AN ABSTRACT OF THE THESIS OF

Jennifer L. Burnaford for the Degree of Doctor of Philosophy in Zoology presented on May 15, 2001. Title: Evaluating the Relative Roles of Positive and Negative Interactions in Communities: Shade, Herbivory and Physiological Stress in the Rocky Intertidal Zone.

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Evaluating the relative influences of positive and negative interactions in shaping communities is a major topic in modern ecology. Facilitative interactions between basal species are important in habitats with intense predation pressure or severe abiotic stresses. However, few studies address the potential for positive interactions between trophic levels to influence community structure by altering patterns of predation.

I investigated whether the association between the canopy-forming alga *Hedophyllum sessile* and the herbivorous chiton *Katharina tunicata* was due to the provision of a refuge from predation, a preference for *Hedophyllum* as a food item, or amelioration of abiotic conditions. In a field experiment, *Katharina* were not affected by predation or *Hedophyllum* thalli, but showed a strong behavioral

selection for shaded areas during summertime low tides. By providing shade, *Hedophyllum* controls the distribution of the system's major herbivore.

In a second field experiment I evaluated the relative effects of shade and *Katharina* on the rest of the community. Shade had strong positive effects on a suite of consumers, increasing abundances of seven animal groups relative to unshaded areas. Shade and *Katharina* had quantitatively equal negative effects on the abundance of basal species, but their effects were qualitatively very different. The positive interaction between *Hedophyllum* and *Katharina* affects the entire community by altering patterns of herbivory. Such complex networks of positive, negative, direct and indirect interactions can produce deceptively simple patterns in natural systems.

I used field experiments and laboratory analyses to evaluate potential physiological benefits of this positive interaction on *Katharina*. Levels of heat shock protein 70 isoforms in field populations were greater in summer than in winter, suggesting that *Katharina* are experiencing seasonal sub-lethal stress. Although shade did not affect Hsp70 levels in *Katharina* maintained in field enclosures, amelioration of abiotic stresses through positive biotic interactions could have direct physiological consequences for beneficiary species.

These studies provide strong evidence that positive interactions between trophic levels can profoundly affect the physiology of individuals, the distribution and abundance of populations, and the structure of communities. I present a conceptual model to summarize predictions of the importance of these multi-level positive interactions in structuring communities.

Evaluating the Relative Roles of Positive and Negative Interactions in Communities: Shade, Herbivory and Physiological Stress in the Rocky Intertidal Zone

by

Jennifer L. Burnaford

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Jennifer L. Burnaford, Author

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Evaluating the Relative Roles of Positive and Negative Interactions in Communities: Shade, Herbivory and Physiological Stress in the Low Rocky Intertidal Zone

CHAPTER 1

GENERAL INTRODUCTION

Evaluating the role of positive interactions in shaping the patterns and processes in natural communities has become a major issue in modern ecology. Positive interactions between species are those in which the presence of one species positively affects the growth, survival, or reproduction of another (Bertness and Callaway 1994) and the benefits usually exceed the costs for both organisms (Bronstein 1994). Positive and negative mechanisms may operate simultaneously in any given interaction between individuals, and the overall effect of one species on another can vary along a number of environmental or physiological gradients (Bronstein 1994, Callaway 1997). The net effect of one individual or species on another depends on which mechanisms are most important at a given point along a gradient; and the strength of any positive interaction can therefore change in time and space (Bronstein 1994, Callaway 1997).

Mutualisms are positive interactions in which both partners benefit. This includes both symbiotic mutualisms between physically connected partners, such as those between unicellular algae and anemones or corals, and interactions between free-living species, such as those between plants and their animal

pollinators or seed dispersers. Commensalisms are positive interactions in which one partner benefits while the other is not affected; for example, the indirect commensalism between the chiton *Katharina tunicata* and limpets in the rocky intertidal zone, in which the foraging activity of *Katharina* increases the main food source for limpets, but *Katharina* are not affected by the presence of limpets (Dethier and Duggins 1984). Many mutualisms and commensalisms are trophic interactions, in which one partner directly or indirectly provides a substance with nutritional benefit for the other partner (for example, pollinators often receive nectar from plants). These positive trophic interactions are distinguished from herbivory or predation, both negative interactions, because in the latter the food provider receives no benefit from the interaction; while in the former, the food provider either receives some benefit (e.g. nectar producers receive the benefit of gamete dispersal) or is not affected (e.g. *Katharina* – limpet interaction).

Facilitation and habitat modification are non-trophic positive interactions between free-living species. I define facilitation as a general term describing any non-trophic positive interaction between species: for instance, branched red algae facilitate the establishment of seagrasses on rocky intertidal benches by providing attachment sites for the seagrass seeds (Turner 1983). Habitat modification is a type of facilitation in which one organism (known as the habitat modifier, facilitator, or benefactor) alters the physical or physiological environment for other organisms, therefore increasing their fitness and/or survivorship; for example, by creating refugia from predation and/or environmental stress (Bruno and Bertness 2001). Such non-trophic positive interactions have been found to have important effects in several different environments, such as alpine habitats (Carlsson and Callaghan

1991), cobble beaches (Bruno 2000), deserts (Turner et al. 1969, McAuliffe 1984a), grasslands (McNaughton 1978, Greenlee and Callaway 1996), riparian areas (Levine 2000), rocky intertidal habitats (Bertness and Leonard 1997, Bertness et al. 1999b, Leonard 1999, 2000), semi-arid shrubland (Pugnaire et al. 1996a, 1996b), salt marshes (Bertness and Hacker 1994, Bertness and Yeh 1994, Callaway 1994, Hacker and Bertness 1995b, 1999), sand dunes (Shumway 2000), and temperate woodlands (Callaway et al. 1991, Callaway 1992). In this dissertation, I describe a non-trophic positive interaction between a canopy-forming alga and a major herbivore in the low rocky intertidal zone of the Pacific Northwest, and evaluate the consequences of this interaction to the physiology of the herbivore and the structure of the low intertidal zone community.

POSITIVE INTERACTIONS: PAST AND PRESENT

Historical Background

The idea that facilitative processes between species can structure communities is not new. In his 1893 review article on symbioses and mutualisms, Roscoe Pound cited numerous examples of positive interactions in the 'vegetable kingdom,' including lichens, mycorrhizae, nitrogen fixing bacteria and legumes, and the association between the *Yucca* plant and the moth *Pronuba* (Pound 1893). Throughout the 1900s, numerous publications documented important positive interactions in communities (for a comprehensive list, see Callaway 1995). For example, Shreve (1931) observed an association between shrubs and the Saguaro cactus in the Sonoran Desert, a positive interaction that was later termed

Saguaro cactus in the Sonoran Desert, a positive interaction that was later termed the 'nurse plant effect' (Niering et al. 1963). Dayton (1972, 1975) described the importance of the canopy-forming alga *Hedophyllum sessile* to the assemblage of obligate understory algae species in the Pacific Northwest rocky intertidal zone, introducing the term "foundation species" into our vocabulary. McNaughton (1978) described associational refugia from herbivory in the grasslands of the African Savanna. However, despite such studies, positive interactions were not part of the general conceptual framework of ecological theory for most of the 20th century, as ecologists emphasized the determination of the role of negative interactions in structuring communities.

The shift from early interest and conceptual emphasis on positive interactions (e.g. Pound 1893) to the mid 20th century emphasis on negative interactions has been attributed to both social and scientific factors. One author has proposed that the subject of positive interactions was avoided because it became laden with unpopular left-wing social and political overtones during the early 20th century (Boucher et al. 1982). Other authors have suggested that this shift was primarily caused by an ideological split between 'supporters' of Fredrick Clements and Henry Gleason, two early 20th century plant ecologists (Kingsland 1991, Callaway 1997). Frederic Clements introduced his concept of the plant community as "a complex organism inseparably connected with its climate" in the early 1900s (Clements 1916, 1936). Particularly in his papers on succession in plant communities, Clements presented what is now interpreted as a 'holistic' view, in which the function of species is interconnected, and the distribution or performance of a species depends on the species that surround it (Callaway

1997). A few years later, the ecologist Henry Gleason proposed that plant communities were a coincidence of similar responses to abiotic conditions, or a collection of individual species structured by the fluctuations of the environment at a particular time and place (Gleason 1926). Over time, these two complex bodies of work have been cited as simple opposing arguments "for" and "against" the recognition of the influence of positive interactions in communities (e.g. Kingsland 1991, Callaway 1995, 1997). As criticism for the "Clementian doctrine" of viewing a community as an organism increased, this artificial dichotomy between the acceptance of positive or negative interactions as important in community structure seemed to discourage the consideration of positive interactions in ecological theory, despite the numerous studies which documented that positive interactions were important in communities (Kingsland 1991, Callaway 1995, 1997, Bruno and Bertness 2001).

The founding of the science of modern experimental ecology is largely attributed to the field studies conducted in the early 1960s by scientists such as Joe Connell and Bob Paine (Connell 1961, Paine 1966). These studies profoundly influenced the way that ecologists thought about communities and the forces important in structuring them (Lubchenco and Real 1991), and further focused the attention of ecologists on the community-level effects of negative interactions (competition and predation). This emphasis was fostered by the growing interest in mathematical models of community structure, and the efforts of scientists such as Robert MacArthur to quantify the strength and importance of (negative) species interactions (MacArthur 1972). Mathematical models dismissed mutualism and other positive interactions as destabilizing to communities because positive

feedback on populations tended to produce models with "silly" solutions (May 1981) in which populations increase without bound (for discussion see May 1973, 1981, Pianka 1994). Until the mid 1990s, positive interactions were not included in most conceptual models of community structure (e.g. Menge and Sutherland 1976, 1987, Menge and Olson 1990) nor presented as important community processes in textbooks (e.g. Pianka 1994, Begon et al. 1996, Campbell 1996).

Another factor that is cited as contributing to the 'neglect' of the study of positive interactions is the restrictive vocabulary often used by ecologists to describe species interactions. Historically, the standard presentation of all two-species interactions is a single pair of signs, in which an interaction is described according to the outcome for each species: i.e. predation (+/-), competition (-/-), mutualism (+/+), or commensalism (+/0) (Bronstein 1994, Pianka 1994, Campbell 1996). This presentation carries a number of unstated assumptions. First, it assumes that interactions are static, remaining constant through time and space. However, recent work has shown that most non-trophic positive interactions (i.e. facilitation and habitat modification) are 'conditional' and change over time and space (e.g. Bertness and Shumway 1993, Bertness and Hacker 1994, Bronstein 1994, Bertness and Leonard 1997, Callaway and Walker 1997, Holmgren et al. 1997, Bertness et al. 1999a, 1999b). Thus these static pairwise descriptions of species interactions discourage the recognition of a whole suite of important positive interactions (Bronstein 1994). Second, these traditional definitions equated the effect of an interaction with the mechanism behind it, defining each category as trophic (e.g. +/- = predation) or non-trophic (e.g. -/- = competition) but not both. However, this again precludes the recognition of non-trophic direct and

abundance of competitors of their prey by directly consuming prey species (Paine 1966, Menge 1995). The interaction between the prey competitor and keystone predator is +/-, but is not predation. In recognition of the wide variety of the possible nontrophic +/- and +/0 interactions within and between trophic levels, some authors have proposed re-labeling +/- interactions as 'contramensalisms' (Arthur 1986, Arthur and Mitchell 1989). Although seemingly irrelevant, such restrictive definitions of species interactions can have pervasive influence on ecological thought (Arthur 1986, Arthur and Mitchell 1989, Bronstein 1994).

Modern Conceptual Models

One major factor that set the stage for the revival of interest in positive interactions in the 1980s was the re-assertion of the importance of abiotic factors in determining the outcome of biotic interactions among species (Connell 1975, Menge and Sutherland 1976, 1987, Menge and Olson 1990). These conceptual 'Environmental Stress Models' (ESMs; Menge and Olson 1990) highlighted two important phenomena for the general ecological consciousness. First, biological interactions can change across gradients of abiotic stress. The Menge-Sutherland model predicts that organisms will be limited by environmental stress in harsh environments, by competition in intermediate environments, and by consumers in benign environments (Menge and Sutherland 1976, 1987). Second, abiotic (environmental) stresses can impact different components of the interaction web differently. Menge and Olson (1990) distinguish two types of ESMs: Consumer Stress Models (CSMs), which predict that consumers are more susceptible than prey, and Prey Stress Models (PSMs), which predict that prey are more

susceptible than consumers. By providing testable predictions about the role of abiotic forces in structuring communities, these papers laid the groundwork for subsequent studies that documented the role of positive interactions in high stress areas.

In the late 1980s and early 1990s, the topic of positive interactions finally began to receive more experimental and theoretical attention. In the mid and late 1990s, a series of conceptual models were published which, in a manner similar to the Menge-Sutherland and Menge-Olson models, further pushed the subject of positive interactions into the general ecological consciousness by providing testable predictions about where and when positive interactions should be important structuring forces in communities.

The Bertness - Callaway Model (Bertness and Callaway 1994), the first of these models, is built upon the CSM, and assumes a linear gradient of intense consumer pressure in benign habitats, and intense abiotic stress in harsh habitats. They predict that communities will be structured primarily by positive interactions at either end of this gradient: interactions that either reduce the impact of predation (in benign environments) or ameliorate environmental stresses (in harsh environments). In intermediate environments, they predict, the majority of interactions will be competitive. Later conceptual models have incorporated concepts such as plant size or density (Callaway and Walker 1997), orthogonal stress gradients (Holmgren et al. 1997), and species diversity (Hacker and Gaines 1997) into this basic framework. All of these models address the effects of positive interactions between 'basal species' (plants or sessile animals), which are both benefactor and beneficiary of the positive interaction.

Current context: Facilitation and Habitat Modification

Although the investigation of non-trophic positive interactions in communities has expanded greatly in the last ten years, some important topics remain unexplored. Possibly because of the focus of conceptual models on basal species, the vast majority of studies on non-trophic positive interactions address interactions between plants (e.g. Callaway et al. 1991, Callaway 1992, Bertness and Yeh 1994, Callaway 1995, Callaway et al. 1996, Greenlee and Callaway 1996, Hacker and Bertness 1999, Bruno 2000, Levine 2000) or between algae and sessile invertebrates (Menge 1978b, Leonard 1999, 2000). A much smaller subset of studies addresses positive interactions between consumers (Dethier and Duggins 1984, Martinsen et al. 2000, Van Der Wal et al. 2000). The potential for non-trophic positive interactions between trophic levels, i.e. for positive, non-food effects of plants on consumers, is virtually ignored (for rare exceptions, see Hacker and Bertness 1995a, 1996, Stachowicz and Hay 1996).

Consumer Stress Models, which are the foundation of most models of positive interactions, predict that consumers will be relatively more affected by abiotic stresses than their prey (Menge and Olson 1990). Therefore, predation is predicted to be unimportant in harsh environments because predators either cannot persist in the environment long enough to have a substantial impact on prey populations or because their performance is reduced to such an extent that their impacts, though constant, are weak. Mobile consumers can avoid stress through behavioral adjustments, such as exploiting less stressful microhabitats (Menge 1978a, Levings and Garrity 1983, Garrity 1984, Lasiak and Dye 1986, Huey et al. 1989, Williams and Morritt 1995, Schwarzkopf and Alford 1996) or

altering temporal behavioral patterns to avoid stressful conditions (Garrity 1984, Wehner et al. 1992). Such habitat selection can strongly influence the physiological and ecological performance of animals, particularly of ectotherms (Stevenson 1985, Huey 1991, Jones and Boulding 1999). Obligatory use of refugia in harsh habitats reduces consumer foraging time and can lead to a spatially or temporally limited predator distribution (e.g. Menge 1978a, Suchanek 1979, Garrity and Levings 1981, Levings and Garrity 1983, Witman 1987, Fairweather 1988). Plant canopies influence a number of environmental variables for understory species, including temperature (Bertness et al. 1999b, Leonard 2000), substratum moisture levels (Brawley and Johnson 1991, 1993, Bertness and Shumway 1993, Callaway et al. 1996, Pugnaire et al. 1996a, Bertness et al. 1999b), and soil salt concentrations (Hacker and Bertness 1995b). If small consumers can also benefit from these benign understory conditions, this could result in important consumer effects in areas from which they would normally be excluded. Such non-food interactions between trophic levels must be addressed if we are to fully understand the community-level effects of positive interactions between species.

Habitat Modification: Potential Physiological Effects of Positive Interactions

Improving our understanding of community-level processes requires studies that investigate the links among responses at various levels of organization (Wiens 1989, Lubchenco et al. 1991, Levin 1992, 1994). Positive interactions in which one organism alters abiotic conditions for another organism can have real fitness consequences for the beneficiary species. Evaluating the effects of positive non-food interactions between trophic levels requires investigations of how abiotic stress affects individuals (on both the whole-animal and molecular level), and how these individual-level responses scale up to populations and communities. To evaluate the degree to which consumer performance is improved by these positive interactions, we must use an integrative approach to quantify the physiological effects of positive interactions to consumers and the ecological effects of altered consumer performance on their prey.

In cases where positive interactions involve the amelioration of harsh abiotic conditions, evaluating the benefits of these interactions must start with an assessment of the physiological response to the stressor. One of the most important environmental variables for ectotherms, and one of the most widely relevant (to both aquatic and terrestrial organisms), is temperature. "Whole-animal traits" such as locomotor capacity, feeding success, and rates of growth, reproduction, respiration and digestion can all vary with temperature (Huey 1991). On a more basic level, all organisms depend on proteins for the essential functions that underlie cell maintenance, growth, and reproduction; and because their three-dimensional structures are largely stabilized by noncovalent interactions which are

strongly affected by temperature change, proteins are some of the most thermally sensitive cell components (Somero 1997). Temperature changes can cause protein denaturation, affect binding events between enzymes and their substrates, alter protein solubility, and affect protein charge. While gross unfolding of protein structure (resulting in protein precipitation or total loss of enzymatic activity) generally only occurs at temperatures outside the thermal tolerance range for any individual (i.e. above the upper lethal temperature), minor changes in protein structure that occur well below lethal limits can disrupt cellular function to a degree which is still biologically significant (Somero 1997).

The heat-shock response (HSR), in which the expression of a small set of genes coding for heat shock proteins (Hsps) is activated in response to stressors, is one mechanism to increase tolerance to physiological stress (Pigliucci 1996). Hsps limit cellular damage by binding and stabilizing denatured proteins until they can either be refolded or degraded (Feder and Hofmann 1999, Fink 1999, Wickner et al. 1999). It seems, therefore, that organisms in variable thermal environments should show frequent and intense expression of Hsps. However, the HSR carries its own costs. Hsps can interact improperly with properly folded proteins, and increased expression of Hsps has been shown to significantly reduce rates of cell growth and division (Feder et al. 1992, Wickner et al. 1999). The cumulative energy investment required for the HSR (transcription of chaperone mRNA, translation and production of chaperone proteins, provision of ATP for proteases, and repair, replacement and recycling of damaged proteins) could be substantial for animals experiencing chronic heat stress. In laboratory populations of *Drosophila*, repeated exposure to sub-lethal thermally stressful conditions

decreases survivorship (Krebs and Feder 1998). Therefore positive interactions that result in the provision of more benign habitats could have tractable benefits on a molecular and cellular level. The next logical step in the study of positive interactions is to undertake integrative studies to simultaneously investigate the physiological and ecological effects of facilitative interactions in communities.

DISSERTATION RESEARCH

Few studies to date have examined the potential for habitat modifying basal species to alter abiotic conditions for consumer species, and therefore we know very little about how these inter-trophic level interactions can affect community structure. In this dissertation, I present evidence of a strong non-trophic positive interaction between trophic levels that affects both the physiology of the beneficiary species and the structure of the community as a whole. Several previous studies have examined the community-level effects of the canopy-forming alga *Hedophyllum sessile* and the herbivorous chiton *Katharina tunicata* in the low rocky intertidal zone of the Pacific Northwest (Dayton 1975, Dethier and Duggins 1984, Duggins and Dethier 1985). As a result, this can be considered a model system for the investigation of the effects of inter-trophic level interactions on community structure.

In Chapter 2, I examine the potential for plant species to modify the environment for mobile consumer species by investigating the mechanism behind the association between *Hedophyllum sessile* (hereafter, *Hedophyllum*) and *Katharina tunicata* (hereafter, *Katharina*). Earlier studies documented that the abundance of *Katharina* dropped precipitously following the removal of the

Hedophyllum canopy (Dayton 1975, Dethier and Duggins 1984, Duggins and Dethier 1985). I tested three hypotheses regarding the association between *Katharina* and *Hedophyllum*: 1) the chiton gains protection from bird predation from the algal canopy, 2) the chiton requires *Hedophyllum* as a food source, and 3) the chiton requires the shade provided by the *Hedophyllum* canopy as a refuge from the physiological stresses that accompany emersion during daytime low tides in the spring and summer. My results stand out as a rare example of a non-trophic positive interaction between trophic levels in which the plant modifies the abiotic environment for the consumer.

In Chapter 3, I examine the community-level effects of this inter-trophic level positive interaction by investigating the relative importance of shade and *Katharina* herbivory on the composition of the understory assemblage. By manipulating both chiton abundance and the presence or absence of shade, I evaluated the strength and sign of many positive and negative direct and indirect interactions that are important structuring forces in the absence of either *Hedophyllum* or *Katharina*, but that are obscured by the examination of the net interaction results when both are present. These results indicate that the positive interaction between *Hedophyllum* and *Katharina* affects the entire understory assemblage.

In Chapter 4, I examine the physiological benefits of this inter-trophic level positive interaction by investigating the effects of season and shade on production of one family of heat-shock proteins in field populations of *Katharina*. Using a combination of monthly population sampling and experimental enclosure of chitons in different microhabitats, I quantified seasonal variation in Hsp70 levels that

indicate that these chitons experience sub-lethal physiological stress at the molecular level during the warm summer months. Although I did not find evidence of substantial stress reduction underneath artificial shades, these results suggest that the amelioration of abiotic stresses by algal canopies can have direct physiological consequences for understory species.

Taken together, the results of these studies provide strong evidence that non-trophic positive interactions between trophic levels can have profound effects on community structure. In Chapter 5, I present a conceptual model that incorporates such multi-level positive interactions into existing conceptual models of the roles of positive interactions and environmental stresses in communities. As the studies presented here show, incorporating higher trophic levels into the theoretical and practical consideration of positive interactions is crucial if we are to understand how these processes work at the level of the entire community.

CHAPTER 2

POSITIVE INTERACTIONS BETWEEN TROPHIC LEVELS: THE IMPORTANCE OF SHADE TO COMMUNITY STRUCTURE

ABSTRACT

In addition to their role as food for herbivores, canopy-forming plants can positively affect other species by providing protection from predation or stressful abiotic conditions. Such positive interactions are common between plant species in habitats with heavy consumer pressure or harsh abiotic conditions. However, positive interactions are rarely examined between trophic levels, and are often not considered in benign habitats. In the low rocky intertidal zone of the Pacific Northwest, the chiton *Katharina tunicata* is closely associated with the canopy-forming alga *Hedophyllum sessile*. In a 27-month field experiment, I demonstrated that this association is due to selection of shaded microhabitats by *Katharina*, and that neither predation nor *Hedophyllum per se* influenced the distribution of the chiton. This association is a rare example of a positive interaction between trophic levels, in which a plant affects the distribution of a major herbivore through modification of the abiotic environment. Despite the traditional classification of the low intertidal zone as a 'low-stress' habitat, the strength of the positive interaction was correlated with environmental variables, becoming more positive in warm summers and more neutral in a cool summer. The shade provided by the *Hedophyllum* canopy affected the entire community, influencing the distribution

and abundance of other mobile animals and of the understory algal and encrusting invertebrate assemblage. These results give new dimension to the classification of *Hedophyllum* as a 'foundation species.' The shade provided by the *Hedophyllum* canopy is critical for the continued maintenance of the understory community in this system.

INTRODUCTION

Plant canopies play important roles in structuring communities through habitat modification. Canopies can negatively affect understory species directly by altering the quantity and quality of light reaching the understory (Reed and Foster 1984, Kennelly 1989, Carson and Root 2000), and in marine systems, by increasing rates of sedimentation (Eckman et al. 1989), altering flow patterns (Duggins et al. 1990) or by "whiplash" effects caused by algal fronds sweeping the substratum (Black 1974, Dayton 1975, Menge 1976, Velimirov and Griffiths 1979, Leonard 1999, 2000). Canopies can also positively affect understory plant species by providing protection from herbivory (Atsatt and O'Dowd 1976, McAuliffe 1986, Rousset and Lepart 2000) and ameliorating stressful abiotic conditions (Brawley and Johnson 1991, Callaway 1995, Callaway et al. 1996, Bertness and Leonard 1997, Holmgren et al. 1997, Raffaele and Veblen 1998, Bertness et al. 1999b). Such non-food positive interactions are thought to have major structuring influences on communities (Bertness and Callaway 1994) but have only recently been widely addressed by experimental ecologists.

Positive Interactions in Communities

Positive, non-food interactions have been found to have important effects in several different environments, such as alpine habitats (Carlsson and Callaghan 1991), cobble beaches (Bruno 2000), deserts (Niering et al. 1963, McAuliffe 1984a), grasslands (Greenlee and Callaway 1996), riparian areas (Levine 2000), rocky intertidal habitats (Bertness and Leonard 1997, Bertness et al. 1999b, Leonard 2000), semi-arid shrubland (Pugnaire et al. 1996a, 1996b), salt marshes (Bertness and Hacker 1994, Bertness and Yeh 1994, Callaway 1994, Hacker and Bertness 1995b, 1999), and sand dunes (Shumway 2000). The vast majority of studies on positive interactions address interactions within the same level: herbivores on herbivores (Dethier and Duggins 1984, Martinsen et al. 2000, Van Der Wal et al. 2000), plants on plants (e.g. Callaway et al. 1991, 1996 Callaway 1992, 1995, Bertness and Yeh 1994, Greenlee and Callaway 1996, Hacker and Bertness 1999, Bruno 2000, Levine 2000) or (in marine systems) plants on sessile animals (Bertness and Leonard 1997, Leonard 1999, 2000). However, in order to understand the role of positive interactions in structuring communities, we must address their effects on the entire community. Surprisingly, research on the effects of positive non-food interactions between plants and consumers is very rare, despite the fact that those few studies that have addressed the topic have found positive interactions that affect many different species.

Higher trophic levels can benefit indirectly from positive interactions among plants or directly from the positive effects of plant habitat modification. Positive interactions between New England salt marsh plants were found to have indirect positive effects on an aphid herbivore by extending the tidal range of their host

plant and providing refugia from their ladybird beetle predators, which were only attracted to the healthy (non-facilitated) host plants (Hacker and Bertness 1995a, 1996). Plants have also been found to have direct non-food positive effects on consumers both in terms of reducing predation and ameliorating stressful abiotic conditions. Numerous studies have documented that small consumers can receive protection from predation through association with plants that are either structurally complex (Stachowicz and Hay 1996) or chemically defended (Hay et al. 1989, Hay et al. 1990a, Hay et al. 1990b, Duffy and Hay 1991, 1994). Fewer studies have been concerned with the beneficial physiological effects of plant canopies on small consumers. In the New England rocky intertidal zone, feeding rates of the predatory whelk *Nucella lapillus* were higher under algal canopies due to the amelioration of desiccation by the canopy (Menge 1978a) and predation pressure on understory barnacles was more intense underneath the canopy due to the combination of higher feeding rates and predator microhabitat selection (Menge 1978a, b, Leonard 2000). Despite the frequent mention of patterns suggesting that the amelioration of physiological stresses by algal canopies can affect the distribution of mobile consumers (e.g. McCook and Chapman 1991, Bertness et al. 1999b, Leonard 2000), and theoretical recognition of the importance of evaluating the effects of physiological and physical stresses on both predators and prey (Menge and Olson 1990), most studies have viewed canopy effects on mobile consumers as merely nuisance effects which obscure the pattern of facilitation on understory plant / sessile animal species (e.g. Callaway 1992, Leonard 1999, 2000, Leonard et al. 1999). This lack of attention to higher trophic levels limits our understanding of the community-level effects of positive

interactions between species. I used field manipulations in a model system to examine the relative importance of shade, protection from predation, and food provided for a mobile consumer by a canopy-forming alga, in order to assess the importance of positive interactions between trophic levels on the consumer population and on the community as a whole.

System and Hypotheses

On moderately exposed rocky shores in the Pacific Northwest, the perennial kelp *Hedophyllum sessile* (hereafter *Hedophyllum*) forms continuous canopies over extensive areas of the low rocky intertidal zone. Several previous studies in this system have documented that removal of the *Hedophyllum* canopy was immediately followed by the dramatic reduction in density of the system's major herbivore, the chiton *Katharina tunicata* (hereafter, *Katharina*) (Dayton 1975, Dethier and Duggins 1984, Duggins and Dethier 1985, Markel and DeWreede 1998). In 1998 I established a field experiment to determine whether this positive effect of *Hedophyllum* on *Katharina* abundance was due to a trophic (i.e. the provision of food) or non-trophic (i.e. facilitation or habitat modification) interaction. I designed the experiment to evaluate three hypotheses:

(1) *Predation hypothesis (non-trophic positive interaction)*: The *Hedophyllum* canopy protects *Katharina* from visual predators; *Katharina* numbers are low in *Hedophyllum* removal areas because they suffer heavy predation from birds due to their increased apparency following canopy removal. Seagulls (*Larus* spp.) are predators of *Katharina* (Dethier and Duggins 1984, Irons et al. 1986, Wootton 1997, J. Burnaford, personal observation). Bird predation can have major

impacts on intertidal community dynamics and structure (Mercurio et al. 1985, Marsh 1986a, b, Lindberg et al. 1987, Wootton 1992, 1995, Wootton 1997, Hamilton 2000). Patchy predation by gulls and oystercatchers on intertidal limpets can affect microhabitat partitioning among species in the field (Mercurio et al. 1985, Hahn and Denny 1989).

(2) *Food hypothesis (trophic / consumption interaction)*: *Hedophyllum* serves as an important food source for *Katharina*, and the chiton leaves canopy removal areas in search of this food item. *Katharina* are reported to prefer *Hedophyllum* over other macroalgae (Himmelman and Carefoot 1975) although they do consume other macroalgae, microalgae, and encrusting invertebrate species (Chapter 3, Padilla 1981, Dethier and Duggins 1984, 1988, Gaines 1985, Piercy 1987).

(3) *Stress hypothesis (non-trophic positive interaction)*: The *Hedophyllum* canopy modifies some abiotic variable for *Katharina*, and *Katharina* numbers are low in *Hedophyllum* removal areas because the chitons either die from physiological stress or actively seek shaded areas as refugia. *Katharina* habitat ranges from approximately +0.5m to -1.0m mean lower low water (or MLLW); this zone is exposed only on the lowest low tides. Based on a classification system that designates habitats as 'stressful' or 'benign' by the physiological conditions relative to other habitats, the low intertidal zone has been considered to be a low-stress habitat, because low-tide emersion periods are relatively short compared to the more stressful high intertidal zone (Menge and Sutherland 1976, 1987, Bertness et al. 1999b, Menge 2000). Because conceptual models predict that positive interactions are only important in harsh environments (Bertness and

Callaway 1994), the general assumption is that physiological stress and positive interactions are not important for determining community structure in the low intertidal zone (Bertness and Leonard 1997, Bertness et al. 1999b). However, in the San Juan Islands, Washington State (the site of the three previous *Hedophyllum / Katharina* studies) low tides occur during mid-day in the summer, and organisms in the low intertidal zone could experience levels of thermal stress which, although not as high as for animals in the higher intertidal, are still considerable.

Because the *Hedophyllum* canopy is a dominant feature in this zone, and *Katharina* is the major grazer in the system, I also examined the effect of canopy removal on the understory algal and invertebrate assemblage. Specifically, I addressed three questions. (1) After canopy removal, does the prevention of bird predation affect the algal understory and the assemblage of mobile invertebrates (other than *Katharina*)? (2) After canopy removal, does the provision of shade affect the algal understory and mobile invertebrate assemblage? (3) If *Katharina* abundances are positively affected by the provision of refugia (either from bird predation or thermal stress), does this cause a "halo effect" such that grazing pressure is concentrated in the area immediately surrounding the refuge? Grazing halos around predator-exclusion areas (Witman 1987) and thermal refugia (Levings and Garrity 1983) have been documented in temperate and tropical marine communities. By affecting the distribution and foraging patterns of the major herbivore, the *Hedophyllum* canopy could exert significant indirect effects on the low intertidal zone both underneath the canopy and in adjacent non-canopy areas. Indirect effects are predicted to be major structuring forces in communities

(Wootton 1991, 1992, 1993, 1994, Menge 1995). Incorporating analysis of direct and indirect effects into studies on positive interactions is crucial if we are to advance our understanding of community and ecosystem dynamics.

METHODS

Study Site

The study site, Pile Point, is located on the west side of San Juan Island, Washington, at 48°28.9' N, 123°05.7' W, 6 km south of Lime Kiln State Park and 15 kilometers east of Vancouver Island, British Columbia, across Haro Strait. Pile Point is a rocky point with several flat rocky benches separated by shallow subtidal channels, flanked on both sides by small cobble beaches and steep rocky cliffs.

The low zone at Pile Point is dominated by the perennial kelp *Hedophyllum*, with occasional large tidepools containing high cover of the surfgrass *Phyllospadix scouleri* or dense beds of the purple urchin *Strongylocentrotus purpuratus*. Large mobile invertebrates in the low intertidal zone include the chiton *Katharina*, which are very abundant (up to 70 / m^2), the gum-boot chiton *Cryptochiton stelleri*, and the seastar *Pisaster ochraceus*. *Katharina* individuals are primarily found underneath the *Hedophyllum* canopy, but occasionally individuals are found in unshaded areas during low tide.

The tidal cycle in the San Juan Islands, as in the rest of the Pacific Northwest, is mixed semi-diurnal. From March to September, low low tides (those in which the low intertidal zone is exposed) occur during daylight hours, from 7am to 4pm. From October to February, low low tides occur at night, from 10pm to

4am. The low intertidal zone is uncovered from 30 min to ≥ 5 hours during a low tide, depending on nearshore weather conditions and pressure systems that affect actual tide height and wave height. Calm ocean conditions often contribute to long periods of exposure for low intertidal organisms in the spring and summer.

Experimental Design: Katharina *abundance*

In April 1998 I marked 25 1m by 1m plots in the *Hedophyllum* band of the low intertidal zone with stainless steel screws and washers at each of the four corners. Each plot had greater than 70% canopy cover of *Hedophyllum*, did not contain any channels, vertical walls or outcrops, and was centered between approximately –0.5 and +0.5 m tidal height. Plots were at least 1.5 meters apart and 0.7m from the edge of the *Hedophyllum* bed. Plots were assigned to 5 blocks (5 plots per block) based on location (3 blocks were located on isolated rocky benches, 2 blocks were spread along the main rocky point).

Within a block, I randomly assigned each plot to one of five treatments, marked with colored plastic markers at the four corners. In one plot per block, the natural *Hedophyllum* canopy was retained as a 'natural control.' In the other four treatments, I removed all *Hedophyllum* thalli by prying the holdfasts off the rock using a sharp knife. One of these '*-Hedophyllum*' treatments was maintained as a 'negative control' and is designated the 'No Structure' treatment.

For the remaining three '*-Hedophyllum*' treatments, I used experimental structures to establish various treatment combinations of Bird access (+B or -B) and Shade (+S or -S). In all cases, a 'structure' refers to a rectangular vinyl coated wire letterbasket (Cascade Office Supplies) measuring approximately 34 x

29 x 7cm with a 4 x 2.5cm mesh 'roof' and 6 x 3.5 cm mesh on the sides. Letterbaskets were turned upside down and bolted to the rock surface using stainless steel screws and washers at all four corners. I cut 'windows' (8cm wide x 6cm tall) on each of the 4 sides, which allowed chitons to move freely under and out of structures. Cut wire surfaces were sealed with silicon sealant to prevent rusting.

In the Bird Exclosure treatment, the roof of the exclosure (the bottom of the upturned letterbasket), prevents bird predation on benthic organisms by preventing birds from reaching the rock surface with their beaks (Wootton 1992). To control for effects of structure installation or materials, I established a 'Structure Control' treatment, in which I removed the exclosure roofs so that only the sides remained. Birds foraged freely inside these control structures (J. Burnaford, personal observation). To create an 'Artificial Shade' treatment, I installed structures as for the Bird Exclosure treatment, and to the roof of each structure I attached 3 pieces of black vexar mesh, 39x34 cm (Norplex, Inc; ¼" mesh size) with cable-ties. For all three treatments, five structures were arranged within the 1m by 1m plot in an approximate "X" pattern (Fig 2.1).

These five treatment combinations allowed me to test the effects of specific factors by pair-wise treatment contrasts. Treatments are coded by the presence or absence of *Hedophyllum* (+ or –H), Bird access (+ or –B) and Shade (+ or –S).

1) Effects of structure installation or materials: No Structure (-H, +B, -S (NS)) vs. Structure Control (-H, +B, -S (SC)).
2) Effects of bird predation: Structure Controls (-H, +B, -S (SC)) vs. Bird Exclosure (-H, -B, -S).

3) Effects of shade: Bird Exclosure (-H, -B, -S) vs. Artificial Shade (-H, -B, +S).

4) Effects of the *Hedophyllum* thallus: Artificial Shade (-H, -B, +S) vs. *Hedophyllum* (+H (-B, +S)).

Although this does not represent all combinations of these three factors (H, B, S), these treatments were sufficient to address the hypotheses. Limited access time to the low intertidal, combined with the physical properties of *Hedophyllum* and the intertidal zone made other combinations impractical or impossible. For instance, because the natural *Hedophyllum* canopy is opaque, it has the inherent properties of obscuring the chitons from birds (-B) and providing shade (+S); because these could not be manipulated independently from the canopy, (+H, -S was not a feasible combination) this treatment is coded as +H (-B, +S). Due to the water motion and log bashing in the intertidal zone, it was not possible to construct artificial shades large enough to allow bird access (+B, +S treatments). Treatments that combined *Hedophyllum* and experimental structures (e.g. +H, +S) did not address the hypotheses, and the added installation and survey time would have made the experiment logistically impossible.

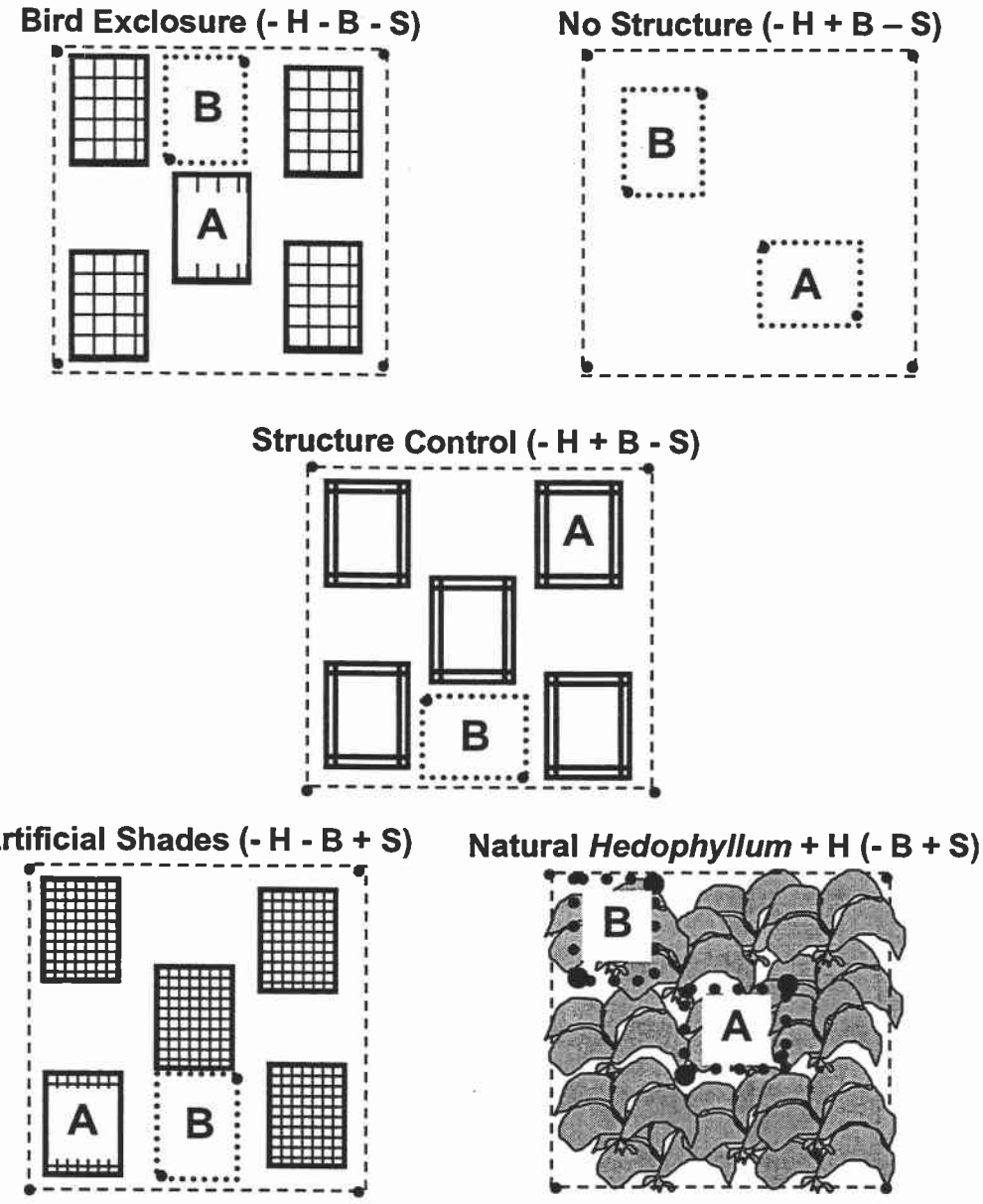

Figure 2.1 Design of experimental plots. Plots are 1m by 1m (dotted lines represent unmarked plot perimeters). Plots were assigned to one of five treatments with various combinations of *Hedophyllum* (present = + H, removed = - H); bird access (accessible = + B, not accessible = - B); and shade (shaded = + S, unshaded = -S). Bird Exclosures = wire mesh roofs. Structure Controls = 'roofs' removed (sides only). Artificial Shade structures = roofs + mesh. Two permanent understory survey plots were marked within each 1m by 1m plot by stainless steel screws at two corners. A = 'Under Experimental Structure' understory survey plots (or the comparison plot from No Structure and Natural *Hedophyllum* Plots); B = plots adjacent to experimental structures.

I established experimental treatments by manipulating plots on the day after taking initial *Katharina* counts and 'baseline' survey data (Time 0 data). Each block was set-up over two or three consecutive days of a low tide series. All *Hedophyllum* regrowth (which was very limited) or new *Hedophyllum* recruits were recorded and removed in the *–Hedophyllum* treatments over the course of the experiment. For all *Hedophyllum* removal plots, *Hedophyllum* thalli were removed from a 50cm area around the perimeter of the plots to prevent edge effects such as overgrowth.

Structures were left in the field from March or April through December, but were removed in December because increased wave forces and log bashing caused severe structure loss and damage. Since low tides occur at night (10pm to 5am) from October through March, neither bird predation nor heat stress was thought to be significant during the period of structure removal. Structures were replaced by the end of the second daylight tide series in the spring (usually in March).

Experimental Design: Understory Assemblage

Because time limitations also made detailed surveys of community structure in the 1m by 1m experimental plots impossible, I established smaller survey plots (34cm by 29cm) within each experimental plot. In the three experimental structure treatments (Structure Control, Bird Exclosure and Artificial Shades) I randomly selected one of the five structures as a survey plot 'under experimental structures,' and randomly located another plot 'adjacent to experimental structures' which I marked with two stainless steel screws at opposite

corners (Fig 2.1). For the *Hedophyllum* and No Structure treatments, I randomly selected locations for two survey plots on a 1m grid and randomly assigned these as controls for comparisons with plots under or adjacent to experimental structures (Fig 2.1).

On the day before I removed the *Hedophyllum* canopy from the experimental plot, I surveyed the abundance of all algae and animal species in these survey plots using a 34cm by 29cm quadrat divided into 25 equal squares. I visually estimated percent cover of all algae and encrusting invertebrates to species, counted the abundance of all mobile animals (invertebrates and fish) and identified all organisms to genus or species.

Data Collection: Katharina *Abundance*

Each month (with the exception of occasions in the fall and winter when I was not able to access plots due to weather conditions) I counted all *Katharina* individuals within the 1m by 1m plot, using a 1m by 1m quadrat divided into 100 squares. *Katharina* in the Natural *Hedophyllum* plots were recorded as being under the canopy (underneath a *Hedophyllum* blade) or in the open. *Katharina* in the experimental structure treatments were recorded as being under the experimental structures or being outside the experimental structures (but still within the 1m by 1m plot). An animal was recorded as being 'under' a structure if >1/2 its body length was inside the structure. In summer 1998, all *Katharina* counted at each survey were individually marked using numbered plastic bee tags (color coded by treatment; Christian Graze KG™) affixed with nail glue (Sally Hansen™). During each monthly survey I also recorded the presence and location

(inside or outside structures) of any individuals of the seastar *Pisaster ochraceus* in my experimental plots. In 1998 and 2000, I measured the length (in mm) of all *Katharina* in experimental plots (1998 = in 2 of 5, 2000 = all 5 blocks).

Data Collection: Understory Assemblage

I re-surveyed the community Survey Plots in May, July, and November 1998, April, May and July 1999, and May and July 2000. I could not conduct surveys in Fall 1999 because of adverse weather conditions. Both survey plots within any $1m^2$ plot were surveyed on the same day, and all plots within a block were surveyed within two consecutive days.

Data Collection: Katharina *Mortality*

The seastar *Pisaster ochraceus* (hereafter *Pisaster*) is a known predator of *Katharina* (Mauzey 1965, 1967, Paine 1966, Menge and Menge 1974). I quantified the diet of actively feeding *Pisaster* at Pile Point on 24 dates from May 1998 through August 2000. After carefully removing *Pisaster* individuals from the rock surface with a screwdriver, I recorded the identity of all prey species being consumed. On each date I examined all *Pisaster* in the low intertidal zone along the accessible rocky benches at Pile Point, and examined *Katharina* prey items for the presence of experimental tags. *Pisaster* digest the foot and internal organs of *Katharina*; the mantle and articulated plates are always still intact after feeding, and therefore tags were readily visible on prey items.

On two occasions in 1999, I conducted site-wide surveys for *Katharina* carcasses over consecutive days of a tide series. On the first day of a tide series I removed all dead *Katharina* from my study site, and I collected dead *Katharina* on each subsequent day as a rough estimate of maximum daily mortality. I assigned each carcass to one of three 'cause of death' categories, based on observations of active seastar and bird predation events: seastar predation (foot and internal organs gone, mantle and articulated valves still intact), bird predation (puncture wounds in foot, varying degrees of foot tissue and internal organs removed or regurgitated pellets on rock), and unknown (no visible signs of predation).

Data collection: Environmental Measurements

Ambient temperatures were recorded every 15 or 30 min by data-loggers (Optic StowAway, Onset Computer Corporation) attached in the low intertidal zone around Pile Point. Data-loggers were secured to stainless steel mesh frames that were bolted to the rocky substratum. Throughout the experiment I experienced a high rate of data-logger failure; temperatures were recorded by one to seven data-loggers at any time. Data-loggers were deployed both in the open and underneath experimental shades for the duration of this study. Data-loggers were deployed underneath *Hedophyllum* thalli only during selected low tide periods, as the abrasion by mesh frames ripped *Hedophyllum* blades during high tides, potentially biasing subsequent low tide measurements.

By combining temperature data with predicted tidal heights for Haro Strait, (Tides & Currents, Nautical Software) I extracted Low Tide Temperatures for each data-logger (temperatures recorded while the data-logger was emersed). Low tide

temperatures reported here are averages of all data-loggers that were active at any one time. I calculated summary statistics to compare temperatures across years. Daily maximum low tide temperature is calculated by extracting the maximum temperature value for each day (averaged across all active data-loggers to get a composite daily maximum). Average daily maximum low tide temperature is the average of this composite value for each calendar month.

I recorded maximum wave force from February to August 2000 by deploying five wave force dynamometers (Bell & Denny 1994) around Pile Point (one in each experimental block). Dynamometers were read and reset every 24 hours during each low tide series.

Statistical Analysis

All statistical analyses were performed using SAS version 8.0 and JMP Version 4 (SAS Institute, Inc). Repeated measures and MANOVA analyses were programmed using Proc GLM in SAS. For all analyses, model fit and heterogeneity of variances were examined by visually inspecting residual by predicted value plots, residual normal QQ plots, and plots of variance between groups. Data were transformed to meet model assumptions if required. For percent cover data, the logarithmic transformation corrected problems of heterogeneity of variance and was selected over the arcsine square-root transformation because the latter requires values < 100%, and due to canopy layering, many plots had cover values > 100%. For counts of mobile animals, both logarithmic and square-root transformations were used. Mahalanobis distances

were calculated to identify multivariate outliers within groups for multivariate analyses, but none were detected.

I identified *a priori* groups of animal and algal species for analysis using a combination of ecological, taxonomic and life history traits (Table 2.1, Table 2.2). I analyzed algae and encrusting invertebrates (AEI) separately from mobile animals. Encrusting invertebrates were analyzed with algae because they can occupy continuous areas of rock surface and thus compete with algae for primary space, they must disperse into new areas in ways ecologically similar to algae, and they are consumed by *Katharina* (Chapter 3).

Some groups were not included in statistical analyses. Cover of crustose algae could not be reliably quantified over time because macroalgal holdfasts and microalgae obscured crusts. Egg masses, sessile worms, and filter feeders were present in very low numbers and could not be logically assigned to either the AEI or mobile animal group.

Data from the start of the experiment (Time 0) were analyzed separately to assess the null hypothesis of no difference between plots before treatments were established. All models were fit with treatment and block as main effects (fixed and random, respectively). There were significant differences between blocks at Time 0 that persisted throughout the experiment; information on block effects is presented in tables but, as they are not of interest, will not be discussed. In all analyses, if the effect of treatment was significant, I tested specific hypotheses with four linear contrasts. Contrasts are labeled in tables as follows:

1) **Structure Effect** = –H +B – S (NS) vs. –H +B –S (SC) = No Structure vs. Structure Controls. There were no detectable effects of experimental

structure installation or materials on any response variable. Results for this contrast are presented in tables but will not be discussed further.

2) **Bird Effect** = –H +B –S (SC) vs. –H –B –S = Structure Control vs. Bird Exclosures

3) **Shade Effect** = –H –B –S vs. –H –B +S = Bird Exclosures vs. Artificial Shades

4) ***Hedophyllum* thallus effect** = –H –B +S vs. + H (-B +S) = Artificial Shades vs. *Hedophyllum*

Because these contrasts were decided *a priori* and low replication provided limited power to detect treatment differences, I did not make adjustments to the α level for contrasts. However, when univariate comparisons were used to examine seasonal treatment differences, I used the Bonferroni adjustment to the α level to evaluate significant treatment effects before performing contrasts. For repeated measures analyses that did not meet Mauchly's criterion for homogeneity of variances, both multivariate and univariate repeated measures statistics were examined. If the interpretations did not differ and Mauchly's criterion was nearly met ($p \geq 0.01$) I present univariate statistics with Huynh-Feldt adjusted probabilities. If there were serious violations of Mauchly's criterion, I present multivariate statistics even if the multivariate and univariate interpretations did not differ.

Table 2.1 Animal groups. Species are listed in order of relative abundances in plots. **Groups included in mobile animal totals and multivariate analysis. *Groups included in mobile animal totals. Unmarked groups were not included in statistical analyses. See text for details.

Mobile Animal Group	Species
Chitons **	*Lepidochitona dentiens*
	Mopalia ciliata
	Mopalia lignosa
	Mopalia mucosa
	Tonicella lineata
	Lepidozona mertensii
	Cryptochiton stelleri
Snails **	*Calliostoma ligatum*
	Margarites pupillus
	Granulina margaritula
	Lacuna vincta
	Lacuna variegata
Whelks **	*Nucella lamellosa*
	Searlesia dira
	Amphissa columbiana
	Amphissa versicolor
	Bittium eschrichtii
	Nucella emarginata
	Nucella canaliculata
	Ocenebra lurida
	Ocenebra interfossa
	Fusitriton oregonensis
Seastars **	*Leptasterias hexactis*
	Henricia sp. (newly described)
	Henricia leviuscula
Crabs **	*Pugettia gracilis*
	Pagurus hirsutiusculus
	Pagurus samuelis
	Pagarus granosimanus
	Cancer oregonensis
	Cancer productus
	Hemigrapsus nudus
	Hemigrapsus oregonensis
	Petrolisthes cinctipes
	Petrolisthes eriomerus
	Pugettia producta
	Cryptolithodes sitchensis

Table 2.1 Continued

Limpets ** (includes one non-limpet species)	*Lottia pelta* *Tectura scutum* *Acmaea mitra* *Diadora aspera* *Onchidella borealis*
Anemones *	*Anthopleura elegantissima* *Epiactis spp.* *Urticina spp.*
Fishes *	*Oligocottus maculosus* *Gobiesox maeandricus* *Artedius lateralis* *Pholis spp.*
Mobile Worms * (annelids, nemerteans, flatworms)	*Nereis spp.* *Amphiporus spp.* *Tubulanus spp.* *Notoplana acticola*
Nudibranchs *	*Hermissenda crassicornis* *Archidoris montereyensis* *Dirona albolineata* *Dendronotus spp.* *Rostanga pulchra*
Urchins *	*Strongylocentrotus droebachiensis* *Strongylocentrotus purpuratus*
Cucumbers / Detritivores *	*Pseudocnus lubrica* *Cucumaria miniata* various sipunculans
Isopods *	*Idotea wosnesenskii* unknown isopod
Filter Feeders	*Crepidula adunca* *Modiolus modiolus* various tunicate species
Sessile Worms	*Dodecaceria fewkesi* *Serpula vermicularis* *Spirorbis spp.* *Schizobranchia spp.*
Egg Masses	*Nucella lamellosa* *Archidoris montereyensis* *Rostanga pulchra* *Hermissenda crassicornis* unknown egg masses

Table 2.2 Algal and encrusting invertebrate groups. Species are listed in order of relative abundances in plots. **Groups included in multivariate analysis. *Groups included in AEI totals but not in multivariate analyses. Unmarked groups were not included in statistical analysis. See text for details.

Algal / Encrusting Invertebrate Group	Species
Green Algae **	*Ulva fenestrata* *Acrosiphonia mertensii* *Codium fragile*
Microalgae **	Diatoms (several species) *Navicula sp.* Cyanobacteria
Finely Branched Red Algae **	*Microcladia borealis* *Polysiphonia hendryi* (several subspecies) *Callithamnion pikeanum* *Scagelia sp.* *Ceramium spp.*
Foliose / Thickly Branched Red Algae **	*Halosaccion glandiforme* *Mastocarpus papillatus* *Cryptopleura lobulifera* *Callophyllis sp.* *Mazzaella splendens* *Mazzaella flaccida* *Delesseria decipiens* *Odonthalia floccosa*
Articulated Coralline Algae **	*Corallina vancouveriensis* *Bossiella plumosa* *Corallina officinalis* *Calliarthron tuberculosum*
Encrusting Invertebrates **	*Halichondria sp.* (at least 2 species) *Schizoporella sp.* unknown hydroid species
Fleshy Brown Algae *	*Alaria marginata* *Egregia menziesii* *Fucus gardneri* *Laminaria setchellii* *Costaria costata* *Nereocystis luetkeana* *Desmarestia ligulata* *Desmarestia viridis*

Table 2.2 Continued

Rare Algae *	*Leathesia difformis* *Colpomenia sp.* *Mesophyllum conchatum*
Crustose Algae	Coralline crusts: *Pseudolithophyllum sp.* *Lithophyllum sp.* *Lithothamnion sp.* *Ralfsia sp.* *Mastocarpus papillatus (Petrocelis)*

RESULTS

Time Zero

At the start of the experiment, there were no detectable differences in *Katharina* abundance or understory assemblage characteristics between plots assigned to different treatments. *Katharina* abundances were almost identical between 1m by 1m plots (Table 2.3a, Treatment effect). There were no detectable differences in total animal abundance, species richness or the composition of the animal assemblage between plots assigned to different treatments (Treatment effect) or between survey plots assigned to 'under experimental structures' and 'adjacent to experimental structures' (Plot (treatment) effect; Table 2.3b). Similarly, there were no detectable differences in total percent cover of algae and encrusting invertebrates ("AEI"), AEI species richness, or the composition of the AEI assemblage (Table 2.3c).

Table 2.3 Analysis of experimental plots before manipulation (Time 0). **(A)** ANOVA on square-root transformed counts of *Katharina* in $1m^2$ plots. **(B)** Analysis of mobile animals in survey plots (under and adjacent to experimental structures) nested within treatment; all data are log-transformed; univariate ANOVAs on total abundance and total number of species, MANOVA analysis on 6 animal groups (seastars, snails, whelks, limpets, chitons, crabs). **(C)** Analysis of algae and encrusting invertebrates in survey plots, details as for Table B; MANOVA groups = green algae, brown fleshy algae, finely branched red algae, foliose red algae, microalgae, articulated coralline algae, and encrusting invertebrates.

