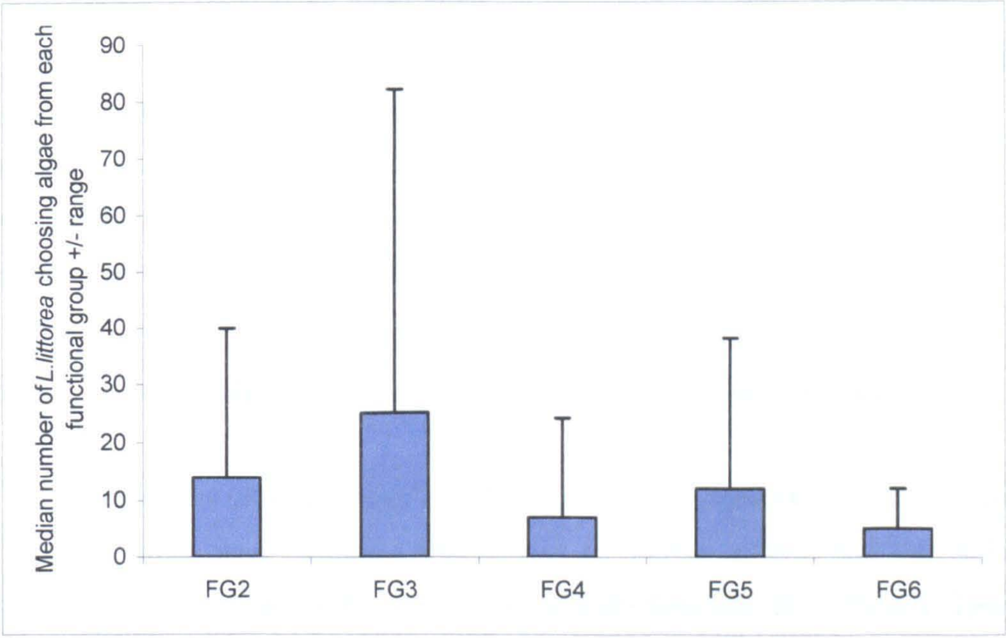

Figure Ib – Median number of *Littorina littorea* choosing algae from each functional group (± range) (n = 80).

The difference in choice exhibited by *L. littorea* showed a general decrease from functional group two to functional group six (Fig. Ib) and this difference was significant (Table Ib).

Table Ib – Two-factor analysis of variance of the effects of FUNCTIONAL GROUP and TIME on algal attractiveness preference of *Littorina littorea*, using arcsine transformed data (level of significance * $P < 0.05$, *** $P < 0.001$, NS not significant).

Source of Variation	df	SS	MS	F	*P*
FUNCTIONAL GROUP	4	476.286	119.072	4.499	0.003*
TIME	4	1157.22	289.431	10.936	<0.001***
FUNCTIONAL GROUP * TIME	10	272.135	27.213	1.028	0.431NS
Error	62	1640.874	26.466		
Total	81	37526.920			

FUNCTIONAL GROUP and TIME were independent variables, and the two-factor ANOVA investigated the effect of each of these, and their interaction, upon the dependent variable (the position of the functional group in the attractiveness hierarchy). Attractiveness differed significantly both over TIME and also between FUNCTIONAL GROUPS (Table Ib). However, as the interaction effect was not significant, *L. littorea* displayed the same pattern of attractiveness for each FUNCTIONAL GROUP through TIME (Table Ib). *Post hoc* Tukey pairwise comparison ($P = 0.05$) revealed that functional group two was significantly different from functional groups four and six, but these groups were not significantly different from each other. Also, functional group three was significantly different from functional groups four, five and six, but again these groups were not significantly different from each other.

I.3.4 Within-group variation in the attractiveness of algal functional groups

The results of the one-way ANOVAs conducted to determine if species within a functional group were similarly attractive to *L. littorea* showed that the individual species of functional group three were not similarly attractive to *L. littorea* (Table Ic). The *post hoc* Tukey pairwise comparison ($P = 0.05$) revealed that *U. intestinalis* and *U. lactuca* were significantly more attractive than *D. contorta*, but were not significantly different from each other. However, species within functional groups two, four and five were similarly attractive to *L. littorea* as the numbers of grazers choosing species within these three groups were not significantly different (Table Ic).

Table Ic – One-way analyses of variance to show the comparison of attractiveness to *Littorina littorea* of algal species within a functional group (level of significance * $P < 0.05$, NS not significant).

