

Medolian C. Pressley. THE ROLE OF DENSITY, PREDATOR DEFENSE, AND MICROHABITAT PREFERENCE ON THE SPATIAL DISTRIBUTION OF *Chromopleustes oculatus* (CRUSTACEA: AMPHIPODA) AT BELL ISLAND, WASHINGTON. (Under the direction of Lisa M. Clough and Roger A. Rulifson) Department of Biology, June 2003.

This study examined abiotic and biotic effects on the spatial distribution of *Chromopleustes oculatus* during mid-May to late-July 2000. *In situ* video of *A. fimbriatum* recorded spatial distributions of the amphipods in the field at high and low slack tide. Testing pools were designed to isolate the roles of density and the presence of predators on spatial distributions. I assessed habitat use by presenting amphipods with five substrates: sea cucumbers, rocks, *Agarum* blades, sand and shell mixtures, and sand. Amphipod numbers were greatest at the base of *A. fimbriatum* (20 individuals per image) and smallest at the tip (eight individuals per image). Mean nearest neighbor distances (MNND) significantly decreased from tip to base. MNND for four densities in testing pools (500, 1000, 2000, and 5000 individuals·0.664 m⁻²) significantly decreased as density increased from 500 to 2000 individuals. MNND in pools containing 5000 and 1000 individuals were not statistically different. The MNND in the presence of a naïve fish predator, *Oligocottus maculosus*, was higher than the MNND in the predator's absence. Amphipods occupied the highest density per area on sea cucumbers (*Cucumaria miniata*). Amphipods attempting to mate in testing pools, in addition to possible cannibalism, were most likely the result for the increased MNND in density and predator experiments. Multiple abiotic and biotic factors influenced the spatial distribution of *C. oculatus*, however, this study would argue that chemical defense does

not play an important role for *C. oculatus* with respect to amphipod position on algal blades, habitat choices, predators, reproduction, and in some cases cannibalism.

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PREFERENCE ON THE SPATIAL DISTRIBUTION OF *Chromopleustes oculatus*
(CRUSTACEA: AMPHIPODA) AT BELL ISLAND, WASHINGTON

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By

Medolian C. Pressley

APPROVED BY:

DIRECTOR OF THESIS



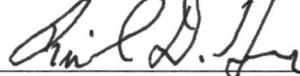
Lisa M. Clough, Ph.D.

DIRECTOR OF THESIS



Roger A. Rulifson, Ph.D.

COMMITTEE MEMBER



Richard D. Hauser, Ph.D.

COMMITTEE MEMBER



Kyle D. Summers, Ph.D.

COMMITTEE MEMBER



Terry L. West, Ph.D.

CHAIR OF THE DEPARTMENT OF BIOLOGY



Ronald J. Newton, Ph.D.

INTERIM DEAN OF THE GRADUATE SCHOOL



Paul D. Tschetter, Ph.D.

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TABLE OF CONTENTS

LIST OF TABLES.....	v
LIST OF FIGURES.....	vii
INTRODUCTION.....	1
Abiotic Factors.....	1
Biotic Factors.....	2
HYPOTHESES.....	18
Distributions and Patterns.....	18
Density.....	19
METHODS.....	20
Study Site.....	20
Standardization.....	20
Nearest Neighbor Calculations.....	21
Field Observations.....	24
Laboratory Experiments.....	28
Spatial distributions and patterns of aggregates.....	29
Predators and spatial distributions.....	30
Habitat choice experiments.....	31
RESULTS.....	35
Standardization.....	35
Field Observations.....	35

Laboratory Experiments.....	53
Spatial distributions and patterns of aggregates	56
Predators and spatial distributions.....	63
Habitat choice experiments.....	68
DISCUSSION.....	72
Standardization.....	72
Field Observations.....	72
Spatial distributions	74
Density.....	74
Laboratory Experiments.....	77
Spatial distributions and patterns of aggregates.....	77
Predators and spatial distributions.....	81
Habitat choice experiments.....	82
CONCLUSIONS.....	87
LITERATURE CITED.....	89

LIST OF TABLES

1.	Percentage of nearest neighbor distances < 0.5 pixels (<0.56 cm for field observations and < 0.22 cm for laboratory manipulations) that were eliminated from nearest neighbor distance calculations due to possible mating or cannibalism by <i>Chromopleustes oculatus</i>	23
2.	Amphipod variability, expressed as Mean \pm SE, from counts of 12 algal blades taken as a field survey <i>in situ</i> at Bell Island 13 June 2000 through 25 June 2000 during high and low slack tide prior to beginning field observations.....	38
3.	ANOVA of the differences in mean number of individuals at three positions on <i>Agarum fimbriatum</i> (base, center, and tip) using images taken at high and low slack tides of natural populations of <i>Chromopleustes oculatus</i> at Bell Island.....	44
4.	Bonferroni Post Hoc test comparing the mean abundances of <i>Chromopleustes oculatus</i> at three locations on <i>Agarum fimbriatum</i> ; base, center, and tip.....	46
5.	ANOVA of the differences in mean nearest neighbor distances at three positions on <i>Agarum fimbriatum</i> (base, center, and tip) using images taken at high and low slack tides of natural populations of <i>Chromopleustes oculatus</i> at Bell Island.....	54
6.	Bonferroni Post Hoc interaction results for mean nearest neighbor distances that shifted as a function of tidal state (High / Low Slack tide) at a given blade position (Base / Center / Tip) for <i>Chromopleustes oculatus</i>	55
7.	ANOVA of the relative change in mean nearest neighbor distances of <i>Chromopleustes oculatus</i> at 2 and 24 hours in experiments manipulating four densities (500, 1000, 2000, and 5000 individuals per 0.664m ²).....	58
8.	Bonferroni Post Hoc comparisons of mean nearest neighbor distances analyzed from images of <i>Chromopleustes oculatus</i> taken at five preset locations (north, south, east, west, and center) in testing pools for four densities, respectively (500, 1000, 2000, and 5000 individuals 0.664m ²).....	58
9.	Bonferroni Post Hoc interaction results for mean nearest neighbor distances that shifted as a function of density (500, 1000, 2000, and 5000 individuals 0.664m ²) at a given time (2 hr and 24 hr) for <i>Chromopleustes oculatus</i> (comparisons are sorted by increasing probabilities).....	64

10. Table of values used in Chi-Square analyses: two-dimensional percentage of cover for each habitat in aquaria, expected number of *Chromopleustes oculatus* with respect to habitat percentages, and observed numbers of individuals in aquaria..... 69

LIST OF FIGURES

1.	Map of San Juan Islands National Wildlife Refuge at Friday Harbor Laboratories in the state of Washington, USA.....	8
2.	Patterns of distribution for <i>Chromopleustes oculatus</i> from photoquadrats taken summer 1999 based on an Index of Aggregation (R).....	12
3.	Detailed map featuring Bell Island, the primary field site, located south of Orcas Island and north of Shaw Island.....	14
4.	Image of the Colander Weed, <i>Agarum fimbriatum</i> , the dominant spatial habitat at Bell Island, Washington.....	16
5.	Lateral photo of the gammarid amphipod (<i>Chromopleustes oculatus</i>) used for all laboratory and field experiments.....	26
6.	Diagram of habitat positioning in amphipod choice experiments for aquaria containing the dominant environments found at Bell Island: sand and shell mixture, <i>Laminarian</i> algal blade, rock, and sea cucumber (<i>Cucumaria miniata</i>).....	33
7.	Distribution of mean body lengths for 36 images in testing pools containing 500 individuals.....	36
8.	Comparison of mean body lengths of <i>Chromopleustes oculatus</i> observed in the field and those that were selected according to body size in the laboratory experiments.....	39
9.	Mean number of amphipods (abundance) observed for images taken in the field at three locations (base, center, and tip) at high and low slack tides on the algal blade, <i>Agarum fimbriatum</i>	42
10.	Mean nearest neighbor distances (expressed as body lengths) of <i>Chromopleustes oculatus</i> at high and low slack tide on the algal blade, <i>Agarum fimbriatum</i>	47
11.	Mean nearest neighbor distances (expressed as body lengths) of <i>Chromopleustes oculatus</i> at three locations (base, center, and tip) on the algal blade, <i>Agarum fimbriatum</i>	49
12.	Mean nearest neighbor distances (expressed as body lengths) of <i>Chromopleustes oculatus</i> at three locations (base, center, and tip) at high and low slack tides on the algal blade, <i>Agarum fimbriatum</i>	51

13.	Mean nearest neighbor distances (expressed as body lengths) of <i>Chrompleustes oculatus</i> at four densities (500, 1000, 2000, and 5000 individuals/0.664 m ²) for testing pools in the laboratory to determine impacts of density on spatial distributions.....	59
14.	Mean nearest neighbor distances (expressed as mean body lengths) of <i>Chrompleustes oculatus</i> at two time intervals (2 hr and 24 hr) at each of the four densities for testing pools in the laboratory (500, 1000, 2000, and 5000 individuals/0.664 m ²).....	61
15.	Mean nearest neighbor distances (expressed as mean body lengths) of <i>Chrompleustes oculatus</i> in 0.664 m ² testing pools with and without predators (excluding all distances < 1.37 body lengths, or 0.54 cm for mating.....	66
16.	Two-dimensional percentage of cover for each habitat in aquaria used for habitat choice experiments; <i>Cucumaria miniata</i> , <i>Agarum fimbriatum</i> , rock, sand and shell debris, and sand only.....	70

INTRODUCTION

Many species use aggregate behavior for certain life history functions such as reproduction, feeding, migration, and survival. Aggregations are defined as large numbers of conspecifics in a particular area (e.g., swarms found on substrates or in the water column). Determining distributions and patterns within an aggregated community is crucial in understanding how the species maintains itself (Davis et al. 1991).

Crustaceans, especially amphipods of the Family Pleustidae, exhibit aggregate behavior, which may be altered by abiotic and biotic factors such as temperature, salinity, life cycles, water flow, suitable habitats, food, reproduction, in a few cases cannibalism, predation, and inter- and intraspecific competition.

Abiotic Factors

Temperature regulates many aspects of marine species through habitat preferences, migration, mating, and larval development (Maravelias 1997, Graham et al. 2001, Prince and Griffin 2001). Among gammarid amphipods, brood productivity and time of development are directly related to temperature (Welton and Clark 1980, Borowsky 1991). For example, female amphipods having a predicted longevity of 17-23 months, produce as many as six broods during a summer, with brood development time decreased by more than half at increased temperatures (Mottram 1933, Hynes 1955, Kinne 1960, Steele and Steele 1973, Welton and Clark 1980).

Changes in salinity do not appear to be a limiting factor in amphipod distributions in their normal environments. For example, when two species of gammarid amphipods

are exposed to extreme salinity differences (above and below ambient), they show survival times equal to experimental controls, suggesting that at least some amphipods should be classified as euryhaline (Hopper 1960; also see Van Dolah 1978). However, zooplankton (including amphipods) are positively affected by density discontinuities and will aggregate at salinity fronts (Harder 1968). Fronts may act to concentrate food, leading to aggregations, which may also impact mating (Straty 1972, Uye and Fleminger 1977, Facey and Van-Den-Avyle 1987, Wippelhauser and McCleave 1987, Parker 1995, Graham et al. 2001, Grudemo and Andre 2001, Kingsford et al. 2002, Meade et al. 2002).

Biotic Factors

Biotic factors, in conjunction with abiotic factors, also can modify amphipod behavior. For example, Van Dolah (1978) found that peak amphipod abundance is correlated with both environmental and biological factors, with the former most influential during the winter and the latter dominating other times of the year. Winter declines in abundance at intertidal habitats correlate with amphipod subtidal migrations. Additional causes for declines in intertidal populations include mortality due to freezing. In contrast, mortality during the summer months coincides with limited food resources. When food resources are limited, it is believed that adults displace juveniles from preferred substrata. Mortality during the summer and fall also may be a result of omnivorous fish predation on those amphipod species inhabiting palatable microhabitats that are consumed by these fish.

Many gammarid amphipods swim upstream in currents (Macan and Mackereth 1957, Minckley 1964, Muller 1966, Kureck 1967, Lehman 1967, Hultin 1968, Elliot 1971). *Gammarus bousfieldi* is the only species (of six known to occur in the stream system of Doe Run, Kentucky) that appears to swim upstream in organized migrations (Minckley 1964). A separate study on *Gammarus pulex* in the English Lake District notes amphipods swim upstream in large quantities (Elliot 1971). Aggregates swim into the current at this stony stream that ranges in turbidity from $7.1 \text{ cm}\cdot\text{s}^{-1}$ in June to $95.0 \text{ cm}\cdot\text{s}^{-1}$ in November (Elliot 1971).

Amphipods, like other species of crustaceans, physically adapt to areas of various water flow. The literature suggests that amphipods found in extreme hydrodynamics (e.g., swift currents) are excellent swimmers and specialize in their foraging mechanisms (e.g., graze on algae itself or host organisms of the substratum) (Krapp 1993). Swimming speeds typically show a linear relationship with body weight: larger amphipods swim faster and vice versa (Takeuchi and Watanabe 1998). Swarms of small deep-sea amphipods (< 5 cm compared with deep-sea amphipods much larger than 5 cm) are found in the East Pacific Rise vent field from 2520 m to 2580 m (Kaartvedt et al. 1994). Increased swimming speeds provide the individuals with the ability to maintain a position in the current (Kaartvedt et al. 1994). The author of this study does mention that “observations contrast with the general concept of low swimming activity in deep-sea crustaceans ” (Kaartvedt et al. 1994).

Amphipods inhabiting slower moving waters with prevailing orbital motions cling to substrate (Krapp 1993). These areas of oscillation zones are generally coupled with

hydroids and bryozoans; it is possible these omnivorous amphipods are commensal and catch their prey out of the hydranths or polyps, and in some cases even biting off tentacles of the host (Krapp 1993, David et al. 1996). Their ability to cling to the substrate is excellent but they have limited swimming abilities with only short bursts of speed for small distances (Krapp 1993).

For small herbivorous amphipods that live on algae or plants, food and habitat are closely linked. Food choices in nature are constrained by the need to choose sites that provide safe living. Many amphipods prefer to live on algae that are distasteful to omnivorous fishes (Duffy and Hay 1991, Duffy and Hay 1994, Sotka et al. 1999, Poore et al. 2000). The luxury of living and feeding on noxious plants allows amphipod species such as *Ampithoe longimana* to reach their highest abundances during seasons when predatory fish are most prevalent, while other amphipod species not living on noxious plants decrease to near extinction (Duffy and Hay 1994). Therefore, there has been selection for less mobile herbivores to be most tolerant of plant chemical defenses (Duffy and Hay 1994).

Non-herbivorous amphipods living on chemically defended plants may also benefit from the association. These commensal amphipods inhabiting macroalgae typically do not forage on their defended hosts. Amphipods such as *Erichthonius brasiliensis* wrap themselves in pieces of seaweed to create domiciles in which they filter-feed (Sotka et al. 1999).

Aggregation facilitates reproduction. In organisms with external fertilization, aggregation during gamete release increases fertilization success (Levitan and Petersen

1995, Coma and Lasker 1997, Levitan 1998, Styan 1998). Even for species with internal fertilization, aggregation brings otherwise dispersed individuals together (e.g., Aguilar-Perera and Aguilar-Davila 1996, Domier and Colin 1997, Sauer et al. 1997). These aggregations may occur in areas in which no resources critical for reproductive success, except mates, may be at stake; e.g., in a breeding system known as leks (e.g., Vehrencamp and Bradbury 1984, Anderson 1997).