A. *Katharina* Abundance

Source	Df	MS	F	P
Treatment	4	0.003	0.008	0.9999
Block	4	1.758	3.514	0.0306
Error	16	0.500		

B. Understory Assemblage: Mobile Animals

Source	Df	MS	F	P
Univariate Analysis: Total Abundance				
Treatment	4	0.29	0.80	0.5331
Plot (Treatment)	5	0.18	0.48	0.7909
Block	4	3.72	10.13	< 0.0001
Error	36	0.37		
Univariate Analysis: Species Richness				
Treatment	4	0.11	0.45	0.7744
Plot (Treatment)	5	0.13	0.57	0.7262
Block	4	7.06	7.42	0.0002
Error	36	0.24		

Multivariate Analysis	Ndf	Ddf	Wilks λ	F	P
Treatment	24	109	0.47	1.10	0.3538
Plot (Treatment)	30	126	0.50	0.80	0.7562
Block	24	109	0.14	3.47	< 0.0001

C. Understory Assemblage: Algae and Encrusting Invertebrates

Source	Df	MS	F	P
Univariate Analysis: Total Abundance				
Treatment	4	0.71	0.96	0.4400
Plot (Treatment)	5	0.30	0.41	0.8413
Block	4	0.71	0.96	0.4440
Error	36	0.74		
Univariate Analysis: Species Richness				
Treatment	4	0.07	0.90	0.4754
Plot (Treatment)	5	0.14	1.76	0.1458
Block	4	0.07	0.91	0.4699
Error	36	0.08		

Table 2.3c Continued

Multivariate Analysis	**Ndf**	**Ddf**	**Wilks λ**	**F**	**P**
Treatment	28	109	0.40	1.24	0.1052
Plot (Treatment)	28	128	0.45	0.78	0.8124
Block	28	109	0.23	1.98	0.0068

Katharina *Abundance: Effect of Predation*

Predation did not explain the patterns of *Katharina* abundance. Despite the fact that birds (gulls) were observed to prey upon *Katharina* at Pile Point (J. Burnaford, personal observation), excluding birds from experimental plots had no effect on *Katharina* abundances (Fig 2.2; Table 2.4; bird and Time x bird effects). *Pisaster,* the primary non-bird predator of *Katharina,* were more abundant in +Shade than –Shade plots (total number of *Pisaster* observed in experimental treatments: +Shade = 55, -Shade = 9). *Pisaster* were more frequently observed under Artificial Shades than Bird Exclosure structures (n = 46 and 0 observations, respectively).

Katharina *Abundance: Effect of the* Hedophyllum *Thallus*

The *Hedophyllum* thallus did not affect patterns of *Katharina* abundance either. *Katharina* abundances in Artificial Shade plots (– H + S) plots were not different from *Hedophyllum* plots overall (Fig 2.2; Table 2.4, *Hedophyllum* thallus effect). *Katharina* abundances in Artificial Shade plots were equal to those in *Hedophyllum* plots throughout 1998 and 1999, and were higher in Artificial Shade

plots than *Hedophyllum* plots in 2000 (Fig. 2.2; Table 2.4, Time x *Hedophyllum* thallus effect p = 0.03).

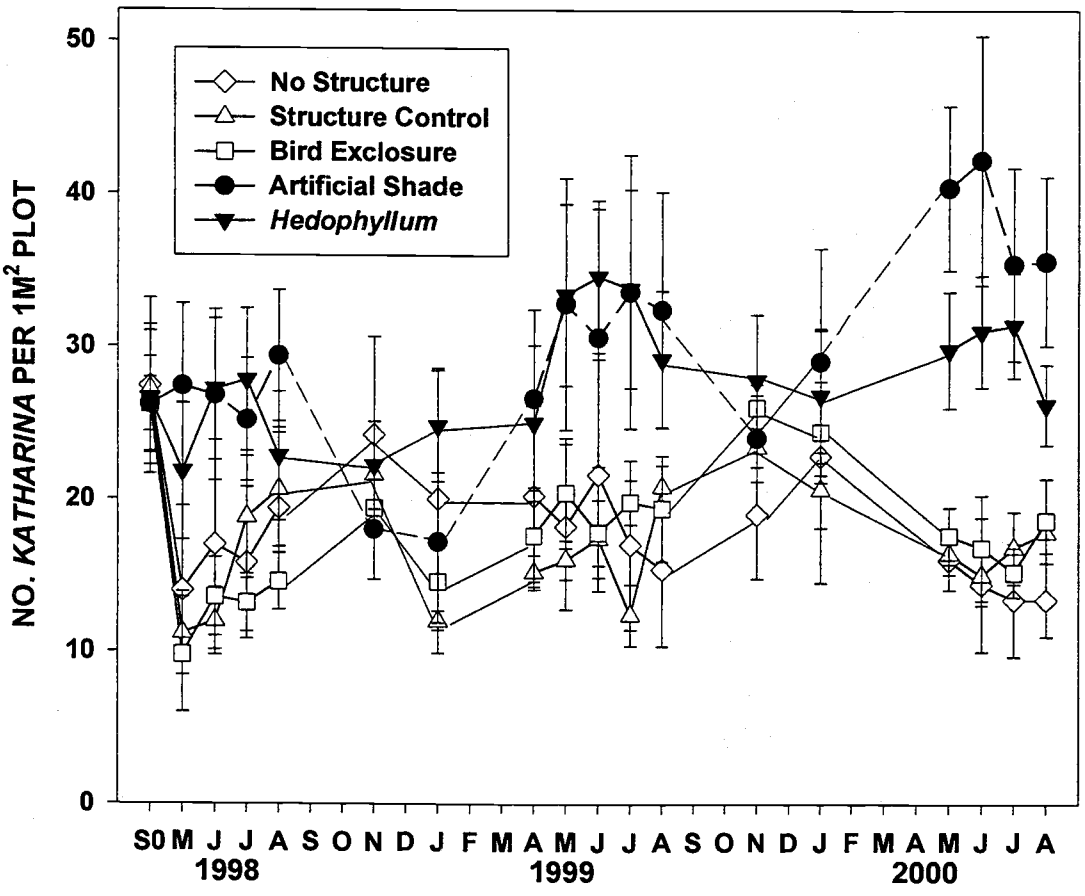

Figure 2.2 Effect of treatment on *Katharina* abundance. Mean (±SEM) counts of *Katharina* individuals in $1m^2$ plots (n = 5 / treatment in 5 blocks) April 1998 (Survey 0) through August 2000. Treatments are coded with various combinations of *Hedophyllum* (H), Bird Access (B), and Shade (S). No Structure and Structure Control = -H +B –S; Bird Exclosure = -H –B –S; Artificial Shade = -H –B +S; and *Hedophyllum* = +H (-B +S). See Figure 2.1 and text for details.

Table 2.4 Repeated measures analysis of the effect of birds, shade, and *Hedophyllum* on total *Katharina* abundance per 1m by 1m plot, 1998 – 2000. Abundances (number *Katharina* / m^2 plot) were compared using RM ANOVA over five periods: Summer 1998 (average of abundances in May, June, July and August), November 1998, Summer 1999, November 1999 and Summer 2000. Data were square-root transformed prior to analysis. Mauchly's criterion for homogeneity of variance-covariance matrices (or 'sphericity' – necessary to validate the univariate approach to repeated-measures ANOVA) was nearly met ($p = 0.048$) so p-values from univariate analyses are presented with Huynh-Feldt adjusted probabilities. Hypotheses were tested using a series of *a priori* contrasts (see text for details). Bold-faced p-values indicate time or treatment significance (or very near significance) at the $\alpha = 0.05$ level.

Source	Df	MS	F	P
Between Subjects (average effect over time)				
Treatment	4	6.772	3.34	**0.0362**
Block	4	10.386	5.12	0.0075
Error	4	2.030		
contrasts				
1. – H + B – S (NS) vs. – H + B – S (SC) (structure effect)	1	0.473	0.23	0.6358
2. – H + B – S (SC) vs. – H – B – S **(bird effect)**	1	0.003	0.00	0.9688
3. – H – B – S vs. – H – B + S **(shade effect)**	1	9.080	4.47	**0.0505**
4. – H – B + S vs. + H(– B + S) **(*Hedophyllum* thallus effect)**	1	0.005	0.00	0.9603
Within Subjects (change in effect over time)				
Time	4	1.259	6.07	**0.0003**
Time x Treatment	16	1.324	6.38	**< 0.0001**
Time x Block	16	0.308	1.48	0.1344
Error	64	0.207		
contrasts				
Time x 1 (structure effect)	4	0.206	1.00	0.4168
Time x 2 **(bird effect)**	4	0.204	0.99	0.4220
Time x 3 **(shade effect)**	4	2.702	13.02	**< 0.0001**
Time x 4 **(*Hedophyllum* thallus effect)**	4	0.584	2.81	**0.0324**

Katharina *Abundance: Effect of Shade*

In contrast to the lack of predation or *Hedophyllum* thallus effects, shade increased *Katharina* abundances, but only in the spring and summer (Fig 2.2; Table 2.4, Time x shade effect $p < 0.0001$). *Katharina* abundances were up to 49 individuals (per $1m^2$ plot) higher in Artificial Shade plots than in Bird Exclosure

plots in the summer; average between-treatment differences from May through August were 14.4 (1998), 13.0 (1999) and 21.4 (2000). In the fall, however, *Katharina* abundances were equal to or lower in Artificial Shade plots than in Bird Exclosure plots; average between-treatment differences in November were –1.4 (1998) and –2.0 (1999). Removal of Artificial Shades in December did not affect *Katharina* abundance, which did not differ between November and January in either year (paired t-tests; 1998/1999, $t = -0.37$, $p = 0.73$; 1999/2000, $t = 0.58$, $p = 0.59$).

The shade effect can be more effectively evaluated by comparing the number of chitons found directly underneath the five experimental structures (as opposed to the total number in the 1m x 1m plot; see Fig 2.1 for design details). This analysis suggested that the effects of shade changed not only with season, but also between years (Fig 2.3a, Table 2.5; Time x shade effect $p < 0.0001$). Shade greatly increased *Katharina* abundances under experimental structures in summers 1998 and 2000 (Table 2.6a,e; shade effect), but not in summer 1999 (Table 2.6c, shade effect).

This lack of treatment effect in 1999 may be due to between-year differences in ambient temperature. 1999 had lower average daily temperatures and fewer warm days than either 1998 or 2000, indicating that temperatures were consistently cooler on several different time scales (Fig 2.3b, Table 2.7a, b).

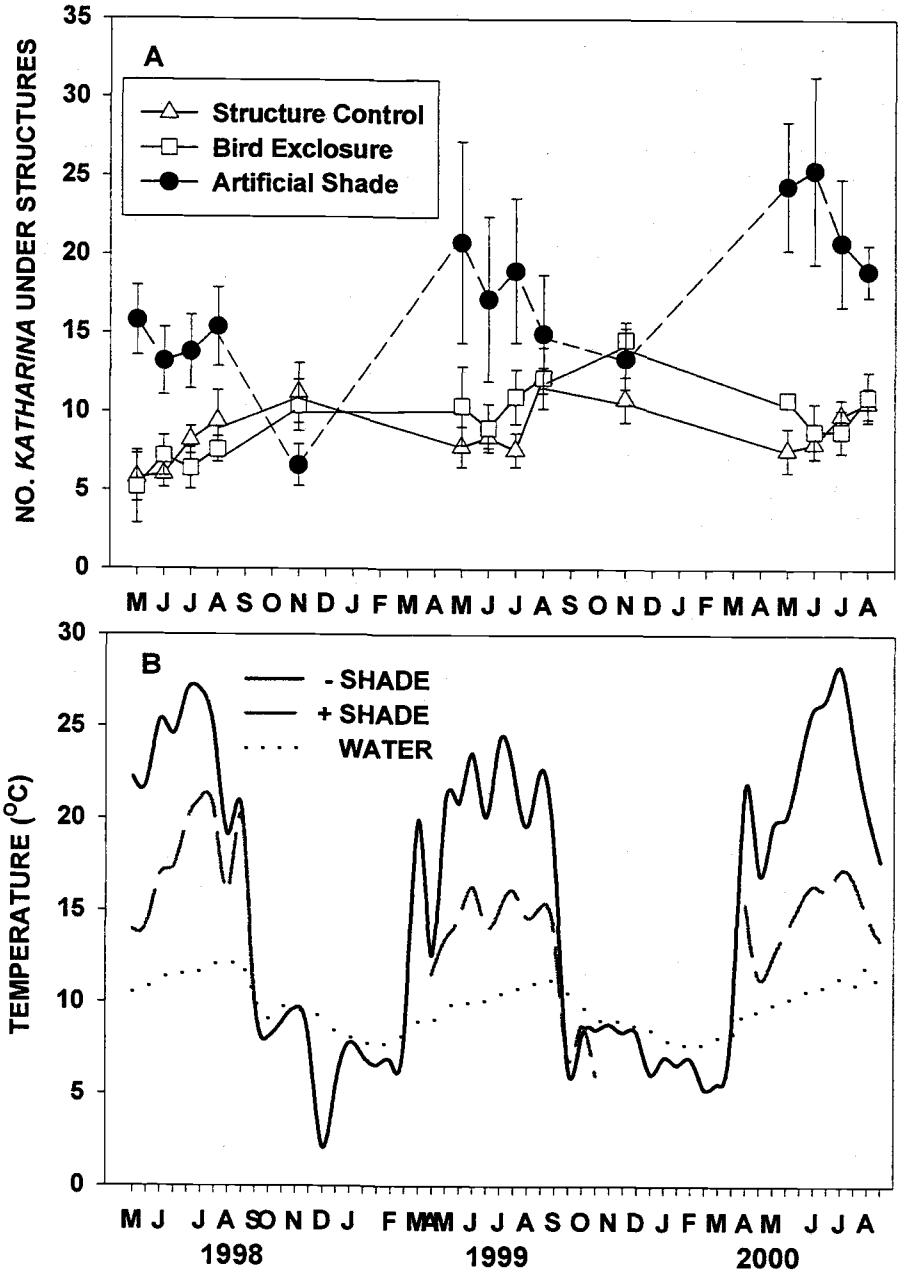

Figure 2.3 Effect of shade, birds, and ambient temperature on *Katharina* abundance under experimental structures. **(A)** Mean (±SEM, n = 5 blocks) counts of *Katharina* under 5 structures per 1m^2 plot, May 1998 through August 2000. Structure Control = -H +B –S; Bird Exclosure = -H –B –S; Artificial Shade = -H –B +S. See Figure 2.1 and text for details. **(B)** Mean daily maximum low tide temperature for each low tide series. Temperature data were recorded every 15 min with submersible data-loggers: -Shade and +Shade = low tide temperatures in respective treatments; Water = water temperature at high tide.

Table 2.5 Repeated measures analysis of the effect of birds and shade on *Katharina* abundances under experimental structures. RM ANOVA on total abundance under experimental structures (5 structures / m^2 plot) in Summer 1998, November 1998, Summer 1999, November 1999 and Summer 2000 (details in Table 2.3). Data were square-root transformed prior to analysis. Mauchly's criterion was nearly met ($p = 0.027$) so p-values from univariate tests are presented with Huynh-Feldt adjusted probabilities. The two experimental contrasts are numbered in accordance with equivalent contrasts in other tables. Bold-faced p-values indicate significance at the $\alpha = 0.05$ level.

Source	Df	MS	F	P
Between Subjects (average effect over time)				
Treatment	2	3.315	4.85	**0.0418**
Block	4	2.322	3.39	0.0664
Error	8	0.684		
contrasts				
3. – H + B – S (SC) vs. – H – B – S **(bird effect)**	1	0.212	0.31	0.5932
4. – H – B – S vs. – H – B + S **(shade effect)**	1	3.857	5.64	**0.0449**
Within Subjects (change in effect over time)				
Time	4	1.327	6.92	**0.0004**
Time x Treatment	8	1.555	8.11	**< 0.0001**
Time x Block	16	0.131	0.68	0.7875
Error	32	0.192		
contrasts				
Time x 3 **(bird effect)**	4	0.209	1.09	0.3781
Time x 4 **(shade effect)**	4	2.250	11.73	**< 0.0001**

Table 2.6 Univariate analysis of *Katharina* abundance under experimental structures in summer and fall. Analyses are 2-factor ANOVAs on squareroot-transformed data. **(A)** Summer 1998 **(B)** November 1998 **(C)** Summer 1999 **(D)** November 1999. **(E)** Summer 2000. Bold-faced p-values indicate significance at the $\alpha = 0.01$ level (adjusted for multiple comparisons).

A. Summer 1998

Source	Df	MS	F	P
Treatment	2	2.338	8.86	**0.0094**
Block	4	0.295	1.12	0.4128
Error	8	0.264		
contrasts				
3. – H + B – S (SC) vs. – H – B – S **(bird effect)**	1	0.069	0.26	0.6629
4. – H – B – S vs. – H – B + S **(shade effect)**	1	3.961	15.01	**0.0047**

B. November 1998

Source	Df	MS	F	P
Treatment	2	0.875	2.80	0.1197
Block	4	0.483	1.55	0.2778
Error	8	0.312		
contrasts				
3. – H + B – S (SC) vs. – H – B – S **(bird effect)**	1	0.029	0.09	0.7687
4. – H – B – S vs. – H – B + S **(shade effect)**	1	1.105	3.54	0.0968

C. Summer 1999

Source	Df	MS	F	P
Treatment	2	1.581	3.06	0.1033
Block	4	0.990	1.91	0.2018
Error	8	0.517		
contrasts				
3. – H + B – S (SC) vs. – H – B – S **(bird effect)**	1	0.145	0.28	0.6106
4. – H – B – S vs. – H – B + S **(shade effect)**	1	1.726	3.34	0.1052

D. November 1999

Source	Df	MS	F	P
Treatment	2	0.392	2.08	0.1879
Block	4	0.373	1.97	0.1918
Error	8	0.189		
contrasts				
3. – H + B – S (SC) vs. – H – B – S **(bird effect)**	1	0.762	4.03	0.0796
4. – H – B – S vs. – H – B + S **(shade effect)**	1	0.093	0.49	0.5025

E. Summer 2000

Source	Df	MS	F	P
Treatment	2	4.349	25.80	**0.0003**
Block	4	0.707	4.20	0.0403
Error	8	0.169		
contrasts				
3. – H + B – S (SC) vs. – H – B – S **(bird effect)**	1	0.043	0.26	0.6269
4. – H – B – S vs. – H – B + S **(shade effect)**	1	5.973	35.44	**0.0003**

Table 2.7 Summary statistics of low-tide temperature conditions at Pile Point, 1998 to 2000. **(A)** Mean of daily temperature maxima for each month **(B)** Total number of days per month on which the temperature exceeded the mean daily maximum temperature for 1999, the coolest year (i.e. for May = number of days on which temperatures exceeded 17.98°C). See text for details.

A. Mean Daily Maximum Temperature (°C)

	1998	1999	2000
May	22.64	17.98	19.59
June	24.44	21.74	23.99
July	26.19	23.16	26.83
August	22.30	20.79	20.84

B. Total Number of Days Exceeding 1999 Mean Daily Maximum Temperature

	1998	1999	2000
May	13	12	12
June	14	11	17
July	15	13	16
August	11	9	11

Effect of Shade and Experimental Structures on Abiotic Conditions

Temperatures were substantially lower in the shade than in unshaded areas during low tides in spring and summer (Fig 2.3b). Artificial shades closely approximated the temperature effects of the natural *Hedophyllum* canopy; temperatures were less than 1°C different between these two treatments (Chapter 4). Temperatures recorded by submersible data-loggers are highly correlated with *Katharina* body temperatures ($r^2 = 0.79$, $p < 0.0001$) and therefore seem representative of the actual thermal experience of the chiton. The cooling effect of shade increased with increasing ambient (-Shade) temperature, reducing temperatures, on average, 6.6 degrees at ambient temperatures 20-25°C, 10.41 degrees from 25-30°C, and 13.33 degrees above 30°C (Chapter 4).

Although the experimental structures undoubtedly altered water flow, there is no evidence that they provided *Katharina* with refugia from wave action. Wave action was greatest during the fall and winter (personal observation) yet *Katharina* abundances in Artificial Shade plots were not different between November and January, despite the removal of shades in December. Wave force in the summer at Pile Point is low; on many days, the water simply rises and falls with no lapping motion (J. Burnaford, personal observation). The shear force required to dislodge an adult (53mm) *Katharina* from the substratum is 102 N (Irons et al. 1986). Mean maximum wave force in summer 2000, the time of greatest treatment differences, only exceeded 15 N on one occasion (Fig 2.4). The absolute maximum wave forces recorded in 2000 were: May = 6.7 N, June = 19.9 N, July = 13.3 N, August = 9.8 N. These data suggest that wave forces high enough to dislodge *Katharina* from the substratum are unlikely at Pile Point. Although logs do occasionally strike the substratum and could cause *Katharina* mortality or dislodgement, there is no evidence that artificial shades provided protection from this stress. Log bashing is much worse in winter, and, as noted, *Katharina* abundances did not differ between November and January of either year.

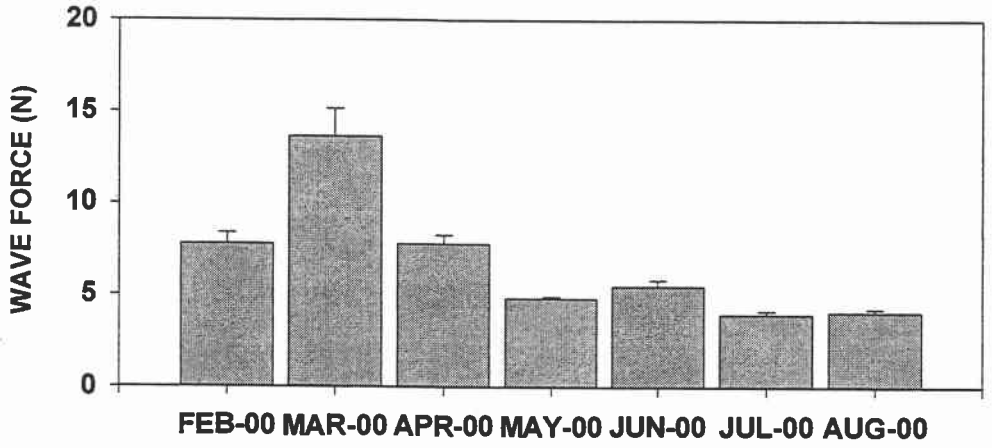

Figure 2.4 Maximum wave force at Pile Point, 2000. Average (± SEM, n = 5 dynamometers) maximum wave force measured from February through August. Dynamometers, placed in the approximate center of each of the five treatment blocks, were read and reset daily. For days on which dynamometer springs registered no movement, I conservatively estimated wave force as the minimum force required to move the spring (Feb–April = 5.84N, April-May = 5.29N, June – August = 3.17N).

Katharina *Size Distribution*

There was no overall pattern of *Katharina* size distribution with respect to experimental treatment or the presence or absence of shade (Fig 2.5). Although mean *Katharina* size differed between treatments in 1998 and 2000, (1998, $p = 0.02$; 2000, $p < 0.0001$) differences were not consistent between years. There was a weak trend for animals in the shade to be larger than animals in unshaded areas (Fig 2.5b, d), but this trend was only evident in 1 of 2 blocks in 1998 and 3 of 5 blocks in 2000, and is therefore not considered a general pattern.

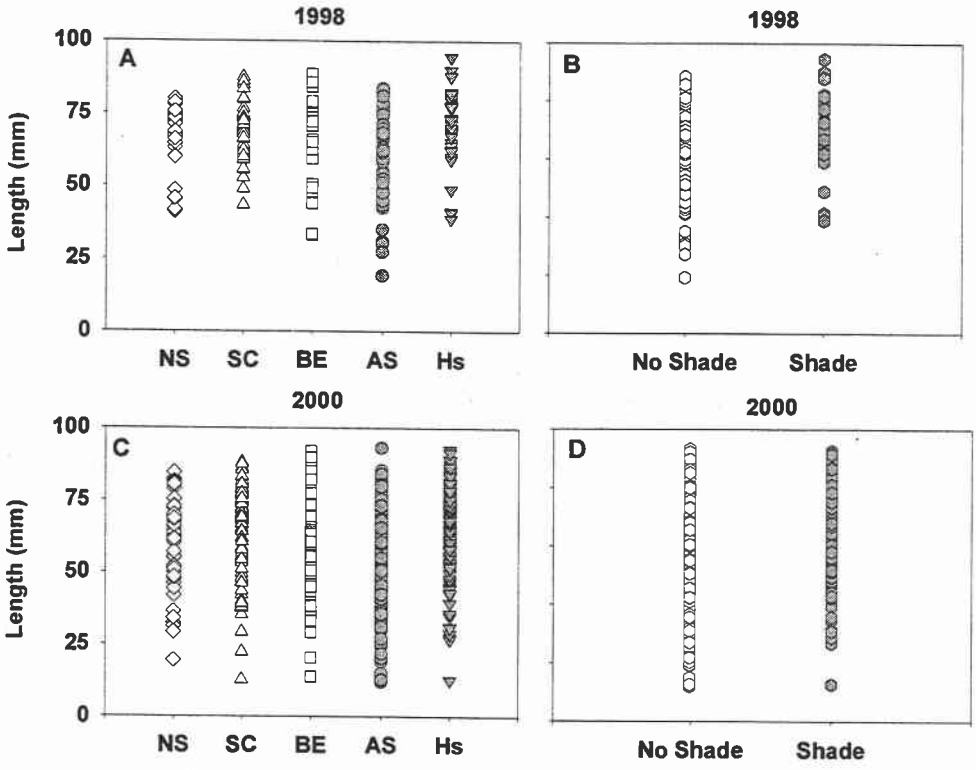

Figure 2.5 *Katharina* size distribution by experimental treatment. Length (in mm) of individual *Katharina* in experimental plots in August 1998 (2 blocks) and June 2000 (5 blocks). **(A)** Experimental treatments, 1998. NS = No Structure, SC = Structure Control, BE = Bird Exclosure, AS = Artificial Shade, Hs = *Hedophyllum* **(B)** –Shade (NS + SC + BE) vs. +Shade (AS + Hs). **(C)** Experimental treatments, 2000 **(D)** –Shade vs. +Shade. See text for details.

Understory Assemblage Under Experimental Structures: Mobile Animals

I evaluated the response of the animal assemblage by examining three statistics: total abundance (of all individuals of all groups), the total number of animal species and the abundance of animals within six major functional groups. In addition to examining individual responses of functional groups, I assessed the composition of the assemblage by examining the patterns of abundance within and

between these six groups in the experimental treatments for selected surveys using MANOVA.

Bird predation did not affect any aspect of the animal assemblage. No differences were detected between +B and –B plots in total number of mobile animals (Fig 2.6; Table 2.8; bird effect), the number of animal species in July 2000 (Fig 2.7a, contrast 2, $p = 0.64$), the abundances of individual animal groups (Fig 2.8a – f, compare BE to SC), or the composition of the animal assemblage (Table 2.10, bird effect).

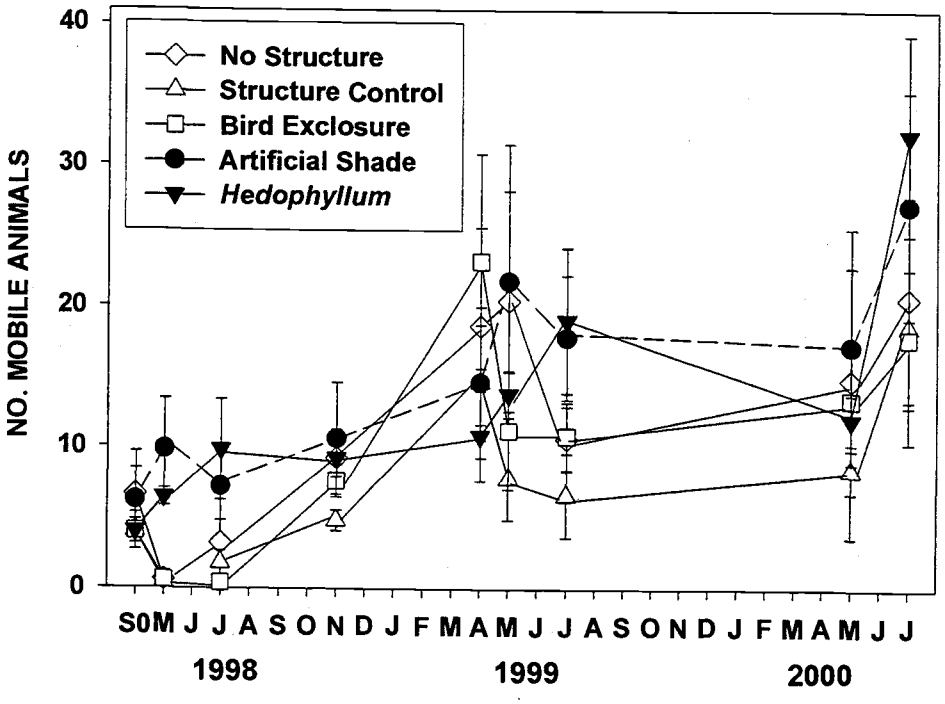

Figure 2.6 Effect of treatment on total mobile animal abundance under experimental structures. Mean (±SEM, n = 5) counts of all mobile animals in experimental survey plots, April 1998 (Survey 0) through July 2000. No Structure and Structure Control = -H +B –S; Bird Exclosure = -H –B –S; Artificial Shade = -H –B +S; and *Hedophyllum* = +H (-B +S). See Fig 2.1 for design details.

Table 2.8 Repeated measures analysis of total mobile animal abundance under experimental structures. See Fig 2.1 for design details. Data are log-transformed counts for May, July, and November 1998, April, May, and July 1999, and May and July 2000. Mauchly's criterion was met (p = 0.31) so univariate analyses are presented with un-adjusted p-values. Bold-faced p-values for treatment effects indicate significance at $\alpha = 0.05$ level.

Under Experimental Structures

Source	Df	MS	F	P
Between Subjects (average effect over time)				
Treatment	4	5.28	5.35	**0.0063**
Block	4	4.86	4.92	0.0088
Error	16	0.99		
contrasts				
1. –H +B –S (NS) vs.–H +B –S (SC) (structure effect)	1	1.86	1.88	0.1889
2. –H +B –S (SC) vs. –H –B –S **(bird effect)**	1	1.24	1.26	0.2784
3. –H –B –S vs. –H –B +S **(shade effect)**	1	6.29	6.37	**0.0226**
4. –H –B +S vs.+H(–B +S) **(*Hedophyllum* thallus effect)**	1	0.001	0.00	0.9706
Within Subjects (change in effect over time)				
Time	7	11.20	32.01	< 0.0001
Time x Treatment	28	0.77	2.21	**0.0019**
Time x Block	28	0.62	1.76	0.0201
Error (Time)	112	0.35		
contrasts				
Time x 1 (structure effect)	7	0.11	0.33	0.9410
Time x 2 **(bird effect)**	7	0.42	1.19	0.3123
Time x 3 **(shade effect)**	7	1.57	4.48	**0.0002**
Time x 4 **(*Hedophyllum* thallus effect)**	7	0.06	0.17	0.9907

The *Hedophyllum* thallus had only weak effects on the animal assemblage. No differences were detected between *Hedophyllum* and Artificial Shade plots in total animal abundance (Fig 2.6, Table 2.8, *Hedophyllum* thallus effect) or in the number of animal species in July 2000 (Fig 2.7a, contrast 4, p = 0.53). The abundance of crabs, however, was consistently higher in *Hedophyllum* plots than in Artificial Shade plots (Fig 2.8b), while seastars and snails differed in the opposite direction (Fig 2.8a, d). These overall group differences were largest in July 1999, the only survey in which there was a detectable effect of the

Hedophyllum thallus on the overall composition of the assemblage (Table 2.10, *Hedophyllum* thallus effect, $p = 0.006$ for July 1999; all other seasons, $p > 0.12$).

Previous researchers had observed that limpet abundances were negatively correlated with the abundance of *Hedophyllum* (Dethier and Duggins 1984, Duggins and Dethier 1985). A similar relationship was detected in this study, but the effect was very weak (Fig 2.8f; comparison of limpet abundances in *Hedophyllum* vs. all four non-*Hedophyllum* treatments, May 1998 – July 2000, RMANOVA, Between subjects, $p = 0.07$). Limpet abundance was not related to the abundance of non-*Hedophyllum* algae; percent cover macroalgae in –Shade plots only explained 25% of the variance in limpet abundance (linear regression) and a polynomial fit only improved the R^2 value slightly (to 0.33). Limpet abundances also were not correlated with microalgal abundance ($R^2 = 0.14$).

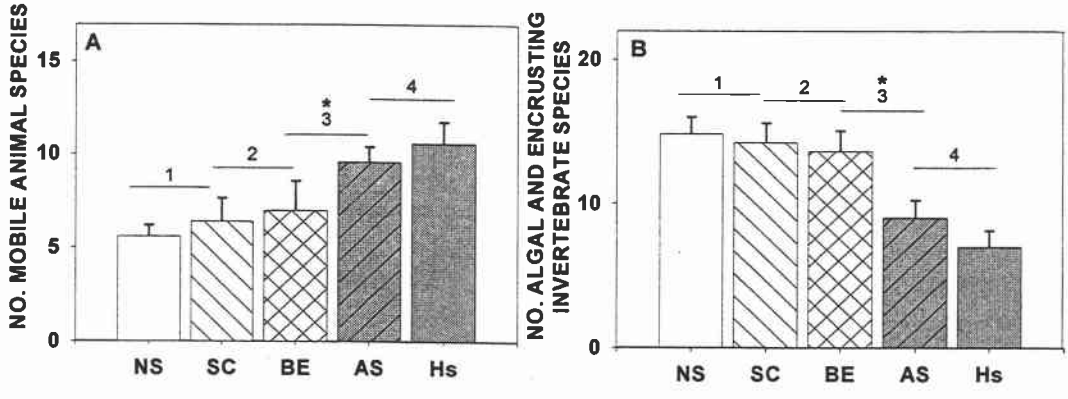

Figure 2.7 Effect of treatment on species richness under experimental structures, July 2000. Number of species (mean ±SEM, n = 5 plots per treatment). NS = No Structure, SC = Structure Control, BE = Bird Exclosure, AS = Artificial Shade, Hs = *Hedophyllum* **(A)** Mobile animals **(B)** Algae and encrusting invertebrates. Treatment effects were evaluated with *a priori* pairwise contrasts (numbered as in tables, indicated by a line above the contrasted treatments) *indicates $p < 0.05$.

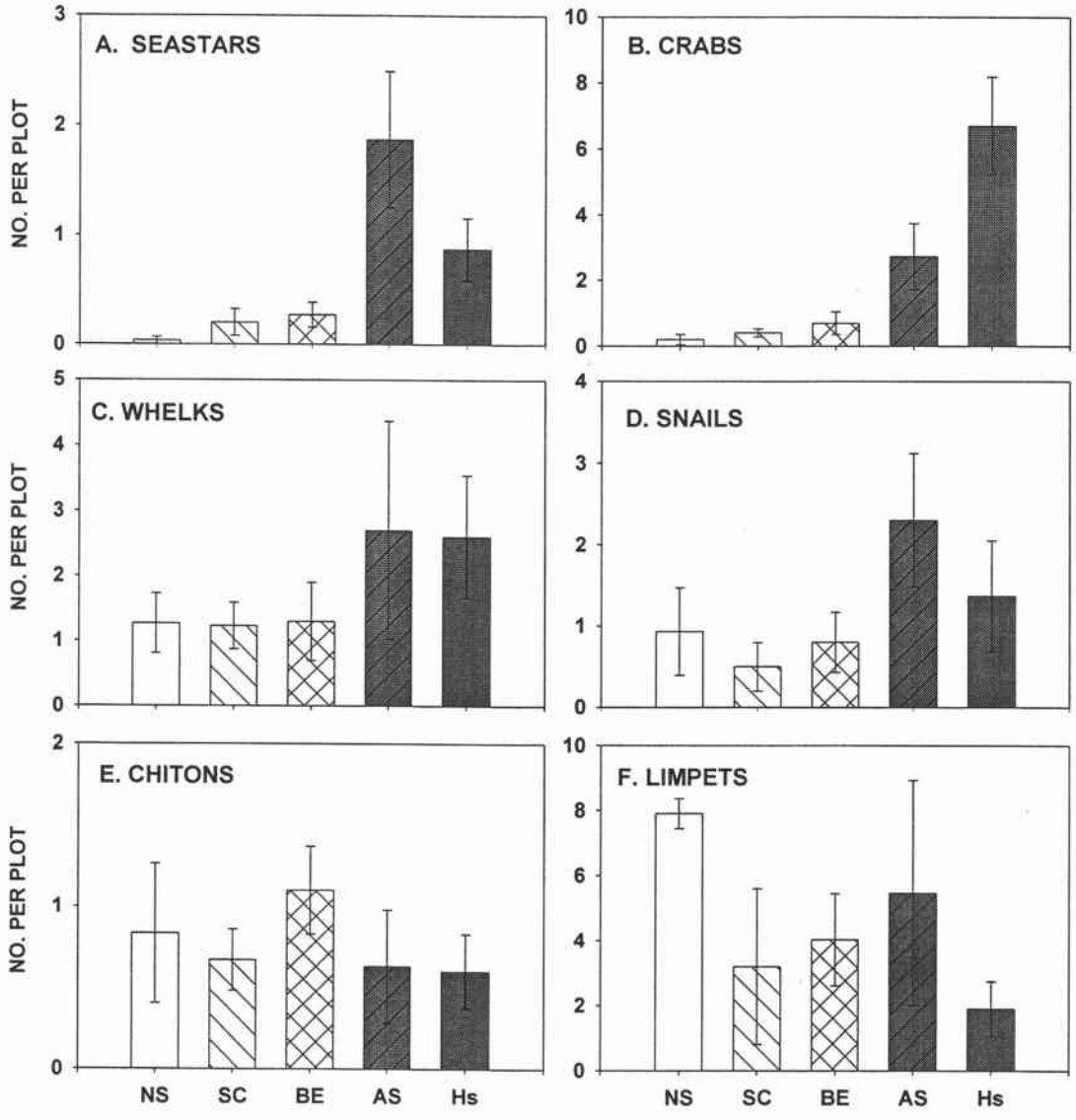

Figure 2.8 Mean summertime abundance of six animal groups under experimental structures. Mean (±SEM, n = 5 plots / treatment) number of animals per survey plot, averaged across summertime sample dates (May and July, 1998, 1999, 2000). NS = No Structure, SC = Structure Control (-H +B –S); BE = Bird Exclosure (-H –B –S); AS = Artificial Shade (-H –B +S); and Hs = *Hedophyllum* (+H (-B +S)). See Fig 2.1 for design details and Table 2.1 for species listings.

Table 2.9 Univariate analysis of total mobile animal abundance under experimental structures in summer and fall. See Figure 2.1 for design details. Data are log-transformed counts for *a priori* selected surveys (see text for details). Bold faced p-values for treatment effects indicate significance at $\alpha = 0.01$ (adjusted for multiple contrasts).

Under Experimental Structures

Source	Df	MS	F	P
JULY 1998				
Treatment	4	2.71	6.30	**0.0030**
Block	4	1.21	2.82	0.0601
Error	16	0.43		
contrasts				
1. –H +B –S (NS) vs. –H +B –S (SC) (structure effect)	1	0.20	0.47	0.5007
2. –H +B –S (SC) vs. –H –B –S **(bird effect)**	1	0.84	1.95	0.1819
3. –H –B –S vs. –H –B +S **(shade effect)**	1	6.75	15.73	**0.0011**
4. –H –B +S vs. +H(–B +S) **(*Hedophyllum* thallus effect)**	1	0.03	0.06	0.8036
NOVEMBER 1998				
Treatment	4	0.26	1.63	0.2146
Block	4	0.42	2.59	0.0767
Error	16	0.16		
JULY 1999				
Treatment	4	0.98	3.14	0.0439
Block	4	0.43	1.37	0.2892
Error	16	0.31		
JULY 2000				
Treatment	4	0.50	2.16	0.1200
Block	4	1.46	6.28	0.0031
Error	16	0.23		

In marked contrast to the bird and *Hedophyllum* thallus effects, shade had strong effects on all three characteristics of the assemblage, and seasonal trends similar to those seen with *Katharina* were also evident. Overall, animal abundance was higher in +Shade plots than in –Shade plots (Fig 2.6, Table 2.8; Between subjects shade effect), and the effect differed with season (Within subjects shade effect). Shade effects on total mobile animal abundance were greatest in July 1998 (Table 2.9, $p = 0.001$), but there was no effect of shade on either total animal

abundance or the composition of the animal assemblage in the fall (Table 2.9, 2.10, no significant treatment effects in November 1998). Although shade affected total mobile animal abundance only in summer 1998, the composition of the assemblage was affected both in 1998 and 1999 (Table 2.10; shade effects, July 1998 and July 1999, $p < 0.03$). Summertime abundances of seastars, crabs, and snails (Fig 2.8a, b, d), and species richness in July 2000 (Fig 2.7a; contrast 3, $p = 0.02$) were all higher in +Shade than –Shade plots.

Table 2.10 Multivariate analyses of mobile animal assemblage under experimental structures. Data are log-transformed counts for six animal groups: crabs, seastars, whelks, snails, limpets and chitons. See Table 2.1 for species listings. Bold faced p-values for treatment effects indicate significance at $\alpha = 0.01$ (adjusted for multiple comparisons).

Under Experimental Structures

JULY 1998	N df	D df	Wilks λ	F	P
Treatment	16	40.4	0.15	2.16	0.0243
Block	16	40.4	0.29	1.26	0.2714
contrasts					
1. –H +B –S (NS) vs. –H +B –S (SC)	4	13	0.82	0.71	0.5978
2. –H +B –S (SC) vs. –H –B –S **(bird effect)**	4	13	0.90	0.37	0.8240
3. –H –B –S vs. –H –B +S **(shade effect)**	4	13	0.47	3.66	0.0331
4. –H –B +S vs.+H (–B +S) **(*Hedophyllum* thallus effect)**	4	13	0.59	2.22	0.1236

NOVEMBER 1998	N df	D df	Wilks λ	F	P
Treatment	24	39.6	0.10	1.51	0.1230
Block	24	39.6	0.04	2.62	0.0036

JULY 1999	N df	D df	Wilks λ	F	P
Treatment	24	39.6	0.02	3.71	**0.0001**
Block	24	39.6	0.10	1.51	0.1240
contrasts					
1. –H +B –S (NS) vs. –H +B –S (SC)	6	11	0.69	0.84	0.5673
2. –H +B –S (SC) vs. –H –B –S **(bird effect)**	6	11	0.70	0.78	0.6045
3. –H –B –S vs. –H –B +S **(shade effect)**	6	11	0.11	15.08	**< 0.0001**
4. –H –B +S vs.+H (–B +S) **(*Hedophyllum* thallus effect)**	6	11	0.24	5.81	**0.0060**

JULY 2000	N df	D df	Wilks λ	F	P
Treatment	24	39.6	0.05	2.30	**0.0097**
Block	24	39.6	0.02	3.73	0.0001
contrasts					
1. –H +B –S (NS) vs. –H +B –S (SC)	6	11	0.32	0.59	0.7351
2. –H +B –S (SC) vs. –H –B –S **(bird effect)**	6	11	0.46	0.84	0.5675
3. –H –B –S vs. –H –B +S **(shade effect)**	6	11	0.89	1.63	0.2283
4. –H –B +S vs.+H (–B +S) **(*Hedophyllum* thallus effect)**	6	11	1.09	13.25	0.1506

Understory Assemblage Under Experimental Structures: Algae and Encrusting Invertebrates

I also evaluated the response of the understory algal and encrusting invertebrate assemblage using three metrics: total abundance (macroalgae and encrusting invertebrates ("macro-AEI") + microalgae), species richness (number of AEI species) and the response of individual functional groups. As in the mobile animal analysis, the combined response of the seven AEI groups (six macro-AEI groups and microalgae) can be used to evaluate the treatment effect on the composition of the assemblage using MANOVA.

As with *Katharina* and mobile animals, bird predation did not affect any component of the AEI assemblage. No differences were detected between +B and –B plots in either total macro-AEI cover or microalgal cover (Fig 2.9a, b; Table 2.11, 2.12, bird effect); abundances of individual macro-AEI groups (Fig 2.10, compare BE to SC), the composition of the AEI assemblage (Table 2.13; bird effect) or species richness in July 2000 (Fig 2.7b; contrast 2, $p = 0.64$).

The *Hedophyllum* thallus affected AEI abundance, but did not affect species richness or composition. Overall, total macro-AEI and microalgal abundances were slightly lower in *Hedophyllum* plots than under Artificial Shades (Fig 2.9a, b; Tables 2.11, 2.12; *Hedophyllum* thallus effect). The effect on the macro-AEI assemblage was primarily due to negative effects of the *Hedophyllum* thallus on the abundance of green and fleshy brown algae (Fig 2.10b, d). Although *Hedophyllum* negatively affected the abundance of these two groups, it did not affect species richness or the overall composition of the assemblage (Fig 2.7b, contrast 4, $p = 0.79$; Table 2.13, *Hedophyllum* thallus effect).

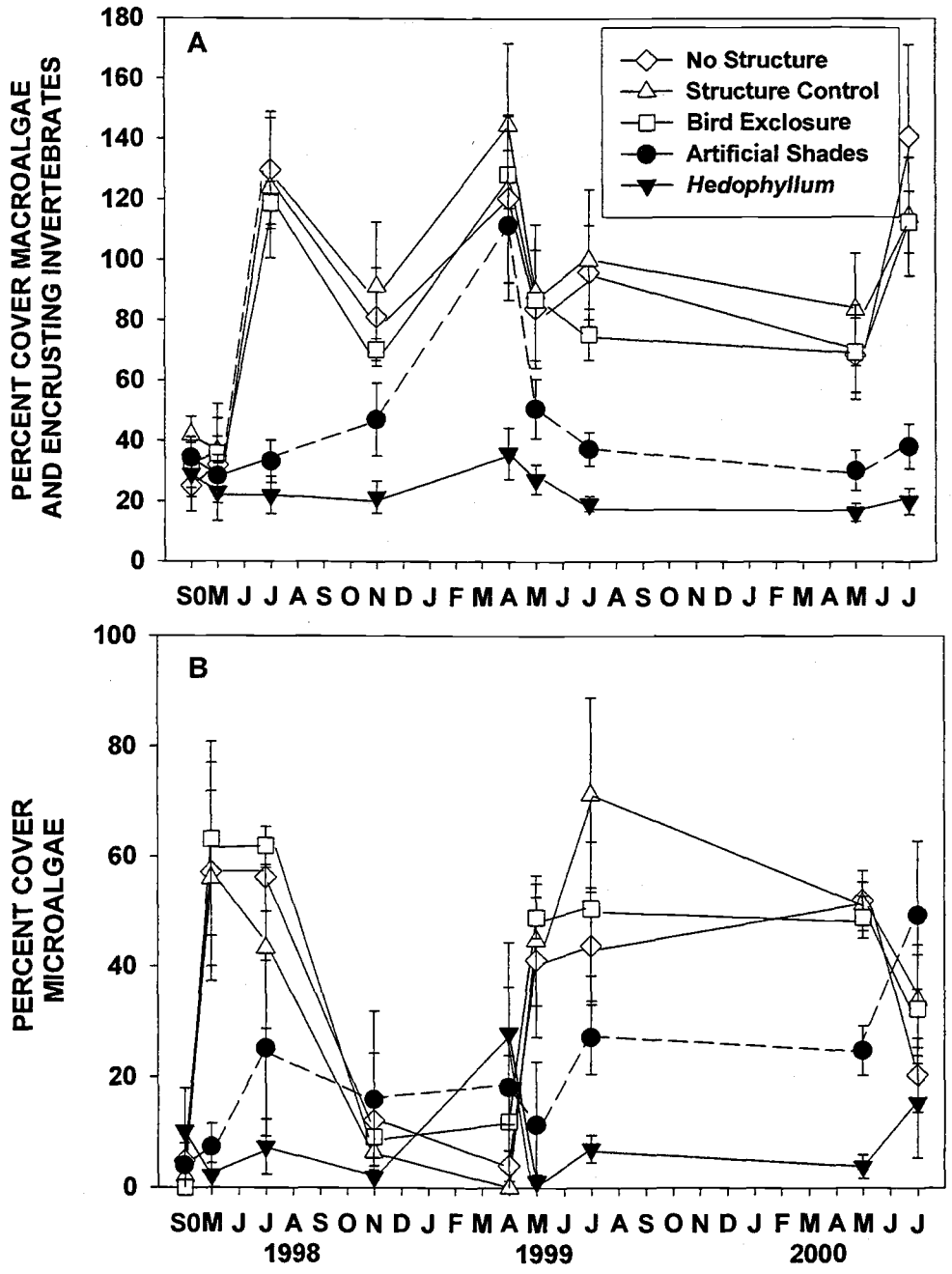

Figure 2.9 Effect of treatment on algal and encrusting invertebrate abundances under experimental structures. Mean (±SEM, n = 5) percent cover (primary + secondary) per survey plot, April 1998 through July 2000. No Structure and Structure Control = -H +B –S; Bird Exclosure = -H –B –S; Artificial Shade = -H –B +S; and *Hedophyllum* = +H (-B +S) **(A)** Macroalgae and encrusting invertebrates **(B)** Microalgae (primarily diatoms). See Fig 2.1 for design details.

Table 2.11 Repeated measures analysis of total macroalgal and encrusting invertebrate cover under experimental structures. See Figure 2.1 for design details. Data are log-transformed percent cover values, (primary + secondary cover), for May, July, and November 1998, April, May, and July 1999, and May and July 2000. As Mauchly's criterion was not met (p = 0.00002) multivariate analyses are presented.

Under Experimental Structures

Source	Df	MS	F	P
Between Subjects (average effect over time)				
Treatment	4	15.03	14.55	**< 0.0001**
Block	4	1.00	0.97	0.4501
Error	16	1.03		
contrasts				
1. –H +B –S (NS) vs. –H +B –S (SC) (structure effect)	1	0.20	0.19	0.6665
2. –H +B –S (SC) vs. –H –B –S **(bird effect)**	1	0.14	0.14	0.7171
3. –H –B –S vs. –H –B +S **(shade effect)**	1	8.52	8.25	**0.0111**
4. –H –B +S vs. +H(–B +S) **(*Hedophyllum* thallus effect)**	1	9.66	9.35	**0.0075**

Within Subjects (change in effect over time)	N df	D df	Wilks λ	F	P
Time	7	10	0.107	11.96	**0.0004**
Time x Treatment	28	37.5	0.186	0.80	0.7323
Time x Block	28	37.5	0.100	1.20	0.3002
contrasts					
Time x 1 (structure effect)	7	10	0.835	0.28	0.9467
Time x 2 **(bird effect)**	7	10	0.848	0.26	0.9578
Time x 3 **(shade effect)**	7	10	0.522	1.31	0.3384
Time x 4 **(*Hedophyllum* thallus effect)**	7	10	0.858	0.24	0.9657

Shade strongly affected both AEI abundance and the composition of the assemblage. Total macro-AEI and microalgal abundances were lower under shades than in unshaded areas (Fig 2.9a, b, Tables 2.11, 2.12; shade effect). This effect of shade was constant over time for macro-AEI groups (Table 2.11, Time x shade effect $p > 0.3$) but was only important during the spring and summer for microalgae (Fig 2.9b, Table 2.12; Time x shade effect $p = 0.0036$). Macro-AEI abundance fluctuated over time as abundance of certain algal groups increased

with recruitment events in April, grew over the course of the summer 'growing season', and decreased in the fall (Fig 2.9a).

Table 2.12 Repeated measures analysis of microalgal abundance under experimental structures. See Figure 2.1 for design details. Data are log-transformed percent cover values, for May, July, and November 1998, April, May, and July 1999, and May and July 2000. Although Mauchly's criterion was not met ($p = 0.008$) interpretations did not differ between multivariate and univariate analyses, and univariate analyses are presented with Huynh-Feldt adjusted probabilities.

Under Experimental Structures

Source	Df	MS	F	P
Between Subjects (average effect over time)				
Treatment	4	25.15	18.99	**< 0.0001**
Block	4	4.94	3.73	**0.0249**
Error	16	1.32		
contrasts				
1. –H +B –S (NS) vs. –H +B –S (SC) (structure effect)	1	0.01	0.00	0.9729
2. –H +B –S (SC) vs. –H –B –S **(bird effect)**	1	1.08	0.81	0.3803
3. –H –B –S vs. –H –B +S **(shade effect)**	1	23.56	17.79	**0.0007**
4. –H –B +S vs. +H(–B +S) **(*Hedophyllum* thallus effect)**	1	13.16	9.94	**0.0062**
Within Subjects (change in effect over time)				
Time	7	22.41	17.73	**< 0.0001**
Time x Treatment	28	3.36	2.66	**0.0002**
Time x Block	28	3.50	2.77	**< 0.0001**
Error (Time)	112	1.26		
contrasts				
Time x 1 (structure effect)	7	0.88	0.69	0.6782
Time x 2 **(bird effect)**	7	0.49	0.39	0.9089
Time x 3 **(shade effect)**	7	4.10	3.24	**0.0036**
Time x 4 **(*Hedophyllum* thallus effect)**	7	2.57	2.03	0.0573

Three macro-algal groups were less abundant in + Shade than –Shade plots overall: finely branched red algae, green algae, and foliose red algae (Fig 2.10a, b, c, compare AS to BE). The response of encrusting invertebrates to shade was the opposite of the macroalgal groups, as abundances of these

colonies were higher in + Shade than –Shade plots (Fig 2.10f). The abundance of encrusting invertebrates showed a unique seasonal pattern, increasing dramtically in Artificial Shade plots in November 1998 (to 84% cover in some plots), then dropped to near 0% by April 1999 (Fig 2.11). As expected from these different group responses to the presence of shade, at the end of the experiment, the assemblage composition differed between +Shade and –Shade plots (Table 2.13, shade effect) and species richness was lower in +Shade than -Shade plots (Fig 2.7b; contrast 3, p = 0.03).

Table 2.13 Multivariate analysis of algal and encrusting invertebrate assemblage, July 2000, under experimental structures. Data are log-transformed percent cover values (primary + secondary); GRE = green algae, BRO = fleshy brown algae, FNR = finely branched red algae, FOL = foliose and corticated red algae, MIC = microalgae, RARE = rare algae, COR = articulated coralline algae, ENC = encrusting invertebrates (see Table 2.1). The magnitude of a group's canonical coefficient (analogous to a partial regression coefficient) indicates the relative contribution of each group to the differences among treatments. Similar signs of coefficients indicate positive correlation between groups.

Under Experimental Structures

Source	N df	D df	Wilks λ	F	P
Treatment	32	34.8	0.013	2.46	**0.0052**
Block	32	34.8	0.059	1.23	0.2540
contrasts					
1. –H +B –S (NS) vs. –H +B –S (SC)	8	9	0.348	2.11	0.1434
2. –H +B –S (SC) vs. –H –B –S **(bird effect)**	8	9	0.816	0.25	0.9668
3. –H –B –S vs. –H –B +S **(shade effect)**	8	9	0.201	4.46	**0.0193**
4. –H –B +S vs.+H (–B +S) **(*Hedophyllum* thallus effect)**	8	9	0.452	1.36	0.3256

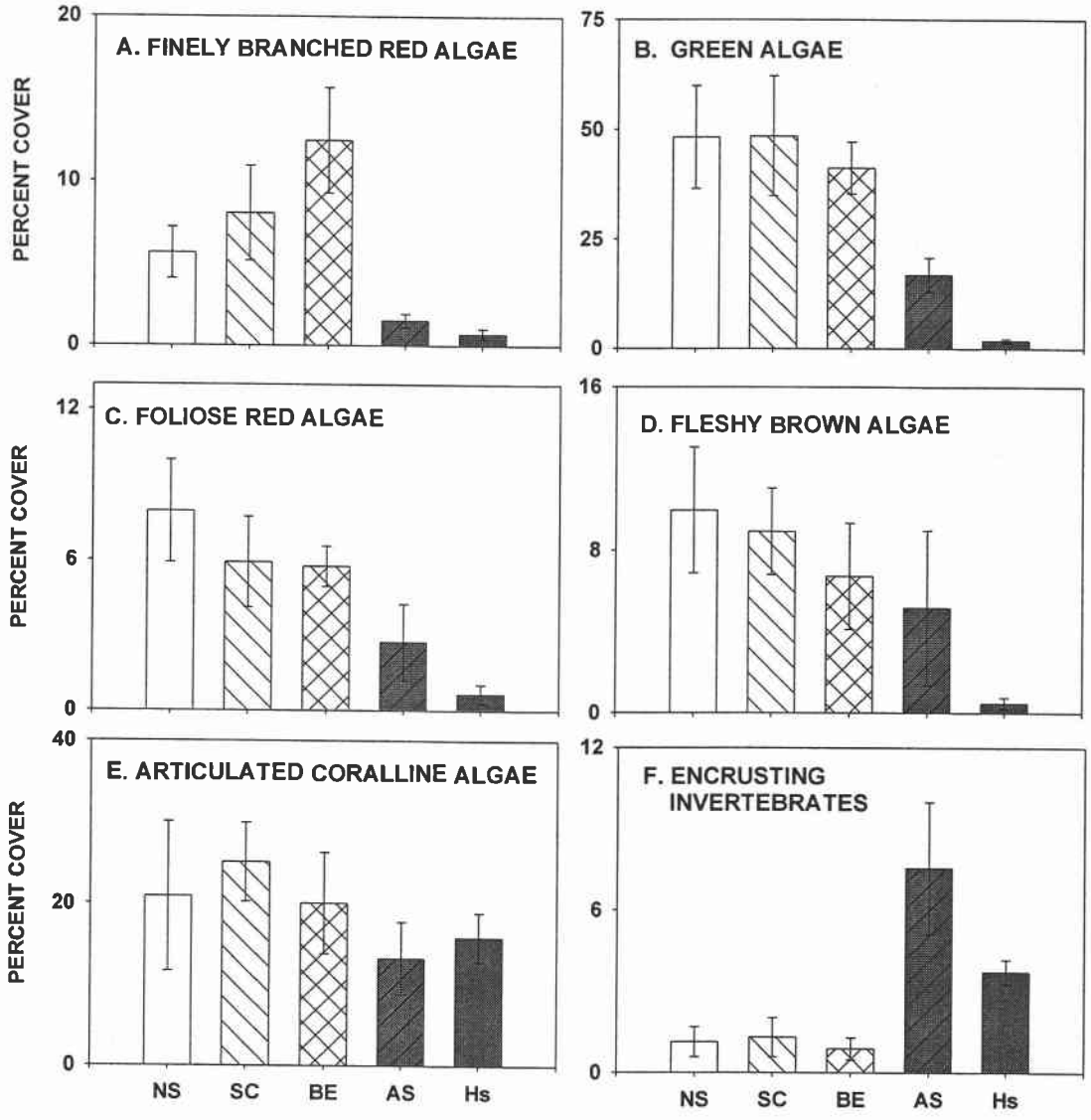

Figure 2.10 Overall abundance of six algal and encrusting invertebrate groups under experimental structures. Mean (±SEM, n = 5 plots / treatment) percent cover (primary + secondary) per survey plot, averaged over sampling dates (May 1998 through July 2000). No Structure and Structure Control = -H +B –S; Bird Exclosure = -H –B –S; Artificial Shade = -H –B +S; and *Hedophyllum* = +H (-B +S) See Fig 2.1 for design details and Table 2.2 for species listings.