Source of Variation	df	SS	MS	F	*P*
Functional Group 2					
Between Groups	2	24.731	12.366	0.351	0.709NS
Within Groups	19	670.107	35.269		
Total	21	694.839			
Functional Group 3					
Between Groups	2	867.148	433.574	8.959	0.01*
Within Groups	25	1209.899	48.396		
Total	27	2077.047			
Functional Group 4					
Between Groups	1	19.092	19.092	0.660	0.432NS
Within Groups	12	346.871	28.906		
Total	13	365.964			
Functional Group 5					
Between Groups	2	7.968	3.984	0.061	0.942NS
Within Groups	10	657.472	65.747		
Total	12	665.440			

I.4 Discussion

Preliminary analysis of the data proved difficult to interpret due to the high number of combinations between algal species. Further preliminary experiments and analysis suggested that meaningful results were still collected with fewer combinations. The initial analysis also showed that the attractiveness of both functional groups and algal species to *L. littorea* was consistent with time *i.e.* it did not change over the course of a year (Table Ia, Ib). Taking both of these points into consideration, it was decided to conduct the experiment with fewer algal species from each functional group, and not include time as a factor in the analysis. The method outlined in section 2.2.2.1 of chapter 2 was therefore used to determine if the attractiveness of algae to *L. littorea* differed both between and within functional groups.

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Comparison of nutritive chemistry of a range of temperate seaweeds

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Abstract

Eleven species of macroalgae (including four species from commercially important genera) were analysed for moisture, ash, fat, protein, neutral detergent fibre, crude fibre, calorific value, and calcium content. At the extremes of the nutritional values, *Corallina officinalis* had low calorific value (2.7 ± 0.3 MJ kg^{-1}), high ash content (77.8 ± 0.2% dw), low protein (6.9 ± 0.1% dw) and high calcium content (182 ppm); whereas the exploited *Porphyra* sp. had high calorific value (18.3 ± 1.8 MJ kg^{-1}), low ash content (9.3 ± 0.2% dw), high protein (44.0 ± 1.2% dw) and low calcium content (19.9 ppm). The other species considered had intermediate values, but tended to be more similar to *Porphyra* than to *Corallina*. When possible our data were also compared with those of other workers; they were found to be broadly similar.

Keywords: Seaweeds; Ash; Protein; Fibre; Calorific value; Calcium

1. Introduction

In 2003, it was estimated that approximately 1 millions tonnes of wet seaweed were harvested in 35 countries as a source of food; as sources of agar, alginate and carrageenan; as a fertilizer; as fuel; and, for use in cosmetics annually (McHugh, 2003). However, it is as a dietary supplement that seaweed has had the longest and perhaps most significant use. Seaweed has been an important dietary component since at least the fourth century in Japan and the sixth century in China (McHugh, 2003). Recently, both these and other countries, such as the Republic of Korea, the United States of America, South America, Ireland, Iceland, Canada and France have significantly increased the consumption, production and marketing of seaweeds (McHugh, 2003). As demand has increased, natural stocks have been unable to meet market requirements, and now more than 90% of seaweed that is used commercially is cultivated (McHugh, 2003). Seaweeds are a valuable food source as they contain protein, lipids, vitamins and minerals (Norziah & Ching, 2000; Sánchez-Machado, López-Cervantes, López-Hernández, & Paseiro- Losada, 2004; Wong & Cheung, 2000). Seaweeds are not only a useful food source to humans, whole plants and seaweed mixes have been used in animal nutrition (Chapman & Chapman, 1980; Indergaard & Minsaas, 1991; Ventura & Castanon, 1998) and fish feed (McHugh, 2003). However, very few of the world's available seaweed species are used commercially. This may be because they cannot be harvested or cultivated on a commercially viable scale, or because their composition simply makes them unsuitable.

This study aims to compare aspects of the nutritional composition of seaweeds from genera that are traditionally used in the food industry with other (currently not exploited) commonly occurring temperate macroalgae. The data will also be compared with available data on the same species reported by other workers.

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2. Materials and methods

2.1. Materials

Samples comprising of up to 15 individual plants of 11 species of algae (the commercially important *Ulva lactuca*, *Porphyra* sp., *Fucus serratus* and *Laminaria digitata*, and the unexploited *Cladophora rupestris*, *Ceramium* sp., *Polysiphonia* sp., *Dumontia contorta*, *Mastocarpus stellatus*, *Osmundea pinnatifida* and *Corallina officinalis*) were collected from Holbeck, North Yorkshire, UK (54°16′N, 0°25′W) and washed to remove all epifauna and epiphytes.

2.2. Nutrient analysis

Seaweeds were analysed for moisture content, ash content, fat content, protein content, fibre (neutral detergent fibre and crude fibre), and calorific value. Although the authors recognise that other components of seaweed (e.g. amino acids, soluble fibre, fatty acids and vitamins) play an important role in the human diet, this study focuses on a range of general nutritional components as outlined above. For all analyses (with the exception of moisture content) algal material was dried in an oven at 100 °C for 24 h and ground into a fine powder prior to use. Each analysis was replicated three times.

2.2.1. Moisture content

Percentage dry matter of fresh algal material was measured using Oxford and Ohaus moisture balances.

