

ABSTRACT

Marcy E. Hutchinson. CHEMICAL DEFENSE IN THE GAMMARID AMPHIPODS *CHROMOPLEUSTES OCULATUS*, *CH. LINEATUS*, AND *CRYPTODIUS KELLERI* (ARTHROPODA: CRUSTACEA). (Under the direction of Dr. Stephen F. Norton). Department of Biology, November 2002.

Organisms that use chemical compounds as defense must either produce these compounds *de novo* or sequester them from another source, typically something in their diet. The central focus of my research was to determine if the source of chemical defense used by three gammarid amphipods *Chromopleustes oculatus*, *Ch. lineatus*, and *Cryptodius kelleri* (Arthropoda: Crustacea) is endogenous or exogenous. Three approaches were used: an investigation of diet by fecal analysis, an examination of ontogeny of chemical defense and an experimental analysis of regeneration of chemical defense after depletion. Consumption of chemically defended prey is a prerequisite for sequestration of defensive chemicals. *Ch. oculatus* was found to consume a chemically defended sea cucumber, *Cucumaria miniata*. Chemically defended prey were not identified in the diets of *Ch. lineatus* or *Cr. kelleri*. Eggs recovered from the brood pouch of any of the three species were not chemically defended. Ontogenetically, chemical defense occurred in *Cr. kelleri* at the pre-feeding juvenile stage when palatability was tested using a predatory fish, *Clinocottus acuticeps*. *Ch. oculatus* and *Ch. lineatus* were not significantly rejected by predators until adults; however, a high frequency of "cough" responses by predators when eating these amphipods at the juvenile stage may indicate a developing chemical defense. The most conclusive evidence for endogenous production of chemical defense was the regeneration of chemical defense within two days by *Ch. oculatus* that had been depleted of defensive chemicals and deprived of food. Access to

chemically defended prey did not effect the regeneration of defensive ability. The compilation of evidence from these experiments indicated an endogenous source of defensive chemicals in these crustaceans.

CHEMICAL DEFENSE IN THE GAMMARID AMPHIPODS *CHROMOPLEUSTES*
OCULATUS, *CH. LINEATUS*, AND *CRYPTODIUS KELLERI*
(ARTHROPODA: CRUSTACEA)

A Thesis

Presented to

The Faculty of the Department of Biology
East Carolina University

In Partial Fulfillment

Of the Requirements for the Degree
Master of Science in Biology

by

Marcy Hutchinson

November 2002

QL
444
M315
H88
2002

CHEMICAL DEFENSE IN THE GAMMARID AMPHIPODS *CHROMOPLEUSTES*

OCULATUS, *CH. LINEATUS*, AND *CRYPTODIUS KELLERI*

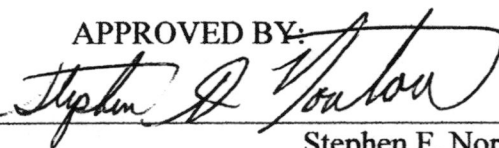
(ARTHROPODA: CRUSTACEA)

by

Marcy E. Hutchinson

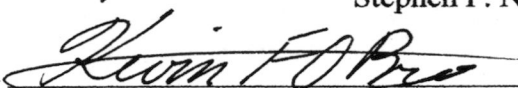
APPROVED BY:

DIRECTOR OF THESIS



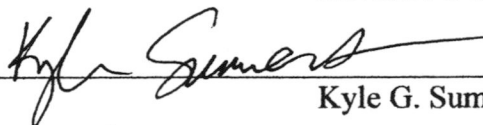
Stephen F. Norton, Ph.D.

COMMITTEE MEMBER



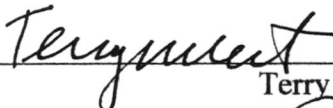
Kevin F. O'Brien, Ph.D.

COMMITTEE MEMBER



Kyle G. Summers, Ph.D.

COMMITTEE MEMBER



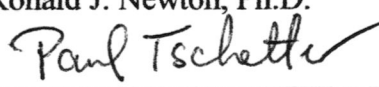
Terry L. West, Ph.D.

CHAIR OF THE DEPARTMENT OF BIOLOGY



Ronald J. Newton, Ph.D.

INTERIM DEAN OF THE GRADUATE SCHOOL



Paul D. Tschetter, Ph. D.

ACKNOWLEDGEMENTS

I sincerely thank my thesis advisor Dr. Stephen Norton for guidance and expertise throughout this research. I also sincerely thank Dr. Kevin O'Brien, Dr. Kyle Summers and Dr. Terry West, my thesis committee, for their advice and contributions to this research through revisions. I especially thank Dr. West for his willingness to offer guidance in the absence of my thesis advisor. I also thank Dr. Gerhard Kalmus for his assistance and support during my graduate career at East Carolina University.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES.....	iv
---------------------------------	----

CHAPTER ONE: DIET OF THE CHEMICALLY DEFENDED GAMMARID AMPHIPODS *CHROMOPLEUSTES OCULATUS*, *CH. LINEATUS* AND *CRYPTODIUS KELLERI*

INTRODUCTION.....	1
Chemical defense in crustaceans.....	2
Amphipod trophic ecology.....	2
Chemically defended amphipods.....	3
Objectives of the research.....	5
MATERIALS AND METHODS.....	6
Study sites and collections.....	6
Analysis.....	7
RESULTS.....	8
Sources of variation in the diet of <i>Ch. oculatus</i>	11
DISCUSSION.....	17
Diet differences among species of amphipod.....	17
Season, substrate and spatial variation in the diet of <i>Ch. oculatus</i>	19

CHAPTER TWO: ONTOGENY AND REGENERATION OF CHEMICAL DEFENSE IN THE GAMMARID AMPHIPODS *CHROMOPLEUSTES OCULATUS*, *CH. LINEATUS* AND *CRYPTODIUS KELLERI*

INTRODUCTION.....	23
-------------------	----

Characteristics of chemically defended insects.....	23
Chemical defense and marine organisms.....	25
Objectives of the research.....	29
MATERIALS AND METHODS.....	30
Field collections and maintenance of prey and predators.....	30
Ontogeny of chemical defense: are eggs defended?.....	32
Analysis.....	34
Ontogeny of chemical defense: are newly released juveniles defended?..	35
Ontogeny of chemical defense: are adults defended?.....	37
Can <i>Ch. oculatus</i> regenerate defensive chemicals?.....	38
Preparation of amphipods.....	39
Do starved <i>Ch. oculatus</i> regenerate their defense?.....	39
Does diet influence regeneration?.....	40
Analysis.....	41
RESULTS.....	42
Ontogeny of chemical defense.....	42
Regeneration of chemical defense by <i>Ch. oculatus</i>	50
DISCUSSION.....	56
Ontogeny of chemical defense.....	56
Regeneration of chemical defense by <i>Ch. oculatus</i>	59
Evaluation of endogenous versus exogenous chemical defense.....	60
REFERENCES.....	64

APPENDIX A.....74

APPENDIX B.....76

LIST OF TABLES AND FIGURES

Table 2-1.	Variation in predator consumption of <i>Chromopleustes oculatus</i> broods.....	48
Figure 1-1.	Diet of <i>Chromopleustes oculatus</i> , <i>Ch. lineatus</i> and <i>Cryptodius</i> <i>kelleri</i>	9
Figure 1-2.	Effect of season on the diet of <i>Ch. oculatus</i>	12
Figure 1-3.	Effect of location on the diet of <i>Ch. oculatus</i>	15
Figure 2-1.	Ontogeny of chemical defense.....	43
Figure 2-2	Gradient of predator feeding responses to juvenile <i>Ch. oculatus</i> and <i>Ch.</i> <i>lineatus</i>	45
Figure 2-3.	Regeneration of chemical defense by <i>Ch. oculatus</i> when starved.....	52
Figure 2-4.	Regeneration of chemical defense by <i>Ch. oculatus</i> when offered a “non- defended” diet and “defended” diet.....	54

CHAPTER ONE

DIET OF THE CHEMICALLY DEFENDED GAMMARID AMPHIPODS *CHROMOPLEUSTES OCULATUS*, *CH. LINEATUS* AND *CRYPTODIUS KELLERI*

INTRODUCTION

Many organisms that use chemical defenses to escape predation, acquire their chemical defense from their own prey. Works by insect chemical ecologists indicate that consumption of prey that produce secondary chemicals, and often specialization on a single chemically defended prey species, is a necessary evolutionary step in the adaptation to sequester chemical defenses from food sources (Bowers 1992). A secondary chemical is defined as any chemical substance not directly involved in fundamental life processes (Berenbaum and Seigler 1992). The occurrence of predator sequestration of defense from prey is well documented among terrestrial arthropods (Blum 1981). Many insects readily regurgitate or defecate when disturbed and it has been emphasized that these enteric discharges can constitute highly effective repellents because of the plant secondary chemicals that they contain (Esiner 1970).

Among marine organisms there are relatively few documentations of sequestered chemical defense. Some nudibranchs acquire defensive compounds from the sponges, coelenterates, bryozoans, or tunicates that constitute their diet (Paul and Pennings 1991, Rogers and Paul 1991, Paul 1992, Slattery et al. 1998, Johnson and Willows 1999). The butter clam, *Saxidomus giganteus*, sequesters dinoflagellate toxins from its planktonic diet as a means to deter predators (Kvitek 1989). Ascoglossans feed by sucking algal sap

mainly from *Caulerpa* spp. and are widely assumed to derive their chemical defense from algal metabolites; however, this interaction has not been rigorously tested (Hay 1992).

Chemical defense in crustaceans

Evidence for chemical defense among crustaceans is sparse. This is surprising given the diversity of marine organisms that are chemically defended (Pawlik 1993), the speciose nature of the Crustacea, and the wide-spread occurrence of chemical defenses in other arthropods (Berenbaum and Seigler 1992). Lucken and Orth (1990) suggest that the higher rejection rate of larval pea crabs *Pinnotheres ostreum* versus larval blue crabs (*Callinectes sapidus*) by the mummichog (*Fundulus heteroclitus*) was due to an unidentified chemical defense. In another case, de Freitas (1990) reported that a digestive secretion regurgitated by some brachyuran decapod crustaceans affected the feeding behavior of the predatory marine gastropod *Nassarius vibex*. Other studies indicate that amphipod crustaceans may employ an “indirect” chemical defense. For example, some amphipods reduce their predation risk by living on chemically defended algae (Hay et al. 1987), and Antarctic amphipod *Hyperielle dilatata* uses a chemically defended pteropod as a shield against fish predators (McClintock and Janssen 1990). In light of the common occurrence of prey-derived chemical defenses in other organisms, a likely source of secondary compounds in crustaceans, if discovered, would be their diet.

Amphipod trophic ecology

Various gammarid amphipods have been classified as shredders, detritivores, scavengers, predators and omnivores (Clemens 1950, Barlocher and Kendrick 1973, Ladle 1974, Moore 1975, Willoughby and Sutcliffe 1976, Marchant 1981, DeLong et al. 1993). These designations are based on observations of mouth parts, direct observations of feeding in the laboratory, or examination of gut contents or fecal contents. Although generalist feeding behavior is apparently most common among amphipod species, some dietary specialists have been reported. Diet studies have revealed amphipod species that specialize on coelenterates or sponges (Coleman 1989a), bryozoans (Coleman 1989b), and holothurians (Coleman 1990). These species possess specialized mouthpart morphologies which may represent adaptations to utilize their specialized food source.

Even if an organism consumes chemically defended prey, it may not become chemically defended as a result. For example, while a variety of gammarid amphipods do feed on the chemically defended alga, *Dictyota dichotoma*, they remain palatable to predatory fish (Hay et al. 1987). This is also true of insects which may feed on chemically defended host plants but not acquire chemical defenses as a result (Isman et al. 1977, Blum 1983, Bowers and Puttick 1986, Rowell-Rahier and Pasteels 1986, Brattsten 1986).

Chemically defended amphipods

Recent research indicates that three species of gammarid amphipods (*Chromopleustes oculatus*, *Chromopleustes lineatus* and *Cryptodius kelleri*) are chemically defended against predatory invertebrates and fish (Norton and Stallings 1999,

Zalewski 2001). All three species are brightly colored and occur conspicuously in the open and at high densities on a variety of substrates in the subtidal zone of the Strait of Juan de Fuca, WA (Norton and Stallings 1999). Yet they are rarely consumed by common amphipod predators (Norton, personal communication). Zalewski (2001) showed, through prey choice experiments, that adults of *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* were not eaten by two predatory fish (*Artedius harringtoni* and *Jordania zonope*) which had prior experience with these amphipods. Laboratory observations reveal that when physically disturbed all three amphipod species expel fluids from their mouths that are distasteful to fish and invertebrates that feed on amphipods (Norton, personal communication). These defensive compounds are stored in the digestive tract, particularly the midgut caecae. A similar phenomenon has been commonly observed in insect-plant interactions, in which the herbivore sequesters secondary compounds from the plants it consumes and then regurgitates these compounds for its own defenses (Harborne 1993). For example, Eisner (1970) demonstrated that the enteric regurgitates of two grasshoppers, *Romalea microptera* and *Brachystola magna*, effectively repel aggressive worker ants.

Although the two *Chromopleustes* species and *Cryptodius kelleri* are conspicuous, very little is known about their biology. Bousfield and Hendrycks (1994) describe the family Pleustidae, to which *Chromopleustes* belongs, as benthic detritivores and micro-predators. The mouth parts and gnathopods of the Pleustidae tend to show specialization for carnivory and raptorial feeding behavior (Bousfield and Hendrycks 1994). The superfamily Stegocephaloidea, to which *Cryptodius kelleri* belongs, was referred to as

“parasitic” by Barnard and Karaman (1991) based on the conically projecting mouthpart bundle observed in members of this group. More recent research by Moore and Rainbow (1984, 1989, 1992) and by Coleman (1989a, b, 1990) indicate that stegocephaloids are carnivorous, existing on diets of coelenterates, sponges or bryozoans.

While chemically defended organisms appear to be more abundant in the tropics, many temperate organisms have been discovered to harbor chemical defenses against predators (Bakus 1974, 1981). In San Juan Islands, WA, *Chromopleustes oculatus*, *Ch. lineatus* and *Cryptodius kelleri* were observed on organisms known to be chemically defended. These organisms include holothurians (Bingham and Braithwaite 1986), kelps, tunicates, bryozoans, and sponges (Scheuer 1982, Paul 1992). However, the diets of these amphipods have not been studied, and it is therefore unclear if there is any potential mechanism of acquiring defensive chemicals via their diet.