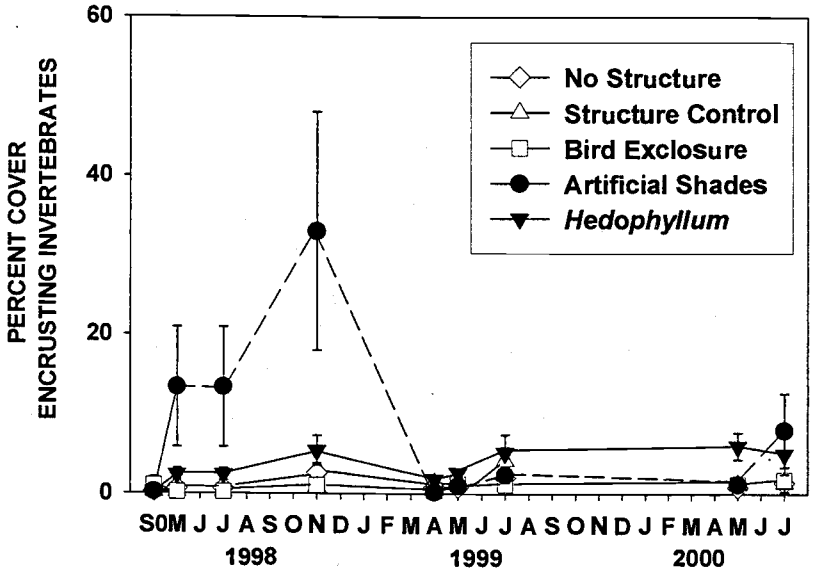

Figure 2.11 Percent cover of encrusting invertebrates over time under experimental structures. Mean (±SEM, n = 5 plots / treatment) percent cover per survey plot, April 1998 (S0) through July 2000. No Structure and Structure Control = -H +B –S; Bird Exclosure = -H –B –S; Artificial Shade = -H –B +S; and *Hedophyllum* = +H (-B +S) See Table 2.2 for species, Fig 2.1 for design details.

Understory Assemblage Adjacent to Experimental Structures: Halo Effects

Because shade had the largest influence on the understory community, I assessed possible 'halo' effects by contrasting plots adjacent to Artificial Shades to those adjacent to –Bird experimental structures ('B' plots in Fig 2.1, contrast 3 in tables). Mobile animal abundance was not enhanced by the proximity of shade (Table A.I, halo effect); none of the individual animal groups were more abundant next to Artificial Shades than next to other structures (Fig 2.12, compare AS to BE). Accordingly, neither the composition of the mobile animal assemblage nor species richness differed between +Shade and –Shade plots (Table A.II; halo effects $p > 0.15$; Fig 2.13, contrast 3, $p = 0.35$).

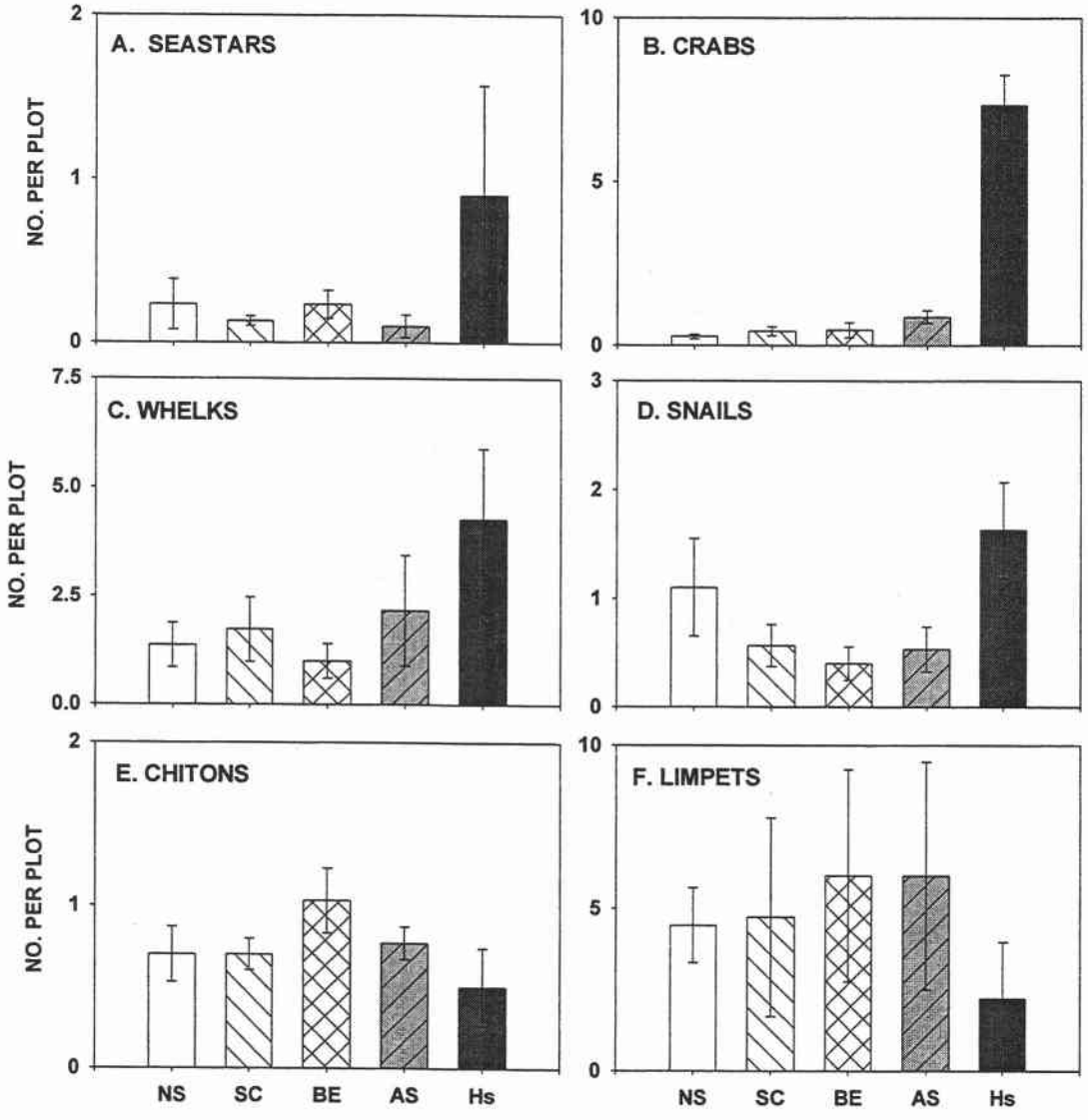

Figure 2.12 Summertime abundance of six animal groups in plots adjacent to experimental structures. Mean (±SE, n = 5 plots / treatment) number of animals per plot, averaged over summer sampling dates (May and July 1998, 1999, 2000). NS = No Structure, SC = Structure Control (-H +B –S) BE = Bird Exclosure (-H – B –S); AS = Artificial Shade (-H –B +S); Hs = *Hedophyllum* (+H (-B +S)).

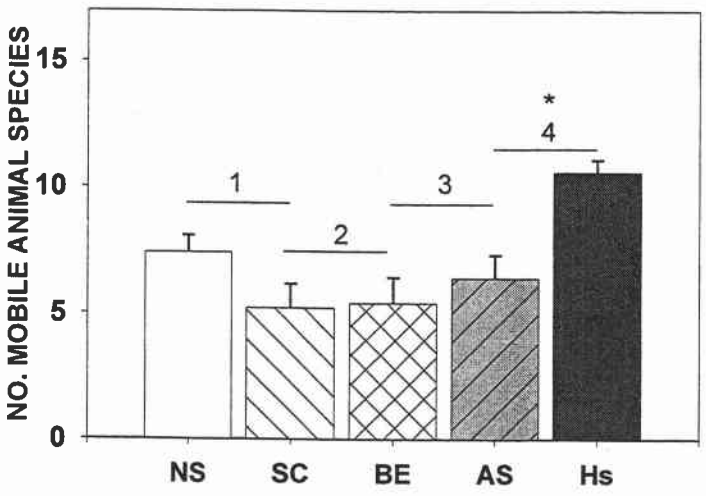

Figure 2.13 Species richness of mobile animals in survey plots adjacent to experimental structures, July 2000. Mean (+SEM, n = 5 plots / treatment) number of species per plot. See Table 2.2 for species. NS = No Structure, SC = Structure Control (-H +B –S); BE = Bird Exclosure (-H –B –S); AS = Artificial Shade (-H –B +S); and *Hedophyllum* = (+H (-B +S)) Hypotheses were tested using *a priori* pairwise contrasts (numbered as in tables; indicated by lines above contrasted treatments). * indicates $p < 0.05$.

Despite higher grazer abundances underneath artificial shades, there were no detectable 'halo effects' on any attribute of the AEI assemblage in –Shade plots immediately adjacent to these grazer aggregations. Neither macro-AEI abundance nor microalgal abundance was reduced in –Shade plots adjacent to Artificial Shades compared to those in –Shade treatments (Fig 2.14a,b; Table A.III, A.IV; halo effect). No individual AEI group was less abundant in plots next to Artificial Shades than in "B" plots in –Shade treatments (Fig 2.15, compare AS to BE) and, accordingly, species richness and the composition of the assemblage in July 2000 were not different between plots next to Artificial Shades and next to Bird Exclosure structures (Fig 2.14c; $p = 0.55$; Table A.V, halo effect).

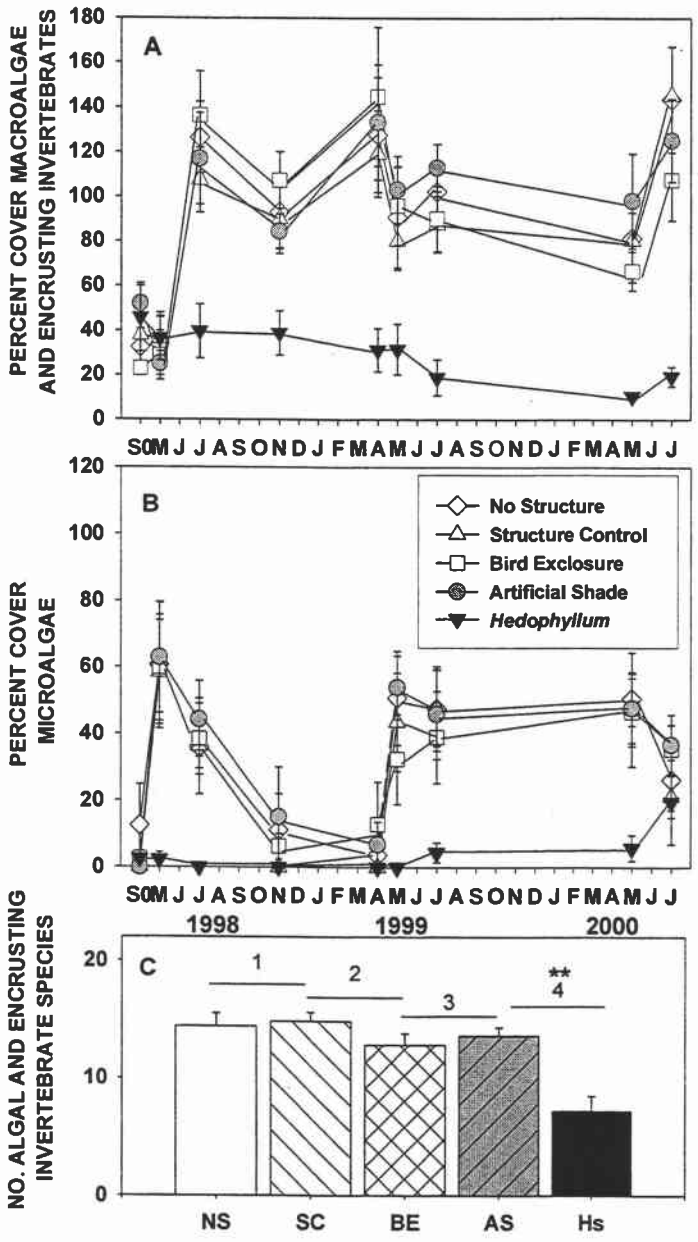

Figure 2.14 Effect of treatment on the algal and encrusting invertebrate assemblage in plots adjacent to experimental structures. NS = No Structure, SC = Structure Control (-H +B –S); BE = Bird Exclosure (-H –B –S); AS = Artificial Shade (-H –B +S); Hs = *Hedophyllum* (+H (-B +S)) **(A)** Percent cover of macroalgae and encrusting invertebrates (primary + secondary; mean ± SEM, n = 5). **(B)** Percent cover microalgae **(C)** Species richness measured in July 2000 (mean ± SEM). Hypotheses were tested using *a priori* pairwise contrasts (numbered as in tables, indicated by a line above the contrasted treatments). ** indicates $p < 0.01$.

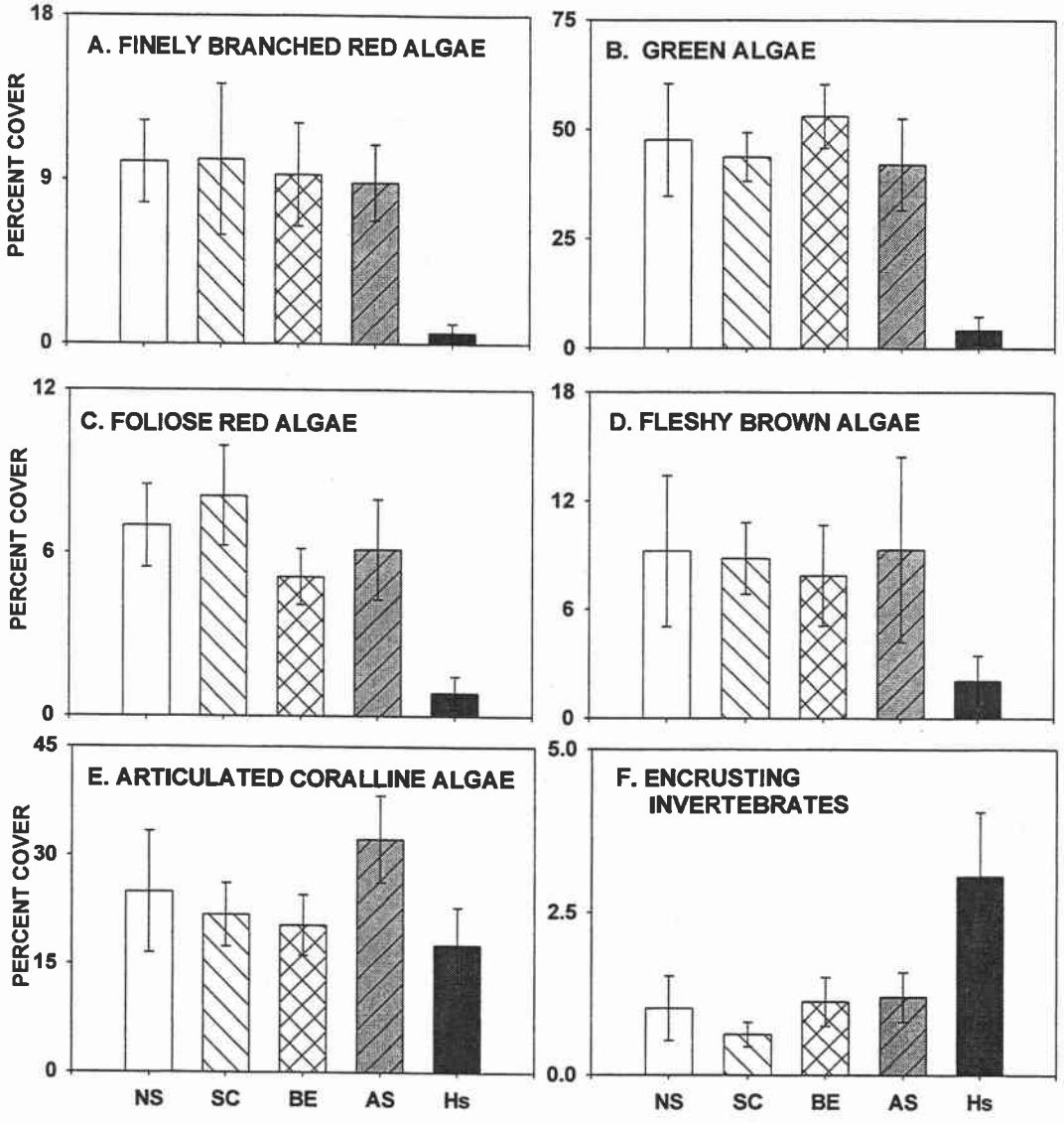

Figure 2.15 Overall abundance of six groups of macroalgae and encrusting invertebrates in plots adjacent to experimental structures. Mean (±SEM, n = 5 plots / treatment) percent cover (primary + secondary) per survey plot, averaged over all sampling dates (May 1998 through July 2000). No Structure and Structure Control = -H +B –S; Bird Exclosure = -H –B –S; Artificial Shade = -H –B +S; and *Hedophyllum* = +H (-B +S) See Fig 2.1 for design details and Table 2.2 for species listings.

DISCUSSION

Katharina / Hedophyllum *Association: Shade Hypothesis*

These results indicate that the provision of shade by the *Hedophyllum* canopy, and not the presence of the *Hedophyllum* thallus or bird predation, explains the positive association between *Katharina* and *Hedophyllum*. There are several potential physiological benefits to the association with shade during daytime low tides. Despite the classification of the low intertidal zone as a 'low-stress' environment (Menge and Sutherland 1987, Bertness et al. 1999b), temperature fluctuations can be large even over the course of a single low tide. At high tide, the temperature of intertidal ectotherms approximates the temperature of the surrounding water (Helmuth 1998). High tide temperatures never rose above 14°C during the course of this study, yet I measured low tide *Katharina* body temperatures (at haphazard times) as high as 29°C, and recorded data-logger temperatures (which are highly predictive of *Katharina* body temperatures) above 33°C (Chapter 4). In all three summers, the difference between water temperatures and the daily maximum temperature at low tide constitutes a substantial change (Fig 2.3b). Repeated fluctuations over consecutive days of a tide series are likely to result in significant physiological effects. An examination of the production of proteins that play a role in reducing cellular damage from heat stress indicates that *Katharina* at Pile Point experience sub-lethal thermal stress during the summer, to which they respond at the molecular level (Chapter 4). Over time, active selection of shaded areas could substantially reduce levels of physiological stress.

The shade provided by the *Hedophyllum* canopy positively affected *Katharina* abundances, but the effect differed slightly between years. In the summer of 1999, levels of chronic stress (average temperatures, daily maxima and number of hot days) were lower than in 1998 and 2000 (Fig 2.3b, Table 2.7). Numerous studies have shown that the strength of a positive interaction increases with increasing abiotic stress (e.g. Walker and Chapin 1987, Bertness and Shumway 1993, Bertness and Hacker 1994, Bertness and Yeh 1994, Callaway 1997). However, only two other studies have documented a positive interaction that changed with temporally changing abiotic conditions (Greenlee and Callaway 1996, Leonard 2000). Such temporal shifts in the sign and intensity of interactions may be common in nature, but their detection depends on long-term high-resolution studies that can distinguish annual and seasonal trends. Such long-term studies are necessary to advance our understanding of complex species interactions.

Katharina / Hedophyllum *Association: Predation Hypothesis*

There was no evidence to support the hypothesis that increased mortality from bird predation was the cause of low *Katharina* abundance in canopy removal areas. However, the threat of predation alone can influence microhabitat associations of mobile herbivores (Garrity and Levings 1981, Duggins 1983, Mercurio et al. 1985, Wootton 1992, Beckerman et al. 1997); and this must be taken into account when evaluating the effects of predators on a system. If *Katharina* are moving out of canopy removal areas to avoid bird predation, then birds are an important factor influencing chiton abundance, even if actual predation

rates are low. However, several pieces of evidence suggest that the risk of predation by *Pisaster* is greater than the risk of predation by gulls, especially at Pile Point, where *Katharina* make up a far greater proportion of the *Pisaster* diet than in other locations; 31% (Fig 2.16) vs. 0-6 % elsewhere (Feder 1959, Mauzey 1965, 1967, Paine 1966, Mauzey et al. 1968, Menge and Menge 1974, Sanford 1999a). This heavy predation pressure is reflected in rough mortality estimates; of 69 recovered dead *Katharina* over 7 days, 57% had been killed by *Pisaster*, while only 23% showed signs of bird predation. The threat of seastar predation has also been shown to produce patchy distributions of other marine herbivores due to strong escape responses of the prey (Dayton 1975, Duggins 1983). If *Katharina* were actively choosing microhabitats to avoid predators, –Shade areas should be selected over +Shade areas, because *Pisaster* were more frequently found in +Shade plots throughout the study (55 individuals in +Shade plots vs. 9 in –Shade plots). However, *Katharina* actively selected +Shade areas despite the higher risk of predation.

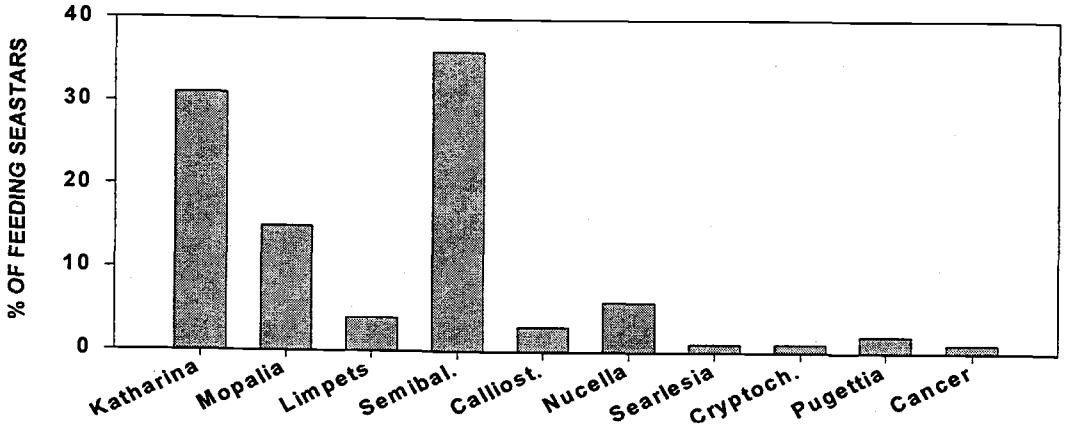

Figure 2.16 Diet of *Pisaster ochraceus* at Pile Point 1998 – 2000. The % of feeding seastars is the percent of actively feeding individuals (n = 165 of 756 *Pisaster* observed on 24 dates) observed feeding on prey species: Semibal. = *Semibalanus*, Calliost. = *Callisotoma*, Cryptoch. = *Cryptochiton*.

Katharina / Hedophyllum *Association: Food Hypothesis*

The association of *Katharina* with *Hedophyllum* is not influenced by the presence of the *Hedophyllum* thallus *per se*, but instead by physical properties that are mimicked by the Artificial Shades. *Katharina* do consume *Hedophyllum* in the field; the kelp comprised approximately half of the diet of animals collected at Pile Point in summer 2000 (Chapter 3; 95% confidence interval for the mean proportion of *Hedophyllum* in total diet, 18 to 53%, n = 6). This fact, combined with reports that *Katharina* prefer *Hedophyllum* as a food source, (Himmelman and Carefoot 1975, Dethier and Duggins 1984), may suggest that *Katharina* would select microhabitats based on the presence of *Hedophyllum*. However, the reported feeding preference for *Hedophyllum* is based on experiments in which *Katharina* individuals were offered the choice of either *Hedophyllum* or *Iridaea* (now *Mazzaella*) in field enclosures from which all other algae were removed

(Himmelman and Carefoot 1975), and therefore only demonstrates a preference for *Hedophyllum* over *Iridaea*. At Pile Point, over 50% of the *Katharina* diet consisted primarily of the green alga *Ulva* and diatoms, a result that is consistent with other field surveys (Piercy 1987, Dethier and Duggins 1988). As the abundance of these other algae is consistently low in areas with high abundance of *Hedophyllum* (this study, Duggins and Dethier 1985), the high proportion of *Hedophyllum* in the *Katharina* diet is as likely to be due to availability as to preference. *Katharina* abundance remained high in Artificial Shade plots despite the absence of *Hedophyllum*, indicating that the selection of microhabitats is not based on characteristics of *Hedophyllum* but rather on the ability of the alga to provide shade.

Community-Level Effects of Shade: Grazers and their Prey

The high abundance of grazers, including *Katharina*, under Artificial Shades did not appear to affect abundances of their prey items in –Shade areas directly adjacent to these shades. Predation or grazing halos surrounding refugia are well-documented spatial patterns in intertidal communities (Menge 1978a, Suchanek 1979, Garrity and Levings 1981, Levings and Garrity 1983, Witman 1987, Fairweather 1988) and the lack of a grazing halo in this system is surprising for two reasons. First, *Katharina* have been classified by several experimental studies as "strong interactors" in the low rocky intertidal zone, with strong negative effects on algae both as a population (Dethier and Duggins 1988) and as individuals (Paine 1992). Yet despite significant aggregation of *Katharina* underneath the artificial shades, plots a few centimeters away were not different

from plots a few meters away from shade. Second, previous studies found a weak effect of *Katharina* grazing in areas of *Hedophyllum* canopy removal despite the relative absence of *Katharina* in these areas during low tide (Duggins and Dethier 1985). The different results between studies are even more puzzling because the clearance areas in this study were much smaller than in the previous experiment ($4m^2$ plot + border randomly located in *Hedophyllum* beds vs. $12m^2$ clearance areas) and the surveys in this experiment were taken directly adjacent to shaded areas (with high *Katharina* abundances) vs. surveys in the center of the large canopy clearance areas.

Are *Katharina* simply exerting grazing pressure evenly over the whole – Shade area? From the high number of chitons in +Shade plots, this seems unlikely. An alternative hypothesis is that the strong selection shown by *Katharina* for shaded areas results in differential patterns of movement across microhabitats. Under this hypothesis, *Katharina* in shaded areas would be essentially sedentary, remaining almost exclusively in areas under shades. In contrast, *Katharina* in open areas may travel rapidly until they encounter shade. Such differential activity in response to shade would result in aggregations under shades, and thus concentrated feeding on the organisms directly underneath the canopy. Chitons 'in transit' through –Shade areas may feed little, and thus have little grazing impact. Preliminary observations are consistent with these postulated patterns of movement in + Shade and –Shade areas (A.S. Flecker, unpublished data) but the effects on grazing patterns and algal consumption have yet to be determined.

Reciprocal Effects: Katharina *on* Hedophyllum

Hedophyllum plays an interesting role in this community. Although the primary effect of *Hedophyllum* on the distribution and abundance of *Katharina* is through the provision of non-food resources (habitat modification), the chiton does consume the alga. What is the effect of *Katharina* on *Hedophyllum*?

Several pieces of evidence suggest that the effects of *Katharina* grazing on the *Hedophyllum* population vary from neutral to negative, and that the strength of the interaction depends on the degree of environmental (physical) stress. In a separate simultaneous experiment, I found that enclosing *Katharina* in plots with *Hedophyllum* over 14 months did not reduce *Hedophyllum* canopy cover, the number of adult *Hedophyllum*, or abundance of new *Hedophyllum* recruits relative to *Katharina* exclusion plots, despite the fact that *Katharina* were consuming *Hedophyllum* in the enclosure plots (Chapter 3). However, evidence from previous work indicates that *Katharina* can negatively affect the *Hedophyllum* population by consuming juvenile thalli and thus preventing population recovery after the loss of adults (Dayton 1975, Markel and DeWreede 1998). *Katharina* can also affect the abundance of small adult thalli (1 – 4 cm holdfast size class) by degrading holdfast integrity, subsequently increasing the frequency of thallus removal by wave action (Markel and DeWreede 1998). These previous investigators concluded that *Hedophyllum* populations were generally more affected by year-to-year variation in abiotic variables (such as wave force) than by the effects of *Katharina* (Duggins and Dethier 1985). Their observations suggest that, just as the intensity of the positive interaction of *Hedophyllum* on *Katharina* depends on the degree of abiotic physiological (temperature) stress, the degree or even the sign of the effect of

Katharina on *Hedophyllum* depends on the degree of abiotic physical stress (wave exposure).

In years or sites in which wave action is low and thus loss of adult thalli is patchy or rare, the overall effect of *Katharina* on *Hedophyllum* may be to produce a constantly changing patchy landscape, in which consumption of *Hedophyllum* recruits underneath the established *Hedophyllum* canopy reduces recovery rates following winter storm losses, but avoidance of –Shade (-Canopy) areas allows *Hedophyllum* recruits to attain a 'size escape' from predation and leads to the establishment of a *Hedophyllum* canopy in these areas. *Hedophyllum* recruits were frequently observed in (and subsequently removed from) experimental –Shade plots, which had low *Katharina* abundances over all three years of this study; and similar patterns of herbivore aggregation under canopies, followed by canopy removal and grazer dispersal, have been noted with limpets in higher intertidal zones (Southward and Southward 1978, Thompson 1980, Hartnoll and Hawkins 1985). Although evaluation of this hypothesis is beyond the scope of this study, the results suggest that in this system, the effects of *Katharina* on the *Hedophyllum* population at Pile Point are, at most, weakly negative. Therefore, the association of *Katharina* with the kelp *Hedophyllum* is a commensalism, a net positive interaction which provides benefits to the chiton and is, over time, essentially neutral for the *Hedophyllum* population.

The Effect of Hedophyllum *on the Low Zone Community*

The *Hedophyllum* canopy affects not only the abundance and distribution of *Katharina*, but the entire understory community, primarily due to the provision of shade. The effect of shade on the mobile animal assemblage changed with season in a manner predicted by theoretical considerations of positive interactions in communities: shade affected animal abundances in the summer, but not in the fall. This seasonal pattern suggests that the *Hedophyllum* canopy plays an important role in ameliorating seasonal abiotic stress for a suite of low intertidal consumers, including, but not limited to, the chiton *Katharina*.

As a result, the *Hedophyllum* canopy seems to affect understory algal and encrusting invertebrate abundance both directly and indirectly (through the concentration of consumers: Chapter 3). In addition, the canopy may relieve grazing pressure in –Shade areas by concentrating grazers underneath the canopy, and thus indirectly affect the algal and encrusting invertebrate assemblage throughout the low intertidal zone.

Hedophyllum has previously been termed a 'foundation species,' which is disproportionately important to the continued maintenance of existing community structure (Dayton 1972). The results of this study confirm this classification and provide more evidence for the diversity of mechanisms by which the kelp influences the low zone community. In addition, this study shows that the primary mechanism through which *Hedophyllum* affects the community is through the provision of shade. *Hedophyllum* therefore fits the description of a physical autogenic ecosystem engineer (Jones et al. 1994, 1997b, Lawton and Jones 1998), an organism that causes physical state changes in abiotic conditions,

modifying, maintaining or creating habitats with its own physical structures (rather than by transforming other materials). Algal canopies can directly affect the understory AEI assemblage through a number of mechanisms, including mechanical interference of settlement or growth by blades, (Black 1974, Menge 1976, Velimirov and Griffiths 1979, Vadas Sr. et al. 1992); chemical interference of settlement or growth (Fletcher 1975, Inderjit and Dakshini 1994, Schmitt et al. 1995, de O. Figueiredo et al. 1997, Suzuki et al. 1998); or by altering flow regimes and affecting propagule settlement (Duggins et al. 1990). However, in this system, the influence of any of these effects was very weak; the presence of shade was a much greater influence on the structure of the algal and encrusting invertebrate assemblage (Fig 2.9; mean percent cover in –Shade plots, 122.6; in Artificial Shade plots = 38.2; in *Hedophyllum* plots = 20); and the presence of the *Hedophyllum* thallus had no effect on the mobile animal assemblage or the distribution and abundance of *Katharina*.

The determination of the characters that make *Hedophyllum* a 'foundation species' is important because it allows us to make predictions about how the community might change in response to long-term or large-scale elimination of *Hedophyllum*. There has been much debate in recent years about the amount of functional redundancy or functional compensation in communities, and about the hypothesis that species within a "functional group" overlap with each other such that the loss of one species from a system has only a small effect (Walker 1992, Lawton and Brown 1993, Menge et al. 1994, Chapin et al. 1995, Frost et al. 1995). Our ability to evaluate this hypothesis depends on our ability to identify "functional groups" which are ecologically relevant, a task which has led to considerable

debate in its own right (e.g. Steneck and Dethier 1994, 1995, Grime 1995, Lavorel et al. 1997, Sullivan and Zedler 1999). That the primary force in structuring this community is not related to *Hedophyllum per se* but is in fact merely an artifact of the shade-producing nature of the algal canopy suggests that any shade-producing alga could fill this role.

However, in this system, no species is functionally equivalent to *Hedophyllum*. Other kelps (such as *Alaria* and *Desmarestia*) recruit into the intertidal in high abundance during the late winter and early spring, but their abundances plummet rapidly following the onset of the intense sunlight and desiccation that accompanies mid-day low tides in May and June (Fig. 2.17a, Duggins and Dethier 1985). *Hedophyllum* is the only canopy-forming kelp that persists in this system year-round (Fig 2.17b). Several species of green and red algae (e.g. *Acrosiphonia* or *Mazzaella* spp.) that persist through the summer could potentially provide a minimal canopy, but they do not; the abundance of *Katharina* was not correlated with the secondary cover of non-*Hedophyllum* alga at any time during this study (J. Burnaford, unpublished data). Therefore *Hedophyllum* is unique in this system as the only species capable of providing shade throughout the period when it is most important to the rest of the community.

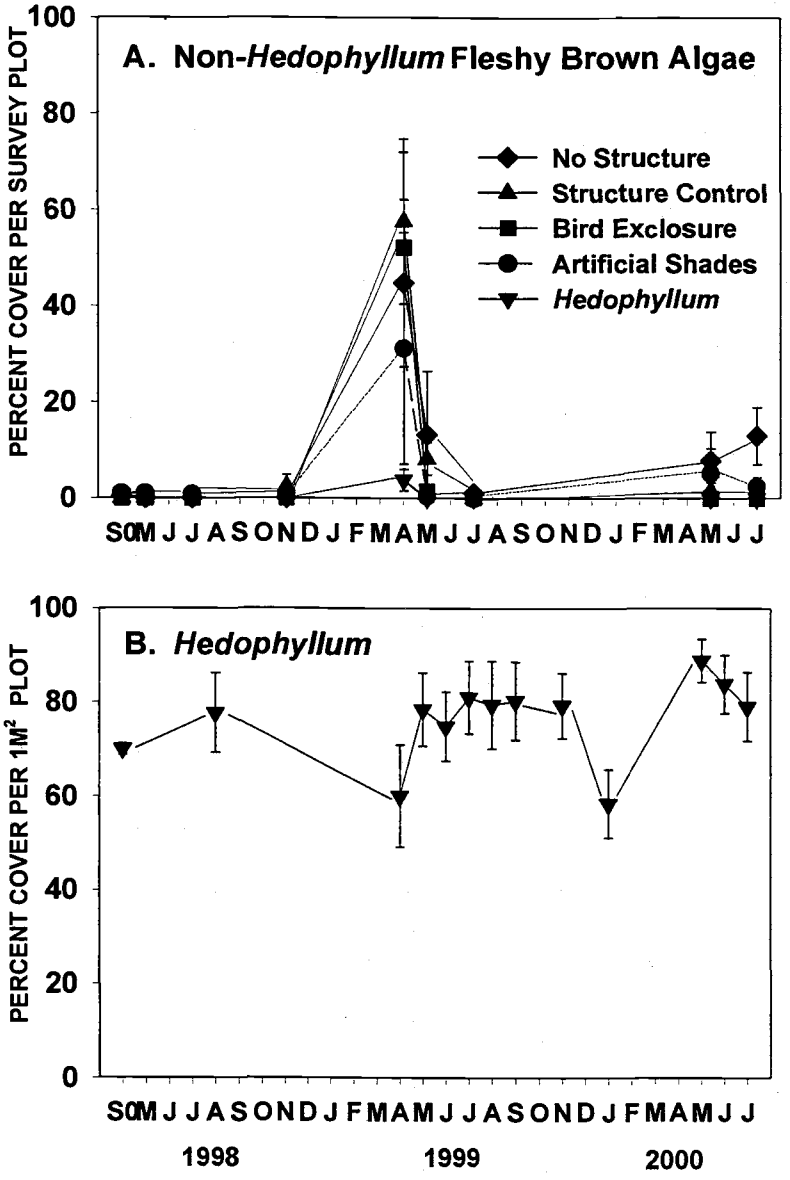

Figure 2.17 Percent cover fleshy brown algae over time at Pile Point. Mean (±SEM, n = 5 plots / treatment) percent cover per plot, April 1998 (S0) through July 2000. **(A)** Non-*Hedophyllum* fleshy brown algae (see Table 2.2 for species), primary + secondary cover, under experimental structures (see Fig 2.1 for design details). **(B)** *Hedophyllum* cover (secondary only) in $1m^2$ experimental plots. Because *Hedophyllum* was manually removed from four of the five experimental treatments (see methods for details), only the data from the natural *Hedophyllum* treatment are shown.

The results of this study can aid in forming predictions about how large-scale, long-term changes in the *Hedophyllum* population may affect the low intertidal zone community. Most conceptual models predict that positive interactions will only be important in high stress areas or times (Bertness and Callaway 1994, Callaway and Walker 1997, Holmgren et al. 1997). Therefore, we could predict that the loss of *Hedophyllum* would result in the disappearance of *Katharina*, and several other consumer groups in the low zone community, from systems in which low tide conditions were physiologically stressful (i.e. areas with low tides during mid-day, such as the San Juans) but that the timing of the disappearance may depend on annual variations in environmental conditions. Communities in which low tide conditions are less stressful (i.e. in which low tides occur at less stressful times of day, or in which weather conditions are frequently foggy) might be less affected.

Understanding how environmental changes affect and alter interactions between species, and not only how they affect geographical distributions, is an important component of understanding the potential impacts of climate change on natural systems (Lubchenco et al. 1991, Sanford 1999a). Studies such as this one provide crucial information that can increase our ability to predict these impacts, while also suggesting new directions on which to focus future empirical and theoretical work.

Community-structure models predict that the specific response of communities to physiological stress will depend on the relative susceptibility of predators and prey to increased stress levels (consumer or prey stress models: Menge and Olson 1990). These models assume a single stress axis (or parallel

axes), such that predator and prey are both responding to the same stressor (i.e. temperature) or stressors that increase in parallel (i.e. temperature stress and desiccation stress increase from low to high intertidal). In this system, however, *Katharina* seems to respond primarily to low-tide air temperatures, while *Hedophyllum* is susceptible to changes in high-tide water conditions. Kelps in general, and *Hedophyllum* in particular, are susceptible to the increased water temperatures and decreased nutrient levels that accompany El Niño events and the longer term regime shifts predicted to result from global climate change. Studies following the 1987 El Niño determined that increased water temperatures and low nutrient levels decreased growth and standing stocks of kelps from California to British Columbia (Germann 1988, Tegner et al. 1997, Dayton et al. 1998). After the 1997 El Niño, *Hedophyllum* canopy cover at two central Oregon field sites dropped below a total of $2m^2$ from pre-El Niño values of 31.8 and $24.1m^2$ per site (G. W. Allison, pers. comm.).

Atmospheric models predict that global warming will cause average surface temperatures to rise another 1.4 – 5.8°C during the next century (IPCC Report 2001). While this translates into relatively straightforward predictions for low-tide temperature conditions (affecting *Katharina*), short-term events such as ENSO and regional upwelling (in which nutrient-rich cold water is drawn onshore for short periods) can alter the local near-shore oceanic conditions that affect *Hedophyllum* at high tide. Sea surface temperature has been negatively correlated with nutrient availability in a number of studies on kelp population dynamics (Dayton et al. 1998), and long-term data sets have shown that sea surface temperatures off the California coast have increased an average of 1.5°C since 1951 (Roemmich and

McGowan 1995). Predicting how nearshore oceanic events, which may reduce (upwelling events) or compound (ENSO events) the effects of general sea-surface temperature changes on local communities, will be affected by global climate change is very difficult (reviewed in Sanford 1999a). Therefore, the stress gradients to which *Hedophyllum* and *Katharina* are responding could be parallel, opposite, or orthogonal, and could vary with time and/or geographic location. At present, we have no conceptual basis with which to make predictions about a system such as this. In order to predict the potential for major alteration of community interactions over the long term, we must carry out more community-level experiments that incorporate interactions between trophic levels. We must also recognize the potential for positive, as well as negative, interactions to play major roles in structuring communities.

Conclusions

Hedophyllum sessile structures the entire low intertidal zone through the provision of shade by controlling the distribution and abundance of understory species, including the chiton *Katharina tunicata*. Despite the classification of the low intertidal zone as a 'low stress' habitat, the association of *Katharina* with *Hedophyllum* was due to a behavioral selection of *Katharina* for shaded habitats, which ameliorated stressful temperature conditions during summertime low tides. The strength of this positive interaction varied with the level of environmental stress between years. *Hedophyllum* is a 'foundation species' in this community, and appears to influence the understory community both directly and indirectly

through the concentration of grazers underneath the canopy. This is a rare example of a non-food positive interaction between trophic levels that has significant effects on the entire community.

CHAPTER 3

COMPLEX INTERACTIONS BETWEEN TROPHIC LEVELS: POSITIVE AND NEGATIVE EFFECTS OF AN 'ECOLOGICAL DOMINANT' AND A MAJOR HERBIVORE ON COMMUNITY STRUCTURE

ABSTRACT

Natural communities are structured by complex combinations of interactions that affect the distribution and abundance of species. Because of the multiple processes that act simultaneously within and between trophic levels, examining only the net effects of an interaction may hide important details. In the low rocky intertidal zone of the Pacific Northwest, the perennial, canopy-forming kelp *Hedophyllum sessile* is considered an 'ecological dominant,' which affects community structure through the competitive suppression of understory algal species and the concentration of grazing effects of the herbivorous chiton *Katharina tunicata*. The combined effects of *Hedophyllum* and *Katharina* produce consistent patterns in the distribution and abundance of understory species, but mask important individual effects that define the community in the absence of one or the other.

To evaluate the separate and combined effects of the shade provided by the *Hedophyllum* canopy and the grazing effects of *Katharina*, I followed the understory assemblage over 14 months in 48 plots in a factorial design with two levels of shade (+ Shade or –Shade) and two levels of *Katharina* (present or

absent). I separated the effects of the *Hedophyllum* thallus from the effects of shade using artificial shades, and manipulated *Katharina* abundances with short fences. The individual effects of *Hedophyllum* (through the provision of shade) and *Katharina* (through foraging activities) on the understory algal and encrusting invertebrate assemblage are quantitatively equivalent but qualitatively very different. The presence of shade negatively affected algal abundance, but positively affected the abundance of encrusting invertebrates and mobile consumers. The presence of *Katharina* exerted strong negative effects on algae and encrusting invertebrates, but did not affect most mobile consumers. In the natural situation with both *Hedophyllum* and *Katharina*, complex chains of direct and indirect interactions masked these qualitatively different individual effects. Ultimately, the maintenance of this low zone community depends on the presence of *Hedophyllum*, which, through the provision of shade, controls the distribution and abundance of organisms on all trophic levels. Understanding the complex mechanisms that underlie seemingly straightforward patterns must be a priority if ecologists are to make predictions about community and ecosystem-level patterns in natural systems that are under increasing threats of modification on small and large scales.

INTRODUCTION

In recent years, ecologists have begun to emphasize the complex nature of species interactions, in the attempt to tease apart the network of direct, indirect, positive and negative interactions that combine to influence the abundance and distribution of species (Callaway and Walker 1997, Callaway 1997, Holmgren et al.

1997, Brooker and Callaghan 1998, Levine 2000). This appreciation for the composite nature of species interactions is partly the result of the recent focus by ecologists on the influence of positive interactions and indirect effects on community structure. By investigating the effects of positive interactions in communities, we have begun to re-evaluate old questions about the forces acting on communities and rise to the challenge of dissecting and interpreting the numerous synergistic and antagonistic forces which act simultaneously to produce patterns in nature.

Positive interactions (e.g. mutualisms, commensalisms, and habitat modification) are interactions in which the presence of one species positively affects the growth, survival, or reproduction of another (Bertness and Callaway 1994) and the benefits usually exceed the costs for both organisms (Bronstein 1994). Habitat modification, a positive interaction in which one species modifies the abiotic environment, buffering other species from harsh conditions, is now widely recognized to be important and common among plants in physically stressful environments such as deserts (Niering et al. 1963, Turner et al. 1966), alpine habitats (Carlsson and Callaghan 1991), semi-arid shrubland (Pugnaire et al. 1996a, 1996b), salt marshes (Bertness and Hacker 1994, Bertness and Yeh 1994, Callaway 1994, Hacker and Bertness 1995b, 1999), sand dunes (Shumway 2000), and rocky intertidal habitats (Bertness and Leonard 1997, Bertness et al. 1999b). Both theoretical and empirical work has emphasized, however, that the strength and even sign of species interactions varies according to external factors. Life stages (McAuliffe 1984b, Callaway 1998b, Rousset and Lepart 2000), plant density (Bertness and Yeh 1994), species-specific attributes (Callaway 1992,

1994, 1998a, Rousset and Lepart 2000), and abiotic stress levels (Bertness and Grosholz 1985, Lively and Raimondi 1987, Bertness 1989, 1991, Callaway 1992, 1998b, Bertness and Shumway 1993, Greenlee and Callaway 1996, Bertness et al. 1999a, Leonard 2000) are factors that can tip the balance, resulting in a greater influence, at least at that point in time or space, of negative or positive interactions (Bronstein 1994, Callaway 1997).

In the past 15 years, there has also been renewed interest in determining the relative importance of direct and indirect species interactions in structuring communities (Wootton 1993, 1994, Menge 1995, Callaway and Pennings 2000). Direct effects are those in which changes in abundance of a given species result from its interaction with another species (Schoener 1993, Menge 1995). Direct effects can be negative (for example, herbivory or inhibition of recruitment) or positive (such as the provision of habitat or shelter, or enhancement of recruitment). Indirect effects are those in which one species, though direct interaction with a second species, indirectly alters the abundance of a third species, although species one and three do not interact directly (Schoener 1993, Menge 1995). Both positive (i.e. indirect commensalism (Dethier and Duggins 1984), keystone predation (Paine 1966, Menge et al. 1994), and trophic cascades (Carpenter et al. 1985)) and negative (i.e. exploitation competition (Kerfoot and Sih 1987) and apparent competition (Schmitt 1987)) indirect effects have been documented in natural systems. With the blending of these two foci in ecology, an increasing number of studies are providing experimental evidence that patterns in community structure result from the net effects of simultaneous positive and

negative, direct and indirect interactions (Olff et al. 1999, Callaway and Pennings 2000, Levine 2000, Rousset and Lepart 2000).

On moderately exposed rocky shores in the Pacific Northwest, the perennial kelp *Hedophyllum sessile* forms continuous canopies over extensive areas of the low rocky intertidal zone. Dayton (1975) classified *Hedophyllum* as an 'ecological dominant,' which exerted strong effects on the understory algal community by competitively displacing a number of 'fugitive' algal species, and furnishing a protective habitat for a suite of obligate understory algal species. However, removal of the *Hedophyllum* canopy in these experiments was immediately followed by the departure of the system's major herbivore, the chiton *Katharina tunicata*, and thus attributing causation to changes in the algal assemblage was difficult. In a later study, Duggins and Dethier (1985) manipulated *Katharina* abundance and the presence of the *Hedophyllum* canopy and examined changes in the algal understory assemblage. They concluded that both *Katharina* and *Hedophyllum* had independent negative effects on the algal understory assemblage. However, because of low statistical power and the continued presence of low numbers of *Katharina* in removal areas, quantifying the relative importance of *Hedophyllum* and *Katharina* or investigating the mechanisms behind these interactions was still difficult.

In a three-year experiment in the low rocky intertidal zone in the San Juan Islands, Washington State, I documented that the *Hedophyllum* canopy maintained high *Katharina tunicata* abundances by providing shade (Chapter 2); thus localizing high grazer abundances *via* a direct positive interaction between trophic levels. I also documented several community-level patterns that suggested that

the mechanisms structuring the natural understory algal assemblage were considerably more complex than previous studies had been able to detect. In the presence of *Katharina*, shade, and not the *Hedophyllum* thallus *per se*, was the most important factor in determining the algal and encrusting invertebrate understory assemblage. In addition, although *Katharina* distribution was strongly positively affected by the presence of shade, there was no detectable 'halo' effect of increased *Katharina* abundances in -Shade areas directly adjacent to shaded areas. Lastly, a seasonal drop in *Katharina* abundance in shaded plots was accompanied by a dramatic peak in the abundance of encrusting invertebrates, a functional group that was universally rare in previous studies (Duggins and Dethier 1985).

In this study, I set out to examine the influence of the positive and negative effects of the shade provided by the *Hedophyllum* canopy and the grazing exerted by *Katharina* on low-zone community structure. I designed a factorial experiment with two levels of shade (present = *Hedophyllum* canopy or Artificial Shades) or absent (open or structure control plots) and two levels of *Katharina* (present or absent) in order to address four major questions. (1) What is the effect of shade in the absence of *Katharina*? (2) Does *Katharina* affect the understory community in the absence of shade? Since *Katharina* exhibit a dramatic behavioral preference for shaded areas, I wondered if *Katharina* maintained in –Shade areas would be able to significantly affect the algal and encrusting invertebrate assemblage, or if their importance to community structure would be reduced by physiological stress. (3) Does *Katharina* affect the understory community in the presence of shade? Algae are photosynthetic organisms, and the reduction in algal growth or

abundance by shade alone could 'swamp out' any effects of *Katharina* grazing. (4) Is the effect of Shade (shade 'only,' in the absence of *Katharina*) on community structure the same as the effect of *Katharina* (*Katharina* 'only,' in the absence of shade)? And, ultimately, what are the relative importances of positive and negative interactions in maintaining documented patterns of community structure in this system?

METHODS

Study Site and Organisms

Pile Point is located on the west side of San Juan Island, Washington, at 48°28.9' N, 123°05.7' W, 6 km south of Lime Kiln State Park and 15 kilometers east of Vancouver Island, British Columbia, across Haro Strait. The site is a rocky point with several flat benches separated by shallow subtidal channels, flanked on both sides by small cobble beaches and steep cliffs, and is the same site used for the field studies of the *Hedophyllum* / *Katharina* association in the 1980s (Dethier and Duggins 1984, Duggins and Dethier 1985). Plots used in this study were on the main point, at –0.3 m to +0.3 m tidal height.

Hedophyllum sessile (hereafter, *Hedophyllum*) dominates the low zone at Pile Point, reaching densities of 20 thalli per square meter. Each thallus consists of numerous 'dimpled' blades, up to 30cm in length, attached to a root-like holdfast. *Katharina tunicata*, (hereafter *Katharina*) the black chiton, is the major herbivore in this system. *Katharina* are generalist consumers, primarily feeding on micro and macroalgae, but also occasionally ingesting encrusting invertebrates

(Dethier and Duggins 1984, 1988, Gaines 1985, Piercy 1987). *Katharina* grow to 10cm in length as adults, and occur at densities of up to 70 individuals per square meter at Pile Point.

Experimental Enclosure Design

In June and July of 1999 I marked 48 plots, approximately 50 by 44 cm, in three blocks (16 plots each) around the main rocky bench at Pile Point. Each plot was centered on at least one mature *Hedophyllum* thallus that provided 100% canopy cover over the plot. Before manipulating plots, I conducted a baseline data survey (Time Zero) of all algal and invertebrate species. To control chiton abundances, I constructed fences (4-5 cm high) of black Vexar mesh (Norplex, Inc, ¼" mesh size) embedded in a layer of Z-spar™ marine epoxy putty (Seattle Marine) around the perimeter of each plot, after clearing the perimeter strip using a paint scraper and a scrub brush. Following fence construction, I removed all *Katharina* from inside the plots and waited at least 24 hours to allow complete polymerization of the epoxy putty.

Plots within blocks were randomly assigned to eight treatments, in a factorial design with two levels of Shade (+ Shade = *Hedophyllum* canopy or Artificial Shades, - Shade = Structure Control or No Structure plots) and two levels of *Katharina* grazing (+ or -*Katharina*), with two replicates of each treatment per block (n = 16 plots / block, Fig 3.1). +*Katharina* plots were stocked with six adult (6.5 – 8.5cm) *Katharina* individuals (the median number of *Katharina* individuals in the plots during the Time 0 survey). *Katharina* were 'transplanted' into experimental plots after prying them off the rock using a table knife wedged under

the foot; individuals injured during the transplant process were not used for the experiment.

For the *Hedophyllum* treatments, *Hedophyllum* thalli were not disturbed; for the other treatments, all *Hedophyllum* adults and recruits were removed by prying the holdfast off the rock surface with a knife. Artificial shades were assembled from vinyl-coated wire letterbaskets (34 x 29 x 7cm, Cascade Office Supply Co.) that were turned upside down and bolted to the rock surface (using washers and stainless steel screws screwed into wall anchors sunk into holes drilled into the rock). Three sheets of black Vexar™ mesh (¼" inch mesh size) were attached to the top of the structure (the bottom of the upturned letterbasket) using cable-ties, to create a shade-producing roof. Structure controls were constructed by removing the structure roofs (i.e. the basket bottoms), to control for confounding effects of the letterbasket materials or of the physical structure of the metal frame. Both Artificial Shades and Structure Controls had 'windows' (5 cm wide by 5cm high) on 2 sides to allow *Katharina* to move through them. Exposed metal edges were prevented from rusting by the application of silicon sealant. 'No Structure' plots were not manipulated following *Hedophyllum* removal.

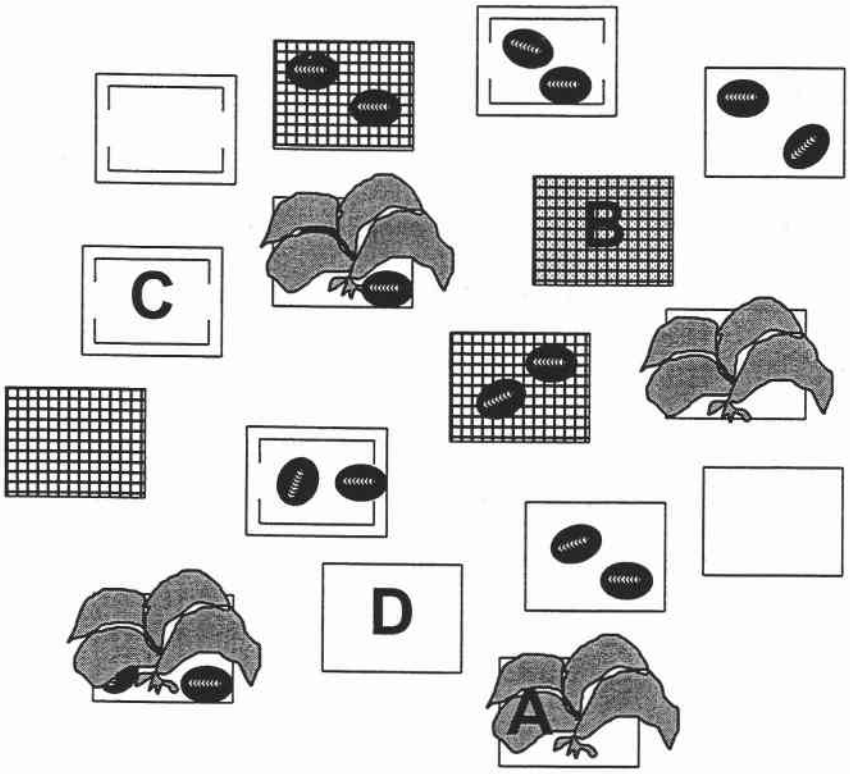

Figure 3.1 Experimental design. Plots (not to scale) were surrounded by mesh fences to enclose or exclude *Katharina* (6 per plot). Pictured is one block (of three). **(A)** *Hedophyllum* **(B)** Artificial Shade **(C)** Structure Controls **(D)** No Structure. A and B = +Shade, C and D = -Shade. See text for details.

Surveys and Experimental Maintenance

Blocks were established in June (Blocks 1 and 2) and July (Block 3) 1999. Beginning in August 1999, I conducted surveys approximately every 30 days, as weather conditions permitted. I marked a smaller survey area, 34cm by 29cm, in the center of each enclosure or exclosure plot, and using a 34cm by 29cm quadrat divided into 25 equal squares, I visually estimated percent cover of all algal and encrusting invertebrates, identified to species (or genus in some cases where individual species were difficult to reliably identify). Percent cover of primary

space (rock surface covered by a holdfast or colony) and canopy (secondary cover = portion of plot covered by the erect portion of an algal thallus) were estimated separately. Percent cover of microalgae (mostly diatoms) was visually estimated using the quadrat, and each plot was assigned a measure of microalgal thickness: light, medium, heavy, and very heavy (all algal crusts completely obscured). Because the abundance of fleshy algal crusts could not be sampled reliably over time without removing microalgae, they were not quantified. At each survey, all mobile animal individuals were identified to genus or species and counted; limited time in the low intertidal zone precluded measurement of sizes.

