PLASTICITY OF CONSUMER-PREY INTERACTIONS IN THE SEA: CHEMICAL SIGNALING, LEARNED AVERSION, AND ECOLOGICAL CONSEQUENCES

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Plasticity of Consumer-prey Interactions in the Sea: Chemical Signaling, Learned Aversion, and Ecological Consequences

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To my mother, for her strength and encouragement

To Heidi, for her love and support

Thank you both

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SUMMARY

My research focused on consumer-prey interactions in the ocean; especially the underlying mechanisms and ecological consequences of these dynamic interactions. Aquatic chemical signals were important to consumers and prey from a broad range of taxonomy (phytoplankton to fishes), sizes (microscopic to macroscopic), and habitats (pelagic to benthic). Chemical signals produced by these organisms or their activities provided information that allowed for surprisingly complex, induced responses in the receiving organisms. In addition to affecting specific consumer-prey interactions, chemical signals can play critical roles in the structure and function of marine communities and ecosystems.

To understand the plastic, behavioral responses of consumers to novel prey, I studied benthic fishes and their responses to the chemical defenses of a marine nudibranch (Chapter 1). Using bioassay-guided fractionation, I isolated and identified the feeding deterrent, polygodial from the nudibranch, *Doriopsilla pharpa*. Two fishes, *Chasmodes bosquianus* and *Fundulus heteroclitus*, rapidly learned to avoid palatable foods treated with lipid-soluble, nudibranch extracts. *C. bosquianus* also learned to avoid foods treated with natural concentrations of polygodial. However, the physiological effects of consuming these foods and the cues used to recognize aversive foods varied considerably between these species. Learned aversions could affect the outcome of species introductions in marine environments.

The remaining experiments examined the interactions of the bloom-forming phytoplankton genus, *Phaeocystis*, with its consumers and competitors. Most *Phaeocystis* species alternate between a solitary cell and a colonial form. *Phaeocystis* spp. are globally-distributed, play a critical role in the structure of pelagic communities during these blooms, and significantly affects carbon sequestration at high latitudes.

To understand the importance of intraspecific variability to consumer-prey interactions in the plankton, I examined the effects of variability of *Phaeocystis globosa* morphology and growth phase on grazing zooplankton (Chapter 2). The responses of three copepod species to variable *P. globosa* were species-specific. In some cases, the variability in how grazers responded to different types of *P. globosa* exceeded the variability measured for how grazers responded to different species. These observations suggest that intraspecific variability can strongly determine the outcome of zooplankton-phytoplankton interactions.

Because zooplankton response to *Phaeocystis globosa* depended on both the identity of the zooplankton and the type of *P. globosa* they encountered, we examined whether *P. globosa* could recognize and respond defensively to grazers (Chapter 3). *P. globosa* displayed a remarkable specificity in their response to grazer-associated chemical cues; ciliates (microzooplankton) enhanced colony formation and copepods (macrozooplankton) suppressed colony formation. These responses are adaptive because microzooplankton grew poorly on colonies while macrozooplankton grazed colonies more than solitary cells. Induced responses in phytoplankton could affect pelagic food webs, phytoplankton blooms, and the movement of energy and nutrients through these systems.

To study the importance of chemical signals between planktonic organisms, other than consumers and their prey, I also studied interactions between phytoplankton (Chapter 4). Cell-free filtrates from a mesocosm bloom of *Phaeocystis pouchetii* suppressed the growth of the phytoplankton *Rhodomonas baltica*. Similar results using a laboratory culture of *Phaeocystis globosa* grown under different conditions suggest that *Phaeocystis* species are general producers of allelopathic chemicals. These

interactions could contribute to the formation and persistence of *Phaeocystis* spp. blooms.

CHAPTER 1

LEARNED AVERSION BY MARINE CONSUMERS: NUDIBRANCH CHEMICAL DEFENSE AND REGURGITATION BY FISHES

Abstract

Consumers can learn to avoid foods that produce physiological distress. However, the speed of learning, the cues used, and the duration of aversion without reinforcement are poorly investigated in marine systems. We found that the nudibranch *Doriopsilla pharpa* was rejected as a food in field assays and by 2 species of fishes and 2 species of crabs in laboratory assays. When the fishes were offered tuna-based artificial foods containing extracts of the nudibranch, both species initially consumed these foods, but rapidly developed aversions in subsequent feedings. The striped blenny (*Chasmodes bosquianus*) regurgitated following the initial feeding and then avoided any food made of tuna, whether it contained the extract or not. In contrast, the mummichog (*Fundulus heteroclitus*) did not regurgitate, but rapidly learned to avoid foods containing the extract; it still consumed tuna-based foods without the extract. By making treatment and control foods of differing colors and tastes, we determined that the blennies were using taste rather than visual cues to avoid the aversive food. The blennies initially spent considerable periods of time sampling treated foods before rejecting them, but the mummichogs made these decisions an order of magnitude more rapidly. After 2 days of exposure to treated foods, the sampling time of blennies dropped to the low levels typical of the mummichogs. Once experienced with aversive foods, blennies retained this aversion for at least 2 weeks without reinforcement. However, if constantly exposed to a palatable food that had previously been aversive, some blennies would learn to consume this food after 1 week, others still rejected these,

now undefended, foods after 3 weeks of exposure. The sesquiterpene polygodial was responsible for the deterrent effects of the nudibranch extract.

Introduction

Animals can select favorable foods and avoid harmful foods via innate or learned preferences (Provenza 1995). Under some conditions, innate preferences may be too restrictive, causing consumers to miss opportunities during variation in food composition and availability (Laska and Metzker 1998) or allowing the ingestion of toxins as prey evolve resistance or as new, chemically-defended prey enter the habitat (Provenza et al. 2003). Learning provides an ecological response to changing prey availability by allowing consumers to add novel foods to their diets while reducing the risk of consuming noxious foods (Provenza 1995). Consumer learning and behavior can significantly affect population dynamics and community structure (Bernstein et al. 1988, Provenza et al. 2003) and incorporating learning into ecological models increases their predictive power (Hughes 1979). Thus, the paucity of investigations focused on how marine consumers learn to avoid defended foods constrains our understanding of consumer-prey interactions in marine systems.

Because human activities are introducing non-native species into new ecosystems at unprecedented rates (Mack et al. 2000), learned aversions to novel prey could be critical to the consequences of these introductions. This may be especially true for coastal areas overrun with introduced species (Ruiz et al. 2000), and for “open” marine systems where noxious prey produced in 1 habitat can be transported over large distances to affect naïve consumers in other habitats and geographic regions. As an example, in 1987-1988, satellite images showed a bloom of the toxic dinoflagellate *Karenia brevis* initiating along the Gulf coast of southern Florida, being transported around Florida towards the Gulf Stream, and delivered to inshore North Carolina by a

loop current from the Gulf Stream (Tester and Fowler 1990). This brevetoxin producing phytoplankton had never before been seen this far north of the tropics, so local consumers had not been selected to tolerate its compounds or avoid consuming it. This algal bloom caused massive scallop mortality, closure of shellfish harvesting, and an estimated loss of $24,000,000 to coastal economies due to the loss of shellfish harvest alone (Tester and Fowler 1990). Such impacts could select for consumers that learn to recognize and reject this new species.

Learned food aversions occur in a diverse array of consumers including insects (Berenbaum and Miliczky 1984), mollusks (Gelperin 1975), fish (Gerhart 1991, Lindquist and Hay 1995), reptiles (Burghardt et al. 1973), birds (Nicolaus et al. 1983), and mammals (Garcia et al. 1974, Bernstein 1978, Yoerg 1991), with the majority of research focusing on terrestrial species. Learned aversions in terrestrial consumers develop rapidly (Bernays and Lee 1988, Ratcliffe et al. 2003), visual or chemical cues can maintain these aversions (Wilcoxon et al. 1971, Burghardt et al. 1973), and aversions can persist for long periods in the absence of reinforcement (Bernstein 1978, Kimball et al. 2002). Compared to terrestrial systems, we understand little regarding learned food aversions in marine systems. Although fishes are well-known for their learning ability (Coble et al. 1985, Crossland 2001) and quickly develop aversions to foods that cause regurgitation (Gerhart 1984, 1991, Lindquist and Hay 1995), there is minimal information regarding the timing, cues, retention, and potential ecological importance of these behaviors. Thus, the relative importance of innate and learned aversions in marine systems remains unclear.

In this investigation, we determined that a common nudibranch was unpalatable to many co-occurring consumers and then conducted a comparative study of learned food aversions using 2 marine fishes. Our goal was to study learned feeding preferences within an ecologically realistic context by offering different consumers co-

occurring prey, prey extracts, and deterrent prey compounds at natural concentrations. We evaluated: 1) how learned aversions varied between different consumer species, 2) whether aversive learning relied more on chemical or visual cues from the prey, and 3) which prey compounds caused aversive learning.

Materials and Methods

Nudibranchs are well-known for their chemical defenses against consumers (Cronin et al. 1995, Avila and Paul 1997), so we used a local nudibranch as a model prey capable of stimulating learned aversions in co-occurring predators. The lemon drop sea slug, *Doriopsilla pharpa*, is bright orange, reaches lengths of 2.5 cm, and occurs on oyster shell communities along the east coast of the U.S.A. Eggs, juveniles, and adults inhabit loose shells colonized by their reported prey, the yellow boring sponge, *Cliona celata* (Guida 1976, Eyster and Stancyk 1981). *D. pharpa* is usually found on subtidal shell rubble in tidal rivers and creeks with high flow and steep banks (J. Long, personal observation). All *D. pharpa* used in these experiments were collected from shallow subtidal areas of House Creek (31°56'51''N, 80°55'26''W) and Mud Island (31°57'58''N, 80°57'34''W) near Savannah, GA, U.S.A.

Palatibility of the Nudibranch - Nudibranch susceptibility to a natural suite of consumers was assessed by tethering nudibranchs and similar sized portions of squid mantle in the field. Tethers consisted of fishing hooks taped every 10 cm along weighted 220 cm long PVC pipes. Weights kept tethered prey close to their benthic habitats. On 12 and 13 September 2002, 10 nudibranchs and 10 squid pieces were randomly interspersed at 10 cm intervals along each of 4 weighted PVC pipes. On both days, 2 PVC pipes were placed 5 m apart at known nudibranch habitats at Mud Island. Presence or absence of foods was recorded after 2-3 hours, and squid pieces and nudibranchs were examined

for evidence of partial predation. Because many pieces of squid were removed from the tethers in the field, we tested if loss of squid pieces would have occurred due to flow alone. We tethered 20 squid pieces on each of 2 PVC pipes and placed these in a racetrack flume at flow rates typical of tidal creeks like our field test site (M. Ferner, personal communication). Flow durations and rates were 1 h at a flow rate of 5 cm sec^{-1}, then 1 h at 30 cm sec^{-1}, and then 24 h at 0 cm sec^{-1}.

To determine the palatability of nudibranchs to different co-occuring consumers, we collected lesser blue crabs [*Calinectes similus*, N=7], Atlantic mud crabs [*Panopeus herbstii*, N=31], mummichogs [*Fundulus heteroclitus*, N=14], and striped blennies [*Chasmodes bosquianus*, N=29] from oyster reefs and used these consumers in laboratory feeding assays. Consumers were maintained individually in 0.5 or 2.4 L plastic containers in a flow-through seawater system, except Atlantic mud crabs which were maintained in closed containers because of space limitations. During laboratory acclimation, consumers were trained to feed on palatable foods offered via a pipet or forceps. Consumers not previously fed *Doriopsilla pharpa* tissue or extracts were considered "naïve," although they may have fed on the nudibranchs in the field before collection. If we previously fed *D. pharpa* tissue or extracts to consumers, these consumers were considered "experienced." A few hours before feeding assays, naïve crabs and fishes were fed to satiation on commercial crayfish food or frozen brine shrimp, respectively, thus preventing the consumption of defended foods by starved consumers (Cronin and Hay 1996, Thacker et al. 1997).

During feeding assays, individual consumers were first offered a palatable control food. One minute later, consumers were offered a nudibranch. Finally a second control was offered to confirm that avoidance of nudibranchs was not due to satiation. A different procedure was adopted for Atlantic mud crabs because they would not train to accept food from forceps or a pipet. Each Atlantic mud crab was simultaneously offered

a control food and a small nudibranch, and consumption was assessed 13 h later. For all assays, control foods were created by incorporating freeze-dried squid (5% total wet mass) into a sodium alginate solution (2% total wet mass; see Hay et al. [1998] for procedures), and dying this with food coloring to increase visibility to the observer. This mixture was injected into a 0.25 M $CaCl_2$ bath with a syringe, thereby creating a "noodle" with the consistency of cooked pasta which was cut into pellets of several millimeters in length. Consumer behaviors were scored as accepted (consumer ingested food), rejected (consumer took food into its mouth, forcefully expelled the food, and failed to reingest it after 1 minute), or ignored (consumer never tasted the food). After assays, containers were checked periodically for regurgitation of accepted foods.

To confirm that our artificial foods were nutritionally similar to nudibranchs, we measured the caloric content of squid-based pellets, tuna-based pellets, and *Doriopsilla pharpa* (N = 5 for each). Four individual *D. pharpa* were combined per replicate.

Tests of Chemical Defenses - We assessed potential chemical defenses of the nudibranch by evaluating the palatability of nudibranch extracts. The same procedures used to assess palatability of whole nudibranchs in the lab were used to assess palatability of chemical extracts with 2 exceptions: 1) treatment foods consisted of control foods with nudibranch extracts added to them and 2) because extracts were dissolved in ether when added to foods, an equivalent volume of extract-free ether was added to control foods. Extracts were prepared by homogenizing and soaking 1-2 ml (wet volume) of nudibranchs in acetone for several hours. Removal of acetone via rotary evaporation was followed by a partition between dichloromethane (DCM) and water. After removing solvents via rotary evaporation (DCM) and freeze-drying (water), the lipid-soluble (DCM) extract was re-dissolved in a known amount of ether and the water-soluble extract was re-dissolved in a known amount of water so that appropriate

amounts of each extract could be added to experimental foods. For preparing foods, the ether-soluble extract was placed in a small vial, the ether was removed by gentle aeration with nitrogen, and the remaining extract was mixed with squid paste. For water-soluble extracts, the water with the extract replaced part of the water normally used in the recipe for the food. For this experiment, extracts were added to squid pellets at 2x natural volumetric concentrations in order to counteract possible losses of compound in the initial extraction and separation procedures. After finding initial deterrence in this test, all subsequent tests were conducted at natural (not 2x natural) volumetric concentration. To test extracts at natural volumetric concentrations, extracts from a given volume of nudibranchs were added to an equivalent volume of treatment food and offered to consumers as described above. Naïve striped blennies (N=16) were first offered lipid-soluble extracts and controls on day 1. Water-soluble extracts and controls were offered the next day. Fish were watched for regurgitation or other unusual behaviors for several minutes after accepting pellets. They were checked again after several hours and on the following day.

To purify the compound responsible for defending nudibranchs from consumers, we used bioassay-guided fractionation. Lipid-soluble extracts from the nudibranch's mantle margin, prepared as described above, were separated using preparative, normal phase, thin layer chromatography (TLC). These fractions were added to squid pellets and fed to experienced mummichogs (N = 11-13) because this species learned to avoid the nudibranch extract itself rather than the taste of the food with which the extract was associated. Unpalatable fractions were further purified by preparative TLC until 1 deterrent compound was apparent using TLC. ^{1}H-NMR and mass spectroscopy were used to identify the structure of this deterrent compound.

Learned Aversion Assays - When striped blennies were offered foods treated with lipid-soluble extracts, we observed regurgitation, suggesting the potential for the development of learned aversions. To test whether fish would learn to avoid these foods, we conducted short-term learning assays with striped blennies and mummichogs. Lipid-soluble extracts were incorporated into squid-based pellets at natural volumetric concentrations. Palatability of these pellets versus control pellets without extracts (solvent only) was measured daily for naïve mummichogs (N = 20) and striped blennies (N = 10) for 3 consecutive days. All fish were fed brine shrimp until satiation several hours before experimental feedings. Control pellets were offered first and a treatment pellet was offered 90 seconds later. After both pellets were offered, each fish was watched for 5 minutes before proceeding to the next fish. Regurgitation was monitored for an additional 45 minutes and rejected and regurgitated pellets were removed.

To understand if learning decreased handling time for foods that were ultimately rejected, we analyzed the handling time for treated foods that were rejected. Handling time was the time between the first tasting of a treated food until the last rejection of that food. We also recorded the number of times a fish sampled and expelled a treated food before finally ignoring it. Fish accepting treated foods were not included in these analyses. Thus, sample sizes for handling time and rejection number are always less than or equal to the total number of fish assayed. Fishes ignoring treated foods were given a score of 0 for both handling time and rejection number. Handling time was not recorded for 1 striped blenny on day 1 (we decided to take this measurement while watching this fish's behavior), any mummichogs on day 2 (we decided to measure the change of fish behavior with time after day 2 for mummichogs), and 1 mummichog on day 3 (due to a failure to start the stopwatch on time).

Striped blennies that consumed extract-containing foods soon began avoiding both treatment and control foods, suggesting that they were avoiding foods based on

cues from the base foods rather than from the extract itself. To identify the relative importance of visual and chemical cues for learned aversions, we set up a long-term feeding assay. Naïve striped blennies were divided into 2 groups (treatment fish and control fish, N = 17 in each group), placed individually in 0.5 L containers, and randomly interspersed in a flow-through seawater table. As with the short-term learning assay, fish were fed brine shrimp until satiation in the morning (0830-0930h) and late afternoon (1730-1830h). Test foods thus represented only a small fraction of each consumer's total daily diet. Containers were cleaned after each feeding. Fish were trained to feed on palatable brine shrimp pellets for 5 days before the experiment started. Thus, assay foods containing tuna or squid represented novel foods. After the acclimation period, each fish was fed 2 pellets, 1 dyed red and 1 dyed orange (to produce a visual cue that they could associate with food type), daily for 30 days during the early afternoon (1400-1500h). On days 1-7, 15, and 30, freeze-dried tuna was used as the base food for pellets; freeze-dried squid was used on all other days. For treatment fish, lipid-soluble, nudibranch extracts were added at natural volumetric concentrations to orange pellets (producing a visual cue) for treatment fish only on those days when tuna (producing a taste cue) was the base food. On days when extracts were not added to the orange pellets, fishes were fed squid-based pellets. Solvent was added to all other pellets as a control. The feedings at days 15 and 30 tested whether striped blennies could remember aversive foods after 1 and 2 weeks without exposure to these foods.

This design allowed us to compare the relative importance of visual versus chemical cues for fish developing learned aversions. If striped blennies learned to avoid foods based on color, treatment fish should have avoided all foods colored orange. If chemical cues were more important, treatment fish should have avoided all foods tasting like tuna. On each day, the order of pellet presentation was determined randomly except for days 15 and 30 when red tuna pellets without extract were offered first. This

bias provided a test of whether aversive fish remembered their aversion to tuna or to the lipid-soluble extract. During the assay, the second pellet was offered 1 minute after the first pellet was accepted or ignored. One minute after the second pellet was accepted or ignored, we proceeded to the next fish and repeated this procedure until all fish were assayed. Containers were checked for regurgitation every 15 minutes for 2 hours and then again before the start of the feeding the following day.

To determine the persistence of aversion in the absence of reinforcement, we measured how long it took striped blennies that were averse to tuna to begin accepting undefended tuna when it was offered daily. We compared the feeding on tuna-based foods by striped blennies that were averse to tuna to striped blennies that were not averse to tuna. Striped blennies with learned aversions to tuna created during the long-term assay were used as treatment fish (N=10). Striped blennies lacking learned aversions to tuna were used as control fish (N=8). All fish were fed brine shrimp until satiation in the morning and late afternoon. For 2 days after the long-term learning assay ended, all treatment fish from that assay were offered a single red tuna pellet containing lipid-soluble, nudibranch extract at natural volumetric concentration to ensure aversive conditioning. After this 2 day period of reinforcement, control and treatment fish were offered a single red tuna pellet lacking nudibranch extract once per day for 3 weeks (N = 8-10). Pellets not accepted after 1 minute were removed.

The deterrent compound purified from the nudibranchs (^{1}H NMR estimate of purity = 99%) was offered to naïve striped blennies to determine whether they would develop aversions to foods treated only with this compound. Fish were collected late in the season when they were rare, so only eleven fish could be collected. To increase the power of our analyses, each fish was tested both as a control and treatment fish. In the first experiment, fish were fed tuna-based foods, and 6 fish were assigned to the treatment group and 5 to the control group. In the second experiment, which

immediately followed the first experiment, the assigned groups were switched, the base food was switched to squid, and the procedure was repeated. For 3 days in both experiments, all fish were trained to feed on pellets without nudibranch extracts. For 5 days after this preconditioning period, red pellets fed to treatment fish contained the purified compound at natural volumetric concentration and all other pellets had solvent added as a control. To simplify data presentation, these data are shown combined in 1 graph.