2.2.2. Ash content

Two grams of dried algal material were added to a preweighed crucible and weighed, placed in a furnace at 400 °C for 4 h, cooled in a desiccator and reweighed. The ash content was determined using Eq. (1):

$$\%\text{ash} = \text{weight of ash}/\text{weight of sample} \times 100. \qquad (1)$$

2.2.3. Fat content

Crude fat content was determined using the Soxtherm method. One hundred and forty millilitres of petroleum ether was poured over 5 g of algal material in an extraction thimble. The thimble was placed in a pre-weighed beaker containing anti-bumping granules and placed in a Soxtherm for 80 min, after which the beaker was dried in an oven, cooled and reweighed. The fat content of each sample was calculated using Eq. (2):

$$\begin{aligned}\%\text{crude fat} = {} & ([\text{weight of dried beaker} + \text{fat}] \\ & - [\text{weight of dried beaker} \\ & + \text{granules}]/\text{weight of sample}) \times 100. \qquad (2)\end{aligned}$$

2.2.4. Protein content

Crude protein content was determined using the Kjeldahl method. One gram of algal material was digested in 15 ml of sulphuric acid in the presence of 2 kjeltec Ck catalyst tablets by placing in a turbosog fume scrubber for 1 h. Digestion was complete on production of a clear, coloured solution. After digestion, samples were analysed for nitrogen content by placing digested material into a Vapodest 33 distilling unit. The digested sample was then titrated against standard (0.1 M) hydrochloric acid until a colour change from blue to straw colour occurred. Nitrogen content was calculated using Eq. (3):

$$\begin{aligned}\%N = {} & [14.01 \times (\text{ml titrant for sample} \\ & - \text{ml titrant for blank}) \\ & \times \text{molarity of acid}]/\text{weight of sample} \times 100. \qquad (3)\end{aligned}$$

The crude protein content was then calculated using Eq. (4):

$$\%\text{protein} = N \times 6.25 \text{ (protein factor specific to sample).} \qquad (4)$$

The average $\%N$ in plant proteins is 16%. The general conversion factor to convert N to protein is $100/16 = 6.25$.

2.2.5. Neutral detergent fibre

Neutral detergent fibre (NDF) was determined using fat-free samples. A half gram of algal material was placed in a fibre bag and boiled with 360 ml of neutral detergent solution for 30 min. One hundred and eighty millilitres of this hot neutral detergent solution was added to 180 ml of cold neutral detergent solution and 12 ml of amylase solution and boiled for a further 30 min. Fibre bags were then washed in four portions of hot, distilled water, patted dry and dried in an oven at 100 °C for 4 h, desiccated, cooled and weighed. They were then ashed in a furnace at 600 °C for 6 h, desiccated, cooled and reweighed. NDF content was determined using Eq. (5):

$$\begin{aligned}\%\text{NDF} = {} & [(\text{beaker} + \text{residue weight} - \text{fibrebag weight}) \\ & - (\text{beaker} + \text{ash weight})/\text{sample weight}] \times 100. \qquad (5)\end{aligned}$$

2.2.6. Crude fibre

Crude fibre was determined using fat-free samples. One gram of algal material was placed in a fibre bag, boiled firstly with 360 ml of 0.128 M sulphuric acid for 30 min and then with 360 ml of 0.313 M hydrochloric acid for a further 30 min. Fibre bags were washed once with hot distilled water, once with 0.1 M hydrochloric acid and twice more with hot distilled water, patted dry and dried in an oven at 100 °C for 4 h, desiccated, cooled and weighed. They were then ashed in a furnace at 600 °C for 6 h, desiccated, cooled and reweighed. Crude fibre content was determined using Eq. (6):

$$\begin{aligned}\%\text{crude fibre} = {} & [(\text{beaker} + \text{residue weight} \\ & - \text{fibrebag weight}) - (\text{beaker} \\ & + \text{ash weight})/\text{sample weight}] \times 100. \qquad (6)\end{aligned}$$

2.2.7. *Calorific value*

A half gram of algal material was placed in a bomb calorimeter (Parr 1351 calorimeter). A spike of benzoic acid was added to the samples of *C. officinalis* to aid ignition of the material.

2.3. *Calcium content*

Algal samples were also analysed for calcium content using atomic absorption spectrophotometry (AAS). Ashed algal samples were used and samples were prepared by weighing out the amount of ash obtained from 2 g of algae, to which 10 ml of concentrated acid mix (2 parts hydrochloric acid, 1 part nitric acid and 3 parts distilled water) was added. This solution was filtered and made up to 100 ml with distilled water. Two millilitres of lanthanum chloride was added to stabilise the calcium atoms. Hundred millilitres standard solutions of 1, 5, 10, 15, 20, 25, 50, 75 and 100 ppm were prepared using a calcium stock solution in nitric acid, distilled water and 2 ml of lanthanum chloride. Standard solutions were analysed in the AAS to prepare a calibration curve, against which the algal samples were compared to calculate calcium levels.