Katharina abundances were assessed every low tide series (approximately once every 2 weeks in the summer, at least once a month in the winter). Individuals were removed from or added to plots as necessary to maintain the original experimental densities, which were maintained at 6 (± 2) *Katharina* for the +*Katharina* treatments and effectively 0 in the –*Katharina* treatments for the duration of the experiment. The number of *Katharina* 'invaders' into –*Katharina* plots was very low: Artificial Shade plots = total of 9 individuals, *Hedophyllum* = 6, and –Shade (No Structure and Structure Control) plots = 26, over 14 months.

Taxonomic and Functional Groupings

For data analysis, I grouped species into *a priori* classes on the basis of a combination of ecological, taxonomic and life history traits (Table 3.1, Table 3.2). Separate analyses were carried out for algae and encrusting invertebrates ("AEI") and mobile animals (MA). Encrusting invertebrates were analyzed with algae because they can occupy continuous areas of rock surface and thus compete with

algae for primary space, they must disperse into new areas in ways that are ecologically similar to algae, and they are consumed by *Katharina* (Dethier and Duggins 1984, 1988). Three classes (egg masses, sessile worms, and filter feeders) were not included in any statistical analysis, because they were rare and could not be logically assigned to either the mobile animals or AEI group.

Algal abundance was analyzed as total percent cover (primary + secondary + tertiary) in order to best estimate total abundance (for example, canopy forming thalli which also took up large amounts of primary space have higher values than canopy forming thalli with small holdfasts). Due to layering, total cover for an individual plot can exceed 100%. Microalgal abundance was calculated as total space occupied (percent cover) multiplied by a numerical value assigned to the field estimate of the thickness of diatom cover (Light = 1, Medium = 1.5, Heavy = 2, Very Heavy = 2.5).

Table 3.1 Algal and encrusting invertebrate groups. Species are listed in order of relative abundances in plots. **Groups included in totals and multivariate analyses. *Groups included in abundance totals. Unmarked groups were not analyzed. See text for details.

Algal / Encrusting Invertebrate Group	Species
Green Algae **	*Ulva fenestrata* *Acrosiphonia mertensii* *Codium fragile*
Microalgae **	Diatoms (several species) Cyanobacteria *Navicula sp.*
Finely Branched Red Algae **	*Microcladia borealis* *Polysiphonia hendryi* (several subspecies) *Callithamnion pikeanum* *Scagelia sp.* *Ceramium spp.*
Foliose / Thickly Branched Red Algae **	*Halosaccion glandiforme* *Mastocarpus papillatus* *Cryptopleura lobulifera* *Callophyllis sp.* *Mazzaella splendens* *Mazzaella flaccida* *Delesseria decipiens* *Odonthalia floccosa*
Articulated Coralline Algae *	*Corallina vancouveriensis* *Bossiella plumosa* *Corallina officinalis* *Calliarthron tuberculosum*
Encrusting Invertebrates **	*Halichondria sp.* (at least 2 species) *Schizoporella sp.* unknown hydroid
Crustose Algae	*Ralfsia sp.* *Mastocarpus papillatus (Petrocelis)* Coralline crusts including: *Pseudolithophyllum sp.* *Lithophyllum sp.* *Lithothamnion sp.*

Table 3.1 Continued

Fleshy Brown Algae*	*Alaria marginata* *Egregia menziesii* *Fucus gardneri* *Laminaria setchellii* *Costaria costata* *Nereocystis luetkeana* *Desmarestia ligulata* *Desmarestia viridis*
Rare Algae*	*Leathesia difformis* *Colpomenia sp.* *Mesophyllum conchatum*

Katharina *Diet Data*

In July and August 2000, I studied the diet of *Katharina* removed from each of the four experimental treatments (Artificial Shade, *Hedophyllum*, Open and Structure Controls) and compared it to *Katharina* in the general population (not enclosed in experimental plots). On several sequential low tide series, I marked chitons in experimental plots with colored nail polish on the exposed portion of one dorsal valve. On the following low tide series, I collected three marked individuals from each enclosure plot (total n over 5 tide series = 72 individuals, 18 from each experimental treatment). Collecting marked individuals ensured that they had been maintained in a given treatment for at least two weeks. Since preliminary observations indicated that gut passage time was no more than 3 days (J. Burnaford, personal observation), this was considered long enough to provide an unbiased estimate of diet in a given treatment. Immediately after collection, *Katharina* were transported in individual flow-through containers in a seawater-filled cooler to Friday Harbor Labs where they were held in flowing seawater tanks.

Within 6 hours of collection, one *Katharina* individual was removed for analysis of gut contents (n = 24 individuals, 2 each in 3 blocks in each of 4 treatments). The stomach and intestines were dissected and the contents spread out on glass slides to (approximately) uniform thickness. The slides were examined under a dissecting microscope against a background of a 1cm x 1cm grid. Gut contents were classified into groups corresponding to those used for the analysis of survey data, and quantified by visually estimating the portion of each 1cm by 1cm square that was covered by each group. These data were summed for each individual and divided by the total number of squares covered by gut contents for that individual; data are thus expressed as percent of the total gut contents for an individual chiton.

In order to increase sample sizes without substantially impacting the *Katharina* population, I maintained the remaining two chitons from each plot in the lab for 4 days in individual containers, and collected fecal pellets from the containers twice daily. Fecal pellets were broken apart, spread on glass slides, and data were collected as for gut contents. *Katharina* digestion appears to be extremely inefficient. Numerous algal genera could be identified in fecal pellets (e.g. *Ceramium*, *Polysiphonia*, *Microcladia,* and *Ulva*) and on several occasions, live chironomid larvae and gravid copepods were discovered in fecal pellets. Because food items are only minimally processed during the digestion process, I am confident that my ability to categorize fecal contents is no less accurate than my ability to categorize gut contents. Since my categories were broad, I believe these data are comparable. For analysis, data from all three individuals within a plot were treated as equivalent replicates.

Table 3.2 Animal groups. Species are listed in order of relative abundances in plots. **Groups included in multivariate analysis and mobile animal totals. *Groups included in abundance totals. Unmarked groups were not included in analysis. See text for details.

Mobile Animal Group	Species
Chitons **	*Lepidochitona dentiens*
	Mopalia ciliata
	Mopalia lignosa
	Mopalia mucosa
	Tonicella lineata
	Lepidozona mertensii
	Cryptochiton stelleri
Snails **	*Calliostoma ligatum*
	Margarites pupillus
	Granulina margaritula
	Lacuna vincta
	Lacuna variegata
Whelks **	*Nucella lamellosa*
	Searlesia dira
	Amphissa columbiana
	Amphissa versicolor
	Bittium eschrichtii
	Nucella emarginata
	Nucella canaliculata
	Ocenebra lurida
	Ocenebra interfossa
	Fusitriton oregonensis
Seastars **	*Leptasterias hexactis*
	Henricia sp. (newly described)
	Henricia leviuscula
Crabs **	*Pugettia gracilis*
	Pagurus hirsutiusculus
	Pagurus samuelis
	Pagarus granosimanus
	Cancer oregonensis
	Cancer productus
	Hemigrapsus nudus
	Hemigrapsus oregonensis
	Petrolisthes cinctipes
	Petrolisthes eriomerus
	Pugettia producta
	Cryptolithodes sitchensis
Anemones **	*Anthopleura elegantissima*
	Epiactis spp.
	Urticina spp.

Table 3.2 Continued

Limpets ** (includes one non-limpet species)	*Lottia pelta* *Tectura scutum* *Acmaea mitra* *Diadora aspera* *Onchidella borealis*
Fishes*	*Oligocottus maculosus* *Gobiesox maeandricus* *Artedius lateralis* *Pholis spp.*
Mobile Worms* (annelids, nemerteans, flatworms)	*Nereis spp.* *Amphiporus spp.* *Tubulanus spp.* *Notoplana acticola*
Nudibranchs*	*Hermissenda crassicornis* *Archidoris montereyensis* *Dirona albolineata* *Dendronotus spp.* *Rostanga pulchra*
Urchins*	*Strongylocentrotus droebachiensis* *Strongylocentrotus purpuratus*
Cucumbers / Detritivores*	*Pseudocnus lubrica* (prev. *Cucumaria lubrica*) *Cucumaria miniata* various sipunculans
Isopods*	*Idotea wosnesenskii* unknown isopod
Filter Feeders	*Crepidula adunca* *Modiolus modiolus* various tunicate species
Sessile Worms	*Dodecaceria fewkesi* *Serpula vermicularis* *Spirorbis* *Schizobranchia spp.*
Egg Masses	*Nucella* *Archidoris* *Rostanga pulchra* *Hermissenda crassicornis* unknown egg masses

Statistical Analysis

All analyses were carried out using SAS Version 8.0 and JMP Version 4 (SAS Institute, Inc). Repeated Measures, MANOVA, and doubly-multivariate analyses were programmed using Proc GLM in SAS. For all analyses, model fit and heterogeneity of variances were examined by visually inspecting residual by predicted value plots, residual normal QQ plots, and plots of variance between groups. Shapiro-Wilk and Kolmogorov-Smirnov tests for normality of data within groups were conducted for most analyses. If required, data were transformed to meet model assumptions. For percent cover data, the logarithmic transformation corrected most problems of heterogeneity of variance. I chose the logarithmic transformation instead of the arcsine square-root transformation because the latter requires that all cover values are truncated at 100%, and many of my experimental plots had cover values exceeding 100% due to layering. For count data, both logarithmic and square-root transformations were used. Mahalanobis and Jackknife distances (an alternative outlier distance measure which uses estimates of the mean, standard deviation and correlation matrix that do not include the observation itself) were calculated in JMP to identify multivariate outliers within groups for multivariate analyses, and groups were examined using 3-D plots. When outliers were identified, analyses were run with and without the outliers. In every case, exclusion of the outlier did not change the results, and as there is no reason to assume that any of the experimental plots are not part of the population of interest, results are presented with all plots included.

I analyzed baseline survey data (Time 0) using analysis of variance techniques to assess the null hypothesis of no difference between treatments at

the start of the experiment. Models were fit with treatment and block as main effects (fixed and random, respectively).

I first tested for significant differences between treatments within the two shade classes (+ Shade = Artificial Shade and *Hedophyllum* plots, -Shade = No Structure and Structure Control plots) by comparing the two sets of treatments in separate repeated measures analyses. If group abundances did not differ between treatments within a shade class, treatments were combined for subsequent tests of the effect of shade. Green algae were the only group to differ among –Shade treatments. Green algal abundance was consistently lower in Structure Control plots than in No Structure plots (contrast $F = 6.30$, $p = 0.02$). Abundances of all other AEI and animal groups were similar between –Shade no structure and structure control plots (all Treatment and Block x Treatment tests, $p \geq 0.11$). Abundances of green algae and encrusting invertebrates were different between Artificial Shade and *Hedophyllum* plots (see Results). Since running analyses (to test for shade effects) using only Artificial Shade and Structure Control plots did not change any conclusions, to maintain equal sample sizes across response variable groups, I still combined these two groups into +Shade (Artificial Shade + *Hedophyllum*) and -Shade (No Structure and Cage Control) plots for analysis. Abundances of all other AEI or animal groups were similar between Artificial Shade and *Hedophyllum* plots (all Treatment and Block x Treatment effect tests, $p \geq 0.12$).

Subsequent models were fit using main effects (Shade, *Katharina* and Block) and interactions (Shade x *Katharina*, Shade x Block, *Katharina* x Block, and Shade x *Katharina* x Block). Shade and *Katharina* were considered fixed factors,

and Block a random factor, using the Method of Moments (Expected Mean Squares) technique (Milliken and Johnson 1992, SAS Institute 1994). One invertebrate and one algal group exhibited significant block by treatment interactions (whelks and articulated coralline algae, respectively) and were not included in multivariate analyses. In all other models, block by treatment interaction terms were not significant ($p >> 0.1$), and were dropped, since the effect of block was not of interest. Dropping the interaction terms either did not affect model fit or, in most cases, dramatically improved it.

Individual repeated measures analyses of the major AEI ($n = 6$) and mobile animal groups ($n = 7$) were carried out following a significant repeated measures MANOVA analysis of each class. Significance levels were adjusted using the Bonferroni method ($\alpha = 0.05/6 = 0.008$, $0.05 / 7 = 0.007$). For multivariate analyses, all reported values are based on Wilks' Lambda statistics unless otherwise noted. In some cases, due to high heterogeneity of variances, Pillai's Trace statistic values are reported as noted. Standardized canonical coefficients are presented for groups included in MANOVA analysis (Scheiner 1993). These coefficients, calculated from eigenvectors, indicate the unique contribution of a given variable to the differences among groups, and are analogous to partial regression coefficients. The magnitude and sign of these coefficients can change depending on what other response variables are included in the analysis, and therefore the coefficients should be interpreted cautiously and are used only for illustrative purposes.

RESULTS

Survey Zero

Mean *Katharina* density at the start of the experiment was 5.5 animals per $0.099m^2$ plot (range = 1 to 11). Because enclosure areas were larger than survey plots (enclosures = $0.22m^2$), the density in experimental enclosures (6 animals / enclosure) was slightly lower than the mean value.

There were no detectable differences between experimental plots at the start of the experiment for most response variables. *Hedophyllum* canopy cover, *Katharina* abundance, total algal and encrusting invertebrate (AEI) cover, total number of mobile animals, total number of mobile animal species, and the composition of the AEI and mobile animal assemblage (MANOVA on 7 (AEI) or 6 (animals) major groups) were all similar between plots assigned to different treatments ($p \geq 0.12$). Algal and encrusting invertebrate species richness was marginally different between plots assigned to different shade treatments ($p = 0.04$). The mean number of AEI species in plots assigned to +Shade treatments was 9.5, vs. 10.8 in plots assigned to -Shade treatments. However, AEI species richness did not differ among plots assigned to different *Katharina* treatments or to a *Katharina* treatment within a shade treatment (*Katharina* effect, $p = 0.16$; Shade x *Katharina* interaction, $p = 0.67$). With the exception of total AEI cover, all response variables differed between blocks ($p < 0.01$).

Effect of Hedophyllum *Thallus*

In order to separate effects of the *Hedophyllum* thallus (i.e. mechanical or chemical effects) from the effects of the shade cast by the *Hedophyllum* blades, I compared the understory in the *Hedophyllum* treatment to that in the Artificial Shade treatment. The effects of the *Hedophyllum* thallus *per se* on the understory community were very weak, a result consistent with earlier experiments (Chapter 2). Only two AEI groups were less abundant in *Hedophyllum* plots than Artificial Shade plots. The *Hedophyllum* thallus negatively affected the abundance of green algae (Fig 3.2a; RM ANOVA, Between Subjects effect, $F = 6.78$, $p = 0.018$) and encrusting invertebrates (Fig 3.3a; RM ANOVA, Between Subjects Treatment effect, $F = 64.2$, $p < 0.0001$). The negative effect of the *Hedophyllum* thallus on green algae was only evident in the absence of *Katharina* (Fig 3.2b, contrast *Hedophyllum* vs. Artificial Shade plots; *+Katharina*, $p = 0.37$; *-Katharina*, $p = 0.013$). However, for encrusting invertebrates, the negative effect of *Hedophyllum* was evident both in the presence and absence of *Katharina* (Fig 3.3b, Hs vs. AS plots, both + and *–Katharina*, $p \leq 0.0019$). No other algal or encrusting invertebrate groups were affected by the presence of the *Hedophyllum* thallus ($p \geq 0.12$).

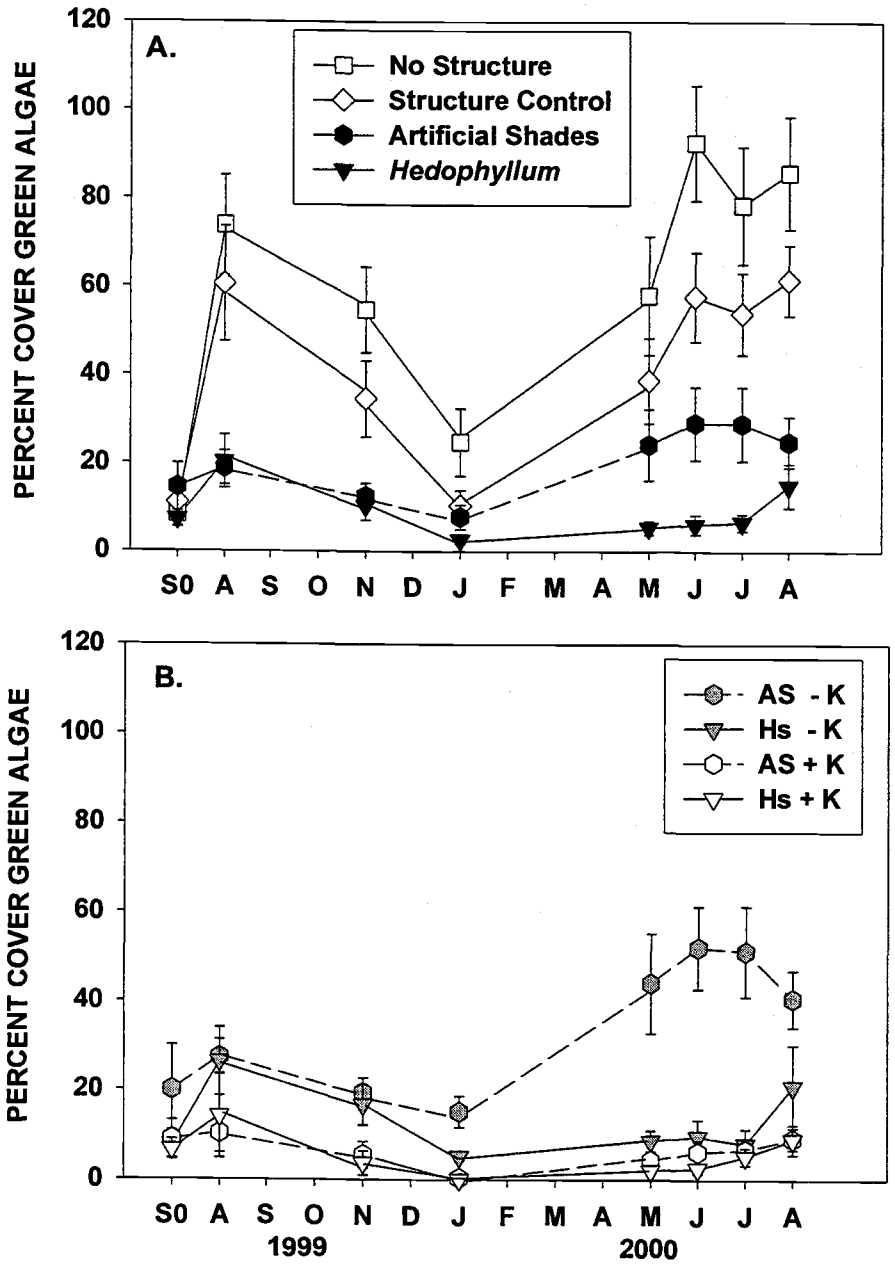

Figure 3.2 Green algal abundance in individual treatments. Percent cover (primary + secondary, mean ± SEM). S0 = Baseline survey, before manipulation. **(A)** Four 'shade' treatments (n = 12 plots / treatment; see Fig 3.1). **(B)** Artificial Shade (AS) and *Hedophyllum* (Hs) treatments. K = *Katharina* (n = 6 plots per treatment combination).

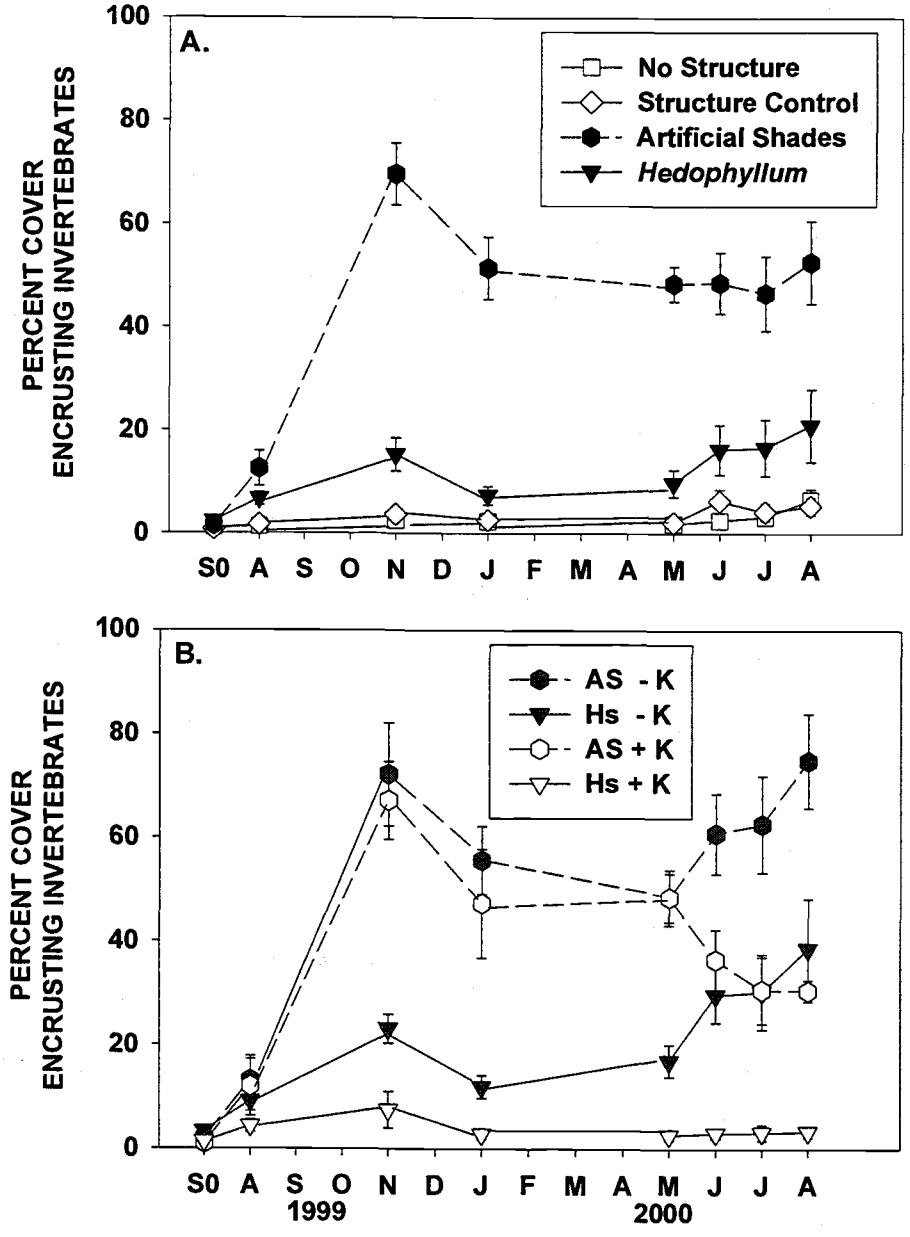

Figure 3.3 Encrusting invertebrate abundance in individual treatments. Percent cover (mean ± SEM). S0 = Baseline survey, before manipulation. **(A)** Four 'shade' treatments (n = 12 plots / treatment; see Fig 3.1). **(B)** Artificial Shade (AS) and *Hedophyllum* (Hs) treatments. K = *Katharina* (n = 6 plots per treatment combination).

Algal and Encrusting Invertebrate (AEI) Assemblage: Abundance

I assessed the effects of shade and *Katharina* on the AEI assemblage can using three metrics: the effect on the total macroalgal and encrusting invertebrate (macro-AEI) and microalgal cover, the effect on the total number of species (species richness) and the effect on the abundance of individual AEI groups. In addition, I evaluated the effects of shade and *Katharina* on the structure of the assemblage by examining the combined response of several AEI groups over time using repeated measures MANOVA.

Total macro-AEI cover was negatively affected by both shade and *Katharina* (Fig 3.4a; Table 3.3a, Between subjects Shade and *Katharina* effects). The effect of shade was strong in both summers but weak in the winter (Table 3.3a, Time x Shade $p < 0.0001$; Table 3.3b, Shade effect). The effect of *Katharina* was strongly negative and constant over time in –Shade plots, and became more negative over time in +Shade plots (Fig 3.4a; Tables 3.3a, b; *Katharina* effects). However, despite these different temporal trajectories, the overall effect of *Katharina* was similar in both shade treatments (Table 3.3a, Between subjects, Shade x *Katharina* $p = 0.55$); at the end of the experiment *Katharina* reduced cover (relative to –*Katharina* plots) by 64% in the absence of shade, and 66% under shades (Fig 3.4a).

Surprisingly, the individual effects of shade and *Katharina* on AEI cover were not distinguishable overall (Fig 3.4a, Table 3.3a; contrast –S +K vs. +S -K). Across all surveys, AEI cover in 'Shade only' plots averaged 80% to 103% (95% C.I.) and in '*Katharina* only' plots, 86% to 102%.

AEI species richness, measured at the end of the experiment, showed the same patterns as algal abundance: highest in –Shade –*Katharina* plots, lowest in +Shade +*Katharina* plots (Fig 3.4b) and negatively and independently affected by both Shade and *Katharina* (ANOVA; Shade effect $p = 0.008$; *Katharina* effect, $p < 0.0001$; Shade x *Katharina* interaction $p = 0.4084$). Again, the individual effects of *Katharina* and Shade were similar (Fig 3.4b; 95% C.I. for mean species richness; 'Shade only' = 9 – 11 species, '*Katharina* only' = 9-13 species; $p > 0.05$).

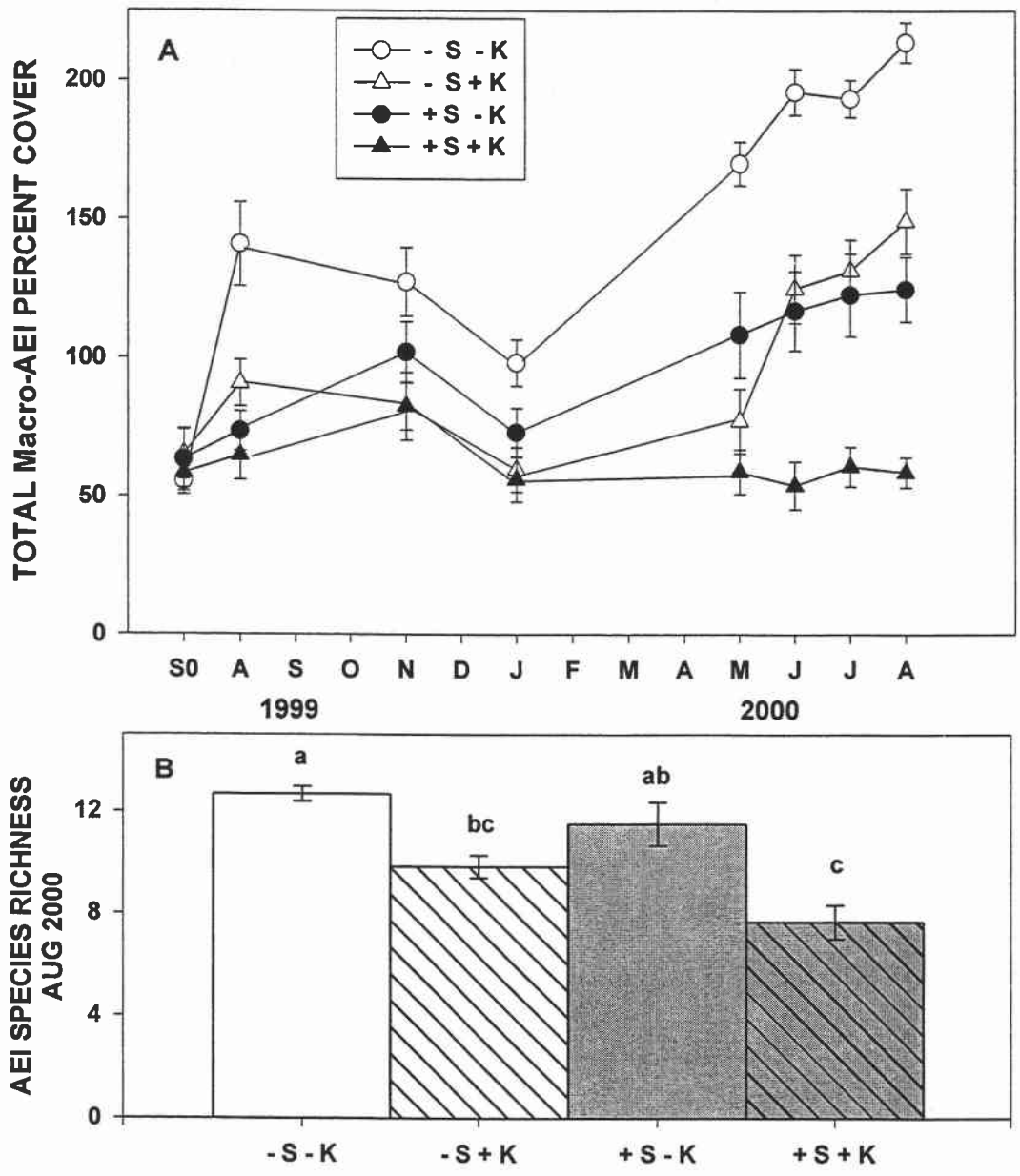

Figure 3.4 Effect of shade and *Katharina* on total macroalgal and encrusting invertebrate (AEI) abundance and species richness. S = Shade, K = *Katharina*. **(A)** Percent cover (secondary + primary) July 1999 (Survey 0) through August 2000 (mean ± SEM, n = 12 plots per treatment combination). **(B)** Number of AEI species: August 2000 (mean ± SEM, n = 12 plots per treatment combination). Treatments with the same letter are not significantly different (Tukey-Kramer, $p > 0.05$).

Table 3.3 Repeated measures analysis of variation in total macroalgal and encrusting invertebrate cover as a function of shade and *Katharina* grazing. Log-transformed percent cover data (secondary + primary) from August and November 1999, and January, May, and August 2000. Mauchly's criterion for homogeneity of variance-covariance matrices (or "sphericity" – necessary to validate the univariate approach to repeated measures ANOVA) was not met ($p << 0.05$), so only multivariate analyses are presented. **A.** Bold-faced p-values for treatment effects indicate significance at $\alpha = 0.05$. **B.** Univariate tests of treatment differences in *a priori* selected surveys. Bold-faced p-values for treatment effects indicate significance at $\alpha = 0.017$, adjusted for multiple comparisons.

A. Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	10.16	15.31	**0.0003**
Katharina	1	15.10	22.76	**< 0.0001**
Block	2	0.89	1.34	0.2722
Shade x *Katharina*	1	0.24	0.36	0.5505
Error	42	0.66		
Contrast –S +K vs. +S -K	1	0.24	0.37	0.5473

Within Subjects (change in effect over time)	N df	D df	Wilks λ	F	P
Time	4	39	0.28	25.29	**< 0.0001**
Time x Shade	4	39	0.43	13.16	**< 0.0001**
Time x *Katharina*	4	39	0.70	4.23	**0.0062**
Time x Block	8	78	0.37	6.34	< 0.0001
Time x Shade x *Katharina*	4	39	0.59	6.89	**0.0003**
Error (Time)	16				
Time x (-S +K vs. +S –K)	4	39	0.60	6.47	**0.0004**

B. Univariate Analyses: Effect of Season

Survey	Source	df	MS	F	P
August 1999	Shade	1	3.12	22.26	**< 0.0001**
	Katharina	1	1.16	8.29	**0.0063**
	Block	1	1.21	8.62	0.0007
	Shade x *Katharina*	2	0.18	1.28	0.2651
	Error	42	0.14		
January 2000	Shade	1	0.49	1.41	0.2409
	Katharina	1	2.77	8.03	**0.0071**
	Block	2	0.04	0.10	0.9036
	Shade x *Katharina*	1	0.27	0.78	0.3817
	Error	42	0.34		
August 2000	Shade	1	7.20	68.36	**< 0.0001**
	Katharina	1	4.03	38.26	**< 0.0001**
	Block	2	0.12	0.17	0.8431
	Shade x *Katharina*	1	0.43	4.12	0.0488
	Error	42	0.11		

AEI Assemblage: Composition

In addition to their effects on AEI abundance, Shade and *Katharina* also strongly affected the structure of the AEI assemblage (Table 3.4; Shade and *Katharina* effects) and their effects were again independent (Shade x *Katharina* p = 0.44). However, although the individual effects of Shade and *Katharina* on AEI abundance were equivalent, they affected the structure of the assemblage in different ways (Table 3.4; contrast –S +K vs. +S -K, $p < 0.0001$).

Table 3.4 Effect of shade and *Katharina* on the algal and encrusting invertebrate assemblage. RM MANOVA on log-transformed percent cover of five AEI groups (GRE = green algae, FTR = foliose /thickly branched red algae, FNR = finely branched red algae, MIC = microalgae, ENC = encrusting invertebrates). Bold-faced p-values for treatment effects indicate significance at $\alpha = 0.05$. The magnitude of a group's canonical coefficient (analogous to partial regression coefficients) indicates its relative contribution to the differences among treatments. Similar signs of coefficients indicate positive correlation between groups.

Multivariate Analysis

Source	**N df**	**D df**	**Wilks' λ**	**F**	**P**
Between Subjects (average effect over time)					
Shade	5	38	0.16	40.28	**< 0.0001**
Katharina	5	38	0.18	34.72	**< 0.0001**
Block	10	76	0.49	3.26	0.0015
Shade x *Katharina*	5	38	0.89	0.98	0.4403
Contrast (-S +K vs. +S –K)	5	38	0.35	13.94	**< 0.0001**
Canonical Coefficients	***GRE***	***FTR***	***FNR***	***MIC***	***ENC***
Shade	1.67	0.42	0.46	0.21	- 0.75
Katharina	2.18	0.65	0.18	- 0.60	- 0.09
Within Subjects (change in effect over time)	**N df**	**D df**	**Wilks' λ**	**F**	**P**
Time	20	23	0.05	23.17	**< 0.0001**
Time x Shade	20	23	0.16	6.28	**< 0.0001**
Time x *Katharina*	20	23	0.11	9.67	**< 0.0001**
Time x Block	40	46	0.12	2.18	0.0057
Time x Shade x *Katharina*	20	23	0.39	1.82	0.0845
Time x (-S +K vs. +S –K)	20	23	0.13	7.46	**< 0.0001**

AEI Composition I: Effects of Shade

All four algal groups (green algae, foliose red algae, finely branched red algae and microalgae) were negatively affected by the presence of shade (Fig 3.5a, b, c; Fig 3.6; Tables 3.5-3.8, Between subjects Shade effects). While the abundance of all three macroalgal groups increased during the summer 'growing season' in –Shade plots, abundance fluctuated little in +Shade plots. This 'damping' resulted in a seasonal pattern of increased shade effects during the summer for all three groups, although the effect did not manifest itself until the second year of the experiment for finely branched red algae, which recruited into plots over the fall and winter (Fig 3.5a,b,c; Table 3.5 – 3.8; all Time x Shade $p \leq 0.0053$).

Microalgae have much higher rates of growth and production than macroalgae (Duggins and Dethier 1985), a difference that is reflected in the greater temporal variability of microalgal abundances. Although the effect of shade on microalgae was negative overall, the effect seemed to flip from a clear negative effect to a potentially positive effect in May 2000 (Fig 3.6, Table 3.8, Shade and Time x Shade effects). The effect of shade on microalgae contributed little to the overall quantification of the effect of shade on the assemblage (Table 3.4, canonical coefficient MIC = 0.21).

Figure 3.5 Effect of shade and *Katharina* on percent cover of three macroalgal groups. Percent cover (primary + secondary) July 1999 – August 2000 (mean ± SEM, n = 12 plots per treatment combination; see Fig. 3.1). S0 = Baseline survey. S = Shade, K = *Katharina*. **(A)** Green Algae **(B)** Foliose and Thickly Branched Red Algae **(C)** Finely Branched Red Algae (See Table 3.1 for group details).

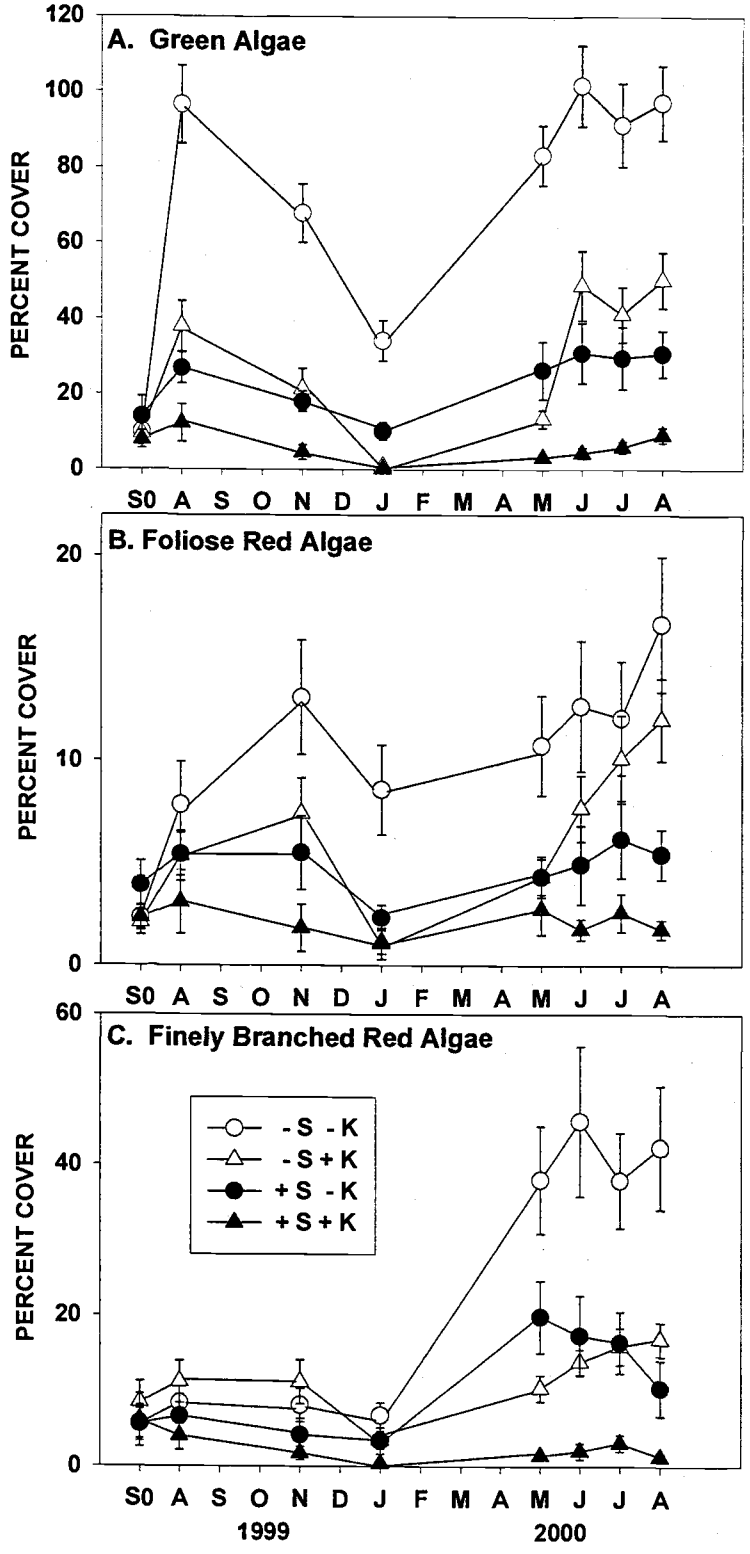
A. Green Algae
B. Foliose Red Algae
C. Finely Branched Red Algae
PERCENT COVER
120
100
80
60
40
20
0
20
10
0
60
40
20
0
S0 A S O N D J F M A M J J A
1999
2000
- S - K
- S + K
+ S - K
+ S + K

Figure 3.5

Table 3.5 Repeated measures analysis of effects of shade and *Katharina* grazing on green algal abundance, August 1999 – August 2000. Mauchly's criterion was not met (p = 0.003) so multivariate analyses are presented. Bold face p-values indicate that the factor is significant at α = 0.008 for repeated measures analysis (adjusted for comparisons of multiple algal species).

Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	106.07	93.73	**< 0.0001**
Katharina	1	149.22	131.86	**< 0.0001**
Block	2	4.14	3.66	0.0343
Shade x *Katharina*	1	0.06	0.05	0.8227
Error	42	1.13		
Contrast (-S +K vs. +S –K)	1	1.84	1.62	0.2097

Source	N df	D df	Wilks' λ	F	P
Within Subjects (change in effect over time)					
Time	4	39	0.10	86.01	**< 0.0001**
Time x Shade	4	39	0.69	4.35	**0.0053**
Time x *Katharina*	4	39	0.38	16.09	**< 0.0001**
Time x Block	8	78	0.60	2.87	0.0074
Time x Shade x *Katharina*	4	39	0.81	2.23	0.0831
Time x (-S +K vs. +S –K)	4	39	0.36	17.04	**< 0.0001**

Encrusting invertebrate cover was higher in +Shade plots than –Shade plots throughout the experiment (Fig 3.7; Table 3.9, Between subjects Shade effect). Therefore, in contrast to the negative effects on the algal groups, shade had a strong positive effect on the abundance of encrusting invertebrates (Table 3.4, canonical coefficient ENC = -0.75, opposite in sign to the algal coefficients, and second largest). Encrusting invertebrate colonies covered up to 94% of the substratum in +Shade plots (38 – 94% in Artificial Shade plots, 1 – 61% in *Hedophyllum* plots), but were virtually absent from –Shade plots for the duration of the experiment. Those colonies that did begin to grow in –Shade plots in 2000 were limited to the spaces between dense algal holdfasts (of upright coralline

algae or finely branched red algae such as *Microcladia*; J. Burnaford, personal observation).

Table 3.6 Repeated measures analysis of effects of shade and *Katharina* grazing on the abundance of foliose and thickly branched red algae, August 1999 – August 2000. Mauchly's criterion was not met (p = 0.001) so multivariate analyses are presented. Bold face p-values indicate that the factor is significant at $\alpha = 0.008$.

Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	38.20	26.99	**< 0.0001**
Katharina	1	26.68	18.85	**< 0.0001**
Block	2	10.11	7.14	0.0021
Shade x *Katharina*	1	0.13	0.09	0.7673
Error	42	1.42		
Contrast (-S +K vs. +S –K)	1	0.52	0.36	0.5495

Source	N df	D df	Wilks' λ	F	P
Within Subjects (change in effect over time)					
Time	4	39	0.28	25.76	**< 0.0001**
Time x Shade	4	39	0.46	11.44	**< 0.0001**
Time x *Katharina*	4	39	0.82	2.08	0.1014
Time x Block	8	78	0.79	1.22	0.3002
Time x Shade x *Katharina*	4	39	0.65	5.28	**0.0017**
Time x (-S +K vs. +S –K)	4	39	0.55	7.98	**< 0.0001**

AEI Assemblage Composition II: Effects of Katharina

Katharina had strong negative effects on the cover of all three macroalgal groups and encrusting invertebrates, while having more variable effects on the abundance of microalgae. *Katharina* affected the cover of green algae most strongly (Fig 3.5a; Table 3.4, canonical coefficient 2.18 (largest); Table 3.5, *Katharina* effect). Foliose and finely branched red algae were generally less abundant than green algae, but were also reduced by *Katharina* (Fig 3.5b, c; Table

3.4, coefficients same sign as GRE, Tables 3.6, 3.7, *Katharina* effect). The effects of *Katharina* on the abundance of green and finely branched red algae increased with increasing cover in –*Katharina* plots (Fig 3.5a, c; Tables 3.5, 3.7; Time x *Katharina* $p < 0.0001$).

Table 3.7 Repeated measures analysis of effects of shade and *Katharina* grazing on finely branched red algal abundance, August 1999 – August 2000. Mauchly's criterion was just met ($p = 0.06$) so univariate analyses are presented with Huynh-Feldt Epsilon adjusted probabilities. Bold face p-values indicate that the factor is significant at $\alpha = 0.008$.

Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	79.98	36.86	**< 0.0001**
Katharina	1	27.17	12.52	**0.0010**
Block	2	5.85	2.69	0.0793
Shade x *Katharina*	1	4.25	1.96	0.1692
Error	42	2.17		
Contrast (-S +K vs. +S –K)	1	6.96	3.21	0.0805
Within Subjects (change in effect over time)				
Time	4	15.19	30.78	**< 0.0001**
Time x Shade	4	2.72	5.50	**0.0003**
Time x *Katharina*	4	5.87	11.90	**< 0.0001**
Time x Block	8	1.66	3.37	0.0013
Time x Shade x *Katharina*	4	0.25	0.51	0.7294
Error (Time)	168	0.49		
Time x (-S +K vs. +S –K)	4	1.99	4.03	0.0038

Katharina reduced the abundance of microalgae and encrusting invertebrates (Fig 3.7, 3.8, Tables 3.8 and 3.9, Between subjects *Katharina* effect). However, unlike the constant negative effect of *Katharina* on macroalgae, the effect of *Katharina* changed over time for both of these groups (Table 3.4, negative canonical coefficients indicate that responses differ from those of macroalgae;

Tables 3.8, 3.9; Within Subjects, Time x *Katharina* effect). The effect of *Katharina* on microalgae seemed to flip from negative to positive in early 2000 (Fig 3.6). The effect of *Katharina* on encrusting invertebrate abundance was negative throughout the course of the experiment, but increased in strength over time, until in August 2000, encrusting invertebrate abundances in +Shade +*Katharina* plots were very similar to those in –Shade –*Katharina* plots (Fig 3.7).

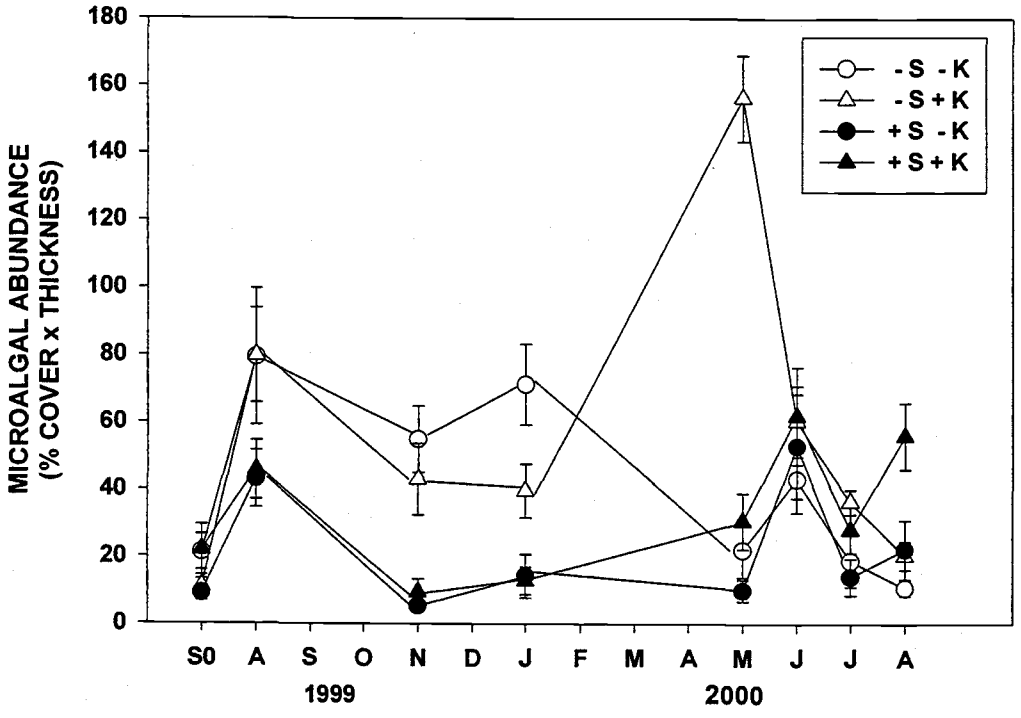

Figure 3.6 Effect of shade and *Katharina* on microalgal abundance. Abundance (percent cover x thickness rating; see Methods for details) July 199 – August 2000 (mean ± SEM, n = 12 plots per treatment combination; See Fig 3.1). S0 = Baseline survey. S = Shade, K = *Katharina*.

In this system, non-*Hedophyllum* fleshy brown algae (primarily *Alaria marginata* and *Desmarestia* spp.) are rare throughout most of the year, but increase dramatically (from 0% to 100% cover of the low intertidal zone) in early spring (Chapter 2). This annual recruitment event is followed by a dramatic drop to near zero-levels by May (J. Burnaford, personal observation; Chapter 2). Although the timing of surveys in this experiment did not capture the recruitment event, the data indicate that *Katharina* affect the rate of disappearance of this group from the low intertidal zone. Fleshy brown algae persisted in –*Katharina* plots through August 2000, while the group was virtually absent from +*Katharina* plots by May (Fig 3.8).

Articulated (upright) coralline algae were present in all experimental plots, but the response of this group to manipulations varied across blocks with no detectable pattern (RM MANOVA, Block x Shade x *Katharina*, $p = 0.0004$). This result is consistent with previous studies in this system (Duggins and Dethier 1985). Percent cover of crustose corallines was not different between treatments overall ($p > 0.5$); their abundance fluctuated over time as microalgae and the holdfasts of macroalgae alternately covered them and were removed by grazers or died back.

Table 3.8 Repeated measures analysis of effects of shade and *Katharina* grazing on microalgal abundance, August 1999 – August 2000. Mauchly's criterion was not met ($p = 0.03$) univariate analyses are presented with Huynh-Feldt Epsilon adjusted probabilities. Bold face p-values indicate that the factor is significant at $\alpha = 0.008$ (adjusted for comparisons of multiple algal groups).

Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	69.27	47.37	**< 0.0001**
Katharina	1	13.72	9.38	**0.0038**
Block	2	2.35	1.61	0.2121
Shade x *Katharina*	1	0.12	0.08	0.7791
Error	42	1.46		
Contrast (-S +K vs. +S –K)	1	72.32	49.46	**< 0.0001**
Within Subjects (change in effect over time)				
Time	4	9.96	11.79	**< 0.0001**
Time x Shade	4	15.60	18.47	**< 0.0001**
Time x *Katharina*	4	7.18	8.50	**< 0.0001**
Time x Block	8	0.98	1.16	0.3254
Time x Shade x *Katharina*	4	2.26	2.68	0.0337
Error (Time)	168	0.85		
Time x (-S +K vs. +S –K)	4	7.88	9.33	**< 0.0001**

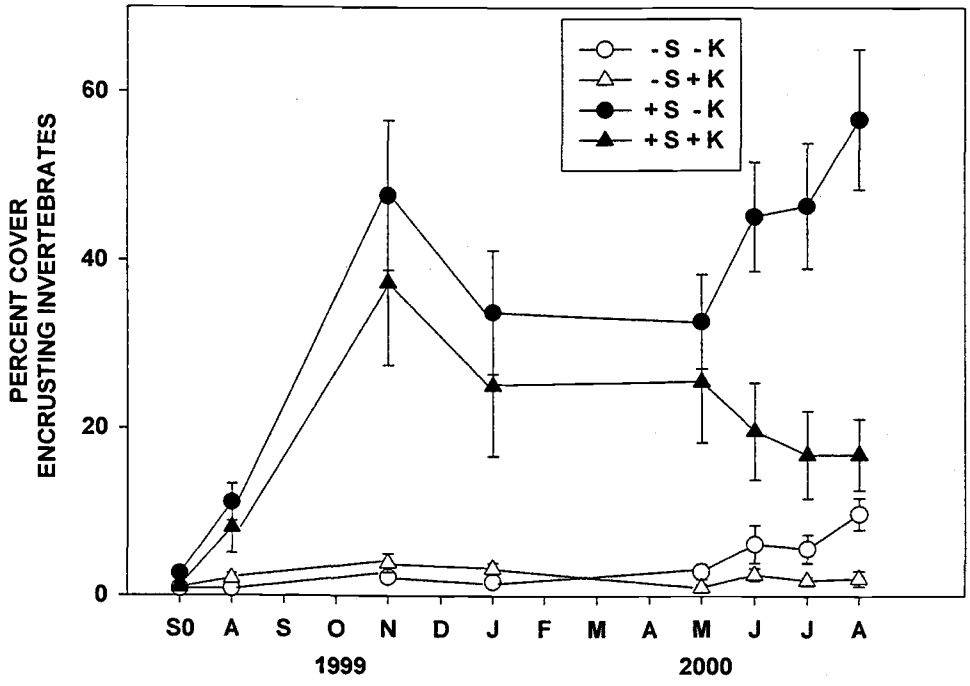

Figure 3.7 Effect of shade and *Katharina* on the abundance of encrusting invertebrates. Percent cover, July 199 – August 2000 (mean ± SEM, n = 12 plots per treatment combination; See Fig 3.1). S0 = Baseline survey. S = Shade, K = *Katharina*. See Table 3.1 for species.

AEI Assemblage Composition III: Effect of Shade vs. Effect of Katharina

As with total AEI abundance, the effects of Shade and *Katharina* on the abundance of individual AEI groups were independent (Tables 3.4 – 3.9, all Shade x *Katharina* $p \geq 0.1456$). The individual effects of Shade and *Katharina* on the abundance of the three macroalgal groups were roughly equivalent overall (Fig 3.5a, b, c; Tables 3.5 – 3.7, contrast –S +K vs. +S –K). However, the individual effects of Shade and *Katharina* were not equivalent for microalgae or encrusting invertebrate abundances. Over time, the presence of shade seemed to depress

microalgal abundance (relative to the –Shade –*Katharina* 'release condition') more strongly than did the presence of *Katharina* (Fig 3.6, Table 3.8; contrast -S+K vs. +S –K). For encrusting invertebrates, the antagonism between the positive effects of Shade and the negative effects of *Katharina* resulted in substantially greater abundances in 'Shade only' plots than in '*Katharina* only' plots, and intermediate abundances in +S +K and –S –K plots (Fig 3.7, Table 3.9; contrast -S+K vs. +S –K).

Table 3.9 Repeated measures analysis of effects of shade and *Katharina* grazing on encrusting invertebrate abundance, August 1999 – August 2000. Mauchly's criterion was not met (p = 0.001) so multivariate analyses are presented. Bold face p-values indicate that the factor is significant at α = 0.008 (adjusted for comparisons of multiple groups)

Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	187.32	62.53	**< 0.0001**
Katharina	1	16.90	5.64	0.0222
Block	2	0.43	0.14	0.8667
Shade x *Katharina*	1	6.58	2.20	0.1456
Error	42	2.99		
Contrast (-S +K vs. +S –K)	1	158.38	52.87	**< 0.0001**

Source	N df	D df	Wilks' λ	F	P
Within Subjects (change in effect over time)					
Time	4	39	0.37	16.63	**< 0.0001**
Time x Shade	4	39	0.66	5.10	**0.0021**
Time x *Katharina*	4	39	0.49	10.32	**< 0.0001**
Time x Block	8	78	0.76	1.47	0.1823
Time x Shade x *Katharina*	4	39	0.81	2.42	0.0643
Time x (-S +K vs. +S –K)	4	39	0.62	5.93	0.0008

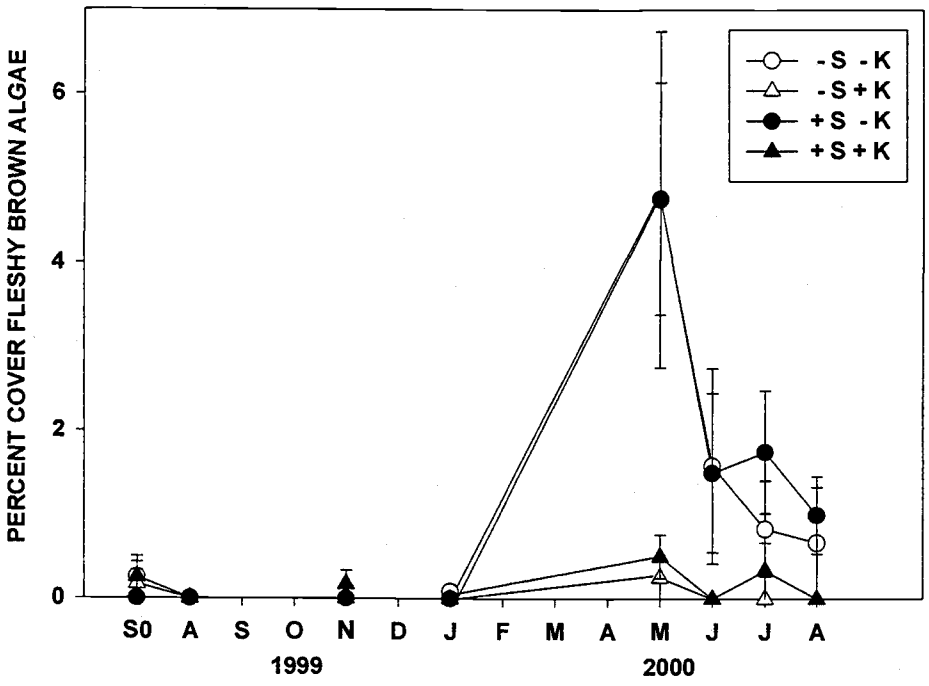

Figure 3.8 Effect of shade and *Katharina* on the abundance of fleshy brown algae. Percent cover, July 199 – August 2000 (mean ± SEM, n = 12 plots per treatment combination; See Fig 3.1). S0 = Baseline survey. S = Shade, K = *Katharina*. See Table 3.1 for species.