Objectives of the research

The central focus of this research was to determine whether the chemical defenses employed by *Chromopleustes oculatus*, *Ch. lineatus* and *Cryptodius kelleri* to deter predatory fish could be sequestered from prey. I will attempt to ascertain potential external sources of defensive chemicals by delineating the diet of *Chromopleustes oculatus*, *Ch. lineatus*, and *Cryptodius kelleri* through fecal pellet analysis and laboratory observations of feeding behavior.

MATERIALS AND METHODS

Study sites and collections

The primary study area was Bell Island, a small rocky island (0.03 km²), in Wasp Passage, San Juan Islands, WA. A secondary collection area was established at a rocky outcrop (Twin Peaks) located 500m west of Bell Island and southeast of Crane Island. At both sites rocky slopes and walls drop rapidly to a maximum depth of 35m. While both sites are swept by strong currents driven by mixed semi-diurnal tides, currents are especially strong at the north and south ends of Bell Island and Twin Peaks.

The diet study began during December 2000 with the collection of fecal pellets from individuals of *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri*. Fecal pellets of these species were also collected during May – July, 2001. *Ch. oculatus*, *Ch. lineatus*, and *Cr. kelleri* were collected from the subtidal using SCUBA. These amphipods are commonly found on the fronds of *Agarum fimbriata* and *Laminaria saccharina*. Intact fronds bearing the amphipods were each enclosed in a large (70cm x 95cm) plastic bag. The bag was then tied shut and brought to the surface. Adults of all three amphipod species were also collected from a variety of substrates (e.g. rocks, sea cucumbers) using a turkey basting bulb as an aspirator to capture them.

All amphipods were returned to the lab and kept in individual containers of filtered seawater for 24-48 hours. Fecal pellets produced during this period of time were removed with a pipette and preserved in 70% ethyl alcohol. Fecal pellets were examined microscopically for the presence of identifiable items which were limited to the hard,

non-digested parts of prey. A list was compiled of prey identified in the fecal pellets of each amphipod species and the frequency of occurrence of each prey item.

Analysis

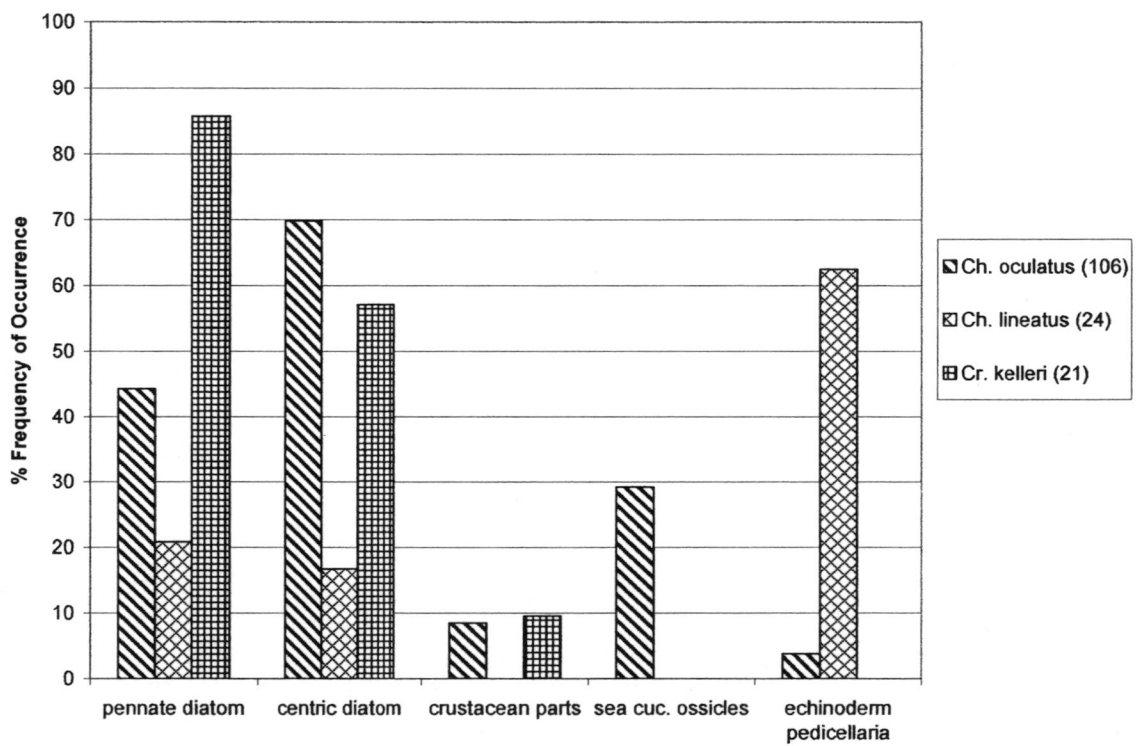
Data, consisting of the frequency of occurrence of identified prey, were analyzed for evidence of differences in diet among *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri*. A homogeneity Chi-square was used to identify those prey that differed in their frequency of occurrence among the three species. For example, a Chi-square analysis was used to address the null hypothesis that centric diatoms occurred with similar frequencies among the three amphipod species. Because of the larger sample sizes, I also analyzed the data from *Ch. oculatus* for differences in the frequency of occurrence of identified prey a) between seasons (December 2000 versus May - July 2001), b) between collection sites, location of collection (Bell Island versus Twin Peaks), and c) between different substrates (rock versus sea cucumber). The observed and expected values used in the Chi-square calculations are reported in appendix B.

RESULTS

The only clearly identifiable prey in the fecal pellets were those with distinctive hard parts that survived digestion. Five prey types: pinnate diatoms, centric diatoms, crustacean pieces, sea cucumber ossicles and echinoderm pedicellaria dominated the diets of these amphipod species. Prey items found in only single individuals included a dinoflagellate, filamentous algae and a foraminiferan; these rare prey types were not included in the subsequent quantitative analyses.

There were significant deviations from a homogenous frequency of occurrence of prey among the three species of amphipods. While pinnate and centric diatoms were common in the diets of all three species there were significant differences in their frequency of occurrence (minimum $X^2 = 19.5$, $df = 2$, $P < 0.001$) (Figure 1-1). Pinnate diatoms had the highest frequency of occurrence in the feces of *Cr. kelleri*; they were present in 86% of fecal pellets collected from this species. Centric diatoms had a lower than expected frequency of occurrence (present in 17 % of samples) in the feces of *Ch. lineatus* and had the highest frequency in the feces of *Ch. oculatus* (present in 70% of samples). Crustaceans were present in the diet of *Ch. oculatus* and *Cr. kelleri* but did not comprise a major portion of identifiable prey. There was no significant difference in the frequency of occurrence of crustacean parts among the three species ($X^2 = 2.3$, $df = 2$, $P > 0.25$). There were significant differences among the three species in the occurrence of sea cucumber ossicles ($X^2 = 16.6$, $df = 2$, $P < 0.001$) and echinoderm pedicellaria ($X^2 = 64.9$, $df = 2$, $P < 0.001$). Sea cucumber ossicles, most likely belonging to *Cucumaria miniata* or *Cu. piperata* (Lambert 1997), were found exclusively in fecal pellets from *Ch.*

Figure 1-1. Percent frequency of occurrence of prey recovered from the fecal pellets of *Chromopleustes oculatus*, *Ch. lineatus* and *Cryptodius kelleri*. All collections during December 2000 and May – July 2001 from the Bell Island and Twin Peaks sites are combined in this graphic. Numbers in parentheses indicate the number of fecal pellets examined that had identifiable prey.



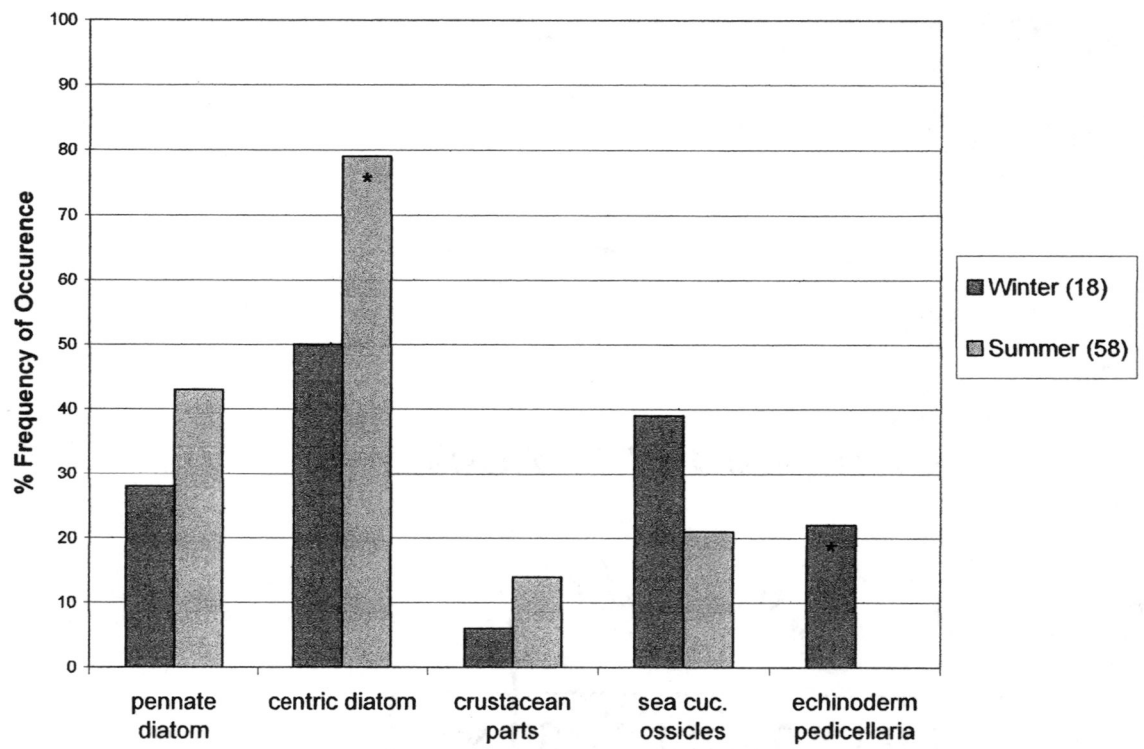
oculatus. They were present in 29% of the fecal samples from this species. Echinoderm pedicellaria (not identified) were found in the fecal contents of *Ch. oculatus* and *Ch. lineatus*. Echinoderm pedicellaria were most common in the fecal contents of *Ch. lineatus*; they were present in 58% of fecal pellets collected from this species. Evidence of consumption of echinoderm tissue was absent from fecal pellets of *Cr. kelleri*.

Sources of variation in the diet of *Ch. oculatus*

The larger sample size for *Ch. oculatus* allowed for a more detailed analysis of the diet of this species. I looked for differences in the frequency of occurrence of prey between collection seasons (winter 2001 versus May – July, 2001) for the Bell Island population, substrate (rocks or sea cucumber) at the Twin Peaks site, and location of the collection (Bell Island collections from algae versus Twin Peaks collections from algae). Seasonal differences in diet were limited to the presence of echinoderm pedicellaria ($X^2 = 14.95$, $df = 1$, $P < 0.001$) in winter and a 29% higher abundance of centric diatoms during May – July, 2001 ($X^2 = 5.9$, $df = 1$, $P < 0.025$) (Figure 1-2). There were no seasonal differences in the frequency of occurrence of centric diatoms, pinnate diatoms, crustacean pieces or sea cucumber ossicles (maximum $X^2 = 2.43$, $df = 1$, $P > 0.5$).

Because two of the dominant substrates occupied by *Ch. oculatus* were rocks and sea cucumbers, I compared the fecal contents of individuals collected from these two substrates during May – July, 2001 at Twin Peaks for homogeneity of frequency of occurrence of prey. *Ch. oculatus* from both substrates showed similar diets, consisting of pinnate and centric diatoms and sea cucumber ossicles. The substrate from which an

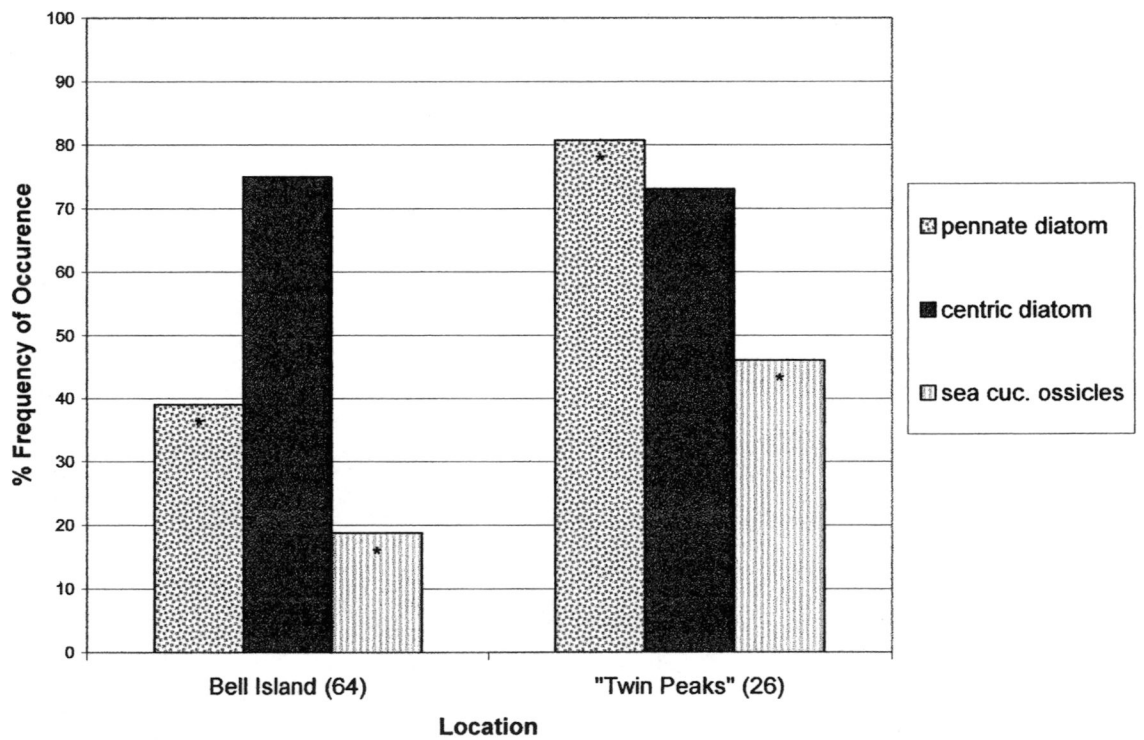
Figure 1-2. Percent frequency of occurrence of prey recovered from the fecal pellets of *Chromopleustes oculatus*. *Ch. oculatus* were collected during December 2000 and May – July 2001. Numbers after the collection season indicate the number of fecal pellets examined that had identifiable prey. * indicate categories that were significantly different (minimum $X^2 = 4.14$, $df = 1$, $P < 0.05$).



amphipod was collected had no significant effect on the number of identifiable prey in the fecal contents (maximum $X^2 = 2.85$, $df = 1$, $P > 0.05$). When sea cucumber ossicles were present in fecal pellets from *Ch. oculatus* collected on rocks or sea cucumbers, they were found in high density.