Aggregation also affords the opportunity for cannibalism. There are numerous reports of cannibalistic acts by marine animals including copepods (Anderson 1970). Many species of freshwater *Gammarus* amphipods will become cannibalistic in laboratory studies (Embody 1911, Sexton 1924, Sexton 1928, Clemens 1950, Jones 1951, Schmitz 1967, Kostalos and Seymour 1976, Jenio 1980, Dick 1995, MacNeil et al. 1997). Unfortunately, detailed feeding records for marine gammarid amphipods are rare due to the complexity of the marine environment and size of the organisms (e.g., Muspratt 1951, Larkin 1956, Macan 1965, Paine 1965, Nikolskii 1969, Manzi 1970). Some authors have suggested that cannibalism erupts in individuals under severe stress, especially when alternatives, such as dispersal, are not available (Colinvaux 1973, but see Fox 1975). These stresses may include containment in laboratory systems (e.g., under severe resource depletion or in situations where conspecifics are injured, diseased, and dead), cannibalism of juveniles by adults, and cannibalism of smaller males by larger males during mating season (MacNeil et al. 1999).

Gregarious behaviors serve many functional roles in defense against predators including early warning, confusion, cooperative defense, and a dilution effect.

Individuals in an aggregation benefit from their own abilities to detect predators and also from the aggregated sensory abilities of the group (Vulinec 1990, McCartt et al. 1997). This early warning permits these organisms to take appropriate actions to reduce their predation risk. For mobile organisms this may take the form of coordinated movements. Predator confusion strategies are often observed within the masses by school circles, splits, and flashes (e.g., Domenici and Batty 1994). In cooperative defense, aggressive acts by groups drive off potential predators, a behavior that would be very risky if performed by individuals (e.g., Vulinec 1990, Mueller 1994). Hamilton's (1971) selfish herd hypothesis proposed that individuals in the center of groups may suffer lower per capita predation than solitary individuals or individuals on the periphery; a variety of tests have provided some level of support for this theory (Treisman 1975; Turner and Pitcher 1986; Sillen-Tullberg and Leimar 1988; Vulinec 1990, Krause 1993, but see Parish 1989).

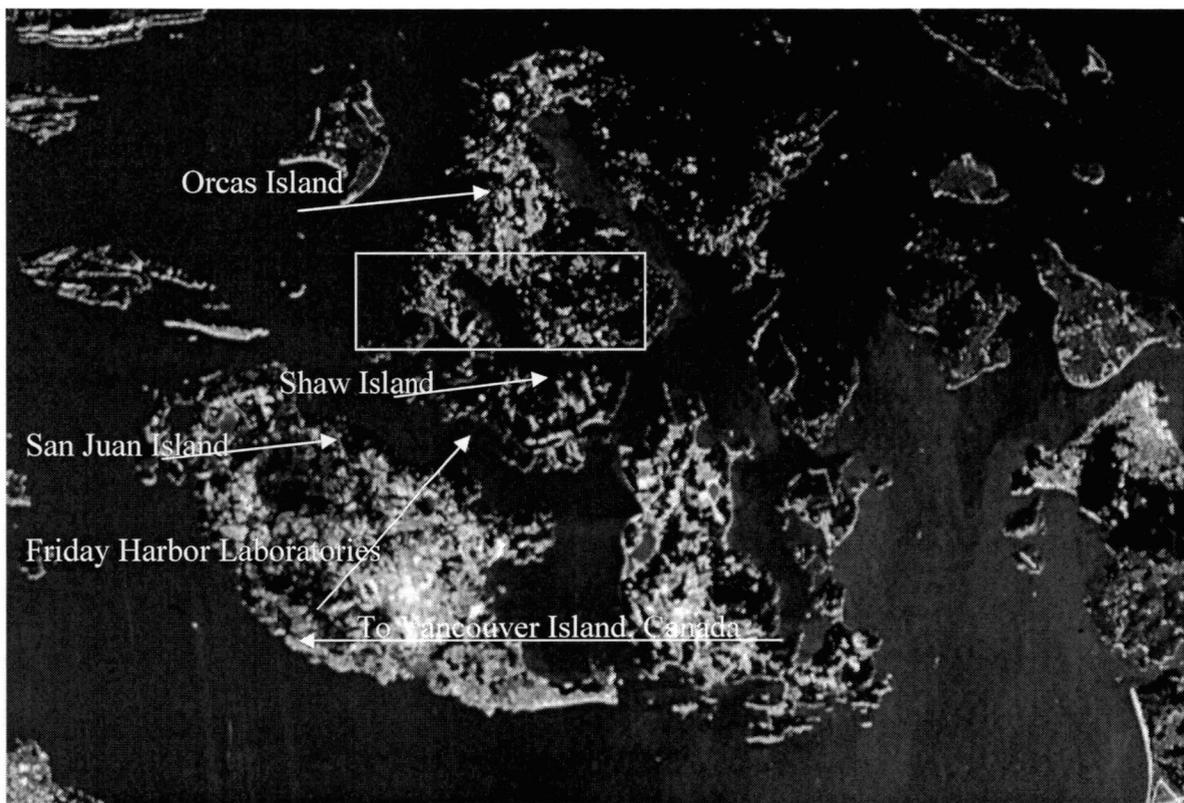
Aggregations may also benefit chemically defended prey and/or prey having aposematic coloration. Mathews (1977) suggests that a predator's consumption of large numbers of a mildly noxious prey in rapid succession might build toxin levels to induce an aversion not otherwise associated with that prey. In this case, aggregation reinforces prior negative interactions between chemically defended organisms and predators. Guilford (1985) updates an argument first developed by Fisher (1958) that aposematic coloration and chemical defense are most likely to evolve in the presence of kin groups (or under green beard selection).

Experimental tests demonstrate the value of aggregation in reducing predation pressure on aposematic, chemically defended artificial prey (Alatalo and Mappes 1996). In terrestrial insects in which it has been best studied, aggregation of conspecifics often co-occurs with aposematic (poster) coloration and chemical defense (Sillen-Tullberg and Leimar 1988). Larval aggregations are usually the result of clusters of eggs laid by single females, thus creating kin groups (Sillen-Tullberg and Leimar 1988, Vulinec 1990, Bowers 1993). Gammarid amphipods, like other paracarid crustaceans, have direct development; juveniles emerge from the marsupium without experiencing a planktonic larval stage so common in other crustacean groups. Thus, for juvenile gammarid amphipods at least kin groups may be present.

Recently, three species of chemically defended, aposematic, gammarid amphipods (*Chromopleustes oculatus*, *Chromopleustes lineatus*, and *Cryptodius kelleri*) have been discovered in the San Juan Islands, Washington (Figure 1) (Norton and Stallings 1999). All species are sympatric at several locations. They occupy a variety of habitats and can reach densities greater than 24,500 individuals·m⁻² (Norton and Stallings 1999, Aikins and Kikuchi 2001).

Fecundity in these amphipods may exceed 75 offspring per female (Hutchinson 2002), creating immediate kin groups. Amphipods, especially those in the family Pleustidae, to which *Chromopleustes* belongs, also use color patterns for predator defense, e.g., crypsis (Edmunds 1990, Stamp and Wilkens 1993), startle colorations (Sargent 1990), and warning coloration (Bowers 1993). Pleustids also appear to use coloration to mediate predator-prey interactions. For example, three species of pleustid

Figure 1. San Juan Islands National Wildlife Refuge in Washington.



amphipods are known as Batesian mimics. These species mimic chemically defended marine gastropods in coloration and locomotory behavior (Crane 1969, Field 1974, Carter and Behrens 1980). Experiments with predatory fish demonstrate that mimicry reduces attack rate (Field 1974). Similar mimicry of chemically defended organisms has been described for other amphipod species (Goddard 1984). These marine amphipods also exhibit behaviors comparable to many terrestrial insects; e.g., they share the limited mobility characteristic of several caterpillar species.

A variety of field and laboratory data indicates that *Chromopleustes oculatus*, *Chromopleustes lineatus*, and *Cryptodius kelleri* are chemically defended and aposematic. Fishes that commonly prey upon other amphipod species avoid these three species in the field. In laboratory experiments, fishes that capture these amphipods typically release them unharmed (Hutchinson 2002). Predators appear to learn to recognize and avoid these amphipods; experienced fishes will abort attacks on these species (Zalewski 2001). The chemical defense that produces these effects appears to be stored in the mid-gut caecae of these amphipods. This material is emitted when the animal is stressed (e.g., when attacked, grabbed, or placed in a harsh environment). Niels Lindquist (personal communication) at the Institute for Marine Studies (UNC-Chapel Hill) tentatively identified the key compound as a terpene.

Chromopleustes oculatus, *Chromopleustes lineatus*, and *Cryptodius kelleri* are found on available habitats by species-specific swarms. Tests to determine patterns of distribution indicate at one spatial scale (comparisons of abundance patterns among 0.1-m² visual quadrats) all three species demonstrate high levels of aggregations, or clumping

(Norton and Stallings 1999). Tests using an Index of Dispersion - R (Donnelly 1978, Krebs 1989) on *C. oculatus* at a finer spatial scale (within 0.0065-m² photoquadrats) indicate *C. oculatus* exhibits a more uniform or regular pattern on a multitude of habitats found at Bell Island, Washington: rocks, sand-shell, and *Agarum fimbriatum*. *C. oculatus* maintains a minimum inter-individual spacing of 1 cm at high densities (60 individuals•65cm⁻²), a distance more than double the body length of an individual amphipod (Figure 2). These earlier findings concur with Leising and Yen's (1997) studies performed on crustacean swarms. Leising and Yen (1997) note that several species of invertebrate crustaceans will cohabitate in an area (swarm) but will remain separated from conspecifics without any body-to-body contact.

This study concentrates on the spatial distributions of *C. oculatus*, the most common amphipod found at Bell Island, Washington. At a fine spatial scale it is known that large densities in excess of 60 individuals•65 cm⁻² of *C. oculatus* maintain a uniform pattern of dispersion with inter-individual spacing of 1 cm among conspecifics on various habitats at Bell Island (rocks, kelp, sea cucumbers, and sand-shell debris) (Norton and Stallings 1999). No studies on the spatial distribution of *C. oculatus* have solely been conducted on the dominant kelp habitat at Bell Island, *Agarum fimbriatum*, otherwise known as the Colander Weed (Figures 3 and 4). Is *A. fimbriatum* the preferred substrate? Or do *C. oculatus* aggregate on this kelp in response to distribution of food resources? Additionally, the exact density that forces *C. oculatus* into this minimum inter-individual spacing of 1 cm is not known.

Figure 2. Patterns of distribution for *Chromopleustes oculatus* from photoquadrats taken summer 1999 at Bell Island on various microhabitats. Distributions are based on an Index of Aggregation (R). n = 420 photoquadrats

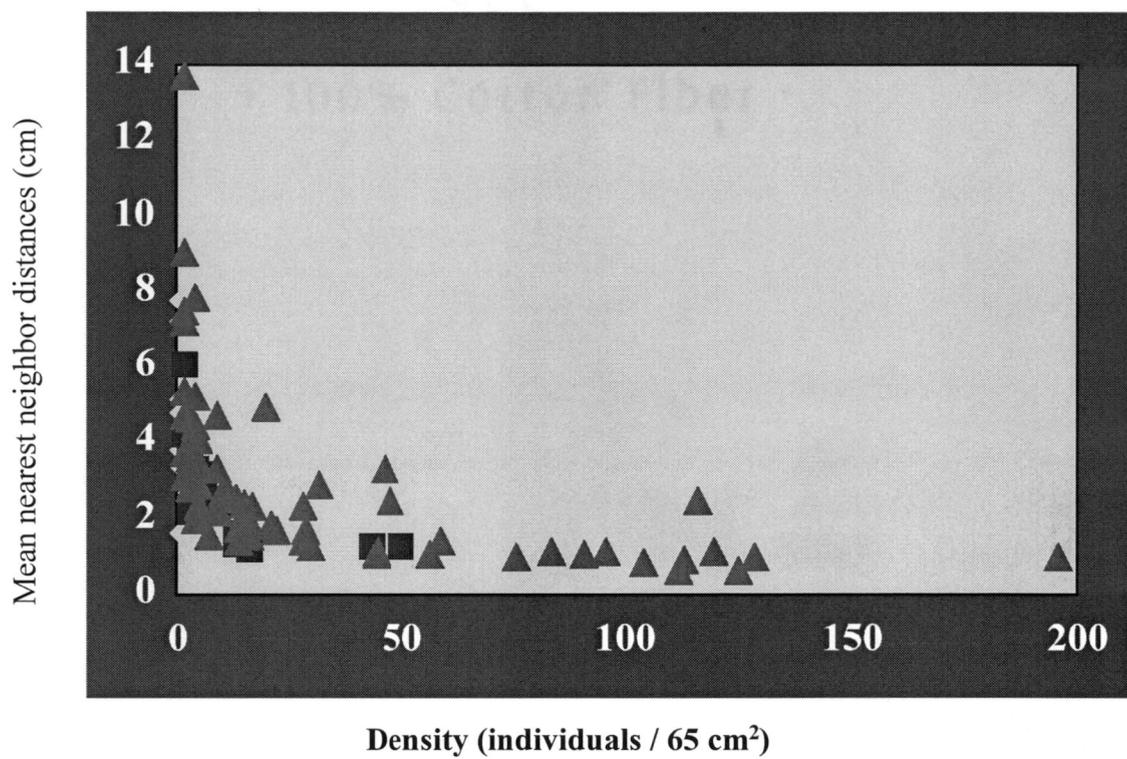


Figure 3. Detailed section taken from Figure 1 featuring Bell Island, located south of Orcas Island and north of Shaw Island. Courtesy of USGS.