Data Analysis - Tethering data were pooled and analyzed using a G-test with a Yates correction for continuity (Zar 1999). Acceptance frequencies of consumers feeding on *Doriopsilla pharpa*, extracts, and purified compound compared to feeding on controls were analyzed by Fisher exact tests. Caloric content of nudibranchs and control foods were compared using ANOVA. For short-term learning, a changing percent acceptance over time was tested for a linear trend using chi-square (Zar 1999). For handling time and the number of times a pellet was rejected, homogeneity of variances were tested with the Bartlett's test. Groups with homogeneous variances were analyzed with a two-tailed ANOVA while non-homogeneous groups were analyzed using a Kruskal-Wallis test with a correction for ties (Zar 1999). When the null hypothesis was rejected using the Kruskal-Wallis test, nonparametric multiple comparisons for unequal sample sizes were made (Zar 1999).

Results

Nudibranch Palatability - The nudibranch *Doriopsilla pharpa* was unpalatable to mixed species of consumers in the field and to 2 crab and 2 fish species in laboratory assays (Figure 1.1). When tethered in the field, 78% of squid pieces, but only 10% of the *D. pharpa* individuals, were removed ($P < 0.001$). Neither remaining squid nor remaining *D.*

pharpa showed signs of partial predation. Because all squid pieces remained hooked under realistic flow speeds in flume trials, it is unlikely that field losses were due to physical processes alone. Laboratory assays corroborated field patterns. Living *D. pharpa* were rejected by all naïve lesser blue crabs ($P = 0.001$), mummichogs ($P < 0.001$), and striped blennies ($P < 0.001$). These consumers accepted all control foods. No Atlantic mud crabs consumed nudibranchs, but 48% of the crabs consumed control foods ($P < 0.001$).

The control foods used in our assays were similar to *Doriopsilla pharpa* in caloric value. The caloric content per volume of *D. pharpa* (346 ± 11 cal ml^{-1}; mean $\pm$ 1 SE) and both artificial diets (tuna pellets = 329 ± 3 cal ml^{-1}; squid pellets = 326 ± 2 cal ml^{-1}) were not significantly different ($P = 0.127$).

Nudibranch Chemical Defense - Neither lipid-soluble nor water-soluble fractions of nudibranch extracts initially deterred feeding by striped blennies (Figure 1.2, $P > 0.999$). However, 67% of the blennies accepting foods containing lipid-soluble extracts regurgitated; no control pellets were regurgitated (Figure 1.2, $P < 0.001$). Water-soluble, nudibranch extracts were palatable to striped blennies experienced with lipid-soluble extracts (Figure 1.2, $P > 0.999$), and none of these pellets were regurgitated. All fish accepted control foods with the exception of the experiment with water-soluble extracts where 5 fish avoided control foods. These 5 fish may have avoided control foods due to satiation or learned aversion associated with regurgitation of lipid-soluble foods on the previous day. They were therefore excluded from analyses, reducing the sample size from 16 to 11.

Short-term Learning - When control food and food treated with lipid-soluble extract were fed to striped blennies and mummichogs for 3 consecutive days, striped blennies

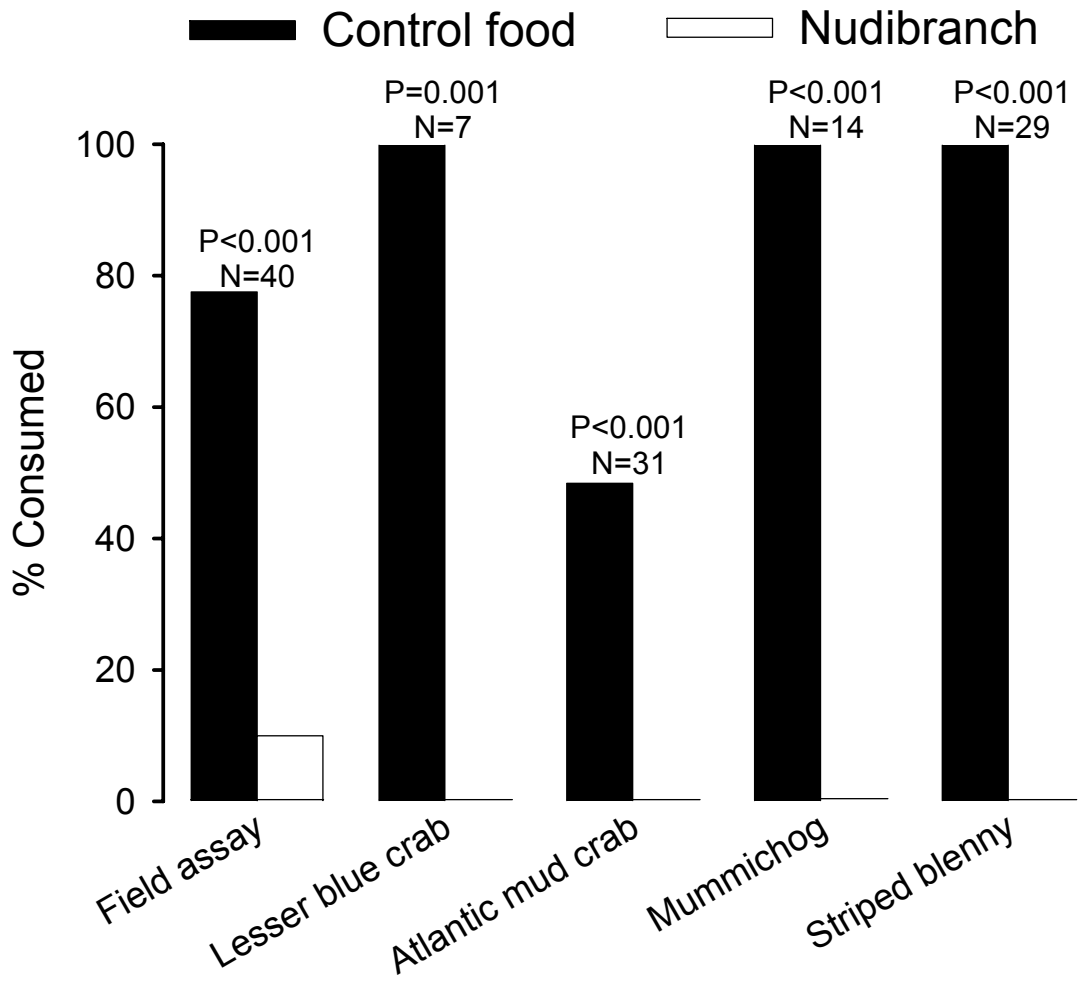

Figure 1.1. Percentage of nudibranchs consumed relative to palatable controls. Analyses were by two-tailed Fisher exact tests.

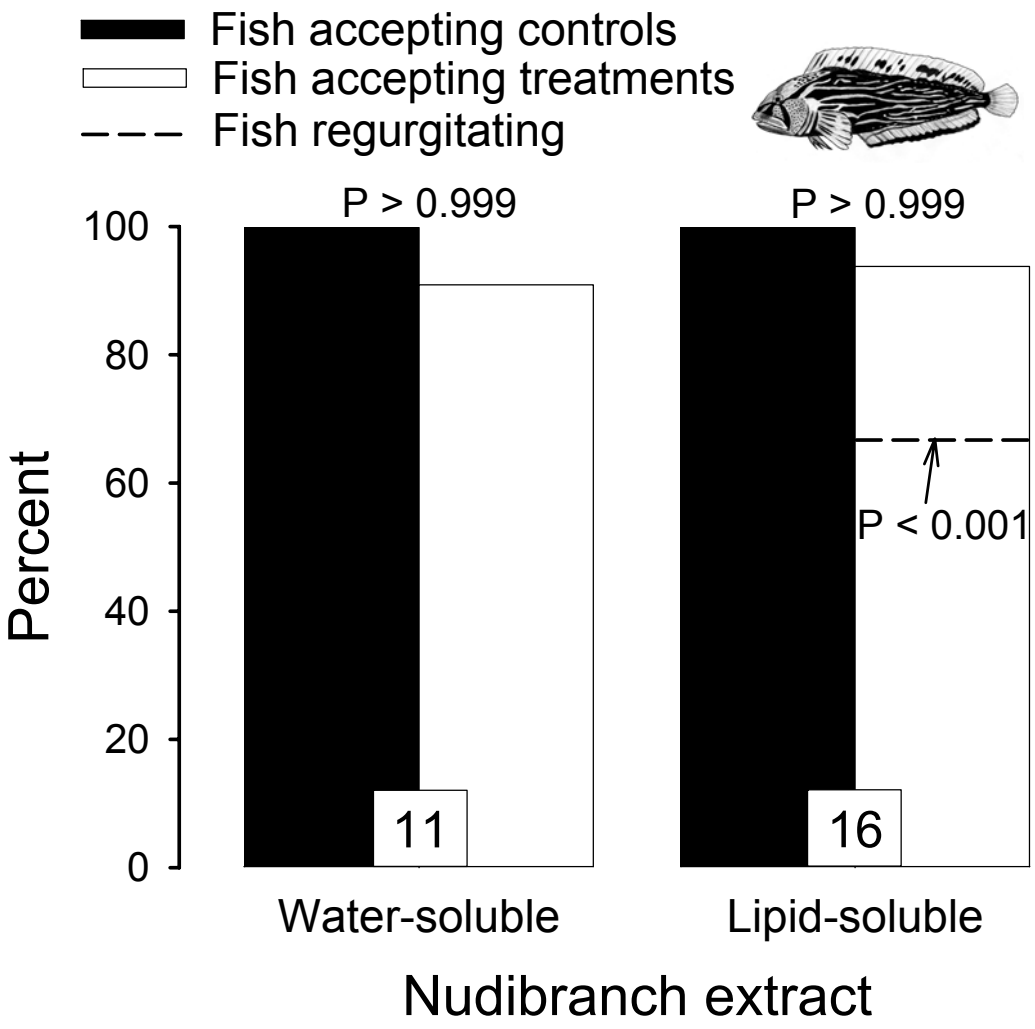

Figure 1.2. Palatability of water-soluble and lipid-soluble nudibranch extracts fed at 2x natural concentration to striped blennies relative to palatable control foods. The dashed line indicates the percentage of treatment fish regurgitating accepted foods (no control fish regurgitated). Sample size is given within bars. Analyses were by two-tailed Fisher exact tests.

consumed both foods on day 1 (Figure 1.3A, P = 0.21). However, 71% of the fish accepting treatment pellets regurgitated these after an average of 12.7 ± 5.8 minutes (mean ± 1 SE), with a range from 2.5 to 39.3 minutes. The willingness of striped blennies to accept either treatment or control foods declined dramatically after 1-2 days of exposure to this food ($P < 0.005$ and $P<0.05$ for the treatment and control foods, respectively; chi-square; Figure 1.3A). Three striped blennies died after day 1, reducing N from 10 to 7. Handling time of treated foods for striped blennies not accepting foods decreased with experience (Figure 1.3C, P = 0.002; Kruskal-Wallis). The number of times that striped blennies sampled and rejected treatment pellets before finally rejecting these as foods decreased with experience (Figure 1.3D, P = 0.008; Kruskal-Wallis).

Feeding patterns for mummichog differed from those for striped blennies. On day 1, significantly more mummichogs accepted control than treatment pellets (Figure 1.3B, P = 0.02), and mummichogs never regurgitated any pellets. The willingness of mummichogs to accept foods containing lipid-soluble extracts declined after repeated feedings (Figure 1.3B, $P<0.005$; chi-square). The willingness of mummichogs to accept control pellets did not change with time (Figure 1.3B, $0.10 < P < 0.26$; chi-square). There was a trend for handling time and number of rejections of treated foods to decrease with experience for mummichogs but this trend was not significant in either case (Figure 1.3C, P = 0.233 and Figure 1.3D, P = 0.168, respectively; ANOVA). However, mummichog were as fast at rejecting treatment pellets on day 1 as blennies became after 2 additional days of learning (Figure 1.3C and D); thus, mummichogs were initially good at this and had less scope for improvement.

Retention of Aversive Memory – Once aversion to tuna tasting foods was learned by striped blennies, they retained this behavior for at least 2 weeks without reinforcement. Pellets containing lipid-soluble, nudibranch extracts were initially eaten by most naïve

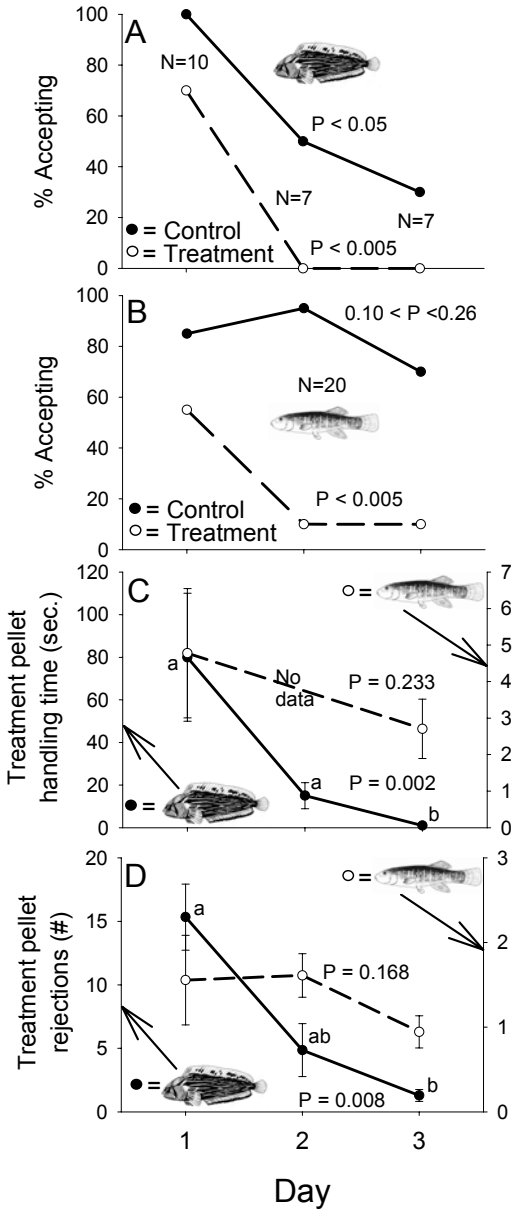

Figure 1.3. Short-term learning. Palatability of treated foods (palatable foods containing lipid-soluble, nudibranch extracts at natural concentrations) and untreated foods fed to (A) striped blennies and (B) mummichogs relative to palatable control foods over 3 consecutive days. Changing response over time was evaluated using chi-square and significant changes are indicated by P-values < 0.05. Handling time before final rejection (C) and the number of tastes and rejections before final rejection (D) of pellets treated with lipid-soluble, nudibranch extracts at natural concentrations for striped blennies and mummichogs (note the >10X difference in y-axes values for the 2 fishes). Values are means +/- 1 SE. Analyses were by ANOVA or Kruskal-Wallis.

striped blennies (Figure 1.4A, P = 0.148). However, after 3 days of exposure, only 0-12% of treatment fish would accept tuna based foods containing nudibranch extracts while 71% of control fish continued to accept tuna based foods without extracts (Figure 1.4A and B, P < 0.001). Thus, learned aversions were present after 1 week (and actually became apparent after 1-3 days of exposure – Figure 1.4A). When base foods were switched from tuna to squid and nudibranch extracts were not added to any foods, averse fish immediately accepted these new foods. There were no differences between the proportion of averse fish versus control fish feeding on squid-based pellets, throughout the 30 day period (Figures 1.4A and B, all P > 0.071). After 1 week without exposure to tuna or nudibranch extracts, most averse fish immediately rejected tuna-based foods whether or not they contained nudibranch extracts (Figure 1.4A, P = 0.002). All averse fish also immediately rejected both treatment and control foods tasting of tuna after 2 weeks without exposure (Figure 1.4A, P = 0.003).

Striped blennies learned to avoid foods using chemical rather than visual cues. During the first week, treatment fish developed aversions to foods containing extracts (tuna pellets dyed orange; Figure 1.4A) and to extract-free foods (tuna pellets dyed red; Figure 1.4A), indicating that fish were avoiding tuna rather than just the extract-containing tuna. Striped blennies did not develop aversions to food color; extract-free squid foods dyed the same color (orange) as aversive foods were readily accepted (Figure 1.4A). Additionally, fish did not develop aversions in response to the feeding procedure or alginate foods since squid foods were consumed at high rates by averse fish. Thus, striped blennies developed aversions to chemical cues associated with tuna rather than to the color orange or the taste of the extract itself. Direct contact with foods was not always necessary for avoidance of aversive foods; treatment fish would sometimes bite at the water near tuna-based pellets and then ignore them. This

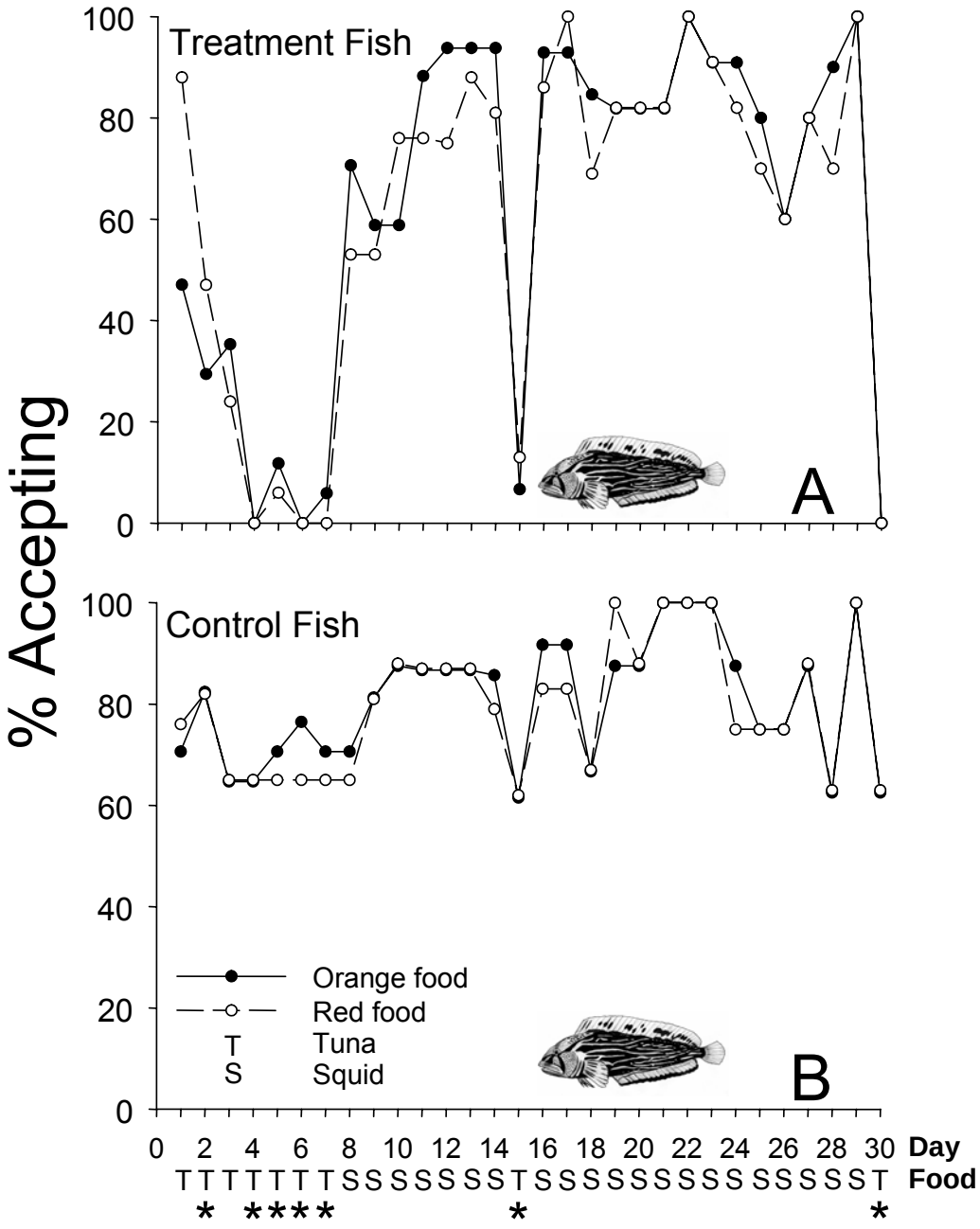

Figure 1.4. Long-term learning and an assessment of chemical vs. visual cues. For each of 30 days, the acceptability of orange and red pellets fed to separate groups of treatment (A) and control (B) striped blennies. Pellets consisted of a tuna based food on days 1-7, 15, and 30. Pellets consisted of a squid based food on all other days. Only orange tuna pellets fed to treatment fish contained lipid-soluble extracts from the nudibranch at natural concentrations. Analyses were by one-tailed Fisher exact tests. Significant differences ($P < 0.05$) between treatment fish and control fish feeding on orange foods are indicated by an asterisk below the day of comparison.

suggests that striped blennies may have used both olfactory and gustatory cues to avoid aversive foods.

All striped blennies averse to tuna continued to avoid undefended tuna for at least 7 days, even when offered undefended tuna on a daily basis (Figure 1.5, $P < 0.020$). On day 8, 3 of 10 averse fish started accepting these foods. The other 7 averse fish continued rejecting these foods after 14 days of exposure to the undefended food. Fish deaths and the resulting small sample size of only 8 and 10 fish (control and treatment, respectively) decreased statistical power in this assay.