Mobile Animal Assemblage: Abundance

In contrast to the strong effects of both shade and *Katharina* on the AEI assemblage, only shade affected the abundance of mobile animals. Shade had strong positive effects on total mobile animal abundance, but only during the summer (Fig 3.9a, Table 3.10a; Time x Shade effect). Summertime animal abundances in +Shade plots averaged 19 to 27 animals (95%C.I., averaged May to August), compared to a mean of 6 to 10 animals in –Shade plots. The effect of shade was similar in summer 2000 and 1999 (Table 3.10b; Shade effects in May and August 2000 vs. August 1999, both $p > 0.4$). However, the effect of shade

differed between summer and winter (Table 3.10b, Shade effects in November and January vs. August 1999 both $p \leq 0.001$). Wintertime animal abundances did not differ between +Shade and –Shade plots; averaging 15 to 23 animals in +Shade plots and 10 to 27 animals in –Shade plots (95% C.I., averaged November and January). In addition to the positive effects of shade on total animal abundance, summertime species richness was also higher in +Shade than –Shade plots (Fig 3.9b, $p < 0.0001$).

Katharina had no effect on overall mobile animal abundance (Fig 3.9a; Table 3.10a, Between subjects *Katharina* effect) or species richness (Fig 3.9b; $p = 0.1$). As with the AEI assemblage, the effect of shade and *Katharina* were independent (Table 3.10a, Between subjects, Shade x *Katharina* $p = 0.6258$).

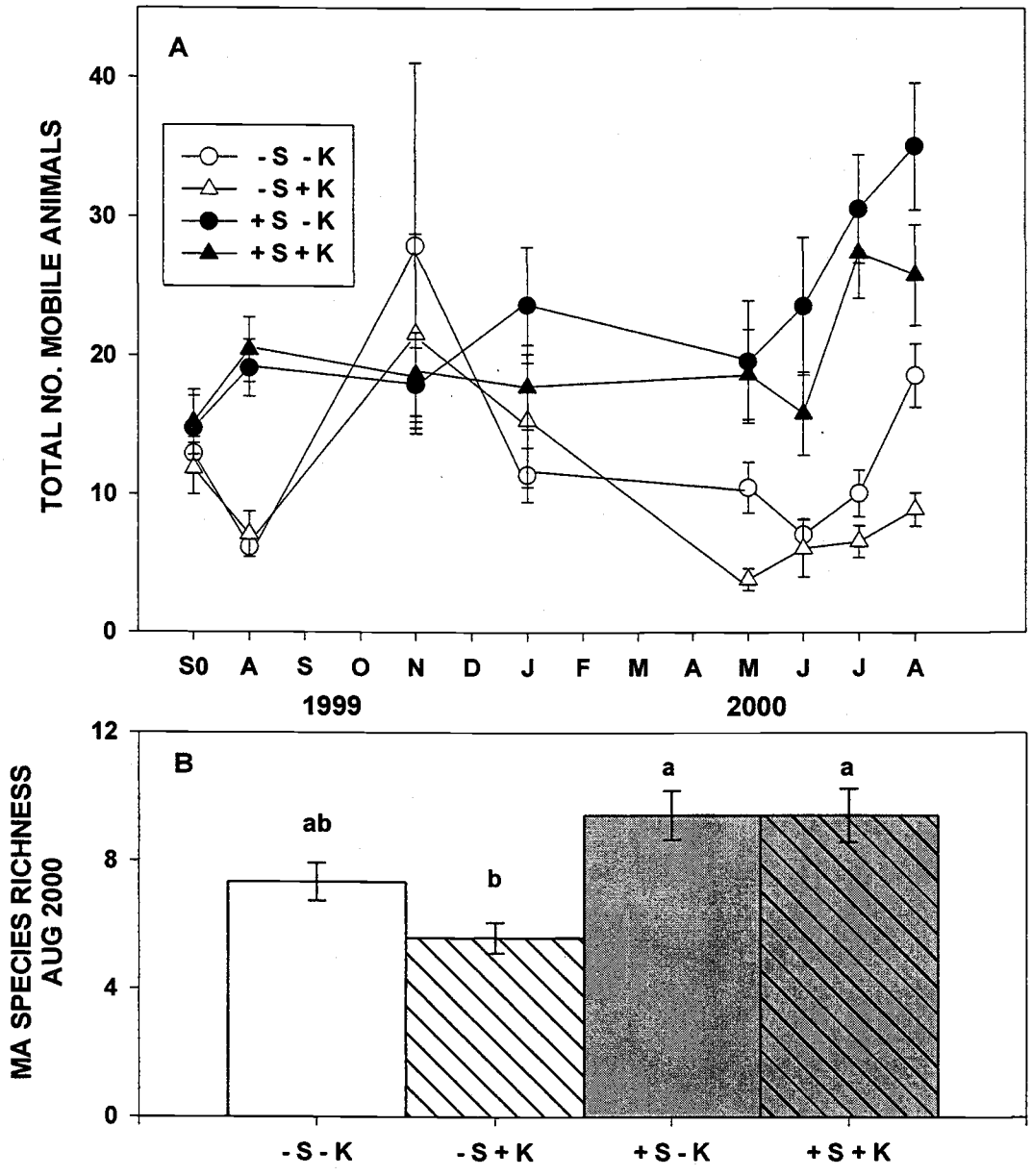

Figure 3.9 Effect of shade and *Katharina* on total mobile animal (MA) abundance and species richness. S0 = Baseline survey, S = Shade, K = *Katharina*. **(A)** Total counts, July 1999 through August 2000 (mean ± SEM, n = 12 plots per treatment combination). **(B)** Number of MA species, August 2000 (mean ± SEM, n = 12 plots per treatment combination). Treatments with the same letter are not significantly different (Tukey-Kramer, $p > 0.05$).

Table 3.10 Repeated measures analysis of total mobile animal abundance as a function of shade and the presence of *Katharina*. Mauchly's criterion was not met ($p = 0.0004$) so only multivariate analyses are presented. Data are squareroot transformed summed animal abundance for surveys in August 1999, November 1999, January 2000, May 2000 and August 2000. **A.** Bold-faced p-values are significant at $\alpha = 0.05$ level. **B.** Contrasts comparing the effect of Shade and *Katharina* at each survey with the effect in August 1999. Because each contrast tests only two time periods, the univariate and multivariate results are identical, and testing for sphericity is not necessary. Significant p-values for any factor indicate that the effect of that factor is different in a given survey than the effect in August 1999. Bold-faced p-values indicate significance at $\alpha = 0.013$ level, adjusted for multiple contrasts.

A. Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	91.52	29.07	**< 0.0001**
Katharina	1	8.12	2.58	0.1159
Block	2	10.29	3.27	0.0479
Shade x *Katharina*	1	0.76	0.24	0.6258
Error	42	3.15		

Source	N df	D df	Wilks' λ	F	P
Within Subjects (change in effect over time)					
Time	4	39	0.52	9.02	**< 0.0001**
Time x Shade	4	39	0.67	4.73	**0.0033**
Time x *Katharina*	4	39	0.82	2.17	0.0905
Time x Block	8	78	0.43	5.19	< 0.0001
Time x Shade x *Katharina*	4	39	0.88	1.41	0.2487

B. Survey Contrasts with August 1999

Survey	Source	df	MS	F	P
November 1999	Shade	1	47.96	12.61	**0.0010**
	Katharina	1	0.39	0.10	0.7502
	Block	2	28.56	7.51	0.0016
	Shade x *Katharina*	1	0.17	0.04	0.8344
	Error	42	3.80		
January 2000	Shade	1	11.01	7.51	**0.0090**
	Katharina	1	0.69	0.47	0.4963
	Block	2	22.64	15.46	< 0.0001
	Shade x *Katharina*	1	3.31	2.26	0.1404
	Error	42	1.47		
May 2000	Shade	1	0.76	0.52	0.4764
	Katharina	1	6.34	4.34	0.0434
	Block	2	2.64	1.80	0.1771
	Shade x *Katharina*	1	3.86	2.64	0.1116
	Error	42	1.46		

Table 3.10, Continued

August 2000	Shade	1	0.18	0.08	0.7739
	Katharina	1	17.21	8.07	**0.0069**
	Block	2	1.27	0.60	0.5561
	Shade x *Katharina*	1	0.37	0.17	0.5250
	Error	42	2.13		

Mobile Animal Assemblage: Composition

While only shade affected the total abundance of mobile animals, both shade and *Katharina* independently affected the make-up of the animal assemblage (Table 3.11, Between subjects, Shade, *Katharina*, and Shade x *Katharina* effects). The response to shade was generally positive, and loosely correlated across animal groups; no group was less abundant in the +Shade than –Shade plots (Table 3.11, all canonical coefficients for Shade have the same sign) but the magnitude of the response varied widely across groups, from a strong positive effect (crabs, canonical coefficient = 1.18) to no effect (chitons, canonical coefficient = 0.11). The response of individual groups to *Katharina* also varied, from strongly positive (limpets, canonical coefficient for *Katharina* = 1.05) to weakly negative (anemones, coefficient = -0.6). In general, most groups responded either to the presence of shade or the presence of *Katharina*.

Table 3.11 Variation in the composition of the mobile animal assemblage as a function of shade and *Katharina* grazing. Analysis is a repeated measures MANOVA with log-transformed counts of six response variables (CHI = chitons, CRA = crabs, SEA = seastars, SNA = snails, ANE = anemones, LIM = limpets). Bold faced p-values indicate significance at $\alpha = 0.05$. The magnitude of a group's canonical coefficient indicates the relative contribution of each group to the differences among treatments. Similar signs of coefficients indicate positive correlation between groups. See text for details.

Multivariate Analysis

Source		N df	D df	Wilks' λ	F	P
Between Subjects (average effect over time)						
Shade		6	37	0.33	12.42	**< 0.0001**
Katharina		6	37	0.65	3.26	**0.0112**
Block		12	74	0.35	4.26	< 0.0001
Shade x *Katharina*		6	37	0.79	1.60	0.1735
Canonical Coefficients	***CHI***	***CRA***	***SEA***	***SNA***	***ANE***	***LIM***
Shade	0.11	1.19	0.50	0.12	0.30	0.31
Katharina	- 0.18	- 0.17	0.15	0.16	- 0.64	1.05
Within Subjects (change in effect over time)						
Time		24	19	0.08	8.95	**< 0.0001**
Time x Shade		24	19	0.29	1.93	0.0738
Time x *Katharina*		24	19	0.33	1.58	0.1574
Time x Block		48	38	0.04	3.40	< 0.0001
Time x Shade x *Katharina*		24	19	0.35	1.46	0.2030

Two animal groups, crabs and seastars, were positively affected by shade and not affected by *Katharina*. Both groups were consistently more abundant in +Shade plots than in –Shade, regardless of season (Fig 3.10a, b; Table 3.12, 3.13; Shade $p < 0.0001$; Time x Shade, $p \geq 0.1210$). Overall, neither group responded to *Katharina* (Tables 3.12, 3.13, Between subjects *Katharina* effects $p \geq 0.0826$).

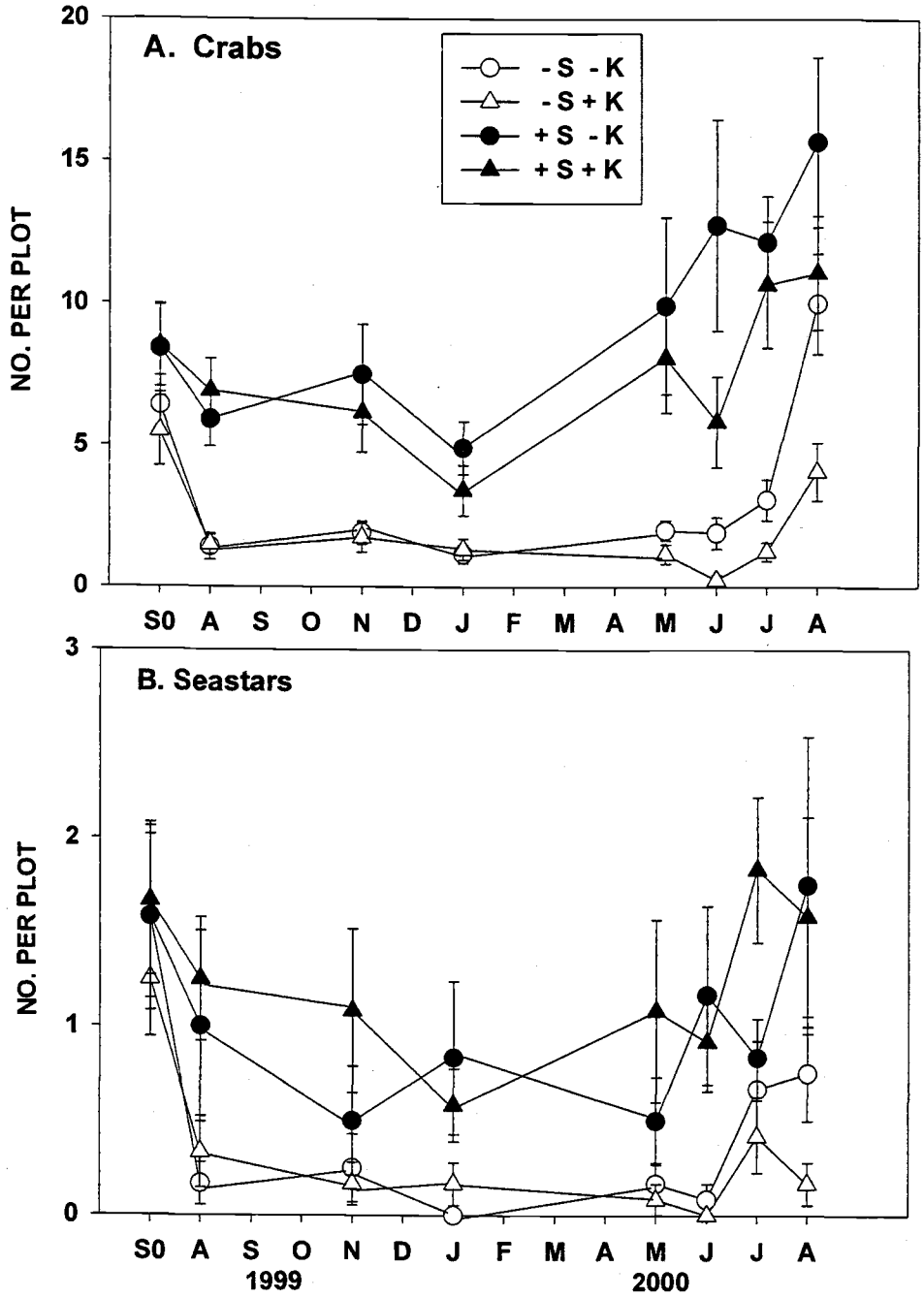

Figure 3.10 Effect of shade and *Katharina* on the abundance of crabs and seastars. Counts in experimental plots, July 1999 – August 2000 (mean ± SEM, n = 12 plots per treatment combination; Fig. 3.1) **(A)** Number of crabs. **(B)** Number of seastars. S0 = Baseline survey. S = Shade, K = *Katharina*. See Table 3.2 for species listings.

Table 3.12 Repeated measures analysis of effect of shade and *Katharina* on crab abundance August 1999 – August 2000. Mauchly's criterion was met (p = 0.93), so univariate analyses are presented with un-adjusted probabilities. Bold-faced p-values indicate significance at $\alpha = 0.007$.

Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	52.83	61.48	**< 0.0001**
Katharina	1	2.72	3.16	0.0826
Block	2	0.33	0.38	0.6846
Shade x *Katharina*	1	0.72	0.08	0.7738
Error	42	0.86		
Within Subjects (change in effect over time)				
Time	4	7.78	24.72	**< 0.0001**
Time x Shade	4	0.59	1.85	0.1210
Time x *Katharina*	4	0.88	2.80	0.0278
Time x Block	8	0.45	1.43	0.1879
Time x Shade x *Katharina*	4	0.40	1.26	0.2880
Error (Time)	168	0.32		

Two animal groups responded seasonally to shade. Herbivorous snails were more abundant in +Shade plots than in –Shade plots during the summer, but not in the fall and winter (Fig 3.11a; Table 3.14a, Shade and Time x Shade effects; Table 3.14b; Shade effect). Snail abundances did not respond to *Katharina* (Table 3.14a, Between and Within subjects, *Katharina* effect). The overall pattern of anemone abundance seems driven primarily by the presence or absence of shade (Fig 3.11b, Table 3.15; Shade effect, p = 0.0068; *Katharina* effect is not significant at $\alpha = 0.007$). Although there is an annual pattern of temporary anemone abundance peaks in +Shade, -*Katharina* plots in August (Fig 3.11b), the effects of *Katharina* and shade are still independent overall (Table 3.15, Shade x *Katharina* not significant at $\alpha = 0.007$).

Table 3.13 Repeated measures analysis of effect of shade and *Katharina* on seastar abundance August 1999 – August 2000. Mauchly's criterion was not met ($p = 0.004$), but conclusions from multivariate and univariate analyses did not differ, so univariate analyses are presented with Huynh-Feldt adjusted probabilities. Bold faced p-values indicate significance at $\alpha = 0.007$.

Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	7.75	30.67	**< 0.0001**
Katharina	1	0.11	0.57	0.4540
Block	2	0.28	1.12	0.3365
Shade x *Katharina*	1	0.51	2.03	0.1617
Error	42	0.25		
Within Subjects (change in effect over time)				
Time	4	0.64	3.16	0.0182
Time x Shade	4	0.06	0.31	0.8580
Time x *Katharina*	4	0.21	1.06	0.3773
Time x Block	8	0.11	1.85	0.0771
Time x Shade x *Katharina*	4	0.20	0.54	0.6925
Error (Time)	168			

Limpets were unique among the animal groups in responding to *Katharina* but not to shade (Fig 3.12, Table 3.16a; Between subjects *Katharina* effect, $p = 0.0005$, Shade effects $p > 0.7$). The positive effect of *Katharina* on limpet abundance increased over time (Table 3.16a, Time x *Katharina* $p = 0.0003$, Table 3.16b, *Katharina* effect).

Two groups did not respond to the experimental manipulations. Chitons other than *Katharina* (which was manipulated experimentally) did not respond to either shade or *Katharina* (RM ANOVA, Shade, *Katharina*, and Shade x *Katharina* tests $p \geq 0.2$; data not shown). The abundance of whelks in experimental plots varied across blocks (RM ANOVA, Block x Shade x *Katharina* test $p = 0.001$) and could not be attributed to any experimental factor.

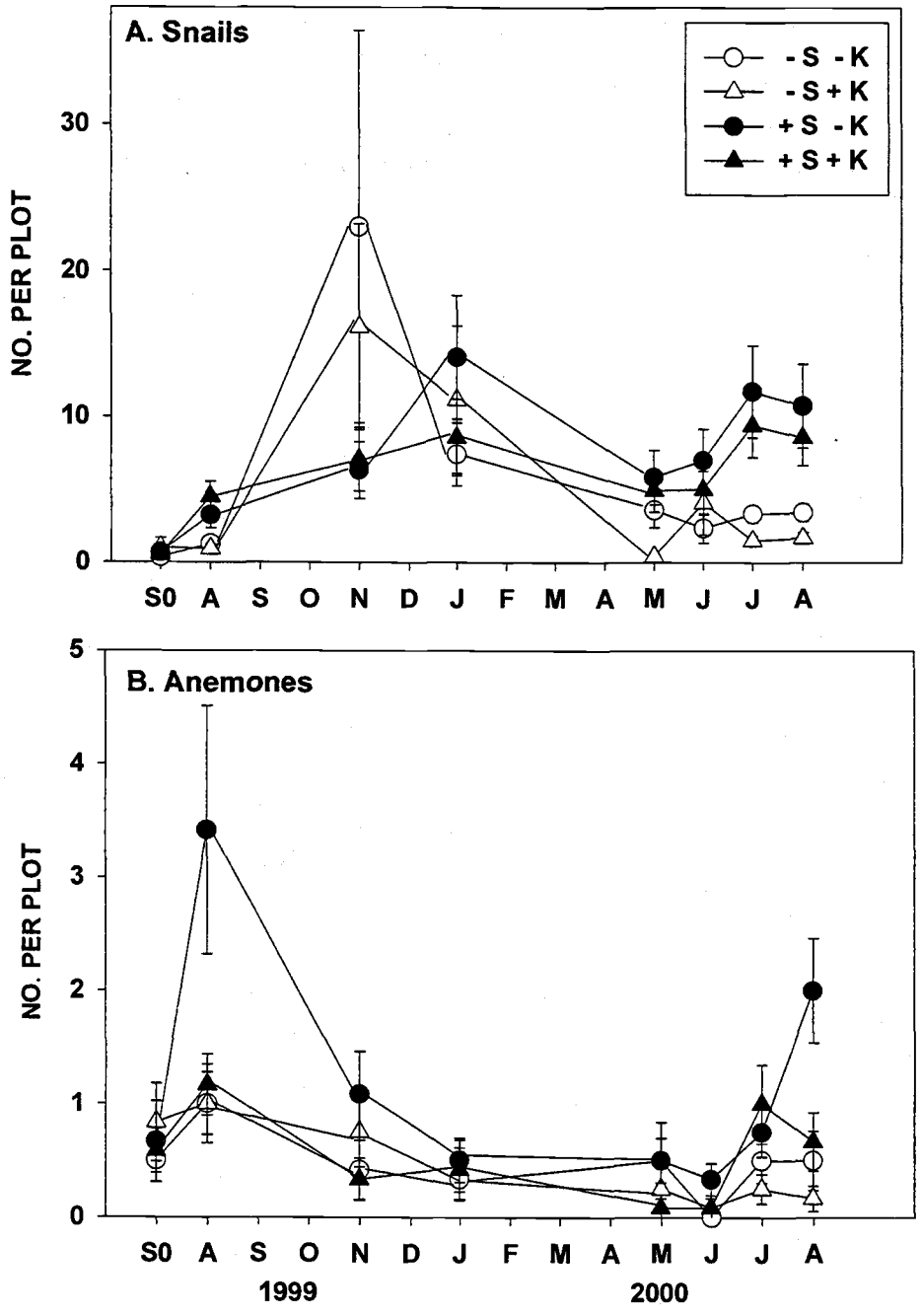

Figure 3.11 Effect of shade and *Katharina* on the abundance of herbivorous snails and anemones. Counts in experimental plots, July 1999 – August 2000 (mean ± SEM, n = 12 plots per treatment combination) **(A)** Number of snails. **(B)** Number of anemones. S0 = Baseline survey. S = Shade, K = *Katharina*.

Table 3.14 Repeated measures analysis of effect of shade and *Katharina* on snail abundance August 1999 – August 2000. Mauchly's criterion was not met ($p = 0.006$), but interpretations from multivariate and univariate analyses did not differ, so univariate analyses are presented with Huynh-Feldt adjusted probabilities. **A.** Bold-faced p-values indicate significance at $\alpha = 0.007$. **B.** Univariate analyses comparing treatments in a single survey. Bold-faced p-values indicate significance at $\alpha = 0.002$ (adjusted for multiple contrasts).

A. Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	13.86	16.60	**0.0002**
Katharina	1	0.95	1.14	0.2920
Block	2	20.79	24.89	< 0.0001
Shade x *Katharina*	1	0.84	1.00	0.3220
Error	42	0.84		
Within Subjects (change in effect over time)				
Time	4	8.40	13.91	**< 0.0001**
Time x Shade	4	3.06	5.07	**0.0009**
Time x *Katharina*	4	0.65	1.08	0.3691
Time x Block	8	3.79	6.27	< 0.0001
Time x Shade x *Katharina*	4	1.05	1.73	0.1494
Error (Time)	168	0.60		

B. Univariate Analyses: Effect of Season

Survey	Source	df	MS	F	P
August 1999	Shade	1	7.69	18.87	**< 0.0001**
	Katharina	1	0.17	0.42	0.5199
	Block	2	1.28	3.15	0.0533
	Shade x *Katharina*	1	0.42	1.03	0.3157
	Error	42	0.41		
January 2000	Shade	1	1.33	1.97	0.1679
	Katharina	1	0.25	0.37	0.5459
	Block	2	10.49	15.54	< 0.0001
	Shade x *Katharina*	1	1.28	1.90	0.1752
	Error	42	0.68		
August 2000	Shade	1	9.83	17.18	**0.0002**
	Katharina	1	1.07	1.87	0.1790
	Block	2	0.48	0.84	0.4398
	Shade x *Katharina*	1	0.55	0.96	0.3330
	Error	42	0.57		

Table 3.15 Repeated measures analysis of effect of shade and *Katharina* on anemone abundance August 1999 – August 2000. Mauchly's criterion was not met (p = 0.002), but conclusions from multivariate and univariate analyses did not differ, so univariate analyses are presented with Huynh-Feldt adjusted probabilities. Bold faced p-values indicate significance at $\alpha = 0.007$.

Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	2.23	8.09	**0.0068**
Katharina	1	1.89	6.89	0.0120
Block	2	0.21	0.77	0.4672
Shade x *Katharina*	1	1.78	6.47	0.0147
Error	42	0.27		
Within Subjects (change in effect over time)				
Time	4	2.28	10.67	**< 0.0001**
Time x Shade	4	0.52	2.43	0.0535
Time x *Katharina*	4	0.21	0.98	0.4192
Time x Block	8	0.37	1.73	0.0998
Time x Shade x *Katharina*	4	0.14	0.66	0.6109
Error (Time)	168	0.21		

Katharina *diet: Gut and Fecal Pellet Contents*

Katharina consume a wide range of diet items; several algal and angiosperm genera were identified from *Katharina* gut and fecal contents (Fig 3.13, Table 3.17). *Katharina* gut contents and fecal pellets also included a large number of non-algal items (Table 3.18), including chironomid larvae and copepods that were still alive after passage through the gut.

There were no apparent cage-related effects of enclosure on *Katharina* diet composition. Gut contents from *Katharina* maintained in experimental *Hedophyllum* plots did not differ from those of general population *Katharina* (MANOVA, arcsine-squareroot transformed data; Ndf, Ddf = 10, Pillai's Trace = 0.66, F = 0.77, p = 0.66).

Gut contents from *Katharina* collected from –Shade plots (No Structure and Structure Control) and +Shade plots (Artificial Shade) did not differ (Fig 3.16, Table 3.19; No Structure vs. Structure Control and Artificial Shade vs. –Shade plots, $p > 0.3$). *Katharina* in these plots were primarily consuming diatoms (mean percent of diet = 37%) and *Ulva* (mean = 29%), and smaller proportions of finely branched red algae (mean = 14%).

Table 3.16 Repeated measures analysis of effect of shade and *Katharina* on limpet abundance August 1999 – August 2000. Mauchly's criterion was not met ($p = 0.012$) so univariate analyses are presented with Huynh-Feldt adjusted probabilities. **A.** Bold faced p-values indicate significance at $\alpha = 0.007$. **B.** Univariate analyses comparing the effects of shade and *Katharina* in selected surveys. Bold-faced p-values indicate significance at $\alpha = 0.002$ (adjusted for multiple comparisons). See text for details.

A. Multivariate Analysis: Repeated Measures

Source	df	MS	F	P
Between Subjects (average effect over time)				
Shade	1	0.13	0.09	0.7599
Katharina	1	19.06	14.46	**0.0005**
Block	2	8.24	6.25	0.0042
Shade x *Katharina*	1	0.44	0.34	0.5652
Error	42	1.32		
Within Subjects (change in effect over time)				
Time	4	2.81	14.52	**< 0.0001**
Time x Shade	4	0.07	0.35	0.8438
Time x *Katharina*	4	1.00	5.69	**0.0003**
Time x Block	8	0.31	1.59	0.1307
Time x Shade x *Katharina*	4	0.18	0.91	0.4564
Error (Time)	168	0.19		

B. Univariate Analyses: Effect of Season

Survey	Source	df	MS	F	P
August 1999	Shade	1	0.01	0.03	0.8735
	Katharina	1	0.74	5.53	0.0235
	Block	2	0.44	3.28	0.0474
	Shade x *Katharina*	1	0.07	0.50	0.4830
	Error	42	0.13		
January 2000	Shade	1	0.27	0.46	0.5027
	Katharina	1	4.81	8.22	**0.0064**
	Block	2	2.41	4.12	0.0233
	Shade x *Katharina*	1	0.27	0.46	0.5027
	Error	42	0.59		
August 2000	Shade	1	0.04	0.07	0.8000
	Katharina	1	7.60	14.00	**0.0005**
	Block	2	1.86	3.42	0.0419
	Shade x *Katharina*	1	0.15	0.28	0.6014
	Error	42	0.54		

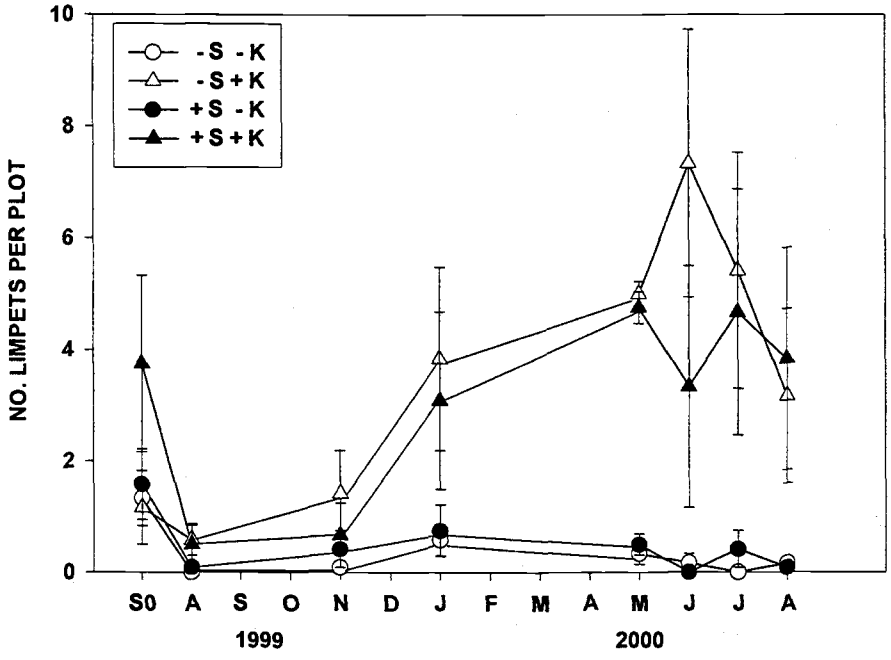

Figure 3.12 Effect of shade and *Katharina* on the abundance of limpets. Counts in experimental plots, July 1999 – August 2000 (mean ± SEM, n = 12 plots per treatment combination) S0 = Baseline survey. S = Shade, K = *Katharina*. See Fig 3.1 and Table 3.2 for details.

Diets of *Katharina* in the +Shade treatments (Artificial Shades and *Hedophyllum*) differed due to the high percentage of *Hedophyllum* in the diet of chitons in +*Hedophyllum* plots (mean = 35% vs. 0 % in Artificial Shade plots; $F = 37.29$, $p < 0.0001$). Proportions of all other individual algal groups were similar between these two treatments ($p > 0.05$). Although *Katharina* in *Hedophyllum* plots were actively consuming the kelp, this did not affect *Hedophyllum* canopy cover (RM ANOVA on un-transformed percent cover data; Between subjects *Katharina* effect, $p = 0.42$; Within subjects, Time x *Katharina* effect, $p > 0.73$), and at the end of the experiment, the number of *Hedophyllum* thalli did not differ between +*Katharina* and –*Katharina* plots ($p = 0.89$).

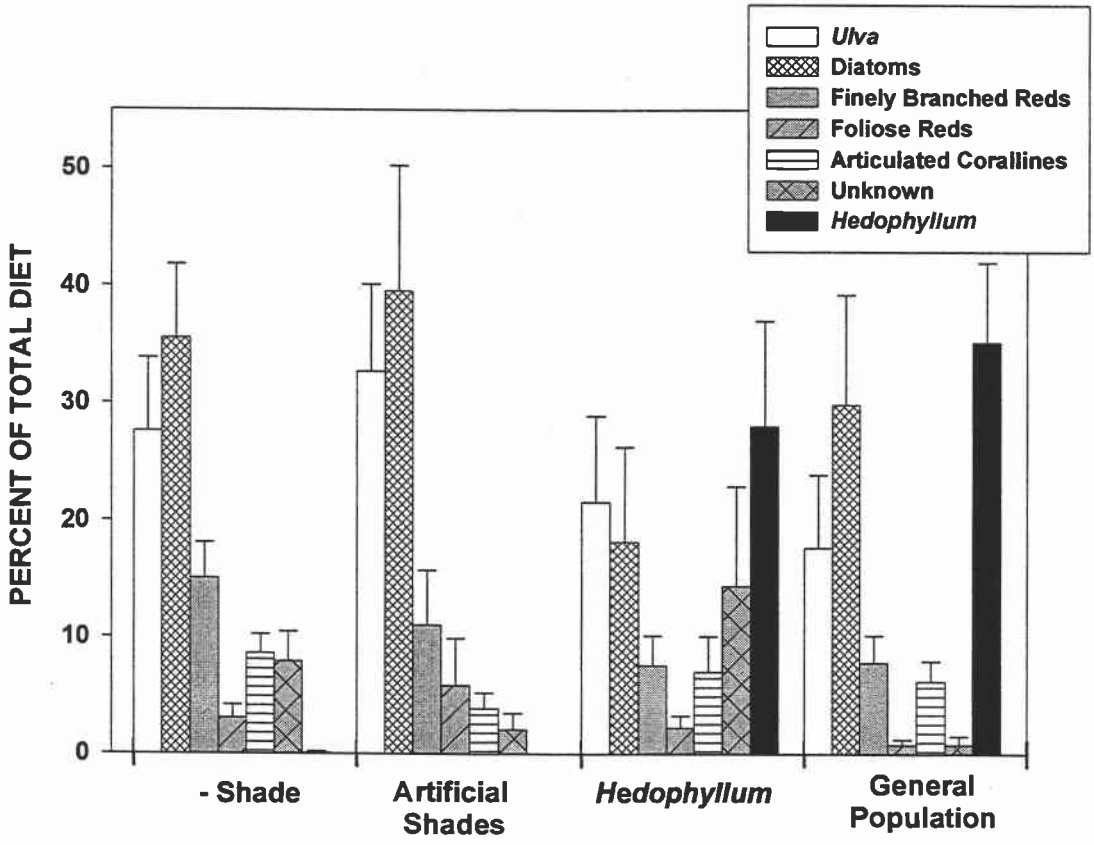

Figure 3.13 Diet of *Katharina tunicata* at Pile Point, July and August 2000. Proportion of total *Katharina* diet (mean ± SEM, n = 36 individuals from – Shade plots, 18 individuals from + Shade plots, 18 individuals from *Hedophyllum* plots and 6 from the general population). See text and Table 3.17 for details.

Table 3.17 Algal and encrusting invertebrate groups present in *Katharina* gut contents and fecal pellets. In most cases contents could be identified only to group; genera listed were identified in at least one specimen.

Diet Class	Genera
Green Algae	*Acrosiphona, Ulva*
Microalgae	Diatoms
Finely Branched Red Algae	*Microcladia, Polysiphonia*
Foliose and Thickly Branched Red Algae	*Odonthalia, Mazzaella*
Articulated Coralline Algae	*Bossiella, Corallina*
Hedophyllum	*Hedophyllum*
Encrusting Invertebrates	
Rare algae	*Porphyra*
Crustose Coralline Algae	
Unknown	
Seagrasses	

DISCUSSION

The maintenance of the 'natural' assemblage underneath the *Hedophyllum* canopy and the dramatic change in the understory assemblage following canopy removal are due to a complex network of direct, indirect, positive and negative interactions. *Katharina* affects the understory AEI assemblage both in the presence and absence of shade, but shade also exerts strong independent effects. Therefore, the *Hedophyllum* canopy influences the structure of the understory assemblage through direct competition, direct facilitation, and indirect effects mediated through direct effects on the suite of mobile animals, including the chiton *Katharina tunicata*, which exert major structuring forces on the understory community themselves.

Table 3.18 Counts of non-algal items found in *Katharina* guts and feces. Data are combined counts from all *Katharina* examined (N = 42 chitons; 9 each from Open, Structure Control, Artificial Shade and *Hedophyllum* plots, and 6 chitons from the general *Katharina* population). Total Number Observed = sum over all *Katharina* individuals (items listed as N/A were recorded as % total gut contents). Frequency = Number of *Katharina* individuals found with that prey item.

"Prey" Item	Total Number Observed	Frequency
Bryozoans (encrusting)	N/A	1
Chironomid Larvae (alive)	5	4
Chironomid Larvae (dead)	125	26
Copepod (alive)	8	5
Copepod (dead)	22	12
Crepidula	2	2
Egg masses	14	9
Foraminiferans	102	25
Hydroids	N/A	10
Katharina teeth	35	11
Lacuna	1	1
Limpet	9	9
Nematodes (alive)	7	5
Rocks	N/A	9
Sponge Spicules	N/A	15

Table 3.19 Multivariate analysis of gut contents of *Katharina* collected from experimental plots. Response variables are arcsine-squareroot transformed percent of total gut contents for 9 groups (diatoms, green algae, *Hedophyllum,* articulated corallines, coralline crusts, finely branched red algae, encrusting invertebrates, foliose red algae, and unknown). Because of significant heterogeneity of variance among treatments (due to the fact that *Hedophyllum* was only present in one treatment) Pillai's Trace statistics are presented. Bold-faced p-values indicate significance at the $\alpha = 0.05$ level.

Multivariate Analysis

Source	N df	D df	Pillai's Trace	F	P
Treatment	30	69	1.43	2.10	**0.0057**
Block	20	44	1.03	2.31	0.0103
No Structure (NS) vs. Structure Control (SC)	10	21	0.03	0.83	0.6081
Artificial Shade vs. –S (NS + SC)	10	21	0.36	1.16	0.3669
Artificial Shade vs. *Hedophyllum*	10	21	0.81	9.08	**< 0.0001**

Effects of Hedophyllum *on the Understory AEI Assemblage*

The *Hedophyllum* canopy primarily affects the AEI understory through the provision of shade. Only two AEI groups (green algae and encrusting invertebrates) responded differently to +*Hedophyllum* and Artificial Shade (–*Hedophyllum* +Shade) treatments. In both cases, these groups were less abundant underneath the natural *Hedophyllum* canopy than under Artificial Shades, indicating that some factor other than shade was also important in controlling their abundance in the natural condition. Algal canopies can directly disturb understory species through mechanical abrasion of the rock surface by algal blades, reducing settlement and/or growth of algae and invertebrates (Black 1974, Menge 1976, Velimirov and Griffiths 1979, Duggins et al. 1990, Vadas Sr. et al. 1992, van Tamelen et al. 1997, Jenkins et al. 1999, Leonard 1999). Several algal species release allelopathic chemicals, which directly inhibit the growth,

settlement, survival or maturation of other algae (Fletcher 1975, Inderjit and Dakshini 1994, Suzuki et al. 1998) and of encrusting invertebrates (Schmitt et al. 1995).

However, even for these two groups, the negative effect of the *Hedophyllum* thallus effects was weak in comparison to the effect of shade. Artificial shades reduced the abundance of green algae by an average of 35% (per plot) overall from levels in –Shade plots, and the *Hedophyllum* thallus only further reduced cover by an average of 11% reduction (Fig 3.2). Although the presence of a *Hedophyllum* thallus reduced the cover of encrusting invertebrates, the positive effect of the shade provided by the canopy was stronger. Encrusting invertebrate cover was far higher in +*Hedophyllum* plots than in –Shade plots (in August 2000, in *Hedophyllum* plots, 6 replicates = 1%, 20%, 42%, 50%, 58%, 61%; range in – Shade plots = 5-20%). Therefore the negative effects of the canopy reduced, but did not eliminate, the positive effects of shade.

Effects of Shade on the Understory AEI Assemblage

The presence of shade reduced total AEI abundance and species diversity relative to unshaded areas (Fig 3.4). Shade negatively affected the abundance of all algal groups, but positively affected the abundance of encrusting invertebrates (Fig 3.5-3.7). Shade alters a number of abiotic variables that could contribute to these differential effects on AEI groups. Algae gain energy through photosynthesis and therefore require sunlight for growth and maintenance (for a review of algal photosynthetic physiology, see Lobban and Harrison 1994; for field experiments that document canopy shading effects, see Reed and Foster 1984,

Kennelly 1989). Therefore, by altering the quality and reducing the quantity of light, the algal canopy or artificial shades could negatively affect photosynthesis, growth, and reproductive rates for understory algae. Algal diversity could therefore be reduced by the presence of shade as those species less tolerant to low light levels (or altered light quality) are eliminated from the assemblage. However, reduced light levels positively affect non-photosynthetic understory species. Some bryozoan larvae become negatively phototactic at the settling stage (Ryland 1977, Reed 1987, Wendt and Woollacott 1999), therefore shade could enhance settlement rates of encrusting invertebrates in the field. Shade also reduces desiccatory stress for understory organisms at low tide by reducing evaporation caused by exposure to sunlight and wind. The rock surface beneath the *Hedophyllum* canopy and Artificial Shades remained moist throughout low tide emersion on warm summer days, while the rock surface in –Shade plots was dry and algae became distinctly 'crispy' (personal observation). Studies with intertidal sponges have found that the distribution of *Halichondria*, the most common sponge in this experiment, was highly correlated with interspecific associations that decreased desiccation (Palumbi 1985). Such amelioration of stressful abiotic conditions can be an important direct positive effect of the presence of shade for understory sessile invertebrates (Leonard 1999, 2000).

Shade could also affect the understory AEI assemblage through a number of indirect pathways. Shade could have indirect negative effects on the abundance of macro and microalgal species by increasing consumer pressure in shaded areas. Abundances of two groups of mobile herbivores, crabs and herbivorous snails, were increased in +Shade plots. Shade can also have indirect

negative effects on algal diversity if these herbivores differentially consume algal species. Shade could have indirect positive effects on encrusting invertebrates by reducing the abundance of their algal competitors. Slow-growing colonies of encrusting invertebrates can be out-competed, or 'swamped out' by rapidly growing macroalgae in this low intertidal zone system (Palumbi 1985). Such indirect effects can have large impacts on the structure of communities (Menge 1995).

One striking result from this experiment is the important role of shade in the control of microalgae by the *Hedophyllum* canopy. Although previous researchers hypothesized that both shade and whiplash effects were important components of the direct *Hedophyllum* / diatom interaction (Dethier and Duggins 1984, Duggins and Dethier 1985), I found no detectable negative effects of the *Hedophyllum* thallus *per se* on diatom abundance. Overall, microalgal abundance did not differ between *Hedophyllum* and Artificial Shade plots (RM ANOVA $p = 0.15$); and in +Shade plots, *Hedophyllum* canopy cover only explained more than 7% of the variation in microalgal cover on one sampling date (August 1999, linear regression, $R^2 = 0.21$). Therefore, the primary negative direct effect of the *Hedophyllum* canopy on microalgal abundance is the reduction of light thorough shading. Clearly, however, the negative direct effect of shade is only one factor affecting microalgal abundance. In +Shade plots, the abundance of microalgae was weakly negatively correlated with the abundance of encrusting invertebrates ($R^2 = 0.19$), a relationship which could be due to direct consumption of microalgae by colonies of invertebrates, by direct competition for space on the rocky substratum, or both. Shade could also have negative indirect effects on the

abundance of microalgae; mobile herbivores, which are more abundant in +Shade than –Shade areas, could reduce microalgal cover by direct consumption.

Effects of Shade on Mobile Animals

The shade provided by the *Hedophyllum* canopy exerts strong positive effects on the mobile animal assemblage. The presence of shade increased overall animal abundance, species richness, and the abundance of seven animal groups (crabs, seastars, snails, anemones, mobile worms, detritivores, and fish) relative to unshaded areas.

There are several direct physiological benefits for small animals that can access shaded areas during emersion at low tide. Mid-day summertime low tide temperatures at Pile Point regularly reach 37°C in –Shade areas (Chapter 2, Chapter 3). During these low tides, animals could also be stressed by UV exposure and desiccation. All of these potential stress factors are ameliorated by the presence of shade, and any or all could be important in determining animal distributions. Temperatures underneath *Hedophyllum* canopies and these Artificial Shades are up to 13°C cooler than in -Shade areas (the actual temperature difference increases as days get warmer; Chapter 2, Chapter 4). Numerous studies of mobile molluscs have demonstrated that the use of crevices or other refugia from thermal stress can play a major role in determining abundances in the field (Chapter 2, Garrity and Levings 1981, Garrity 1984, Williams 1994). Although I did not measure UV levels, they are likely to be reduced by the presence of an opaque algal canopy or the mesh of Artificial Shades. Studies in freshwater lakes have found that UV intolerant zooplankton species avoid high UV habitats (Leech

and Williamson 2000), and although other experiments in freshwater systems have demonstrated that snails were relatively resistant to UV exposure compared to invertebrates without shells (McNamara and Hill 1999), shades could provide refugia from UV exposure for the many shell-less organisms that were distributed differentially between treatments. Shade also reduces desiccatory stress for animals at low tide. The seastar *Leptasterias*, which responded strongly and positively to shade, is highly susceptible to desiccation stress during summer low tides (Menge 1972). The increase in mobile animal diversity in shaded areas could be a direct result of this suite of abiotic conditions. As shaded areas simultaneously provide refugia from many physiological stresses, they are likely to be important for a wide range of animals that are differentially susceptible to different stresses.

As was the case with the AEI assemblage, shade could have indirect effects on the mobile animal assemblage. Higher animal densities may be self-perpetuating as carnivorous species, such as the seastar *Leptasterias* (observed consuming snails, crabs, and chitons in +Shade plots), are attracted to increased prey densities in shaded areas. The sign of this effect is obviously different depending on whether one focuses on the effect of the canopy on predator (+) or prey (-), but such effects on higher trophic levels could contribute to increased species richness in shaded areas. Shades could also function as refugia for consumers from some predators, especially large visual predators, which may pose the most risk during periods of high light availability (spring and summer months). Although a separate simultaneous experiment found no effect of bird predation on the abundance or composition of the animal assemblage in similar

plots (Chapter 2), this could be an important effect in other systems. As with the AEI assemblage, the net effect (+ or neutral) of shade on any one animal group, or on the composite assemblage, is most likely a combination of these positive and negative, direct and indirect factors.

Effect of Katharina *on the AEI Assemblage*

Katharina exerted strong direct negative effects on the abundance of the three macroalgal understory groups in this experiment. These results confirm earlier conclusions that *Katharina* are "strong interactors" in this low intertidal system, both in terms of individual (Paine 1992) and population level effects (Dethier and Duggins 1984,1988, Duggins and Dethier 1985, Markel and DeWreede 1998). With their versatile radula (Steneck and Watling 1982), *Katharina* consumed organisms from all of the algal and seagrass groups present at Pile Point (Table 3.17). The high proportion of the green alga *Ulva* in the diet of *Katharina* in all treatments is consistent with the heavy negative effect of *Katharina* grazing on green algal abundance. The smaller proportions of finely branched and foliose red algae also correlate with the patterns of abundance of these groups. These diet data confirm that *Katharina* negatively affect these three groups directly, by consuming them.

Katharina also exerted strong negative direct effects on microalgae. Diatoms made up a large proportion of the *Katharina* diet in all treatments. *Katharina* could also indirectly affect microalgal abundance. By decreasing the abundance of macroalgae and encrusting invertebrates and thus freeing up substratum space for microalgal growth, *Katharina* indirectly positively affect

microalgae. However, by indirectly increasing the abundance of limpets (Fig 3.12), which feed primarily on microalgae (Nicotri 1977), *Katharina* also exert indirect negative effects on microalgal abundance. However, although diatoms made up a larger percentage of the *Katharina* diet than any macroalgal group (Fig 3.13), and the presence of Katharina also increased the abundance of limpets, the effect of *Katharina* on the abundance of microalgae was weaker and more variable than on macroalgae (Fig 3.5, 3.6). The high rate of diatom production may reduce the impact of even high grazing pressure relative to groups that recover more slowly from biomass removal (such as finely branched red algae).

Katharina is an extremely general, highly versatile consumer (this study, (Padilla 1981, Dethier and Duggins 1984, Piercy 1987, Dethier and Duggins 1988). In addition to consuming food items, the high number of poorly digested and, even, living invertebrates found in gut contents and fecal pellets (Table 3.19) suggests that the chiton has a high level of 'incidental take' which could substantially impact populations of ingested items, whether or not those items were "intended" as prey. This non-selective grazing behavior could contribute to the strong negative effect of *Katharina* on AEI diversity. If *Katharina* are removing species regardless of their nutritional value, they would be expected to have a larger impact on species richness than if they were selecting particular food items.

Encrusting invertebrates made up a very small proportion of total gut and fecal contents of animals from +Shade plots (95% C.I. for the mean; Artificial Shade treatments = 0.1 to 2.5%, *Hedophyllum* treatments 0 to 0.6% of total diet), despite cover levels exceeding 50% in some plots by the end of the experiment (Fig 3.3, Fig 3.7). Despite this lack of correlation between grazing effect and diet

proportion, it is possible for consumers to have a strong negative effect on prey items that make only small contributions to their total food intake (Abrams 1992). *Katharina* could negatively impact the abundance of this group by removing settlers before colonies have a chance to establish, or could physically disrupt colony growth as they move over the substratum to forage, in an interaction similar to the 'bulldozing' of barnacles by limpets (Connell 1961, Dayton 1971, Menge 1976, Farrell 1991). Although *Katharina* did not appear to be directly consuming large quantities of encrusting invertebrates, the chiton clearly plays a major role in maintaining low encrusting invertebrate abundances in the natural condition, as abundances in *+Hedophyllum -Katharina* plots had reached 40% by August 2000, versus an average of 0% cover in *+Hedophyllum, +Katharina* plots (Figure 3.2). After one year, the negative effects of *Katharina* had almost entirely eliminated the positive effects of shade in *+Shade, + Katharina* plots (Fig 3.7). *Katharina* is a major consumer which has strong negative effects on all measured AEI groups, even those which make a small contribution to their total diet.

The strong response of encrusting invertebrates to the Shade treatments in this study was a major departure from previous studies in this system, which noted that encrusting invertebrates were very rare, even in *Katharina* removal areas (Duggins and Dethier 1985). The evidence suggests that the negative effect of even low abundances of *Katharina* is sufficient to cancel out the positive effect of shade on encrusting invertebrate abundances, and thus that differences in experimental design affected the ability to detect treatment effects on this group.

Previous experiments used manual *Katharina* removals at 4-5 month intervals to keep *Katharina* abundances low in removal areas (approximately 3.2 to

6.8 *Katharina* / m^2; (Duggins and Dethier 1985). Through the use of exclosure fences and twice-monthly maintenance of *Katharina* removals, I was able to more completely exclude them from my experimental plots (approximately 0.06 *Katharina* per plot over the 14 month duration of the study, for a density of 0.23 *Katharina* / m^2). Thus this lower density of *Katharina* allowed me to detect treatment effects that were not seen in previous studies. There is no evidence that the use of vexar mesh fences affected encrusting invertebrate abundance. Despite the presence of fences in –Shade, -*Katharina* plots, encrusting invertebrate cover remained low, and despite the extensive surface area provided by the mesh for invertebrate settlement, none of these groups (sponges, bryozoans or hydroids) were ever observed settled on vexar mesh in the field in 3 years of experimental work (J. Burnaford, personal observation). In addition, similar large increases in the cover of this group were recorded in a simultaneous experiment in which enclosure fences were not present (Chapter 2; encrusting invertebrate cover in similarly sized –*Hedophyllum* +Shade plots reached up to 84% in November 1998). This peak was presumably caused by a relaxation in *Katharina* grazing pressure, as it occurred during a period of low *Katharina* abundance in plots but had disappeared after several months of increased *Katharina* abundances. No similar patterns were seen in +*Hedophyllum* plots, in which *Katharina* abundances were low but steady throughout this period (Chapter 2). Therefore, these two data sets strongly suggest that the negative effect of even low abundances of *Katharina* is sufficient to cancel out the positive effect of shade on encrusting invertebrate abundances.

Despite the fact that under natural conditions, *Katharina* avoid open areas at low tide (Chapter 2) they are clearly able to exert strong effects on AEI abundance in –Shade plots under sustained conditions of physiological stress (see Chapter 4 for data on summertime physiological stress levels). In fact, the magnitude of the *Katharina* effect (measured as the difference in the abundance of AEI groups between + and –*Katharina* plots) was greater in open plots than in shaded plots for algae, although this pattern is most likely due to a 'zero truncation' pattern; as algal abundances in +Shade plots, already low, could not be reduced below 0% cover.

Effect of Katharina *on the Mobile Animal Assemblage*

In contrast to the strong negative effects of *Katharina* on the AEI assemblage, only two groups of mobile animals showed evidence of a response to the presence of *Katharina*. Primarily because of their low overall abundance and the limited information available on anemone behavior in the field, the annual peak in anemone abundance August in shaded plots without *Katharina*, although distinct, is difficult to interpret. Limpets, however, showed a strong positive association with the presence of *Katharina*, and were virtually absent from plots without the chiton (Fig 3.12). The positive interaction between these two consumers has been previously described as an indirect commensalism, where *Katharina*, in removing macroalgae, have an indirect positive effect on limpets, whose primary food source (diatoms) increases in response to macroalgal reduction (Dethier and Duggins 1984).

Relative Effects of Shade and Katharina *on The Low Zone Assemblage*

Earlier studies in this system found that 'releasing' the understory assemblage from the negative effects of shade and *Katharina* herbivory resulted in a dramatic increase in species diversity and in the abundance of green and red macroalgal groups (Dayton 1975, Dethier and Duggins 1984, Duggins and Dethier 1985). These studies also found that that 'releasing' the assemblage from either shade or *Katharina* grazing resulted in an assemblage of macroalgal abundance and species diversity intermediate between the 'release' and 'natural' conditions (Duggins and Dethier 1985). What this study adds to the previous work are the striking results that: 1) the effects of *Hedophyllum* on the AEI assemblage are primarily through the provision of shade, and not through any effect of the *Hedophyllum* thallus *per se;* 2) the presence of shade has strong positive effects on consumer species (and therefore, the *Hedophyllum* canopy affects species in all trophic levels) and 3) the effects of Shade and *Katharina* on the algal and encrusting invertebrate understory assemblage are quantitatively similar, but qualitatively very different.

The individual effects of shade (+Shade, -*Katharina* plots) or *Katharina* (-Shade, + *Katharina* plots) on total AEI abundance or species diversity were not, overall, distinguishable (Fig 3.4, Table 3.3). As noted in previous studies, plots with either of these treatments were intermediate, in total abundance and species richness, between 'release' plots (high abundance and richness) and 'natural' plots (+ Shade, + *Katharina*, low abundance and richness). However, despite these quantitatively similar effects on both abundance and species number, these two factors have fundamentally different effects on the structure of that assemblage in

terms of the specific identity of species. Plots with Shade (+Shade, -*Katharina* plots) are dominated by encrusting invertebrates. However, plots with *Katharina* (-Shade, + *Katharina* plots) are characterized by intermediate abundances of macroalgae and near 0% cover of encrusting invertebrates. Therefore, because of differential effects of shade and *Katharina* on the various AEI groups, these two factors exert strong, qualitatively different effects on the understory assemblage.

The importance of complex interactions to community structure

The outcome of one component of a complex network of interactions often depends on the outcome of another (Levine 2000). The data from this study elucidate the strong, independent effects of *Hedophyllum* and *Katharina* on this community. In a community with *Hedophyllum* but no *Katharina* (Fig 3.14), the understory assemblage is dominated by encrusting invertebrates, which benefit directly from the *Hedophyllum* canopy through the positive effects of shade and indirectly through the negative effects on algal abundance (both direct (mechanical, chemical and shading) and indirect (increased abundance of herbivores). However, the positive effects of shade on encrusting invertebrates are limited by the negative effects of the *Hedophyllum* thallus. The abundance of mobile herbivores, while increased by *Hedophyllum*, may also be 'controlled' by higher predation rates under the algal canopy due to increased carnivore abundance (an indirect effect of *Hedophyllum*).

In a community with *Katharina* but no *Hedophyllum* (Fig 3.15), the assemblage is dominated by moderate cover of macroalgae and microalgae, with very low abundance of encrusting invertebrates. *Katharina* is the strongest

interactor in the system, although other consumers are present in low numbers. *Katharina* has strong negative effects on macroalgae and encrusting invertebrates. Encrusting invertebrate cover is further reduced by the absence of shade.

However, in a community with both *Hedophyllum* and *Katharina*, these complex combinations of direct and indirect interactions are obscured by a few strong interactions (Fig 3.16). These strong interactions produce a sparse (in terms of cover and species richness) understory AEI assemblage, which in turn leads to the assumption that this sparse assemblage is the result of a simple interaction web. The combination of synergistic (both *Hedophyllum* and *Katharina* have negative effects on algae) and antagonistic (the effects on encrusting invertebrates are opposite, but the negative effect of *Katharina* is stronger than the positive effect of *Hedophyllum*) effects obscures the network of connections that are important for the maintenance of community structure but which only become apparent by removing one or the other of these strong interactors.

Hedophyllum has been labeled a 'foundation species' (Dayton 1972); an 'ecological dominant' (Dayton 1975), and a physical autogenic ecosystem engineer (Jones et al. 1994, 1997b, Lawton and Jones 1998). The results of this experiment, which highlights the network of positive, negative, trophic and non-trophic interactions through which *Hedophyllum* structures the community, strengthen and add a new dimension to these classifications. Ultimately, community structure in this system is more strongly affected by the presence of shade than by the presence of *Katharina*, because the presence of shade strongly affects all trophic levels, including the abundance and distribution of *Katharina*.

Only through a composite perspective can we truly evaluate the effects of such ecological dominants on communities and ecosystems.