While there were strong similarities in the types of prey in the diet of *Ch. oculatus* collected at Bell Is. Versus Twin Peaks, there were differences in frequency of occurrence of pinnate diatoms and sea cucumber ossicles. Pinnate diatoms ($X^2 = 12.9$, $df = 1$, $P < 0.001$) and sea cucumber ossicles ($X^2 = 7.1$, $df = 1$, $P < 0.01$) made up a greater proportion of the diet of *Ch. oculatus* collected at Twin Peaks where 81% of fecal pellets contained pinnate diatoms and 46% of fecal pellets contained sea cucumber ossicles. While at Bell Island, 40% of fecal pellets contained pinnate diatoms and 19% of fecal pellets contained sea cucumber ossicles (Figure 1-3).

Figure 1-3. Percent frequency of occurrence of prey recovered from the fecal pellets of *Chromopleustes oculatus*. *Ch. oculatus* was collected from algae (*Agarum fimbriata*) at either Bell Island or Twin Peaks. Numbers after each location indicate the number of fecal pellets examined that had identifiable prey. * indicate categories that were significantly different between the two locations (minimum $X^2 = 7.1$, $df = 1$, $P < 0.01$).



DISCUSSION

Diet differences among species of amphipod

The groups to which *Chromopleustes oculatus*, *Ch. lineatus* and *Cryptodius kelleri* belong have previously been described as benthic detritivores and carnivores (Bousfield and Hendrycks 1994, Moore and Rainbow 1984, 1989, 1992, Coleman 1989a, b, 1990). However, the types of food ingested by these three species have not been determined before this study. Analyses of fecal pellets indicate *Ch. oculatus*, *Ch. lineatus* and *Cryptodius kelleri* are omnivorous. All three species consumed diatoms and either small crustaceans or echinoderm tissue.

Many species of macroalgae are known to be chemically defended (e.g. Hay et al. 1988, Hay and Fenical 1988, Duffy and Hay 1994, Paul et al. 1987), and they are a potential source of secondary compounds. However, there were no indications from field or laboratory observations that *Ch. oculatus*, *Ch. lineatus* or *Cr. kelleri* consume macroalgae even though they are commonly found on this substrate in the wild. Macroalgae cells were not identifiable in the fecal contents nor was there any evidence of damage to algal blades in the field, in spite of amphipod densities as high as 24,500 ind/m² (Norton and Stallings 1999). In the laboratory, amphipods were never observed to feed on or take any interest in pieces of macroalgae offered to them.

The mechanism by which *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* capture diatoms is not known. However, scrapings of algal blades when viewed microscopically revealed pinnate and centric diatoms similar to those found in the fecal contents of all three species. Schram (1986), noted that some amphipods feed by scraping the nutritive

films off surfaces, such as sand grains, or from a host, such as an echinoderm.

Gammarideans can also use setose anterior pereopods to extract a filtrate from the respiratory current produced by the pleopods. The maxillipeds clean these pereopodal setae and pass the food particles forward to the mouth (Schram 1986). While *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* were commonly found aggregated on algal blades, they were also found on a variety of other substrates, such as rocks and sea cucumbers (Norton and Stallings 1999). They were often observed grooming themselves. Scraping of surfaces on which the amphipod is found or filter feeding from respiratory currents are both possible mechanisms for the capture of diatoms.

Both *Ch. oculatus* and *Ch. lineatus* consumed echinoderm tissue, as indicated by the presence of pedicellaria ossicles and dermal ossicles. The dermal ossicles observed in the feces of *Ch. oculatus* were most likely from the holothurians *Cucumaria miniata* or *Cu. piperata* according to the shape of the ossicles (Lambert 1997). These holothurians produce secondary compounds that deter predators (Bingham and Braithwaite 1986), yet they are consumed by *Ch. oculatus*. Based on this observation this species was a possible candidate for sequestration of chemical defense. Other diet studies have reported several species of gammarid amphipods and caprellids that feed on echinoderm tissue, especially body fluids or epidermis, but this food source is uncommon (Vader 1978, Comely and Ansell 1988, Coleman 1990).

Vader (1978) compiled information from diverse studies documenting associations between gammarids and caprellids from 15 families with echinoderms of all classes. Most observations of such associations are restricted to the northern hemisphere.

The kinds of associations are diverse. Some amphipods use echinoderms merely as a substratum, which also provides some protection from predation, e.g. *Dulichia rhabdoplastis* (Vader 1978). Others live as “energy commensals” with burrowing echinoderms, which deliver oxygenated water and food. Several amphipods, living on the surfaces of starfishes, ingest secretions from their hosts. The lysianasids *Ambasia atlantica* and *Menigrates obtusifrons* are commensals stealing partly digested food from an asteroidean host (Vader 1978). In the field, *Ch. oculatus* was observed on the tentacles and near the mouth of *Cu. miniata* and may in engage in commensal behavior like that described for *Ambasia atlantica* and *Menigrates obtusifrons* in addition to consuming the tissue of sea cucumbers.

While specific identification of the crustaceans in the diet was not possible, *Ch. oculatus* were observed to capture and consume mysid shrimp in the laboratory when the prey was repeatedly passed in front of the amphipod. In no case did the amphipod pursue the prey, even though the amphipod was apparently hungry based on the fact the prey was consumed once captured. This feeding behavior was similar to predaceous caprellids that passively wait for prey to pass by, whereupon they grab the prey with the gnathopods rather than actively stalk the prey (Schram 1986). The “sit and wait” hunting tactic may be advantageous in the strong currents that occur with each tidal exchange in the San Juan Archipelago.

Season, substrate and spatial variation in the diet of *Ch. oculatus*

The more detailed analysis of the diet of *Ch. oculatus* revealed seasonal and spatial variation in diet. The presence of echinoderm pedicellaria in winter was the only significant effect of season on the composition of this species diet. The presence of echinoderm pedicellaria in winter collections of *Ch. oculatus* may be evidence of a seasonal expansion of diet in response to differences in food availability or abundance. Relative abundances of identifiable prey were lower in winter than in summer collections (43% of individuals with identifiable prey in December, 2000 versus 67% of individuals with identifiable prey May – July, 2001). This may be indicative of slower feeding rate during the winter. Also, the decline of diatoms in winter diet may reflect a seasonal decline in primary producers which accompanies the shorter day length in winter.

Other studies of the diet of amphipods have also demonstrated seasonal changes in the frequency and types of prey in the diet. Summers *et al.* (1997) noted that the relative abundance of prey items in the diet of *Gammarus fasciatus*, an omnivorous amphipod, changed seasonally. This species shows relatively low levels of feeding during the winter and switches to a diet consisting mainly of filamentous algae, diatoms and animal matter in early spring and summer.

Because of the strong currents at the collecting sites, it would not be surprising if these amphipods were not very mobile and that their diets would reflect the local prey spectrum found on a particular substrate. However, amphipods collected from rocks and sea cucumbers at the field site Twin Peaks demonstrated no difference in the frequency of occurrence of various prey types. This may indicate that the amphipods are moving from one substrate to another in spite of the strong currents and the risk of dislodgment.

In the comparison between the two field sites (Bell Island versus Twin Peaks), there were significantly lower numbers of pinnate diatoms and sea cucumber ossicles found in the fecal contents of *Ch. oculatus* collected from Bell Island. At both sites amphipods were collected from algae; however, the field site at Bell Island was characterized by more macroalgae and fewer sea cucumbers overall than the Twin Peaks site. The differences in dominate substrate type between these two sites may indicate that substrate does in fact have an effect on the amphipods feeding behavior but that because the amphipods are mobile, these differences can only be detected on a larger spatial scale.

Some of the prey in the diets of *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* may be potential exogenous sources of chemical defense. The diet of *Ch. oculatus* included a chemically defended sea cucumber (Bakus 1974, Bingham and Braithwaite 1986). Thus, chemical defense in *Ch. oculatus* may be exogenous, provided by the saponins in the sea cucumbers in its diet. *Ch. lineatus* consumed echinoderm tissue as indicated by the presence of pedicellaria ossicles, but dermal ossicles were absent. Because pedicellaria are not found on holothurians, it is likely *Ch. lineatus* was feeding on starfish or urchins. Some asteroids are known to produce a chemical defense (Lucas et al. 1979, Scheuer 1982, Paul 1992), but I was unable to identify the echinoderm from which the observed pedicellaria ossicles originated from. Thus, it is uncertain if there is a potential source of chemical defense in the diet of *Ch. lineatus*. Evidence for consumption of echinoderm tissues was absent from the diet of *Cr. kelleri*. There was no potential exogenous source of defensive chemicals identified for this species. However, it is important to note the limitations of fecal analysis: that the importance of soft bodied prey is often overlooked

or underrepresented (Quigley and Vanderploeg 1991). It is possible soft bodied prey may be a potential source of defensive chemicals in any of the three amphipod species.

CHAPTER TWO

ONTOGENY AND REGENERATION OF CHEMICAL DEFENSE IN THE GAMMARID AMPHIPODS *CHROMOPLEUSTES OCULATUS*, *CH. LINEATUS* AND *CRYPTODIUS KELLERI*

INTRODUCTION

Characteristics of chemically defended insects

The use of exogenous (i.e., sequestered from other chemically defended organisms) and endogenous (i.e., produced internally) chemical defenses by insects against their predators has been investigated extensively (e.g., Blum 1981, Roitberg and Isman 1992). Unpalatable insects often exhibit a suite of associated characteristics to advertise their unpalatability, such as aposematic ("warning") coloration and gregarious behavior (Bates 1862, Cott 1940, Brower 1984). Aposematic coloration is thought to enhance avoidance behavior of predators by making the prey more recognizable to the potential predator and thus aiding learning (Gittleman and Harvey 1980, Roper and Wistow 1986). Gregarious behavior is thought to serve a similar function by increasing the magnitude of the warning signal and may immediately reinforce predator avoidance-learning (Cott 1940, Edmunds 1974, Sillen-Tullberg and Leimar 1988, Guilford 1990). There are, however, exceptions to the general characteristics of chemically defended insects. Some cryptic insects are also chemically defended (Jarvi et al. 1981, Turner 1984), and some species of chemically defended insects, such as larvae of danaid butterflies, are solitary (Ackery and Vane-Wright 1984).

Several features are required for the evolution of exogenous chemical defense. Feeding on prey that contain defensive chemicals is a prerequisite for sequestering those compounds (Bowers 1992). Detoxification mechanisms that would normally break down ingested secondary metabolites must be circumvented and the secondary metabolites stored in such a way that they can be delivered by the new host when attacked (Bowers 1992). A secondary metabolite is defined as any chemical substance not directly involved in fundamental life processes. They are often assumed to have a defensive function (Berenbaum and Seigler 1992). In most cases, organisms that sequester their defense from chemically defended prey also specialize on that prey type (Futuyma and Gould 1979, Price 1983, Strong et al. 1984) because the predator of defended prey first must have physiological processes to render it immune to the effect of the chemical defense of the prey. Further, to use the defenses of the prey for protection, the predator must possess even more specialized physiological adaptations to incorporate the defensive compound (Bowers 1992). This presumably requires considerable co-evolution.

In addition to exogenous sources of chemical defense, many chemically defended insects are capable of synthesizing secondary compounds *de novo*. Insects which use an endogenous chemical defense typically produce the defensive chemicals in exocrine glands (Tschinkel 1975, Deroe and Pasteels 1982). In some species, the glands do not make the final product but only precursor components of the defensive chemical which are mixed just prior to discharge (Eisner 1970, Eisner et al. 1977). Ontogenetically, such glands originate as epidermal invaginations (Noirot and Quennedy 1974). Pasteels et al.

(1990) suggest that in the Chrysomelidae (leaf beetles), sequestration of chemical defenses was a derived feature and that *de novo* synthesis was ancestral. In addition to Chrysomelids, a variety of arthropods are capable of biosynthesizing secondary compounds (Blum 1981). Examples include tenebrionids (darkling beetles) (Tschinkel 1975), Carabid beetles (Schildknecht 1970), millipedes (Esiner et al. 1967), and even cockroaches (Roth and Stay 1958).

Chemical defense and marine organisms

In contrast to terrestrial arthropods, specialized feeding by marine herbivores is rare (Lubchenco and Gaines 1981, Hay et al. 1988 a, c, Hay and Fenical 1988b). Hay (1992) proposed that sequestering of chemical defenses from prey is less common in the marine environment when compared to the terrestrial environment because there are fewer dietary specialists in the marine environment. Some nudibranchs acquire defensive compounds from the sponges, coelenterates, bryozoans, or tunicates that constitute their diet (Paul and Pennings 1991, Rogers and Paul 1991, Paul 1992, Slattery et al. 1998, Johnson and Willows 1999). The butter clam, *Saxidomus giganteus*, sequesters dinoflagellate toxins from its planktonic diet as a means to deter predators (Kvitek 1989). Ascoglossans feed by sucking algal sap mainly from *Caulerpa* spp. and are widely assumed to derive their chemical defense from algal metabolites; however, this interaction has not been rigorously tested (Hay 1992).

Endogenous chemical defense in marine organisms appears relatively common. The most potent producers of endogenous defensive compounds in the marine environment are typically soft bodied and sessile. They include such diverse groups as corals (Pawlik et al. 1987, Coll et al. 1982, Coll and Sammarco 1983), sponges (Pawlik 1993, Pawlik et al. 1995, Lindquist and Hay 1996, Chanas et al. 1997), holothurians (Bakus 1974, Burnell and Apsimon 1983), and algae (Hay et al. 1988, Hay and Fenical 1988, Duffy and Hay 1994, Paul et al. 1987). It is commonly assumed that because these organisms lack other defensive abilities, such as evasion or mechanical defense like armor, natural selection has favored the development of chemical defense using endogenous secondary metabolites.