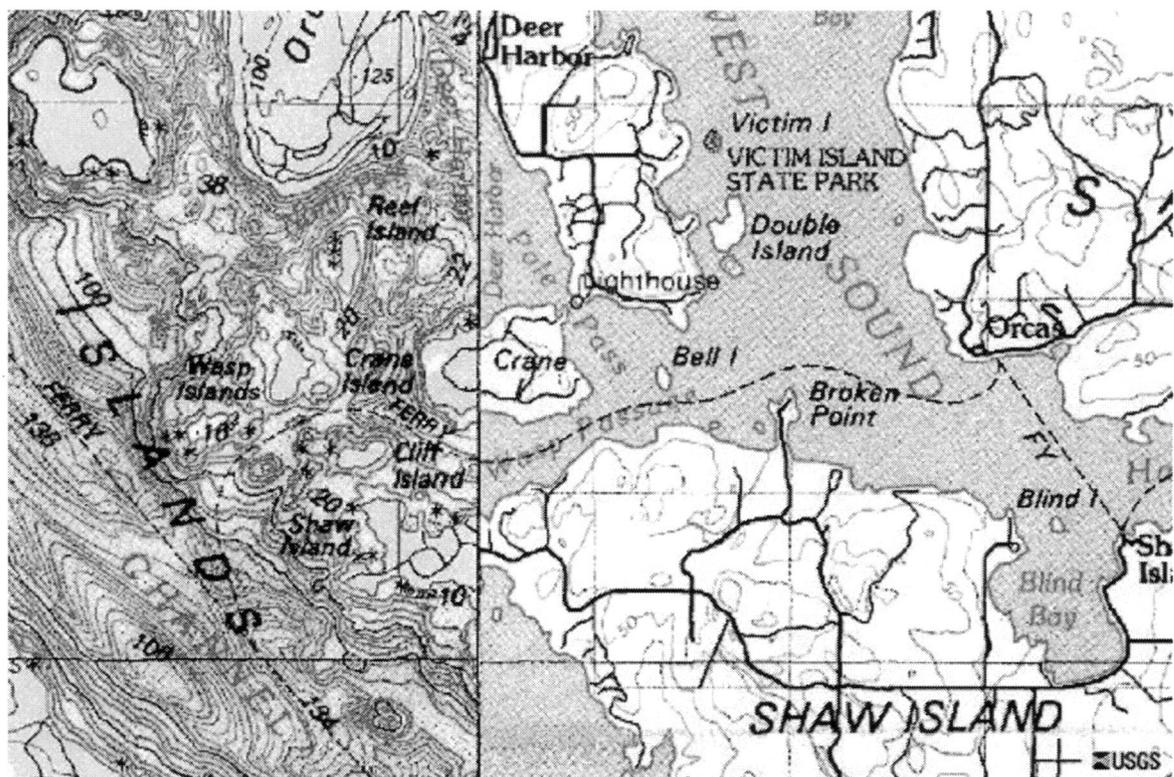
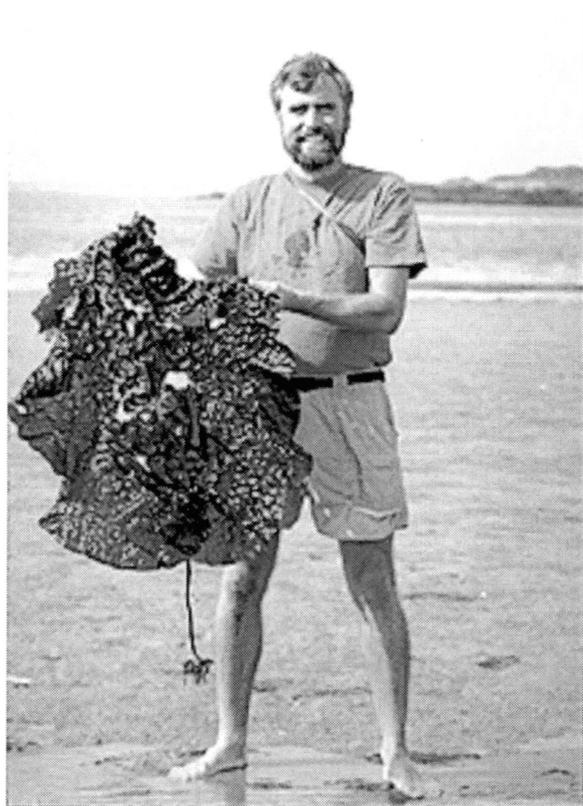


Figure 4. Image of the Colander Weed, *Agarum fimbriatum*, the dominant spatial habitat at Bell Island, Washington. Photographer unknown. Image courtesy of www.life.bio.sunysb.edu/marinebio/kelpforest.html.



HYPOTHESES

The research described herein focuses on understanding aggregation in *Chromopleustes oculatus*, the most common of the three defended species found in the San Juan Islands. I examine inter-individual spatial distributions of amphipods in the field using *in situ* underwater video. I focus on inter-individual distances of amphipods on local kelp, investigating microhabitat of the blade at three locations (base, center, and tip) at high and low slack tide. From the video, I look at density of amphipods in images, in addition to amphipod spatial distributions using nearest neighbor distances. Laboratory experiments further isolate the roles of density, the presence of predators, and habitat availability on spatial distributions of *C. oculatus*.

The term *distribution* is defined here as inter-individual spacing among a group of *C. oculatus*, calculated as nearest neighbor distances and further represented as the number of body lengths. *Density* refers only to the number of individuals in a group (swarm), on an image, or on a particular habitat. *Patterns* are the statistical nature of the shape of the aggregate: clumped, uniform, or random. I asked the following specific questions with regard to density and inter-individual distributions:

Distributions and Patterns

What is the *in situ* distribution of *C. oculatus* on its most abundant kelp habitat, *A. fimbriatum*, at Bell Island? Is the distribution of amphipods affected by observation times (high vs low slack tides)? Does distribution and/or patterns of the aggregate

change with changing density? Do differences exist in pattern and/or inter-individual spacing in the presence of predators?

H1: There is no difference in mean amphipod nearest neighbor distance on *A. fimbriatum* in the field as a function of amphipod location on the blade (base, center, tip), tide state (high and low slack), or any interaction between the two variables.

H2: There is no difference in mean nearest neighbor distance for densities ranging from 753 individuals·m⁻² to 7,530 individuals·m⁻².

H3: There is no difference in the mean nearest neighbor distance in the presence of predators in the laboratory.

Density

At what density will *C. oculatus* maintain a regular spacing pattern of 1 cm in the laboratory? Does density change within microhabitats on the blade of *A. fimbriatum* (base, center, and tip)? Given an array of habitats, would amphipods inhabit the same preferred macroalgae in a controlled environment? Is habitat a possible reason for aggregation?

H4: There is no difference in mean amphipod density on *A. fimbriatum* in the field as a function of amphipod location on the blade (base, center, tip), tide state (high and low slack), or any interaction between the two variables.

H5: The response of amphipods to habitat choices will be the same for each of the four habitats: sea cucumbers, sand, rock, and sand-shell debris.

METHODS

Study Site

My base of operation was Friday Harbor Laboratories, a 484-acre facility on San Juan Island administered by the University of Washington. The primary study site was Bell Island (48° 35' 47'' N, 122° 58' 46'' W) located in Wasp Passage, south of Orcas Island and north of Shaw Island in the San Juan Islands, Washington (Figure 2). Bell Island is characterized by mixed semidiurnal tidal cycles and swift currents due to extreme changes in high and low tide (tidal differences of greater than 4 m in a 6-hour period are common). Bell Island also is characterized by high levels of spatial heterogeneity; abiotic conditions such as current, depth, and physical substrate vary at fine spatial scales. As a result, the relative abundances of the dominant space-holding organisms (e.g., laminarian algae, colonial tubeworms, sea anemones, and sea cucumbers) also vary dramatically within microhabitats.

Chromopleustes oculatus, *Chromopleustes lineatus*, and *Cryptodius kelleri* have patchy distributions at this site and can occur at relatively high densities (up to 24,500 individuals•m², personal observation) in subtidal habitats. In addition, they occupy a variety of habitats including blades of laminarian algae, tentacles of sea cucumbers, other benthic invertebrates, and shell debris (Norton and Stallings 1999).

Standardization

The spatial distributions of *Chromopleustes oculatus* analyzed in images taken at various densities and in the presence of predators in the laboratory were determined using

amphipods pre-selected for average body sizes of 0.39 cm to control for biological effects with various cohorts. For all density experiments, a ruler was placed in the center of each testing pool and photographed for scale after each image of the center was taken at 2 and 24 hours.

Nearest Neighbor Calculations

All images analyzed were captured *in situ* with a SONY DCR-PC 100 digital video camera then downloaded from videotape using iMovie on a Macintosh G3. The images were used to determine nearest neighbor distances using the T-square method (Ludwig and Reynolds 1988, Krebs 1989). The T-square technique evaluated the distribution of distances of amphipods from randomly selected points compared to distances between each amphipod and its nearest amphipod neighbor. Specifically, the coordinates of a point were chosen at random and the distance from the point to the nearest amphipod was recorded. The distance from that amphipod to its nearest amphipod neighbor was considered the nearest neighbor distance (NND). This process was repeated for all amphipods for each of the randomly selected points.

Random coordinates (X_1, Y_1) were generated in Microsoft Excel using a random number formula and did not exceed the size of each image (length = 8.8 pixels, width = 6.6 pixels). A random number was produced from 0 to 8.8 pixels for X_1 . An additional random number was created between 0 and 6.6 pixels for Y_1 . For each image, a maximum of 30 newly generated random numbers was created (X_1, Y_1) and plotted on a grid in Adobe Photoshop 5.5. From each random X_1, Y_1 coordinate I determined the

closest *Chromopleustes oculatus* and its coordinates (X_2, Y_2). From (X_2, Y_2) the nearest neighbor coordinates (X_3, Y_3) were determined using the T-square technique. Then nearest neighbor distances (pixels) were calculated using the equation:

$$\text{Distance} = \sqrt{(x_2 - x_3)^2 + (y_2 - y_3)^2}$$

For each image, I used a maximum of 30 random numbers. In cases where I found fewer than 30 amphipods on an image, the total of random numbers equaled the total number of individuals visible (n).

Determination of nearest neighbor distances was complicated by the fact that *C. oculatus* appeared to mate (and possibly cannibalize) during my sampling season. Therefore, all nearest neighbor distances less than 0.5 pixels were eliminated from the calculations (Table 1).

Distances in pixels were converted to centimeters using images of a ruler taken *in situ* for field observations and laboratory manipulations, respectively. For field observations, a ruler was glued to an underwater slate to ensure all images were in focus. The number of the blade was recorded on the slate below the ruler and was photographed using the underwater video camera prior to photographing the base, center, and tip of the preset algal blades. One cm equaled 0.889 pixels for field observations of *Agarum fimbriatum*. For lab experiments, a ruler was photographed in the center of the testing pools once images had been taken of the five locations (north, south, east, west, and center). One cm equaled 0.931 pixels for laboratory experiments.

Table 1. Percentage of nearest neighbor distances < 0.5 pixels (< 0.56 cm for field observations and < 0.22 cm for laboratory manipulations) that were eliminated from nearest neighbor distance calculations due to possible mating or cannibalism for *Chromopleustes oculatus*.

FIELD OBSERVATIONS

AGARUM	% Mating
Base	21.1
Center	17.3
Tip	13.2
Hi Slack	20.0
Low Slack	17.3

LAB MANIPULATIONS

DENSITY	% Mating
500	11.9
1000	19.4
2000	42.9
5000	23.8

2hr vs 24 hr	% Mating
500 2 hour	14.9
24 hour	6.5
1000 2 hour	14.6
24 hour	21.9
2000 2 hour	36.2
24 hour	57.5
5000 2 hour	23.8
24 hour	23.7

PREDATOR	% Mating
with predator	7.5
without predator	11.9

Nearest neighbor distances (cm) were later expressed as body length measurements (BL) for all graphs with respect to the field and laboratory results. I chose body lengths because the anterior and posterior ends of amphipods were visibly clear in all images, and average body sizes were relatively consistent for comparisons. Body widths were not used because amphipods are laterally compressed, making width difficult to measure.

Field Observations

A field survey was conducted on the mobility of *Chromopleustes oculatus* on *Agarum fimbriatum* to determine if *C. oculatus* remained stationary during tidal cycles on fixed blades. Blades were selected at random prior to beginning the field experiments and counted for abundance from 13 June to 25 June 2000. A total of 12 blades were selected along a 15-m transect at the northwest corner of Bell Island in water 8.0 m to 15.0 m deep. Divers on SCUBA counted whole algal blades for the number of *C. oculatus* present on the dorsal and ventral side of the blade. Each blade was counted a minimum of five times.

Field observations were made on the distribution of *C. oculatus*, the dominant amphipod at Bell Island (Figure 5). Observations were made from 5 July to 23 July 2000 using blades of *Agarum fimbriatum*, a laminarian kelp, as habitat. Blades were selected at random from a population on the northeast corner of Bell Island in water 11.7 m to 15.0 m deep along a 13-m transect. All observations were made using self-contained underwater breathing apparatus (SCUBA). A total of 17 selected algal blades were

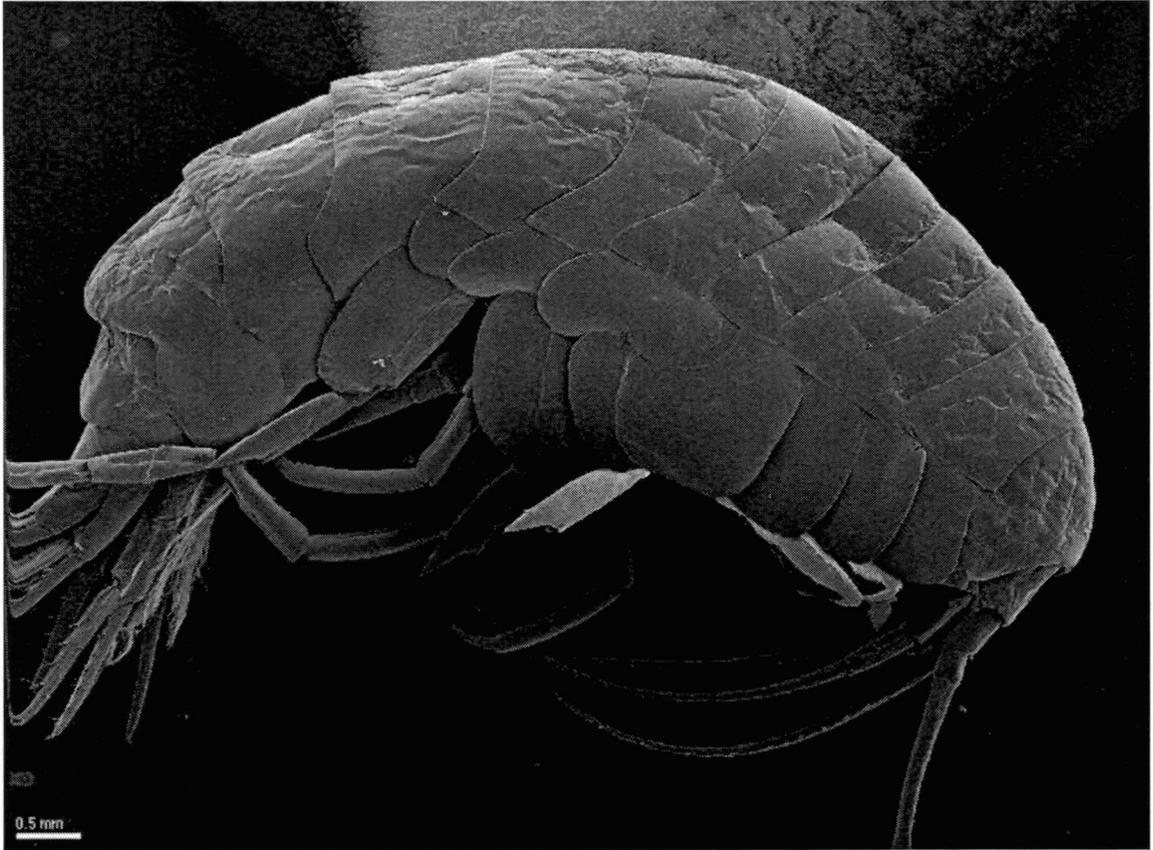
marked by attaching brightly colored flagging tape to the holdfast. Inter-individual spacing was recorded *in situ* using a SONY DCR-PC 100 digital video camera in a Light and Motion underwater housing. A small frame was attached to the camera housing to ensure that images were standardized to a distance of 28 cm from the blade. Images were taken during the day at high and low slack tides from three separate locations on each *A. fimbriatum* blade: base, center, and tip. Each blade was observed at least five times over the 18-day period, but it was not possible to census all 17 algal blades during each dive due to time limitations for slack water intervals. Additionally, five blades were lost over the course of observations.

Digital images were downloaded from the videotape and processed according to the nearest neighbor calculations. The effects of tidal state and location on algal blades on inter-individual amphipod nearest neighbor distances (\pm SE) were evaluated by a univariate analysis of variance (ANOVA). For all one-way ANOVAs, treatment means were analyzed with an experiment-wise error rate of 0.05 using a data analysis feature in Microsoft Excel 98. The interactions between tidal state and position on the blade were studied using Two-way ANOVA. For two-way ANOVAs, Data Desk 6.0 was used for both analysis and post-hoc comparisons.



Figure 5. Photo of *Chromopleustes oculatus* using scanning electron microscope (SEM).

Courtesy of Tim Charles.