2.4. *Data analysis*

Data were analysed using principle components analysis (PCA), to identify the variables important in separating the algal species by nutritional composition. Prior to PCA percentage data were arc-sine transformed (James & McCulloch, 1990) and the data set was examined for outliers (Pallant, 2001). Bartlett's test of sphericity was performed to determine if the matrices in the data set contained adequate correlation coefficients (greater than 0.3) (Tabachnick & Fidell, 2001). The Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy was also performed, to test for significance of correlations within the data matrices (Tabachnick & Fidell, 2001). The factors within the analysis were not correlated (determined by running an oblique rotation and examining the factor correlation matrix for correlations of 0.32 and above) and so data were subjected to orthogonal Varimax rotation (Tabachnick & Fidell, 2001). Two PCAs were performed. The first included all algal species and all nutrient variables. After interpretation of these results *C. officinalis* was found to form a discrete out-group. A second PCA of all nutrients was therefore carried out with the exclusion of *C. officinalis* to permit further elucidation of the relationships between the other species. All PCAs were performed using the SPSS version 11.5 for Windows statistical package.

3. Results and discussion

The nutritional composition of the seaweeds considered in this study are reported in Table 1. The table also presents data previously reported in the literature, where such data are available it would appear that our data are broadly similar to them. The eight nutritional variables for all algal species were subjected to PCA. Data screening of transformed nutrient data prior to PCA identified no outliers in the variables. Bartlett's test of sphericity was significant (all species: $\chi^2_{28} = 285.288$, $P < 0.001$; excluding *C. officinalis*: $\chi^2_{28} = 155.154$, $P < 0.001$). The KMO measure of sampling adequacy was 0.616 for all species ($P < 0.05$), thus indicating factor analysis was appropriate. When *C. officinalis* was excluded, the KMO measure of sampling adequacy was 0.474 ($P > 0.05$), which is lower than the recommended KMO value of 0.6 (Tabachnick & Fidell, 2001). However, coupled with the significant Bartlett's test for these data it was considered appropriate to proceed with factor analysis.

PCA revealed the presence of three components with eigenvalues exceeding 1, which accounted for 85.8% of the total variance (Table 2). Inspection of the screeplot (not presented) showed a clear break after the second component, so two components were retained for further investigation (Pallant, 2001). The factor loadings of the eight nutrient variables for all algal species on the first two components, and their communalities are shown in Table 3. The two factor extraction explained a total of 72.9% of the variance (see Table 3 for the % explained by each component). The main nutrients loading on component 1 were calorific value and ash content, whereas fat loaded strongly on component 2 (Table 3).

The plot of the regression factor scores for the first two principle components (Fig. 1) showed that most of the separation of the data points occurred across the first principle component axis, with some separation across the second principle component axis. Data points for *C. officinalis* were strongly displaced along the first principle components axis (Fig. 1), which suggests that this species has a different nutritional composition, which is likely to be caused by its relatively low calorific value and high ash content (Table 1). Along the second principle components axis, data points for *M. stellatus* were displaced (Fig. 1), which could be due to its relatively high fat content and low calcium content (Table 1).

As *C. officinalis* displaced strongly across the first principle component axis, PCA was performed excluding this species in order to permit further elucidation of the variation that exists between the other species considered. This analysis also produced three components with eigenvalues exceeding 1, accounting for 81.3% of the total variance (Table 4). Inspection of the screeplot (not presented) showed a clear break after the fifth component, however, as only three components had eigenvalues over 1 it was decided to retain three components for further investigation. The factor loadings of the eight nutrient variables for ten algal species (excluding *C. officinalis*) on the first three components, and their communalities are shown in Table 5. The three factor extraction explained a total of 70.2% of the variance (see Table 5 for the % explained by each component). The main nutrients loading on component 1 were calcium content and ash content, whereas