Research by Fraenkel (1959) and Ehrlich and Raven (1965) provided the first evidence that secondary metabolites are a defensive adaptation to predation pressure (Harborne 1982). Since that time, this area of research has gained much attention and the diversity of known secondary metabolites now includes over 30,000 unique compounds (Harborne 1982). Especially vulnerable structures tend to be better defended (Bingham and Braithwaite 1986), and the production of secondary compounds can be induced by the presence of or damage by predators (Van Alstyne 1988, Harvell 1990).

Evidence for chemical defense among crustaceans is sparse. This is surprising given the diversity of marine organisms that are chemically defended, the speciose nature of the Crustacea, and the wide-spread occurrence of chemical defenses in other arthropods (Berenbaum and Seigler 1992). Lucken and Orth (1990) suggest that the higher rejection rate of larval pea crabs *Pinnotheres ostreum* versus larval blue crabs

(*Callinectes sapidus*) by the mummichog (*Fundulus heteroclitus*) was due to an unidentified chemical defense. In another case, de Freitas (1990) reported that a digestive secretion regurgitated by some brachyuran decapod crustaceans affected the feeding behavior of the predatory marine gastropod *Nassarius vibex*. However, amphipod crustaceans may employ an “indirect” chemical defense. For example, some amphipods reduce their predation risk by living on chemically defended algae (Hay et al. 1987). The Antarctic amphipod *Hyperielle dilatata* uses a chemically defended pteropod as a shield against fish predators (McClintock and Janssen 1990).

Recent research indicates three species of gammarid amphipods (*Chromopleustes oculatus*, *Ch. lineatus* and *Cryptodius kelleri*) are chemically defended against predatory invertebrates and fish (Norton and Stallings 1999, Zalewski 2001). All three species are brightly colored, occur conspicuously in the open and at high densities on a variety of substrates in the subtidal zone of the Strait of Juan de Fuca, WA (Norton and Stallings 1999), yet they are rarely consumed by common amphipod predators (Norton, personal communication). Zalewski (2001) showed, through prey choice experiments in the laboratory, that the adults of *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* were not eaten by predatory fish (*Artedius harringtoni* or *Jordania zonope*) that had prior experience with these amphipods in the wild. Laboratory observations reveal that when physically disturbed, all three amphipod species expel fluids from their mouths that are distasteful to fish and invertebrates that feed on amphipods (Norton, personal communication). These defensive compounds are stored in the digestive tract, particularly the midgut caecae. A similar phenomenon has been observed in insect-plant interactions, in which the insect

herbivore sequesters secondary compounds from plants in its diet and then uses these compounds for its own defenses (Harborne 1993). For example, Eisner (1970) demonstrated that the enteric regurgitates of two grasshoppers, *Romalea microptera* and *Brachystola magna*, effectively repel aggressive worker ants.

The presence of chemically defended prey in the diet of a chemically defended species provides circumstantial evidence that the source of chemical defense in this species is exogenous. In previous research, *Chromopleustes oculatus* was found to consume chemically defended prey (*Cucumaria miniata*) (see chapter 1). The results for *Ch. lineatus* and *Cryptodius kelleri* did not positively identify a potential source of chemical defense in the diet; however, these dietary investigations were complicated by the limits of the means of analysis, fecal samples, from such small organisms (Quigley and Vanderploeg 1991). For this reason, few prey items (only those with hard body parts) were identifiable in the amphipod fecal pellets. Soft bodied prey were not detected by this method of diet analysis. Thus, there is a potential for chemically defended prey to have been overlooked in the diets of *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri*.

Even if *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* consume chemically defended prey, they may not become chemically defended themselves as a result. For example, while a variety of gammarid amphipods do feed on the chemically defended alga, *Dictyota dichotoma*, they remained palatable to predatory fish (Hay et al. 1987). This is also true of insects which may feed on chemically defended host plants but not acquire chemical defenses as a result (Isman et al. 1977, Blum 1983, Bowers and Puttick 1986, Rowell-Rahier and Pasteels 1986, Brattsten 1986). For this reason, I conducted

laboratory experiments to gain insight into the source of the chemical defenses observed in *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri*.

Objectives of the research

This research addresses two questions: (1) When during the life cycle of *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* is a chemical defense first acquired? (2) Are the defensive chemicals synthesized by the amphipods (i.e., endogenous) or do they acquire them from their prey (i.e., exogenous)? I will exploit the life history pattern of amphipods to address the first question. Amphipods and other paracarid crustaceans (isopods, mysids, etc.) have direct development. The female amphipod retains her fertilized eggs in a brood pouch made from modified thoracic appendages. The larvae hatch but remain in the brood pouch until they become juveniles, thereby eliminating an extensive pelagic larval phase that is characteristic of most other crustacean taxa (Bousfield 1995). Therefore, I can collect all life cycle stages (fertilized eggs, brooded juveniles and adults) and subject each stage to predatory fish in order to determine the ontogeny of chemical defense for each of the three species of amphipods. To test the ability of the amphipod to regenerate defensive chemicals, I will compare the palatability of adult amphipods that have been depleted of their chemical defenses and subsequently starved with those allowed to feed on chemically defended prey.

MATERIALS AND METHODS

Field collections and maintenance of prey and predators

Ch. oculatus, *Ch. lineatus* and *Cr. kelleri* were collected from the subtidal using SCUBA off of Bell Island during May - July of 2001. Bell Island is a small rocky island (0.03 km²) located in Wasp Passage, San Juan Islands, WA. It is characterized by rocky slopes that drop rapidly to a maximum depth of 35m and strong currents driven by mixed semi-diurnal tides. *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* are commonly found on the fronds of *Agarum fimbriata* and *Laminaria saccharina*. Intact fronds bearing the amphipods were each enclosed in a large (70cm x 95cm) plastic bag. The bag was then tied shut and brought to the surface. At the lab, each bag was emptied into a sorting tray with seawater. Gravid females were removed and placed in individual containers of filtered seawater. These females provided a source of eggs and newly released juveniles for subsequent predation experiments. Adults from these collections were also retained for use in experiments on the regeneration of defensive chemicals.

Several types of palatable prey were used as controls in the fish feeding experiments or during "training" and maintenance of predatory fish between experiments. Control eggs were obtained from gravid female glass shrimp (*Hippolyte clarki*). Gravid *H. clarki* were collected from the same algal blades as the three species of defended amphipods, returned to the lab, and maintained in an aquarium. Eggs were siphoned from the pleopods by pipette for use in the predation experiments. The palatable gammarid amphipod, *Allorchestes* spp., and the harpacticoid copepod, *Tigriopus californicus*, were used as a source of food for maintaining fish in the lab and as controls

in fish feeding experiments. Both species were collected from the intertidal zone at Eagle Cove, San Juan Is., WA. *Allorchestes* were found in high densities under detrital algae. *T. californicus* were dip netted from small tide pools that filled rock crevices in the supra littoral zone. In the lab, *Allorchestes* and *T. californicus* were held separately in large plastic containers partially submerged in flow-through sea water tables at ambient temperatures, 9 - 12°C.

Young of the year *Clinocottus acuticeps* were used as predators in the feeding experiments with eggs and juveniles. Young of the year *Oligocottus maculosus* were used as predators in the feeding experiments with adult amphipods. These fish were collected by dip net from tidepools at Eagle Point on San Juan Island. *Cl. acuticeps* and *O. maculosus* were chosen as the predators in my experiments because they are abundant, available at an appropriate size for consumption of *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri*, and are common amphipod predators in the field (Cross 1981). However, *Cl. acuticeps* and *O. maculosus* occur intertidally and are therefore unlikely to encounter subtidal species like *Ch. oculatus*, *Ch. lineatus* or *Cr. kelleri* in the wild. Thus, *Cl. acuticeps* and *O. maculosus* are naive to the chemical defense of these amphipods and do not avoid them when initially encountered.

Cl. acuticeps were held in plastic containers (20 x 25 x 7 cm or 15 x 20 x 7 cm) that were partially submerged in a flow-through sea water table to maintain ambient seawater temperature. The bottoms of the holding containers were covered with sand and small rocks to provide hiding places. *O. maculosus* were placed in individual plastic containers (31 x 18 x 12 cm) that also served as feeding arenas for these fish. All fish

were acclimated to lab conditions for at least one week before feeding experiments began and were maintained on a diet of *Allorchestes* spp. and copepods. Prey were introduced to the holding containers twice a day by pipette so that the fish would become accustomed to this feeding method. Most *Cl. acuticeps* ate readily within a day of being brought into the lab, while *O. maculosus* generally needed a week to become acclimated to the lab.

Ontogeny of chemical defense: are eggs defended?

To assess if the chemical defense in the three defended gammarid amphipod species is passed from mother to offspring when the egg is provisioned, I compared the feeding response of *Cl. acuticeps* when fed eggs removed from the brood pouch of the three amphipods with that of eggs removed from the pleopods of the palatable shrimp *H. clarki*. To collect fertilized eggs from a female amphipod, she was first placed in a finger bowl and beheaded to prevent her from releasing copious amounts of defensive chemicals. Eggs were then siphoned from the brood pouch using a pipette, washed three times with approximately 100 ml of filtered seawater (approximately 5µm pore size) and placed in a finger bowl containing filtered seawater. Five to eight broods of eggs from each of the three species of defended amphipods were used in these feeding trials. The individual broods for a particular species were labeled and used in random order during a feeding trial to account for potential inter-brood differences in level of defense.

The feeding experiments assessing the palatability of amphipod versus shrimp egg by *Cl. acuticeps* were carried out in 8 x 8 cm plastic containers. The bottoms of these

containers were not covered with sand because sand made it difficult to see the eggs during feeding trials. Fish (each approximately 20 mm total length) were transferred to feeding arenas the day before feeding trials to allow them time to adjust to their new containers. Fishes were not starved prior to a feeding experiment; however, the number of maintenance prey (*Allorchestes* spp.) was reduced to 2 – 4 individuals on the day of an experiment to minimize complications resulting from starvation or satiation.

During a feeding experiment, a *Cl. acuticeps* was first offered four *H. clarki* eggs to determine if the fish was willing to eat. If the fish ate, it was then offered an egg from *Ch. oculatus*, *Ch. lineatus* or *Cr. kelleri*. The presentation of eggs to *Cl. acuticeps* then alternated between one from the non-defended *H. clarki* and one from a different brood of the same “defended” amphipod species, until an egg from each available amphipod brood had been presented to the fish. This process was repeated with a different fish until a total of at least eight eggs from broods of the same amphipod species had been presented to each fish for consumption or rejection. Ten fish were normally used to complete the feeding trials for a single life cycle stage of each amphipod species. This entire process was repeated on subsequent days using eggs from one of the other two species of “defended” amphipods.

The reaction of *Cl. acuticeps* to control and amphipod eggs was assigned to one of three categories: 1) consumed, 2) rejected, or 3) ignored prey. Consumed prey were those that the fish ingested. Rejected prey were captured but then spit out by the fish. An ignored prey item (prey that were obviously noticed by the fish but not eaten) was removed and presented again. If a prey item was ignored twice, it was recorded as an

ignore. Ignore reactions were excluded from the statistical analysis. My analysis thus examined whether amphipods were rejected once tasted, not whether they were avoided without being sampled by the fish.

Analysis

The central question is do eggs of the amphipods *Ch. oculatus*, *Ch. lineatus* or *Cr. kelleri* elicit a rejection response by the predator? The categories, accept or reject, reflect the ultimate outcome for the amphipod and therefore represent the most relevant data for determining defended or non-defended status. During a feeding bout, a fish was presented a series of alternating prey types (e.g., amphipod egg, control egg, amphipod egg, ...) until it had been exposed to a total of eight “defended” prey and eight control prey. Accordingly, the responses by the fish within a feeding bout cannot be used as independent data points in a statistical analysis because exposure to defended prey early in the bout may have influenced the response by the fish to defended prey later in the bout. Therefore, a scoring system was developed to generate an average response score for “defended” prey and control prey for each fish for each feeding bout.

To convert my raw data into a form that could be tested statistically, a numeric value was assigned to each response category (1 = consumed, and 2 = rejected). The number of responses a fish made in a particular category was multiplied by the appropriate numeric value. Therefore, if a “defended” prey score for an individual fish was 4 consumed and 4 rejected, its total score would have been $(4 \times 1) + (4 \times 2) = 12$, with an average response score of 1.5 (12/8 prey). In this scoring system, a higher value

for the response score is indicative of a higher frequency of rejection responses. A response score for control prey was calculated by the same method. The “defended” and control prey response score were compared for statistically significant differences by a Wilcoxon signed rank test.

Ontogeny of chemical defense: are newly released juveniles defended?

To assess if the chemical defense in the three defended gammarid amphipod species is present as the brood is released from the brood pouch of the mother, I compared the feeding response of *Cl. acuticeps* when fed newly released broods (hereafter juveniles) from the three amphipods with that of comparably sized non-defended amphipod, *Allorchestes*. The juvenile amphipod feeding trials were based on the assumption that juvenile *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* do not feed exogenously while in the brood pouch, and do not return to the brood pouch after leaving it. Both are rare behaviors in amphipods (Bousfield 1973) thus, free swimming juveniles collected from brooding females maintained in the lab were assumed to have received no nutrition other than that originally supplied in the egg.

Data on the palatability of newly released juveniles of each species were collected from May to late June, 2001. The timing of brood releases by *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* is not simultaneous; therefore, the two month time span was necessary such that juveniles of all three species could be collected. Average, minimum, and maximum brood size and the time frame over which juveniles were collected is recorded in Appendix A.

Individual gravid *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* were placed in separate containers containing filtered seawater. These were kept partially submerged on a flow-through sea water table. When the female released her brood, the juvenile amphipods were removed from the parent container with a plastic pipette. Broods of newly-released amphipods were collected daily. Each brood of juveniles was placed in a new container of filtered seawater separate from its mother. Experiments began when a total of five to eight separate broods of juveniles from each species were collected. The broods for a particular species were labeled and used in random order during a feeding trial to mitigate against potential inter-brood differences in level of defense.

Palatability was tested using *Cl. acuticeps* approximately 35 mm in length in round feeding arenas measuring 12 cm in diameter and 10 cm in height. The bottom of the feeding arena was covered with a thin layer of sand such that the new container resembled the holding containers. Fish were transferred to feeding arenas the day before feeding trials to allow sufficient time for the fish to adjust to their new containers. Feeding trials with newly released juveniles followed the same procedure and analysis used in experiment described above for eggs with the exception that the palatability of juvenile *Ch. oculatus*, *Ch. lineatus*, and *Cr. kelleri* was compared with that of comparably sized juveniles of *Allorchestes* spp., a non-defended, palatable amphipod. The reaction of *Cl. acuticeps* to each food item presented was recorded using the categories of predator response described in the eggs feeding experiments.