The Chemistry of Deterence – When the organic extract of the nudibranch was fractionated and tested for effects on mummichog feeding, 2 adjacent fractions showed deterrence with most of the activity associated with fraction B (Figure 1.6, $P < 0.001$). Some deterrence was associated with Fraction C, possibly because of incomplete separation of compounds in B and C. Fraction B was further separated into 2 fractions with fraction B_1 being palatable ($P = 0.500$) and fraction B_2 being unpalatable ($P < 0.001$). TLC analysis suggested that B_2 could be a pure compound. Mass spectral analysis (using electron impact ionization) suggested a molecule with a molecular weight of 234.16287, corresponding to a molecular formula of $C_{15}H_{22}O_2$ (expected mass 234.1619). The ^1H NMR spectrum, recorded in deuterated chloroform at 300 MHz, indicated chemical shifts at 9.52 (doublet), 9.44 (singlet), 7.15 (multiplet), 2.81 (multiplet), 2.56-2.22 (multiplet), 1.90-1.10 (multiplet), 0.94 (singlet), 0.92 (singlet), and 0.90 (singlet). These data were consistent with identifying this compound as the known sesquiterpene, polygodial (Ohsuka 1962; structure shown in Figures 1.6 and 1.7). The yield of polygodial from the extraction of whole nudibranchs was 0.39 mg/ml of wet nudibranch tissue.

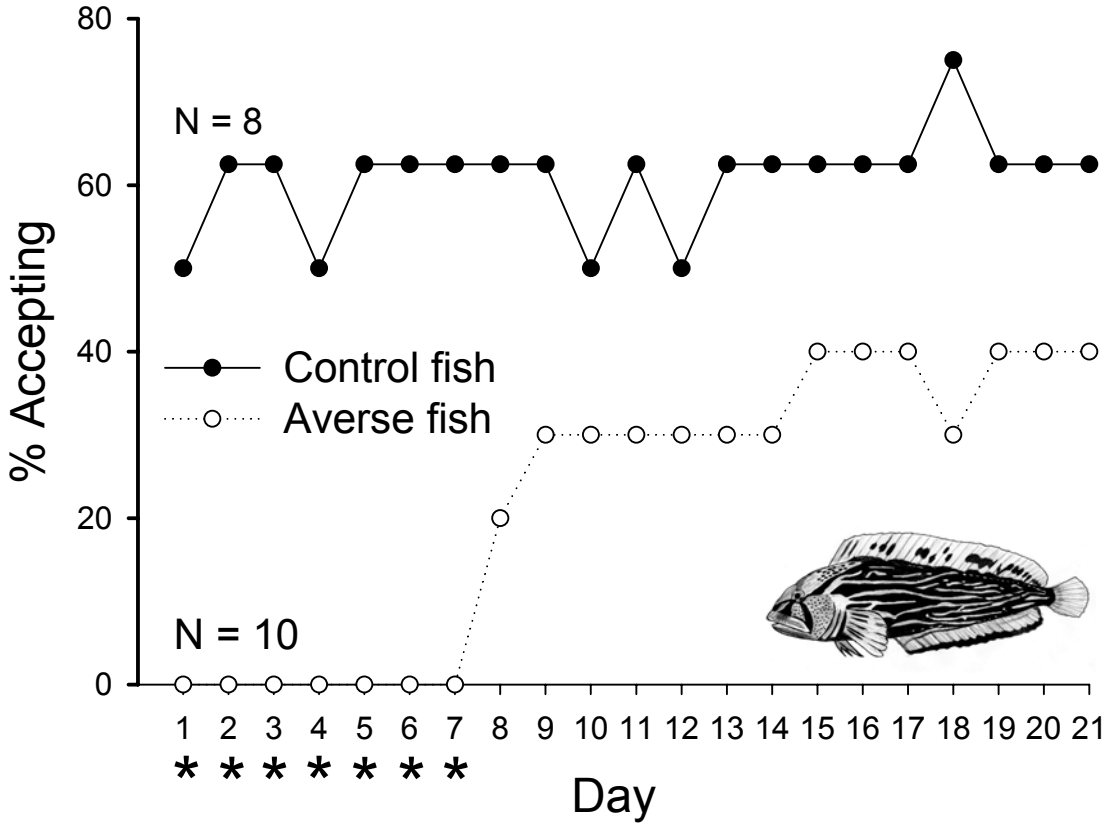

Figure 1.5. Persistence of aversion without reinforcement. For each day, the palatability of tuna pellets lacking extracts to striped blennies that had not previously received tuna with nudibranch extract (=control fish) or that had previously received tuna with extract on 11 days during the preceding 32 days (=averse fish). Analyses were by one-tailed Fisher exact tests. Significant differences ($P < 0.05$) are indicated by an asterisk below the day of comparison.

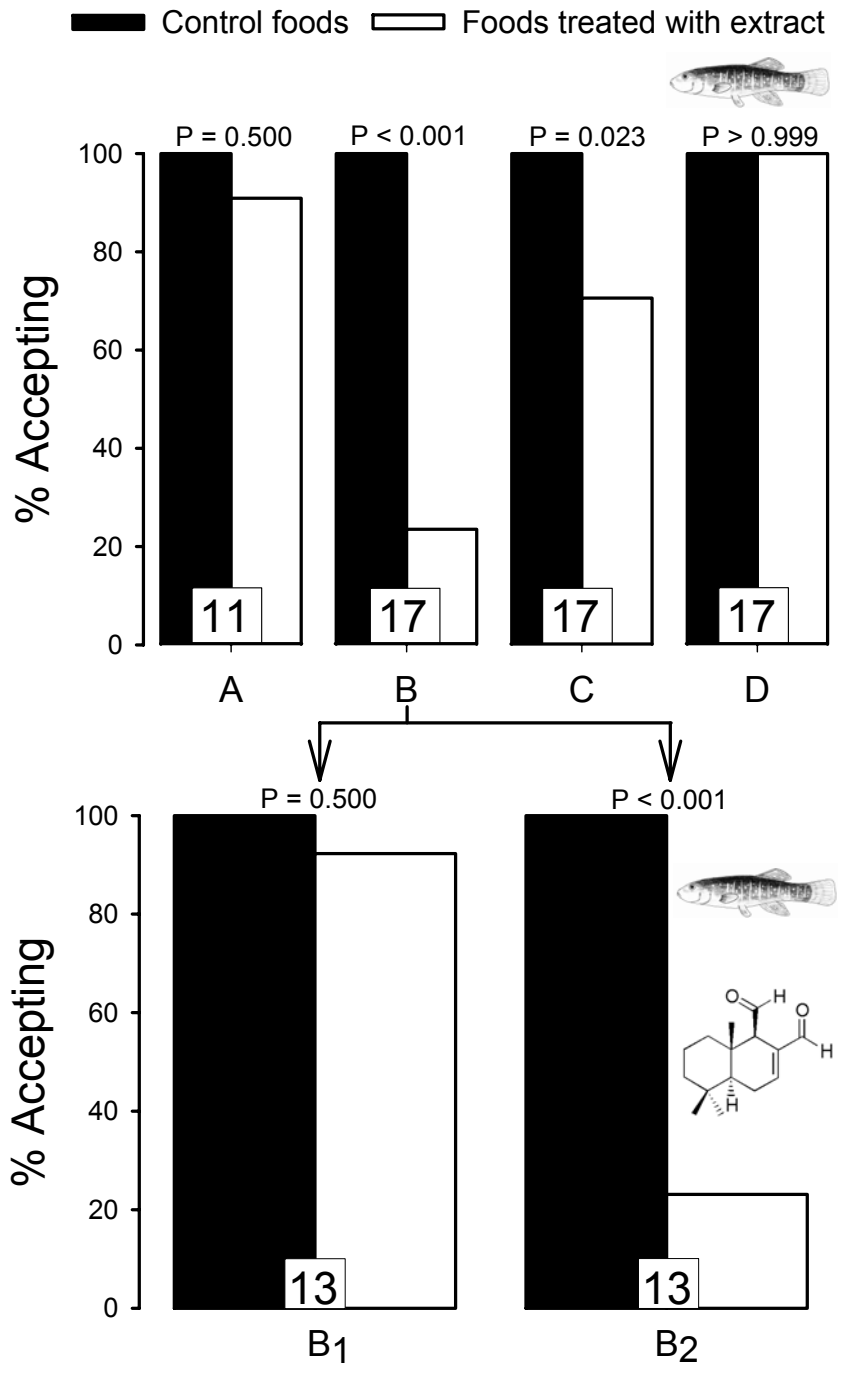

Figure 1.6. Palatability of fractions from the lipid-soluble extract of *Doriopsilla pharpa* fed at natural concentrations to mummichogs. Sample size is given within bars. Analyses were by one-tailed Fisher exact tests.

We then tested this compound (acquired via isolation from the nudibranch) at natural concentration to see if it alone could account for the effects of the lipid-soluble extract on fish feeding, we detected patterns similar to those seen in tests of the lipid-soluble extract. On the first day that food with polygodial was offered, fish ate treatment and control food with equal frequency (Figure 1.7A, $P > 0.999$). However, on days 2-5, feeding by treatment fish decreased, while feeding by control fish did not. After 2 days of exposure to foods with polygodial, treatment fish significantly avoided these foods relative to control fish (Figure 1.7A and B, $P = 0.045$). For feeding on extract-free foods, the difference between treatment fish and control fish was significantly different on day 4 (Figure 1.7A and B, $P < 0.018$). Similar to assays where striped blennies learned to avoid foods treated with lipid-soluble extracts, the striped blennies in this assay appeared to avoid the general taste of foods containing polygodial, not just foods treated with this compound. Unlike assays with the crude extract, striped blennies never regurgitated foods treated with polygodial alone.

Discussion

The nudibranch *Doriopsilla pharpa* was unpalatable to all co-occurring consumers tested in the lab and to a natural suite of consumers in the field (Figure 1.1). Two generalist, marine fish species, striped blennies and mummichogs, developed rapid aversions to foods containing lipid-soluble extracts (Figure 1.3) or the specific compound polygodial (Figure 1.6 and 1.7) from the co-occurring nudibranch. These patterns of learned food aversion are similar to those reported for terrestrial consumers (Burghardt et al. 1973, Provenza et al. 1995, Crossland 2001, Kimball et al. 2002). Learned aversions developed after as few as 1 presentation (Figures 1.3 and 1.4) and striped blennies retained this aversion without reinforcement for at least 2 weeks (Figure 1.4).

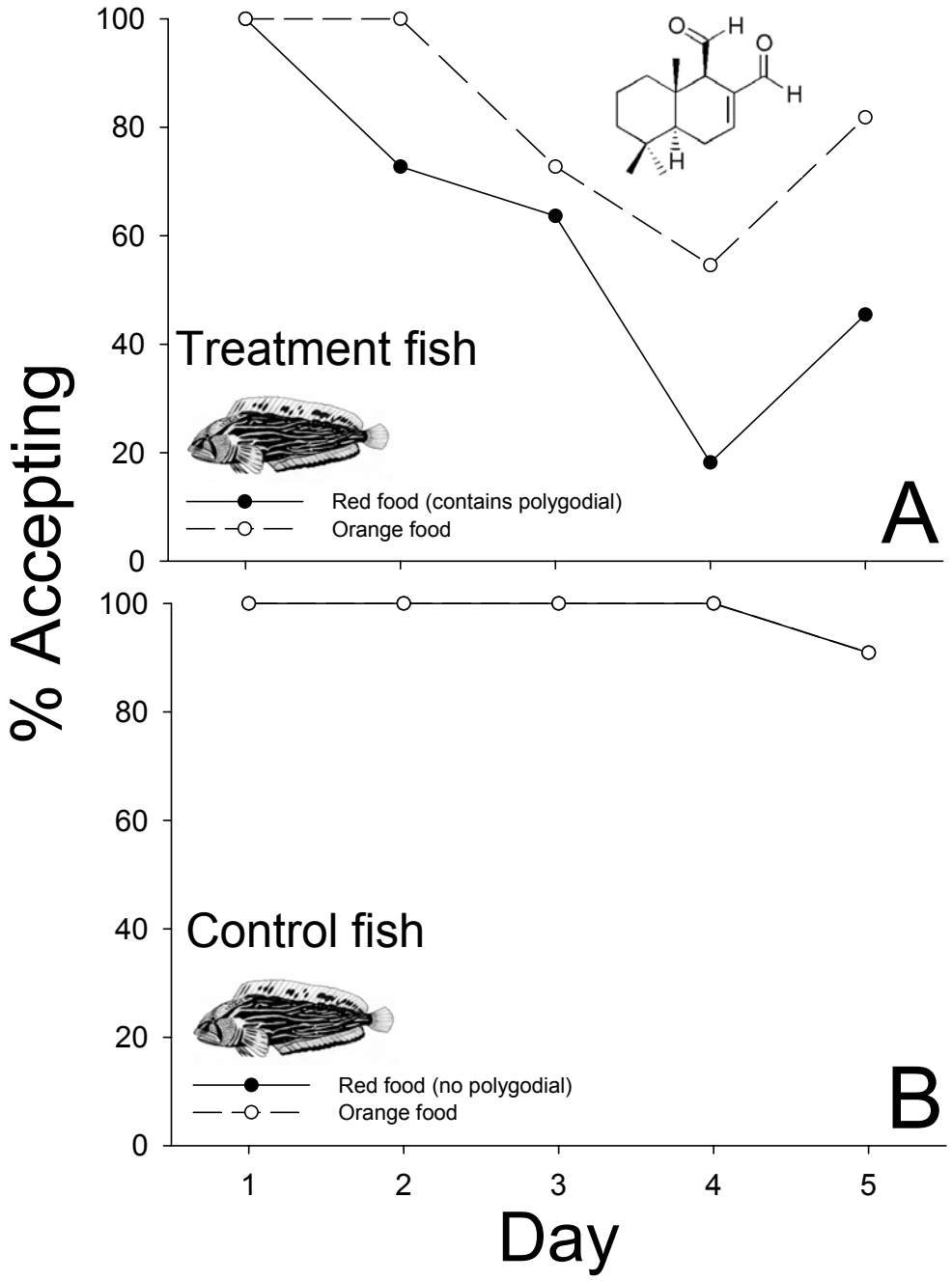

Figure 1.7. Aversive learning caused by polygodial. For each day, the palatability of red and orange pellets to treatment (A) and control (B) striped blennies. Red pellets fed to treatment fish contained polygodial at natural concentrations while red pellets fed to control fish did not. Orange pellets never contained polygodial for either fish group.

The extracts affected the 2 fish species differently, inducing regurgitation in striped blennies but not mummichogs.

Both fishes developed aversions to chemical cues associated with aversive foods but the specific cues varied between the 2 consumers. Striped blennies learned to avoid foods tasting and smelling like aversive foods, even when foods lacked aversive compounds, while mummichogs only avoided foods containing the aversive compound or extract. After 2 days of exposure to aversive foods, striped blennies dramatically decreased the handling time and the number of times foods were sampled before final rejection. For mummichogs, handling time and rejection number did not change significantly over time (Figure 1.3), but their initial handling times were as low as those reached by blennies after 2 days of exposure to the aversive food. When the aversive compounds were removed from foods and these new palatable foods were presented to fish daily, food aversions still persisted for at least 1 week for striped blennies (Figure 1.5).

Doriopsilla pharpa is protected from fish predation by polygodial. This is the first study to isolate polygodial from *D. pharpa* and to report polygodial's role in learned aversions. Several organisms contain polygodial, including the terrestrial plant from which this compound was first discovered *Polygonum hydropiper* (Ohsuka 1962), Canallaceae trees (Kubo and Ganjian 1981), several species of Dendrodorid nudibranchs, including several members of the *Doriopsilla* genus (Cimino et al. 1983, Okuda et al. 1983, Cimino et al. 1985, Gavagnin et al. 2001a, Gavagnin et al. 2001b), and a member of the sponge genus *Dysidea* (Paul et al. 1997). While previous studies with fish suggest possible ecological functions of polygodial (Cimino et al. 1982), they are ecologically unrealistic because they used consumers that would never encounter these prey in nature. Ecological functions are best demonstrated when natural compounds are presented in realistic ways to co-occurring consumers (Pawlik et al.

1995, Hay 1996, Bernays and Chapman 2000). In our study, 2 co-occurring fishes would not feed on the nudibranch (Figure 1.1) and learned to avoid foods treated with natural concentrations of polygodial and lipid-soluble nudibranch extracts (Figures 1.3, 1.4 and 1.7). Polygodial alone did not induce regurgitation in striped blennies, but lipid-soluble extracts did, suggesting that other chemicals play additional, or synergistic, roles in defense and in causing regurgitation.

Although both fishes learned to avoid foods containing these compounds, their responses to aversive foods differed in 3 major aspects. First, consuming extracts induced regurgitation in striped blennies but not mummichogs. Second, the chemical cues used during development of learned aversions differed between species (striped blennies avoided the general taste of food [tuna] that caused regurgitation while mummichogs recognized and avoided the taste of the extract and of polygodial). Third, although both fishes learned to avoid aversive food, handling time for naïve striped blennies was high but dropped by 95% after 2 days of experience with aversive foods. In contrast, handling time was initially low and did not change for mummichogs, suggesting that these fish were immediately more efficient at detecting and rejecting unpalatable foods.

The short-term physiological effects of consuming these aversive foods differed between the 2 fish species. Mummichogs never regurgitated foods treated with nudibranch extracts, while most striped blennies regurgitated treated foods after an average of ~13 minutes, a time similar to previous reports for other marine fishes - within 1-2 hours (Lindquist and Hay 1995), 1.52 minutes (Gerhart 1984), and 7.25 minutes (Gerhart 1984). Short delays between ingestion and illness create strong learned aversions (Gelperin 1975). Our results disagree with the hypothesis that materials that induce regurgitation in a given fish species tend also to induce emetic responses in other fish species (Gerhart and Coll 1993).

Our test fishes used different chemical cues when developing learned aversions, possibly as a function of how the compounds affected the 2 fishes. Although both fishes learned to avoid foods with these extracts, striped blennies learned to avoid foods tasting or smelling like aversive foods but mummichogs learned to avoid the taste of the aversive extract or compound alone. Striped blennies may be unable to detect these compounds forcing them to exploit other cues, such as general taste of the food (i.e., tuna). Alternatively, the use of different cues may be related to how the compounds affected the consumers (Nicolaus et al. 1983). Nudibranch extracts induced a strong regurgitation response in striped blennies. These fish then took a conservative strategy by associating the taste of food with aversive compounds and then avoiding all foods tasting or smelling like defended foods, even when these lacked aversive compounds. Mummichogs, however, never regurgitated. They learned to detect and respond to the specific taste of the extract, or of polygodial, alone. This interspecific difference was not a simple issue of dosage (because both fish were of similar size and received equal concentrations) and was not due to an inability of mummichogs to regurgitate foods; mummichogs regurgitated foods treated with defensive extracts from marine worms (C. Kicklighter, pers. comm.).

Striped blennies rapidly learned to spend less time handling aversive foods, and by the third day of exposure to treated foods, their handling time had dropped to similar levels as mummichogs that could taste the deterrent extract (Figure 1.3C and D; note the different scales for the different consumers on these graphs). Decreased handling of aversive foods occurs in other consumers developing aversions including mantid insects (Berenbaum and Miliczky 1984), hyenas (Yoerg 1991), and freshwater fishes (Crossland 2001). Learning to spend less time handling harmful foods could increase consumer foraging efficiency by increasing the time available for encountering beneficial foods (Hughes 1979) and by reducing physiological exposure to harmful foods. In contrast,

mummichogs did not significantly change these behaviors during the development of aversions, but they were able to better sense the extracts or compounds responsible for deterrence, and thus respond more directly to the defensive traits of the foods.

Learned food aversions should be common in marine habitats given the relative prevalence of generalist consumers in marine versus terrestrial systems (Hay 1992) and the predominance of learned food aversions in generalist consumers. In terrestrial systems, generalist feeders, such as polyphagous insects (Lee and Bernays 1988) and frugivorous bats (Ratcliffe et al. 2003), learn to avoid foods after a negative experience while more specialist feeders, such as oligophagous insects (Dethier and Yost 1979) and vampire bats, do not (Ratcliffe et al. 2003). Bioassays that account for learned aversions by repeatedly offering foods to consumers for several days should improve our ability to further evaluate this hypothesis in marine systems. For example, we documented learned food aversions only after observing consumer behavior on successive days. If we had only offered foods to consumers once, we might have incorrectly concluded that these nudibranchs lack a chemical defense. With 1-time feeding trials, which are the most commonly used assays (Hay et al. 1998), we would not have been able to detect these ecologically important learned aversions.

Learned food aversions provide consumers with a response to changing prey populations and changing prey communities. For example, range shifts, range expansions, and species introductions create novel interactions between consumers and prey. Consumer learning may control the outcomes of such interactions by allowing consumers to add beneficial foods to their diet and to avoid harmful foods containing cues that are not recognized during initial exposure. By conducting comparative studies of learning in consumers, we can improve our understanding of the underlying causes of interspecific differences in learned aversions. Given that the rates of novel species introductions into marine communities are rising dramatically (Ruiz et al. 2000) and that

the noxious chemicals produced by some of these species may cause large-scale mortality of species that have not previously contacted them (e.g., Tester and Fowler 1990), an improved understanding of the learning potential of local consumers could be critical to more accurately predicting effects of species introductions.