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Laboratory Experiments

The impacts of amphipod density, presence of predators, and habitat availability on the distribution of *Chromopleustes oculatus* were tested in laboratory experiments. Intact fronds of *Agarum fimbriatum* containing large numbers of *C. oculatus* were collected from the southern area of Bell Island using SCUBA. Clear plastic bags (70 cm x 95 cm) were carefully placed over the tip and pulled to the base, where the holdfast was removed from the rock and inserted into the bag. Collection bags were tied underwater and taken to the surface to be transported to the laboratory for immediate separation. The contents of each bag, including the seawater, were placed into a large tray (1 m² x 0.3 m deep) where *C. oculatus* individuals were caught using a turkey-basting bulb as a pipette. Individuals were sorted by body sizes (mean body sizes used in the laboratory experiments were approximately 0.39 cm).

All experiments were conducted outdoors in a 20-m² arena containing static seawater 0.25 m deep. A large plastic hood was constructed over the main area to block direct sunlight and to prevent algal and diatom growth. A maximum of five round 0.664-m² x 23-cm deep plastic pools were placed in the arena to maintain ambient temperatures of 9-12° C for each pool; the pools will be referred to as testing pools. Each testing pool was filled with a homogenous sand substrate taken from a nearby hardware store. It was not known if *C. oculatus* had preferential sites on *A. fimbriatum*; therefore, this sand substrate was used as a control in the testing pools to test for changes in nearest neighbor distances with respect to density and the presence of predators without complications of habitat choices. Additionally, *A. fimbriatum* was not used in experiments because of the

rapid decomposition of the algal blade when removed from the study site and its possible negative effects on *C. oculatus* in static waters. Between trials (after 24 hours), both the sand and testing pools were rinsed with freshwater and the exposed pool edges were scrubbed with a nylon brush. In addition to cleaning the testing pools between trials, *C. oculatus* were moved to a single holding pool and fed brine shrimp pellets for a period of 24 hours to again be retested in subsequent trials.

Spatial distributions and patterns of aggregates. Amphipod densities were manipulated to determine changes in nearest neighbor spacing. The testing pools with homogeneous sand substrate were filled with seawater in a static system. Four density treatments were chosen to depict a higher and lower range of densities most commonly observed in the field on various habitats: 500, 1000, 2000, and 5000 individuals per pool, for densities of 753 individuals·m⁻² to 7,530 individuals·m⁻². Testing pools were not saturated with amphipods. In order to completely fill a testing pool to saturation, approximately 43,681 amphipods would need to be added. To fill the testing pool to the highest density recorded at Bell Island, approximately 16,268 individuals would need to be added to the testing pool. Numbers of this magnitude were not replicated in testing pools due to the ecological impacts of removing large quantities of the population at Bell Island, since amphipods were not released after each density experiment. Amphipods remained in holding pools and were fed brine shrimp to be used in subsequent experiments. The maximum number of amphipods kept in a holding pool at any time was approximately 6,000 individuals. Individuals were removed from the holding pools using a turkey-basting bulb and the appropriate numbers of individuals were placed into

testing pools. Trials in the testing pools lasted 24 hours. The testing pools remained in the larger holding arena to maintain ambient temperatures of 9-12° C. Replicates did not all occur on the same day; the four density treatments were conducted during a period of several weeks (14 June to 11 July 2000) and were conducted at random times during the day, in some cases to allow for time to dive at the field site.

Images were recorded on digital videotape at 2-hour and 24-hour intervals after initial introductions of the amphipods to each pool. At each time interval, five images were taken at non-overlapping locations in the pools: the four compass directions (north, south, east, west) and the center. There was a minimum of four trials per density treatment. A trial was defined as two images taken within a 24-hour period (n = 21 total trials: 5 trials at 500, 8 trials at 1000, 4 trials at 2000, and 4 trials at 5000).

Nearest neighbor distances were calculated using the method outlined previously. Patterns in the arrangement of amphipods in testing pools were assessed using the plotless technique outlined in Holgate (1965) and Brower et al. 1998. A single univariate ANOVA was used to test changes in average nearest neighbor distances (\pm SE) in density. An additional univariate ANOVA examined the density distributions over time. Results from the ANOVAs were compared using an error rate of 0.05.

Predators and spatial distributions. The testing pools with sand substrate were again placed in the larger holding arena to maintain ambient temperatures. This time, testing pools contained 500 amphipods, taken from holding pools, and one *Oligocottus maculosus*, a naïve predatory fish captured from a nearby tide pool. *O. maculosus* is a predatory sculpin that frequently feeds on palatable amphipods and had not been

previously exposed to the chemically defended amphipod species, *Chrompleustes oculatus*. Prior to adding the predator, amphipods were given 15 minutes in each pool to establish equilibrium. After 15 minutes, the single *O. maculosus* was introduced and inter-individual distributions were again recorded on digital videotape. Recording continued thereafter every 15 minutes for a total of six images per trial. At each recording, five images were taken at the four compass directions and center in non-overlapping locations. The experiment was repeated 13 times (n=390 images: 13 trials x 5 locations x 6 images per trial). The density treatment experiment using 500 individuals per testing pool was considered the control in the absence of predators. The methods for the control are outlined previously for testing pools in *Spatial Distributions and Patterns of Aggregates*.

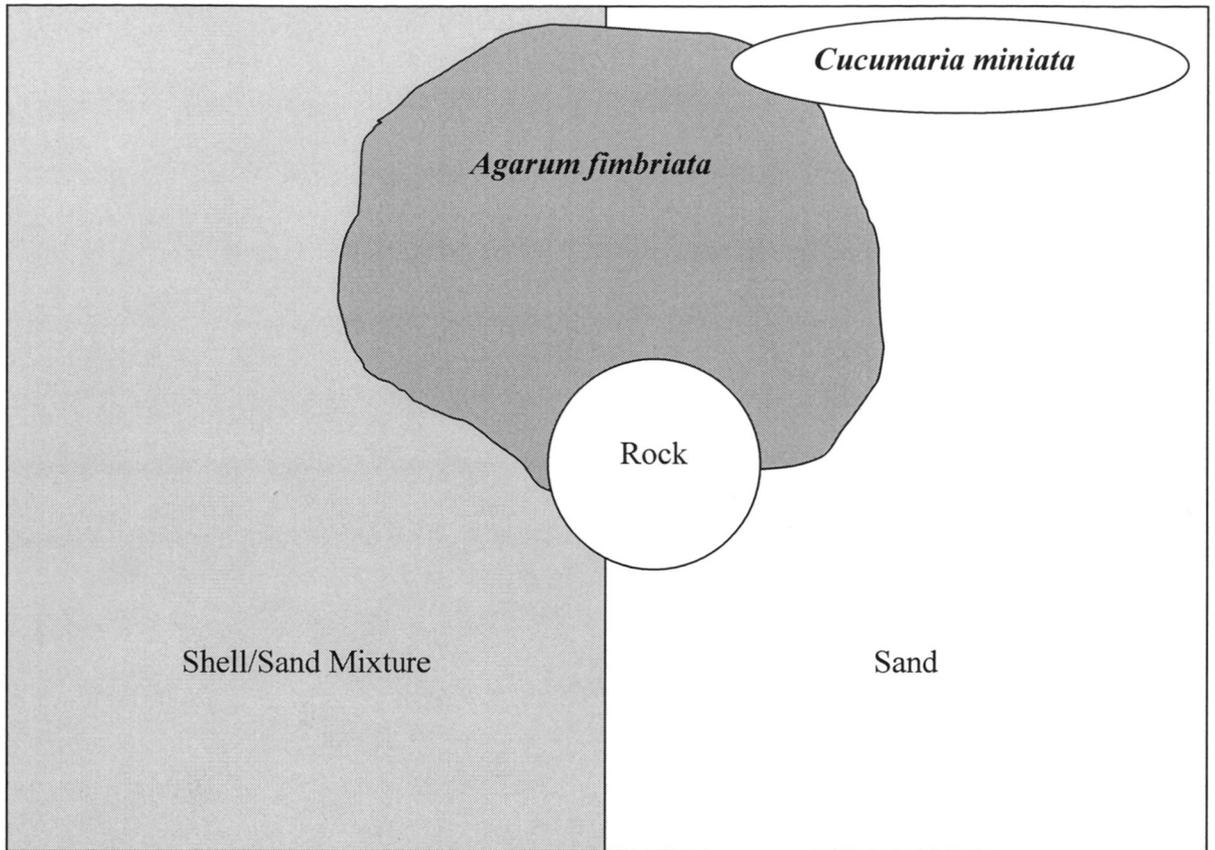
Nearest neighbor distances of amphipods in the presence of predators were calculated using the method outlined previously. Then, nearest neighbor distances were compared with the images taken from the testing pools containing 500 individuals $\cdot 0.664 \text{ m}^{-2}$ (n = 50 images) and the images of pools containing the predator (n = 390 images). A single univariate ANOVA was used to test differences among average nearest neighbor distances (\pm SE) in the presence or absence of a predator. Results from the ANOVA were analyzed using an error rate of 0.05.

Habitat choice experiments. I examined habitat choice by introducing *Chrompleustes oculatus* into two different sized aquaria (Aquarium A: 0.119 m^2 x 25 cm deep, Aquarium B: 0.139 m^2 x 28 cm deep), each contained the dominant field habitats: sea cucumber (*Cucumaria miniata*), *Agarum fimbriatum*, sand and shell

mixture, and rock (Figure 6). Habitats were collected from Bell Island prior to the beginning of the experiment and placed in flow-through sea water tables at ambient temperatures of 9 - 12° C. Before initiating each test the aquaria were cleaned with a nylon brush, and the sand and sand-shell mixtures were rinsed with seawater and placed so that each covered 50% of the bottom. I then added to each aquarium a fresh piece of algae, a fresh-collected *C. miniata*, and a rock rinsed with seawater.

Approximately 50% of the bottom of each aquarium was covered with sand (used in the previous laboratory experiments) and the other 50% was a natural sand and shell mixture from Bell Island. The rock and the piece of algae were then placed in the center of the tank while the *C. miniata* was positioned in a corner. Areas for each habitat were measured two-dimensionally in NIH-Image 1.63. All aquaria were set up with once-through flows of seawater. Habitat choices of 50 amphipods were monitored at 2, 6, and 24 hours after introduction. The experiment was repeated four times (n = 21 images: 4 trials x 3 time intervals x 2 aquaria). Three images were lost due error (image was too dark or was not taken). The expected amphipod values were calculated by multiplying the percentage of the total area occupied by each of the five habitats with the 50 amphipods placed in each aquarium (number of expected values, n = 105; 21 images x 5 habitats). Choices were compared for the number of amphipods found on each habitat using a Chi-Square analysis in Microsoft Excel 98.

Figure 6. Diagram depicting an example of habitat positioning used in Aquarium A and B in habitat choice experiments.



RESULTS

Standardization

Ninety-two percent of images in testing pools containing 500 individuals had body lengths within 0.1 pixels of the average body size used for laboratory experiments. Average body length per image (based on 173 individuals) ranged from 0.160 pixels to 1.566 pixels (Figure 7). The mean body length for the 36 images was 0.313 ± 0.04 pixels (\pm values are standard error). The average body length of individuals selected for testing pool experiments was 0.39 cm, which is equivalent to body lengths of 0.363 pixels (1 cm = 0.931 pixels for laboratory calculations).

Field Observations

Results from the field survey, conducted prior to the field observations, suggested *Chromopleustes oculatus* were highly mobile on individual blades. The mean number of amphipods on any blade ranged from approximately 14 ± 2.7 individuals to 358 ± 23.4 individuals (Table 2).

The average body length for adults and juveniles in the field, determined from six individuals in three random pictures, was approximately 0.46 ± 0.03 cm (Figure 8). Populations of similar sizes of *Chromopleustes oculatus* (referred to hereafter as cohorts) varied in body sizes throughout the summer at Bell Island. Late May cohorts were

Figure 7. Distribution of mean body lengths for 36 images in testing pools containing 500 individuals. Distribution is shown according to number of observations for each body length.

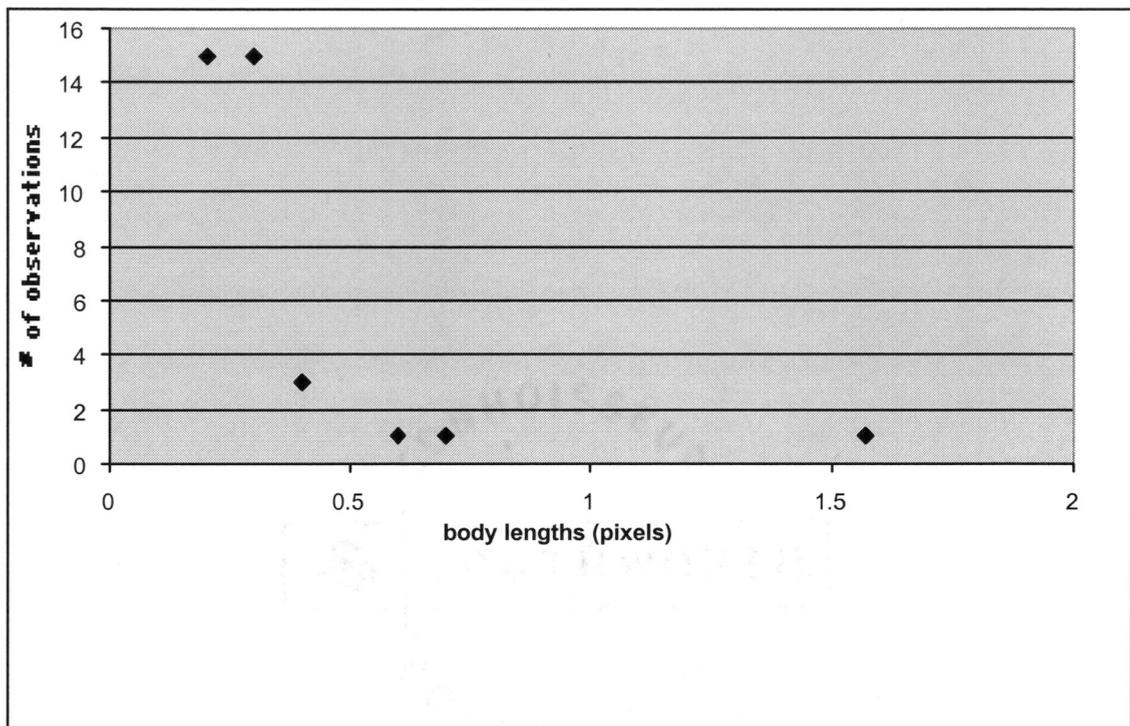
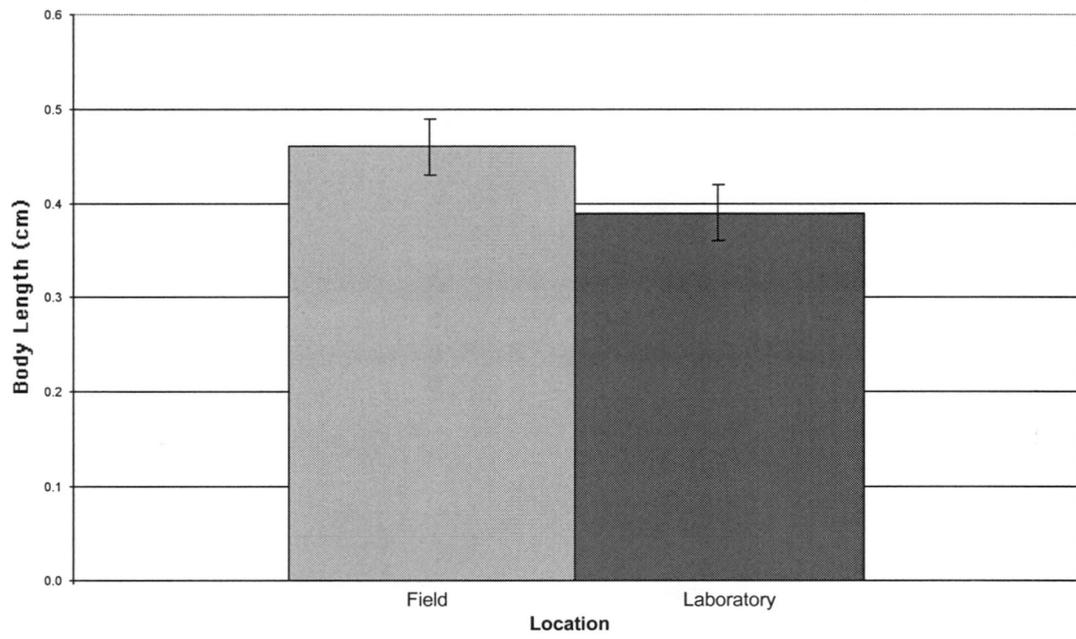


Table 2. Amphipod variability, expressed as Mean \pm SE, from 12 algal blades. Counts were taken as a field survey *in situ* at Bell Island 13 June 2000 through 25 June 2000 during high and low slack tide prior to beginning field observations. Blade numbers represent the assigned number given to each blade in the field. n = # of observations per blade

Blade	<i>n</i>	Mean \pm SE
12	7	358.4 \pm 23.4
11	5	215.8 \pm 22.4
9	7	292.4 \pm 17.4
s5	5	38.0 \pm 10.8
s4	5	115.4 \pm 6.1
7	5	169.6 \pm 19.0
6	7	13.9 \pm 2.7
10	6	282.7 \pm 18.1
s2	5	83.4 \pm 7.0
s1	5	34.2 \pm 3.5
5	6	86.0 \pm 5.9
8	8	220.8 \pm 28.6

Figure 8. Comparison of mean body lengths of *Chromopleustes oculatus* observed in the field and those that were selected according to body size in the laboratory experiments (n = 18 individuals; 6 individuals per image x 3 images).



approximately twice as large as cohorts selected in mid-June. By mid-June average body sizes seen in the field were proportional to those used in the laboratory observations. All individuals were clearly visible in the pictures; absolute underwater visibility ranged from limited (~ 1 m) to moderate (~ 4 m) at slack tide. Best visibility occurred at high slack tide. Amphipods remained in place at slack tide unless touched by another organism (e.g., amphipod, cohabitant fish, etc.) or a diver disturbed the blade.