Recordings of fish behavior during feeding trials were amenable to analysis that included a gradient of predator responses. Predator responses were recorded as one of

four categories: swallowed prey, coughed and swallowed prey, coughed and spit out the prey and spit out prey without coughing. Coughing described a forceful movement where the fish thrusts its mouth open and flares the opercula. In this gradient, a direct swallow (1) was considered a response to a non-distasteful prey. Coughed and swallowed (2) was viewed as a reaction to weak distastefulness. The cough and spit reaction (3) was viewed as weaker distastefulness than an immediate spit (4). In analyses, these four response categories were converted into a response score similar to that used in the egg and juvenile feeding experiments described above with the exception that each response was weighted to represent the gradient of distastefulness (1 = swallow, 2 = cough and swallow, 3 = cough and spit, 4 = spit). Control prey response scores were compared to these data for significant differences by a Wilcoxon signed rank test.

The use of up to eight individuals from the same brood on multiple *Cl. acuticeps* allowed for a comparison of responses by multiple predators to a single brood. A homogeneity Chi-square was used to test for consistent responses by predators among broods to identify broods that were significantly different from others in the frequency of consume or reject responses by predators. Although this type of analysis is confounded by the possibility that some individual *Cl. acuticeps* may be more sensitive to the defensive chemical than others, this possibility was controlled for by only comparing broods that were exposed to the same set of predators and is an ecologically relevant comparison of variation in defense.

Ontogeny of chemical defense: are adults defended?

To complete the analysis of ontogenetic patterns in chemical defense among the three defended amphipod species, data on the response of *O. maculosus* to post release, field-caught individuals were collected in June-July 2000 (Norton and Hutchinson in prep.). These experiments were conducted using the same procedure described above for experiments with eggs and juveniles. Because individuals of *O. maculosus* reach a larger size than those of *Cl. acuticeps*, they are a better match in size for the adult amphipods. This predator species demonstrates the same behavioral responses as *Cl. acuticeps* (swallow, cough/swallow, cough/spit, and spit). These responses were used to calculate predator response scores as described for egg and juvenile feeding experiments and were analyzed similarly. To examine changes in predator response across life stages, a Kruskal-Wallis test was used to compare predator response scores among eggs, juveniles and adults for each amphipod species. This was followed by Wilcoxon-Mann-Whitney tests to identify which life stages showed significant differences. Significantly rejected life stages indicated the onset of chemical defense in that species; P-values for these *a posteriori* test were adjusted using a Bernoulli correction.

Can *Ch. oculatus* regenerate defensive chemicals?

Because *Ch. oculatus* was the most abundant of the three species during my field season and are also more easily induced to release their defensive chemical, this species was tested for its ability to regenerate defensive chemicals. This work consisted of two parts. The first part was designed to test the ability of starved *Ch. oculatus* to regenerate the defensive chemical. The second part was designed to test the role of diet in the

regeneration of defensive chemicals. Both parts of the experiment were conducted over the same six-day period during the summer of 2001.

Preparation of amphipods

To deplete the amphipods of their defensive compounds, a group of 300-mid sized *Ch. oculatus*, approximately 5 mm in length, were transferred into a mesh container and placed in fresh water for one minute and then returned to sea water and allowed to recover for at least 30 minutes. Exposure to fresh water causes the amphipods to release defensive chemicals from the midgut caecae. This process was repeated three more times, for a total of four one-minute “dunks” for this group of amphipods. Preliminary trials indicated that four one-minute dunks are sufficient to deplete the supply of defensive chemicals in a *Ch. oculatus* individual and render it palatable. A control group of 300 *Ch. oculatus* were transferred to tanks of filtered seawater but not dunked.

Do starved *Ch. oculatus* regenerate their defense?

A group of depleted amphipods and a group of control amphipods were held for up to six days during the duration of this experiment. Two plastic aquariums (40 x 20 x 30 cm) were filled with filtered sea water, placed in a flow-through sea water table to maintain temperature and aerated to ensure adequate dissolved oxygen. In these aquariums, 96 of the 300 depleted amphipods and 96 of the control amphipods were isolated and not provided with food during the six days of this experiment. The

palatability of these amphipods was tested using the predator, *Cl. acuticeps*, measuring approximately 35mmTL, on the same day as dunking occurred (day zero), and two and six days after the dunking. These predation trials were carried out as described previously for predation experiments on eggs, juveniles and adults.

Does diet influence regeneration?

The second part of this work assessed the role that food may play in regenerating chemical defenses in *Ch. oculatus*. Both laboratory observations and fecal collections from the field (see results, chapter 1) indicate that the diet of *Ch. oculatus* includes both crustaceans and sea cucumbers, including *Cucumaria miniata*. *C. miniata* is known to possess defensive compounds (saponins) that deter predation by fishes (Bingham and Braithwaite 1986), while *P. danae* is not known to produce defensive chemicals.

Accordingly, I set up the following groups: (1) 32 depleted *Ch. oculatus* that were provided with pieces of tail meat from *P. danae*; (2) 32 control *Ch. oculatus* with pieces of *P. danae*; (3) 32 depleted *Ch. oculatus* in a holding tank with a live sea cucumber, *Cu. miniata*; and (4) 32 control *Ch. oculatus* in a holding tank with a live *Cu. miniata*.

Amphipods were allowed to feed for five days and then offered to *Cl. acuticeps* on day six in feeding experiments like those described for eggs and juveniles: However, in these experiments, only four prey were offered to the fish since amphipods used in these experiments were large enough to cause satiation of the predators when offered in higher numbers. A feeding trial consisted of a palatable control (*Allorchestes* spp.), two depleted *Ch. oculatus* alternated with palatable control amphipods (*Allorchestes* spp.),

and a non-depleted *Ch. oculatus* followed by a final palatable control amphipod (*Allorchestes* spp.). Prey were offered in this order so that a distasteful experience with non-depleted *Ch. oculatus* would not effect the response by the fish to depleted *Ch. oculatus*.

Analysis

Response scores (consumed vs. rejected) of predatory fish feeding on starved or fed *Ch. oculatus* were determined as described earlier for the feeding trials on eggs, juveniles and adults. A Wilcoxon signed-rank test was used to evaluate the feeding responses of fish on starved depleted *Ch. oculatus* and on the palatable amphipod *Allochestes* spp. for day zero of the experiment. A Kruskal-Wallis test was used to compare feeding responses on starved depleted *Ch. oculatus* among days 0, 2 and 6 for equality of response scores. This analysis, if significant, was followed by an *a posteriori* Wilcoxon-Mann-Whitney tests with a Bernoulli correction to identify those days in which significantly different feeding responses occurred. A Kruskal-Wallis test was used to assess differences in the response of predatory fish on day 6 among starved depleted *Ch. oculatus*, depleted *Ch. oculatus* that had subsequently been allowed to feed on *P. danae* (not chemically defended) and dunked *Ch. oculatus* that were allowed to feed on *C. miniata* (chemically defended).

RESULTS

Ontogeny of chemical defense

While a wide variety of marine and terrestrial invertebrates pass chemical defense to their offspring via materials packaged in the egg, there is no evidence that the eggs of chemically-defended gammarid amphipods received this type of chemical defense. A total of 193 eggs were tested for palatability (67 for *Ch. oculatus*, 63 for *Ch. lineatus* and 63 for *Cr. kelleri*). All of the eggs were consumed with no indication of distastefulness to the predators; hence, there was no indication that the eggs from any of the three species of amphipods were chemically defended. (Figure 2-1).

In palatability tests with pre-feeding juveniles, *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* did not evoke equivalent responses from the predators. While juvenile *Ch. oculatus* experienced 20 % rejection by predators, this was not significant when compared to *Allorchestes* spp. (Wilcoxon signed rank test, $P = 0.125$). All juvenile *Ch. lineatus* were consumed. Juvenile *Cr. kelleri*, however, were rejected significantly more often than the palatable control prey (Wilcoxon signed rank test, $P = 0.002$) (Fig. 2-1).

Analysis that incorporated the gradient of predator responses to prey (1 = swallow, 2 = cough and swallow, 3 = cough and spit, 4 = spit) showed that juvenile *Ch. oculatus* evoked a higher frequency of cough and swallow, cough and spit, and spit than the palatable control, *Allorchestes* (Wilcoxon signed rank test, $P = 0.008$) (Fig. 2-2). Similarly, juvenile *Ch. lineatus* showed a significantly higher frequency of cough and swallow responses by predators but did not produce any response above this level

Figure 2-1. Percent consumption by the predator *Cl. acuticeps* for eggs (E) and juveniles (J) and the predator *O. maculosus* for adults (A) of the three “defended” amphipod species. Values are means $\pm$ standard error.

Percent consumed (mean $\pm$ s.e.)

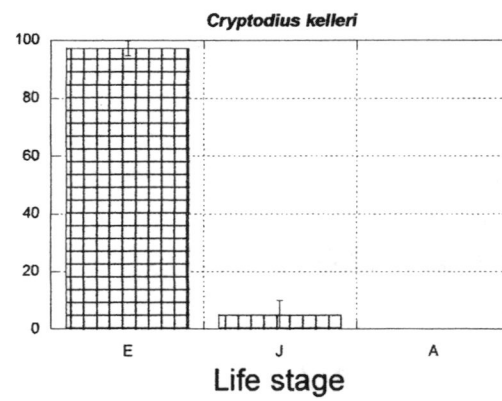
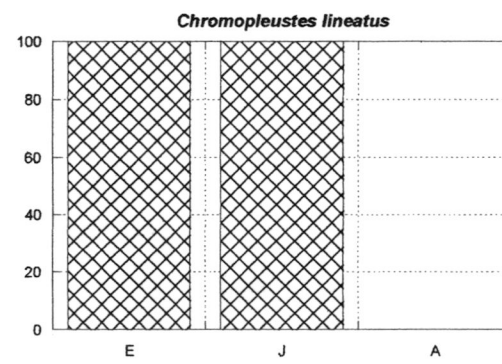
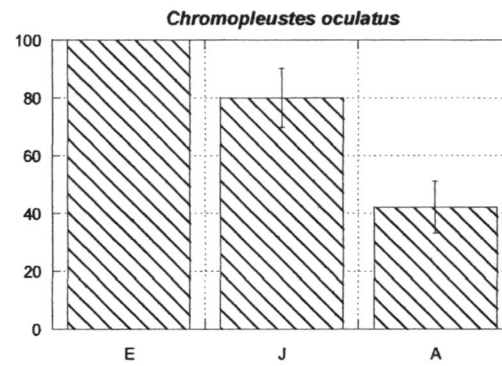
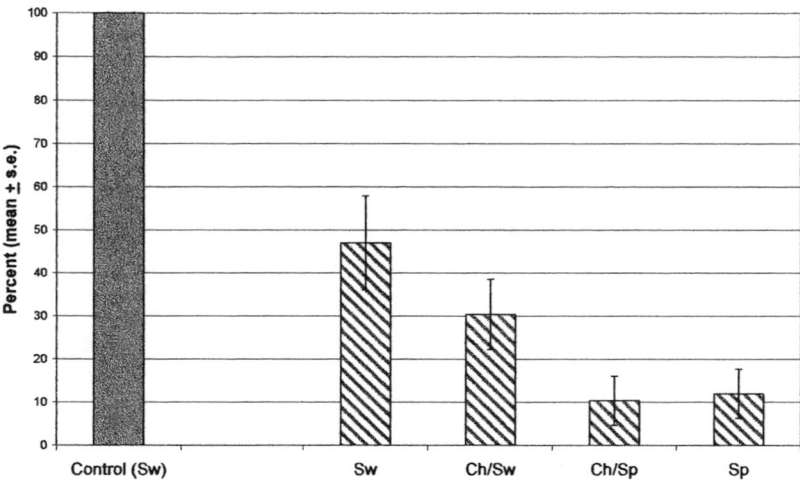
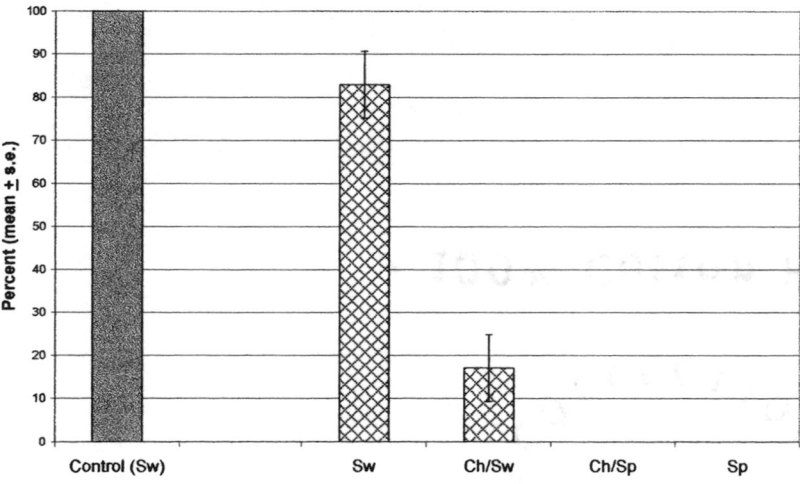


Figure 2-2. Behavioral responses of *Cl. acuticeps* to pre-feeding juvenile *Ch. oculatus* and *Ch. lineatus* compared to their behavioral response to the control amphipods, *Allorchestes* spp. Responses are abbreviated as follows: Swallow = Sw, cough and swallow = Ch/Sw, cough and spit = Ch/Sp and spit = Sp. Values are means $\pm$ standard error.

Chromopleustes oculatus



Chromopleustes lineatus



(Wilcoxon signed rank test, $P = 0.031$) (Fig. 2-2). All but one juvenile *Cr. kelleri* were spit out immediately.

There was some evidence for variation in effectiveness of the chemical defense against predatory fish among broods of juveniles. There was a significant difference in the categories “consume” vs. “reject” among juvenile *Ch. oculatus* ($X^2 = 13.59$, $df = 3$, $P < 0.005$). That is, some broods of this species experienced greater consumption by the predators than others (Table 2-1). The consumption of *Ch. lineatus* by *Cl. acuticeps* was not different from their consumption of the non-defended control amphipods as both juvenile *Ch. lineatus* and *Allorchestes* spp. were always consumed. Juvenile *Cr. kelleri* were always rejected by *Cl. acuticeps*; thus there was no observable difference in reactions by predators to this species.

Naïve *O. maculosus* rejected the adults of all three defended amphipods (Wilcoxon signed rank test, all P -values < 0.008). Adult *Ch. oculatus* were rejected in a little over half (58%) of the trials compared to the control, comparably sized *Allorchestes*, which were never rejected. Adult *Ch. lineatus* and *Cr. kelleri* were never consumed during feeding trials with the predator *O. maculosus* (Fig. 2-1).