Table 1
Nutritional composition of 11 species of macroalgae

Species	Moisture	Ash	Protein[a]	Fat	Crude fibre	NDF	Calorific value	Calcium
Cladophora rupestris	68.5 ± 2.7 59[c]	16.8 ± 0.6 55[d]	29.8 ± 0.6	1.0 ± 0.4	24.7 ± 0.5	45.7 ± 6.1	15.9 ± 0.3	49.0
Ceramium sp.	87.4 ± 1.5	27.1 ± 0.5	31.2 ± 0.5	0.6 ± 0.3	5.1 ± 1.3	33.7 ± 2.3	14.4 ± 0.06	95.1
Polysiphonia sp.	77.2 ± 2.2 86.3[c]	19.2 ± 0.1 31[c]	31.8 ± 0.2	0.05 ± 0.0 7	4.3 ± 0.6	52.8 ± 19.5	16.1 ± 0.1	104
Ulva lactuca	79.6 ± 2.6 78[f] 10.6[i] 79.6[j]	17.8 ± 0.1 13–22[f] 21.3[i] 23.6[j] 21[c] 20[d]	29.0 ± 0.1 15–25[f] 10–21[h] 7.06[i]	0.5 ± 0.03 0.6–0.7[f]	2.8 ± 0.7	32.9 ± 0.1	15.7 ± 0.1	53.7
Porphyra sp.	77.1 ± 4.4 86[f]	9.3 ± 0.2 8–16[f] 21[k] 12[d] 19.07[m]	44.0 ± 1.2 33–47[f] 28.29[k] 24.11[m] 30–50[l]	0.7 ± 0.09 0.7[f]	1.1 ± 0.6	33.5 ± 0.6	18.3 ± 1.8	19.9
Dumontia contorta	87.7 ± 0.6	17.8 ± 0.1	31.7 ± 0.4	0.12 ± 0.2	2.0 ± 0.2	34.3 ± 0.3	15.6 ± 0.06	51.6
Mastocarpus stellatus	64.9 ± 3.9	15.6 ± 0.2	25.4 ± 0.2	3.0 ± 4.8	1.8 ± 0.5	16.6 ± 0.8	15.5 ± 0.06	38.7
Osmundea pinnatifida	86.4 ± 3.7	32.3 ± 0.3	27.3 ± 0.1	4.3 ± 6.38	6.5 ± 1.7	25.6 ± 0.4	13.6 ± 0.2	89.1
Fucus serratus	81.1 ± 3.0	18.6 ± 0.3	17.4 ± 0.2 3–11[e]	1.8 ± 0.3	16.0 ± 0.8	26.2 ± 2.8	15.5 ± 0.1	44.26
Laminaria digitata	86.1 ± 0.3 73–90[f]	23.6 ± 2.2 21–35[f] 33[b] 37.6[k]	15.9 ± 0.4 8–15[f] 9.3[b] 10.7[k]	0.5 ± 0.3 1–2[f]	7.7 ± 1.6	16.6 ± 0.5	13.0 ± 0.3	73.4
Corallina officinalis	31.5 ± 3.7	77.8 ± 1.2 80[g]	6.9 ± 0.1 6.1[g]	0.3 ± 0.2	8.3 ± 3.2	9.4 ± 3.5	2.7 ± 0.3	182

All values are expressed as mean % dry weight ±SD, except calorific value (MJ kg^{-1} ± SD) and calcium content (ppm).

[a] Estimated using $N \times 6.25$ as a conversion factor, though this may over-estimate protein content, especially if samples contain high levels of non-protein nitrogen (Salo-Väänänen & Koivistoinen, 1996; Crossman et al., 2000). For each species, values in the first row were obtained during this study. Other values have been referenced from the literature as a comparison.

[b] Averaged from Black (1950).

[c] Paine and Vadas (1969).

[d] Carefoot (1973).

[e] Munda (1977).

[f] Indergaard and Minsaas (1991) and references therein.

[g] Foster and Hodgson (1998).

[h] Fleurence (1999) and references therein.

[i] Wong and Cheung (2000).

[j] Lamare and Wing (2001).

[k] Rupérez and Saura-Calixto (2001).

[l] McHugh (2003).

[m] Sánchez-Machado et al. (2004).

Table 2
Eigenvalues and % of total variance for the first three principle components extracted from PCA using nutritional data for all algal species

Component	Eigenvalues	% of Variance	Cumulative %
1	4.542	56.8	56.8
2	1.292	16.2	72.9
3	1.034	12.9	85.8

Table 3
Post extraction communalities and factor loadings of the eight nutrient variables for all algal species for the first two principle components with orthogonal Varimax rotation

Variable	Communalities	Rotated first component	Rotated second component
Calorific value	0.980	0.955	
Ash	0.956	−0.921	0.306
Protein	0.843	0.884	
NDF	0.775	0.784	0.384
Moisture	0.617	0.782	
Calcium	0.861	−0.777	0.468
Fat	0.847		−0.872
CF	0.988		0.361
%Variance		55.2	17.7

NB only loadings above 0.3 are displayed.

NDF and protein loaded strongly on component 2, and crude fibre on component 3 (Table 5).

The plot of the regression factor scores for the first two principle components (Fig. 2) showed that most of the separation of the data points occurred across the first principle component axis, with some separation across the second principle component axis. Data points for *Porphyra* sp.