The mean abundance of amphipods found on the base of the 17 *Agarum fimbriatum* blades was 20 ± 2.91 SE (standard error) at high slack tide and 20 ± 4.07 individuals at low slack tide (Figure 9). This mean abundance was equal to a mean density of 3,076 amphipods per m^2 for the base of the blade. Mean abundance at the center of the blade was 13 ± 1.77 individuals at high slack tide and 12 ± 1.63 individuals at low slack tide, for a mean density of 1,923 amphipods per m^2 . Mean amphipod abundance at the tip of the blade was 8 ± 2.23 at high slack tide and 7 ± 1.86 at low slack tide, for a mean density of 1,153 amphipods per m^2 .

Amphipod abundance was influenced by position on the blade but not by tidal state. A 2-way ANOVA showed abundances did not vary as a function of tide state (Table 3, $p = 0.7$; Figure 9). The interaction between position and tide state also was non-significant (Table 3, $p = 1$, Figure 9). Further analysis using a Bonferroni Post Hoc

Figure 9. Mean number (\pm SE) of *Chromopleustes oculatus* for images taken in the field on *Agarum fimbriatum* at three locations -- base, center, and tip -- at high and low slack tides. n = number of images.

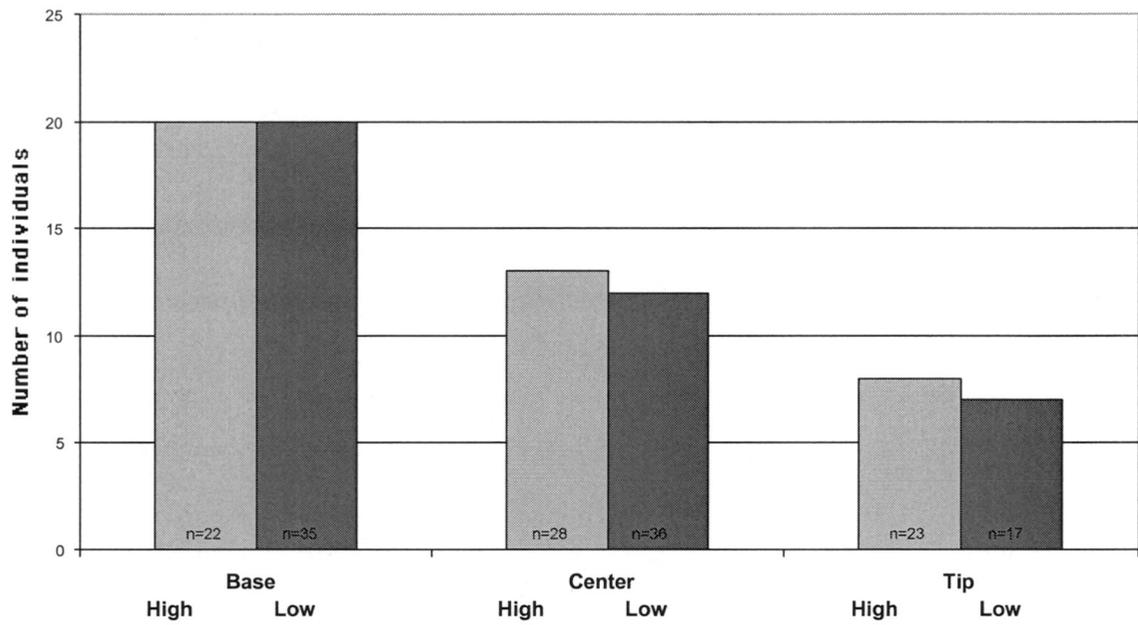


Table 3. ANOVA of the differences in mean number of individuals at three positions on *Agarum fimbriatum* using images taken at high and low slack tides of natural populations of *Chromoleustes oculatus* at Bell Island. n = number of images.

Source	df	n	MS	F	P
Blade Position (Base / Center / Tip)	2	161	2117.8000	8.99	0.0002
Tide (High Slack / Low Slack)	1	161	34.0864	0.14	0.7041
Interaction (Tide / Blade Position)	2	161	1.2507	0.01	0.9947

test indicated densities observed at the base were significantly different from the center ($p = 0.01$) and tip ($p = 0.0002$; Table 4). The center and tip did not show any statistical differences ($p = 0.3$).

Mean nearest neighbor distances (MNND) in graphs were expressed as distance in body lengths, calculated for the average body length seen in the field ($0.46 \text{ cm} = 1$ body length). The mean nearest neighbor distance between amphipods on kelp blades ranged between 3.24 and 4.63 body lengths (BL). Excluding blade positions, the MNND at high slack tide and low slack tide was 3.56 ± 0.08 BL and 3.71 ± 0.08 BL, respectively (Figure 10). Excluding tidal state, the MNND at the base was 3.41 ± 0.07 BL, at the center was 3.66 ± 0.09 BL, and the tip was 4.46 ± 0.18 BL (Figure 11). The MNND found on the base of the *A. fimbriatum* blade was 3.24 ± 0.09 BL at high slack tide and 3.54 ± 0.12 BL at low slack tide (all \pm values are standard errors; Figure 12). The MNND at the center of the blade at high slack tide was 3.73 ± 0.14 BL and MNND at low slack tide was 3.59 ± 0.11 BL. The MNND at the tip of the blade was 4.29 ± 0.21 BL at high slack tide and 4.63 ± 0.29 BL at low slack tide.

A 2-way ANOVA showed MNND did not vary as a function of tidal state (Table 5, $p = 0.7$; Figure 10), but MNND shifted as a function of position on the blade (Table 5, $p \leq 0.0001$; Figure 11). The interaction between blade position and tide state also was

Table 4. Bonferroni Post Hoc test comparing the mean abundances of *Chromopleustes oculatus* at three locations on *Agarum fimbriatum*. n = number of images.

Comparison	n	Difference	Std. Error	P
Base - Center	121	-8.2954	2.845	0.0122
Base - Tip	97	-13.1961	3.222	0.0002
Center - Tip	104	-4.9007	3.124	0.3157

Figure 10. Nearest neighbor distances (NND) expressed as mean body lengths (\pm SE) of *Chromopleustes oculatus* on *Agarum fimbriatum* at high and low slack tides. Body lengths <1.22 (0.56 centimeters) were removed from the calculations. n = number of NND.

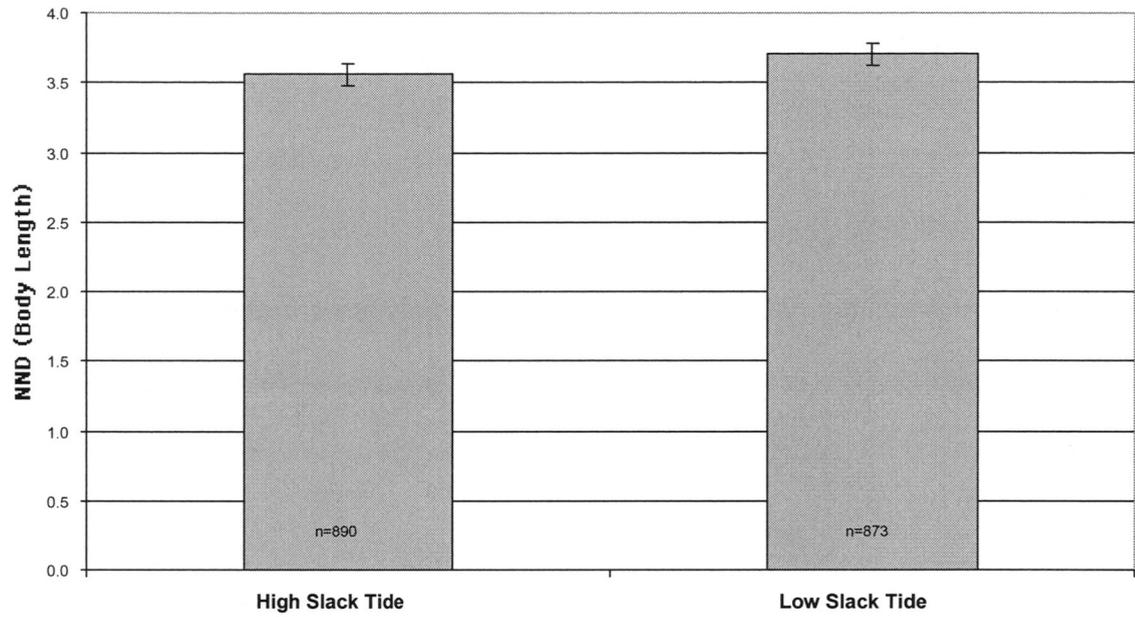


Figure 11. Nearest neighbor distances (NND) expressed as mean body lengths (\pm SE) of *Chromopleustes oculatus* on *Agarum fimbriatum* at three locations -- base, center, and tip -- independent of tidal cycle. Body lengths <1.22 (0.56 centimeters) were removed from the calculations. n = number of NND.

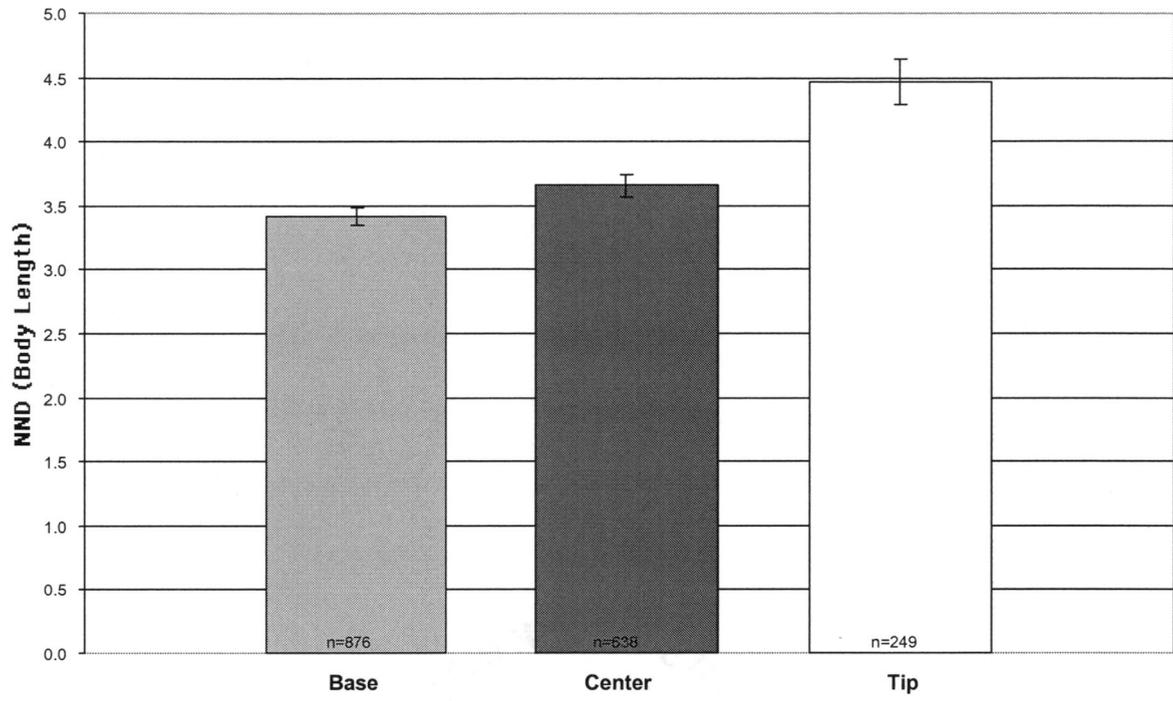
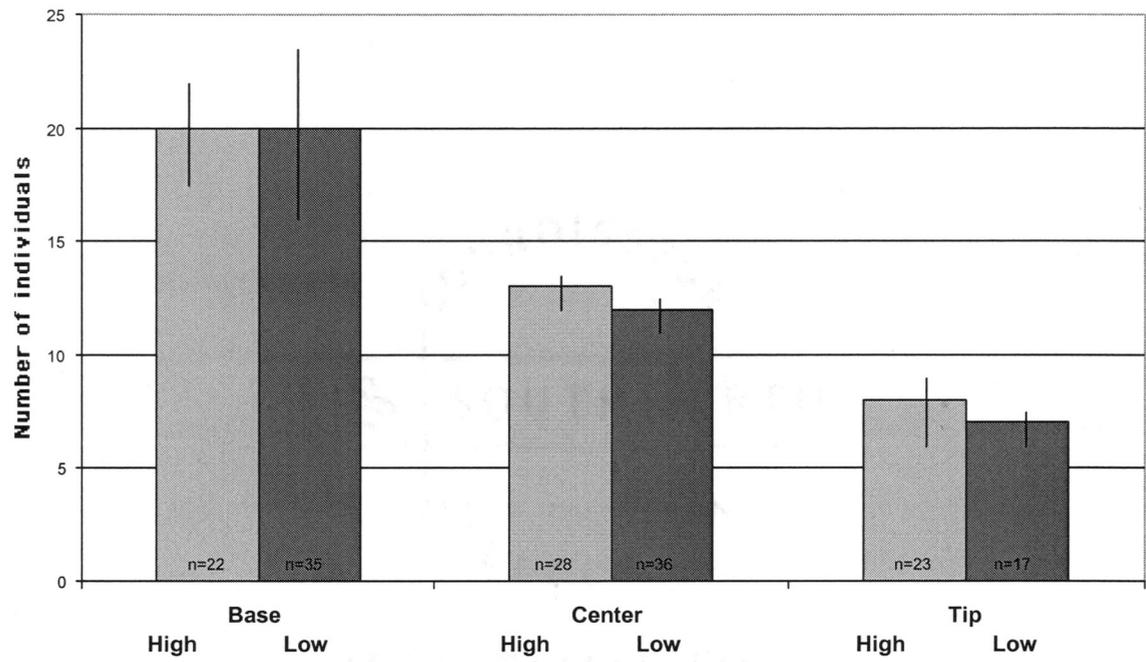


Figure 12. Nearest neighbor distances expressed as mean body lengths (\pm SE) of *Chromopleustes oculatus* on *Agarum fimbriatum* at three locations -- base, center, and tip -- at high and low slack tides. Body lengths <1.22 (0.56 centimeters) were removed from the calculations. n = number of NND.



significant (Table 5, $p = 0.0009$; Figure 12). In further analysis using a Bonferroni Post Hoc, I found the following specific interactions to be significant; interactions between the base and tip of the blade during tidal state (high and low, and high versus low, respectively), interactions between tidal state at the given position, center ($p = 0.04$), and interactions between the position, center and tip, of the *Agarum* blade at high and low slack tides, in addition to low slack tide (Table 6). There was no interaction between the center and tip at high slack tide ($p = 0.3$). Additional Bonferroni comparisons specifically for blade position showed the tip was statistically different from the center (Table 6, $p \leq 0.001$) and also from the base (Table 6, $p \leq 0.001$), but the base and center were not statistically different ($p = 0.9$).