CHAPTER 2

ZOOPLANKTON DO NOT EAT LATIN BINOMIALS: INTRASPECIFIC VARIABILITY IN PHYTOPLANKTON MORPHOLOGY AND GROWTH PHASE DRAMATICALLY AFFECTS COPEPOD FEEDING AND FITNESS

Abstract

Copepod feeding on a single clone of the Haptophyte *Phaeocystis globosa* can vary by nearly 2 orders of magnitude depending on changes in growth phase and morphology of the prey. This within-clone variance can considerably exceed variance between different species of phytoplankton and may explain why some studies find *Phaeocystis* to be palatable and others find it to be avoided as a food. When confined with different cell types, the copepods *Acartia tonsa, Pseudodiaptomus pelagicus*, and *Eucalanus pileatus* all fed more heavily on colonies than on solitary cells. *A. tonsa* consumed more stationary phase than exponential phase cells. *P. pelagicus* exhibited the opposing preference and *E. pileatus* did not distinguish as a function of growth phase. Growth phase and morphology interacted to affect feeding by *A. tonsa*; this copepod consumed 16-92 times more *P. globosa* when feeding on stationary phase colonies than when feeding on any other cell type. Effects of diet on copepod fitness were variable and not related to amount of food consumed. Survivorship of *A. tonsa* and *E. pileatus* did not differ on any of the *P.* globosa cell types, but survivorship of *P. pelagicus* was significantly suppressed on colonies (which they consumed more of) versus solitary cells. Although *A. tonsa* consumed 30X more stationary phase colonies than exponential phase solitary cells, egg production on the lesser consumed food was 2X higher. The dramatic consumption of stationary phase colonies may occur because this is a low quality, but non-toxic, food, and *A. tonsa* is attempting to compensate for low food quality by consuming more quantity. The limited consumption of other *P. globosa* colonies (i. e., exponential phase) suggests that chemical defenses may be

limiting their consumption by *A. tonsa* and that these defenses are compromised when colonies enter stationary phase. The within-species variability we documented could affect bloom dynamics and biogeochemical cycles.

Introduction

Phytoplankton, seaweeds, and higher plants exhibit intraspecific variance in morphology and chemistry that alters how herbivores use these resources as foods (Denno and McClure 1983, Butler et al. 1989, Karban 1992, Bolser and Hay 1996). While previous studies emphasized the importance of interspecific differences among plants and the effects of this variance on herbivores, recent studies suggest that intraspecific variation within plants may be equally important to plant-herbivore interactions (Lawrence et al. 2003, Taylor et al. 2003). Intraspecific variance in plant traits can strongly determine herbivore preference (Jones 1962, Cronin et al. 1998, Stout et al. 1998), as well as herbivore growth, survivorship, and fitness (Lill and Marquis 2001, Taylor et al. 2003, Barrett and Agrawal 2004). This intraspecific variance in plants can lead to significant differences in natural rates of herbivory (Ruel and Whitham 2002, Lawrence et al. 2003), herbivore population densities (Ylioja et al. 1999, Moon and Stiling 2002), and community structure and ecosystem function (Whitham et al. 2003, Bailey et al. 2004). Although relatively well-described for terrestrial plants and a few seaweeds, the effects of variable phytoplankton traits on zooplankton herbivores are understudied despite the potential for cascading affects of this variance on food webs and energy flow in pelagic ecosystems (Hay and Kubanek 2002).

Phytoplankton encounter diverse environmental conditions on ecological and evolutionary timescales that create genetic and phenotypic variability in traits such as physiological status and morphology (Hessen and Van Donk 1993, Taroncher-Oldenburg et al. 1997, Johansson and Graneli 1999). Some phytoplankton are even

capable of rapidly altering morphology as an induced defense against planktonic consumers (Hessen and Van Donk 1993, Tang 2003, Chapter 3). Despite the substantial intraspecific variance in how some species of phytoplankton are utilized by consumers (Butler et al. 1989, Teegarden 1999), previous studies frequently ignored this variance (e.g., Huntley et al. 1986, Koski et al. 1999). This has led to the common use of adjectives such as "palatable or toxic," "defended or undefended," and "good or bad" to describe the food quality of a given phytoplankton species to herbivores. This focus on the Latin binomial as an adequate descriptor of the prey being studied will be useful if interspecific variance of phytoplankton predictably exceeds intraspecific variance in how these prey are treated by consumers. However, if intraspecific variance equals, or exceeds, interspecific variance, then appreciating such variance will be critical for our understanding of pelagic communities.

There are seemingly conflicting studies of whether particular phytoplankton species are readily consumed by, or avoided by, zooplankton (reviewed in Weisse et al. 1994). For example, some studies report that copepods readily feed on *Phaeocystis pouchetii* (Huntley et al. 1987, Tande and Bamstedt 1987) while other studies report that copepods feed only reluctantly and at low rates on this alga (Verity and Smayda 1989). If the species name alone is an adequate descriptor of *P. pouchetii* food value, then these studies conflict. However, grazing zooplankton, like other herbivores, "do not eat Latin binomials" (Janzen 1979) but instead encounter individual phytoplankton cells whose histories, nutritional status, defensive traits, and thus values as foods may vary. Recent studies highlight the importance of within species variability of plants on herbivory, especially compared to interspecific plant variability (Lawrence et al. 2003, Taylor et al. 2003). If a single phytoplankton species can vary enough to occupy both palatable and unpalatable ends of the food spectrum, then these apparently conflicting

reports could be explained by an unappreciated role of intraspecific variance in traits affecting utilization by zooplankton.

Some phytoplankton species form massive blooms during which they comprise much of the local standing stock, and thus the available food, for herbivorous zooplankton. For example, *Phaeocystis* spp. can represent over 85% of total phytoplankton biomass during blooms (Hansen and van Boekel 1991, Davies et al. 1992, Hamm et al. 2001). Intraspecific variance in bloom-forming phytoplankton could, therefore, affect how consumers respond to these phytoplankton and this change could significantly alter food web structure and energy flow within planktonic ecosystems. Most *Phaeocystis* spp. alternate between solitary cell and colonial forms (Peperzak et al. 2000), and these may be susceptible to different grazing pressures. Microzooplankton are assumed to graze solitary cells and mesozooplankton are assumed to graze primarily colonies (Verity 2000). Although morphological and physiological variability within *Phaeocystis* spp. might affect food webs dominated by this phytoplankton, direct comparisons of grazing on different types of *Phaeocystis* spp. are rare. In the few studies that measured grazing on multiple morphologies, copepods grazed colonies at higher rates than solitary cells (Huntley et al. 1987, Tande and Bamstedt 1987). However, these studies tested only 1 copepod species, *Calanus hyperboreus*, so it is unclear if copepods in general prefer colonies or if this is a species-specific trait of the copepod that was studied. Similarly, few grazing studies directly controlled the growth phase of *Phaeocystis* spp. (Huntley et al. 1987, Turner et al. 2002) and none of these studies measured grazing on stationary phase algae. However, growth phase might be a key factor regulating grazing of *Phaeocystis* spp. For example, Estep et al. (1990) observed higher grazing of *Phaeocystis pouchetii* colonies by *Calanus finmarchicus* during later stages of blooms when colonies appeared less “healthy” (Estep et al. 1990) – possibly due to changes in nutrient availability and the resources available for

allocation to defenses against consumers. In addition, the potential interactive effects of *Phaeocystis* spp. growth phase and morphology on algal palatability are unknown despite the demonstrated importance of such complex interactions on the susceptibility of seaweeds and terrestrial plants to herbivory, and on how such interactive traits affect herbivore fitness (Hay et al. 1994, Cruz-Rivera and Hay 2003, Barrett and Agrawal 2004). Considering that *Phaeocystis* spp. can represent the majority of phytoplankton biomass during blooms (Davies et al. 1992, Hamm et al. 2001) and that these blooms sequester large amounts of carbon (Smith et al. 1991, Arrigo et al. 1999), the dynamics of *Phaeocystis*-grazer interactions could affect *Phaeocystis*-dominated communities and the ecosystems in which they occur.

To assess how the utilization of a phytoplankton as a food varied with growth phase and morphology of the phytoplankton as well as with the species of the consumer, we offered 3 copepod species 1 of 4 types of *Phaeocystis globosa* in a 2 factor design, with *P. globosa* growth phase (stationary or exponential) and morphology (solitary cells or colonies) as the 2 factors. We assessed how these different *Phaeocystis* cell types affected copepod survivorship for all 3 copepods and how the foods affected egg production for *Acartia tonsa*.

Materials and Methods

Plankton Cultures - *Phaeocystis globosa* (CCMP 627), originally isolated from the Gulf of Mexico, was grown xenically in L1-Si medium (CCMP recipe) at 20^{o}C under a light:dark cycle of 14:10 h. Illumination was provided by a combination of cool white and daylight fluorescent bulbs at ~100-150 µE m^{-2} sec^{-1}. Batch cultures of *P. globosa* were transferred each week into fresh media at a 1:10 dilution, for a starting concentration of approximately 50,000 cells ml^{-1}. *Acartia tonsa* and *Pseudodiaptomus pelagicus* were collected in Wilmington River near the Priest Landing dock (31^{o}57.736' N, 81^{o}00.710'

W), Skidaway Island, Savannah, Georgia, USA. *Eucalanus pileatus* were collected on the middle continental shelf off Savannah, GA. All animals were acclimated to 20°C for at least 10 h. After acclimation, adult female copepods were sorted into GF/F filtered seawater to allow gut evacuation for at least 1 hour.

Fecal Pellet Production as a Measure of Feeding on Different Diets - Quantifying grazing of heteromorphic phytoplankton, such as *Phaeocystis* spp., is more complicated than quantifying grazing of other algae because grazers can induce transformations between algal morphologies (Hessen and Van Donk 1993, Hansen et al. 1994b). Copepods can physically disrupt *Phaeocystis* colonies (Hansen et al. 1994b) and copepod-associated chemical cues can suppress colony formation (Chapter 3). Thus, a decrease in colonies or colony cell concentration can result from indirect chemical cues rather than direct grazing. These indirect effects could increase the apparent grazing rates on colonies and, likewise, decrease the apparent grazing rates on solitary cells. Therefore, measuring grazing on different morphologies should rely on methods other than counts of cells or colonies. We estimated grazing indirectly by measuring fecal pellet volume; this approach avoided problems associated with *Phaeocystis* cell counts (Hansen et al. 1994b, Chapter 3) and facilitated a survey of multiple copepod species grazing on multiple types of *P. globosa*. This approach is not novel. Fecal pellet production frequently is used as a proxy for ingestion rates for copepods, including *Acartia* (Gaudy 1974, Reeve and Walter 1977, Gamble 1978, Besiktepe and Dam 2002).

We measured fecal pellet production for each copepod species (*Acartia tonsa*, N = 3 separate containers; *Eucalanus pileatus*, N = 4; *Pseudodiaptomus pelagicus*, N = 6) feeding on a monoculture of the 4 different types of *Phaeocystis globosa* (exponential phase solitary cells, exponential phase colonies, stationary phase solitary cells, or stationary phase colonies), as well as on a patatable control food and in a treatment with

no food. Copepods in reatments with food received 22,000 cells ml^{-1} *Phaeocystis globosa*, or an equivalent cellular volume of a control food (5000 cells ml^{-1} *Rhodomonas baltica* for *Acartia tonsa* and *Pseudodiaptomus pelagicus* or 11 cells ml^{-1} *Rhizoselenia* sp. for *Eucalanus pileatus*). The cell density for *P. globosa* represents natural concentrations during peak bloom periods (Claustre et al. 1990, Hansen and van Boekel 1991). For each replicate, several adult female copepods (13 *A. tonsa*, 4 *E. pileatus*, or 10 *P. pelagicus*) were incubated with the test alga in 500 ml jars, systematically interspersed on a plankton wheel (60 cm in diameter), and rotated at ~0.5 r.p.m. Grazing was allowed overnight, and copepods were removed the next day.

Acartia tonsa and *Pseudodiaptomus pelagicus* were removed by passing contents through a 160 µm mesh. *Eucalanus pileatus*, a larger species, were removed individually with a large bore pipet. Fecal pellets were collected by separately sieving the remaining contents of each container through a 25 µm mesh. The retained fecal pellets were counted with a dissecting microscope. Fecal pellet production rates were corrected for the number of surviving copepods and the size of fecal pellets for each diet. To standardize fecal pellet counts by size across diets, we measured the lengths and widths of 20-50 fecal pellets for each copepod species feeding on each diet and calculated an average fecal pellet volume per copepod species per diet assuming a cylindrical shape for fecal pellets. All counts were then adjusted to fecal pellet volume produced per diet.

Assays assessing the effects of various foods for a particular copepod species were all run synchronously to prevent confounding treatment effects with temporal effects, but each copepod species was assayed at a different time. Thus, we can rigorously contrast effects of different foods on a copepod species, but we cannot rigorously contrast effects across different species due to time and species being confounded.

The 4 *Phaeocystis globosa* types were collected from 2 stock cultures; 1 was in exponential phase and 1 in stationary phase. For both cultures, solitary cells were separated from colonies by repeatedly reverse-filtering cultures through a 10 μm mesh. Reverse-filtration gently separates particles according to size. A sieve is partially submerged in a culture, a small tube is placed within the sieve, and a weak suction is applied to the tube. This process separates cultures into 2 volumes, 1 containing solitary cells (the volume passing across the sieve and into the tube) and the other containing colonies (the volume containing particles too large to pass across the sieve).

Prior to assays, exponential phase cultures were frequently diluted with fresh media to maintain their growth phase. Stationary phase cultures were inoculated 10 days prior to feeding assays to ensure that they would reach stationary phase by the start of feeding assays. To confirm that this transfer schedule produced stationary phase cultures, we measured daily chlorophyll *a* concentrations in a similarly inoculated culture for 12 days. Three 10 ml sub-samples were taken from this culture each day, filtered onto GF/F filters, and chlorophyll *a* concentrations were measured using the fluorometric method of Parsons et al. (1984). No chlorophyll data were collected on day 8.

Effects of Diet on Survivorship, Egg Production, and Temporal Patterns of Feeding - To determine if copepods changed their feeding rates with increased exposure to particular diets (due to acclimation or hunger) and to assess the effects of *Phaeocystis globosa* diets on copepod fitness, copepods were transferred to new solutions of the same food type daily for several days (3 days for *Euplotes pileatus*, 4 days for *Pseudodiaptomus pelagicus*, and 3-7 days for *Acartia tonsa*). We depleted our stock of stationary phase colonies after 3 days during the *A. tonsa* assay, so copepods receiving this diet were thereafter offered exponential phase colonies to see how their feeding changed with this

change in diet. We measured fecal pellet production and survivorship each day. For *Acartia tonsa*, we also measured daily egg production as an additional measure of the fitness effects of feeding on different *P. globosa* diets.

Data Analysis - We used a two-factor ANOVA, with growth phase and morphology as separate factors, to examine differences in fecal pellet production across diets on day 1 for each copepod species. Simple linear regression determined whether fecal pellet production changed with time, an indication of acclimating to diets. Only the first 3 days were included in regression analyses for *Acartia tonsa* fed stationary colonies because we exhausted all of this food type by the end of day 3. Survivorship for all copepod species was assessed on the last experimental day by comparing transformed percentages using ANOVA. For *A. tonsa*, this was done on day 3 as well because that is when we exhausted our supply of stationary phase colonies. Post-hoc comparisons of survivorship were made with Tukey tests. The total number of *A. tonsa* eggs produced was determined by adding the daily number of eggs produced for each replicate and these totals were compared using ANOVA followed by Tukey tests. This was done on day 3 for all diets and then on day 7 for all diets but the stationary phase colonies.

Results

Phaeocystis globosa cultures grew rapidly on days 1-7, but were in stationary phase thereafter (Figure 2.1). Thus, our 10 day old cultures used in feeding assays were correctly described as stationary phase cultures. The change in chlorophyll *a* levels during the rapid growth phase were linear, rather than exponential, but this likely reflects changes in chlorophyll levels within cells as nutrients become more limiting rather than a lack of exponential growth in biomass or biovolume (Larson and Rees 1996).

Feeding Rates on the Different Diets - During the first day of feeding, all 3 copepod species fed more on colonies of *Phaeocystis globosa* than on solitary cells (Figure 2.2, *Acartia tonsa* $P < 0.001$; *Eucalanus pileatus* $P = 0.052$; *Pseudodiaptomus pelagicus* $P = 0.050$). *Pseudodiaptomus pelagicus* also fed more on exponential than on stationary phase cells ($P = 0.014$), while *Eucalanus pileatus* did not differentiate as a function of growth phase ($P = 0.592$). Feeding by *A. tonsa* was more complex. For this species there was a significant interaction between *P. globosa* growth phase and morphology (Figure 2.2A, $P < 0.001$). Feeding rates on the different *P. globosa* diets completely bracketed feeding on the positive control diet (Figure 2.2A). This copepod fed 92X more rapidly on colonies that were in stationary phase than on colonies of the same clone that were in exponential phase. When *P. globosa* was undergoing exponential growth, *A. tonsa* fed more on solitary cells than on colonies, but when *P. globosa* reached stationary phase, *A. tonsa* feeding rates reversed and the copepods fed much more heavily on colonies. The patterns seen during day 1 feeding were consistent over longer time periods; feeding was consistently much higher on stationary phase colonies of *P. globosa* than on any other food (Figure 2.3A). Fecal pellet production by *A. tonsa* was 3.5-5 times higher on stationary phase colonies compared to *R. baltica* diets for the 3 days that we offered both algae to *A. tonsa* (Figure 2.3A). When *A. tonsa* being fed stationary phase colonies were switched to a diet of exponential phase colonies on day 4, fecal pellet production immediately decreased by about 95%, and remained low for the next 4 days of the experiment.

Both *Eucalanus. pileatus* and *Pseudodiaptomus pelagicus* fed more on the positive control foods than on any of the *Phaeocystis globosa* diets. In contrast, *Acartia tonsa* feeding on stationary phase colonies consumed about 5X as much *P. globosa* as control food, but when feeding on the other 3 *P. globosa* diets, consumption was much less than consumption of the control food. Thus, *A. tonsa* recognized the intraspecific

Figure 2.1. Growth curve of a *Phaeocystis globosa* culture grown under experimental conditions measured by chlorophyll *a* concentration. Culture reached stationary phase 7 days after inoculation.

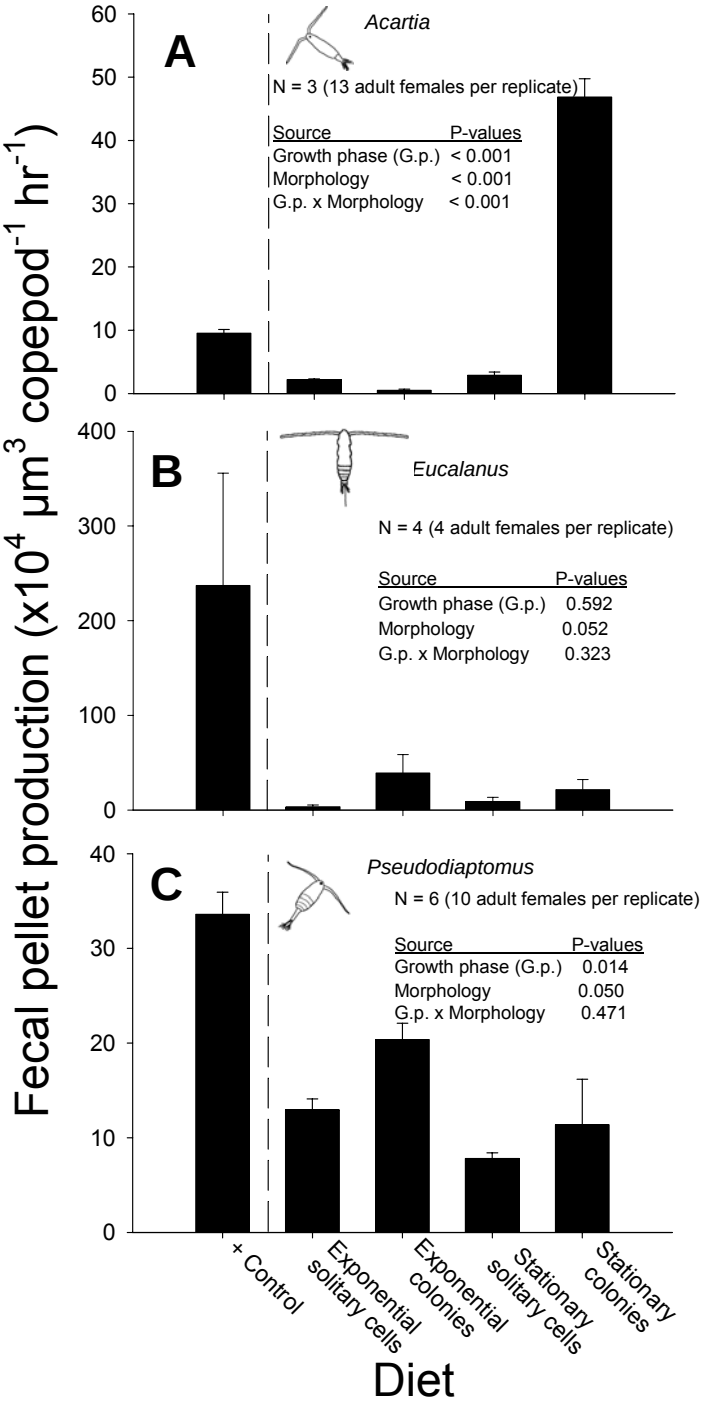

Figure 2.2. Fecal pellet production of (A) *Acartia tonsa*, (B) *Eucalanus pileatus*, and (C) *Pseudodiaptomus pelagicus* on day 1. Copepods were offered equivalent cellular volumes of 1 type of *Phaeocystis globosa* or a positive control phytoplankton. Values are means +1 SEM.