Unpalatability to predatory fish developed at different life stages among *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* (Kruskal-Wallis test, all P -values < 0.00001). *Ch. oculatus* and *Ch. lineatus* did not evoke a significant rejection response by predatory fish until the adult stage (Wilcoxon-Mann-Whitney test, P -values < 0.004). Of the three species, *Cr. kelleri* showed evidence of chemical defense earliest in the life history,

Table 2-1. Percent consumption by *Cl. acuticeps* of broods of juveniles released from female *Ch. oculatus*. Each brood was exposed to the same set of seven *Cl. acuticeps*. Each predator tasted 10 newly-released *Ch. oculatus*.

Species	Brood	% Consumed	% Rejected	# of predators
<i>Ch. oculatus</i>	#134	64	36	7
	#140	79	21	7
	#25	79	21	7
	#113	86	14	7
	mean	77	23	
	std.err.	± 8.9	± 8.9	

evoking a rejection response by predatory fish as a newly released juvenile (Wilcoxon-Mann-Whitney test, $P = 0.0001$).

Regeneration of chemical defense by *Ch. oculatus*

While repeated dunking in freshwater was an effective means to deplete the chemical defense in *Ch. oculatus*, individuals were capable of restoring their defense in as little as two days (Fig. 2-3). *Ch. oculatus* were as palatable as the non-defended control amphipods after four dunks in freshwater on day zero (Wilcoxon signed rank test, $P = 1.0$). After two days without access to food, these depleted amphipods were rejected significantly more often than the palatable control food (Wilcoxon signed rank test, $P = 0.0001$) and were also rejected significantly more often than depleted amphipods on day zero (Wilcoxon-Mann-Whitney test, $P = 0.0015$) (Fig. 2-3). There was no significant difference in rejection between depleted amphipods tested on day two and depleted amphipods tested on day six (Wilcoxon-Mann-Whitney test, $P = 0.2824$). However, even after six days, depleted amphipods were not rejected with the same frequency as *Ch. oculatus* that were never depleted of their chemical defense (non-dunked control) (Wilcoxon signed rank test, $P = 0.0005$) (Fig. 2-3).

The opportunity to feed on defended prey did not enhance the ability of *Ch. oculatus* to regain its defense. There were no significant differences among dunked amphipods that were starved for six days or provided with access to chemically-defended sea cucumbers or with palatable shrimp *Ch. oculatus* (Kruskal-Wallis test, $P = 0.30$)

(Figure 2-4). Thus, diet does not seem to play a role in acquisition of chemical defense in *Ch. oculatus*.

Figure 2-3. Percent consumption (mean $\pm$ standard error) by the predator *Cl. acuticeps* of *Ch. oculatus* that were exhausted of their defensive chemical (day 0) and two days and six days later. These amphipods were not provided with food.

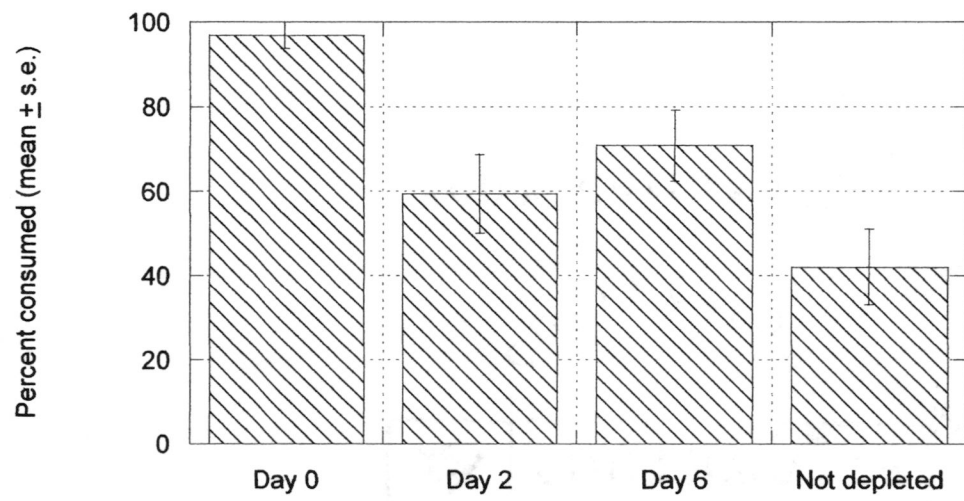
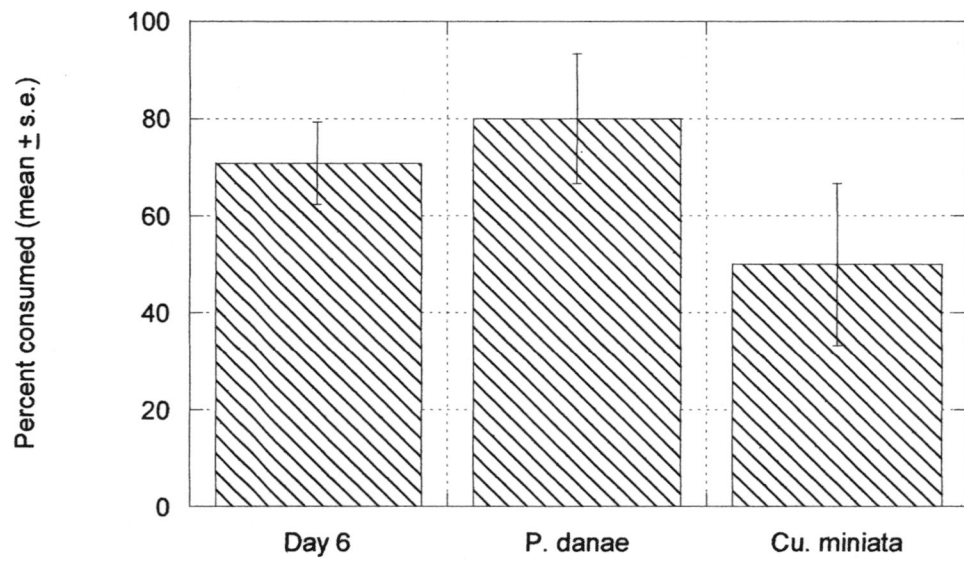


Figure 2-4. Percent consumption (mean $\pm$ standard error) by the predator *Cl. acuticeps* of *Ch. oculatus* after the amphipods were exhausted of defensive chemical on day zero and then starved for six days (starved), fed a non-defended diet of *Pandalus danae* (P. danae) or a defended diet of *Cucumaria miniata* (Cu. miniata) for six days.



DISCUSSION

Ontogeny of chemical defense

Ch. oculatus, *Ch. lineatus*, and *Cr. kelleri* were dissimilar in the schedule of development of chemical defense through the life stages: egg, newly-released-juvenile and adult. The eggs of all three species were consumed. At this point in the life history none were defended against predatory fish. *Ch. oculatus* and *Ch. lineatus* at the juvenile stage were not significantly rejected; however, an analysis that included the gradient of predator responses (swallow, cough and swallow, cough and spit, and spit) showed there were significant differences between controls and these species when “coughing” by the fish was considered an indication of distastefulness. In this analysis, 51% of pre-feeding juvenile *Ch. oculatus* received a response of cough and swallow or higher and 19% of pre-feeding juvenile *Ch. lineatus* received a response of cough and swallow. The differences in predator behavior when tasting these amphipods compared to the palatable control may indicate a weak level of chemical defense and may mark the beginning of chemical defense development in these species. *Cr. kelleri* was rejected by predators as juveniles at the time of release from the brood pouch and therefore showed a fully developed chemical defense at the pre-feeding stage. The evidence of a weakly developed chemical defense in pre-feeding juveniles of the two *Chromopleustes* species, and a fully developed chemical defense in pre-feeding juveniles of *Cr. kelleri* preceded in all three species by palatable eggs, is indicative of an endogenous source of chemical defense.

Transmission of defenses from parents to offspring has been reported in a variety of marine organisms. Chemical defenses have been reported in asteroid eggs (Lucas et al. 1979, McClintock and Vernon 1990), ascidian larvae (Young and Bingham 1987, Lindquist et al. 1992), and the egg masses of a nudibranch (Pawlik et al 1988). In most cases, the chemically defended larvae of these organisms are brooded lecithotrophic larvae (non-feeding), relatively large and conspicuously colored, and quickly settle to the adult habitat (i.e. did not have an extensive planktonic stage) (Lindquist 1996).

In these amphipod experiments, all brooded eggs were palatable as well as the juveniles of the two *Chromopleustes* species. Orians and Janzen (1974) asked “why are embryos so tasty?” after noting that many organisms lose large proportions of their eggs or larval stages to predators and pointed out that the evolution of distasteful eggs and larvae should be favored. They hypothesized that eggs and larvae generally lack chemical defenses due to autotoxicity resulting from a fundamental incompatibility of actively growing tissues and secondary metabolites used in defense (Orrians and Janzen 1974). Energetic constraints may also limit investment in secondary metabolites in a trade off between deterrence and potential development rates (Orrians and Janzen 1974). Interestingly, *Ch. oculatus*, the least well defended as an adult of the three species tested, had the highest fecundity measured during my study (Appendix A). Life history theory predicts higher fecundity for organisms that suffer high mortality from predation (Green 1967). For example, *Daphnia lumholtzi* exposed to fish predation as adults had higher reproductive effort and lower growth rates than individuals that developed in the absence of fish predators (Green 1967).

There is an energetic cost to *de novo* synthesis of chemical defense (Bowers 1992). This cost is derived from the diversion of resources that might otherwise be used for growth, for the production of defensive compounds (Bowers 1992). If an individual experiences a cost for being less palatable than other individuals in the population then it may be to the advantage of some individuals to cheat, to produce less or even no defense, and yet gain protection from predation by predators who have experience with the less palatable individuals (Bowers 1992). Substantial variation in the defensive chemical content of individuals within a population has been documented in a diversity of systems (Brower et al. 1968, 1982, 1984; Bowers 1980; Gardner and Stermitz 1988; Jones et al. 1987, 1989). Because the individuals are the same species, the visual resemblance is exact. This phenomenon has been called automimicry (Brower et al. 1968, 1970). The relative abundance of models and automimics in the population, coupled with the predation pressure and the discriminatory ability of the predators, will determine the efficacy of automimicry.

The palatability experiments with juveniles of *Ch. oculatus* provided possible evidence of automimicry. Broods of pre-feeding juvenile *Ch. oculatus* were not consumed equally by *Cl. acuticeps*. It is useful to use broods in feeding trials with predatory fish to look for evidence of automimicry because genetically similar individuals (siblings) were exposed to multiple predators, and the consistency of the responses by predators to a brood analyzed. One possible explanation for variation in consumption of broods is that individuals of some broods may start to develop the defensive chemical earlier than individuals of other broods. Eventually all broods may

reach the same level of defense. A second possible explanation is that some individuals produce a higher concentration of the defensive chemical. Recall that newly-released juvenile *Ch. oculatus* experienced rejection in 20 % of encounters with predatory fish. Even though this level of rejection was not significantly different from the palatable control amphipods, it showed evidence that some newly-released *Ch. oculatus* are better defended against predatory fish than those which were consumed. When some individuals of the species are chemically defended against predators while other are not or are less defended, this is considered automimicry. Because adults of this species are sometimes consumed there is evidence the phenomenon of automimicry may play an important role in adult populations as well.

Regeneration of chemical defense by *Ch. oculatus*

Ch. oculatus depleted of chemical defense was able to regenerate its chemical defense in a few days regardless of the quantity or quality of food. Starved amphipods were as well defended as fed amphipods regardless of the prey offered after a period of six days. It appears, based on these experiments, that the source of chemical defense in *Ch. oculatus* was independent of diet and was produced *de novo* rather than sequestered from prey items.

For organisms that produce defensive compounds *de novo*, variation in the amount and thus effectiveness of the defense may depend on a variety of factors such as the number of recent discharges (Eisner 1965) and the time since the last use of the defense (Eisner 1965, Abushama 1972). *Ch. oculatus* depleted of their defensive

compound, regained defensive ability within two days however; even after six days, these amphipods were not rejected with the same frequency as *Ch. oculatus* that were never depleted of their chemical defense. When defensive chemicals cannot be replenished quickly, conservation (i.e., reluctance to use the defense) of these chemicals is important for maintaining defensive ability. Repeated dunks in freshwater were necessary to provoke regurgitation to the point of depleting the defensive chemicals of *Ch. oculatus*. This indicated a storage mechanism and an unwillingness to use the entire store of chemical on a single attack/offense.

The general conservativeness of arthropods regarding defensive secretions may reflect this inability to rapidly regenerate defensive compounds. Dethier (1939) observed that the froth produced by the arctiid *Apantes arge* was only partially regenerated 3-12 hours after the initial secretion had occurred. Eisner (1967) examined the cyanogenetic potential of two species of millipedes and demonstrated that after one week, both were able to produce and discharge less than 10% of their initial capacity and even after two months were not able to produce their defensive chemicals at initial levels.

Evaluation of endogenous versus exogenous chemical defense

Based on a review of intensive research of insect chemical ecology, Bowers (1992) proposed several requirements for the evolution of unpalatability due to sequestration of secondary metabolites from prey. The initial requirement is that the organism feeds on chemically defended prey. In chapter one I found that *Ch. oculatus* fed on chemically defended prey (*Cu. miniata* and potentially other sea cucumber

species). *Ch. lineatus* fed on unidentified echinoderms; so it is possible, but not conclusive, that this species fed on chemically defended prey. *Cr. kelleri* did not feed on chemically defended prey based on fecal analysis. Thus, only *Ch. oculatus* meets the first requirement for sequestration of chemical defense, although the possibility *Ch. lineatus* and *Cr. kelleri* may also feed on defended prey cannot be ruled out because of the limitations of fecal analysis.

Not all life stages of *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* were chemically defended. The absence of chemical defense at the egg stage in the life history indicated the mother did not pass the chemical defense to her offspring in any of the amphipod species studied. The rejection by predators of pre-feeding juvenile *Cr. kelleri* is indicative of *de novo* production of defensive compounds, since these individuals never had the opportunity to feed and therefore could not acquire defense through an exogenous source. *Ch. oculatus* and *Ch. lineatus* were not significantly rejected as pre-feeding juveniles and can only be considered defended at the adult stage. However, the frequency of “cough and swallow” reactions by the predators in feeding trials with these species may be an indication of a developing chemical defense at the pre-feeding juvenile stage.