Laboratory Experiments

The MNND values for the laboratory experiments were much lower than the normal avoidance minimum of 1 cm established from previous experiments (Norton and Stallings 1999) because of mating and cannibalism attempts. Amphipods appeared to be attempting to mate in testing pools. One amphipod, presumably the male, would position itself perpendicular to the female, “grabbing” her with his claws. The male would palpate the female by brushing its pleopods rapidly against the female’s body. Additional observations relevant to the following laboratory results were that approximately 10-15

Table 5. ANOVA of the differences in mean nearest neighbor distances at three positions on *Agarum fimbriatum* using images taken at high and low slack tides of natural populations of *Chromopleustes oculatus* at Bell Island. n = number of nearest neighbor distances.

Source	df	n	MS	F	P
Blade Position (Base / Center / Tip)	2	1763	19.9452	22.08	≤0.0001
Tide (High Slack / Low Slack)	1	1763	0.1480	0.16	0.6858
Interaction (Tide / Blade Position)	2	1763	6.3726	7.05	0.0009

Table 6. Bonferroni Post Hoc interaction results for mean nearest neighbor distances that shifted as a function of tidal state (High / Low Slack tide) at a given blade position (Base / Center / Tip) for *Chromopleustes oculatus*.

Comparison 1	Comparison2	Difference	Std.Error	P
High, Center	High, Base	0.2011	0.0712	0.069545
High, Tip	High, Base	0.4270	0.0918	0.000053 ***
High, Tip	High, Center	0.2259	0.0978	0.272335
Low, Base	High, Base	0.1215	0.0643	0.598938
Low, Base	High, Center	-0.0797	0.0726	0.991537
Low, Base	High, Tip	-0.3056	0.0928	0.015135 *
Low, Center	High, Base	-0.0164	0.0650	1.00000
Low, Center	High, Center	-0.2175	0.0732	0.044278 *
Low, Center	High, Tip	-0.4434	0.0933	0.000033 ***
Low, Center	Low, Base	-0.1378	0.0665	0.444284
Low, Tip	High, Base	0.5861	0.1013	0.000000 ***
Low, Tip	High, Center	0.3850	0.1067	0.004768 *
Low, Tip	High, Tip	0.1591	0.1214	0.957833
Low, Tip	Low, Base	0.4647	0.1023	0.000088 ***
Low, Tip	Low, Center	0.6025	0.1027	0.000000 ***

amphipods in each testing pool would die before the 24 hours of testing ended. In testing pools only, I noted several cases of cannibalism where amphipods would attack a single dead or dying individual. At later observation times only the exoskeleton would remain of the injured individual. Therefore, the NNDs < 0.5 pixels (< 1.22 body lengths (0.56 cm) for field observations and < 1.37 body lengths (0.54 cm) for laboratory observations) were not used in the comparisons of measured MNND (Table 1). Any distance less than the minimum 0.5 pixels was regarded as reproductive efforts or cannibalism and consequently was removed from calculations. Additionally it was noted that during July, small juveniles (~ 0.2 cm) could be found in testing pools with adults. These pools had not been replenished with amphipods from the field for several weeks.

Spatial distributions and patterns of aggregates. The average body length amphipods used in the lab, determined from six individuals in three random pictures, was approximately 0.39 ± 0.03 cm (Figure 8). Mean nearest neighbor distances (MNND) in graphs were expressed as distance in body lengths (BL), calculated for the average body length used in the lab (0.39 cm = 1 body length). The mean nearest neighbor distances (MNND) were inversely related to density of amphipods in the testing pools. The MNND in testing pools containing 500 individuals (for a density of 753 individuals \bullet m⁻²) was 5.98 ± 0.2 BL (all \pm values are standard errors), and ranged from 6.42 ± 0.3 BL at two hours after introduction to 5.25 ± 0.3 BL 24 hours after introduction. The MNND in testing pools containing 1,000 individuals (for a density of $1,506$ individuals \bullet m⁻²) was 4.7 ± 0.1 BL, and ranged from 5.34 ± 0.3 BL to 4.36 ± 0.1 BL two and 24 hours after introduction, respectively. The MNND in pools containing 2,000 individuals (for a

density of 3,012 individuals \cdot m⁻²) was 3.7 ± 0.1 BL or 3,012 individuals per m², and ranged from 3.65 ± 0.1 BL to 3.84 ± 0.2 BL at two and 24 hours, respectively. The MNND in testing pools containing 5,000 individuals (for a density of 7,530 individuals \cdot m⁻²) was 4.70 ± 0.1 BL or 7,530 individuals per m², and ranged from 4.50 ± 0.2 BL to 4.92 ± 0.2 BL at two and 24 hours, respectively.

These differences in mean nearest neighbor distance as a function of density were highly significant (Table 7, $p \leq 0.0001$; Figure 13). Comparisons of MNND at two and 24 hours after introduction were also significant (Table 7, $p = 0.0237$; Figure 14). The Bonferroni Post Hoc showed all but one test for the MNND were significantly different for the four densities; the comparison made between the MNND in testing pools containing 1,000 and 5,000 individuals was not significant (Table 8, $p = 0.947$). Bonferroni Post Hoc results demonstrated all but one comparison (contrasts between MNND at 2,000 and 5,000 individuals \cdot 0.664 m⁻²) showed statistical differences when MNND were compared using 2-hour observation times as a constant for the four densities (Table 9). There were no statistical differences in MNND comparisons when the observation time, 24 hours, was used as constant. In comparisons where no constant was used, 2 hours versus 24 hours and vice versa, significant differences were only found for MNND at 500 individuals \cdot 0.664 m⁻² and 2,000 individuals \cdot 0.664 m⁻² (Table 9).

Table 7. ANOVA of the relative change in mean nearest neighbor distances of *Chromopleustes oculatus* at 2 and 24 hours in experiments manipulating four densities (individuals per 0.664m²).

Source	df	n	MS	F	P
Time (2 vs 24)	1	2003	4.15977	5.1250	0.0237
Density (500, 1000, 2000, 5000)	3	2003	15.0444	18.535	≤0.0001
Interaction (Time / Density)	3	2003	7.74631	9.5437	≤0.0001

Table 8. Bonferroni Post Hoc comparisons of mean nearest neighbor distances analyzed from images of *Chromopleustes oculatus* taken at five preset locations in testing pools for four densities, respectively (500, 1000, 2000, and 5000 individuals 0.664m²).

Comparison	Difference	Std.Error	P
1000 - 500	-0.295241	0.0661	0.000050 ***
2000 - 500	-0.544271	0.0735	0.000000 ***
2000 - 1000	-0.249031	0.0575	0.000094 ***
5000 - 500	-0.337438	0.0669	0.000003 ***
5000 - 1000	-0.042197	0.0488	0.947290
5000 - 2000	0.206833	0.0585	0.002501 *

Figure 13. Nearest neighbor distances (NND) expressed as mean body lengths (\pm SE) of *Chromopleustes oculatus* at four densities (individuals/0.664m²). Body lengths <1.37 (0.54 centimeters) were removed from the calculations. n= number of NND.

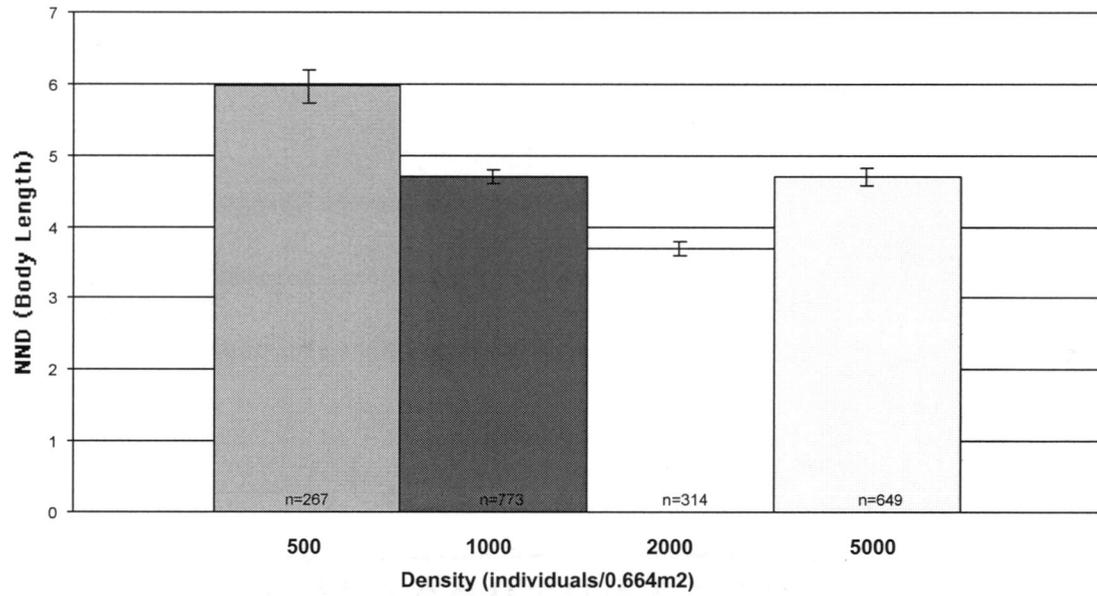
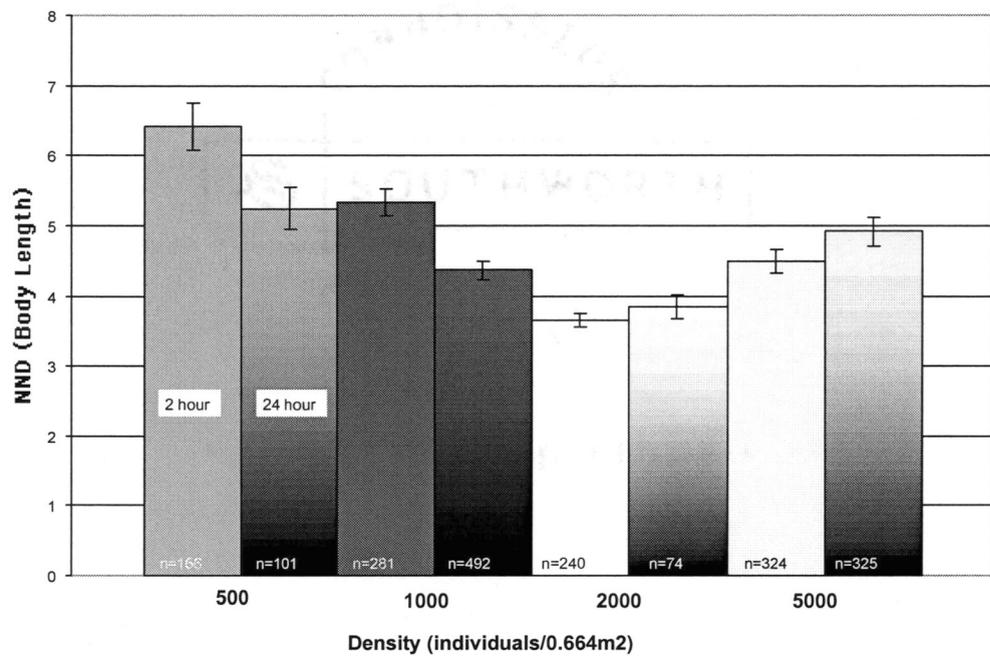


Figure 14. Nearest neighbor distances (NND) expressed as mean body lengths (\pm SE) of *Chromopleustes oculatus* at 2 time intervals--2 hours (unshaded) and 24 hours (shaded)--for 4 densities. Body lengths <1.37 (0.54 centimeters) were removed from the calculations. n= number of NND.



The patterns of the aggregates in testing pools were significantly different than random ($p = 0.05$), with the exception of 2000 individuals·0.664 m⁻². The plotless index of aggregation by Holgate (1965) showed patterns of aggregates in testing pools containing 500, 1000, and 5000 individuals·0.664 m⁻² were contagious, or clumped.

Predators and spatial distributions. *Chrompleustes oculatus* were not visibly disturbed by *Oligocottus maculosus* unless touched. In many cases *C. oculatus* would attach to the fish's tail, and occasionally to its body. This caused the fish to swim violently in efforts to remove the amphipod(s).

Mean nearest neighbor distances (MNND) in graphs were expressed as distance in body lengths (BL), calculated for the average body length used in the lab (0.39 cm = 1 body length). The MNND for testing pools that contained a single *O. maculosus* was 6.48 ± 0.1 BL (all \pm values are standard errors; Figure 15). The MNND for testing pools that did not contain a predator was used from the density experiment (MNND of 500 individuals = 5.98 BL \pm 0.2). A univariate ANOVA found the differences in MNND in the presence or absence of predators was significant ($p = 0.05$).

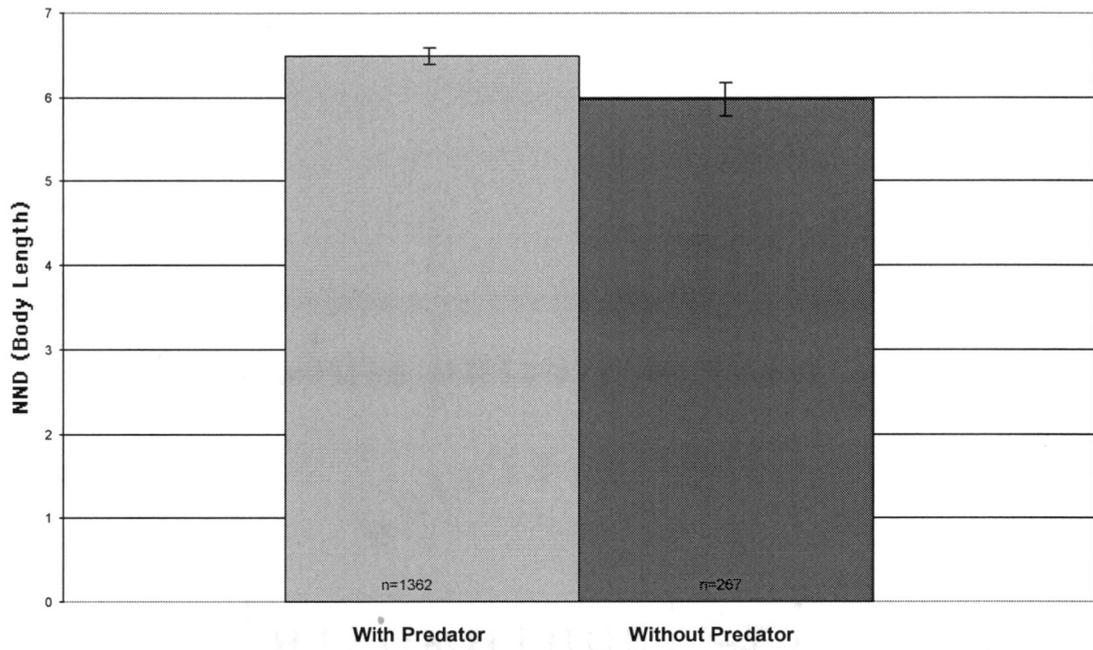
Table 9. Bonferroni Post Hoc interaction results for mean nearest neighbor distances that shifted as a function of density at a given time for *Chromopleustes oculatus* (comparisons are sorted by increasing probabilities).