Figure 3.14 Interaction web in the absence of *Katharina*. Each interaction proceeds as indicated by the direction of the arrow. Solid lines signify direct interactions, dashed lines signify indirect interactions. Arrow thickness indicates relative interaction strength. Font size of each group indicates relative abundance. Labeled lines indicate results from this study. Unlabelled lines indicate results from previous studies or food interactions (i.e. herbivores consume algae, carnivores consume herbivores). See text for details.

Figure 3.15 Interaction web in a *+Katharina*, *-Hedophyllum* plot. Details as in Fig 3.14.

Figure 3.16 Net interaction web in the natural (*+Hedophyllum*, *+Katharina*) system. Examining only the net effects hides details which are illustrated in Fig 3.14 and Fig 3.15. Details as in Fig 3.14.

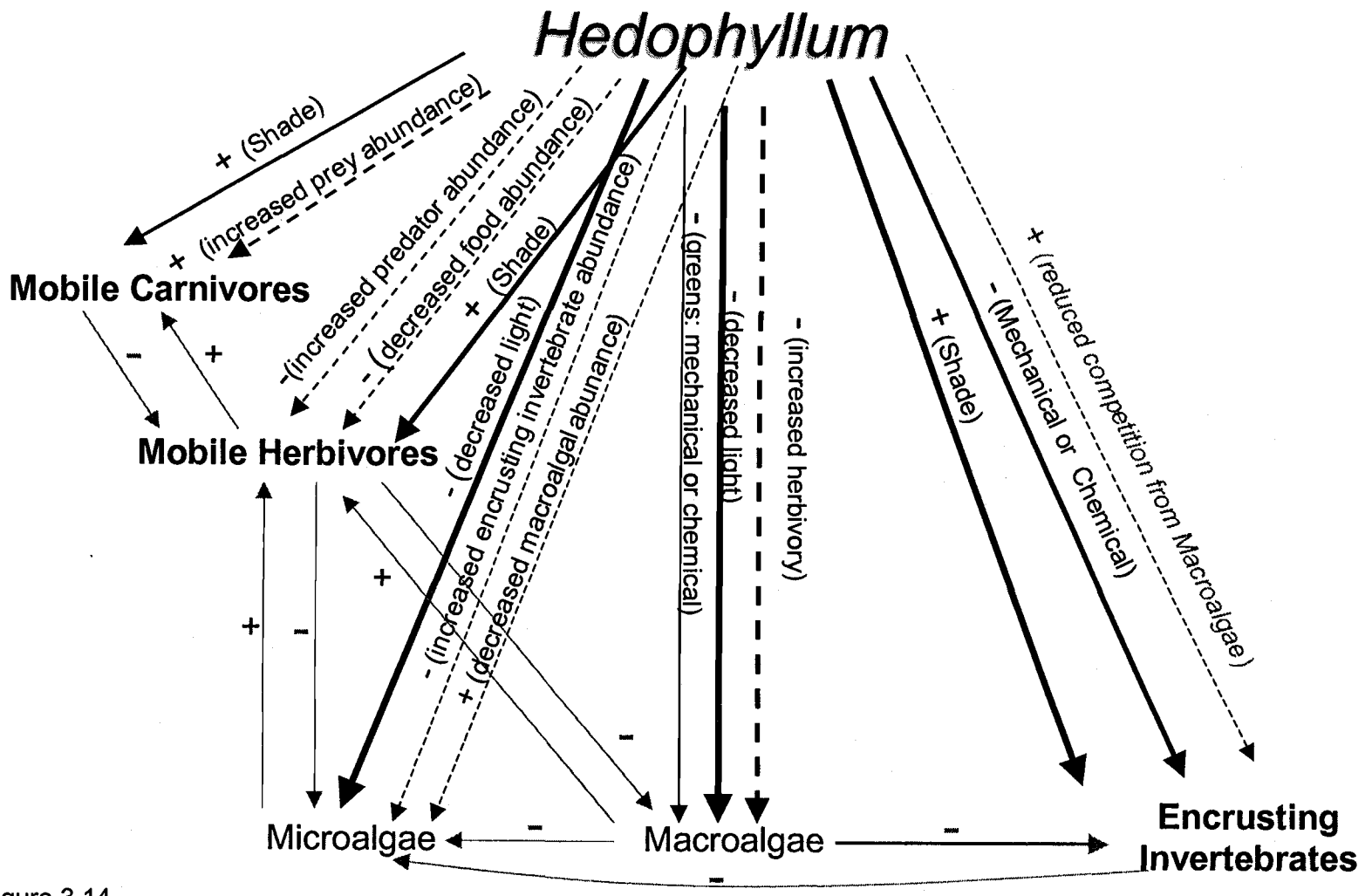
Hedophyllum
+ (Shade)
+ (increased prey abundance)
Mobile Carnivores
- (increased predator abundance)
- (decreased food abundance)
+ (Shade)
- (decreased light)
- (increased encrusting invertebrate abundance)
+ (decreased macroalgal abunance)
- (greens: mechanical or chemical)
- (decreased light)
- (increased herbivory)
+ (Shade)
- (Mechanical or Chemical)
+ (reduced competition from Macroalgae)
Mobile Herbivores
Microalgae
Macroalgae
Encrusting Invertebrates

Figure 3.14

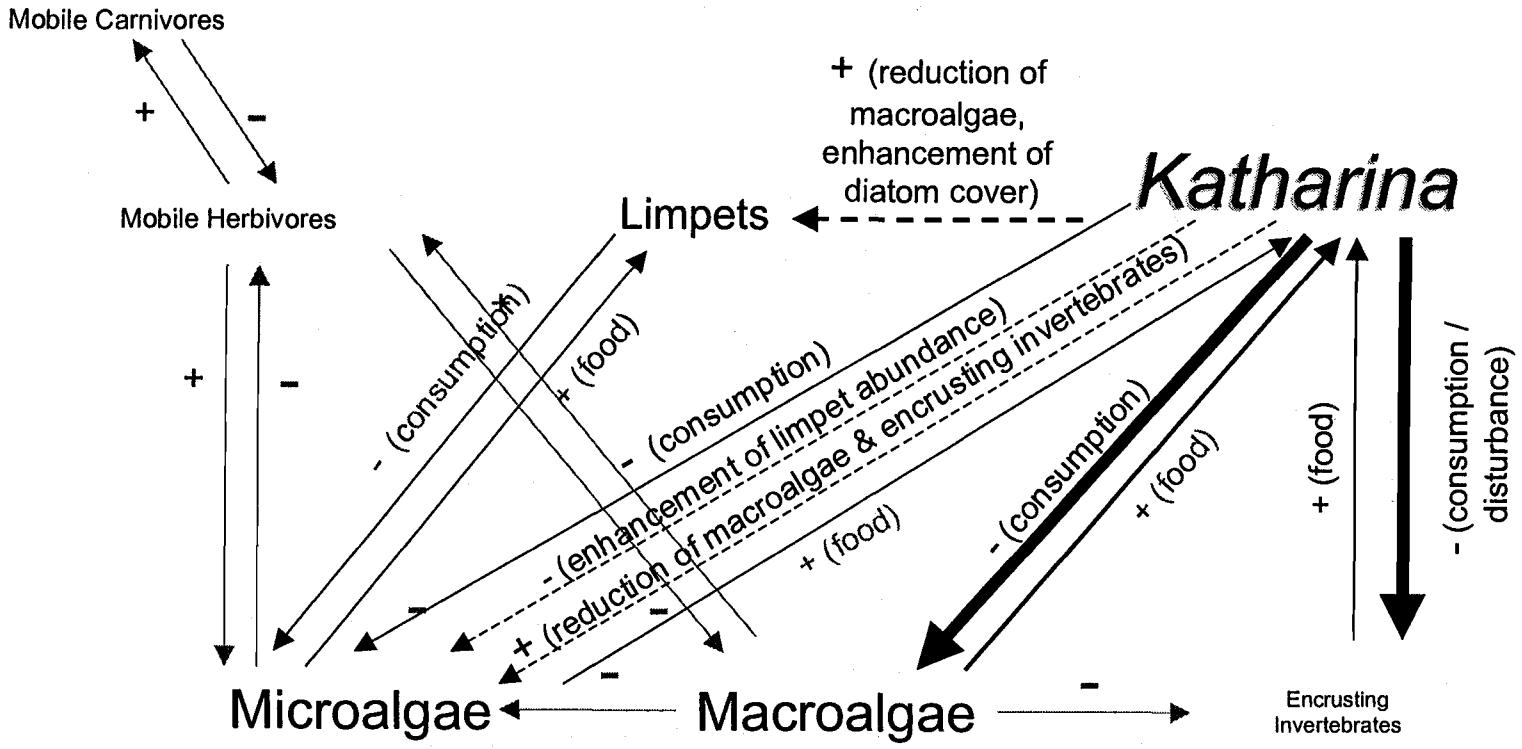
Mobile Carnivores
+
-
Mobile Herbivores
+ (reduction of macroalgae, enhancement of diatom cover)
Limpets
Katharina
+
-
- (consumption)
+ (food)
- (consumption)
- (enhancement of limpet abundance)
+ (reduction of macroalgae & encrusting invertebrates)
+ (food)
- (consumption)
+ (food)
+ (food)
- (consumption / disturbance)
-
-
-
-
Microalgae
Macroalgae
Encrusting Invertebrates

Figure 3.15

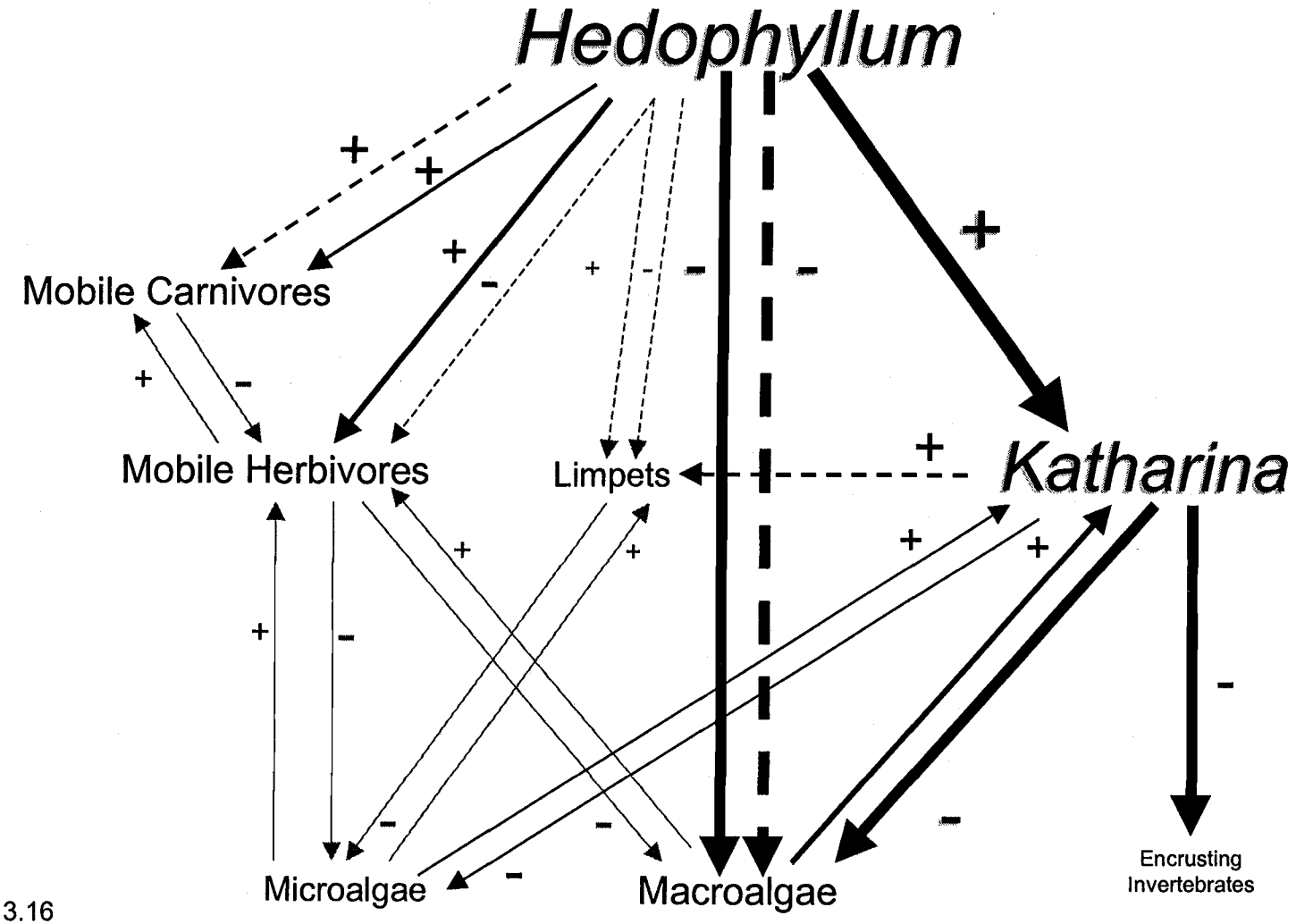
Hedophyllum
Mobile Carnivores
Mobile Herbivores
Limpets
Katharina
Microalgae
Macroalgae
Encrusting Invertebrates

Figure 3.16

Conclusions

The maintenance of the natural low-zone *Hedophyllum*- associated community depends on the combined effects of the presence of shade (provided by the *Hedophyllum sessile* canopy under natural conditions) and the grazing activity of the chiton *Katharina tunicata*. Although *Katharina* is able to exert a strong effect on the understory algal and encrusting invertebrate assemblage both in shaded and unshaded areas, because of strong behavioral selection of the chiton for shaded areas, the ultimate control of the effect of *Katharina* lies in the presence or absence of *Hedophyllum*. In addition to its effects on sessile algae and animals, the shade provided by the *Hedophyllum* canopy was the major factor in determining abundances for most of the mobile animals studied. Overall, the net effect of the *Hedophyllum* canopy is to structure the understory community through direct positive effects on understory mobile animals (including *Katharina*) and direct and indirect negative effects on algae and encrusting invertebrates (through the concentration of *Katharina* and other consumers in canopy areas). However, these net effects are the result of complex layers of positive and negative, direct and indirect effects, which combine to structure the low-zone community.

CHAPTER 4

PHYSIOLOGICAL EFFECTS OF HEAT STRESS: EFFECTS OF SHADE AND SEASON ON HSP70 LEVELS IN FIELD POPULATIONS OF *KATHARINA TUNICATA*

ABSTRACT

In the low intertidal zone of the Pacific Northwest, the chiton *Katharina tunicata* exhibits a strong behavioral preference for shaded areas during the spring and summer, but shows no such preference during the fall and winter. The use of refugia by mobile animals to avoid thermally stressful conditions is widely documented in both terrestrial and aquatic systems, and has been shown to have major effects on community structure. One physiological consequence of temperature stress is the denaturation of cellular proteins, which, because they depend on specific three-dimensional structures for proper function, are some of the most thermally sensitive cellular components.

Heat shock proteins (Hsps) function to limit the cellular damage caused by thermal denaturation of proteins, and are produced in response to thermal stress in almost every animal species tested to date. Few field studies have attempted to characterize the pattern of Hsp expression in any field population of mobile animals; therefore, the effect of behavioral avoidance of thermal stress on the production of heat shock proteins is largely unknown.

I predicted that the strong behavioral selection for shaded areas exhibited by *Katharina* during the summer months would result in lower levels of Hsps in

chitons maintained in shaded areas than in chitons maintained in open areas. I tested this hypothesis by maintaining chitons in the field in experimental plots with and without shade through two summers (1999 and 2000). In addition, I characterized temporal patterns of Hsp expression in the natural *Katharina* population by combining monthly Hsp sampling with long-term intensive monitoring of thermal conditions. Experimental manipulations showed no effect of shade on Hsp70 levels; however, Hsp70 levels in the general population of *Katharina tunicata* showed strong seasonal patterns. Thus, although *Katharina* responds to seasonal sub-lethal stress at the molecular level, Hsp70 production did not respond to field manipulations. This data set represents one of the first investigations of the spatial and temporal pattern of Hsp expression in a field population of mobile animals, and highlights the need for linked laboratory and field studies that document the pattern of Hsp expression in nature while simultaneously investigating the environmental and behavioral factors that influence the pattern.

INTRODUCTION

The important role of physiological stress (defined as the direct effect on organisms of deviations from environmental optima (Menge and Olson 1990)) in natural communities is widely recognized in conceptual models of community structure (Menge and Sutherland 1987, Menge and Olson 1990, Huey 1991, Bertness and Callaway 1994) and widely documented in both terrestrial and aquatic systems. Animals can avoid physiologically stressful conditions through behavioral adjustments, such as exploiting less stressful microhabitats (Menge

1978a, Levings and Garrity 1983, Garrity 1984, Lasiak and Dye 1986, Huey et al. 1989, Huey 1991, Williams and Morritt 1995, Schwarzkopf and Alford 1996) or altering temporal behavioral patterns to avoid stressful conditions (Garrity 1984, Wehner et al. 1992). Behaviors such as these can create distinctive patterns in communities by affecting the spatial or temporal distribution of prey (Menge 1978a, Levings and Garrity 1983, Menge and Sutherland 1987, Fairweather 1988, Sanford 1999b).

In the low intertidal zone of the Pacific Northwest, the chiton *Katharina tunicata* exhibits a strong behavioral preference for shaded areas during the spring and summer, but shows no such preference during the fall and winter (Chapter 2). Results from previous experiments indicated that this habitat association was a response to the presence of shade *per se*, and not a result of differential food availability, predation, or predation avoidance (Chapter 2, Chapter 3). Due to the strong seasonal component of the behavior, I hypothesized that the chitons were using the shades as refugia from the thermal stress caused by the high air temperatures and insolation during daytime low tides.

What are the biological consequences of summertime thermal stress? Proteins are some of the most thermally sensitive cell components because three-dimensional protein structures are largely stabilized by non-covalent interactions, which are strongly affected by temperature change (Somero 1997). Molecular chaperones are a special class of proteins that function to limit the cellular damage caused by stress-induced denaturation of other proteins (Feder and Hofmann 1999). The 70kDa molecular weight family of Heat Shock Proteins (Hsps) is one of the best-characterized groups of molecular chaperones, and plays important

roles in both routine cell maintenance (Bukau and Horwich 1998), and recovery from stress.

Because of their role in cell maintenance, many organisms, including intertidal mussels (Hofmann and Somero 1995, Chapple et al. 1998) and snails (Tomanek and Somero 1999), maintain 'baseline', or constitutive pools of Hsps (Bukau and Horwich 1998). Increased concentrations of these endogenous Hsps have been correlated with increased exposure to high temperatures (Hofmann and Somero 1995, Roberts et al. 1997, Hofmann 1999). In addition to these constitutive forms, the production of pools of 'inducible' Hsp70 isoforms following heat stress has been observed in almost every animal species tested to date (Feder and Hofmann 1999). Under conditions of heat stress, the primary function of Hsps is to bind and stabilize denatured proteins until these proteins can either be refolded or degraded by proteases (Fink 1999, Wickner et al. 1999).

Although Hsp expression is seemingly ubiquitous, the vast majority of studies are conducted in laboratory settings, in which animals experience tightly controlled variations in a single stress factor (Feder and Hofmann 1999). As a result, little is known about the functional significance of Hsps in natural populations, which experience a number of stresses simultaneously. The few studies that have examined natural populations have established that, at least in some circumstances and some taxa, Hsp expression is induced by field conditions (Near et al. 1990, Hofmann and Somero 1995, Roberts et al. 1997, Tomanek and Somero 2000); but our knowledge of the frequency, variability, and intensity of Hsp expression in natural populations remains extremely limited (Brennecke et al. 1998, Hofmann 1999).

In field populations of intertidal invertebrates, variation in Hsp70 expression has been observed both on a seasonal basis and between individuals at different tidal heights. In studies with *Mytilus trossulus*, endogenous concentrations of Hsp70 in gill tissue were significantly elevated in summer versus winter-acclimatized mussels (Hofmann and Somero 1995). Studies with field-collected *Mytilus californianus* individuals showed a similar pattern; endogenous concentrations of Hsp70 were higher in gill tissue of summer-collected specimens than in winter-collected animals (Roberts et al. 1997). In that study, *M. californianus* collected from the high edge of the mussel bed in the summer showed higher levels of Hsp70 isoforms compared to mussels from the lower edge of the mussel bed, a result which complements observational evidence suggesting that thermal stress increases with increasing exposure time in the intertidal. In field studies with *Strongylocentrotus purpuratus* (the purple urchin), animals from intertidal tidepools displayed higher Hsp70 levels, Hsp induction temperatures, and levels of ubiquitin conjugates (indicating elevated levels of irreversibly damaged proteins) than their subtidal conspecifics (Hofmann 1999).

Expression of heat-shock proteins can reduce the short and long-term deleterious effects of thermal stress; however, this expression carries its own costs. Hsps can interact improperly with properly folded proteins, and increased expression of Hsps has been shown to significantly reduce rates of cell growth and division (Feder et al. 1992, Wickner et al. 1999). The energy investment required for Hsp production and the degradation of damaged proteins (transcription of chaperone mRNA, translation and production of chaperone proteins, provision of ATP for proteases, and repair, replacement and recycling of damaged proteins)

could be substantial for animals experiencing chronic heat stress. When Hsp production is induced in *Drosophila* by increasing temperatures, transcription and translation of non-Hsp proteins is almost completely halted, Hsp mRNA is preferentially transcribed and translated, and within one hour Hsp mRNA levels can increase 1,000 to 10,000-fold (Petersen and Lindquist 1989, Lindquist and Petersen 1990). In laboratory populations of *Drosophila*, repeated exposure to sub-lethal thermally stressful conditions decreases survivorship (Krebs and Feder 1998).

The observed patterns of Hsp expression in intertidal invertebrates indicate that these animals are experiencing stressful conditions which have real biological consequences on a number of spatial and temporal scales. All of these studies were conducted on essentially sessile animals (although urchins can move, populations in the Pacific Northwest intertidal zone are primarily sedentary, forming permanent urchin beds by creating depressions in soft rock substrata). Thus the effect of behavioral avoidance of thermal stress on the production of heat shock proteins is largely unknown.

In this study I investigated the effect of shade and season on Hsp70 levels in field populations of the mobile herbivore, *Katharina tunicata*. I predicted that the strong behavioral selection for shaded areas exhibited by *Katharina* during the summer months would result in lower levels of heat shock proteins in chitons maintained in shaded areas than in chitons maintained in open areas, and tested this hypothesis by maintaining chitons in the field in experimental plots with and without shade through two summers (1999 and 2000). In addition, I characterized temporal patterns of Hsp expression in the natural *Katharina* population by

combining monthly Hsp sampling with long-term intensive monitoring of thermal conditions in order to answer two key questions. 1) Are Hsp70 levels higher in *Katharina* during the summer than during the winter? 2) Are differences in Hsp70 levels between treatments and surveys correlated with differences in body temperature?

METHODS

Study Site

The study site, Pile Point, is located on the west side of San Juan Island, Washington, at 48°28.9' N, 123°05.7' W, 6 km south of Lime Kiln State Park and 15 kilometers east of Vancouver Island, British Columbia, across Haro Strait. Pile Point is a rocky point with several flat rocky benches separated by shallow subtidal channels, flanked on both sides by small cobble beaches and steep rocky cliffs. Plots used in this study were on the main point, at +0.6 to +0.9m tidal height.

The low zone at Pile Point is dominated by the perennial kelp *Hedophyllum sessile*, with occasional large tide pools containing high cover of the surfgrass *Phyllospadix scouleri* or dense beds of the purple urchin *Strongylocentrotus purpuratus*. Large mobile invertebrates in the low intertidal zone include the chiton *Katharina tunicata*, which are very abundant (up to 70 / m^2), the gum-boot chiton *Cryptochiton stelleri*, and the seastar *Pisaster ochraceus*. *Katharina tunicata* individuals are primarily found underneath the *Hedophyllum sessile* canopy, but occasionally individuals are found in the open (unshaded areas) during low tide.

The tidal cycle in the San Juan Islands, as in the rest of the Pacific Northwest, is mixed semi-diurnal. From March to September, low low tides (those in which the low intertidal zone is exposed) occur during daylight hours, from 7am to 4pm. From October to February, low low tides occur at night, from 10pm to 4am. The low intertidal zone is uncovered from 30 min to 5+ hours during a low tide, depending on weather conditions such as wind and pressure systems which affect actual tide height and wave height. Pile Point is considered a 'wave protected' site (maximum wave force during the summer of approximately 14 N) and the calm ocean conditions contribute to long periods of exposure for low intertidal organisms in the spring and summer.

Experimental Enclosure Design

In May 1999 I established 12 *Katharina* enclosure plots, 43 by 38 cm, on flat rocky surfaces at approximately +0.5 meters tidal height at Pile Point. I constructed fences of black vexar mesh (¼" mesh size, 4 to 5cm high) embedded in a layer of Z-spar™ marine epoxy putty (Seattle Marine Supply Co.) around the plot perimeter, and removed all *Katharina tunicata* individuals from the plots. Plots (n=12) were assigned to pairs based on location and topography, so that each pair of plots would be covered and uncovered by tidal flow at the same time (immersion or emersion of different pairs varied by a maximum of 20 minutes). One plot in each pair was randomly assigned to a shade treatment (+Shade), the other to an unshaded (-Shade) treatment. For both treatments, I installed vinyl-coated wire mesh letterbaskets (34 x 29 x 7cm, Cascade Office Supplies) with stainless steel screws and washers into plastic wall anchors sunk into holes at the 4 corners

drilled with a Ryobi gas-powered rotohammer. For shaded plots, I attached 3 sheets of black vexar mesh (41 x 36 cm, ¼" mesh, Norplex) to the tops of the letterbaskets by threading cable ties through the mesh at the 4 corners of the basket top. Thus, almost the entire area of the + Shade plots was shaded by the mesh roof.

After letting the experimental plots sit for at least 24 hours (to allow complete polymerization of the epoxy putty) I stocked each plot with 4 adult (6.5 to 8cm) *Katharina*, the median number of adults in the plots at the time of their establishment (J. Burnaford, unpublished data). *Katharina* were transplanted by prying them off the rock surface using a table knife wedged under the foot. *Katharina* that were injured during the transplant process were not used for the experiment. I individually tagged each *Katharina* with 3 separate tags, color-coded for treatment (red for shaded animals, blue for unshaded animals). Each tag was affixed to the exposed portion of a separate valve: valves were first scraped with a scalpel (to remove diatoms) and dried with a piece of cloth. The three tag types were: a) nail polish, b) numbered plastic bee-tags (Christian Graze KG) affixed with nail glue, c) small pieces of electrical tape, affixed with nail glue. Plots were checked every tide series (approximately once every 2 weeks) and missing tags were replaced. If a chiton was missing from an experimental plot, I replaced the chiton with a 'filler' chiton, which was within the original size range and tagged with a uniquely colored tag. Only originally stocked chitons were used for experimental sampling.

Beginning in June 1999 (one month after set-up) I sampled experimental chitons at approximately 30 day intervals (actual sampling dates depended on tidal

and weather conditions). In most cases, I sampled chitons on the last day of a low tide series. On each sampling date, I randomly chose 3 pairs of plots, and randomly chose 1 individual from each plot for sampling (n = 3 individuals per treatment per month). Sampled chitons were removed from the rock with a table knife, and a small (10-13ml) piece of foot tissue was removed using a clean scalpel. The tissue was immediately frozen on dry ice for transport to the lab. Tissue samples were maintained at –80°C until protein analysis. Although tissue removal was not fatal to the chitons (J. Burnaford, personal observation) I did not replace sampled chitons in experimental plots to avoid confounding effects of chemical stress signals on other experimental animals. Instead, sampled chitons were left outside experimental plots, and 'filler' chitons were added as described above to maintain treatment densities. However, only originally stocked chitons were used for experimental sampling.

I sampled plots through November 1999 (until all originally stocked chitons had been sampled or lost). I re-stocked the plots in April 2000 and repeated the experiment through August 2000.

Sampling of Non-Experimental Chitons

In June 1999 I began monthly sampling of 6 *Katharina* from the general population at Pile Point, at the same tidal height as my experimental plots. Tissue sampling procedures were the same as for the experimental chitons. Because tissue removal left a recognizable scar on the foot, I was able to avoid re-sampling individuals. On each date I collected tissue from 3 individuals in the open and 3 individuals underneath the *Hedophyllum* canopy, to avoid any confounding effects

of immediate location on Hsp70 levels. Sampling continued until August 2000, although no samples were taken in December 1999.

Quantification of Hsp70 Levels

For protein analysis, frozen tissue samples were weighed, mixed 1:1 (w/v) with a 2% SDS homogenization buffer solution (50mM Tris-HCl (pH 6.8), 2% SDS, 1mM EDTA, 1mM PMSF) and homogenized for 15 minutes using Kontes Duall ground glass homogenizers. Homogenate (0.4 to 0.7 ml per sample) was transferred to Eppendorf tubes and boiled for 7 minutes at 100°C in a dry bath, then centrifuged for 10 minutes at 10,000g. Supernatant was carefully drawn from the tubes, separated in 15 to 20 µl aliquots and frozen at –80°C.

Protein concentration in each sample homogenate was determined by a Coomassie Plus protein assay (Pierce Chemical Co.). After protein concentration determination, equal amounts of protein (10 µg or 15 µg depending on the individual gel) were mixed 1:1 (v/v) with Laemmli sample buffer (BioRad Laboratories) with 5% 2-mercaptoethanol. The protein-buffer solution was boiled for 3 min and centrifuged at 10,000g for 1 min. The samples were electophoresed on a 10% SDS- polyacrylamide gel using the methods described by Laemmli (1970). In the first lane of each gel, I loaded 10µg of a 73kDA bovine Hsc mixed with Laemmli sample buffer (0.02µg Hsc73 / µl sample buffer). Tissue from *Katharina* individual was run in duplicate (2 separate protein:buffer mixtures loaded on 2 lanes on a gel).

Separated proteins were transferred to nitrocellulose membrane via semi-dry electrophoretic transfer at 30V for 15 hours, using a transfer buffer of 192mM

glycine, 25mM Tris base and 20% methanol. Transfer conditions were optimized to ensure complete transfer of proteins in the 70kDa region of the gel.

Western blotting was then performed using an enhanced chemiluminescent protocol. Following transfer, the membrane was blocked for 1 hour with 5% nonfat dry milk in phosphate-buffered saline (PBS). After three 5 min washes in PBS containing 0.1% Tween-20, the blot was incubated for 1.5 hours in the primary antibody solution composed of a rat anti-hsp70 antibody (7.10, Affinity Bioreagents) diluted 1:2500 in a solution containing 2% nonfat dry milk in PBS, 20% fetal calf serum, 0.02% thimerosal and 1 mM PMSF. The blot was washed three times for 10 min with PBS/0.1% Tween-20, incubated for 30 min in bridging antibody (rabbit anti-rat IgG; Vector Laboratories) diluted 1:2000 in blocking solution, and then washed three times for 5 min with PBS/0.1% Tween-20. The presence of the primary antibody was detected by a 1 hr incubation in horseradish peroxidase (HRP)-conjugated Protein A (diluted 1:5000 in blocking solution) followed by three 5 min washes in PBS containing 0.3% Tween-20 and three 5 min washed in PBS/0.1% Tween-20. The western blot was then developed using ECL detection (Amersham). Each blot was incubated for 1 min in 0.5 ml of the detection reagents (0.25ml of each reagent). Blots were exposed to x-ray film (Kodak X-OMAT AR 5) for 5 to 120 seconds. The developed film was scanned with a Fluor-S MultiImager System (BioRad Laboratories) and band intensities quantified using Quantity One software (BioRad Laboratories).

To compare concentration of Hsp70 in tissue samples run on separate gels, I calculated the Relative Hsp70 Level for each protein band; this is a dimensionless value suitable only for comparison among these samples. The

density of each protein band was calculated by the Quantity One program as optical density units (ODU) / mm^2. Relative Hsp70 level was calculated separately for each band in each lane of each gel, using the intensity of the positive control which was loaded onto each gel.

$$\text{Relative Hsp70 Level} = \frac{\text{(band density / µg sample protein loaded)}}{\text{(density of positive control / µg positive control protein loaded)}}$$

I averaged duplicate sample lanes to get one value of Hsp70 level for each *Katharina* individual.

Katharina *Body Temperature*

I used a hypodermic needle probe (Hyp-1, Omega Engineering) attached to a hand-held microprocessor thermometer (model HH21, Omega Engineering) to record body temperatures of *Katharina* at low tide on a number of summer days in 1999 and 2000. After opening a hole in the dorsal surface of the mantle with a dissecting probe, I inserted the temperature probe into the mantle cavity. I recorded body temperatures of haphazardly selected chitons at haphazardly selected times. Temperature samples were always taken from 3 chitons in any microhabitat (-Shade, under Artificial Shades, or under *Hedophyllum* canopy) at any given time, and measurements were always paired (-Shade vs. Artificial Shades or -Shade vs. *Hedophyllum* canopy) for comparison.

Ambient Temperature Records

Ambient temperatures were recorded every 15 or 30 min by data-loggers (Optic StowAway, Onset Computer Corporation) attached in the low intertidal zone around Pile Point. Data-loggers were secured to stainless steel mesh frames that were bolted to the rocky substratum. Throughout the experiment I experienced a high rate of data-logger failure; temperatures were recorded by one to seven data-loggers at any time. Data-loggers were deployed both in -Shade areas and underneath Artificial Shades for the duration of this study. Data-loggers were deployed underneath *Hedophyllum sessile* thalli only during selected low tide periods, as the abrasion by mesh frames ripped *Hedophyllum* blades during high tides, potentially biasing data.

By combining temperature data with predicted tidal heights for Haro Strait, (Tides & Currents, Nautical Software) I extracted Low Tide Temperatures for each data-logger (temperatures recorded while the data-logger was emersed at low tide). Low Tide Temperatures reported here are averages of all data-loggers that were active at any one time. To compare the temperature regimes of my experimental treatments, I used data from a pair of data-loggers deployed in the center of these experimental enclosures, which recorded temperatures every 15 minutes. After extracting Low Tide Temperatures, I calculated the Treatment Difference by matching recorded temperatures by time and subtracting temperatures underneath the Artificial Shade plots from temperatures in the – Shade plot. I calculated two summary statistics to compare temperatures in 1999 and 2000. Maximum monthly low tide temperature is the maximum value recorded by each active data-logger (averaged across all active data-loggers). Average

Daily Maximum Low Tide Temperature is calculated by extracting the maximum temperature value for each day, averaging these values for all active data-loggers to get a composite daily maximum, then averaging this composite value for each calendar month.

Statistical Analysis

All statistical analysis was performed with SAS/STAT software (version 8, SAS Institute, Inc.). Assumptions of normality and homoscedasticity were assessed by visual inspection of residual plots and normal probability plots of the residuals and original response variables. When necessary, data were log transformed to meet model assumptions. Data presented in figures are always the untransformed means ± SEM for each effect.

Body temperature data were examined using an ANOVA design with the GLM procedure. Because microhabitat body temperature data were paired (-Shade vs. *Hedophyllum* or –Shade vs. Artificial Shades), this allowed two independent comparisons of microhabitat effects on body temperature. The relationship between *Katharina* body temperature and data-logger temperature was examined with the correlation procedure in SAS.

To evaluate how the effect of shade changes with increasing ambient (-Shade) temperature, I used data-logger recorded temperatures to compare the treatment difference (-Shade – Artificial Shades) to ambient temperature using regression analysis. Residual plots and normal probability plots were visually inspected to check for violations of normality assumptions. I have presented the data in 5°C ambient temperature bins as 99% confidence intervals for mean

difference. Because data-logger measurements underneath the *Hedophyllum* canopy were limited in number, I performed a paired t-test to compare temperatures under the *Hedophyllum* canopy to temperatures under artificial shades; Shapiro-Wilk and Kolmogorov-Smirnov statistics were calculated to test for violations of the assumption of normality.

Relative Hsp70 Levels were compared across treatments and seasons by ANOVA, using the GLM procedure in SAS. Since *Katharina* individuals were never re-sampled and experimental plots were randomly selected each month for sampling, the data were not considered repeated measures, and time was included as a main factor in ANOVA after visual examination of plots of residuals versus time revealed no serial effects. Experimental Hsp70 levels were examined using time and treatment as main effects. Because of the unusually high variance of experimental data from June 1999, these data were excluded from statistical analysis. General population Hsp70 levels were compared between animals collected under *Hedophyllum* and animals collected in the –Shade areas; since there was never a statistically significant difference ($p > 0.4$ for all comparisons) these data were pooled. To test for differences in Hsp70 levels between sampling dates in the general population, I fit an ANOVA model with time as the main factor; to test for seasonal differences I used linear contrasts of sampling times and adjusted α levels for the number of non-orthogonal contrasts.

Two distinct bands of Hsp70 isoforms (a high molecular weight band and a low molecular weight band) were detected after Hsp70 quantification (Figure 4.4). As the presence of two distinct molecular weight bands in the 70 kDa range could indicate groups of isoforms which respond differently to environmental conditions

(G. Hofmann, *personal communication*), I examined changes in levels of high and low molecular weight bands separately in addition to examining changes in total Hsp70 levels (the sum of high and low molecular weight bands).

RESULTS

Katharina *Body Temperatures*

Body temperatures of *Katharina* individuals underneath the *Hedophyllum sessile* canopy or Artificial Shades were lower than body temperatures of *Katharina* in –Shade areas (Fig 4.1; Open vs. *Hedophyllum*, ANOVA on log-transformed data: $p < 0.0001$; -Shade vs. Artificial Shades, ANOVA on log-transformed data: $p < 0.0001$). Body temperatures were significantly correlated with data-logger temperature (Fig 4.2; $p < 0.0001$, $r^2 = 0.79$).

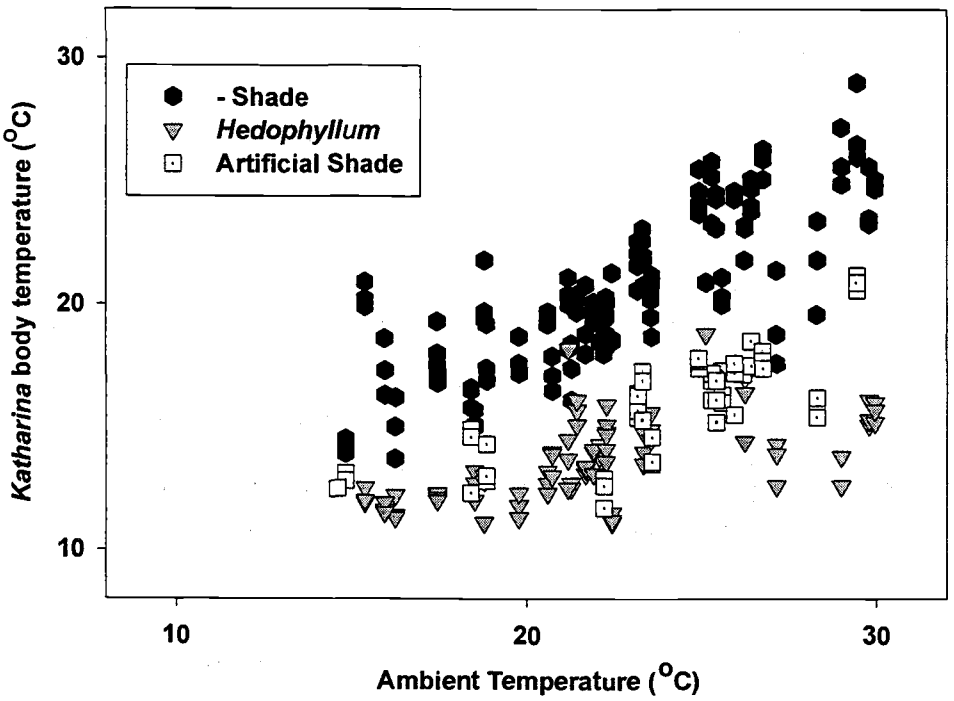

Figure 4.1 *Katharina tunicata* body temperatures in three microhabitats. Points are individual chiton body temperatures recorded in summer 1999 and 2000. Chitons were sampled from three locations in the field; in the –Shade areas (N = 131); underneath *Hedophyllum sessile* (N = 80) and underneath Artificial Shades (N = 51). Ambient temperature is the mean of all active (-Shade) data-loggers at each sampling time.

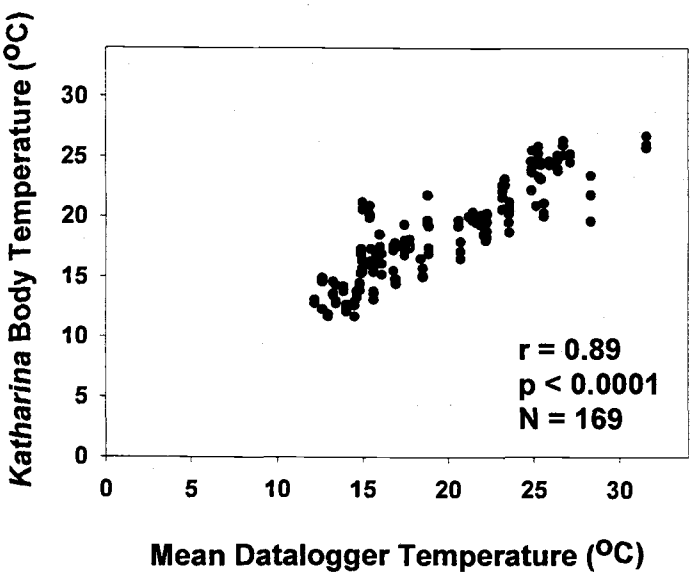

Figure 4.2 Correlation of *Katharina* body temperature with data-logger recorded temperatures. Mean data-logger temperatures are averages of all active data-loggers at the time of the associated body temperature recording. r = Pearson Correlation Coefficient; p-value indicates significance at $\alpha = 0.05$.

Ambient Temperature Records

Maximum monthly and average daily maximum low tide temperatures at Pile Point were similar slightly cooler in 1999 than in 2000 (Table 4.1). At ambient (-Shade) temperatures above 10°C, temperatures under artificial shades are significantly lower than temperatures in the open; and this temperature difference increases with increasing ambient temperature (Fig 4.3, Table 4.2, linear regression $p<0.0001$, $R^2 = 0.90$). Temperatures underneath the *Hedophyllum* canopy are less than 1°C different from those under Artificial Shades (paired t-test, temperatures $p<0.0001$, $T = 6.034$, mean difference 0.97°C). The difference between *Hedophyllum* canopy and -Shade temperatures also increased with increasing ambient temperature (linear regression $p<0.0001$, $R^2= 0.73$).

Table 4.1 Ambient low tide temperatures at Pile Point, 1999 – 2000. Mean (SD) of all active data-loggers. Low tide temperatures were extracted from continuous temperature records by combining data-logger output with predicted tide tables. **(A)** Maximum monthly temperatures are the maximum value recorded by each data-logger for that month. **(B)** Daily maximum temperatures were calculated for each data-logger by extracting the maximum value recorded each day. These values were then averaged for all active data-loggers and these composite values were averaged for each month to produce the Average Daily Maxima.

A. Maximum Monthly Low Tide Temperature (^{o}C)

Month	1999	2000
May	29.23 (1.98)	27.01 (2.02)
June	33.26 (2.68)	34.78 (3.46)
July	33.93 (3.10)	36.23 (1.64)
August	31.55 (2.93)	31.48 (1.94)

B. Average Daily Maximum Low Tide Temperature (^{o}C)

Month	1999	2000
May	17.98 (5.55)	19.58 (4.23)
June	21.74 (4.77)	23.99 (6.14)
July	23.16 (5.53)	26.83 (6.71)
August	20.79 (5.02)	20.84 (5.01)

Table 4.2 Effect of shade on understory temperature. Mean (SD) differences for low tide temperature records from paired data-loggers (-Shade vs. Artificial Shade) from July 1999 through October 2000. Because of the high sample size, upper (UCLM) and lower (LCLM) 99% confidence limits for the mean of each temperature range are given in lieu of statistical tests.

Treatment Difference (^{o}C)

Ambient Temperature	Mean (SD)	UCLM	LCLM	N
Less than 10^{o}C	-0.97 (0.81)	-0.78	-1.16	128
10 to 15^{o}C	1.10 (0.95)	1.22	0.99	464
15 to 20^{o}C	3.72 (1.30)	3.88	3.57	481
20 to 25^{o}C	6.60 (1.62)	6.81	6.39	393
25 to 30^{o}C	10.41 (1.65)	10.80	10.02	121
Greater than 30^{o}C	13.33 (1.79)	14.05	12.62	45

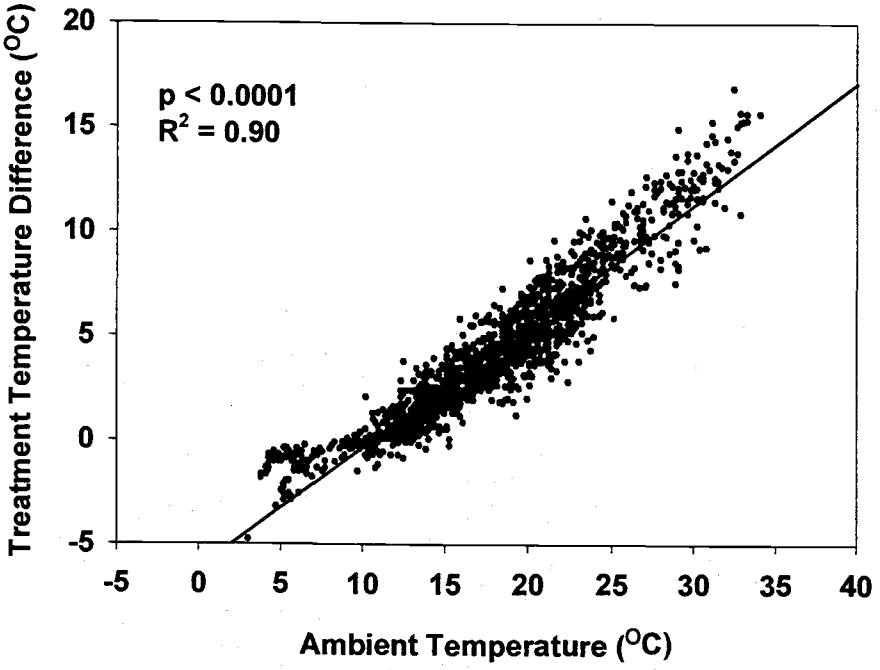

Figure 4.3 Relationship between cooling effect of shade and ambient temperature, July 1999 through October 2000. Temperature difference (-Shade – underneath Artificial Shade) was calculated from matched time recordings from paired data-loggers. Ambient temperature = -Shade temperature.

Experimental Treatment Hsp70 Levels

Two separate bands containing Hsp70 isoforms were detected from *Katharina* (Fig4.4). The concentration of proteins in the high molecular weight band (HMWB) changed over time in both treatments (Fig 4.5a; Table 4.3a; Time, $p = 0.004$) but changed differently with time in the two treatments (Table 4.3a; Time x Treatment, $p = 0.0336$). In contrast, the concentration of proteins in the low molecular weight band (LMWB) did not change significantly over time or with treatment (Fig 4.5a; Table 4.3b; Treatment, Time, and Treatment x Time all $p > 0.1$). Total Hsp70 concentration (HMWB + LMWB) did not differ consistently

between treatments, nor did it differ significantly between sampling periods (Fig 4.3b; Table 4.3c; Time, Treatment, Treatment x Time all $p > 0.05$).

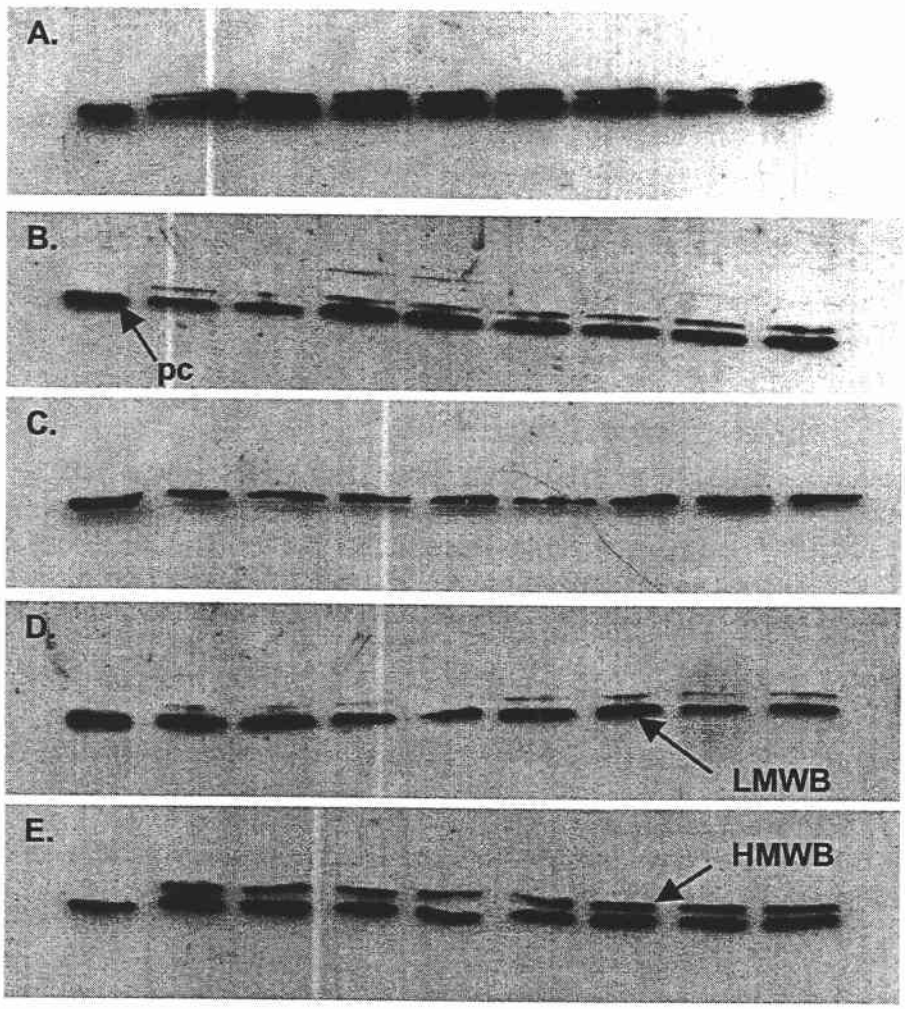

Figure 4.4 X-Ray Film showing Hsp70 protein isoforms as detected by chemiluminescent markers. Samples are from general population *Katharina*. pc = Positive control, a standard concentration of bovine Hsc73 loaded on each gel. HMWB = High Molecular Weight Band (Hsp70 isoforms). LMWB = Low Molecular Weight Band (Hsp70 isoforms). **(A)** July 1999 **(B)** September 1999 **(C)** January 2000 **(D)** March 2000 **(E)** July 2000.

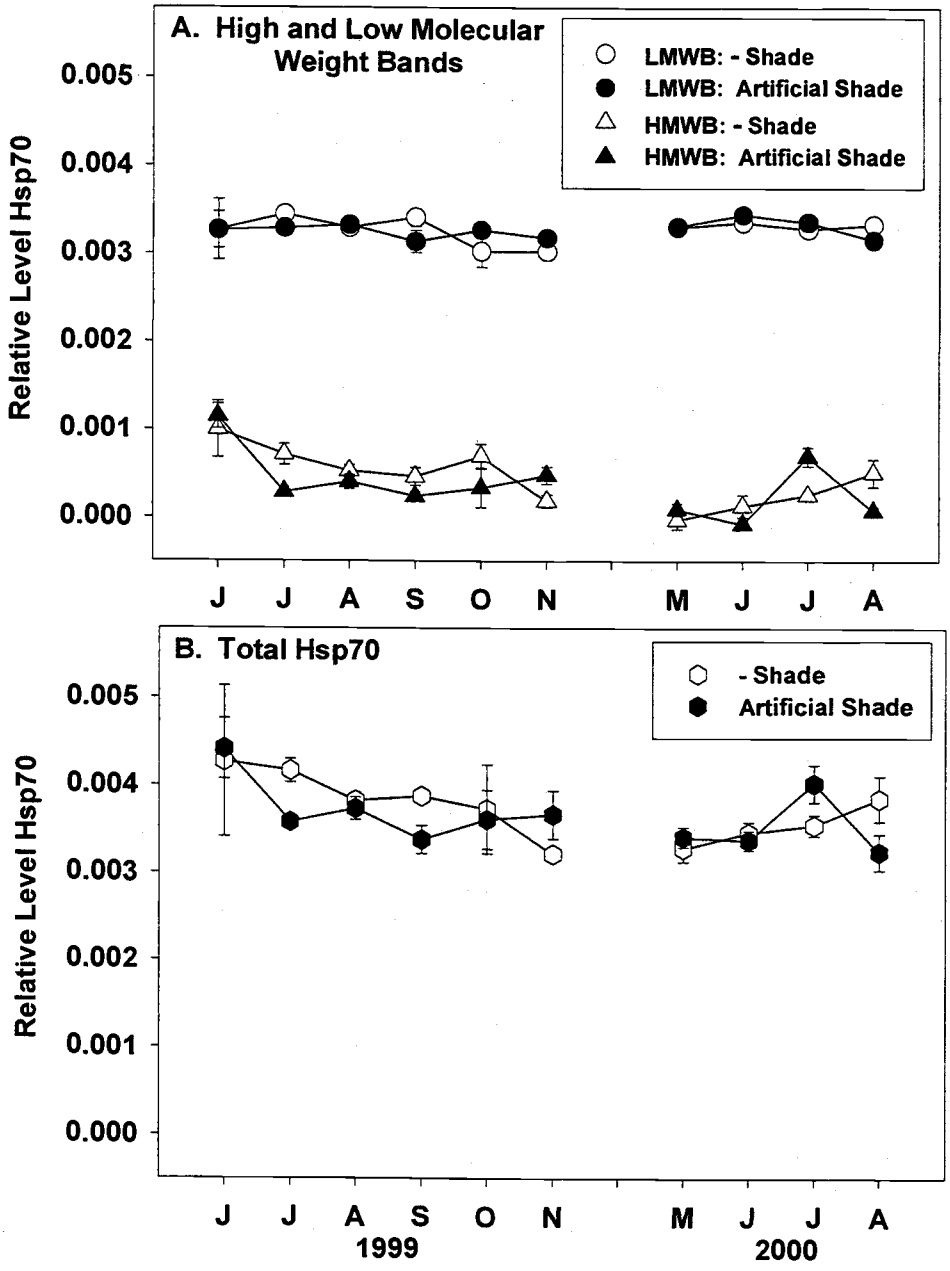

Figure 4.5 Relative level Hsp70 in experimental treatments. Mean (± SEM) Hsp70 concentration for 3 *Katharina* per treatment. Relative level is calculated for each protein band as [density of protein band / µg protein loaded] / [positive control density / µg positive control loaded]. **(A)** High (HMWB) and Low (LMWB) molecular weight bands. **(B)** Total Hsp70 (HMWB + LMWB).

Table 4.3 Variation in Hsp70 concentration with treatment and time in experimental *Katharina*. The analysis is a two-factor ANOVA on untransformed data. **(A)** and **(B)** Relative levels of Hsp70 of the two separate Hsp70 isoform bands (HMWB and LMWB); **(C)** Total Hsp70 (HMWB + LMWB). df: degrees of freedom; MS: mean squares. Bold face p-values indicate that the factor is significant at $\alpha = 0.05$.

A. High Molecular Weight Band

Source	df	MS	F	P
Treatment	1	1.52E-7	2.53	0.1206
Time	8	2.13E-7	3.55	**0.0040**
Treatment x Time	8	1.45E-7	2.41	**0.0336**
Error	36	5.99E-8		

B. Low Molecular Weight Band

Source	df	MS	F	P
Treatment	1	8.04E-11	0.00	0.9601
Time	8	5.44E-8	1.72	0.1273
Treatment x Time	8	4.31E-8	1.36	0.2456
Error	36	3.17E-8		

C. Total Hsp70 Concentration

Source	df	MS	F	P
Treatment	1	1.44E-7	1.22	0.2766
Time	8	2.05E-7	1.73	0.1244
Treatment x Time	8	2.52E-7	2.13	0.0580
Error	36	1.86E-7		

General Population Hsp70 Levels

The concentration of proteins in the high molecular weight band (HMWB) changed significantly over time (Fig 4.6a; Table 4.4a; Time effect $p < 0.0001$). *Katharina* collected in summer 1999 had higher HMWB protein concentrations than chitons collected in summer 2000 (Table 4.4a; $p = 0.0049$). However, HMWB concentrations did not differ between chitons collected in summer 1999 and winter (Table 4.4a; $p = 0.1971$). HMWB concentrations were lower in chitons collected in summer 2000 than in winter, although the difference was not significant ($p = 0.075$). Over both summers, high peaks in HWMB concentration corresponded to

sudden changes in ambient temperatures (Fig 4.7a; July 99 and March 2000). However, despite this correlation, HWMB concentrations cannot be reliably predicted from ambient temperatures (e.g. July and August 2000).

The concentration of proteins in the low molecular weight band (LMWB) changed significantly over time (Table 4.4b, Fig 4.6a; time effect $p < 0.0001$). *Katharina* collected in the summer of 1999 had LMWB protein concentrations which were lower than animals collected in summer 2000, although the difference was not significant (Table 4.4b; $p = 0.076$ for summer 99 vs. summer 00 contrast). *Katharina* collected in either summer, however, did have higher concentrations of the low molecular weight protein band than animals collected in the winter (Table 4.4b; $p = 0.0038$ for summer 99 vs. winter contrast, $p < 0.0001$ for summer 00 vs. winter contrast). The dramatic peak in protein concentration in March 2000 corresponds to the sharp increase in ambient temperature in the first daylight low tide series of the year (Fig 4.7b).