The most conclusive evidence for *de novo* production of chemical defense among the three species studied was the regeneration of chemical defense within two days by *Ch. oculatus* that had been depleted of defensive chemicals and deprived of food. While this provides strong evidence for an endogenous source, it should be noted that even after depletion, there may be diet-derived precursors stored within the body tissues that are not

immediately available for use in defense. To investigate this possibility, future research might include identification of the defensive chemical used by these amphipods and comparison with possible matches found in their diets.

The selective agents for the adaptations of unpalatability and associated characteristics, such as warning coloration and gregariousness, are generally considered to be visually oriented predators (Bowers 1992). The dynamic relationship between the selective agents and the characteristics of chemically defended organisms has been studied extensively for terrestrial arthropods. In 1930, Fisher first suggested that kin selection was a prerequisite for the evolution of unpalatability and warning coloration. Because aposematic animals are often gregarious, the attacked individual could be killed, but in the process, the predator would learn to avoid other similarly-colored individuals, which were likely to be siblings in the same aggregation as the attacked individual. This suggestion became commonly accepted (Benson 1971, Harvey and Greenwood 1978, Harvey and Paxton 1981) until a series of experiments suggested that individual selection could also be invoked to explain the evolution of unpalatability and warning coloration (Jarvi et al. 1981, Wiklund and Jarvi 1982, Sillen-Tullberg 1985). These experiments showed that several species of aposematic insects, including lepidopteran caterpillars, beetles, and true bugs could survive attack by birds. Because the attacked individual survived, it was not necessary to invoke kin selection as an explanation for the evolution of unpalatability and warning coloration. Experiments with adult *Ch. oculatus*, *Ch. lineatus* and *Cr. kelleri* showed a 98% survival rate 24 hours after being attacked (Norton pers. comm.) and may provide additional support for the idea of individual selection.

The presence of chemical defense in adults of all three species and the nearly identical aposematic coloration of *Ch. oculatus* and *Ch. lineatus* creates the possibility of an association where both species benefit from being distasteful and having the same warning coloration since predators may learn to avoid both species after tasting either one or the other (Norton and Hutchinson in prep.). Because *Ch. oculatus* was less well defended than *Ch. lineatus* as an adult, there is the possibility of quasi-Batesian mimicry (Speed 1993). In this case a palatable or presumably more palatable species (*Ch. oculatus*) might benefit from the distasteful experience a predator had with the highly unpalatable species with similar coloration (*Ch. lineatus*). At the same time, because *Ch. oculatus* is far more abundant, it is more likely to function as a model than as a mimic. Also, *Ch. oculatus* may not have to be as defended individually because they occur in dense aggregations and may derive protection from this.

Interest in the chemical ecology of marine organisms has grown tremendously in recent years (Paul 1992). This research provided additional examples of the great potential the marine environment has as an area for research in chemical ecology and that the huge diversity of amphipods, in particular, may provide new opportunities to study the dynamics between predators and chemically defended species.

REFERENCES

- Abushama, F.T.E. 1972. The repugnatory gland of the grasshopper *Poecilocerus hieroglypicus*. J. Ent. A. 47: 95-100.
- Ackery, P.R. and R.I. Vane-Wright. 1984. Milkweed Butterflies: their Cladistics and Biology. Cornell University Press. Ithaca, New York.
- Avila, C. and V.J. Paul. 1997. Chemical ecology of the nudibranch *Glossodoris pallida*: Is the location of diet-derived metabolites important for defense? Mar. Ecol. Prog. Ser. 150: 171-180.
- Bakus, G.J. 1974. Toxicity in holothurians: A geographical pattern. Biotropica 6(4): 229-236.
- Bakus, G.R. 1981. Chemical defense mechanism on the Great Barrier Reef, Australia. Science 211: 497-499.
- Barlocher, F and B. Kendrick. 1973. Fungi and food preferences of *Gammarus psuedolimnaeus*. Arch. Hydrobiol., 72: 501-516.
- Barnard, J.L., and Karaman, G. S., 1991, The families and genera of marine gammaridean Amphipoda (except marine gammaroids), Records of the Australian Museum, Supplement 13 (part 1), 1-417, (part 2), 419-866.
- Bates, H.W. 1862. Contributions to an insect fauna of the Amazon valley. Trans. Linn. Soc. Lond. 23: 495-566.
- Benson, W.W. 1971. Evidence for the evolution of unpalatability through kin selection in the Heliconiinae. Am. Nat. 105: 213-226.
- Berenbaum, M., and D. Seigler. 1992. Biochemicals: engineering problems for natural selection. Pages 89-122 in B.D. Roitberg and M.B. Isman, (eds.), Insect Chemical Ecology, an Evolutionary Approach. Chapman & Hall, New York.
- Bingham, B.L. and L.F. Braithwaite. 1986. Defense adaptations of the dendrochirote holothurian *Psolus chitonoides* Clark. J. Exp. Mar. Biol. Ecol. 98: 311-322.
- Blum, M.S. 1981. Chemical Defenses of Arthropods. Academic Press, New York.
- Blum, M.S. 1983. Detoxication, deactivation, and utilization of plant compounds by insects. Pages 265-275 in P.A. Hedin (ed.), Plant resistance to Insects, ACS Symposium series 208. Amer. Chem. Soc., Washington, DC.

- Bousfield, E.L. 1973. Shallow-water Gammaridean Amphipods of New England. Connell University Press, Ithaca, New York.
- Bousfield, E.L. and E.A. Hendrycks. 1994. A revision of family Pleustidae, (Amphipoda: Gammaridea) Part I. Systematics and biogeography of component subfamilies. *Amphipacifica* 1: 17-57.
- Bousfield, E.L. and E.A. Hendrycks. 1995. The amphipod family Pleustidae on the Pacific coast of North America: Part III. Subfamilies Parapleustidae, Dactyopleustinae, and Pleusirinae. Systematics and Distributional Ecology. *Amphipacifica* 2: 65-133.
- Bowers, M.D. 1980. Unpalatability as a defense strategy of *Euphydryas phaeton* (Lepidoptera: Nymphalidae). *Evolution* 34: 586-600.
- Bowers, M.D. and G.M. Puttick. 1986. The fate of ingested iridoid glycosides, plants and herbivorous insects. Pages 133-165 in K. Spencer (ed.), Chemical Mediation of Coevolution. Academic Press.
- Bowers, M.D. 1992. The evolution of unpalatability and the cost of chemical defense in insects. Pages 216-245 in B.D. Roitberg and M.B. Isman (eds.), Insect Chemical Ecology, an Evolutionary Approach. Chapman & Hall, New York.
- Brattsten, L.B. 1986. Fate of ingested plant allelochemicals in herbivorous insects. Pages 211-255 in L.B. Brattsten and S.Ahmad (eds.), Molecular Aspects of Insect Plant Associations. Plenum Press, New York.
- Brower, L.P., W.N. Ryerson, L.L. Coppinger, and S.C. Glazier. 1968. Ecological chemistry and the palatability spectrum. *Science* 161: 1349-1351.
- Brower, L.P., F.H. Pough, and H.R. Meck. 1970. Theoretical investigations of automimicry. I. Single trial learning. *Proc. Natl. Acad. Sci.* 66: 1059-1066
- Brower, L.P., J.N. Seiber, C.J. Nelson, S.P. Lynch and P.M. Tuskes. 1982. Plant determined variation in the cardenolide content, thin-layer chromatography profiles, and emetic potency of Monarch butterflies, *Danaus plexippus*, reared on the milkweed, *Asclepias eriocarpa* in California. *J. Chem. Ecol.* 8: 579-633.
- Brower, L.P., J.N. Seiber, C.J. Nelson, S.P. Lynch and M.M. Holland. 1984. Plant determined variation in the cardenolide content, thin-layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus plexippus*, reared on the milkweed, *Asclepias eriocarpa* in California. *J. Chem. Ecol.* 10: 601-639.
- Brower, L.P. 1984. Chemical defense in butterflies. Pages 109-132 in R.I. Vane-Wright

- and P.R. Ackery (eds.), The Biology of Butterflies. Academic Press, New York.
- Burnell, D.J. and J.W. Apsimon. 1983. Echinoderm saponins. Pages 287-389 in P.J. Scheuer (ed.), Marine natural products: chemical and biological perspectives, Vol. 5. Academic Press, New York, pp. 287-389.
- Chanas, B., J.R. Pawlik, T. Lindel and W. Fenical. 1997. Chemical defense of the Caribbean sponge *Agelas clathrodes*. J. Exp. Mar. Biol. Ecol. 208: 185-196.
- Cimino, G., S. De-Rosa, S. De-Stefano and G. Sodano. 1985. Observations on the toxicity and metabolic relationships of polygodial, the chemical defense of the nudibranch *Dendrodoris limbata*. Experientia 41: 1335-1336.
- Clemens, H.P. 1950. Life cycle and ecology of *Gammarus fasciatus* Say. Contrib. Franz. Theodore Stone Inst. Hydrol. 12: 63.
- Coleman, C. O. 1989a. On the nutrition of two antarctic acanthonotozomatidae (*Crustacea: Amphipoda*). Polar Biol. 9: 287-294.
- Coleman, C. O. 1989b. *Gnathiphimedia mandibularis* K.H. Barnard 1930, an antarctic amphipod (Acanthonotozomatidae, Crustacea) feeding on bryozoa.. Antarct. Sci. 1(4): 343-344.
- Coleman, C. O. 1990. *Bathypanoploea schellenbergi* Holman & Watling, 1983, an antarctic amphipod (Crustacea) feeding on holothuroidea. Ophelia 31(3): 197-205.
- Coll, J.C., S. La Barre, P.W. Sammarco, W.T. Williams and G.J. Bakus. 1982. Chemical defense of soft corals (Coelenterata: Octocorallia) of the Great Barrier Reef: a study of comparative toxicities. Mar. Biol. Prog. Ser. 8: 271-278.
- Coll, J.C. and P.W. Sammarco. 1983. Terpenoid toxins of soft corals (Cnidaria: Octocorallia): their nature, toxicity, and ecological significance. Toxicon Suppl. 3: 69-72.
- Comely, C. A. and A. D. Ansell. 1988. Invertebrate associations of the sea urchin *Echinus esculentus* L. from the Scottish West Coast. Ophelia 28: 111-137.
- Cott, H.B. 1940. Adaptive Coloration in Animals. Methuen, London, UK.
- Cross, J. N. 1981. Structure of a rocky intertidal fish assemblage. Ph. D. Dissertation. College of Fisheries, University of Washington, Seattle, Washington, USA.
- de Frietas, J.C. 1980. Evidencias de um possivel comportamento de defesa quimica em

- crustaceos decapodes braquiuros. Bol. Fisiol. Anim. Univ. Sao Paulo 4: 153-161.
- Delong, M. D., P. B. Summers and J.H. Thorp. 1993. Influence of food type on the growth of a riverine amphipod *Gammarus fasciatus*. Can. J. Fish. Aquat. Sci. 50: 1891-1896.
- Deroe, C. and J.M. Pastreels. 1982. Distribution of adult defense glands in chrysomelids (Coleoptera: Chrysomelidae) and its significance in the evolution of defense mechanisms within the family. J. Chem. Ecol. 8: 67-82.
- Dethier, V.G. 1939. Prothoracic glands of adult Lepidoptera. J. N.Y. Entomol. Soc. 47: 131.
- Duffy, J.E. and M.E. Hay. 1994. Herbivore resistance to seaweed chemical defense: The roles of mobility and predation risk. Ecology 75: 1304-1319.
- Edmunds, M. 1974. Defense in Animals: A survey of Antipredator Defenses. Longman, Essex. England.
- Ehrlich, P. R. and P. H. Raven. 1965. Butterflies and plants: a study in co-evolution. Evolution 18: 586-608.
- Eisner, T. 1965. Defensive spray of a phasmid insect. Science 148: 966-968.
- Eisner, H.E., D.W. Alsop, and T. Eisner. 1967. Defense mechanisms of arthropods: XX. Quantitative assessment of hydrogen cyanide production in species of millipedes. Psyche. 82: 20.
- Eisner, T. 1970. Chemical defense against predation in arthropods. E. Sondheimer and J.B. Simeone (eds.), Chemical Ecology. Academic Press, New York.
- Eisner, T., T.H. Jones, D.J. Aneshansley, W.R. Tschinkel, R.E. Silberglied, J. Meinwald. 1977. Chemistry of defensive secretions of bombardier beetles. J. Insect Physiol. 23: 1383-1386.
- Faulkner, D.J. and M.T. Ghiselin. 1983. Chemical defense and evolutionary ecology of doris nudibranchs and some other gastropods. Mar. Ecol. Prog. Ser. 13: 295-301.
- Fisher, R.A. 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford.
- Fraenkel, G. 1959. The raison d'être of secondary plant substances. Science 129: 1466-1470.

- Futuyma, D.J. and F. Gould. 1979. Associations of plants and insects in a deciduous forest. *Ecol. Monogr.* 49: 33-50.
- Gardner, D.R. and F.R. Stermitz. 1988. Host plant utilization and iridoid glycoside sequestration by *Euphydryx anicia* (Lepidoptera: Nymphalidae). *J. Chem. Ecol.* 14: 2147-2168.
- Gittleman, J.L. and P.H. Harvey. 1980. Why are distasteful prey not cryptic? *Nature* 286: 149-150.
- Green, J. 1967. The distribution and variation of *Daphnia lumholtzi* (Crustacea: Cladocera) in relation to fish predation in Lake Albert, East Africa. *J. Zool. (London)* 151: 181-197.
- Guilford, T. 1990. The evolution of aposematism. Pages 23-61 in J.O. Schmidt and D.L. Evans (eds.), Defense: Predator-Prey Interactions. SUNY Press, Syracuse, NY.
- Harborne, J. B. 1982. Introduction to Ecological Biochemistry, 2nd. Ed. Academic Press: London.
- Harborne, J. B. 1993. Introduction to Ecological Biochemistry, 4th. Ed. Academic Press: London.
- Harvell, C.D. 1990. The Ecology and Evolution of Inducible Defenses. *Quart. Rev. Biol.* 65: 323-340.
- Harvey, P.H. and P.J. Greenwood. 1978. Anti-predator defense strategies: some evolutionary problems. Pages 129-151 in J.R. Krebs and N.B. Davies (eds.) Behavioral Ecology: an Evolutionary Approach. Blackwell, Oxford.
- Harvey, P.H. and R.J. Paxton. 1981. The evolution of aposematic coloration. *Oikos* 37: 391-393.
- Hay, M.E., J.E. Duffy, C.A. Pfister and W. Fenical. 1987. Chemical defenses against different marine herbivores: are amphipods insect equivalents? *Ecology* 68: 1581-1591.
- Hay, M.E., J.E. Duffy, W. Fenical and K. Gustafson. 1988a. Chemical defense in the seaweed *Dictyopteris delicatula*: differential effects against reef fishes and amphipods. *Mar. Ecol. Prog. Ser.* 48: 185-192.
- Hay, M.E. and W. Fenical. 1988b. Marine plant-herbivore interactions: the ecology of chemical defense. *Ann. Rev. Ecol. Syst.* 19: 111-145.