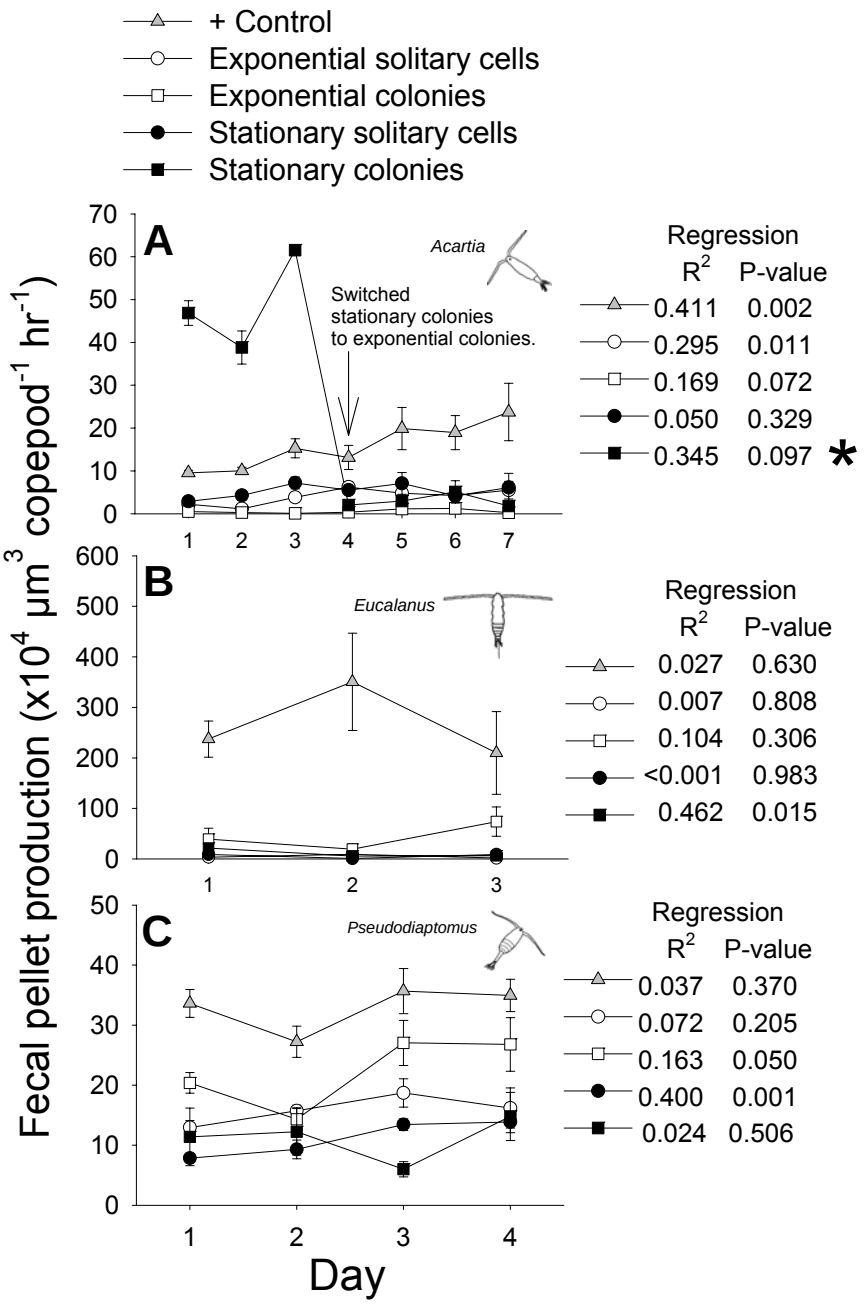

Figure 2.3. Fecal pellet production of (A) *Acartia tonsa*, (B) *Eucalanus pileatus*, and (C) *Pseudodiaptomus pelagicus* during several days of feeding on the same diet type. Copepods were offered equivalent cellular volumes of 1 type of *Phaeocystis globosa* or a positive control phytoplankton. Values are means ± 1 SEM. * Only the first 3 days were included in the regression analysis for stationary colonies fed to *A. tonsa* because these copepods were switched to exponential colonies on day 4.

differences within 1 clone of *P. globosa* as more dramatic than the interspecific differences between *Rhodomonas* and *P. globosa*.

Effects of Preconditioning on Fecal Pellet Production - Because 3 copepod species were each offered either a positive control food or 1 of 4 *Phaeocystis globosa* types, we measured fecal pellet production for a total of 15 copepod-food combinations. Long-term fecal pellet production of *Acartia tonsa* on stationary phase colonies could be assessed for only 3 days. Fecal pellet production decreased with time in 1 combination (Figure 2.3, *Eucalanus pileatus* feeding on stationary phase colonies; $R^2 = 0.462$, $P = 0.015$), and increased with time in 4 combinations [*A. tonsa* feeding on the positive control food ($R^2=0.411$, $P = 0.002$) and on exponential phase solitary cells ($R^2 = 0.295$, $P = 0.011$), and *Pseudodiaptomus pelagicus* feeding on exponential phase colonies ($R^2 = 0.163$, $P = 0.050$) and stationary phase solitary cells ($R^2 = 0.400$, $P = 0.001$)]. Given that most copepods were consuming minimal amounts of the *P. globosa* diets, one might have expected increasing hunger or acclimation behaviors to increase feeding over time. However, fecal pellet production still remained low for copepods feeding on *P. globosa* even for those combinations where feeding increased with time.

Effects on Survivorship and Egg Production - Growth phase of *Phaeocystis globosa* did not affect copepod survivorship (*Acartia tonsa*, $P = 0.789$; *Eucalanus pileatus*, $P > 0.999$; *Pseudodiaptomus pelagicus*, $P = 0.374$; t-tests) so survivorship data for exponential phase and stationary phase *P. globosa* were combined for both morphologies in Figure 2.4. Furthermore, survivorship after 3 days was not significantly different for *A. tonsa* fed any *P. globosa* diet ($P = 0.540$), so we also combined the data for exponential and stationary phase colonies. Survivorship did not differ when feeding on colonies versus solitary cells for *A. tonsa* or *E. pileatus*, but survivorship of *P. pelagicus* was twice as

high on solitary cells as on colonies (Figure 2.4). Survivorship on *P. globosa* diets was higher than on starvation controls for all copepods except *E. pileatus* (Figure 2.4, $P > 0.433$). However, both our sample size and experimental duration for *E. pileatus* were low (4 replicates with 4 copepods per replicate, for 3 days) and probably constrained our statistical power and ability to detect differences in survivorship. Survivorship for both *A. tonsa* and *P. pelagicus* was lower on *P. globosa* colonies than on the positive control foods; survivorship on diets of solitary *P. globosa* cells did not differ from survivorship on positive control foods. After 3 days of feeding, *Acartia tonsa* females produced only 23-51% as many total eggs when fed *Phaeocystis globosa* as when fed *Rhodomonas baltica* (Figure 2.5, $P < 0.001$). After both 3 and 7 days, egg production was a significant 80-185% higher when *A. tonsa* was feeding on exponential phase solitary cells than when feeding on any of the other *P. globosa* diets. Although *A. tonsa* ate stationary phase colonies at about 21X the rate at which it consumed exponential phase solitary cells (Figure 2.2A), this increased feeding resulted in significantly fewer, rather than significantly more, eggs (Figure 2.5). A regression of fecal pellet production on day 1 against *A. tonsa* egg production on day 3 indicated no detectable relationship ($R^2 = 0.002$, $P = 0.942$).

Discussion

It is common for prey species to be considered either palatable to, or defended against, a particular consumer. This basic approach underlies general ecological hypotheses or patterns such as early successional species being more palatable than later successional species (Cates and Orians 1975), tropical prey being better defended than temperate prey (Coley and Aide 1991, Bolser and Hay 1996, Siska et al. 2002), and spatial escapes from consumers being critical for preventing local extinction of palatable prey in areas where consumer activity is high (Hay 1997). However, we show here that

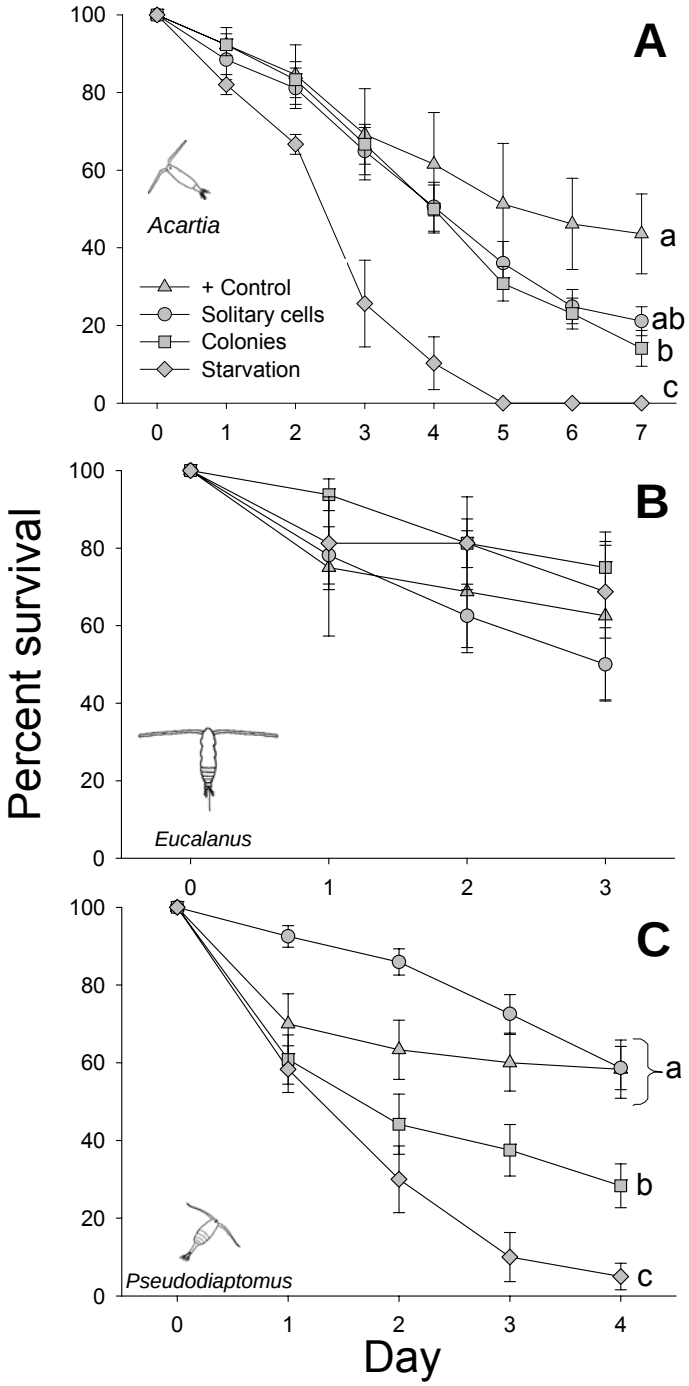

Figure 2.4. Survivorship of (A) *Acartia tonsa*, (B) *Eucalanus pileatus*, and (C) *Pseudodiaptomus pelagicus* during several days of feeding on the same diet type. Copepods were offered equivalent cellular volumes of 1 type of *Phaeocystis globosa* or a positive control phytoplankton. Letters next to values on the last days indicate significant ($P < 0.05$, Tukey) among-treatment differences in survivorship on the last day. Values are means ± 1 SEM.

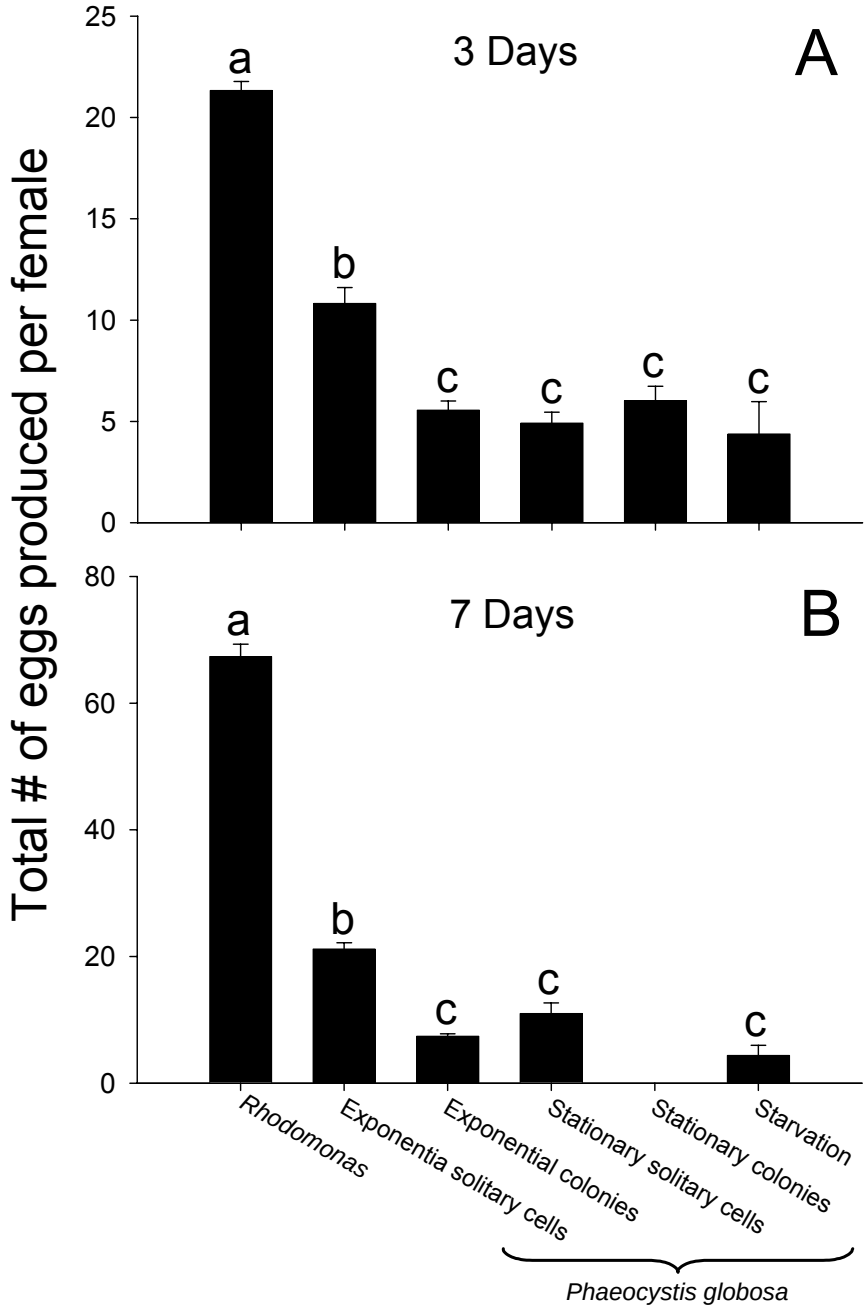

Figure 2.5. Total egg production of *Acartia tonsa* females feeding on the same diet type for 3 (A) or 7 (B) days. Copepods were offered equivalent cellular volumes of 1 type of *Phaeocystis globosa* or a positive control phytoplankton. No data for egg production were collected after 3 days for the diet of stationary colonies. Letters above bars indicate significant ($P < 0.05$, Tukey) among-treatment differences. Values are means + 1 SEM.

a single clone of a simple prey species can become 92X more susceptible to consumption by simply growing a few days older and shifting from a solitary to a colonial form (Figure 2A). The intraspecific variance in palatability of *Phaeocystis globosa* being grazed by *Acartia tonsa* exceeded the interspecific difference between *Rhodomonas* and *P. globosa*. Although the magnitude of this intraspecific difference in grazing seems extreem, the basic finding of intraspecific differences in palatability equalling or exceeding differences among different species also occurs among seaweeds (Bolser and Hay 1996) and terrestrial plants (Lawrence et al. 2003).

An appreciation for the effects of phenotypic plasticity may reconcile the conflicting reports surrounding the palatability of *Phaeocystis* spp. For example, *Phaeocystis* spp. are often referred to as "poor foods" for zooplankton grazers because some studies report low grazing or negative fitness effects on *Phaeocystis* spp. diets (Verity and Smayda 1989, Tang et al. 2001). However, there is variability in this assessment and other studies refer to *Phaeocystis* spp. as useful foods (Huntley et al. 1987, Tande and Bamstedt 1987, Turner et al. 2002). Unfortunately, previous studies rarely assessed the importance of intraspecific variability of *Phaeocystis* spp. to copepod-*Phaeocystis* interactions. If we are to adequately understand consumer-prey interactions and the role of various prey species in affecting food-web dynamics, we will need to appreciate the degree to which intraspecific variance in prey susceptibility to consumers can affect these interactions. For some species, variability among phenotypes may be minimal, but for *Phaeocystis globosa*, it is appreciable. When feeding on stationary phase cultures, *A. tonsa* produced 1500% more fecal volume on colonial versus solitary cell diets, but when feeding on exponential phase cultures, feces production on colonies declined to only about 25% of production on solitary cells.

Intraspecific variance in plant traits can even affect community and ecosystem processes (Whitham et al. 2003, Bailey et al. 2004). For example, beavers are

ecosystem engineers whose behavior can fundamentally change local ecosystems - their foraging is determined, in part, by the concentration of condensed tannins in trees and these concentrations vary strongly with tree genotype (Bailey et al. 2004). Similarly, the intraspecific variation in *Phaeocystis globosa* traits could have broader impacts including altering food webs and biogeochemical processes. For instance, if *Acartia tonsa* was the predominant grazer in a *Phaeocystis globosa* bloom, then grazing by *A. tonsa* would be relatively high if the bloom consisted primarily of colonies in stationary growth phase. Although this grazing would not result in large secondary production of *A. tonsa* eggs, it would lead to high fecal pellet production that could transport carbon and nutrients from surface to deep waters (Besiktepe and Dam 2002). However, much of the production would escape *A. tonsa* consumption if the bloom consisted of *P. globosa* in exponential phase or *P. globosa* solitary cells. In this case, much energy and nutrients might be recycled in the microbial loop.

The significant differences in fecal pellet production frequently failed to predict the effects of *Phaeocystis globosa* diets on copepod fitness measured both by survivorship and egg production. Surprisingly, the 400% increase in fecal pellet production on stationary phase colonies versus *Rhodomonas baltica* did not affect *Acartia tonsa* survivorship or egg production and, in fact, both traits were lower on stationary phase colonies than *R. baltica*. This may indicate that *A. tonsa* finds stationary phase colonies of *P. globosa* to be a low quality, but non-toxic, food and thus tries to compensate for the low quality by increasing consumption. Compensatory feeding also occurs among marine amphipods (Cruz-Rivera and Hay 2000, 2003), marine zooplankton (E. K. Prince et al. submitted), and terrestrial megaherbivores (Sinclair and Norton-Griffiths 1979) when they are confined to foods of differing nutritional quality. Although this behavior is common, it may be an effort of despiration,

compensatory feeding failed to increase survivorship here, as it failed to do for several marine amphipods (Cruz-Rivera and Hay 2000, 2003).

Although *Acartia tonsa* appears to graze stationary phase colonies more readily than exponential phase colonies, some of the differences in fecal pellet production could be attributed to differences in assimilation efficiency rather than grazing rates. We chose to estimate grazing with fecal pellet counts because copepods affect transformations between solitary cells and colonies (Hansen et al. 1994b, Chapter 3), thereby limiting the ability of grazing rates based on cell counts to separate between the direct and indirect effects of grazers. A cost of estimating grazing rates with fecal pellet counts is that high fecal pellet production could be due to low assimilation efficiency. However, for assimilation efficiency alone to produce the differences seen in Figure 2.2A mandates that assimilation efficiency when feeding on the exponential phase colonies is about 9000% higher than when feeding on the stationary phase colonies; this magnitude of change seems unlikely. It is more likely that differences in fecal pellet production are driven primarily by differences in feeding.

Phaeocystis-copepod interactions are complex and generalizations about the palatability of *Phaeocystis* to copepods will thus describe only a subset of these interactions. In this study, intraspecific variation in copepod feeding on *Phaeocystis globosa* was dramatic with some types being consumed 92X more than other types. This variation was greater than the between-species variation we measured. It is clear that copepods do not eat Latin binomials (Janzen 1979); instead, they encounter and choose among individual cells of a certain morphology in a certain growth phase. This underappreciated effect of intraspecific variation could be common for phytoplankton as many phytoplankton species vary temporally and spatially. When intraspecific variability in phytoplankton affects susceptibility to grazers, these effects can scale-up to alter food webs and biogeochemical cycling but also allow for phenotypic plasticity to be used as a

defense against grazers (Lurling and Van Donk 1996, Chapter 3). This variation in palatability, as well as its differential effect on different consumers, needs to be more fully appreciated and understood if we are to gain adequate insight into the processes and mechanisms creating patterns in planktonic ecosystems.