Total Hsp70 concentration (sum of high and low molecular weight bands) changed significantly over time (Table 4.4c, Fig 4.6b; time effect $p < 0.0001$). Total Hsp70 concentration for animals collected in summer 99 was higher than for animals collected in summer 2000, although the difference was not significant (Table 4.4c; $p = 0.0408$ for summer 99 vs. summer 00 contrast: adjusted significance level for multiple contrasts $\alpha = 0.017$). Total Hsp70 concentration for animals collected in summer 1999 was significantly higher than for animals collected in the winter, but animals collected in summer 2000 were not significantly different from animals collected in winter (Table 4.4c; $p = 0.0002$ for summer 99 vs. winter contrast; $p = 0.1003$ for summer 00 vs. winter contrast).

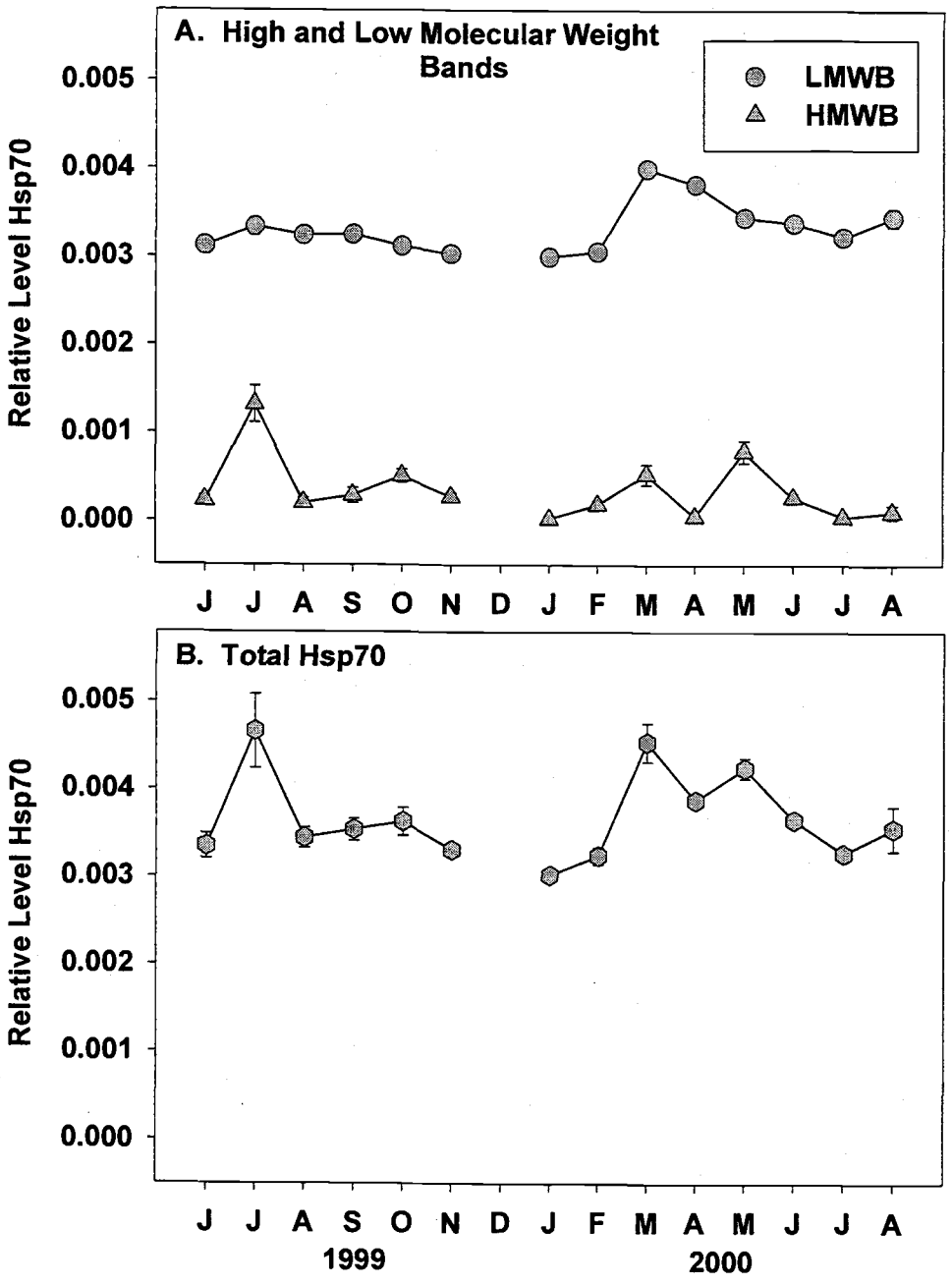

Figure 4.6 Relative level Hsp70 in general population *Katharina*. Mean (± SEM) for 6 *Katharina* per month. Relative level Hsp70 is calculated for each protein band as [density of protein band / µg protein loaded] / [positive control density / µg positive control loaded]. **(A)** High (HMWB) and Low (LMWB) molecular weight bands. **(B)** Total Hsp70 (HMWB + LMWB).

Table 4.4 Variation in Hsp70 concentration with time in the general *Katharina* population. **(A)** and **(B)** Data are relative Hsp70 levels of the two isoform bands (HMWB and LMWB); **(C)** Data are the sum of the Hsp70 levels of the two bands. The analysis is a one-factor ANOVA. Log transformed data (ln y) were used for analysis of HMWB and Total Hsp70 data; untransformed data were used for LMWB analysis. df: degrees of freedom; MS: mean squares. Bold face p-values indicate that the factor is significant at $\alpha = 0.05$ for main effects, $\alpha = 0.017$ for contrasts (adjusted for multiple non-orthogonal contrasts).

A. High Molecular Weight Band

Source	df	MS	F	P
Time	13	4.79	6.18	**< 0.0001**
Error	70	54.33		
Contrast				
Summer 99 vs. Summer 00	1	6.57	8.46	**0.0049**
Summer 99 vs. Winter	1	1.32	1.70	0.1971
Summer 00 vs. Winter	1	2.54	3.27	0.0749

B. Low Molecular Weight Band

Source	df	MS	F	P
Time	13	5.13E-7	13.47	**< 0.0001**
Error	70	3.08E-8		
Contrast				
Summer 99 vs. Summer 00	1	1.24E-7	3.25	0.0759
Summer 99 vs. Winter	1	3.41E-7	8.96	**0.0038**
Summer 00 vs. Winter	1	9.21E-7	24.19	**<0.0001**

C. Total Hsp70 Concentration

Source	df	MS	F	P
Time	13	0.095	8.46	**<0.0001**
Error	70	0.011		
Contrast				
Summer 99 vs. Summer 00	1	0.049	4.34	0.0408
Summer 99 vs. Winter	1	0.171	15.15	**0.0002**
Summer 00 vs. Winter	1	0.031	2.77	0.1003

Figure 4.7 Relationship between general population *Katharina* Hsp70 concentration and ambient low tide temperature. Lines represent maximum daily low tide temperatures (average of all active data-loggers) from May 1999 through August 2000. The number of data-points in a month depends on the number of days on which the low intertidal zone was exposed during low tides. Circles represent relative level of Hsp70 from general population *Katharina* (mean ± SEM of 6 chitons per month). Hsp70 data are plotted on the day of sampling. **(A)** High Molecular Weight Band (Hsp70 isoforms) **(B)** Low Molecular Weight Band (Hsp70 isoforms) **(C)** Total Hsp70 (LMWB + HMWB).

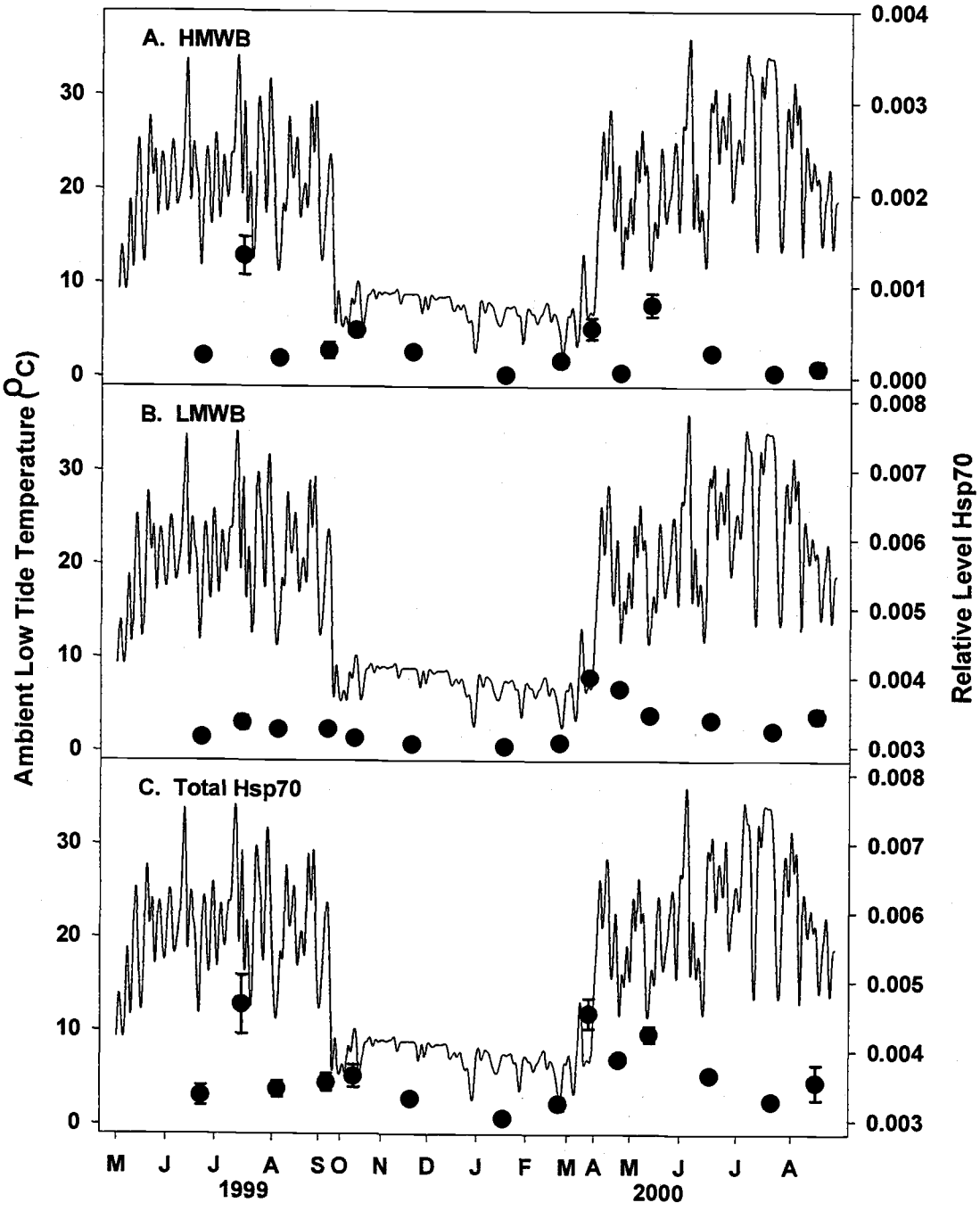
A. HMWB
B. LMWB
C. Total Hsp70
Ambient Low Tide Temperature (°C)
Relative Level Hsp70
0
10
20
30
0.000
0.001
0.002
0.003
0.004
0.003
0.004
0.005
0.006
0.007
0.008
M J J A S O N D J F M A M J J A
1999
2000

Figure 4.7

DISCUSSION

Temperature Data

Location in the intertidal has a strong effect on *Katharina* body temperature. The presence of shade, either from *Hedophyllum sessile* blades or Artificial Shades, significantly reduced body temperatures compared to animals in –Shade areas (Fig 4.1). Because temperature records from data-loggers are good estimates of actual chiton body temperatures (Fig 4.2) these data can be used for a more detailed examination of the potential effects of shade for *Katharina*. At all ambient temperatures above 10°C, shades offer significantly cooler microhabitats in comparison with unshaded areas; and these thermal benefits increase with increasing ambient temperatures (Fig 4.3, Table 4.2). These results show that both Artificial Shades and *Hedophyllum sessile* are effective refugia from high summer low tide temperatures.

Hsp70 Levels: Experimental Data

In a previous experiment, I showed that *Katharina* exploit shaded areas as refugia during the spring and summer (Chapter 2). However, levels of Hsp70 did not differ between *Katharina* maintained in plots with and without shade (Fig 4.5, Table 4.3). These results were surprising, given the large temperature differential between + and –Shade areas (Table 4.2), and there are several possible explanations for this lack of treatment effect.

Previous studies have shown that the magnitude of Hsp synthesis is proportional to the severity of physiological stress (DiDomenico et al. 1982); and it is possible that *Katharina* are not physiologically stressed by the temperatures they experience in unshaded areas. There are no published data on heat tolerances of *Katharina tunicata*, and this is the first study to investigate the physiological effects of field temperatures on *Katharina*, so no data currently exist with which to evaluate this possibility. Other biochemical specializations (such as changes in expression of differentially thermally stable protein isoforms or synthesis of osmotic stress protectants) may act to stabilize organisms so that environmental 'extremes' are not physiologically stressful (Somero 1997, Feder and Hofmann 1999). Alternatively, the lower temperature conditions underneath shades (up to 13°C) may not be low enough to provide real benefits; chitons may still be physiologically stressed at the temperatures underneath the artificial shades. Future experiments will investigate these possibilities.

Hsp70 levels are affected by many stressors, including desiccation, anoxia, toxic chemicals, and UV (Feder and Hofmann 1999) and it is possible that Hsp70 levels in experimental animals were responding to some stressor other than temperature. Studies in freshwater lakes have found significant vertical stratification patterns in zooplankton species that correspond to species-specific UV tolerance (Williamson and Leech 2000) and have shown behavioral avoidance of high UV habitats by UV intolerant species (Leech and Williamson 2000). It is possible that the amount of UVR transmitted through the artificial shades is enough to cause cellular damage (and therefore affect levels of Hsps) but is not enough to trigger movement of the chiton out of the area. Although the

experimental plots seemed to have abundant algal food supplies for the enclosed chitons, nutritional stress could have differentially impacted chitons in the experimental treatments. As fences enclosed chitons in both treatments and Hsp levels for the experimental animals were very similar to general population levels (Fig 4.5 and 4.6) it does not appear that the experimental design *per se* affected Hsp levels. Although I did not make any direct measurements of desiccation potential underneath artificial shades, the environment under the artificial shades was similar to conditions underneath the *Hedophyllum* canopy, which are quite moist compared to areas lacking canopy (J. Burnaford, personal observation). It thus seems unlikely that desiccation caused elevated Hsp70 levels in animals under artificial shades.

As Hsp70 is only one of many components of the Heat Shock Response, the absence of a pattern in this study does not allow the conclusion that shades are not effective physiological refugia for *Katharina*. Examination of other Hsp families (i.e. Hsp90 or Hsp40) or direct quantification of irreversibly damaged proteins (using ubiquitin conjugates) may show a clear pattern of increased physiological stress in chitons held without shade. Examination of Hsp70 levels in different tissue types (i.e. gill or digestive tract) could also provide more insight into the effects of shade; other studies have shown tissue-specific responses of Hsp70 levels to temperature treatments (Near et al. 1990, Sanders et al. 1992). In addition, examination of whole-organism traits such as growth rates and reproductive output could aid in evaluation of the benefits (if any) to *Katharina* of shade refugia during low tides.

Hsp70 Levels: General Katharina *Population*

Data from the general *Katharina* population show interesting patterns of changing Hsp70 levels with season (Fig 4.6, Fig 4.7). Since the patterns of the high and low molecular weight bands differ over time, the isoforms present in these two bands may be responding to different temperature cues. The high temporal variability of the high molecular weight band and the relatively low variability of the low molecular weight band (Fig 4.6) could indicate that these bands contain inducible and constitutive Hsp70 isoforms, respectively. An equally possible alternative is that the HMWB (absent animals collected during the winter months: Fig 4.4) contains inducible forms, and the LMWB contains both constitutive and inducible isoforms. More detailed biochemical work is necessary to fully characterize these bands.

Concentrations of the lower molecular weight isoforms show a highly significant seasonal pattern, with higher concentrations in summers than in winter (Table 4.4). This pattern is consistent with results from studies with *Mytilus californianus*, which found greater standing stock concentrations of Hsp70 in animals collected in the summer versus animals collected in the winter (Roberts et al. 1997). One interesting feature of this seasonal pattern is the dramatic jump in LMWB Hsp70 concentration from February to March of 2000, a jump which corresponds to a temperature spike from the first daylight low tide of the year (Fig 4.7). Although this temperature spike is not particularly large in terms of absolute change, it is the first major temperature increase from a long period of sustained cold temperatures, and could reflect an increase in production of constitutive isoforms of Hsp70 or a triggering of production of inducible isoforms. Previous

studies have shown that the trigger point for the induction of Hsps can change within a range of temperatures, depending on the thermal history of the species (Dietz and Somero 1992, Hofmann and Somero 1996a, Tomanek and Somero 1999) or the thermal history of the individual. In previous studies, the temperature of Hsp induction for intertidal *Mytilus trossulus* fluctuated by as much as 7°C during cold-acclimation of summer-acclimatized mussels (Owen and Hofmann 1998). Similar shifts have been observed for other invertebrates (Roberts et al. 1997, Tomanek and Somero 1999) and fish (Dietz and Somero 1992). In either case, these data show a significant effect of season on *Katharina* Hsp levels, which suggests that the animals are more physiologically stressed in the summer than in the winter. While more work is clearly needed to evaluate the mechanism behind *Katharina*'s behavioral pattern of seeking shade, these data could support the hypothesis that the animals are exploiting shades as refugia from heat stress.

Peaks in concentration of the high molecular weight isoforms seem to correspond to peaks in environmental temperatures in the days preceding collection (Fig 4.7a; see July 1999 and May 2000) but high environmental temperatures are not always followed by high concentrations of this band (Fig 4.7a; see June, July and August 2000). If the HMWB contains inducible forms of Hsp70, two factors must be considered in order to interpret these data. First, the evaluation of concentrations of inducible Hsps is much more sensitive to sampling interval than the evaluation of constitutive forms. Studies with *Mytilus trossulus* have shown that Hsp synthesis following heat stress during low tide occurs primarily after re-immersion and continues for up to 16 hours (Hofmann and Somero 1996b). Studies with *Tegula* examining the time-course of Hsp synthesis

have shown that an intertidal species completes *de novo* synthesis of Hsps after 6 hours of recovery (Tomanek and Somero 2000). These studies, however, did not examine the persistence of Hsps in cells following completion of synthesis. Depending on the time course of Hsp synthesis in *Katharina tunicata* and the persistence of Hsps following completion of synthesis, values of inducible Hsp70 isoforms in these monthly samples could be measuring only the heat stress experienced by the chiton in the one low tide previous to the sampling day. An additional consideration is that induction temperatures (the temperature at which inducible Hsps begin to be produced) could change throughout the year, so that temperatures that would induce production of HMWB isoforms in March would not induce those isoforms in July. The data from this study suggest that environmental temperatures are affecting Hsp70 levels on both fine and large temporal scales. Sampling on both time scales is crucial if we are to determine the ecological importance of heat shock proteins.

Examination of total Hsp70 levels suggests an annual pattern of low Hsp70 levels in the winter, increasing levels in the spring (March, April and May) and mid-range levels in summer with occasional high peaks (Fig 4.7c, Table 4.4c). Whether this is a repeating pattern or merely a characteristic of this sampling period cannot be determined from these data. While the concentrations of the HWMB and LWMB fluctuate over time in a manner which approaches a positive relationship, the concentrations are not strictly correlated (no linear or polynomial equation could explain more than 40% of the variation in the two band concentrations). More extensive laboratory work will address the relative

usefulness of looking at total Hsp vs. various isoform bands to evaluate an animal's response to thermal stress.

Conclusions

This study is one of the first to document patterns of Hsp expression in a field population of mobile animals. The data have shown that levels of Hsp70 vary significantly with season in field populations of *Katharina tunicata,* indicating that *Katharina* are responding to summertime sub-lethal stress at the molecular level. Temperature data indicate that location in the intertidal significantly affects *Katharina* body temperature, and that shades provide a significant cooler microhabitat than unshaded areas at ambient temperatures above 10°C. Although Hsp70 expression was not affected by the presence of shade in experimental manipulations, this study provides preliminary data on which to build future studies of the potential physiological benefits of shade for *Katharina*. Physiological stresses and avoidance behaviors are recognized as important structuring forces in communities. These results highlight the need for linked laboratory and field studies that document the pattern of Hsp expression in nature while simultaneously investigating the environmental and behavioral factors that influence the pattern.

CHAPTER 5

PREDICTING THE IMPORTANCE OF POSITIVE INTERACTIONS BETWEEN TROPHIC LEVELS: REVISING CONCEPTUAL MODELS

Field studies from terrestrial plant assemblages, salt marshes and marine benthic communities have convincingly shown that positive interactions between species are important structuring forces in communities, particularly in areas of high consumer pressure or harsh abiotic conditions (reviewed in (Callaway 1995, Bruno and Bertness 2001, Chapters 1-3). Numerous conceptual papers have recently begun to incorporate positive interactions into models of community structure in order to provide testable predictions regarding when and where these interactions should be important (Bertness and Callaway 1994, Callaway and Walker 1997, Hacker and Gaines 1997, Holmgren et al. 1997). However, the majority of these studies and conceptual models only address positive interactions between "basal species" (individuals on a single level of an interaction web, i.e. plants or sessile animals such as barnacles or mussels). There has recently been a call for an emphasis not only on detecting positive interactions in communities, but for determining how much of a contribution these interactions make to the patterns we see in natural systems (Menge 2000). The potential for positive interactions between trophic levels to influence community structure must be addressed if ecologists hope to evaluate and predict their importance in natural systems. In this chapter I present a series of conceptual models which incorporate the potential for basal species to influence the distribution, abundance or

performance of higher trophic levels through non-trophic mechanisms such as reducing predation pressure or ameliorating harsh abiotic conditions, thereby affecting community structure in ways not predicted by previous models. The capacity for positive interactions between basal species to affect higher trophic levels will depend on the relative sizes of predators and prey, the density of basal species, and the specific mechanism involved in the positive interaction. Incorporating these ideas into conceptual models of community structure will greatly improve our understanding of the structure and function of communities.

BACKGROUND

Environmental Stress Models

Current conceptual models that predict the influence of positive interactions in communities are based on the underlying assumption that biological interactions change across gradients of abiotic stress. This assumption is a prediction of a family of models collectively known as "Environmental Stress Models, " or ESMs (Menge and Olson 1990). The specific predictions of ESMs depend on whether prey (basal species) or consumers are more affected by these abiotic stresses. Consumer Stress Models (CSMs, e.g. Connell 1975, Menge and Sutherland 1976, 1987) predict that consumers are relatively more affected by stress than are prey, thereby giving prey a refuge from predation in areas of high environmental stress (Fig 5.1, Menge and Olson 1990). Therefore, CSMs predict that prey in low stress environments are regulated by predation, and prey in high stress environments are

regulated by competition (under conditions of high prey recruitment) or abiotic stress (low prey recruitment).

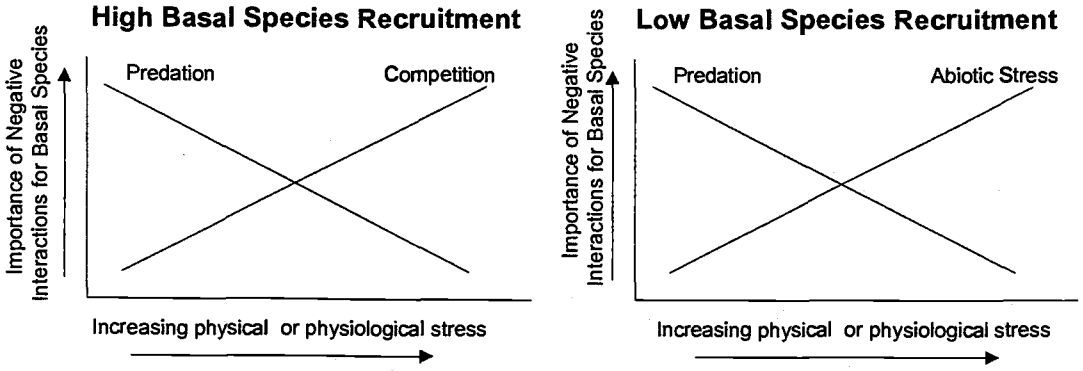

Figure 5.1 Consumer Stress Models (CSMs). The Menge-Sutherland model (Menge and Sutherland 1987) incorporates consideration of prey recruitment levels (prey density) into traditional CSMs. The model predicts that consumers will be inhibited by abiotic stresses relatively more than prey species. Therefore, because consumers are either eliminated from harsh environments or perform poorly, prey in high stress environments are regulated primarily by competition (high prey densities) or directly by abiotic stresses (low prey densities).

Prey Stress Models (PSMs) predict that prey defenses are weakened under conditions of severe environmental stress, and thus that prey in high stress environments are more susceptible to, and controlled more by, predation than prey in low-stress environments (Menge and Olson 1990; Fig 5.2). Therefore PSMs predict that prey in high stress environments are regulated mostly by predation, and in low stress environments are regulated by competition.

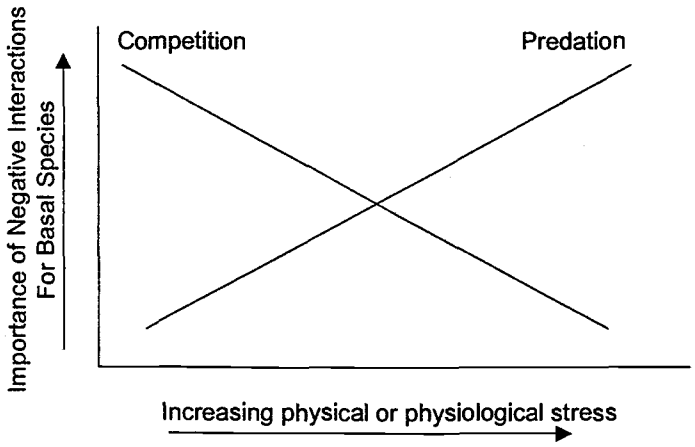

Figure 5.2 Prey Stress Models (PSMs). PSMs predict that predation levels are primarily determined by the strength of prey defenses, which are weakened under conditions of environmental stress. Therefore, in benign environments, prey are regulated by competition between well-defended individuals, and in harsh environments, prey populations are more affected by predation on poorly-defended, susceptible individuals (Menge and Olson 1990). Considerations of prey density are generally not incorporated into PSMs.

Positive Interaction Models

Current conceptual models of positive interactions in communities are primarily based on CSMs. The Bertness-Callaway model (Bertness and Callaway 1994) predicts that positive interactions between basal species will be important in areas of harsh abiotic conditions or intense consumer pressure, and is based on a gradient in which increasing levels of abiotic stress are accompanied by decreasing consumer pressure (Fig 5.3).

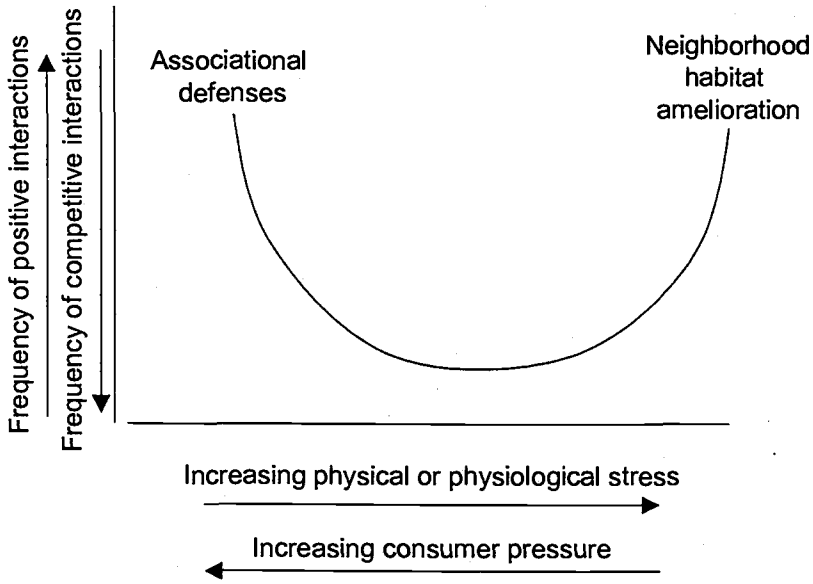

Figure 5.3 The Bertness-Callaway Model. This model is based on traditional CSMs, in which consumer pressure decreases with increasing abiotic stress (see Fig 5.1). Positive interactions that benefit basal species are predicted to be frequent in harsh environments (in which large or dense basal species can modify abiotic variables to create benign microhabitats) or in benign environments with intense consumer pressure (in which basal species which are structurally complex or defended from predation provide protection for other species).

Studies from a number of terrestrial and aquatic systems have provided empirical data to support predictions of the Bertness-Callaway model. Palatable terrestrial plants (Atsatt and O'Dowd 1976, McNaughton 1978, McAuliffe 1986, Rousset and Lepart 2000) and marine algae (Pfister and Hay 1988) can gain protection from herbivores through association with unpalatable plant species that are avoided by herbivores. In harsh environments, there is widening recognition of the fact that plant canopies or high densities of basal species individuals can ameliorate abiotic conditions. Plant canopies influence a number of environmental variables for understory species, including temperature (Chapter 4, Bertness et al. 1999b, Leonard 2000), substratum moisture levels (Brawley and Johnson 1991,

1993, Bertness and Shumway 1993, Callaway et al. 1996, Pugnaire et al. 1996a, Bertness et al. 1999b), and soil salt concentrations (Hacker and Bertness 1995b). The 'nurse-plant syndrome,' first recognized in desert systems, (Niering et al. 1963, Turner et al. 1966, 1969) is a label given to the circumstance of positive spatial association between seedlings of one species and adults of another species (which ameliorate environmental conditions or reduce herbivory on understory seedlings). High neighbor densities, detrimental to individual fitness in benign environments (through increased competitive effects) are often beneficial in harsh environments. High densities of intertidal barnacles decrease desiccation or thermal loading of the rocky substratum, resulting in increased survivorship for individuals in high-density patches in harsh environments (Lively and Raimondi 1987, Bertness 1989, Bertness et al. 1999a). Similar studies of salt marsh mussels have shown increased survivorship in high-density patches due to reduced mortality from wintertime ice scour (Bertness and Grosholz 1985). Models of thermal flux in intertidal mussels demonstrate that mussels living in beds can experience dramatically lower body temperatures than solitary mussels under conditions of intense solar radiation (Helmuth 1998). Studies with intertidal algae have shown that high thallus densities or turf-like morphologies increase individual survivorship under conditions of desiccation or heat stress (Hay 1981, Bertness and Leonard 1997). In salt marsh habitats, marsh elder seedlings surrounded by seedling neighbors grew to larger sizes and showed survivorship rates ten times those of solitary seedlings under (natural) conditions of high soil salinity (Bertness and Yeh 1994). Therefore, the current theory on positive interactions is that the relative importance of positive (facilitative) and negative (competitive) interactions

can be determined by basal species density and abiotic stress: high densities are detrimental in benign environments but beneficial in harsh environments (Fig 5.4; (Callaway and Walker 1997).

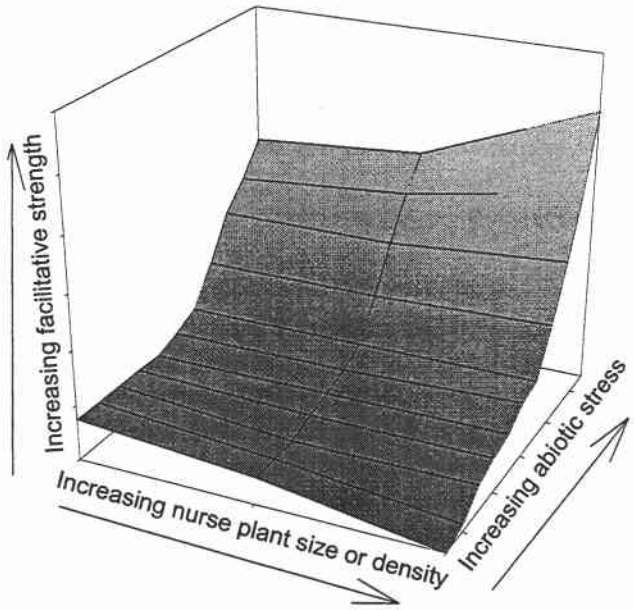

Figure 5.4 The Callaway-Walker Model. This model (Callaway and Walker 1997) summarizes current theory on the effects of the size of the benefactor (nurse plant or canopy-forming) individual, and the relative importance of positive or negative interactions along gradients of abiotic stress. Under benign conditions, increased benefactor size or basal species density is detrimental to the basal species, resulting in increased competition. In harsh habitats, increasing the density or size of basal species results in greater amelioration of harsh conditions through positive interactions.

Assumptions of Existing Positive Interaction Models

In order to generate new predictions about the importance of positive and negative interactions for structuring communities along gradients of abiotic stress, we must examine the implicit assumptions of the role of trophic levels, basal species density, and the importance of negative interactions in current conceptual models of positive interactions (Fig 5.5). Current models of positive interactions in communities consider positive interactions between basal species only: basal species are both the benefactors (i.e. canopy-producing plants or structurally complex sessile invertebrates) and the beneficiaries of the positive interactions (gaining protection from predation or abiotic stress).

As a result, basal species density has important effects on model predictions at both ends of the abiotic stress gradient. In benign habitats, the benefits of associational defenses depend both on the availability and accessibility of predation refugia: it is assumed that lower densities of prey result both in fewer refugia and a low proportion of prey arriving in those refugia. Therefore the effectiveness and importance of associational defenses is predicted to be high under conditions of high basal species density and low under conditions of low basal species density. In harsh environments, habitat amelioration similarly depends on either density-dependent alteration of microhabitat variables, or on the association with a benefactor that depends on accessibility and availability of the benefactor. Therefore, neighborhood habitat amelioration is only predicted to be important (or possible) under conditions of high basal species density.

In these existing models, predation is considered most important in benign habitats (as predicted by traditional CSMs). However, because of the positive

effects of associational defenses in benign habitats, predation is predicted to be less important in benign habitats than in traditional CSMs (contrast Fig 5.5 with Fig 5.1). The importance of predation will depend both on the effectiveness of associational refugia (which increases with increasing basal species density) and the relative mobility and foraging strategy of the predator. Under conditions of low basal species density, if predators are unable to locate isolated prey items, predation will not be important in benign environments, but if predators are capable of locating these prey, predation pressure may strongly impact prey populations that are already low.

Because the models are based on traditional CSMs, competition is only considered to be an important factor under conditions of high basal species density. Under these conditions, competition for associational defenses, or predation refugia, is expected to be intense. In harsh environments, high densities of individuals are predicted to increase individual fitness, and therefore competition is not predicted to be important. Under conditions of low basal species density, habitat amelioration depends on either density-dependent alteration of microhabitat variables, or on the association with a benefactor that depends on accessibility and availability of the benefactor. Because, according to CSMs, consumers are relatively more affected by stress than are prey, the importance of predation is predicted to decrease with increasing abiotic stress. As stress levels increase, the beneficial neighbor effects become stronger than competitive effects, and positive interactions between neighbors are the most important forces in high-stress environments. Under conditions of low basal species density, in harsh environments, prey are controlled directly by abiotic stress as in traditional CSMs.

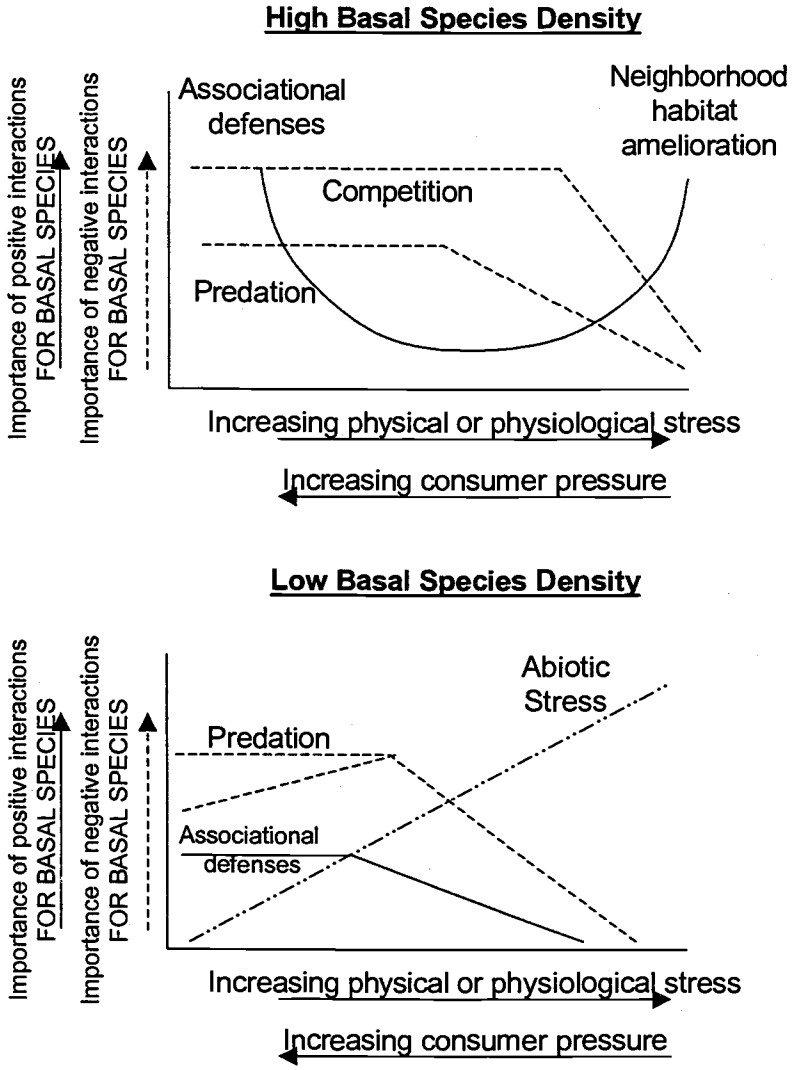

Figure 5.5 Implicit assumptions and predictions of the role of basal species density and negative interactions in the Bertness-Callaway Model. When prey (basal species) densities are high, in benign habitats, associational defenses reduce the importance of predation relative to the predictions of traditional CSMs. As consumers are more affected by stress than are prey, predation is not important in high-stress habitats. In benign habitats, competition for predation refugia can be intense, but as stress levels increase along the gradient, beneficial effects of neighbors become stronger than competitive effects. When prey densities are low, the importance of predation and associational defenses depend on the relative mobility and foraging strategy of the predator and the dispersal rates of prey to refugia. Because habitat amelioration depends on proximity to other prey individuals or to a larger 'benefactor' individual, low-density prey are directly controlled by abiotic stresses in harsh environments.

A NEW CONCEPTUAL MODEL

Updating CSMs

A fundamental feature of all current conceptual models of positive interactions is that they predict the importance of facilitative effects between basal species on basal species. These models assume that consumers cannot take advantage of these positive effects of basal species. In benign habitats, predators cannot access the prey refugia created by associational defenses. In harsh habitats, consumers are either absent or ineffective, and cannot take advantage of the habit amelioration by basal species. This assumption does seem to hold for systems in which consumers are large relative to basal species. Large consumers that cannot utilize structurally complex plant or sessile animal patches cannot gain protection from predators therein. Thermal models of heat flux and body temperature of sessile intertidal animals indicate that while aggregations of mussels reduce individual body temperatures due to mutual shading of individuals within a bed and a reduced rate of solar heat flux, predators (such as the predaceous whelk *Nucella*) which remain on top of the bed during feeding are subject to full solar radiation; therefore, predator and prey in close proximity can experience very different body temperatures and desiccation rates (Helmuth 1998). Similarly, these larger consumers are exposed to physical stresses such as wave forces or (wind or water) shear stress. As is predicted by traditional CSMs, therefore, large consumers in harsh habitats must actively seek refuge from environmental stresses, resulting in a reduction of feeding time and / or a spatially or temporally limited predator distribution (e.g. Menge 1978a, Suchanek

1979, Garrity and Levings 1981, Levings and Garrity 1983, Witman 1987, Fairweather 1988).

However, there is evidence that basal species can facilitate small consumers in ways other than altering food quality or quantity. Numerous studies have documented that small consumers can receive protection from predation through association with plants that are either structurally complex (Stachowicz and Hay 1996) or chemically defended (Hay et al. 1989, Hay et al. 1990a, Hay et al. 1990b, Duffy and Hay 1991, 1994). Similar benefits have been proposed for small consumers associated with structurally complex sessile invertebrate assemblages such as mussel beds (Suchanek 1979). There is also evidence to suggest that consumers can benefit from habitat amelioration by basal species, although fewer studies have been concerned with these potential physiological benefits. In the New England rocky intertidal zone, feeding rates of the predatory whelk *Nucella lapillus* were higher under algal canopies due to the amelioration of desiccation by the canopy (Menge 1978a) and predation pressure on understory barnacles was more intense underneath algal canopies or artificial shades due to the combination of higher feeding rates and predator microhabitat selection (Menge 1978a, b, Bertness et al. 1999a, Leonard 2000). I have documented positive effects of an algal canopy on a suite of mobile consumers, both carnivores and herbivores (Chapters 2, 3). The substratum temperature beneath mussel beds is frequently several degrees cooler than the surface of rocks in adjacent gaps (Helmuth 1998), and mussel beds have been shown to harbor populations of small consumers such as limpets and snails, which create grazing halos on nearby exposed substrata (Suchanek 1979).

Small Primary Consumers, Large Secondary Consumers

In order to understand how positive interactions affect whole communities, therefore, we must incorporate such between-trophic-level interactions into conceptual models. If the predators are large relative to their prey items, the system is expected to follow the predictions of the Bertness-Callaway and Callaway-Walker Models. If primary consumers are small, and benefit from non-trophic positive interactions with basal species, and their secondary consumers are large, then the system would not be expected to follow the predictions of these earlier models. Systems with small primary consumers and large secondary consumers include marine or aquatic habitats with small molluscs and large birds or fish, and small insects or mammals and large birds in terrestrial systems.

Incorporating positive, non-trophic interactions between basal species and consumers into the Bertness-Callaway model increases the importance of predation for basal species at both ends of the abiotic stress gradient (Fig 5.6). In benign habitats, associational refugia are effective only if they are avoided by or inaccessible to consumers. The model predicts that small consumers can access these associational refugia and gain protection from their predators (through associations with chemically defended or structurally complex basal species). Therefore, what would be 'associational refugia' for basal species with large consumers are not beneficial to basal species with small consumers, which are predicted to become concentrated in these areas as a result of heavy predation by secondary consumers. In harsh habitats, habitat amelioration, which increases the fitness or foraging ability of small consumers, can lead to high predation pressure on basal species in habitats from which consumers would otherwise be excluded

or inefficient. Therefore, because of this facilitation of consumer species, predation is an important negative force affecting basal species in both benign and harsh habitats (contrast Fig 5.6 with Fig 5.5). In areas of high predation pressure, competition between basal species is predicted to be weak. At the mid-point of the stress axis, negative interactions (such as predation and competition) are predicted to be important for consumer species, therefore reducing the impact of predation on basal species, and subsequently increasing the impact of competition.

Incorporating these non-trophic positive interactions between basal species and consumers into the Callaway-Walker model changes the model by primarily in the region of high basal species density and high abiotic stress (Fig 5.7). As before, small consumers are predicted to benefit from the effects of 'benefactor' species, and thus to increase predation pressure in areas from which they would previously have been excluded or ineffective. Increased consumer number and/or effectiveness in high stress areas results in heavy predation pressure on basal species, and this increase in the importance of negative interactions results in a much lower predicted importance of positive interactions in this region (compare with Fig 5.4). Therefore, in both of these cases (Fig 5.6 and 5.7), incorporating positive interactions between trophic levels fundamentally changes the predictions of earlier models, in that facilitative effects on primary consumers result in a much higher impact of predation in both benign and harsh habitats than earlier models have predicted.

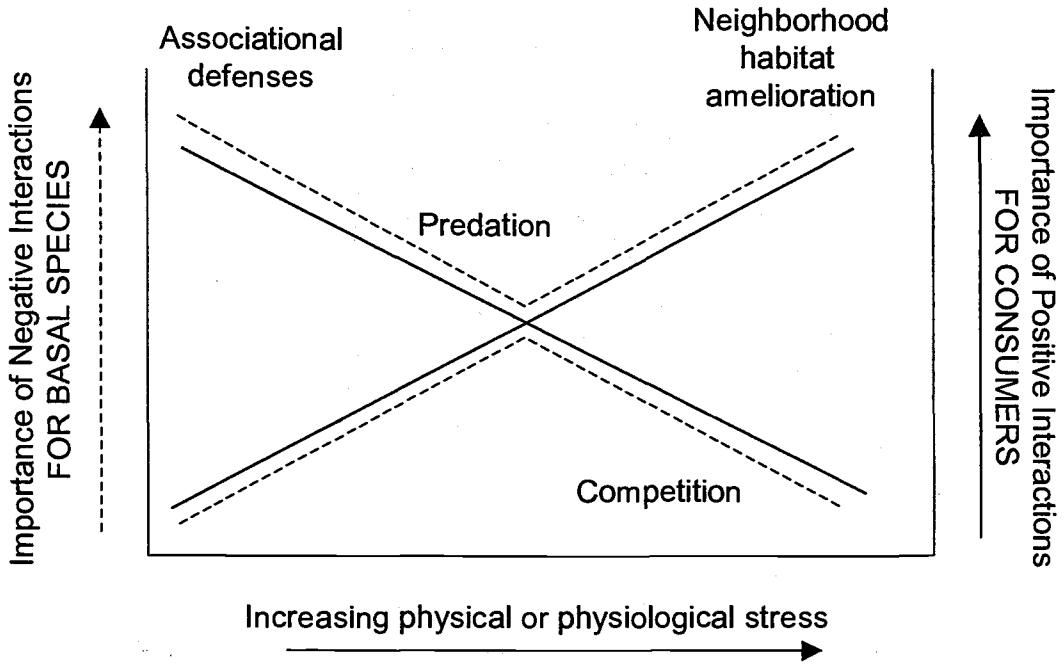

Figure 5.6 Incorporating positive interactions between two trophic levels into a modified Bertness-Callaway model. The model assumes conditions of high prey density (see text). The Bertness-Callaway "smiley face" has been replaced by linear relationships of two types of positive interactions (associational defenses and habitat modification). Small consumers benefit from associational defenses (in benign habitats) or habitat amelioration (in harsh habitats) by basal species. In benign habitats, increased efficiency or density of primary consumers (due to decreased predation from secondary consumers) results in strong predation pressure on basal species. In harsh environments, habitat amelioration increases primary consumer abundance and/or performance, and competitive interactions are weak because heavy predation pressure is the primary regulator of basal species abundance.

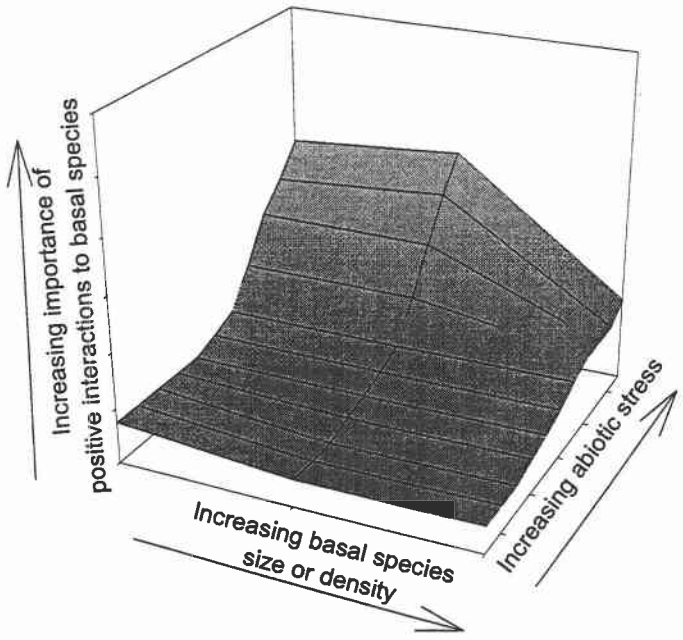

Figure 5.7 Incorporating positive interactions between trophic levels into the Callaway-Walker model. Increasing basal species size or density increases the potential for positive interactions on higher trophic levels (see text). As a result, predation is a strong limiting factor on basal species abundance in both benign and harsh habitats (both ends of the abiotic stress axis).

Because empirical and theoretical evidence suggests that the strength of positive interactions is proportional to the density of the benefactor (basal) species, and consumers have direct effects on basal species density, this variable must also be incorporated into models of two trophic-level interactions (Fig 5.8). If primary consumers cause a reduction in prey species density, this would subsequently eliminate density or size-dependent interactions that affect both basal species and consumers. Even small consumers can remove whole prey individuals, by grazing small recruits of large prey items, or by attacking certain tissues (such as stipes, stems, or holdfasts) that cause subsequent death or

removal of the whole prey individual. Alternatively, the activity of consumers could indirectly result in prey mortality (if crucial light or nutrient gathering tissues are removed, for instance). If prey recruitment rates are not high enough to maintain prey densities above levels required for facilitative benefits, then the system will eventually switch to a low-density system, in which negative interactions and abiotic stress are the primary structuring forces. In such cases, the system could only return to the 'high-density' condition following some 're-setting' event such as a strong recruitment pulse. This cycle of prey establishment, consumer aggregation, prey removal and subsequent consumer dispersal has been noted with fucoid algae and herbivorous limpets in rocky intertidal systems in Britain (Southward and Southward 1978, Thompson 1980). However, if consumers reduce prey fitness without directly causing high levels of prey mortality, or if the rate of prey recruitment is high relative to the rate of predation-induced mortality, then high prey densities should be maintained over time and space, and positive interactions are predicted to remain important structuring forces in the community.

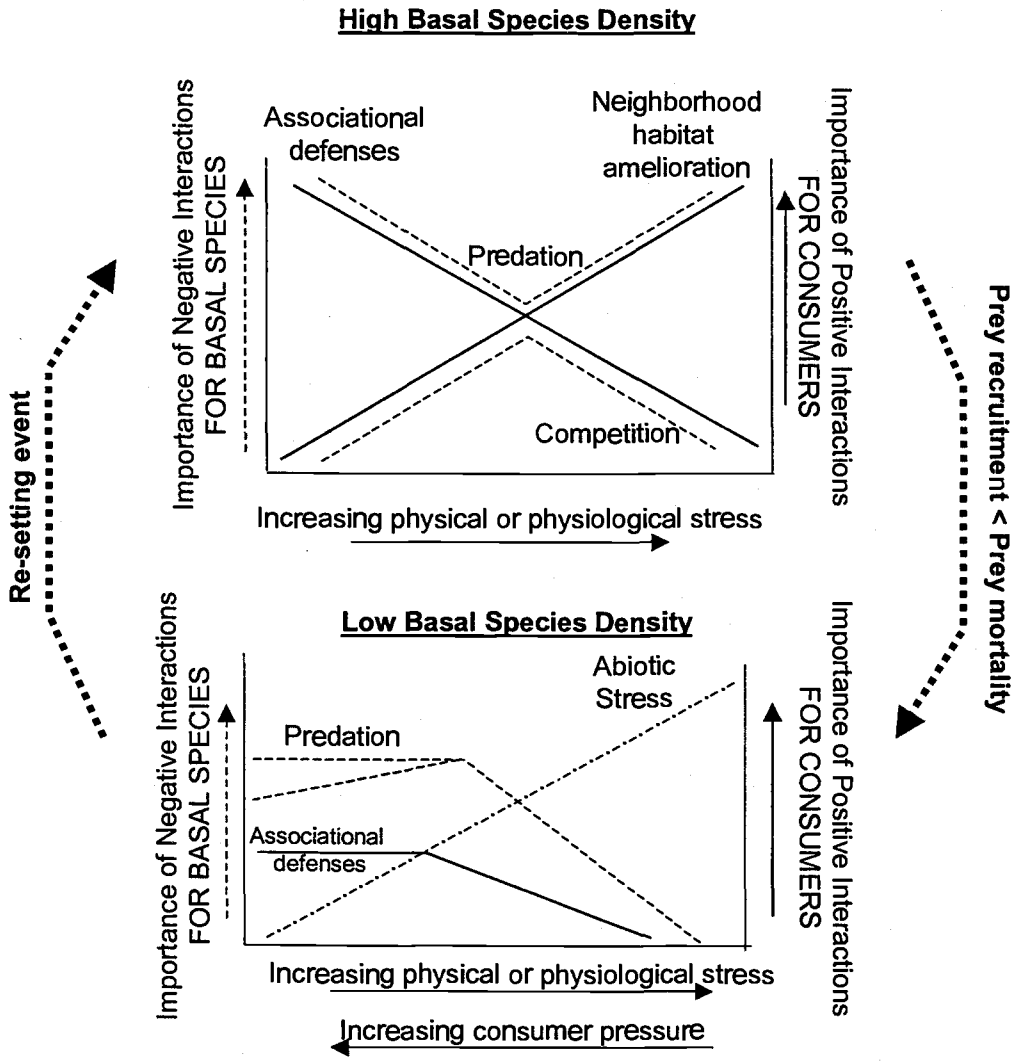

Figure 5.8 Potential spatial or temporal cycles resulting from positive interactions between trophic levels. If small primary consumers remove whole prey individuals or prey recruitment rates are low relative to the rate of predation-induced mortality, then this reduction of prey density is predicted to switch the system to a low-density system in which negative interactions and abiotic stress are the most important structuring forces. In such cases, the system could only return to the 'high-density' condition following a 're-setting event' such as a strong recruitment pulse.

Incorporating Multiple Trophic Levels

The models presented to this point assume that primary consumers are small and secondary consumers are large. Incorporating predictions for the effects of positive interactions on higher levels of the "food chain" (i.e. secondary consumers and above) introduces is necessary in order to evaluate the contribution of positive interactions to community-level patterns.

In cases where secondary consumers are themselves small relative to habitat modifying basal species (for example, intertidal systems in which predatory whelks or seastars consume herbivorous molluscs, or terrestrial systems in which spiders consume herbivorous insects) the model predictions can be logically extended for interactions between higher trophic levels (Fig 5.9). For instance, if both small primary and small secondary consumers benefit from neighborhood habitat amelioration, then heavy predation pressure on primary consumers would outweigh the positive effects of habitat amelioration for this trophic level in harsh environments. This reduction in the abundance or efficiency of primary consumers could alleviate predation pressure on basal species. In this situation, the community-level pattern would resemble that predicted by the one-level conceptual models: habitat amelioration in harsh environments would benefit basal species and would not affect their consumers. However, the process that produced this pattern would be entirely different; the one-level model would predict that primary consumers were rare because of harsh abiotic conditions, while this multiple trophic level model would predict that primary consumers were rare due to increased predation by facilitated secondary consumers. Under this multi-trophic level scenario, positive interactions are more important than one-level models

predict. Therefore, the recognition of multi-level positive interactions is crucial not only for predicting patterns in communities, but also for addressing the need for an evaluation of the overall contribution of positive interactions to those patterns (Menge 2000).

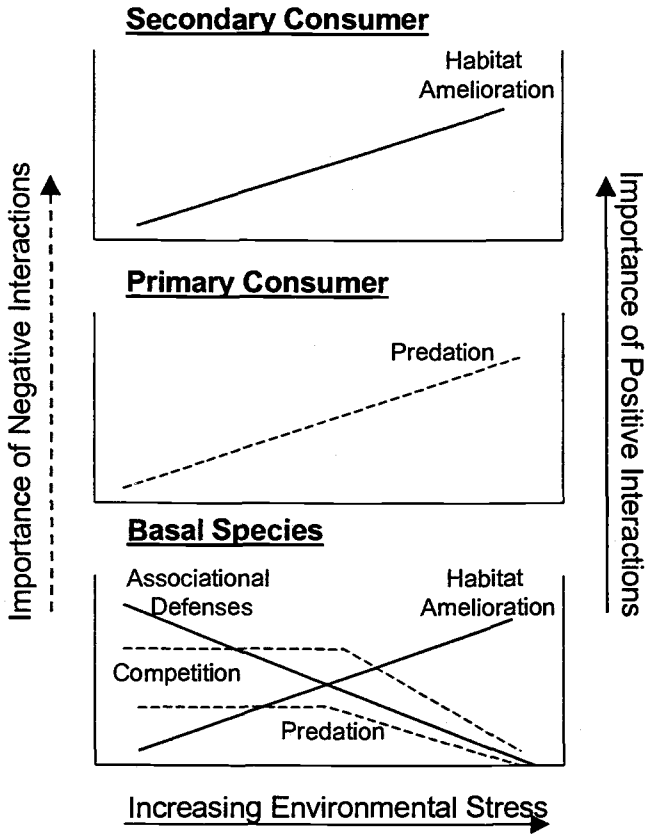

Figure 5.9 Incorporating positive effects on secondary consumers into models of positive interactions in communities. Small secondary consumers benefit from habitat amelioration by basal species in harsh environments, and have strong negative effects on primary consumers. Primary consumer populations are controlled by predation, and populations of basal species in harsh environments will be primarily regulated by positive interactions. Although the predictions for basal species are thus the same as for a one-level model (Fig 5.5), they result from processes that are not predicted by one-level models; namely that basal species facilitate organisms in all three trophic levels.