Comparison 1	Comparison2	Difference	Std.Error	P
1000 - 24	500 - 2	-0.617366	0.0809	0.000000 ***
2000 - 2	500 - 2	-0.779830	0.0875	0 ***
2000 - 2	1000 - 2	-0.454916	0.0752	0.000000 ***
2000 - 24	500 - 2	-0.660510	0.1040	0.000000 ***
5000 - 2	500 - 2	-0.575988	0.0860	0.000000 ***
5000 - 24	500 - 2	-0.450685	0.0859	0.000005 ***
5000 - 24	2000 - 2	0.329144	0.0726	0.000170 **
1000 - 24	1000 - 2	-0.292452	0.0674	0.000415 **
2000 - 2	500 - 24	-0.428033	0.1040	0.001113 **
1000 - 2	500 - 2	-0.324913	0.0882	0.006567 *
2000 - 24	1000 - 2	-0.335597	0.0939	0.009985 *
5000 - 2	1000 - 2	-0.251075	0.0734	0.017796 *
500 - 24	500 - 2	-0.351797	0.1137	0.054476 *
5000 - 2	2000 - 2	0.203841	0.0726	0.132266
1000 - 24	500 - 24	-0.265568	0.0984	0.179089
2000 - 24	500 - 24	-0.308713	0.1182	0.224631
5000 - 24	1000 - 24	0.166680	0.0644	0.239112
2000 - 2	1000 - 24	-0.162464	0.0665	0.337893
5000 - 24	2000 - 24	0.209825	0.0918	0.468647

Table 9. Continued

Comparison 1	Comparison2	Difference	Std.Error	<i>P</i>
5000 - 2	500 - 24	-0.224191	0.1027	0.562627
5000 - 24	5000 - 2	0.125303	0.0707	0.892638
5000 - 24	1000 - 2	-0.125772	0.0734	0.921122
2000 - 24	2000 - 2	0.119319	0.0932	0.998120
5000 - 24	500 - 24	-0.098888	0.1026	0.999989
5000 - 2	2000 - 24	0.084522	0.0918	0.999996
1000 - 2	500 - 24	0.026884	0.1045	1
2000 - 24	1000 - 24	-0.043145	0.0870	1.00000
5000 - 2	1000 - 24	0.041377	0.0645	1.00000

Figure 15. Nearest neighbor distances (NND) expressed as mean body lengths (\pm SE) among 500 *Chromopleustes oculatus* in 0.664m² testing pools with and without predators (excluding all distances < 1.37 body lengths, or 0.54 centimeters for mating). n= number of NND.



Habitat choice experiments. Three images were lost due to error: one image was overexposed and one set of images at 24 hours was not taken of the two aquaria. There were a few cases where all amphipods were not accounted for in images (total \neq 50). This error resulted from individuals attaching to the glue holding the aquarium together. The seams of the aquarium were not accounted for in the two-dimensional technique to assess the percent of space each habitat occupied. In one image taken at 24 hours in Aquarim B, *C. miniata* was coiled into a corner and was not feeding. In this image, no amphipods were found on any of the provided substrates, but were attached to the glue of the aquarium.

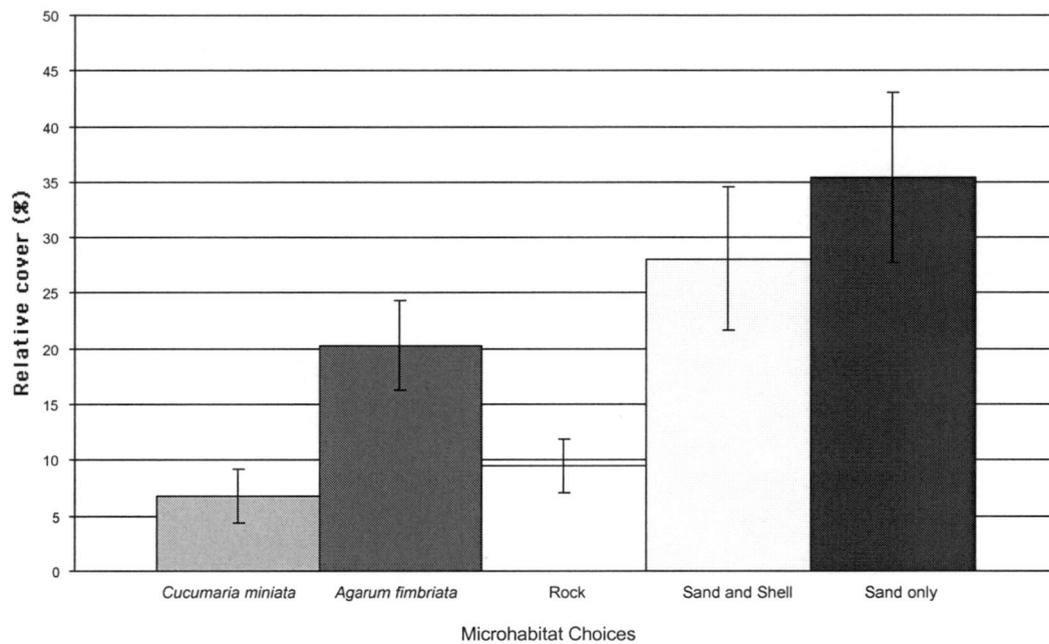
When presented a choice, the amphipods preferred cucumber habitat to kelp blades, sand, rock, or sand and shell mixtures. The average percentage of habitat that *Cucumaria miniata* occupied was $6.8 \pm 2.4\%$ (all \pm values are standard deviations) and the average abundance of amphipods found on *C. miniata* was 18 ± 9 individuals. *A. fimbriatum* represented $20.3 \pm 4.0\%$ of the available habitat, but the average abundance of amphipods found on the blade was only 2 ± 2 individuals. Rock was $9.5 \pm 2.4\%$ of the habitat, and average abundance of amphipods found on rock was only 1 ± 2 individuals. Sand and shell mixture represented $28.1 \pm 7.7\%$ of the habitat and sand represented $35.4 \pm 6.4\%$, yet average abundance on the sand and shell was only 5 ± 5 individuals and 0 ± 1 individual on the sand. A Chi-Square test showed the amphipod population ($n=50$) was not normally distributed on habitats ($X^2 = 2543$, $df = 20$, $p < 0.005$) (Table 10; Figure 16).

Table 10. Table of values used in Chi-Square analyses: two-dimensional percentage of cover for each microhabitat in aquaria, expected number of *Chromopleustes oculatus* with respect to habitat percentages, and observed numbers of individuals in aquaria. Expected and Observed are the average number of individuals for two aquaria taken from introductions of 50 amphipods. (n = 21 images)

Microhabitat	% Coverage	Expected	Observed
<i>C. Miniata</i>	7	3	18
Rock	9	5	1
Sand-Shell	28	14	5
<i>A. fimbriatum</i>	20	10	2
Sand only	35	18	0

Figure 16. Two-dimensional percentage of cover (\pm standard deviation) for each habitat.

n = 21, number of replicates.



DISCUSSION

Standardization

I concluded that no standard was required for processing images taken of amphipods in testing pools. Results indicated that inconsistent camera distances had little variability in average body lengths for images (0.313 pixels) compared with average body lengths selected for laboratory experiments (0.363 pixels; Figure 2). I assumed standardization would not be necessary for other testing pools that invariably had smaller errors in MNND (Figure 10).

Field Observations

In designing the original field experiments, I had assumed that *Chromopleustes oculatus* remained stationary during much of the tidal cycle. In an earlier research design, based on the assumption of little to no mobility, I proposed to introduce and deplete amphipods from existing swarms *in situ* to manipulate densities and monitor nearest neighbor distances in natural populations during a tidal cycle. Given the mobility of *C. oculatus*, the field work was limited to observations at low slack and high slack tide for individual *A. fimbriatum* blades (Table 2). The morphological constraints of the amphipods (apparent limited swimming capabilities and laterally compressed bodies) and swift currents at Wasp Passage likely contributed to prior observations that amphipods were immobile and best suited to reside in more complex habitats offering greater opportunity for good attachment and shelter (Pavia and Aberg 1998). Each blade of *Agarum fimbriatum* has many indentations and the effects of currents on the algal blade

create a whipping motion at the tip of the blade, much like a flag flapping on a windy day. The blades stream easily in the swift current, but remain motionless at slack water. Previous research of water flow effects on *Laminaria* in high-energy systems suggest flow is mostly turbulent around larger macroalgae, and turbulent boundary layers are formed more rapidly among single-bladed kelps (Hurd and Stevens 1997). The swimming speed is not known for *C. oculatus*, nor is the water velocity at which these amphipods can withstand simply by clinging to available substrates. Studies conducted on deep-sea gammarid species have observed smaller amphipods (2-3 cm in length) swimming vigorously at 5-10 cm•s⁻¹ into the current, only to maintain their position in the water column without forward advancement (Kaartvedt et al. 1994). Takeuchi and Watanabe (1998) also report that swimming speeds have a linear relationship with body weight, suggesting the swimming speed of *C. oculatus* (body lengths of ~ 0.4 cm) would be much less than 5-10 cm•s⁻¹.

My field survey on whole blade counts at slack tide suggested amphipods were mobile, either directly by activity or indirectly via currents, given abundance changes between successive observations. Observations of amphipods on whole blades of *A. fimbriatum* taken at various slack tide intervals had shown the number of individuals varied in abundance by as much as 81% on the marked algal blades. This study did not focus on times at which *C. oculatus* were active (slack tide versus tidal exchanges); therefore, it is not known if the amphipods were moving during tidal exchanges. There was no statistical analysis performed on the significance of the amphipod mobility (e.g., tide to tide comparisons) since it was only necessary to determine if individuals would

remain on blades for one tidal cycle to observe changes in their spatial distributions (Table 2).

Spatial distributions. H1: There is no difference in mean amphipod nearest neighbor distance on *Agarum fimbriatum* in the field as a function of amphipod location on the blade (base, center, and tip). Differences in MNND were found to occur among amphipod positions on *A. fimbriatum*. The MNND at the tip of blade was higher than the MNND at the center and base. The MNND at the base approached a minimum separation distance of 1 cm, a distance more than double an individual's body (body lengths of 0.46 cm observed in the field). These results are similar to other experiments conducted on several species of copepods that maintain minimum separation distances (Leising and Yen 1997).

H1: There is no difference in mean amphipod nearest neighbor distance on *A. fimbriatum* in the field as a function of amphipod location at tidal state (high and low slack tide). I failed to reject this null hypothesis. There were no shifts in distribution or abundance in images as a function of tidal state (high and low slack tide) with respect to images taken in the field. The absence of shifts in MNND also suggests that tidal state does not reinforce chemical defenses for *C. oculatus*.

Density. H4: There is no difference in mean amphipod density on *Agarum fimbriatum* in the field as a function of amphipod location on the blade (base, center, tip), tide state (high and low slack), or any interaction between the two variables. The changes in distances noted above were coupled with changes in density. As density increased, MNND decreased. Amphipods were concentrated at the base of the blade and

just as previous research on *Chromopleustes oculatus* has indicated (Norton and Stallings 1999), I found the group's members decreased inter-individual spacing in large populations, but still maintained a minimum separation distance of 1 cm. The base is nearest the holdfast and provides the least movement during tidal exchanges. Mean abundance decreased towards the tip, typically the most actively moving portion of the blade. The reduced abundances seen at the tip may also be influenced by slack tide. Slack tide at the site lasts only for approximately 45 minutes, which may not be enough time to enable amphipods to reposition themselves at the tip, despite rapid colonization (Pavia et. al. 1998). Leising and Yen (1997) reported similar results from copepod experiments testing swarms of individuals. As the density of the copepod swarms increased, the average nearest neighbor distance decreased (Leising and Yen 1997).

One could argue that MNND seen at the base of the blade supports Hamilton's (1971) selfish herd theory. Individuals reduced nearest neighbor distances in the presence of conspecifics in attempts to decrease the probability that any single individual would be attacked. Tighter spacing within the aggregate may also serve to increase the amount of chemical defenses found within a small area. Increased concentrations of *C. oculatus* at the base of the blade supports Mathews (1977) argument on aggregations of noxious individuals. *C. oculatus* are the least of the three defended species of amphipods found at Bell Island, Washington (Hutchinson 2002) and a predator's consumption of large numbers of a mildly noxious prey in rapid succession might build toxin levels to induce an aversion not otherwise associated with that prey, reinforcing negative interactions between chemically defended organisms and predators.

In addition to aggregations of *C. oculatus*, I found that several different species of organisms existed on *A. fimbriata*: for example, colonies of bryozoans, various species of amphipods and gastropods, and small fish. In studies conducted on *A. fimbriatum* from the west coast of Vancouver Island, British Columbia, Canada, some pieces of the kelp blade were completely covered (at least on one side) by bryozoans (e.g., *Lichenopora novaezelandiae* or *Membranipora membranacea*) (Hurd et al. 1994). Previous studies suggest that bryozoans influence the nitrogen (N) and photosynthetic abilities of the kelp (Durante and Chia 1991, Hurd et al. 1994, but see Hurd et al. 2000). In some cases the epiphytic stenolaemate bryozoans limit the ability of the kelp to remove nitrates and ammonium from seawater but provide a source of ammonium to the kelp via excretion (Hurd et al. 1994). According to field surveys by Durante and Chia (1991), bryozoan age distributions are spatially correlated with algal age distributions: younger bryozoans were concentrated on the youngest parts of the kelp (typically near the base), and vice versa. Despite bryozoans limiting chemical uptake for their algal host, adult *L. novaezelandie* has been found to deter gastropod feeding (e.g., herbivorous snail *Tegula pulliog*) on older growth of kelp, revealing an indirect benefit of epiphytic bryozoans (Durante and Chia 1991).

Younger bryozoan settlement on early growth of *A. fimbriata* (Durante and Chia 1991) may provide insight for reasons that *C. oculatus* might be found primarily on new growth (typically the base of the blade). I found no evidence that suggests younger bryozoans limit feeding of gastropods and therefore, may be an indication that younger bryozoans do not limit feeding for *C. oculatus* either. It is possible the older bryozoans

possess deterrents for *C. oculatus* and as a result, large numbers of individuals do not aggregate at the tip of the algal blade.