CHAPTER 3

CHEMICAL CUES FROM GRAZING INDUCE CONSUMER-SPECIFIC DEFENSES IN A MARINE PHYTOPLANKTON

Abstract

Chemical cues from mesozooplankton and a microzooplankton induced adaptive, consumer-specific responses of colony formation in the marine phytoplankton, *Phaeocystis globosa*. The copepod *Acartia tonsa* fed 3x more on colonies than on solitary cells, and chemical cues from this activity suppressed colony formation. In contrast, the ciliate *Euplotes* sp. grew 3x more on solitary cells than on colonies, and signals from this feeding stimulated colony formation. Chemical cues from feeding by a natural, mixed-species collection of mesozooplankton also suppressed colony formation by *P. globosa.* The effects of chemical cues alone accounted for up to 84% of the suppression of colony formation when *P. globosa* was directly grazed by the natural community of mesozooplankton. The colony suppression cue increased with increasing copepod density and was retained in organic fractions of extracts from the water in which copepods had been feeding. Signals affecting colony formation were consistant and strong, but, contrary to previous studies, we found no evidence that colonies enlarged in response to waterborne, grazer cues. Induced responses to grazer cues in *Phaeocystis* dominated systems could affect food web structure, the timing of phytoplankton blooms, and how energy and nutrients move through pelagic ecosystems.

Introduction

Some consumers induce morphological, chemical, life history, or behavioral defenses in their prey (reviewed in Havel 1987, Sih 1987, Tollrian and Dodson 1999). These induced responses have direct consequences for consumer-prey interactions

(Stibor and Luning 1994, Karban et al. 2003) but can also create a powerful cascade of indirect interactions that fundamentally change whole communities, and these community-wide changes can occur due to prey responses to chemical cues alone (i.e., even without the effects of direct consumption or density-dependent effects; Trussell et al. 2002, 2003, 2004). Despite the prevalence and demonstrated importance of inducible defenses in terrestrial and benthic aquatic communities, we know little about these defenses in marine planktonic systems (Lass and Spaak 2003), where chemical defenses and chemical signaling are hypothesized to have strong ecosystem-wide effects (Hay and Kubanek 2002). Induced morphological defenses would be predicted to be advantageous in planktonic systems due to herbivores being spatially and temporally variable and size-selective in their feeding choices (Dodson 1974, Harvell 1990).

We evaluated induced morphological defenses in marine phytoplankton using the haptophyte *Phaeocystis globosa*. This genus commonly dominates phytoplankton blooms in portions of the world's oceans (Hansen and van Boekel 1991, Davies et al. 1992, Hamm et al. 2001, Irigoien et al. 2004), sequesters large amounts of carbon (Verity et al. 1988, Arrigo et al. 1999), and thus produces the major biogeochemical signals within these communities (Smith et al. 1991). This large pulse of production is sometimes described as a palatable input that drives local food webs, and is sometimes described as a resource that is avoided by consumers and thus goes primarily through the microbial loop (van Boekel et al. 1992, Weisse et al. 1994, Gifford et al. 1995, Stelfox-Widdicombe et al. 2004). The effects of this variability on fisheries and local community structure can be considerable, and could be generated by induced morphological changes in *Phaeocystis*.

Most *Phaeocystis* species can change from solitary cells (4-6 μm in diameter) to colonies that can reach 1.5-2.0 mm in diameter (Rousseau et al. 1994) - a change in

diameter of several orders of magnitude. This change profoundly affects *Phaeocystis*-dominated food webs because solitary cells are grazed primarily by microzooplankton while colonies are grazed primarily by meso- and macrozooplankton (Tande and Bamstedt 1987, Hansen et al. 1993, Tang et al. 2001). Furthermore, the transformation from solitary cells to colonies is critical to bloom formation because blooms are dominated by the colonial form while solitary cells dominate pre- and post-bloom periods (Peperzak et al. 2000). Controls on this transformation may indirectly affect biogeochemical processes of entire ecosystems because of the large impact that *Phaeocystis* blooms have on carbon sequestration (Smith et al. 1991, Gifford et al. 1995). Although several studies describe abiotic factors such as temperature, nutrients, and light affecting colony formation (Verity et al. 1991, Riegman et al. 1992, Hegarty and Villareal 1998), the patterns are inconsistent between studies. For example, high light is reported to both suppress (Hegarty and Villareal 1998) and to increase (Peperzak 1993) colony formation. Presently, we lack a firm understanding of critical controls on colony formation (Veldhuis and Admiraal 1987, Rousseau et al. 1994, Hamm 2000). Detecting the threat of grazing and responding by switching to a less susceptible phenotype could decrease *Phaeocystis* losses to consumers as it does for many terrestrial plants (Karban and Baldwin 1997), freshwater phytoplankton (Hessen and Van Donk 1993, Lurling and Van Donk 1996) and freshwater zooplankton (Dodson 1988, Stibor and Luning 1994). This change also could play an important role in promoting *Phaeocystis* blooms.

Alternate prey phenotypes can be differentially susceptible to consumers and this variability may lead to consumer-specific induced responses (Dodson 1974). For example, *Daphnia* body size strongly determines the outcome of *Daphnia*-predator interactions. The size at which *Daphnia* allocate resources to reproduction corresponds to the identity of predator cues that *Daphnia* encounter and this specificity appears to benefit *Daphnia* (Stibor and Luning 1994). Similarly, *Phaeocystis* morphologies vary in

their susceptibility to different herbivores, so selection may favor herbivore-specific responses in *Phaeocystis*. *Phaeocystis globosa* is reported to respond to herbivore cues. Colonies exposed to grazer-associated chemical cues grew up to 300% larger than colonies lacking grazer cues (Tang 2003). This transformation could be advantageous because large *Phaeocystis* colonies can reach millimeters in diameter, a size well beyond the optimal prey size of many potential grazers (Berggreen et al. 1988, Hansen et al. 1994a). Although co-occurring copepods, such as *Acartia tonsa*, do not efficiently graze large colonies (> 200 μm in diameter; Verity and Smayda 1989), they also do not efficiently graze *Phaeocystis* solitary cells (Turner et al. 2002). They efficiently graze particles between 10-80 μm in diameter (Hansen et al. 1994a). Thus, to reach the refuge of large colony size, *Phaeocystis* must grow through a size range where susceptibility to grazers like *Acartia* can be high. Therefore, colony formation may be disadvantageous for *Phaeocystis* solitary cells in the presence of copepods but advantageous in the presence of microzooplankton. This variable susceptibility as a function of the specific types of consumers could select for recognizing and responding differently to different consumers.

Our goal was to test the ability of *Phaeocystis* solitary cells to respond to, and discriminate among, grazer-associated signals by altering colony formation or colony size. We compared colony formation when *Phaeocystis* encountered either microzooplankton or mesozooplankton grazers directly, was exposed to only the chemical signals from these feeding grazers, or received no grazer-associated cues. When grazer-associated cues affected colony formation, we tested the potential adaptive value of such responses.

Materials and Methods

Organisms and Culture Conditions - *Phaeocystis globosa* (CCMP 627), originally

isolated from the Gulf of Mexico, was grown non-axenically in L1-Si medium (Guillard and Hargraves 1993) at 20°C under a light:dark cycle of 14:10 h in all experiments. A combination of cool white and daylight fluorescent bulbs provided illumination at ~100-150 μE m^{-2} sec^{-1}. The ciliate *Euplotes* sp. was isolated from the Wilmington River, Savannah, GA, and fed *Isochrysis galbana* in filtered, autoclaved seawater with f/2 concentrations of vitamins and trace metals added. *Euplotes* cultures were grown at 20°C and at a light level of 25 μE m^{-2} sec^{-1}. *Euplotes* were allowed to graze down remaining prey before being used in the experiments. *Rhodomonas* and *Isochrysis* were maintained in f/2-Si medium at 20°C and at a light level of 35 and 100 μE m^{-2} sec^{-1}, respectively (14:10 L:D cycle).

Effects of a Natural Mix of Mesozooplankton on Colony Formation - To assess the direct and indirect effects of grazing on colony formation, we conducted experiments using a mixed species group of mesozooplankton we collected from the Wilmington River. In these experiments, *Phaeocystis* cells: (1) were directly grazed, (2) received chemical signals from grazed cells and grazers but were not grazed themselves, or (3) received neither signals nor direct contact with grazers. Experimental chambers consisted of 750 ml plastic bottles with ~ 260 cm^2 of 1-μm Nitex mesh making up their sides partially submerged inside 2-L glass beakers (Figure 3.1). The mesh in the walls of the bottles permitted chemical exchange between the bottles and beaker but prevented algae and grazers from moving between compartments. Equal concentrations of *Phaeocystis* cells were added to the bottle and beaker; grazers were added to only the plastic bottles. The volumes of the "+ grazers" (bottles) and "- grazers" (beaker) treatments were 680 ml and 1000 ml, respectively. We assessed the effect of grazer-associated chemicals on algae on the outside, or "- grazers" side, of the plastic bottles in grazer treatments. Prior to

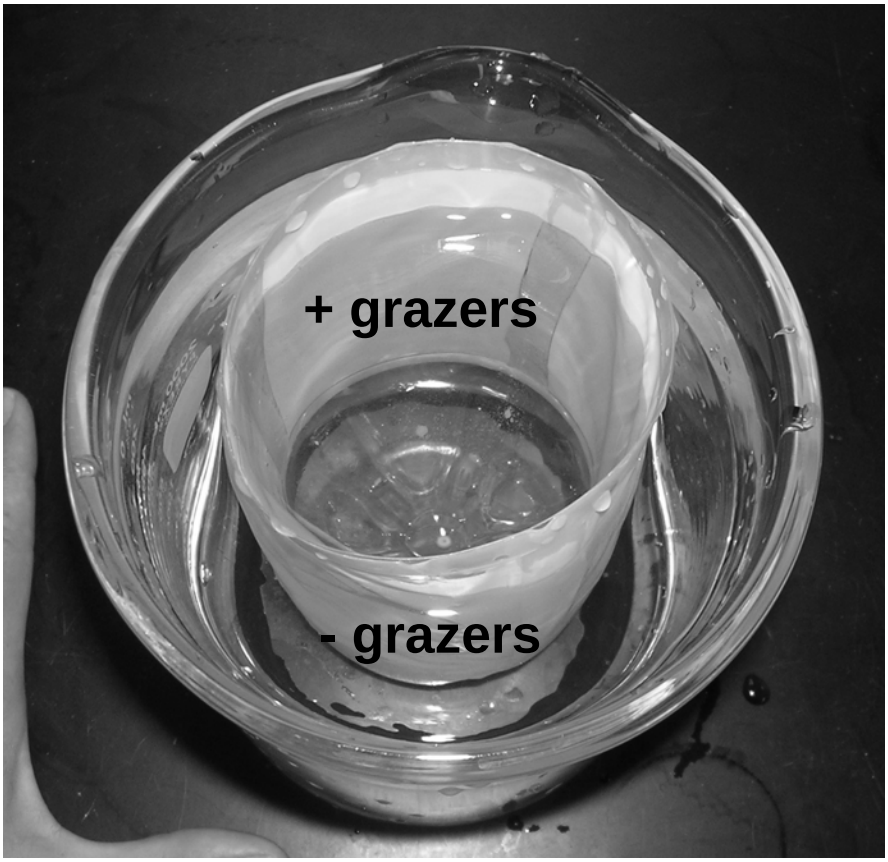

Figure 3.1. Experimental chamber for the mesh bottle experiment.

assays, containers were rinsed and soaked in distilled water for 24 h to remove any harmful residues.

We determined the direct and indirect effects of grazers on *Phaeocystis* colony formation compared to grazer-free controls (N = 3) by comparing colony formation in bottles with grazers and in beakers holding these bottles to colony formation in beakers holding similar bottles without grazers. Grazers were a mixture of mesozooplankton (dominated by grazers between 500 and 1500 µm in length whose optimal prey size is between 11 and 90 µm in diameter; Bartram 1980, Hansen et al. 1994a). Prior to the experiment, *Phaeocystis* cultures were filtered through a 10 µm mesh repeatedly to remove colonies. Solitary cells were diluted with L1-Si medium and added at 50,000 cells ml^{-1} to both compartments in each mesh bottle. Mesozooplankton, collected the previous night via plankton tows in the Wilmington River, Savannah, GA, were filtered through a 100 µm mesh, rinsed in filtered seawater, and then added to the "+ grazers" side of mesozooplankton treatments. Mesozooplankton were dominated by adult copepods (density within "+ grazers" side = 259 +/- 19 L^{-1}), primarily *Acartia tonsa* and *Pseudodiaptomus pelagicus* (formerly *P. coronatus*, [Walter 1989]), and the heterotrophic dinoflagellate, *Noctiluca scintillans* (density within "+ grazers" side = 112 +/- 6 L^{-1}). Natural densities of similar-sized copepods in areas with *Phaeocystis globosa* blooms may reach 17-19 L^{-1} (Martens 1981, Weisse 1983). Thus, our initial grazer density on the "+ grazers" side was ~14x natural densities for adult copepods. However, the overall grazer density in the combined volumes of the 2 compartments was only ~6x natural densities for adult copepods. No smaller eukaryotic organisms, such as ciliates and dinoflagellates, were detected in the mesozooplankton treatments.

Preliminary mesh bottle experiments indicated a low exchange (< 0.5% per hour) of a water-soluble dye across the mesh barrier in the absence of mixing. To enhance exchange of chemical signals from the "+ grazers" side to the "– grazers" side of

containers, mesh bottles in beakers were placed randomly on benchtop orbital shakers and rotated at 50 r.p.m through a 2 cm orbit. In addition, each plastic bottle was gently lifted twice daily until half of its volume was displaced into the "- grazers" side, and then each was gently pushed back down into the larger volume to allow both sides to reach their starting levels. Each lifting process encouraged a net movement of ~20% of a water-soluble dye from the "+ grazers" to the "- grazers" compartment.

To determine cell concentrations and types, containers were gently mixed and both sides were simultaneously subsampled without replacement on day 3 and day 6 of the experiment. At each sampling of each replicate, we collected 10-20 ml samples for each of the following determinations: inorganic nutrients, and *Phaeocystis* solitary cells, total cells, and colonies. Inorganic nutrient concentrations, including ammonium, nitrate, and phosphate were measured using either an autoanalyzer (OI Analytical Flow Solution IV) or manual colorometric assays: ammonium (Koroleff 1976) and phosphate (Strickland and Parsons 1972). Samples for *Phaeocystis* cell counts were preserved separately in Lugol's solution and counted with Palmer-Maloney or Sedgwick-Rafter chambers. Because preserved *Phaeocystis* colonies may degrade and release colonial cells thereby inflating solitary cell counts, colonies were removed and discarded from solitary cell samples before preservation by filtering samples through a 10 μm mesh. A few small colonies passed through this mesh and remained in solitary cell samples so cells within these colonies were excluded from solitary cell counts. Because counting cells within larger colonies can be difficult, samples for total cell counts were shaken vigorously for 30 seconds to disrupt colonies before cells were counted. Cells were counted using a microscope, and these visual observations confirmed that shaking had disrupted most of the colonies. In order to assess concentrations of colonies, we settled 1 ml of live material immediately upon sampling, let this sample settle for at least 1 h, and counted at least 2 transects per sample at 400x with an inverted microscope. For

consistency among experiments, a colony was defined as a group of 4 or more cells whose colony matrix was at least partially visible (Cariou et al. 1994). In order to assess colony diameters and to count the number of cells per colony, we photographed at least 15 colonies per replicate using a Retiga EX Cooled Mono camera. Colonies were photographed multiple times while focusing through the colonies for later analysis of cells per colony. In large, densly-packed colonies, the cells at the top of the colony obscured the underlying cells. In these instances, we counted the cells at the top of the colony that were in focus in the top photograph. We determined the surface area of this spherical cap using Equation 3.1:

$$SA = 2\pi*R*(R-(R^2-r^2)^{1/2}) \qquad (3.1)$$

where R is the colony radius and r is the spherical cap radius. These photographs were analyzed for cells per colony using Skipper Software (http://www.skio.peachnet.edu /research/skipper/). In order to assess concentrations of cells within colonies (i.e. colonial cells), we either calculated the difference between total and solitary cell concentrations or multiplied the colony concentration by the mean number of cells per colony.

Filtrate Experiments - Many copepods died within 6 days, presumably due to the inadequacy of *Phaeocystis* as the sole food source for copepods (Hansen et al. 1993, Chapter 2). Copepod death prevented the unbiased assessment of the effects of live, grazing copepods on colony formation. To avoid this confounding factor, we conducted several experiments using filtrates from containers holding only *Phaeocystis*, or filtrates from containers holding *Phaeocystis* and either microzooplankton or copepods. Filtrates

were acquired from containers that held *Phaeocystis* and copepods for 2-3 days before being filtered; this duration allowed most copepods to survive.

Filtrate Experiments: Acartia tonsa Cues - Using filtrate experiments, we examined whether chemical cues from a single mesozooplankton species, the copepod *Acartia tonsa*, grazing on *Phaeocystis* affected colony formation compared to grazer-free controls (N = 3). We chose *A. tonsa* because it co-occurs with *Phaeocystis* (Weisse 1983, Verity and Smayda 1989) and it was the most abundant copepod in the mesh bottle experiment. For each treatment, 3 cultures of *Phaeocystis* containing both solitary cells and colonies were inoculated at a concentration of 20,000 solitary cells ml^{-1}. Copepods were collected via plankton tows, sorted, and allowed to evacuate their guts for at least 1 h in filtered seawater. Forty adult, female *Acartia* were added to treatment cultures (final density = 200 L^{-1}). Filtrates were collected 2 days later by passing each culture through a GF/F filter using a low vacuum (≤ 200 mm Hg). We measured filtrate pH and then each filtrate was spiked with L1-Si medium nutrients, restocked with *Phaeocystis* solitary cells at 20,000 cells ml^{-1}, and divided among 3-70 ml culture flasks. This provided an experimental design where 3 subsamples were nested within 3 true replicates. All flasks were systematically interspersed on a plankton wheel to disperse any wheel-position effects among the treatments. Flasks were rotated at 0.5 r.p.m. in a 20^{o}C incubator and at a light level of ~100-150 μE m^{-2} sec^{-1}. After 3 days, samples were removed for determination of *Phaeocystis* cell, colony, and nutrient concentrations.

To determine how chemical signals from different densities of *Acartia tonsa* affect colony formation, we incubated triplicate cultures of *Phaeocystis* at a concentration of 20,000 solitary cells ml^{-1} and approximately 5000 colony cells ml^{-1} with *Acartia* (0, 8, 40, 80, or 200 L^{-1}) in 500 ml jars. After allowing grazing for 2 days, each culture was passed through a GF/F filter. Each filtrate was spiked with L1-Si medium nutrients and

restocked with *Phaeocystis* solitary cells at 20,000 cells ml^{-1}. Each replicate was divided into 3-70 ml culture flasks and systematically interspersed on a plankton wheel. Flasks were rotated at 0.5 r.p.m. in a 20°C incubator and at a light level of ~100-150 μE m^{-2} sec^{-1}. Flasks were sampled for nutrients and *Phaeocystis* cells and colonies 3 days later. One replicate of the 0 and 200 *Acartia* L^{-1} treatments were lost due to accidental spillage.

Filtrate Experiments: Euplotes sp. Cues - We also examined whether chemical cues from the microzooplankton *Euplotes* grazing on *Phaeocystis* affected colony formation compared to cues from grazer-free controls (N = 5). We chose *Euplotes* because ciliates can be important grazers during *Phaeocystis* blooms (Weisse and Scheffelmoser 1990). *Euplotes* and solitary *Phaeocystis* were added to L1-Si medium at ~200 cells ml^{-1} and 100,000 cells ml^{-1}, respectively. Natural concentrations of *Euplotes* spp. in Chesapeake Bay may reach 50 ml^{-1} (G. Smalley, *personal observation*). Filtrates were collected 2.5 days later by passing each culture through a GF/F filter using a low vacuum (≤ 200 mm Hg). All filtrates were then spiked with L1-Si medium nutrients, restocked with *Phaeocystis* solitary cells at 20,000 cells ml^{-1}, and added to 70 ml culture flasks. All flasks were systematically interspersed on a plankton wheel and incubated as in the previous experiment. After 3 days, densities of *Euplotes* and of *Phaeocystis* cells and colonies were determined. The diameters of 10 colonies were measured for each replicate using an ocular micrometer to determine an average colony diameter per replicate.

Adaptive Value of Colony Suppression - Because *Acartia tonsa* cues suppressed, and *Euplotes* cues enhanced, *Phaeocystis* colony formation, we wanted to determine if these changes could be adaptive for *Phaeocystis* in the presence of these different

consumers. To accomplish this, we measured fecal pellet production of *Acartia* and growth of *Euplotes* when fed either solitary cells or colonies. We measured fecal pellet production of *Acartia* fed a diet of 22,000 cells ml^{-1} of either solitary or colony *Phaeocystis* cells or an equivalent cellular volume (5000 cells ml^{-1}) of *Rhodomonas*, a palatable food for *Acartia* (N = 9 for *Rhodomonas* and N = 18 for *Phaeocystis* diets). Fecal pellet production is used frequently as a proxy for ingestion rates for copepods, including *A. tonsa* (Reeve and Walter 1977, Gamble 1978), and avoids overestimating grazing based on colony or colony cell counts when grazers physically or chemically affect colony formation (e.g., Hansen et al. 1994b). Solitary cells and colonies were separated by repeated reverse filtration through a 10 µm mesh. For each replicate, 20 adult female *Acartia* were incubated with the test alga in 500 ml jars. All jars were systematically interspersed on a plankton wheel, rotated at 0.5 r.p.m., and grazing was allowed overnight. On the following day, copepods were removed by passing jar contents through a 160 µm mesh. Fecal pellets were collected by gently sieving this filtrate through a 25 µm mesh and the fecal pellets retained on the filter were counted with a dissecting microscope. Five to ten percent of copepods were not recovered in all treatments so fecal pellet production rates were corrected for surviving copepods . Also, the average size of fecal pellets varied between diets so we standardized these counts by multiplying them by a diet-specific fecal pellet volume, determined by measuring the lengths and widths of at least 50 fecal pellets from each diet.