Additional Considerations

Predicting the importance of positive interactions requires evaluating specific characteristics of basal species that affect their ability to provide refugia from predators and ameliorate environmental stresses. No model can be applied to natural systems indiscriminately. There are increasing numbers of field experiments that describe species-specific positive interactions, in which physiological or structural differences between potential benefactor species affect the strength and/or sign of their interactions with potential beneficiary species (reviewed in Callaway 1998a). Whether positive interactions are properties of functional groups or individual species will depend primarily on the mechanism of the interaction. In the case of associational refugia from predation, unique morphological or chemical traits are often important in determining which species are avoided by predators and therefore which species can function as refugia (Atsatt and O'Dowd 1976, Callaway 1998a). Alternatively, in cases such as a canopy-forming species that facilitates understory species by providing shade, presumably the role of benefactor could be filled by any number of shade-producing species. Therefore, identifying 'functional groups' which can act interchangeably as benefactor species requires identifying the specific character that facilitates the beneficiary species (Callaway 1998a, Sullivan and Zedler 1999). This task requires targeted empirical studies in each system. The models presented in this chapter are designed to broaden these studies to incorporate the evaluation of characters that could benefit organisms on all trophic levels. Only by such broad experimental investigations can we evaluate the total importance of positive interactions on both the patterns and processes in natural systems.

A Brief Update of PSMs

Although considerations of the effects of prey density are not generally incorporated into Prey Stress Models, and current models of the effects of positive interactions in communities are based on Consumer Stress Models, incorporating the effects of positive interactions within and between trophic levels into PSMs could still improve our ability to understand natural communities. Because PSMs predict that the importance of predation depends not on characteristics of the predators *per se* but on the strength of prey defenses, which change along an environmental gradient, positive interactions between prey individuals in high stress habitats would be predicted to increase prey defenses and thus reduce the impact of predation by increasing individual prey fitness. Therefore, in high stress habitats, the model would predict increased levels of competition between well-defended prey individuals. If competition in these environments is severe, the outcome (individual mortality and reduced densities, or high densities of weakened individuals) would be predicted to lead to a cycle in which intense competition lowers species densities and the importance of positive interactions, resetting the system to the original PSM (Fig 5.9). As with systems in which the CSM apply, in order to understand the importance of positive interactions in communities, we must recognize the potential for many trophic levels to be affected by positive interactions between basal species in this system.

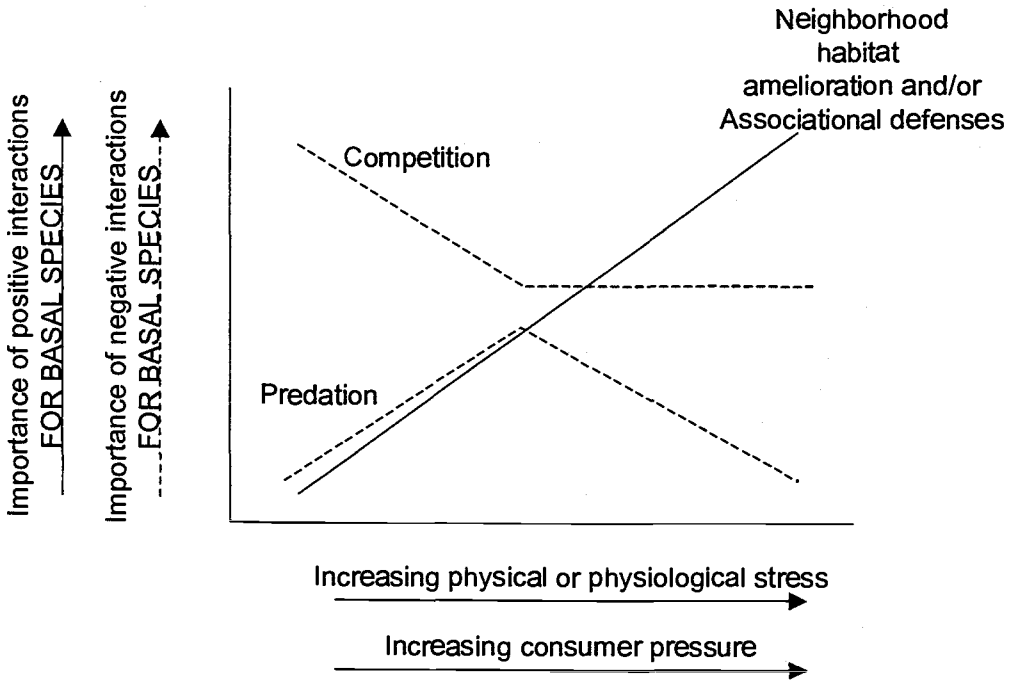

Figure 5.10 Conceptual model incorporating positive interactions into traditional Prey Stress Models. Because these models predict that consumer pressure increases with increasing abiotic stress, both habitat modification and associational defenses are predicted to be important only in high stress habitats. In these habitats, positive interactions are predicted to reduce the importance of predation by increasing individual prey fitness (and thus the effectiveness of prey defenses) and result in increased levels of competition between well-defended prey.

Conclusions

Positive interactions between basal species can also influence the distribution and abundance of higher trophic levels. These multi-level effects can alter the predictions of traditional models of positive interactions in two ways. First, positive effects on primary consumers could significantly alter the distribution and abundance of basal species in ways not predicted by early models. Second, positive effects on secondary consumers can cause patterns that are identical to

those predicted by previous models, but these patterns result from processes that are not considered by most researchers. The capacity for positive interactions between basal species to affect higher trophic levels will depend on the relative sizes of predators and prey, the density of basal species, and the specific mechanism involved in the positive interaction. In order to fully evaluate the importance of positive interactions to communities, we must be willing to investigate their effects at all trophic levels. Incorporating these ideas into conceptual models of community structure is critical step in advancing our understanding of the structure and function of communities.

CHAPTER 6

GENERAL CONCLUSIONS

The studies presented in this dissertation suggest that positive interactions between trophic levels can have profound effects on individuals, populations, and communities. In Chapter 2, I presented evidence that the association between a canopy-forming alga and a major herbivore was due to the positive effects of habitat modification by the alga, and not due to protection from predation or to the selection of the alga as a food source. Positive interactions between basal species (plants or sessile animals) are recognized as important structuring forces in habitats with intense predation pressure or severe abiotic stresses (Bertness and Callaway 1994, Callaway 1995, Callaway and Walker 1997, Bertness et al. 1999b). However, few studies address the potential for these positive interactions to benefit higher trophic levels. In my study, the shade produced by the algal canopy reduced understory temperatures by up to 13°C compared to unshaded areas during spring and summer low tide emersion periods. The positive effect of this habitat modification on *Katharina* densities was strong in two warm summers, but weak during a cool summer. These data show that positive, non-food effects of basal species can benefit higher trophic levels, and suggest that these effects can have broader consequences for communities by controlling foraging patterns of primary consumers over small spatial scales and seasonal and annual time scales.

In Chapter 3, I reported results from a field experiment in which I examined the relative roles of shade and *Katharina* on the understory algal and invertebrate assemblage. In addition to positively affecting the distribution and abundance of *Katharina* (Chapter 2), shade increased the abundance of seven groups of mobile consumers relative to unshaded areas. Shade and *Katharina* had quantitatively equivalent negative effects on the abundance of basal species, but their effects on the assemblage were qualitatively very different. Shade had strong negative effects on the abundance of understory algal groups, but strong positive effects on encrusting invertebrate colonies. *Katharina* had strong negative effects on all basal species. In the natural condition with both shade and *Katharina*, the combination of synergistic and antagonistic effects on individual groups resulted in deceptively simple patterns of species abundance and distribution which masked the complex network of positive, negative, direct and indirect effects which were evident in the absence of one or the other of these strong interactors.

In Chapter 4, I presented results of field manipulations and laboratory analyses designed to evaluate the potential physiological benefits of this positive interaction between *Hedophyllum* and *Katharina* on the physiology of the *Katharina*. Although I found no evidence of substantial stress reduction under artificial shades, I was able to quantify seasonal variation in the levels of the 70kDa family of heat shock proteins in field populations of *Katharina*. Hsp70 levels were higher in summer-collected individuals than in those collected in winter, indicating that *Katharina* experience summertime physiological sub-lethal stress to which they respond at a molecular level. Thus despite the fact that results from field enclosure experiments were inconclusive, these seasonal patterns suggest

that amelioration of abiotic stresses through positive biotic interactions could have direct physiological consequences for beneficiary species.

These results contribute to the growing recognition that the abiotic environment which individual organisms experience may be controlled, to a certain degree, by those same organisms through their morphology, behavior, or spatial arrangement (Helmuth 1998). However, to understand the patterns and processes in natural communities, we must acknowledge the capacity for biological interactions between species to modify the environment for organisms at all levels of the interaction web. In Chapter 5 I presented a conceptual model to summarize predictions of the effects of positive non-food interactions between trophic levels on community structure along gradients of environmental stress. Such multi-level interactions can produce deceptively simple community level patterns that are not recognized by previous conceptual models. Failing to incorporate them into empirical studies could lead researchers to underestimate the total contribution of positive interactions to community structure.

Through the modification of abiotic variables (temperature, desiccation potential, and light levels), the *Hedophyllum* canopy affected organisms on all trophic levels. Species with such wide-ranging effects have been labeled foundation species (Dayton 1972), ecological dominants (Dayton 1975) ecosystem engineers (Jones et al. 1994, 1997a, b, Lawton and Jones 1998) and habitat modifiers (Bruno and Bertness 2001). Identifying foundation species in communities and determining the characters that identify them as such is important because it allows us to make predictions about how the community might change in response to long-term or large-scale disruptions, including the

removal of the foundation species. Understanding how environmental changes affect and alter interactions between species, and not only how they affect geographical distributions, is an important component of understanding the potential impacts of climate change on natural systems (Lubchenco et al. 1991, Sanford 1999a). In order to predict the potential for major alteration of community interactions over the long term, we must recognize the importance of positive and negative, direct and indirect effects on communities.

For systems in which habitat modification is the primary mechanism underlying the effects of a foundation species, knowing how the benefactor and beneficiary species will respond to perturbations is necessary to predict the response of the community. Conceptual models of role of abiotic stress in structuring communities assume that the entire community is responding to stressors that change in parallel along a linear gradient (from non-stressful to stressful conditions) (Menge and Sutherland 1976, 1987, Menge and Olson 1990, Bertness and Callaway 1994). However, in this system, *Katharina* seems to respond primarily to low-tide air temperatures (Chapter 2) while *Hedophyllum* is sensitive to the increased water temperatures and decreased nutrient levels that accompany El Niño events and the longer-term regime shifts predicted to result from global climate change (Germann 1988, Tegner et al. 1997, Dayton et al. 1998). Therefore, the stress gradients to which *Hedophyllum* and *Katharina* are responding could be parallel, opposite, or orthogonal, and could vary with time and/or space. At present, we have no conceptual basis from which to approach systems in which different trophic levels are responding to different stressors that can change on different spatial and temporal scales.

The study of positive interactions in natural communities has only recently benefited from the degree of empirical and conceptual attention necessary for any field to progress. This progression must continue with the identification of new questions to answer and new techniques with which to answer them. Positive interactions are clearly frequent in natural communities. In order to evaluate their importance, however, we must investigate positive interactions between all levels of the interaction web. As a survey of the history of this field has shown, a major factor in driving scientific progress in any field is the development of theoretical predictions that generate testable hypotheses. We must continue to refine and expand conceptual models to incorporate new knowledge about how biological interactions can alter abiotic conditions and how abiotic stress gradients can change relative to one another in both time and space. Integrative studies that evaluate the individual physiological consequences of positive interactions are increasingly important for ecologists and offer exciting new avenues for the evaluation of the importance of positive interactions in communities. We must be willing to take such a comprehensive approach if we are to understand and preserve natural systems at the local, regional and global scale.

BIBLIOGRAPHY

Abrams, P. A. 1992. Predators that benefit prey and prey that harm predators: unusual effects of interacting foraging adaptations. American Naturalist **140**:573-600.

Arthur, W. 1986. On the complexity of a simple environment: competition, resource partitioning and facilitation in a two-species *Drosophila* system. Philosophical Transactions of the Royal Society of London B **313**:471-508.

Arthur, W., and P. Mitchell. 1989. A revised scheme for the classification of population interactions. OIKOS **56**:141-143.

Atsatt, P. R., and D. J. O'Dowd. 1976. Plant defense guilds. Science **193**:24-29.

Beckerman, A. P., M. Uriarte, and O. J. Schmitz. 1997. Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. Proceedings of the National Academy of Sciences **94**:10735-10738.

Begon, M., J. L. Harper, and C. R. Townsend. 1996. Ecology: Individuals, Populations and Communities, Third edition. Blackwell Scientific Publications, Cambridge, MA.

Bertness, M. D. 1989. Intraspecific competition and facilitation in a northern acorn barnacle population. Ecology **70**:257-268.

Bertness, M. D. 1991. Interspecific interactions among high marsh perennials in a New England salt marsh. Ecology **82**:125-137.

Bertness, M. D., and R. Callaway. 1994. Positive interactions in communities. Trends in Ecology and Evolution **9**:191-193.

Bertness, M. D., and E. Grosholz. 1985. Population dynamics of the ribbed mussel, *Geukensia demissa*: The costs and benefits of an aggregated distribution. Oecologia (Berlin) **67**:192-204.

Bertness, M. D., and S. D. Hacker. 1994. Physical stress and positive associations among marsh plants. American Naturalist **144**:363-372.

Bertness, M. D., and G. H. Leonard. 1997. The role of positive interactions in communities: lessons from intertidal habitats. Ecology **78**:1976-1989.

Bertness, M. D., G. H. Leonard, J. M. Levine, and J. F. Bruno. 1999a. Climate-driven interactions among rocky intertidal organisms caught between a rock and a hot place. Oecologia (Berlin) **120**:446-450.

Bertness, M. D., G. H. Leonard, J. M. Levine, P. R. Schmidt, and A. O. Ingraham. 1999b. Testing the relative contribution of positive and negative interactions in rocky intertidal communities. Ecology **80**:2711-2726.

Bertness, M. D., and S. W. Shumway. 1993. Competition and facilitation in marsh plants. American Naturalist **142**:718-724.

Bertness, M. D., and S. M. Yeh. 1994. Cooperative and competitive interactions in the recruitment of marsh elders. Ecology **75**:2416-2429.

Black, R. 1974. Some biological interactions affecting intertidal populations of the kelp *Egregia laevigata*. Marine Biology **28**:189-198.

Boucher, D. H., S. James, and K. H. Keeler. 1982. The ecology of mutualism. Annual Review of Ecology and Systematics **13**:315-347.

Brawley, S. H., and L. E. Johnson. 1991. Survival of fucoid embryos in the intertidal zone depends upon developmental stage and microhabitat. Journal of Phycology **27**:179-186.

Brawley, S. H., and L. E. Johnson. 1993. Predicting desiccation stress in microscopic organisms: the use of agarose beads to determine evaporation within and between intertidal microhabitats. Journal of Phycology **29**:528-535.

Brennecke, T., K. Gellner, and T. C. G. Bosch. 1998. The lack of a stress response in *Hydra oligactis* is due to reduced *hsp70* mRNA stability. European Journal of Biochemistry **255**:703-709.

Bronstein, J. L. 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology and Evolution **9**:214-217.

Brooker, R. W., and T. V. Callaghan. 1998. The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. OIKOS **81**:196-207.

Bruno, J. F. 2000. Facilitation of cobble beach plant communities through habitat modification by *Spartina alterniflora*. Ecology **81**:1179-1192.

Bruno, J. F., and M. D. Bertness. 2001. Habitat modification and facilitation in benthic marine communities. Pages 201-218 *in* M. D. Bertness, S. D. Gaines, and M. E. Hay, editors. Marine Community Ecology. Sinauer Associates, Inc., Sunderland, MA.

Bukau, B., and A. L. Horwich. 1998. The Hsp70 and Hsp60 chaperone machines. Cell **92**:351-366.

Callaway, R., and L. R. Walker. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology **78**:1958-1965.

Callaway, R. M. 1992. Effect of shrubs on recruitment of *Quercus douglasii* and *Quercus lobata* in California. Ecology **73**:2118-2128.

Callaway, R. M. 1994. Facilitative and interfering effects of *Arthrocnemum subterminale* on winter annuals. Ecology **75**:681-686.

Callaway, R. M. 1995. Positive interactions among plants. The Botanical Review **61**:306-349.

Callaway, R. M. 1997. Positive interactions in plant communities and the individualistic-continuum concept. Oecologia (Berlin) **112**:143-149.

Callaway, R. M. 1998a. Are positive interactions species-specific? OIKOS **82**:202-207.

Callaway, R. M. 1998b. Competition and facilitation on elevation gradients in subalpine forests of the northern Rocky Mountains, USA. OIKOS **82**.

Callaway, R. M., E. H. DeLucia, D. Moore, R. Nowak, and W. H. Schlesinger. 1996. Competition and facilitation: contrasting effects of *Artemisia tridentata* on desert vs. montane pines. Ecology **77**:2130-2141.

Callaway, R. M., N. M. Nadkarni, and B. E. Mahall. 1991. Facilitation and interference of *Quercus douglasii* on understory productivity in central California. Ecology **72**:1484-1499.

Callaway, R. M., and S. C. Pennings. 2000. Facilitation may buffer competitive effects: indirect and diffuse interactions among salt marsh plants. American Naturalist **156**:416-424.

Campbell, N. A. 1996. Biology, Fourth edition. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, California.

Carlsson, B. A., and T. V. Callaghan. 1991. Positive plant interactions in tundra vegetation and the importance of shelter. Journal of Ecology **79**:973-983.

Carpenter, C. M., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. BioScience **35**:634-639.

Carson, W., and R. Root. 2000. Herbivory and plant species coexistence: community regulation by an outbreaking phytophagous insect. Ecological Monographs **70**:73-99.

Chapin, F. S., J. Lubchenco, and H. L. Reynolds. 1995. Biodiversity effects on patterns and processes of communities and ecosystems. Pages 289-301 *in* Global Biodiversity Assessment (UNEP). Cambridge University Press, Cambridge.

Chapple, J. P., G. R. Smerdon, R. J. Berry, and A. J. S. Hawkins. 1998. Seasonal changes in stress-70 protein levels reflect thermal tolerance in the marine bivalve *Mytilus edulis* L. Journal of Experimental Marine Biology and Ecology **229**:53-68.

Clements, F. E. 1916. Plant Succession. Carnegie Institute of Washington Publication, Washington, DC.

Clements, F. E. 1936. Nature and structure of the climax. Journal of Ecology **24**:252-284.

Connell, J. H. 1961. Effects of competition, predation by *Thais lapillus*, and other factors on natural populations of the barnacle *Balanus balanoides*. Ecological Monographs **31**:61-104.

Connell, J. H. 1975. Some mechanisms producing structure in natural communities: a model and evidence from field experiments. Pages 460-491 *in* M. L. Cody and J. M. Diamond, editors. Ecology and Evolution of Communities. The Belknap Press of Harvard University Press, Cambridge.

Dayton, P. K. 1971. Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecological Monographs **41**:351-389.

Dayton, P. K. 1972. Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. *in* B. C. Parker, editor. Proceedings of the Colloquium on Conservation Problems in Antarctica. Allen Press.

Dayton, P. K. 1975. Experimental evaluation of ecological dominance in a rocky intertidal algal community. Ecological Monographs **45**:137-159.

Dayton, P. K., M. J. Tegner, P. B. Edwards, and K. L. Riser. 1998. Sliding baselines, ghosts, and reduced expectations in kelp forest communities. Ecological Applications **8**:309-322.

de O. Figueiredo, M. A., T. A. Norton, and K. J. J.M. 1997. Settlement and survival of epiphytes on two intertidal crustose coralline alga. Journal of Experimental Marine Biology and Ecology **213**:247-260.

Dethier, M. N., and D. O. Duggins. 1984. An "indirect commensalism" between marine herbivores and the importance of competitive hierarchies. American Naturalist **124**:205-219.

Dethier, M. N., and D. O. Duggins. 1988. Variation in strong interactions in the intertidal zone along a geographical gradient: a Washington-Alaska comparison. Marine Ecology Progress Series **50**:97-105.

DiDomenico, B. J., G. E. Bugaisky, and S. Lindquist. 1982. The heat shock response is self-regulated at both the transcriptional and posttranscriptional levels. Cell **31**:593-603.

Dietz, T. J., and G. N. Somero. 1992. The threshold induction temperature of the 90-kDA heat shock protein is subject to acclimatization in eurythermal goby fishes (genus *Gillichthys*). Proceedings of the National Academy of Sciences U.S.A. **89**:3389-3393.

Duffy, J. E., and M. E. Hay. 1991. Food and shelter as determinants of food choice by an herbivorous marine amphipod. Ecology **72**:1286-1298.

Duffy, J. E., and M. E. Hay. 1994. Herbivore resistance to seaweed chemical defense: The roles of mobility and predation risk. Ecology **75**:1304-1319.

Duggins, D. O. 1983. Starfish predation and the creation of mosaic patterns in a kelp-dominated community. Ecology **64**:1610-1619.

Duggins, D. O., and M. N. Dethier. 1985. Experimental studies of herbivory and algal competition in a low intertidal habitat. Oecologia (Berlin) **67**:183-191.

Duggins, D. O., J. E. Eckman, and A. T. Sewell. 1990. Ecology of understory kelp environments. II. Effects of kelps on recruitment of benthic invertebrates. Journal of Experimental Marine Biology and Ecology **143**:27-45.

Eckman, J. E., D. O. Duggins, and A. T. Sewell. 1989. Ecology of understory kelp environments. I. Effects of kelps on flow and particle transport near the bottom. Journal of Experimental Marine Biology and Ecology **129**:173-187.

Fairweather, P. G. 1988. Predation creates haloes of bare space among prey on rocky seashores in New South Wales. Australian Journal of Ecology **13**:401-409.

Farrell, T. M. 1991. Models and mechanisms of succession: an example from a rocky intertidal community. Ecological Monographs **61**:95-113.

Feder, H. M. 1959. The food of the starfish, *Pisaster ochraceus*, along the California coast. Ecology **40**:721-724.

Feder, J. H., J. M. Rossi, J. Solomon, N. Solomon, and S. Lindquist. 1992. The consequences of expressing hsp70 in *Drosophila* cells at normal temperatures. Genes & Development **6**:1402-1413.

Feder, M. E., and G. E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual Review of Physiology **61**:243-282.

Fink, A. 1999. Chaperone-mediated protein folding. Physiological Reviews **79**:425-449.

Fletcher, R. L. 1975. Heteroantagonism observed in mixed algal cultures. Nature **253**:534-535.

Frost, T. M., S. R. Carpenter, F. Ives, and T. K. Kratz. 1995. Species composition and complementarity in ecosystem function. Pages 224-239 *in* C. G. Jones and J. H. Lawton, editors. Linking Species and Ecosystems. Chapman and Hall, New York.

Gaines, S. D. 1985. Herbivory and between-habitat diversity: the differential effectiveness of defenses in a marine plant. Ecology **66**:473-485.

Garrity, S. D. 1984. Some adaptations of gastropods to physical stress on a tropical rocky shore. Ecology **65**:559-574.

Garrity, S. D., and S. C. Levings. 1981. A predator-prey interaction between two physically and biologically constrained tropical rocky shore gastropods: direct, indirect, and community effects. Ecological Monographs **51**:267-286.

Germann, I. 1988. Effects of the 1983-El Niño on growth and carbon and nitrogen metabolism of *Pleurophycus gardneri* (Phaeopyceae: Laminariales) in the northeastern Pacific. Marine Biology **99**:445-455.

Gleason, H. A. 1926. The individualistic concept of the plant association. Bulletin of the Torrey Botanical Club **53**:7-26.

Greenlee, J. T., and R. M. Callaway. 1996. Abiotic stress and the relative importance of interference and facilitation in montane bunchgrass communities in western Montana. American Naturalist **148**:386-396.

Grime, J. P. 1995. Functional types: a comment on Steneck and Dethier (1994). OIKOS **73**:120-121.

Hacker, S. D., and M. D. Bertness. 1995a. A herbivore paradox: why salt marsh aphids live on poor-quality plants. American Naturalist **145**:192-210.

Hacker, S. D., and M. D. Bertness. 1995b. Morphological and physiological consequences of a positive plant interaction. Ecology **76**:2165-2175.

Hacker, S. D., and M. D. Bertness. 1996. Trophic consequences of a positive plant interaction. American Naturalist **148**:559-575.

Hacker, S. D., and M. D. Bertness. 1999. Experimental evidence for factors maintaining plant species diversity in a New England Salt Marsh. Ecology **80**:2064-2073.

Hacker, S. D., and S. D. Gaines. 1997. Some implications of direct positive interactions for community species diversity. Ecology **78**:1990-2003.

Hahn, T., and M. Denny. 1989. Tenacity-mediated selective predation by oystercatchers on intertidal limpets and its role in maintaining habitat partitioning by *'Colisella' scabra* and *Lottia digitalis*. Marine Ecology Progress Series **53**:1-10.

Hamilton, D. J. 2000. Direct and indirect effects of predation by Common Eiders and abiotic disturbance in an intertidal community. Ecological Monographs **70**:21-43.

Hartnoll, R. G., and S. J. Hawkins. 1985. Patchiness and fluctuations on moderately exposed rocky shores. Ophelia **24**:53-63.

Hay, M. E. 1981. The functional morphology of turf-forming seaweeds: persistence in stressful marine habitats. Ecology **62**:739-750.

Hay, M. E., J. E. Duffy, and W. Fenical. 1990a. Host-plant specialization decreases predation on a marine amphipod: an herbivore in plant's clothing. Ecology **71**:733-743.

Hay, M. E., J. E. Duffy, V. J. Paul, P. E. Renaud, and W. Fenical. 1990b. Specialist herbivores reduce their susceptibility to predation by feeding on the chemically defended seaweed *Avrainvillea longicaulis*. Limnology and Oceanography **35**:1734-1743.

Hay, M. E., J. R. Pawlik, J. E. Duffy, and W. Fenical. 1989. Seaweed-herbivore-predator interactions: host-plant specialization reduces predation on small herbivores. Oecologia (Berlin) **81**:418-427.

Helmuth, B. S. T. 1998. Intertidal mussel microclimates: predicting the body temperature of a sessile invertebrate. Ecological Monographs **68**:51-74.

Himmelman, J. H., and T. H. Carefoot. 1975. Seasonal changes in calorific value of three Pacific coast seaweeds, and their significance to some marine invertebrate herbivores. Journal of Experimental Marine Biology and Ecology **18**:139-151.

Hofmann, G. E. 1999. Ecologically relevant variation in induction and function of heat shock proteins in marine organisms. American Zoologist **39**:889-900.

Hofmann, G. E., and G. N. Somero. 1995. Evidence for protein damage at environmental temperatures: seasonal changes in levels of ubiquitin conjugates and hsp70 in the intertidal mussel *Mytilus trossulus*. Journal of Experimental Biology **198**:1509-1518.

Hofmann, G. E., and G. N. Somero. 1996a. Interspecific variation in thermal denaturation of proteins in the congeneric mussels *Mytilus trossulus* and *M. galloprovincialis*: evidence from the heat-shock response and protein ubiquitination. Marine Biology **126**:65-75.

Hofmann, G. E., and G. N. Somero. 1996b. Protein ubiquitination and stress protein synthesis in *Mytilus trossulus* occurs during recovery from tidal emersion. Molecular Marine Biology and Biotechnology **5**:175-184.

Holmgren, M., M. Scheffer, and M. A. Huston. 1997. The interplay of faciltation and competition in plant communities. Ecology **78**:1966-1975.

Huey, R. B. 1991. Physiological consequences of habitat selection. American Naturalist **137**:S91-S115.

Huey, R. B., C. R. Peterson, S. J. Arnold, and W. P. Porter. 1989. Hot rocks and not-so-hot rocks: retreat-site selection by garter snakes and its thermal consequences. Ecology **70**:931-944.

Inderjit, and K. M. M. Dakshini. 1994. Algal allelopathy. The Botanical Review **60**:182-196.

IPCC Report, Working Group I. 2001. Summary for Policymakers. http:\\www.usgcrp.gov\ipcc.

Irons, D. B., R. G. Anthony, and J. A. Estes. 1986. Foraging strategies of Glacous-Winged Gulls in a rocky intertidal community. Ecology **67**:1460-1474.

Jenkins, S. R., T. A. Norton, and A. J. S. Hawkins. 1999. Settlement and post-settlement interactions between *Semibalanus balanoides* (L.) (Crustacea: Cirripedia) and three species of fucoid canopy algae. Journal of Experimental Marine Biology and Ecology **236**:49-67.

Jones, C. G., J. H. Lawton, and M. Shachak. 1994. Organisms as ecosystem engineers. OIKOS **69**:373-386.

Jones, C. G., J. H. Lawton, and M. Shachak. 1997a. Ecosystem engineering by organisms: why semantics matters. Trends in Ecology and Evolution **12**:275-275.

Jones, C. G., J. H. Lawton, and M. Shachak. 1997b. Positive and negative effects of organisms as physical ecosystem engineers. Ecology **78**:1946-1957.

Jones, K. M. M., and E. G. Boulding. 1999. State-dependent habitat selection by an intertidal snail: the costs of selecting a physically stressful microhabitat. Journal of Experimental Marine Biology and Ecology **242**:149-177.

Kennelly, S. J. 1989. Effects of kelp canopies on understorey species due to shade and scour. Marine Ecology Progress Series **50**:215-224.

Kerfoot, W. C., and A. Sih, editors. 1987. Predation: direct and indirect impacts on aquatic communities. University Press of New England, Hanover.

Kingsland, S. E. 1991. Defining ecology as a science. Pages 1-13 *in* L. A. Real and J. H. Brown, editors. Foundations of Ecology: Classic Papers with Commentaries. The University of Chicago Press, Chicago.

Krebs, R. A., and M. E. Feder. 1998. Experimental manipulation of the cost of thermal acclimation in *Drosophila melanogaster*. Biological Journal of the Linnean Society **63**:593-601.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **277**:680-685.

Lasiak, T., and R. H. Dye. 1986. Behavioural adaptations of the mangrove whelk, *Telescopium telescopium* (L.) to life in a semi-terrestrial environment. Journal of Molluscan Studies **52**:147-179.

Lavorel, S., S. McIntyre, J. Landsberg, and T. D. A. Forbes. 1997. Plant functional classifications: from general groups to specific groups based on response to disturbance. Trends in Ecology and Evolution **12**:474-478.

Lawton, J. H., and V. K. Brown. 1993. Redundancy in ecosystems. Pages 255-270 *in* E. D. Schulze and H. A. Mooney, editors. Biodiversity and Ecosystem Function. Springer-Verlag, Berlin.

Lawton, J. H., and C. G. Jones. 1998. Linking species and ecosystems: organisms as ecosystem engineers. Pages 141-450 *in* C. G. Jones and J. H. Lawton, editors. Linking species and ecosystems. Chapman and Hall, New York, NY.

Leech, D. M., and C. E. Williamson. 2000. *Daphnia* migrations in the presence and absence of UV radiation: Which way do they go? *in* Ecological Society of America Annual Meeting. Ecological Society of America, Snowbird, UT.

Leonard, G. H. 1999. Positive and negative effects of intertidal algal canopies on recruitment and survival of barnacles. Marine Ecology-Progress Series **178**:241-249.

Leonard, G. H. 2000. Latitudinal variation in species interactions: a test in the New England rocky intertidal zone. Ecology **81**:1015-1030.

Leonard, G. H., P. J. Ewanchuk, and M. D. Bertess. 1999. How recruitment, intraspecific interactions, and predation control species borders in a tidal estuary. Oecologia (Berlin) **118**:492-502.

Levin, S. A. 1992. The problem of pattern and scale in ecology. Ecology **73**:1943-1967.

Levin, S. A. 1994. Patchiness in marine and terrestrial systems: from individuals to populations. Philosophical Transactions of the Royal Society of London B **343**:99-103.

Levine, J. M. 2000. Complex interactions in a streamside plant community. Ecology **81**:3431-3444.

Levings, S. C., and S. D. Garrity. 1983. Diel and tidal movement of two co-occurring Neritid snails; differences in grazing patterns on a tropical rocky shore. Journal of Experimental Marine Biology and Ecology **67**:261-278.

Lindberg, D. R., K. I. Warheit, and J. A. Estes. 1987. Prey preference and seasonal predation by oystercatchers on limpets at San Nicolas Island, California, USA. Marine Ecology Progress Series **39**:105-113.

Lindquist, S., and R. Petersen. 1990. Selective translation and degredation of heat-shock messenger RNAs in *Drosophila*. Enzyme **44**:147-166.

Lively, C. M., and P. T. Raimondi. 1987. Desiccation, predation, and mussel-barnacle interactions in the northern Gulf of California. Oecologia (Berlin) **74**:304-309.

Lobban, C. S., and P. J. Harrison. 1994. Seaweed Ecology and Physiology. Cambridge University Press.

Lubchenco, J., A. M. Olson, L. B. Brubaker, S. R. Carpenter, S. A. Levin, J. A. MacMahon, P. A. Matson, J. M. Melillo, H. A. Mooney, C. H. Peterson, H. R. Pulliam, L. A. Real, P. J. Regal, and P. G. Risser. 1991. The sustainable biosphere initiative: an ecological research agenda. Ecology **72**:371-412.

Lubchenco, J., and L. A. Real. 1991. Experimental manipulations in lab and field systems. Pages 715-733 *in* L. A. Real and J. H. Brown, editors. Foundations of Ecology: Classic Papers with Commentaries. The University of Chicago Press, Chicago.

MacArthur, R. 1972. Strong, or weak, interactions? Transactions, the Connecticut Academy of Arts and Sciences **44**:177-188.

Markel, R. W., and R. E. DeWreede. 1998. Mechanisms underlying the effect of the chiton *Katharina tunicata* on the kelp *Hedophyllum sessile*: size escapes and indirect effects. Marine Ecology Progress Series **166**:151-161.

Marsh, C. P. 1986a. Impact of avian predators on high intertidal limpet populations. Journal of Experimental Marine Biology and Ecology **104**:185-201.

Marsh, C. P. 1986b. Rocky intertidal community organization: the impact of avian predators on mussel recruitment. Ecology **67**:771-786.

Martinsen, G. D., K. D. Floate, A. M. Waltz, G. M. Wimp, and T. G. Whitham. 2000. Positive interactions between leafrollers and other arthropods enhance biodiversity on hybrid cottonwoods. Oecologia (Berlin) **123**:82-89.

Mauzey, K. P. 1965. Feeding behavior and reproductive cycles in *Pisaster ochraceus*. Ph.D. University of Washington, Seattle.

Mauzey, K. P. 1967. The interrelationship of feeding, reproduction habitat variability in *Pisaster ochraceus*. Ph.D. University of Washington, Seattle.

Mauzey, K. P., C. Birkeland, and P. K. Dayton. 1968. Feeding behavior of asteroids and escape responses of their prey in the Puget Sound Region. Ecology **49**:603-619.

May, R. M. 1973. Stability and Complexity in Model Ecosystems. Princeton University Press, Princeton, NJ.

May, R. M. 1981. Models for two interacting populations. Pages 78-104 *in* R. M. May, editor. Theoretical Ecology: Principles and Applications. Sinauer Associates, Sunderland.

McAuliffe, J. R. 1984a. Prey refugia and the distributions of two Sonoran Desert cacti. Oecologia (Berlin) **65**:82-85.

McAuliffe, J. R. 1984b. Sahuaro-nurse tree associations in the Sonoran Desert: competitive effects of sahuaros. Oecologia (Berlin) **64**:319-321.

McAuliffe, J. R. 1986. Herbivore-limited establishment of a Sonoran Desert Tree, *Cercidium microphyllum*. Ecology **67**:276-280.

McCook, L. J., and A. R. O. Chapman. 1991. Community succession following massive ice-scour on an exposed rocky shore: effects of *Fucus* canopy algae and of mussels during late succession. Journal of Experimental Marine Biology and Ecology **154**:137-169.

McNamara, A. E., and W. R. Hill. 1999. Effects of UV-B dose and irradiance: comparison among grazers. Journal of the North American Benthological Society **18**:370-380.

McNaughton, S. J. 1978. Serengeti ungulates: feeding selectivity influences the effectiveness of plant defense guilds. Science **199**:806-807.

Menge, B. A. 1972. Foraging strategy of a starfish in relation to actual prey availability and environmental predictability. Ecological Monographs **42**:25-50.

Menge, B. A. 1976. Organization of the New England rocky intertidal community: role of predation, competition, and environmental heterogeneity. Ecological Monographs **46**:355-391.

Menge, B. A. 1978a. Predation intensity in a rocky intertidal community. I. Relation between predator foraging activity and environmental harshness. Oecologia (Berlin) **34**:1-16.

Menge, B. A. 1978b. Predation intensity in a rocky intertidal community. II. Effect of an algal canopy, wave action and desiccation on predator feeding rates. Oecologia (Berlin) **34**:17-35.

Menge, B. A. 1995. Indirect effects in marine rocky intertidal interaction webs: patterns and importance. Ecological Monographs **65**:21-74.

Menge, B. A. 2000. Testing the relative importance of positive and negative effects on community structure. Trends in Ecology and Evolution **15**:46-47.

Menge, B. A., E. L. Berlow, C. A. Blanchette, S. A. Navarrete, and S. B. Yamada. 1994. The keystone species concept: variation in interaction strength in a rocky intertidal habitat. Ecological Monographs **64**:249-286.

Menge, B. A., and A. M. Olson. 1990. Role of scale and environmental factors in regulation of community structure. Trends in Ecology and Evolution **5**:52-57.

Menge, B. A., and J. P. Sutherland. 1976. Species diversity gradients: synthesis of the roles of predation, competition, and temporal heterogeneity. American Naturalist **110**:351-369.

Menge, B. A., and J. P. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. American Naturalist **130**:730-757.

Menge, J. L., and B. A. Menge. 1974. Role of resource allocation, aggression and spatial heterogeneity in coexistence of two competing intertidal starfish. Ecological Monographs **44**:189-209.

Mercurio, K. S., A. R. Palmer, and R. B. Lowell. 1985. Predator-mediated microhabitat partitioning by two species of visually cryptic, intertidal limpets. Ecology **66**:1417-1425.

Milliken, G. A., and D. E. Johnson. 1992. Analysis of Messy Data, Volume I: Designed Experiments. Van Nostrand Reinhold, New York.

Near, J. C., D. P. Easton, P. S. Rutledge, D. P. Dickinson, and J. R. Spotila. 1990. Heat shock protein 70 gene expression in intact salamanders (*Eurycea bislineata*) in response to calibrated heat shocks and to high temperatures encountered in the field. Journal of Experimental Zoology **256**:303-314.

Nicotri, M. E. 1977. Grazing effects of four marine intertidal herbivores on the microflora. Ecology **58**:1020-1032.

Niering, W. A., R. H. Whittaker, and C. H. Lowe. 1963. The saguaro: a population in relation to environment. Science **142**:15-23.

Olff, H., F. W. M. Vera, J. Bokdam, E. S. Bakker, J. M. Gleichman, K. de Maeyer, and R. Smit. 1999. Shifting mosaics in grazed woodlands driven by the alternation of plant facilitation and competition. Plant Biology **1**:127-137.

Owen, M. E., and G. E. Hofmann. 1998. Resetting the thermostat: Changes in hsp induction temperaures in Mytilus trossulus. American Zoologist **38**:48A.

Padilla, D. K. 1981. Selective agents influencing the morphology of coralline algae. M.S. Oregon State University, Corvallis.

Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist **100**:65-75.

Paine, R. T. 1992. Food-web analysis through field measurement of per-capita interaction strength. Nature **355**:73-75.

Palumbi, S. R. 1985. Spatial variation in an alga-sponge commensalism and the evolution of ecological interactions. American Naturalist **126**:267-274.

Petersen, R. B., and S. Lindquist. 1989. Regulation of HSP70 synthesis by messenger RNA degredation. Cell Regulation **1**:135-149.

Pfister, C. A., and M. E. Hay. 1988. Associational plant refuges: convergent patterns in marine and terrestrial communities result from differing mechanisms. Oecologia (Berlin) **77**:118-129.

Pianka, E. R. 1994. Evolutionary Ecology, Fifth edition. HarperCollins College Publishers, New York, NY.

Piercy, R. D. 1987. Habitat and food preferences in six eastern Pacific chiton species (Mollusca: Polyplacophora). The Veliger **29**:388-393.

Pigliucci, M. 1996. How organisms respond to environmental changes: from phenotypes to molecules (and vice versa). Trends in Ecology and Evolution **11**:168-173.

Pound, R. 1893. Symbiosis and mutualism. American Naturalist **27**:509-520.

Pugnaire, F. I., P. Haase, and J. Puigdefabregas. 1996a. Facilitation between higher plant species in a semiarid environment. Ecology **77**:1420-1426.

Pugnaire, F. I., P. Haase, J. Puigdefabregas, M. Cueto, S. C. Clark, and L. D. Incoll. 1996b. Facilitation and succession under the canopy of a leguminous shrub, *Retama sphaerocarpa*, in a semi-arid environment in south-east Spain. OIKOS **76**:455-464.

Raffaele, E., and T. T. Veblen. 1998. Facilitation by nurse shrubs of resprouting behavior in a post-fire shrubland in northern Patagonia, Argentina. Journal of Vegetation Science **9**:693-698.

Reed, C. G. 1987. Phylum Bryozoa. *in* M. F. Strathmann, editor. Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast. University of Washington Press.

Reed, D. C., and M. S. Foster. 1984. The effects of canopy shading on algal recruitment and growth in a giant kelp forest. Ecology **65**:937-948.

Roberts, D. A., G. E. Hofmann, and G. N. Somero. 1997. Heat-shock protein expression in *Mytilus californianus*: Acclimatization (seasonal and tidal-height comparisons) and acclimation effects. Biological Bulletin **192**:309-320.

Roemmich, D., and J. McGowan. 1995. Climatic warming and the decline of zooplankton in the California Current. Science **267**:1324-1326.

Rousset, O., and J. Lepart. 2000. Positive and negative interactions at different life stages of a colonizing species (*Quercus humilis*). Journal of Ecology **88**.

Ryland, J. S. 1977. Taxes and tropisms of Bryozoans. *in* R. M. Woollacott and R. L. Zimmer, editors. Biology of Bryozoans. Academic Press, New York.

Sanders, B. M., V. M. Pascoe, P. A. Nakagawa, and L. S. Martin. 1992. Persistence of the heat-shock response over time in a common *Mytilus* mussel. Molecular Marine Biology and Biotechnology **1**:147-154.

Sanford, E. 1999a. Oceanographic influences on rocky intertidal communities: coastal upwelling, invertebrate growth rates, and keystone predation. Ph.D. Oregon State University, Corvallis.

Sanford, E. 1999b. Regulation of keystone predation by small changes in ocean temperature. Science **283**:2095-2097.

SAS Institute, 1994. SAS/STAT User's Guide, Version 6, Fourth edition. SAS Institute, Inc., Cary.

Scheiner, S. M. 1993. MANOVA: multiple response variables and multispecies interactions. Pages 94-112 *in* S. M. Scheiner and J. Guerevitch, editors. Design and Analysis of Ecological Experiments. Chapman & Hall, New York.

Schmitt, R. J. 1987. Indirect interactions between prey: apparent competition, predator aggregation, and habitat segregation. Ecology **68**:1887-1897.

Schmitt, T. M., M. E. Hay, and N. Lindquist. 1995. Constraints on chemically mediated coevolution: multiple functions for seaweed secondary metabolites. Ecology **76**:107-123.

Schoener, T. W. 1993. On the relative importance of direct versus indirect effects in ecological communities. *in* H. Kawanabe, J. Cohen, and K. Iwasaki, editors. Mutualism and Community Organization: Behavioural, Theoretical and Food Web Approaches.

Schwarzkopf, L., and R. A. Alford. 1996. Desiccation and shelter-site use in a tropical amphibian: comparing toads with physical models. Functional Ecology **10**:193-200.

Shreve, F. 1931. Physical conditions in sun and shade. Ecology **12**:96-104.

Shumway, S. W. 2000. Facilitative effects of a sand dune shrub on species growing beneath the shrub canopy. Oecologia (Berlin) **124**:138-148.

Somero, G. N. 1997. Temperature relationships: from molecules to biogeography. Pages 1391-1444 *in* E. Danztler, editor. Handbook of Physiology, Section 13: Comparative Physiology. Cambridge University Press, Cambridge.

Southward, A. J., and E. C. Southward. 1978. Recolonization of rocky shores in Cornwall after use of toxic dispersants to clean up the *Torrey Canyon* spill. Journal of the Fisheries Research Board Canada **35**:682-706.

Stachowicz, J. J., and M. E. Hay. 1996. Facultative mutualism between an herbivorous crab and a coralline alga: advantages of eating noxious seaweeds. Oecologia (Berlin) **105**:377-387.

Steneck, R. S., and M. N. Dethier. 1994. A functional group approach to the structure of algal-dominated communities. OIKOS **69**:476-498.

Steneck, R. S., and M. N. Dethier. 1995. Are functional classifications different for marine vs terrestrial plants? A reply to Grime. OIKOS **73**:122-124.

Steneck, R. S., and L. Watling. 1982. Feeding capabilities and limitation of herbivorous molluscs: A functional group approach. Marine Biology **68**:299-319.

Stevenson, R. D. 1985. The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. American Naturalist **126**:362-386.

Suchanek, T. H. 1979. The *Mytilus californianus* community: studies on the composition, structure, organization, and dynamics of a mussel bed. Ph.D. University of Washington, Seattle.

Sullivan, G., and J. B. Zedler. 1999. Functional redundancy among tidal marsh halophytes: a test. OIKOS **84**:246-260.

Suzuki, Y., T. Takabayashi, T. Kawaguchi, and K. Matsunaga. 1998. Isolation of an allelopathic substance from the crustose coralline algae, *Lithophyllum spp.*, and its effect on the brown alga, *Laminaria religiosa* Miyabe (Phaeophyta). Journal of Experimental Marine Biology and Ecology **225**:69-77.

Tegner, M. J., P. K. Dayton, P. B. Edwards, and K. L. Riser. 1997. Large-scale, low-frequency oceanographic effects on kelp forest succession: a tale of two cohorts. Marine Ecology Progress Series **146**:117-134.

Thompson, G. B. 1980. Distribution and population dynamics of the limpet *Patella vulgata* L. in Bantry Bay. Journal of Experimental Marine Biology and Ecology **45**:173-217.

Tomanek, L., and G. N. Somero. 1999. Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (Genus *Tegula*) from different thermal habitats: Implications for limits of thermotolerance and biogeography. Journal of Experimental Biology **202**:2925-2936.

Tomanek, L., and G. N. Somero. 2000. Time course and magnitude of synthesis of heat shock proteins in congeneric marine snails (Genus *Tegula*) from different tidal heights. Physiological and Biochemical Zoology **73**:249-256.

Turner, R., S. Alcorn, G. Olin, and J. Booth. 1966. The influence of shade, soil, and water on saguaro seedling establishment. Botanical Gazette **127**:95-102.

Turner, R. M., S. M. Alcorn, and G. Olin. 1969. Mortality of transplanted saguaro seedlings. Ecology **50**:835-844.

Turner, T. 1983. Facilitation as a successional mechanism in a rocky intertidal community. American Naturalist **121**:729-738.

Vadas Sr., R. L., S. Johnson, and T. A. Norton. 1992. Recruitment and mortality of early post-settlement stages of benthic algae. British Phycological Journal **27**:331-351.

Van Der Wal, R., H. Van Wijnen, S. Van Wieren, O. Beucher, and D. Bos. 2000. On facilitation between herbivores: how Brent Geese profit from brown hares. Ecology **81**:969-980.

van Tamelen, P., M. S. Stekoll, and L. Deysher. 1997. Recovery processes of the brown alga *Fucus gardneri* following the 'Exxon Valdez' oil spill: settlement and recruitment. Marine Ecology Progress Series **160**:265-277.

Velimirov, B., and C. L. Griffiths. 1979. Wave-induced kelp movement and its importance for community structure. Botanica Marina **22**:169-172.

Walker, B. H. 1992. Biodiversity and ecological redundancy. Conservation Biology **6**:18-23.

Walker, L. R., and F. S. Chapin. 1987. Interactions among processes controlling successional change. OIKOS **50**:131-134.

Wehner, R., A. C. Marsh, and R. Wehner. 1992. Desert ants on a thermal tightrope. Nature **357**:586-587.

Wendt, D. E., and R. M. Woollacott. 1999. Ontogenies of phototactic behavior and metamorphic competence in larvae of three species of Bugula (Bryozoa). Invertebrate Biology **118**:75-84.

Wickner, S., M. R. Maurizi, and S. Gottesman. 1999. Posttranslational quality control: folding, refolding, and degrading proteins. Science **286**:1888-1893.

Wiens, J. A. 1989. Spatial scaling in ecology. Functional Ecology **3**:385-397.

Williams, G. A. 1994. The relationship between shade and molluscan grazing in structuring communities on a moderately-exposed tropical rocky shore. Journal of Experimental Marine Biology and Ecology **178**:79-95.

Williams, G. A., and D. Morritt. 1995. Habitat partitioning and thermal tolerance in a tropical limpet, *Cellana grata*. Marine Ecology Progress Series **124**:89-103.

Williamson, C. E., and D. M. Leech. 2000. UV radiation and zooplankton distribution across vertical habitat gradients in three glacial lakes of differing transparency. *in* Ecological Society of America Annual Meeting. Ecological Society of America, Snowbird, UT.

Witman, J. D. 1987. Subtidal coexistence: storms, grazing, mutualism, and the zonation of kelps and mussels. Ecological Monographs **57**:167-187.

Wootton, J. 1997. Estimates and test of *per capita* interaction strength: diet, abundance, and impact of intertidally foraging birds. Ecological Monographs **97**:45-64.

Wootton, J. T. 1991. Direct and indirect effects of nutrients on intertidal community structure: variable consequences of seabird guano. Journal of Experimental Marine Biology and Ecology **151**:139-153.

Wootton, J. T. 1992. Indirect effects, prey susceptibility, and habitat selection: impacts of birds on limpets and algae. Ecology **73**:981-991.

Wootton, J. T. 1993. Indirect effects and habitat use in an intertidal community: interaction chains and interaction modifications. American Naturalist **141**:71-89.

Wootton, J. T. 1994. Predicting direct and indirect effects: an integrated approach using experiments and path analysis. Ecology **75**:151-165.

Wootton, J. T. 1995. Effects of birds on sea urchins and algae: A lower-intertidal trophic cascade. Ecoscience **2**:321-328.

APPENDIX

Appendix: Assemblage Data: Adjacent to Experimental Structures

A. I. Repeated measures analysis of total mobile animal abundance in survey plots adjacent to experimental structures. See Figure 2.1 for design details. Data are log-transformed counts for May, July, and November 1998, April, May, and July 1999, and May and July 2000. Mauchly's criterion was met ($p = 0.07$) so univariate analyses are presented with un-adjusted p-values. Bold-faced p-values for treatment effects indicate significance at $\alpha = 0.05$.

Adjacent to Experimental Structures

Source	Df	MS	F	P
Between Subjects (average effect over time)				
Treatment	4	4.38	3.63	**0.0275**
Block	4	1.22	1.01	0.4296
Error	16	1.21		
contrasts				
1. –H +B –S (NS) vs.–H +B –S (SC)	1	0.01	0.01	0.9279
2. –H +B –S (SC) vs. –H –B –S	1	0.05	0.04	0.8470
3. –H –B –S vs. –H –B +S **(halo effect)**	1	0.61	0.50	0.4895
4. –H –B +S vs.+H(–B +S) **(shade + *Hedophyllum* thallus effect)**	1	7.83	6.48	**0.0216**
Within Subjects (change in effect over time)				
Time	7	12.03	39.72	**< 0.0001**
Time x Treatment	28	0.68	2.23	**0.0017**
Time x Block	28	0.62	2.03	0.0050
Error (Time)	112	0.30		
contrasts				
Time x 1	7	0.13	0.44	0.8743
Time x 2	7	0.08	0.27	0.9643
Time x 3 **(halo effect)**	7	0.08	0.25	0.9700
Time x 4 **(shade + *Hedophyllum* thallus effect)**	7	1.36	4.50	**0.0002**

A.II. Multivariate analyses of mobile animal assemblage in survey plots adjacent to experimental structures. Data are log-transformed counts for six animal groups; crabs, whelks, seastars, limpets, snails, and chitons. See Table 2.1 for species lists. Bold faced p-values for treatment effects indicate significance at $\alpha = 0.01$ (adjusted for multiple comparisons).

Adjacent to Experimental Structures

JULY 1998	N df	D df	Wilks λ	F	P
Treatment	16	40.4	0.08	3.24	0.0013
Block	16	40.4	0.61	0.44	0.9603
contrasts					
1. –H +B –S (NS) vs.–H +B –S (SC)	4	13	0.91	0.33	0.8519
2. –H +B –S (SC) vs. –H –B –S	4	13	0.69	1.48	0.2640
3. –H –B –S vs. –H –B +S (halo effect)	4	13	0.87	0.50	0.7361
4. –H –B +S vs.+H(–B +S) (shade + Hedophyllum thallus effect)	4	13	0.23	10.98	0.0004
NOVEMBER 1998	**N df**	**D df**	**Wilks λ**	**F**	**P**
Treatment	24	39.6	0.20	0.97	0.5203
Block	24	39.6	0.02	3.84	< 0.0001
JULY 1999	**N df**	**D df**	**Wilks λ**	**F**	**P**
Treatment	24	39.6	0.11	1.42	0.1593
Block	24	39.6	0.11	1.49	0.1300
JULY 2000	**N df**	**D df**	**Wilks λ**	**F**	**P**
Treatment	24	39.6	0.16	1.12	0.3648
Block	24	39.6	0.17	1.10	0.3841

A.III Repeated measures analysis of total macroalgal and encrusting invertebrate cover in survey plots adjacent to experimental structures. See Figure 2.1 for design details. Data are log-transformed percent cover values, (primary + secondary cover), for May, July, and November 1998, April, May, and July 1999, and May and July 2000. As Mauchly's criterion was not met ($p = 0.003$) multivariate analyses are presented. Bold-faced p-values for treatment effects indicate significance at $\alpha = 0.05$.

Adjacent to Experimental Structures

Source			Df	MS	F	P
Between Subjects (average effect over time)						
Treatment			4	13.76	16.21	**< 0.0001**
Block			4	1.35	1.59	0.2244
Error			16	0.85		
contrasts						
1. –H +B –S (NS) vs.–H +B –S (SC)			1	0.05	0.05	0.8210
2. –H +B –S (SC) vs. –H –B –S			1	0.01	0.01	0.9213
3. –H –B –S vs. –H –B +S	**(halo effect)**		1	0.05	0.05	0.8205
4. –H –B +S vs.+H(–B +S)	**(shade + *Hedophyllum* thallus effect)**		1	36.21	42.66	**< 0.0001**
Within Subjects (change in effect over time)	**N df**	**D df**	**Wilks λ**	**F**	**P**	
Time	7	10	0.052	26.21	**< 0.0001**	
Time x Treatment	28	37.5	0.089	1.28	0.2378	
Time x Block	28	37.5	0.111	1.12	0.3650	
contrasts						
Time x 1	7	10	0.881	0.19	0.9803	
Time x 2	7	10	0.610	0.91	0.5331	
Time x 3 (halo effect)	7	10	0.705	0.60	0.7464	
Time x 4 **(shade + *Hedophyllum* thallus effect)**	7	10	0.306	3.24	**0.0457**	

A.IV Repeated measures analysis of microalgal abundance in plots adjacent to experimental structures. See Figure 2.1 for design details. Data are log-transformed percent cover values, for May, July, and November 1998, April, May, and July 1999, and May and July 2000. As Mauchly's criterion was met ($p = 0.057$) and interpretations did not differ between multivariate and univariate analyses, univariate analyses are presented with Huynh-Feldt adjusted probabilities. Bold faced p-values for treatment effects indicate significance at $\alpha = 0.05$.

Adjacent to Experimental Structures

Source	Df	MS	F	P
Between Subjects (average effect over time)				
Treatment	4	34.20	37.09	**< 0.0001**
Block	4	5.50	5.96	0.0039
Error	16	0.92		
contrasts				
1. –H +B –S (NS) vs.–H +B –S (SC)	1	1.95	2.12	0.1651
2. –H +B –S (SC) vs. –H –B –S	1	1.27	1.38	0.2571
3. –H –B –S vs. –H –B +S **(halo effect)**	1	0.65	0.70	0.4145
4. –H –B +S vs.+H(–B +S) **(shade + *Hedophyllum* thallus effect)**	1	98.60	106.92	**< 0.0001**
Within Subjects (change in effect over time)				
Time	7	32.55	28.09	**< 0.0001**
Time x Treatment	28	1.62	1.40	0.1107
Time x Block	28	3.29	2.84	< 0.0001
Error (Time)	112	1.16		
contrasts				
Time x 1	7	0.31	0.26	0.9663
Time x 2	7	0.90	0.77	0.6102
Time x 3 **(halo effect)**	7	0.58	0.50	0.8358
Time x 4 **(shade + *Hedophyllum* thallus effect)**	7	3.41	2.94	**0.0073**

A.V. Multivariate analysis of algal and encrusting invertebrate assemblage, July 2000, in survey plots adjacent to experimental structures. Data are log-transformed percent cover values (primary + secondary) of six algal groups; green algae, fleshy brown algae, finely branched red algae, foliose and corticated red algae, microalgae, rare algae, articulated coralline algae, encrusting invertebrates (see Table 2.1 for species). Bold-faced p-values for treatment effects indicate significance at $\alpha = 0.05$.

Adjacent to Experimental Structures

Source	N df	D df	Wilks λ	F	P
Treatment	32	34.8	0.227	1.95	**0.0282**
Block	32	34.8	0.043	1.46	0.1387
contrasts					
1. –H +B –S (NS) vs.–H +B –S (SC)	8	9	0.679	0.53	0.8061
2. –H +B –S (SC) vs. –H –B –S	8	9	0.660	0.58	0.7732
3. –H –B –S vs. –H –B +S **(halo effect)**	8	9	0.898	0.13	0.9959
4. –H –B +S vs.+H(–B +S) **(shade + *Hedophyllum* thallus effect)**	8	9	0.108	9.30	**0.0015**