Laboratory Experiments

Spatial distributions and patterns of aggregates. H2: There is no difference in mean nearest neighbor distance for densities ranging from 753 individuals·m⁻² (500 individuals·0.664m⁻²) to 7,530 individuals·m⁻² (5000 individuals·0.664m⁻²). I rejected this null hypothesis. The trend in nearest neighbor distances in this laboratory experiment was not fully consistent with results observed the field. I expected that as abundances increased in the testing pools, the distribution of amphipods would be forced into tighter spacing minimums, creating a uniform pattern of 1 cm. Most densities followed my hypothesis, with the notable exception of the highest density, 5000 individuals·0.664 m⁻². Results indicated that as densities increased from 500, 1000, and 2000 individuals·0.664 m⁻², nearest neighbor distances declined 38% and approached 1 cm. However, from 2,000 to 5,000 individuals·0.664 m⁻² nearest neighbor distances increased 27% to 1.83 cm. Testing pools containing 5000 individuals were not different than testing pools containing 1000 individuals. The pattern of the aggregation at 5000 individuals was clumped (Holgate 1965). Further review showed that the increased change in the trend of decreasing MNND in the testing pool containing 5,000 individuals was not dependent on the time at which the image was taken in the testing pool (2 hr versus 24 hr). However, it should be noted that the largest number of nearest neighbor distances < 0.5 pixels was eliminated at this density (23.8% or 1,190 NND) compared to

other densities, and it is possible the increased MNND was an outcome of extensive mating and cannibalism efforts for *Chrompleustes oculatus* (Table 1). The clumped pattern of distribution suggests that mating and cannibalistic *C. oculatus* clumped together and thus created additional space in various areas for other opportunistic individuals to disperse. The pattern of dispersion was clumped for testing pools containing 500 individuals and 1000 individuals, which coincides with previous 0.1-m² visual quadrats taken of various habitats. The pattern of dispersion in the testing pool containing 2000 individuals was random, which contradicts previous 0.0065-m² photoquadrats taken on *C. oculatus* at high densities that suggests *C. oculatus* form a uniform pattern.

The time at which images were taken of testing pools (2 and 24 hours) had an effect on mean nearest neighbor distances at various densities. Smaller densities of *C. oculatus* decreased MNND when images were taken at 24 hours. It is known that *C. oculatus* were mating in pools and it is possible the highly variable and large decrease in MNND is the result of individuals trying to locate one another in testing pools containing 500 and 1,000 individuals·0.664 m⁻². Testing pools with higher densities showed an increased trend in MNND for images taken at 2 and 24 hours. It is documented that amphipods can recolonize within large populations in a relatively short period of time; e.g., a period of hours or days (Pavia et al. 1999). It is suggested the increase in MNND (from 2 hours to 24 hours) in testing pools containing 2,000 and 5,000 individuals·0.664 m⁻² is the result of rapid colonization of the larger densities of *C. oculatus* and most likely

occurred to form tighter spacing minimums to maximize space for all individuals in the community.

Laboratory observations suggested that density manipulation experiments were conducted during the mating season for *Chromopleustes oculatus*. Various cohorts could be seen throughout the sampling season from May to July, with July being the warmest period for the Pacific Northwest. Amphipods were observed to initiate pairing with the dominant individual, presumably the male, grasping its partner. After contact, the male would beat its pleopods against the female. This pleopod beating and many other behaviors noted in testing pools were characteristic of those described by Borowsky (1991) on the reproductive biology of gammarid amphipods.

Most gammaridean amphipods are dioecious. Since females do not store sperm, a male must accompany a female each time copulation and fertilization occurs, and females can reproduce many times in succession. Females have four sequential stages: (1) mate location (probably via waterborne pheromones), (2) initiation of pairing (stimulated by contact), (3) pairing (continues until the female molts), and (4) copulation (which occurs shortly after molt). Reproduction is seasonal in most natural populations, but in the laboratory under constant conditions females have been observed to continuously molt and reproduce while males continue to inseminate females (Borosky 1991, Kaestner 1970). Females ovulate minutes after molt, and the eggs pass into a ventral brood pouch, or marsupium.

Results of observations concerning the controversy of the existence of waterborne pheromones in reproductive behaviors have been contradictory. In a study using

Gammarus pulex, Ducruet (1973) concluded waterborne pheromones were present and males were attracted to inter- and intraspecific females, particularly conspecifics at all molt stages. It was not possible to replicate Ducruet's findings in a later study by Hartnoll and Smith (1980).

Temperature and day lengths are two environmental factors that have been shown to affect reproduction in specific amphipods. The time it takes for the eggs to pass into the marsupium (oviposit) varies with latitude and species. Some species oviposit in the fall and release their young in the spring; others oviposit in the spring and release their broods within a few weeks (Steele and Steele 1975).

Results of my study also suggested that *C. oculatus* could be cannibalistic. Approximately 10-15 amphipods in each testing pool would die before the 24 hours of testing ended. In testing pools only, I noted several cases of cannibalism where amphipods would attack a single dead or dying individual. At later observation times only the exoskeleton would remain of the injured individual. In addition to attacks on injured individuals, larger amphipods would attack smaller amphipods if left in holding pools together.

Cannibalism in several freshwater gammarid amphipod species is not uncommon (Embody 1911, Sexton 1924, Sexton 1928, Clemens 1950, Jones 1951, Schmitz 1967, Kostalos and Seymour 1976, Jenio 1980, Dick 1995, MacNeil et al. 1997). Cannibalism has been reported in many laboratory studies 1) as a dietary staple during depleted resources, 2) as predation on injured, diseased, and dead individuals, 3) when adults feed on juveniles, and 4) as a result of mating competition (larger males feed on smaller

males; MacNeil et al. 1999). I personally observed larger, dominant amphipods attacking smaller, injured, or dying amphipods in testing pools, in addition to noting several amphipod remains in pools at testing times (molts not included). To my knowledge, this is the first observation of cannibalism in a marine gammarid amphipod.

Predators and spatial distributions. H3: There is no difference in the mean nearest neighbor distance in the presence of predators in the laboratory. This null hypothesis was rejected. I proposed that *Chrompleustes oculatus* would decrease MNND as defense in the presence of a predator, forming tighter spacing minimums. The opposite held true for this series of experiments. The results indicated amphipods created a significant increase in overall MNND in the presence of predators at a constant density. Palatable amphipods are typically an important prey item (Kaiser et al. 1992, Pavia, Carr, and Aberg 1998, Thiel and Reise 1993), but the chemically defended amphipod, *C. oculatus*, is not a major source of diet for small fish and other predators (Hutchinson 2002, Zalewski 2001). Observations from Bell Island showed several species of small predatory fish (i.e., sculpins) coexisting with *C. oculatus*. Further observations in the laboratory indicated *C. oculatus* did not appear to be threatened by a naïve predator placed in testing pools.

It is possible the increased MNND was a result of eliminating nearest neighbor distances < 0.5 pixels, leaving fortuitously higher mean distances. It cannot be ruled out that the unique distributions seen in testing pools containing predators were a result of mating or cannibalism, although the fact that the percentage of amphipods coming into physical contact was reduced in the presence of predators (Table 1). The change in

distribution may have stemmed from the aggression that was seen from *C. oculatus* when a single *Oligocottus maculosus* was placed in the tank. Individuals that attacked *O. maculosus* increased space throughout the remaining testing pool, allowing amphipods to disperse and increase MNND. One could argue that this latter scenario lends support to the idea that amphipod warning coloration, which advertises their noxious chemicals, is irrelevant in the presence of predators. It is also worth mentioning the p value for this set of experiments ($p = 0.05$) was near the experiment-wise error rate and the number of images used in calculations in the presence ($n = 390$) and absence ($n = 50$) of predators may have biased the results in favor of a slight increase in MNND in the presence of predators.

Habitat choice experiments. H5: The response of amphipods to habitat choices will be the same for each of the four habitats: sea cucumbers, sand, rock, and sand-shell debris. When presented with multiple habitats in the absence of predators, *Chromopleustes oculatus* made distinct environmental choices. Bell Island was very diverse in its habitats. *Agarum* algal blades were dominant at the northwest corner of Bell Island, while a blanket of large starfish (*Pycnopodia*) up to 1 m in body diameter covered the rocky terrain at the southeast portion of the island. Only 50 m west at another rock outcropping known as Twin Peaks, a blanket of sea cucumbers (*Cucumaria miniata*, *Eupentacta quinquesemita*, and *Parastichopus californicus*, to name a few) existed on rock formations in addition to burrowing their trunks into the sand and shell debris. Amphipods flourished in all these habitats, although they were not as dense with the *Pycnopodia* along the southeast corner of Bell Island. When similar choices were

presented in the laboratory, *C. oculatus* actively inhabited *C. miniata* in all aquaria. It was especially notable that *C. miniata* occupied the smallest two-dimensional area for both aquarium A and B.

Evidence presented in Hutchinson (2002) suggests it is possible that selection for *C. miniata* was directly related to foraging attempts. It is not known how *C. oculatus* feeds, or what comprises its diet. Crustaceans in general have exploited many combinations of feeding strategies associated with suspension feeding, filter feeding, or direct feeding by manipulating mouthparts. Gammaridean amphipods are commonly referred to as “sand grazers” and “sand lickers,” indicative of feeding on benthic diatoms, detritus, or microorganisms located on surfaces of sediment particles (Brusca and Brusca 1990). In the study using diet analysis and laboratory behavior to determine if *C. oculatus* sequesters its defenses through endogenous or exogenous mechanisms, Hutchinson (2002) found the amphipod’s diet comprised largely of sea cucumber ossicles, most likely belonging to *Cucumaria miniata* or *Cucumaria piperata*. In addition to cucumber ossicles, echinoderm pedicellaria, centric diatoms, pinnate diatoms, and other crustaceans (although not a major portion of identifiable prey) were found in fecal matter (Hutchinson 2002).

It cannot be ruled out that amphipods may have selected sea cucumbers for other reasons not associated with cucumbers providing food (either directly or indirectly as a result of opportunistic amphipods intercepting the food during filter-feeding by the cucumber). It is possible that sea cucumbers, when elongated, may have presented the largest three-dimensional area compared with other habitats that presented no relief

structures. Aerial views of the aquaria limited analysis to two dimensions. Additionally, three-dimensional views were not practical for this study due to the live nature of the sea cucumber: the sea cucumber would shift positions throughout the 24 hour observations, sometimes feeding with its branched tentacles or other times it would be found in a resting state with its tentacles retracted.

C. oculatus possess multiple deterrents against predation including, but not limited to, chemical defenses, aposematic coloration, gregariousness, and aggressive behaviors. It has been shown that predatory fish in the lab will reject *Chromopleustes oculatus* after previous encounters, suggesting a learning capability possibly in conjunction with the brightly-colored dispositions and chemical defenses (Hutchinson 2002, Zalewski 2001). The hooded nudibranch, *Melibe leonina*, also does not appear to be a potential predator. In a laboratory environment, I personally observed *Melibe* to “regurgitate” individuals of *C. oculatus* when swallowed and to fully consume palatable amphipods (*Allorchestes* spp.). For the invertebrate, there were no avoidance lessons learned from bright coloration and distastefulness since *Melibe* are absent of sight.

The source of chemical defense in *C. oculatus* has recently been studied by Hutchinson (2002). Hutchinson (2002) suggests that as adults, defenses are synthesized *de novo* instead of sequestered from prey, regardless of diet. Starved adults that had their defenses removed were just as defended as those provided with food after a period of six days. Hutchinson’s (2002) study also showed that eggs taken from the marsupium were palatable and eaten by all predators in the lab. Newly released juveniles did not show a statistically different consumption rate in comparisons of palatable prey, but they were

rejected in 20% of trials with predators. Despite the lack of significance in Hutchinson's work (2002), this is other evidence that some juveniles are indeed defended inside the marsupium. Further research on whether juveniles obtain defenses from the adult or create them endogenously is warranted.

One could argue that the field observations from this study supported the concept that aggregation in defended species reinforces chemical defenses (Hamilton 1971, Mathews 1977). Amphipods created dense populations within a small area in the field as a result of decreased spacing. Previous research has not indicated any fish predators for this species (Zalewski 2001, Hutchinson 2002) and it is possible that a swarm of individuals creates a toxic aversion.

Chemical defenses did not play a major role in spacing among individuals. Densities in the laboratory followed similar spacing distributions. However, these density changes were solely among conspecifics and not in the presence of predators. In addition, further investigations in the laboratory in the presence of a potential predator showed that chemical defenses were not reinforced by aggregations, suggesting other biological factors were at work. Recall *C. oculatus* increased MNND and moved away from conspecifics in the presence of predators.

Hutchinson (2002) states that *C. oculatus* does not sequester its defensive compound but produces it *de novo* and therefore aggregations of individuals are not the result of a need to forage on a defended host (e.g., *A. fimbriatum* or *C. miniata*). Various habitats presented in the laboratory in the absence of predators further suggest that chemical defense is not a driving force in habitat choices for *C. oculatus*.

Further studies on various vertebrates (fish) and invertebrates (e.g., predatory nemerteans) would be beneficial in determining if these amphipods are consumed by any predators. Perhaps *C. oculatus* is influenced by other predators that have not been determined. MacNeil et al.'s review (1999) highlights the dangers of focusing only on conventional predators of *Gammarus* spp. such as fish used in this study and other works cited in this paper. It is equally dangerous to assume *Gammarus* spp. have ecologically equivalent behavior patterns, physiochemical tolerances, invasive potentials, and most importantly for this study, resistance to invasion and predatory tendencies (MacNeil et al. 1997).

Subsequent work based on the observations that amphipod numbers are highly variable on algal blades at slack tide would be useful in determining factors for mobility. What water velocities can amphipods withstand before they are swept off an *Agarum* blade? This is a key question in understanding amphipod activity in the field and must be tested in a laboratory environment. With high variability in abundance for each algal blade at different tidal states, in addition to strong currents at Bell Island, it would be surprising if *C. oculatus* were able to create specific domiciles on individual blades without being detached by the flowing waters of the exchange. Additional density studies should focus on research created during the non-mating season for *Chromopleustes oculatus*. This would address whether or not the inconsistencies in experiments manipulating densities and the results from the presence of predators were errors that stem from the distances removed from calculations due to mating and cannibalism.

CONCLUSIONS

A variety of factors influenced the spatial distribution of the chemically defended, aposematic, gammarid amphipod, *Chromopleustes oculatus* in the field and laboratory. Amphipod location on *Agarum* blades affected the distribution of natural populations. Large numbers of individuals could be found at the base of the blade but numbers were reduced at the center and tip. As abundances increased on *Agarum fimbriatum* in the field the mean nearest neighbor distances decreased, reflecting avoidance minimums established by the community. Density, predators, and available habitats affected the spatial distribution of *C. oculatus* in the laboratory. The trend observed for MNND in the field (as density increases MNND decreases) was not seen for all densities in experiments conducted in the laboratory. As density increased in testing pools containing 500 to 2,000 individuals $\cdot 0.664 \text{ m}^{-2}$ MNND decreased to establish minimum spacing among individuals. Further review of the testing pools containing 5000 individuals indicated this density had the highest percentage of nearest neighbor distances eliminated from calculations than any other density as a result of mating. The liberal numbers of amphipods mating in pools created additional space for other individuals and are most likely the result for the increase in MNND. Contrary to expectations, the MNND in the presence of a naïve fish predator was significantly higher than the MNND in the fish's absence. It possible this increase is the result of predator avoidance strategy by the amphipods. Habitat significantly affected spatial distributions of amphipods. A higher percentage of amphipods per area aggregated on the sea cucumber, *Cucumaria miniata* despite various other substrate choices. Mean nearest neighbor distances that did not

coincide with predicted trends were presumably the result of mating, cannibalism, or predator avoidance strategies within amphipod communities.

A variety of abiotic and biotic factors influenced the spatial distribution of *C. oculatus* such as density, proximity of amphipods to the base of algal blades, the presence of predators, and habitat availability. However, it is my beliefs that this study suggests chemical defenses only play a small role for *Chromopleustes oculatus*. Chemical defense did not appear to regulate spacing in the presence of predators in the laboratory: tighter spacing to reinforce toxins was not seen. Other laboratory experiments that evaluated aggregation as a function of density changes and habitat choices were conducted without predators and further suggests that aggregations to reinforce chemical defenses are not an elementary driving force for *C. oculatus*.

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