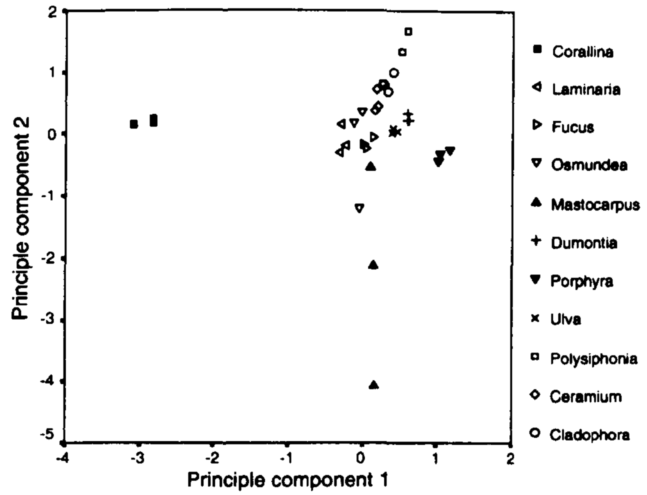

Fig. 1. PCA of transformed nutrient data for all algal species. Scores of the first two principle components are plotted. Algal species are used as markers.

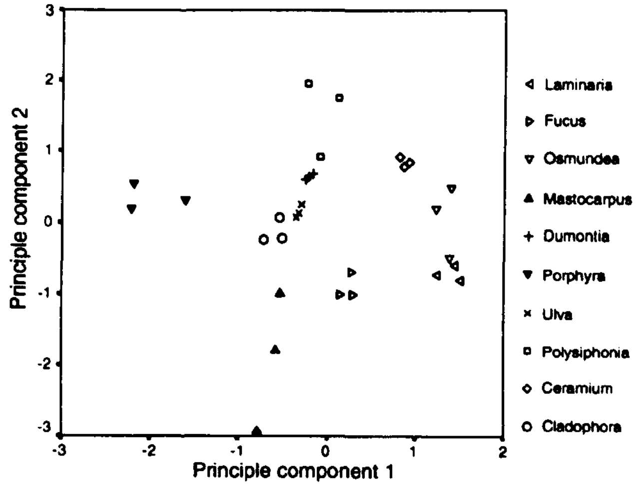

Fig. 2. PCA of transformed nutrient data for 10 algal species, excluding *C. officinalis*. Scores of the first two principle components are plotted. Algal species are used as markers.

Table 4

Eigenvalues and % of total variance for the first three principle components extracted from PCA using nutritional data for 10 algal species (excluding *C. officinalis*)

Component	Eigenvalues	% of Variance	Cumulative %
1	3.260	40.8	40.8
2	1.988	24.9	65.6
3	1.256	15.7	81.3

Table 5

Post extraction communalities and factor loadings of the eight nutrient variables for 10 algal species (excluding *C. officinalis*) for the first three principle components with orthogonal Varimax rotation

Variable	Communalities	Rotated first component	Rotated second component	Rotated third component
Calcium	0.783	0.949		
Ash	0.882	0.880		
Calorific value	0.910	−0.729	0.573	
NDF	0.869		0.915	
Protein	0.810		0.782	−0.465
CF	0.937			0.982
Fat	0.558			
Moisture	0.755			
%Variance		30.6	23.7	16.0

NB only loadings above 0.3 are displayed.

were strongly displaced along the first principle component axis (Fig. 2), which suggests that this species has a different nutritional composition. This displacement is likely to be caused by its relatively low ash content, high calorific value and low protein content (Table 1). Along the second principle component axis, data points for *Polysiphonia* sp. were displaced (Fig. 2), which could be due to its relatively low fat content and high NDF content (Table 1). Data points for *M. stellatus* were also displaced along the second principle component axis (Fig. 2), as reported above for Fig. 1.

Of the species that showed obvious differences in nutritional composition (Figs. 1 and 2), three of them are not currently commercially viable (*C. officinalis, M. stellatus* and *Polysiphonia* sp). The low calorific value and high ash content of *C. officinalis* are likely to be due to the presence of calcium carbonate in the thallus of this species (Steneck & Watling, 1982). In contrast to *C. officinalis, Porphyra* sp., which has a foliose thallus, had a high calorific value and low ash content. These two species illustrate the range of nutritional values for the seaweeds studied. *C. officinalis* had a low protein and high calcium content, whereas *Porphyra* had a high protein and low calcium content (Table 1).

The individual nutritional components of the remaining algal species (both commercial and non-commercial species) generally fell within the range of *Corallina* and *Porphyra*, suggesting there is limited variability between species. It is most likely that it is the relative abundance of a combination of nutritional components for a given species alongside its availability, ease of cultivation and harvesting that results in its commercial value as a food source.

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RESEARCH NOTE

Can juvenile *Fucus* (Phaeophyta) really deter grazing by *Littorina littorea* (Mollusca)?