Adaptive Value of Colony Enhancement - To measure the adaptive value of enhanced colony formation in the presence of *Euplotes*, we measured growth rates of *Euplotes* feeding on either *Phaeocystis* solitary cells, *Phaeocystis* colonies, or *Isochrysis* for 3 consecutive days (N = 5). *Isochrysis* was used as a positive control because it is similarly sized to *Phaeocystis* solitary cells and it supports rapid *Euplotes* growth.

Euplotes (200 cells ml^{-1}) and algal prey (250,000 cells ml^{-1}) were added to 250-ml culture flasks for a final volume of 200 ml and incubated at 20°C and 25 µE m^{-2} sec^{-1}. Solitary cells and colonies were separated by repeated reverse filtration through a 10 µm mesh, but separation was not completely effective. Initially, solitary cells comprised 60 +/- 2% and 11 +/- 1% of the total cell concentration in the solitary cell and colony treatment, respectively. Five ml aliquots for the determination of prey and *Euplotes* concentrations were removed daily and preserved in Lugol's solution. *Euplotes* growth rates on each diet were calculated during exponential growth.

<u>*Nature of Acartia tonsa Cues*</u> - Because filtrates from *Phaeocystis* cultures containing *Acartia* suppressed colony formation in *Phaeocystis*, we conducted additional investigations to determine more about the chemical nature of this signal. Filtrates from *Phaeocystis* cultures (20,000 solitary cells ml^{-1} and 5000 colony cells ml^{-1}) with and without *Acartia* (200 L^{-1}) that had incubated for 2 days were separated into organic and aqueous fractions using hydrophobic polymer columns (Supelco, Diaion HP-20). These materials are synthetic adsorbents based on a cross-linked polystyrenic matrix with a mean particle diameter of 0.5 mm and a predominant pore size of ~35 A°. Filtrates from 3 cultures within each treatment were combined, leaving 2 different filtrates: 1 that previously held only *Phaeocystis* and 1 that held *Phaeocystis* and *Acartia*. Both filtrates were passed through separate HP-20 columns repeatedly. The liquid passing through these columns, hereafter referred to as the aqueous fraction, was spiked with L1-Si medium nutrients. The material adhering to the column, hereafter referred to as the organic fraction, was collected by flushing columns with acetone to remove organic molecules adsorbed to the resin, rotary evaporating and freeze-drying to remove acetone, and redissolving in L1-Si medium with sonication. The following day, solitary cells of *Phaeocystis* were added at 20,000 cells ml^{-1} to the media containing each

fraction. Each fraction was divided among 9-70 ml culture flasks and systematically interspersed on a plankton wheel. Flasks were rotated at 0.5 r.p.m. on a plankton wheel (60 cm in diameter) in a 20^{o}C incubator and at a light level of ~100-150 μE m^{-2} sec^{-1}. *Phaeocystis* cells and colonies and nutrients were sampled after 3 days.

Data Analysis - Two-tailed ANOVA determined differences in colony concentrations, percentages of total cells in colonies after arcsine transformation, colony diameters, total cell concentration, fecal pellet production rates, and *Euplotes* growth rates. Multiple comparisons were made using the Tukey test. Nested designs were analyzed by a Nested ANOVA. When determining whether grazer cues affected colony formation, our rule was to compare changes in the percentage of total cells in colonies, rather than colony concentration, because colony concentration could reflect differences in overall *Phaeocystis* growth (total cell concentration) or differences in the density of cells within colonies. Nonlinear regression compared the effects of filtrates from different *Acartia* densities on colony formation. Since the initial nutrient concentrations were not markedly different between treatments, we compared nutrient concentrations with ANOVA (Zar 1999).

Results

Effects of Mixed Mesozooplankton on Colony Formation - When mesozooplankton had direct access to *Phaeocystis* (+ grazers), their grazing reduced colony concentration by 94% (Figure 3.2A , P = 0.006) and reduced total cell concentration by almost 50% (Figure 3.2B, P = 0.045) but significantly increased the percentage of total cells that were in colonies (Figure 3.2C, P = 0.045) and significantly enhanced colony diameter for the few colonies that persisted (Figure 3.2D, P = 0.016). Furthermore, colonies grown in direct contact with mesozooplankton were more densely packed with cells than other

treatments (Table 3.1, $P < 0.001$). These changes suggest that mesozooplankton were selectively preying on small colonies, but may have avoided large colonies. The few colonies escaping mesozooplankton grazing grew larger and contained a higher percentage of the total cells.

Chemical cues from mesozooplankton feeding suppressed *Phaeocystis* colony formation measured both as a decrease in colony concentration (Figure 3.2A, $P = 0.002$) and as the percentage of total cells in colonies (Figure 3.2C, $P = 0.053$), but did not significantly affect total cell concentration (Figure 3.2B, $P > 0.999$) or colony diameter (Figure 3.2D, $P=0.866$). To estimate the relative importance of direct and indirect effects of mesozooplankton, we compared the suppression of colony concentration when *Phaeocystis* was directly grazed by mesozooplankton (+ grazers) with that observed when *Phaeocystis* received only chemical signals from *Phaeocystis* being grazed by mesozooplankton (- grazers). This contrast showed that 84% of the suppression of colony concentration when mesozooplankton had direct access to *Phaeocystis* could be attributed to grazer-associated chemical cues alone.

<u>*Filtrate Experiments: Acartia tonsa Cues*</u> - Waterborne, chemical cues from *Phaeocystis* being grazed by the single copepod species *Acartia tonsa* had a similar effect on *Phaeocystis* as cues from the mixture of mesozooplankton: *Acartia* cues suppressed colony formation. These cues significantly suppressed colony concentration by 57% (Figure 3.3A, $P < 0.001$) and suppressed the percentage of total cells in colonies by 75% (Figure 3.3C, $P < 0.001$) compared to control filtrates from *Phaeocystis* cultures not being grazed by *Acartia*. Given that *Acartia*-associated cues also enhanced total cell concentrations (Figure 3.3B, $P < 0.001$), the magnitude of the suppression of colony formation is even greater. Most copepods were alive when filtrates were collected after

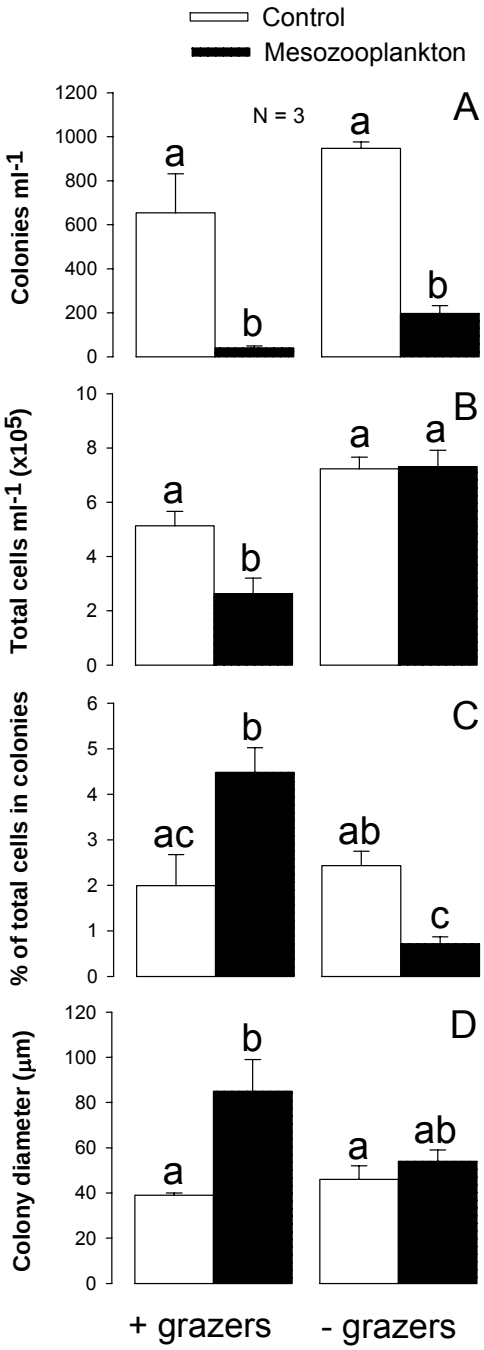

Figure 3.2. Direct and indirect effects of mesozooplankton on *Phaeocystis globosa* (A) colony concentration; (B) total cell concentration; (C) percentage of total cells within colonies; and (D) colony diameter after 6 day incubations in mesh bottles (N = 3). *P. globosa* had direct contact with grazers in the "+ grazers" side but only contact with grazer-associated cues in the "- grazers" side. Controls were grazer-free. Letters above bars indicate significant ($P < 0.05$, Tukey) among-treatment differences. Values are means + 1 SEM.

Table 3.1. *Phaeocystis globosa* colony cell density (cells μm^{-2} of colony surface) when exposed to direct grazing (+ grazers) or just chemical signals from grazing (- grazers). We determined the mean colony size of at least 15 colonies per replicate and then determined the mean of these means for each treatment (N=3). Values are means with SEM in parentheses. Mesozooplankton, + grazers treatment was significantly different from all other treatments ($P < 0.001$).

	+ grazers	- grazers
Control	0.003 (0.000)	0.003 (0.000)
Mesozooplankton	0.013 (0.001)	0.003 (0.000)

2 day incubations suggesting that filtrate experiments avoided the confounding factor of grazer death affecting *Phaeocystis*.

When *Phaeocystis* cultures were subjected to higher densities of grazing *Acartia tonsa,* filtrates from these cultures had a greater inhibitory effect on *Phaeocystis* colony concentration (Figure 3.4A). Total cell concentrations were maximal at intermediate Acartia densities (Figure 3.4B) and the percentage of total cells in colonies was not significantly different among treatments (Figure 3.4C). At natural copepod densities (17-19 copepods L^{-1}), there was a trend for suppression of colony concentration and the percentage of total cells in colonies (Figure 3.4A and C). T-tests comparing either colony concentration or the percentage of total cells in no-copepod controls versus each copepod treatment were significantly different ($P < 0.05$) except for the contrast of colony concentration in no-copepod controls and 8 copepods L^{-1} ($P = 0.203$).

Filtrate experiments: Euplotes sp. Cues - In contrast to the above patterns, filtrates from *Phaeocystis* cultures being grazed by *Euplotes* significantly enhanced the percentage of total cells in colonies (Figure 3.3F, $P = 0.002$). *Euplotes* cues slightly decreased both colony concentration (Figure 3.3D, $P = 0.001$) and total cell concentration (Figure 3.3E, $P = 0.045$). As with mesozooplankton cues, *Euplotes*-associated cues did not affect colony diameter (Figure 3.5, $P = 0.315$).

Adaptive Value of Altered Colony Formation Rates - *Acartia tonsa* feeding on *Phaeocystis* colonies produced more than 3x the fecal pellet volume as did copepods feeding on *Phaeocystis* solitary cells (Figure 3.6, $P = 0.005$), suggesting that *Acartia* will feed more heavily on colonies. In stark contrast, *Euplotes* grew nearly 3x as fast on solitary cells as on colonies (Figure 3.7, $P < 0.05$), indicating that the ciliate was advantaged when it could feed on solitary cells rather than than colonies. Both types of

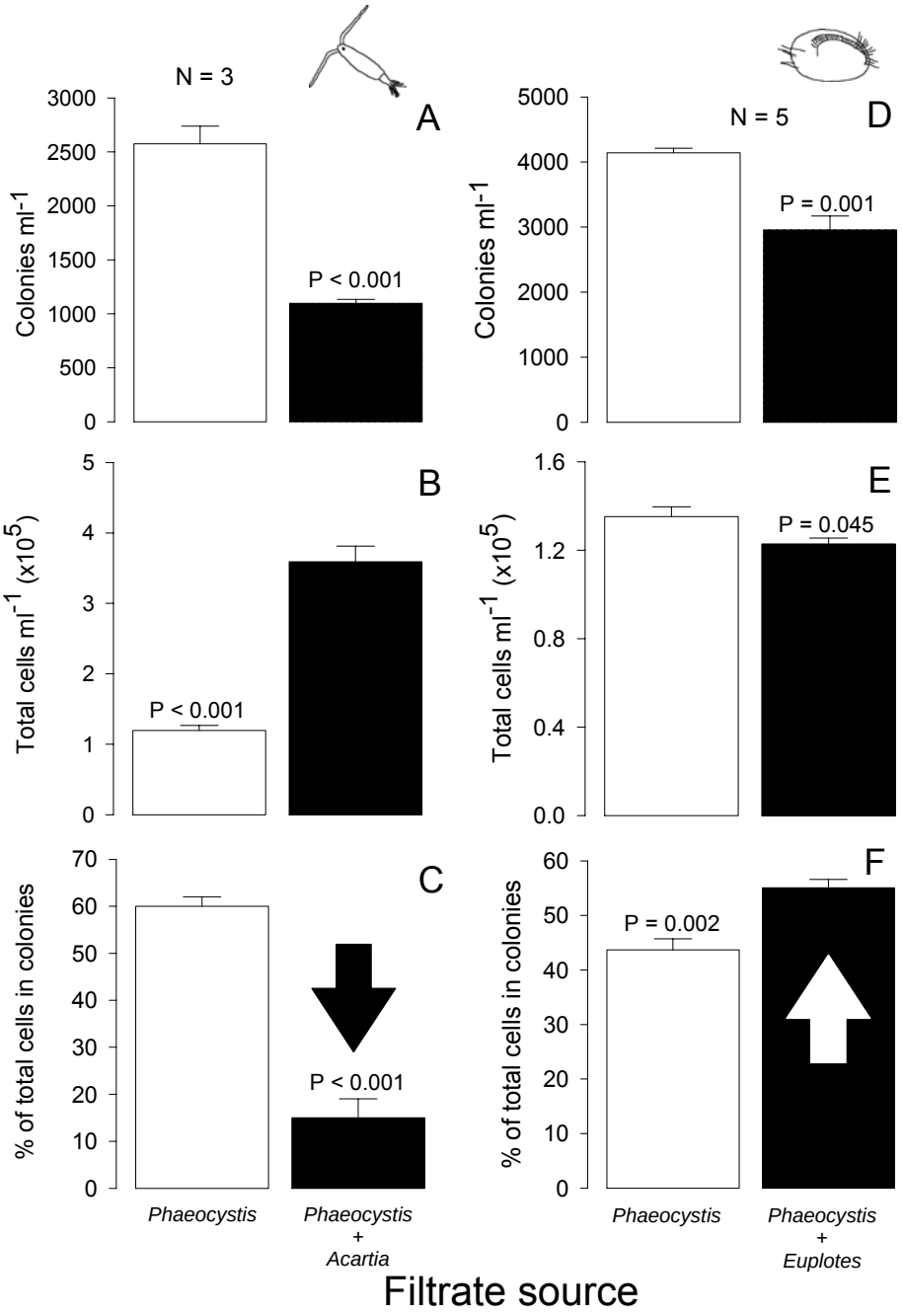

Figure 3.3. Effects of grazer filtrates on *Phaeocystis globosa* (A and D) colony concentration; (B and E) total cell concentration; and (C and F) percentage of total cells within colonies after 3 day incubations. The filtrate sources were either *P. globosa* cultures (white bars), *P. globosa* cultures with *Acartia tonsa* (black bars A-C), or *P. globosa* cultures with *Euplotes* sp. (black bars D-F). Arrows indicate direction of grazer effects on colony formation. P-values designate differences among filtrate source (*A. tonsa* N = 3; *Euplotes* N = 5; ANOVA). Values are means + 1 SEM.

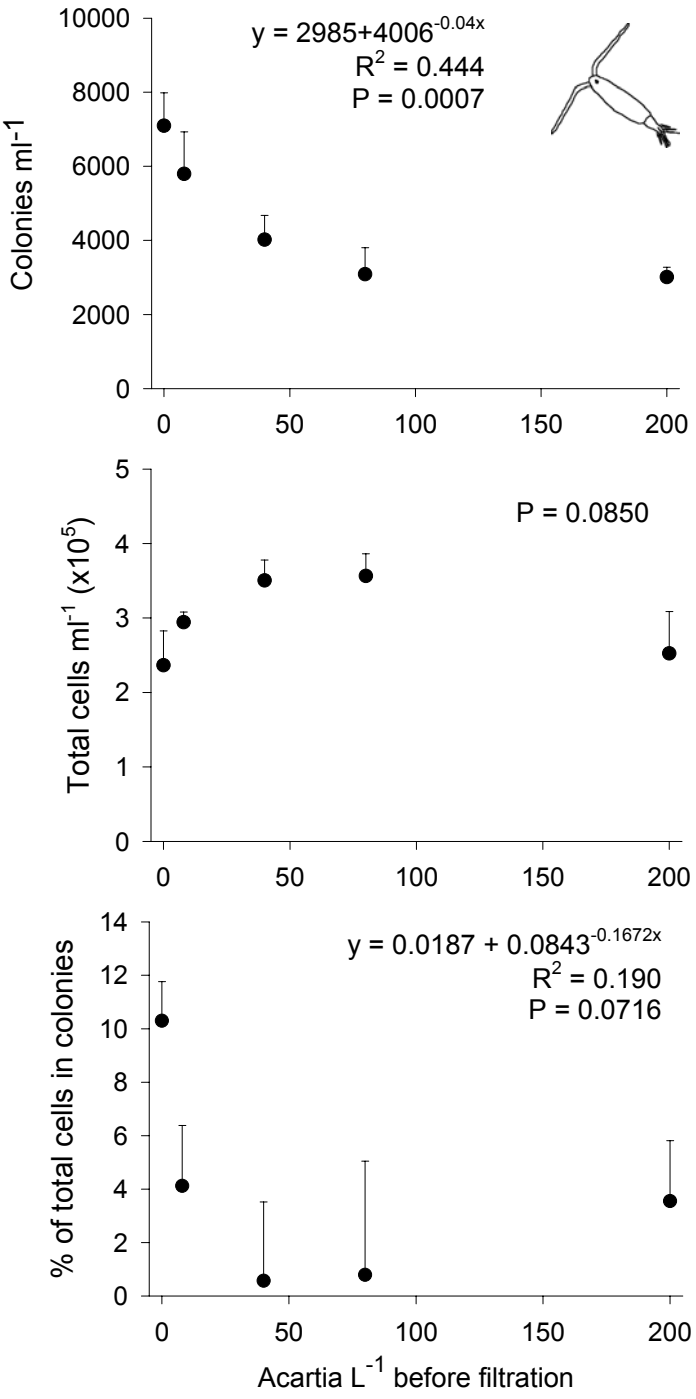

Figure 3.4. Effects of filtrates from *Phaeocystis globosa* cultures with increasing *Acartia tonsa* densities on *P. globosa* (A) colony concentration; (B) total cell concentration; and (C) percentage of total cells within colonies after 3 day incubations. Values are means + 1 SEM.

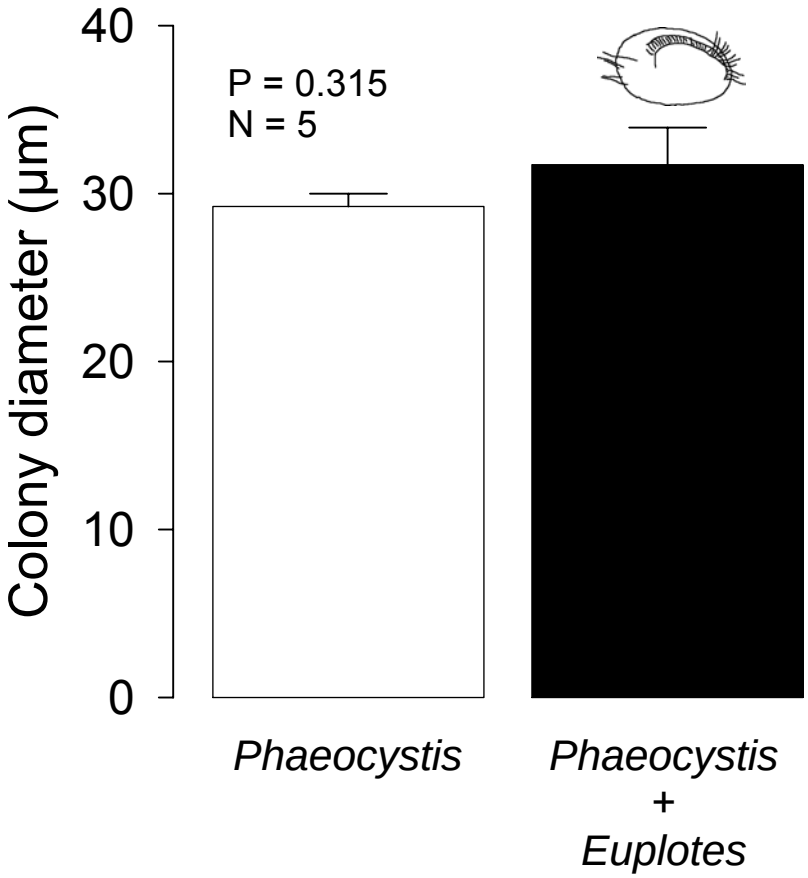

Figure 3.5. Effects of *Euplotes* filtrates on *Phaeocystis globosa* colony diameter after 3 day incubations. The filtrate sources were either *P. globosa* cultures (white bar) or *P. globosa* cultures with *Euplotes* sp. (black bar). Values are means + 1 SEM.