- Hay, M.E., P.E. Renaud, and W. Fenical. 1988c. Large mobile versus small sedentary herbivores and their resistance to seaweed chemical defenses. *Oecologia* (Berlin) 75: 246-252.
- Hay, M. E. 1992. The role of seaweed chemical defenses in the evolution of feeding specialization and in the mediation of complex interactions. Pages 93-119 in Paul, V.J. (ed.), Ecological Roles of Marine Natural Products. Comstock Publishing Associates, Ithaca.
- Isman, M.B., S.S. Duffy, and G.G. Scudder. 1977. Cardenolide content of some leaf- and stem-feeding insects on temperate North American milkweeds (*Asclepias* spp.). *Can. J. Zool.* 55: 1024-1028.
- Jarvi, T., B. Sillen-Tullberg and C. Wiklund. 1981. The cost of being aposematic: an experimental study of predation on larvae of *Papilio machaon* by the great tit *Parus major*. *Oikos* 36: 267-272.
- Johnson, P.M. and D. Willows. 1999. Defense in sea hares (Gastropoda, Opisthobranchia, Anaspidea): Multiple layers of protection from egg to adult. *Mar. Fresh. Behav. Physiol.* 32: 147-180.
- Jones, C.G., T.S. Hess, D.W. Whitman, S.J. Compton, P.J. Silk, M.S. Blum. 1987. Effects of diet breadth on autogenous chemical defense of a generalist grasshopper. *J. Chem. Ecol.* 13: 283-297.
- Jones, C.G., D.W. Whitman, S.J. Compton, P.J. Silk, M.S. Blum. 1989. Reduction in diet breadth results in sequestration of plant chemicals and increases efficacy of chemical defense in a generalist grasshopper. *J. Chem. Ecol.* 15: 1811-1822.
- Kvitek, R.G. 1989. Paralytic shellfish poisoning toxins as a chemical defense in butter clams: The evidence. *J. Shellfish Res.* 8: 441-442.
- Ladle, M. 1974. Aquatic Crustacea. Pages 593-608 in C.H. Dickinson and G.J.F. Pugh (eds.), Biology of Plant Litter Decomposition. Academic Press, London.
- Lambert, P. 1997. Sea Cucumbers of British Columbia, Southeast Alaska and Puget Sound. UBC Press, Vancouver, BC, Canada.
- Lindquist, N., M.E. Hay, and W. Fenical. 1992. Defense of ascidians and their conspicuous larvae: adult vs. larval chemical defenses. *Ecol. Monogr.* 62: 547-568.
- Lindquist, N. and M.E. Hay. 1996. Palatability and chemical defense of marine

- invertebrate larvae. Ecol. Monogr. 66: 431-450.
- Lubchenco, J., and S.D. Gaines. 1981. A unified approach to marine plant-herbivore Interactions. Annual Review of Ecology and Systematics 12: 405-437.
- Lucas, J.S., R.J. Hart, M.E. Howden and R. Salathe. 1979. Saponins in eggs and larvae of *Acanthaster planci* (L.) (Asteroidea) as chemical defenses against planktivorous fish. J. Exp. Mar. Biol. Ecol. 40: 155-165.
- Luchenbach, M. and J. Orth. 1990. A chemical defense in Crustacea? J. Exp. Mar Biol. Ecol. 137: 79-87.
- Marchant, R. 1981. The ecology of *Gammarus* in running waters. Pages 225-249 in M. A. Lock and D. D. Williams (eds.), Perspectives in running water ecology. Plenum Press, New York.
- McClintock, J.B. and J. Janssen. 1990. Pteropod abduction as a chemical defense in a pelagic Antarctic amphipod. Nature 346: 462-463.
- McClintock, J.B., and J.D. Vernon. 1990. Chemical defense in the eggs and embryos of Antarctic sea stars (Echinodermata). Mar. Bol. 105: 491-495.
- Moore, J.W. 1975. The role of algae in the diet of *Asellus aquaticus* L. and *Gammarus pulex* L. J. Anim. Ecol. 44: 719-729.
- Moore, P.G. and Rainbow, P.S., 1984. Ferritin crystals in the gut caeca of *Stegocephaloides christianiensis* Boeck and other Stegocephalidae (Amphipoda: Gammaridea): a functional interpretation. Philos. Trans. R. Soc. Lond. B306: 219-245.
- Moore, P.G. and Rainbow, P.S., 1989. Feeding biology of the mesopelagic gammaridean amphipod *Parandania boeckii* (Stebbing, 1888) (Crustacea :Amphipoda: Stegocephalidae) from the Atlantic Ocean. Ophelia, 30: 1-19.
- Moore, P.G. and Rainbow, P.S., 1992. Aspects of the biology of iron, copper and other metals in relation to feeding in *Andaniexis abyssi*, with notes on *Andaniopsis norlandica* and *Stegocephalus inflatus* (Amphipods: Stegocephalidae), from Norwegian waters. Sarsia 76: 215-225.
- Noirot, C. and A. Quennedey. 1974. Fine structure of insect epidermal glands. Ann. Rev. Entomol. 19: 61-80.
- Norton, S.F. Personal communication. Environmental Studies Program, Department of Biology, University of Puget Sound, Tacoma, WA.

- Norton, S.F. and C.D. Stallings. 1999. The distribution and abundance of the aposematic, chemically defended amphipods off Bell Island, WA. J.N. Heine, D. Canestro, G. Wuttken (eds.), Diving for Science 1999. Proc. Amer. Acad. of Underwater Sciences.
- Orians, G.H., and D.H. Janzen. 1974. Why are embryos so tasty? Amer. Nat. 108: 581-592.
- Pastreels, J.M., S. Duffey, and M. Rowell-Rahier. 1990. Toxins in chrysomelid beetles: possible evolutionary sequence from *de novo* synthesis to derivation from food plant chemicals. J. Chem. Ecol. 16: 211-222.
- Paul, V.J., M.E. Hay, J.E. Duffy, W. Fenical and K. Gustafson. 1987. Chemical defense in the seaweed *Octodes secundiramea* (Montagne) Howe (Rhodophyta): effects of its monoterpenoid components upon diverse coral-reef herbivores. J. Exp. Mar. Biol. Ecol. 114: 249-260.
- Paul, V.J., S.C. Pennings. 1991. Diet-derived chemical defenses in the sea hare *Stylocheilus longicauda* (Quoy et Gaimard 1824). J. Exp. Mar. Biol. Ecol. 151: 227-243.
- Paul, V.J. (ed.) 1992. Ecological Roles of Marine Natural Products. Comstock Publishing Associates, Ithaca.
- Pawlik, J.R., M.T. Burch and W. Fenical. 1987. Patterns of chemical defense among Caribbean gorgonian corals: A preliminary survey. J. Exp. Mar. Biol. Ecol. 108: 55-66.
- Pawlik, J.R., M.R. Kernan, T.F. Molinski, M.K. Harper and D.J. Faulkner. 1988. Defensive chemicals of the Spanish dancer nudibranch *Hexabranhus sanguineus* and its egg ribbons: Macrolides derived from a sponge diet. J. Exp. Mar. Biol. Ecol. 119: 99-109.
- Pawlik, J.R. 1993. Marine invertebrate chemical defenses. Chem. Rev. 93: 1911-1922.
- Pawlik, J.R., B. Charnas, R.J. Toonen and W. Fenical. 1995. Defenses of Caribbean sponges against predatory reef fish. Mar. Ecol. Prog. Ser. 127: 183-194.
- Price, P.W. 1983. Hypotheses on organization and evolution in herbivorous insect communities. Pages 559-598 in R.F. Denno and M.S. McClure (eds.), Variable plants and herbivores in natural and managed systems. Academic Press, New York, New York, USA.

- Quigley, M.A., and H.A. Vanderploeg. 1991. Ingestion of live filamentous diatoms by the Great Lakes amphipod, *Diporeia* sp.: a case study of the limited value of gut contents analysis. *Hydrobiologia* 223: 141-148.
- Rogers, S.D. and V.J. Paul. 1991. Chemical defenses of three *Glossodoris* nudibranchs and their dietary *Hyrtios* sponges. *Mar. Ecol. Prog. Ser.* 77: 221-232.
- Roitberg, B.D. and M. B. Isman, eds. 1992. Insect Chemical Ecology: an Evolutionary Approach. Chapman & Hall, New York.
- Roper, T.J. and R. Wistow. 1986. Aposematic coloration and avoidance learning in chicks. *Quart. J. Exp. Psych.* 38B 141-149.
- Roth, L.M., and B. Stay. 1958. The occurrence of para-quinones in some arthropods, with emphasis on the quinine-secreting tracheal glands of *Diploptera puctata* (Blattaria). *J. Insect Physiol.* 1: 305.
- Rowell-Rahier, M. and J.M. Pasteels. 1986. Economics of chemical defense in chrysomelinae. *J. Chem. Ecol.* 12: 1189-1203.
- Russell, E. 1966. An investigation of the palatability of some marine invertebrates to four species of fish. *Pac. Sci.* 20: 452-460.
- Scheuer, P.J. 1982. Marine Natural Products: Chemical and Biological Perspectives. Vol. 2. Academic Press. New York.
- Schildknecht, H. 1970. The defensive chemistry of land and water beetles. *Angew. Chem. Int. Ed. Engl.* 9: 1-9.
- Schram, F.R. 1986. Crustacea. Oxford University Press. New York.
- Sillen-Tullberg, B. 1985. Higher survival of an aposematic than of a cryptic form of a distasteful bug. *Oecologia* 67: 411-415.
- Sillen-Tullberg, B. and O. Leimar. 1988. The evolution of gregariousness in distasteful insects as a defense against predators. *Am. Nat.* 132: 723-734.
- Slaterry, M., C. Avila, J. Starmer, V. Paul. 1998. A sequestered soft coral diterpene in the aeolid nudibranch *Phyllodesmium guamensis* Avila, Ballesteros, Slaterry, Starmer and Paul. *Journal of Experimental Marine Biology and Ecology.* 226: 33-49.
- Speed, M.P. 1993. Mullerian mimicry and the psychology of predation. *Anim. Behav.* 45: 571-580.

- Summers, R.B., M.D. DeLong and J.H. Thorp. 1997. Ontogenetic and temporal shifts in the diet of the amphipod *Gammarus fasciatus*, in the Ohio River. *Amer. Mid. Natur.* 137: 329-336
- Strong, D.R., J.H. Lawton, and T.R.E. Southwood. 1984. Insects on Plants: Community Patterns and Mechanisms. Blackwell Scientific, Oxford, England.
- Tschinkel, W.R. 1975. A comparative study of the chemical defense system of tenebrionid beetles III. Morphology of the glands. *J. Morph.* 145: 355-370.
- Turner, J.R.G. 1984. Darwin's coffin and Doctor Pangloss – do adaptionist models explaine mimicry? Pages 313-361 in B. Shorrocks (ed.) Evolutionary Ecology. Blackwell, Oxford, UK.
- Turner, J.R.G., E.P. Kearney, and L.S. Exton. 1984. Mimicry and the Monte Carlo predator: the palatability spectrum and the origins of mimicry. *Biol. J. Linn. Soc.* 29: 221-226.
- Vader, W. 1978. Associations between amphipods and echinoderms. *Astarte* 11: 123-134.
- Van Alstyne, K.L. 1988. Herbivore grazing increases polyphenolic defenses in the intertidal brown algae *Fucus distichus*. *Ecology* 69: 655-663.
- Vermeij, G.J. 1987. Evolution and Escalation. Princeton University Press, Princeton NJ.
- Wiklund, C. and T. Jarvi. 1982. Survival of distasteful insects after being attacked by naïve birds: a reappraisal of the theory of aposematic coloration evoking through individual selection. *Evolution* 36: 998-1002.
- Willoughby, L.G. and D.W. Sutcliffe. 1976. Experiments on feeding and growth of the amphipod *Gammarus pulex* (L.) related to its distribution in the River Duddon. *Freshwater Biol.* 6: 577-586.
- Young, C.M., and B.L. Bingham. 1987. Chemical defense and aposematic coloration in larvae of the ascidian *Ecteinascidia turbinata*. *Mar. Bol.* 96: 599-544
- Zalewski, J. A. 2001. (unpublished) Predator-prey interactions between three chemically defended, aposematic gammarid amphipods and cottid fishes. Master of science thesis. Department of Biology, East Carolina University.
- Zar, J.H. 1996. Biostatistical Analysis. 3rd ed. Prentice-Hall, Upper Saddle River, NJ.

APPENDIX A:

Average brood size $\pm$ standard error, minimum and maximum brood size at the time of brood release. Dates through which broods were collected are given.

Species	Mean $\pm$ st. err.	Maximum	Minimum	Collection dates
<i>Chromopleustes oculatus</i>	32 $\pm$ 1.7	67	9	5/24 to 6/17/01
<i>Chromopleustes lineatus</i>	16 $\pm$ 1.7	28	7	6/22 to 6/29/01
<i>Cryptodius kelleri</i>	17 $\pm$ 2.1	23	6	6/22 to 6/30/01

APPENDIX B:

Observed percent frequency of occurrence of prey types and expected values used in Chi-square tests for homogeneity of frequency of occurrence of prey. Numbers in parentheses indicate the number of fecal pellets with identifiable prey.

Species	Pennate diatom	Centric diatom	Crustacean parts	Sea cuc. ossicles	Echinoderm pedicellaria
<i>Ch. oculatus</i> (106)	44	70	9	29	4
<i>Ch. lineatus</i> (24)	21	17	0	0	63
<i>Cr. kelleri</i> (21)	86	57	10	0	0
Expected value	46	60	7	21	13
Season					
Winter (18)	28	50	6	39	22
Summer (58)	43	79	14	21	0
Expected value	40	72	12	25	5
Location					
Bell Island (64)	39	75	12.5	19	
Twin Peaks (26)	81	73	0	46	
Expected value	51	74	9	27	
Substrate					
Sea cucumber (12)	67	58		42	
Rock (14)	93	86		50	
Expected value	81	73		46	