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G.W. SCOTT AND S. MARSHAM. 2005. Can juvenile *Fucus* (Phaeophyta) really deter grazing by *Littorina littorea* (Mollusca)? *Phycologia* 45: 000–000

Grazing by herbivores has been shown to induce chemical defences in algae. To investigate the possibilities that these chemical defences are a property of the plant or of the water surrounding the plant we assigned *Littorina littorea* to one of four experimental treatments and measured the amount of alga consumed. We also used the results to determine if juvenile *Fucus* sp. can deter grazing by snails. Our results show that previously grazed plants are just as palatable as ungrazed plants and chemical deterrents do not seem to accumulate in the seawater surrounding the plant. We suggest that juvenile *Fucus* sp. plants do not produce chemicals that effectively deter the herbivore.

A number of algal species are known to produce chemical defences that are either constitutive or induced in response to environmental stress and disturbance (Agrawal & Karban 1999). Perhaps one of the most significant disturbances affecting macroalgae is grazing by herbivores, and a number of authors have reported that herbivores can induce the chemical defences of algae (see review by Paul *et al.* 2001). *Fucus* sp. Linnaeus produce a number of chemicals that may inhibit grazing (Van Alstyne 1988; Deal *et al.* 2003; Kubank *et al.* 2004), which have been shown to be induced by grazing *Littorina* (Yates & Peckol 1993; Rohde *et al.* 2004). Despite this the herbivorous gastropod *Littorina littorea* Linnaeus consumes *Fucus* sp. both on the shore and in laboratory experiments (Barker & Chapman 1990).

Investigations by other authors tend to involve either long-term assessment of grazing (measured at intervals of weeks and months (Sotka *et al.* 2002)) or artificially damaged plant tissue to 'mimic' grazing effects (Toth & Pavia 2000, 2002; Hemmi *et al.* 2004), or both (Jormalainen *et al.* 2003; Borell *et al.* 2004). In this study we employ a methodology that we consider to be more biologically relevant, by using intact plants and measuring grazing over a relatively short time scale. Specifically we investigate the possibility that any observed deterrent is a property of the plant or of the surrounding seawater (in this case our presumption would be that deterrent chemicals pass into the seawater from the plant tissue). We also test the prediction that, if juvenile *Fucus* sp. plants do produce inducible defences, they should be able to progressively deter grazing *L. littorea*, i.e. consumption of a particular plant by the snails will decrease over time.

Mature *L. littorea* (mean shell height 2.02 ± 0.01 cm SE) and juvenile *Fucus* sp. plants (6–7 cm long and not yet branched) were collected from Holbeck, North Yorkshire, during April 2004. Juvenile plants were presumed to be more vulnerable to grazing by *L. littorea* than mature plants. Six snails were placed in one of four experimental treatment tanks with 200 ml of filtered seawater (four replicates of each): (1) alga and water unchanged for the duration of the experiment; (2) alga unchanged and water changed every three days; (3) alga and water changed every three days; and (4) alga changed every three days and water unchanged. Individual *Fucus* sp. plants were blotted dry, weighed and one plant was added to each tank. Plants were weighed every three days for a total of 21 days. It was assumed that a decrease in weight of algal material recorded in the experimental tanks equated to the amount of the plant material consumed by the grazing *L. littorea*. Alongside the experimental tanks, two grazer-free control treatments (four replicates of each) were set up to allow for autogenic changes: (5) alga and water changed every three days; and (6) alga and water unchanged for the duration of the experiment. Data were analysed for treatment effect and day-of-measurement effect on snail consumption with repeated-measures ANOVA (treatment and day-of-measurement were both repeated factors) using controls in accordance with Peterson & Renaud (1989). They suggested that rather than use control data as a 'correction factor' to be subtracted from the weight lost in experimental tanks (as in the case of Sousa [1979] and Hay *et al.* [1987]), grazer presence/absence should be a factor in a two-factor ANOVA, to allow interactions among experimental variables to be seen.

Grazing of *Fucus* sp. by *L. littorea* was evident in all of the experimental replicates. In some instances plants having an approximate surface area of 16 cm^2 had several 'holes' of approximately 1.5 cm diameter after just a few days. The amount (weight) of alga consumed by *L. littorea* did not differ between treatments (treatment effect: $F_{3,12} = 1.70$, $P > 0.05$, multivariate eta squared = 0.36). The snails therefore consumed the alga in similar amounts regardless of whether the

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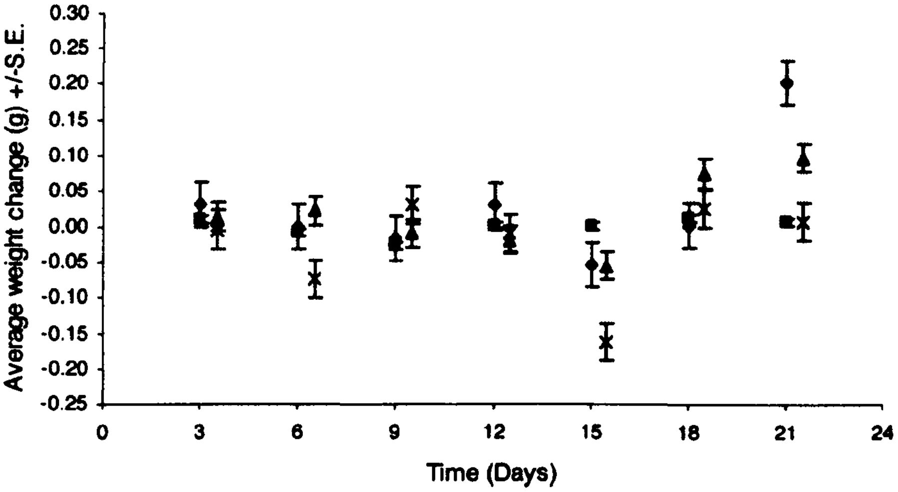

Fig. 1. Average amount of algae consumed by *L. littorea* over 21 days across six treatments (◆ treatment 1, ■ treatment 2, ▲ treatment 3, × treatment 4, ∗ treatment 5, ● treatment 6). NB: treatments 3 and 4 are offset by 0.5 of a day, and 5 and 6 by 1 day, to increase the clarity of the figure.

plants and/or water in experimental tanks had been changed. There was a significant difference in the amount of alga consumed over time (time effect: Wilks' Lambda = 0.217, $F_{6,7}$ = 4.21, $P < 0.05$, multivariate eta squared = 0.78). Consumption by snails in treatments 1 and 3 peaked toward the end of the experiment, while consumption of alga in treatment 2 remained relatively constant throughout the time period (Fig. 1). ?2 The amount of alga consumed by snails in treatment 4 fluctuated over the time period, but began to level out after day 18 (Fig. 1). Algal weight loss across the two control treatments was relatively constant throughout the experiment (Fig. 1). However, the amount of alga consumed in each treatment did not depend upon the day of measurement (day-of-measurement by treatment interaction: Wilks' Lambda = 0.081, $F_{18,20}$ = 1.61, $P > 0.05$, multivariate eta squared = 0.57). Although different amounts of alga were consumed over time, the amounts consumed in each treatment were statistically similar throughout the time period.

Frequent water changes do not appear to alter the level of grazing carried out by *L. littorea*. As snails do not consume greater amounts of plant material when regularly re-supplied with fresh water, or conversely lower amounts when they remain in 'old' water, we suggest that is it unlikely that a grazing deterrent chemical builds up in the water around the plant, especially as in the natural environment accumulation of chemicals would be prevented by water motion. Similarly, regular introduction of new plant material does not result in predictable periodic increases in snail feeding levels (expressed as amount consumed over three days), suggesting that plants previously exposed to grazing are as palatable as newly grazed plants. These findings are surprising given that Pavia & Toth (2000) and Borell *et al.* (2004) have shown that in the case of *Littorina obtusata* Linnaeus and *Ascophyllum nodosum* (Linnaeus) Le Jolis snails are more likely to graze previously ungrazed plants. However Borell *et al.* (2004) also report that in the case of *A. nodosum* young growing tissue has less need for high levels of defence than older parts of the plant. If juvenile *Fucus* sp. plants produce defensive chemicals, there should be a trade-off between production of chemicals and plant growth. Juvenile plants can either allocate resources to producing chemicals that deter grazing by herbivores, or they can concentrate on growth to become mature plants more quickly (Van Alstyne *et al.* 2001). The results of this study suggest to us that *Fucus* sp. juveniles may assign more resources to growth rather than to producing chemical deterrents (although at this stage this hypothesis is highly speculative). Van Alstyne *et al.* (2001) suggest that grazer preference for juvenile algae do not fit predicted patterns (i.e. juvenile plants are not necessarily more palatable than adults) and the palatability of a particular species is dependent upon the herbivore species studies. For *Fucus spiralis* Linnaeus there was no difference in grazer preference or chemical characteristics between adult and juvenile plants; however, juvenile *Fucus gardneri* Linnaeus plants had higher concentrations of defensive chemicals than adult plants (Van Alstyne *et al.* 2001).

Our prediction, that juvenile *Fucus* sp. plants are able to progressively deter grazing by *L. littorea*, is not supported by our data. Snails consume similar amounts of plant material when feeding on the same plant for 21 days or a new plant every three days for 21 days, which suggests that if deterrence does occur it does not have a cumulative effect on the grazer. Our conclusion, based upon these findings, is that either the generalist herbivore *L. littorea* is able to graze young *Fucus* sp. plants in spite of any defensive mechanisms the plants may employ, or juvenile *Fucus* sp. plants do not produce chemical defences against grazing.

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