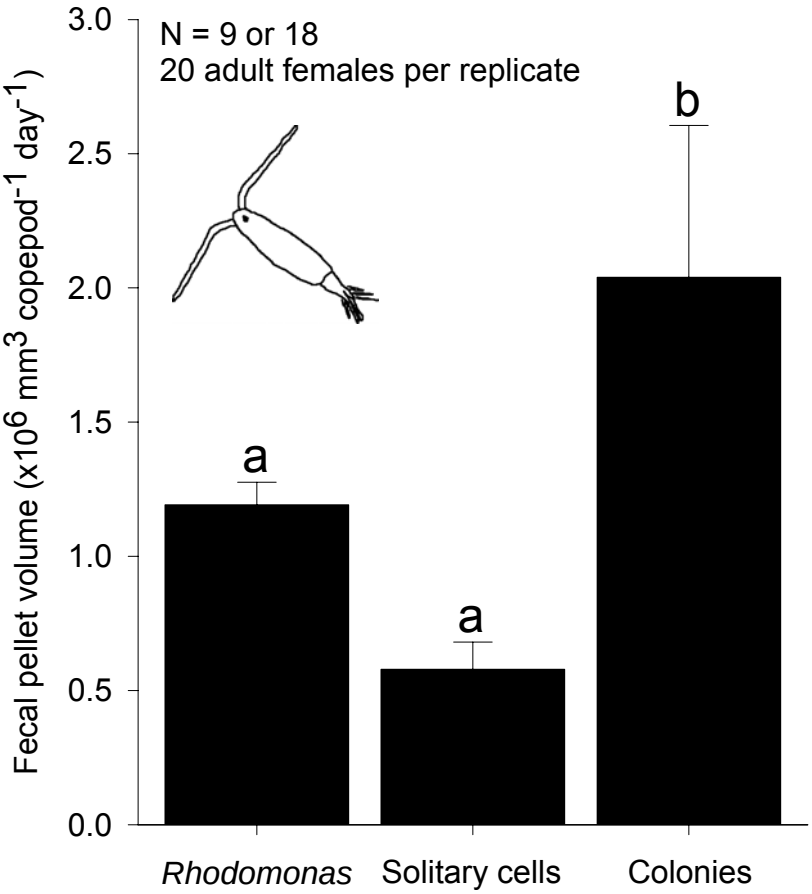

Figure 3.6. Fecal pellet production of *Acartia tonsa* fed 1 of 3 diets: *Rhodomonas* sp., *Phaeocystis globosa* solitary cells, or *P. globosa* colonies (N = 9). Each replicate contained 20 adult female copepods. Production was measured after a 24 h incubation on a plankton wheel. Measurements were corrected for numbers of surviving copepods as well as differences in fecal pellet volume among diets. Letters above bars indicate significant ($P < 0.05$, Tukey) among-treatment differences. Values are means + 1 SEM.

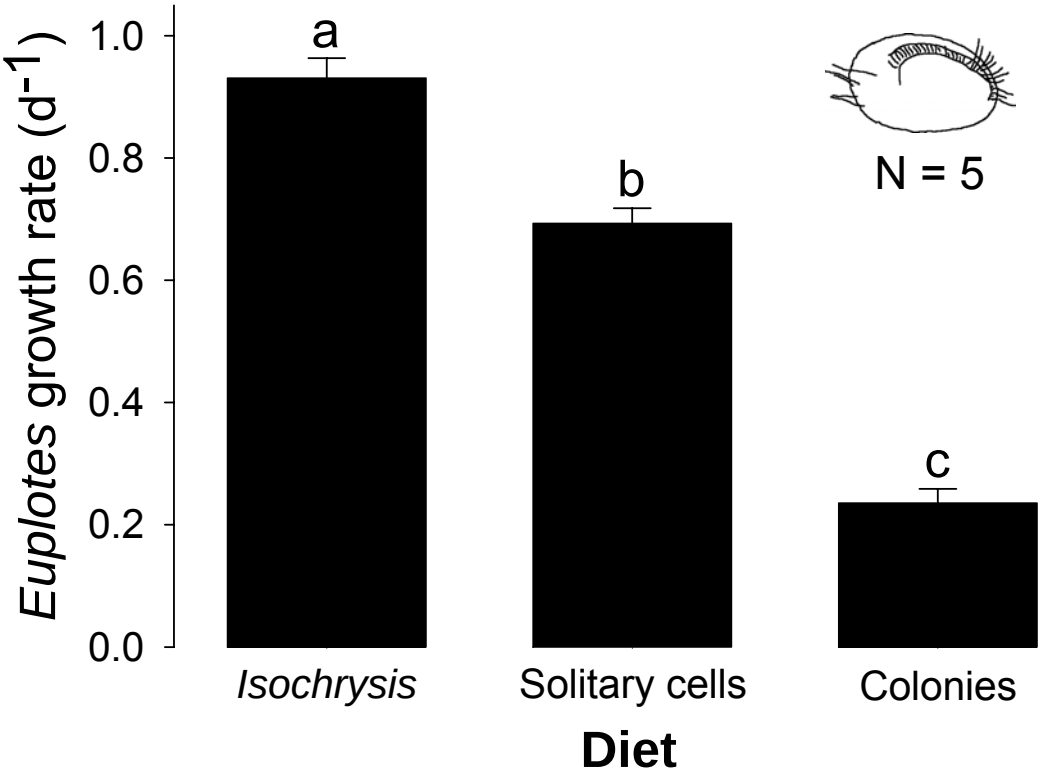

Figure 3.7. *Euplotes* sp. growth rates on 1 of 3 diets: *Isochrysis galbana*, *Phaeocystis globosa* solitary cells, or *P. globosa* colonies (N = 5). Growth rates were measured during exponential growth. Letters above bars indicate significant ($P < 0.05$, Tukey) among-treatment differences. Values are means + 1 SEM.

Phaeocystis supported slower *Euplotes* growth than did *Isochrysis* (Figure 3.7, $P < 0.05$).

Nature of *Acartia tonsa* Cue - The organic fraction of filtrates from *Phaeocystis* cultures with grazing *Acartia* suppressed colony concentration compared to the organic fraction of filtrates from grazer-free *Phaeocystis* cultures (Figure 3.8A, $P < 0.001$), but a similar decrease was also evident for total cell concentrations (Figure 3.8B, $P < 0.001$). Thus, colony concentration alone could not be used to assess the effects of the organic fraction of filtrates on colony formation. However, the percentage of total cells in colonies was significantly suppressed by the organic fraction from cultures with *Acartia* (Figure 3.8C, $P < 0.001$) suggesting that an organic compound, or compounds, is produced when *Acartia* graze *Phaeocystis* and that this compound(s) suppresses colony formation. The aqueous fraction of filtrates did not affect colony formation (Figure 3.8D and F, $P > 0.257$).

Ammonium and phosphate concentrations and pH never differed significantly between controls and treatments (Table 3.2). Nitrate concentrations were significantly controls and treatments (Table 3.2). Nitrate concentrations were significantly different in the mesh bottle experiment but not the filtrate experiment with *Acartia* (Table 3.2).

Discussion

Phaeocystis regularly forms massive blooms (Hansen and van Boekel 1991, Davies et al. 1992, Hamm et al. 2001) that sequester large amounts of carbon (Smith et al. 1991), and that determine the structure of local food webs and the cycling of nutrients and energy within affected ecosystems (Weisse et al. 1994, Biddanda and Benner 1997, Hamm et al. 2001, Tang et al. 2001). Although morphological transformations in *Phaeocystis* are an important part of its life cycle and these transformations may be

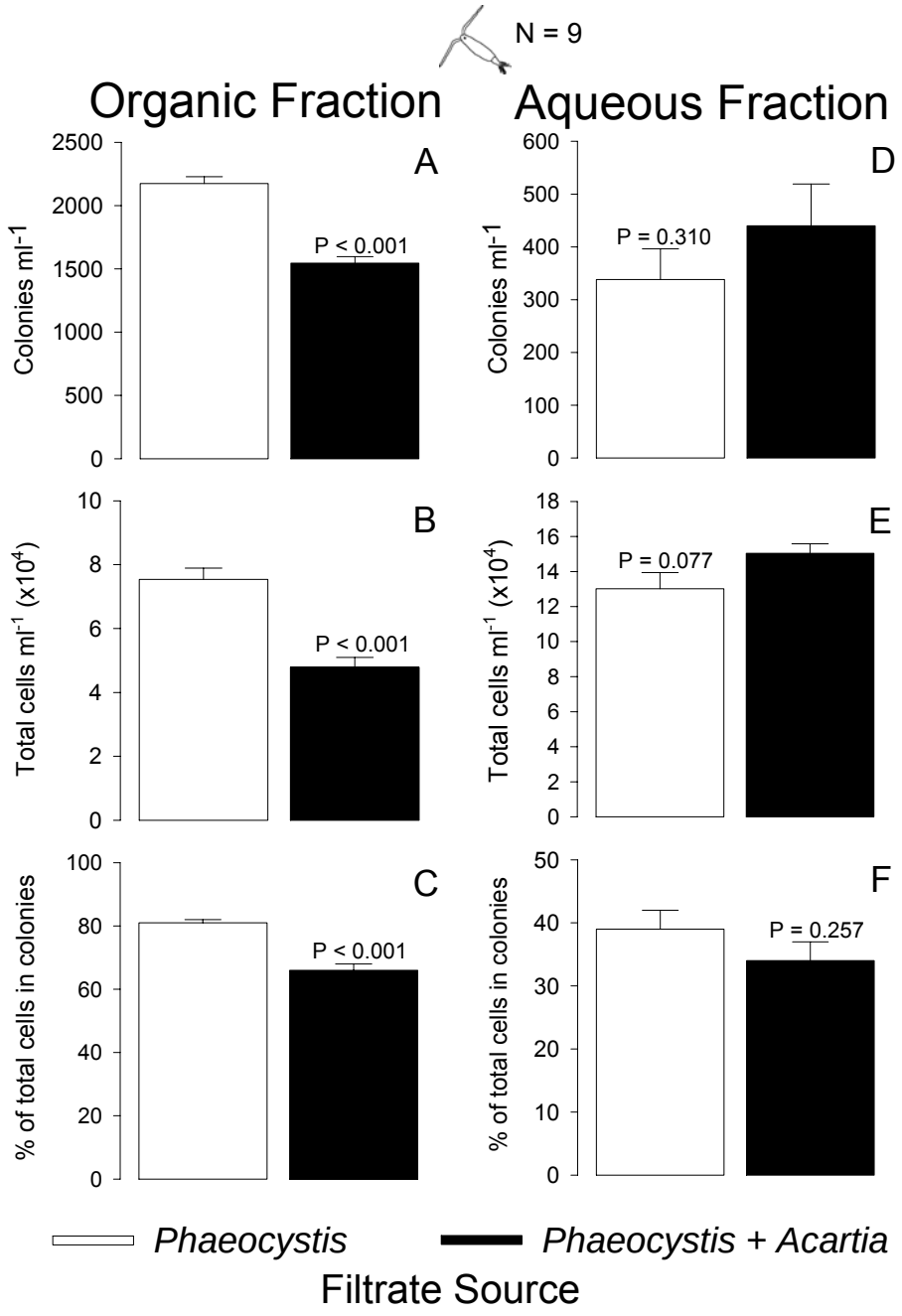

Figure 3.8. Effects of organic (A-C) and aqueous (D-F) fractions of filtrates on *Phaeocystis globosa* (A and D) colony concentration; (B and E) total cell concentration; and (C and F) percentage of total cells within colonies after 3 day incubations. The filtrate sources were either *P. globosa* cultures (white bars) or *P. globosa* cultures with *Acartia tonsa* (black bars). Filtrates were separated into organic and aqueous fractions using column chromatography. P-values indicate significant differences among filtrate source (N = 9; ANOVA). Values are means + 1 SEM.

Table 3.2. Nutrient concentrations and pH in – grazer and filtrate experiments. SEM in parentheses.

Nutrient or pH	Corresponding experiment	Treatment (- Grazers)	Nutrient conc. [µM; X (SE)] or pH	ANOVA P-value
Ammonium	Figure 3.2	Control Mesozooplankton	1.88 (0.22) 1.74 (0.34)	0.582
	Figure 3.3	*Phaeocystis* *Phaeocystis+Acartia*	1.40 (0.33) 1.51 (0.42)	0.841
Nitrate	Figure 3.2	Control Mesozooplankton	928 (20) 1074 (26)	0.002
	Figure 3.3	*Phaeocystis* *Phaeocystis+Acartia*	203 (4) 206 (1)	0.622
Phosphate	Figure 3.2	Control Mesozooplankton	33.7 (4.5) 32.7 (2.3)	0.749
	Figure 3.3	*Phaeocystis* *Phaeocystis+Acartia*	39.2 (1.6) 42.7 (4.4)	0.490
pH	Figure 3.3	*Phaeocystis* *Phaeocystis+Acartia*	8.27 (0.00) 8.25 (0.02)	0.519
	Figure 3.8	*Phaeocystis* (Organic) *Phaeocystis+Acartia* (Organic) *Phaeocystis* (Aqueous) *Phaeocystis+Acartia* (Aqueous)	8.19 8.20 8.14 8.09	n/a (N=1)

critical to bloom formation (Peperzak et al. 2000), the controls on these transformations are poorly known. Our experiments indicate that *Phaeocystis* changes morphology as a way of inducing defenses against consumers, and that it uses chemical cues from nearby grazers to recognize the consumer and respond in an adaptive way. When confronted with direct grazing by a mixture of mesozooplankton, by *Acartia tonsa* alone, or only the chemical signals from neighbors being grazed by these consumers, *Phaeocystis* suppressed colony formation. Because copepods graze colonies at higher rates (Tande and Bamstedt 1987, Figure 3.6), suppressing colony formation in the presence of mesozooplankton is adaptive for *Phaeocystis*.

In contrast, chemical signals from the ciliate *Euplotes* sp. enhanced colony formation. Enhancing colony formation in the presence of *Euplotes* should be adaptive for *Phaeocystis* because *Euplotes* grew slower on a diet of colonies than on a diet of solitary cells. Thus, *Phaeocystis* senses grazer-associated cues, senses the type of consumer, and responds adaptively to these grazer-specific cues.

Although induced responses of prey were originally believed to be a generalized response to abiotic and biotic stress (Karban and Myers 1989), more recent studies indicate a high level of specificity in some of these responses (Karban and Baldwin 1997, De Moraes et al. 1998, Van Zandt and Agrawal 2004). Terrestrial plants, zooplankton, and benthic invertebrates display consumer-specific induced responses including alterations of life history (Stibor and Luning 1994), chemical defenses (Van Zandt and Agrawal 2004), migrations (Dodson 1988), and tritrophic signaling (De Moraes et al. 1998). Induced chemical defenses are herbivore specific for a variety of plants including milkweed (Van Zandt and Agrawal 2004), wild radish (Agrawal 2000), and tomato (Stout et al. 1998). The volatile signaling of plants varies as a function of the herbivore species and these cues can affect visitation rates of herbivore-specific parasitoids (De Moraes et al. 1998). *Daphnia* allocates energy to growth and

reproduction partially as a function of the identity of nearby predators (Stibor and Luning 1994, Riessen 1999). Our results show that even the simple marine phytoplankton can sense, and induce responses to, grazer-specific cues. These observations support the conclusion that specificity of induction is likely to be a common, if not "universal," phenomenon (Van Zandt and Agrawal 2004).

The opposing nature of the consumer-specific responses we documented presents a potential conflict for *Phaeocystis*. Colony formation should be enhanced in response to ciliates like *Euplotes* but suppressed in response to copepods. The conflict exists because *Phaeocystis*-dominated communities commonly consist of a mixture of ciliates and copepods (van Boekel et al. 1992). This apparent conflict may be resolved by the feeding preferences of copepods; they typically prefer ciliates and other microzooplankton prey over *Phaeocystis* (Hansen et al. 1993, Tang et al. 2001). Thus, copepods may not present a significant threat to *Phaeocystis* when alternate prey are available. In these situations, *Phaeocystis* should enhance colony formation to escape ciliate grazing. While copepods preferentially consume microzooplankton, *Phaeocystis* colonies may grow through the intermediate size range where they are most susceptible to copepod-grazing. If copepods switch to *Phaeocystis* grazing after depleting microzooplankton populations, *Phaeocystis* may already have reached a size that is too large for copepods to handle. These responses may partially explain the observed stimulation of *Phaeocystis* blooms by some grazers (Fernandez and Acuna 2003).

How *Phaeocystis* detects the threat of specific grazers and alternates between colony enhancement and suppression is unknown. The chemical signals could come from neighboring *Phaeocystis*, from grazers, or from a combination of both. Signals released by *Phaeocystis*-grazer interactions could be used to identify the predominant morphotype being grazed, and these signals would vary with the dominant grazer. Alternatively, signals released by grazers before they graze might allow a longer time for

Phaeocystis to respond, but might also result in inducing costly defenses before they are needed. Signals released by a combination of grazers and the specific prey being grazed might contain the most useful information in that they could signal both the identity of the consumer and the prey it prefers in the present environmental context. In the case of induced defenses in the freshwater phytoplankton *Scenedesmus*, the inducing signal originates from such combined effects of actively feeding grazers; neither damaged *Scenedesmus* alone nor starved grazers induce a response in *Scenedesmus* (Lampert et al. 1994, Lurling 1998).

Several alternative hypotheses fail to account for the altered colony formation in the presence of grazers. First, when one runs longer-term experiments to investigate copepod effects on *Phaeocystis* morphology, the poor food quality of *Phaeocystis* leaves most copepods dead within a few days. When one gets responses in *Phaeocystis* under such conditions, it is possible that the responses are to chemicals released by dead copepods, not signals eminating from their grazing activity. Our high grazer survivorship in filtrate experiments eliminated problems associated with dead grazers providing cues normally absent with live grazers (see Jakobsen and Tang 2002, Tang 2003). Second, the alternative hypotheses of inorganic nutrients and pH suppressing colony formation were not supported; treatments and controls did not differ significantly in these variables in 6 out of 7 analyses. Furthermore, the *Acartia* cue was present in organic extracts indicating that there is a specific lipid-soluble signal being released and that this signal can produce colony suppression independent of other changes in the physical environment. Had differences in inorganic nutrients been responsible for colony suppression, we would have seen these differences in the aqueous fractions of filtrates, and such differences did not occur.

We only observed colony enlargement when *Phaeocystis globosa* was in direct contact with mesozooplankton - not just the chemical cues from either microzooplankton

or mesozooplankton grazers as previously reported for *P. globosa* (Tang 2003). We present 4 hypotheses to resolve this discrepancy between our study and the experiments of Tang. First, significant differences in colony size were not evident until at least 9 days after the start of Tang's experiments. We limited our experimental duration to only 3-6 days to avoid the potentially confounding factor of copepod death. However, a preliminary study revealed no evidence of colony enlargement after 12 days with filtrates from either *Acartia* or *Euplotes* (J. Long and G. Smalley, personal observation). Second, Tang found significant grazer death in 4 out of 5 experiments documenting colony enlargement and therefore, *Phaeocystis* were exposed to signals from copepod grazing in the early stages of the experiment, but also to signals of copepod death in the majority of the time frame of the experiment – thus, possibly confounding signals of grazing with less natural signals. We observed similar mortality during incubations longer than 3 days which is why we switched to shorter bioassays, thus assuring cues from living, as opposed to dead, grazers. Third, inter-strain differences could produce the differing responses because the *P. globosa* strains used by Tang (CCMP 1528) and this study (CCMP 627) were isolated from 2 separate regions. These 2 strains may respond differently to grazers given the recent discoveries that *P. globosa* is a multispecies complex (Lange et al. 2002) and that phytoplankton response to grazers can be strain-specific (Lurling 1999, Lurling and Beekman 1999). Fourth, *Phaeocystis* may use multiple strategies to escape grazing including adjusting both colony formation and colony size. Colony formation appears to be an ineffective defensive strategy in the presence of mesozooplankton but colonies that grow rapidly through a susceptible, intermediate size range may escape mesozooplankton grazing once they become too large to handle.

The strength of the *Acartia* cue depended on the density of *Acartia* used to generate the cue. The retention of the cue on hydrophobic polymer columns indicates

that it was lipid-soluble and non-polar. Unfortunately, attempts to further follow the chemistry of the copepod cue met with unexpected variability. Despite our efforts to follow a regular transfer schedule and to conduct experiments under the same physical conditions, colony formation in controls was great in some experiments (60% of total cells in colonies) but minimal in others (2% of total cells in colonies). Although we detected grazer cue effects during both low (Figure 3.2) and high (Figure 3.3) colony formation, this variability was disturbing. Similar between-experiment variability has been found by researchers working with induced defenses of other phytoplankton genera (G. Pohnert, H. Pavia, and G. Toth, pers. comm.). Such variability might be expected for *Phaeocystis,* which displays a complex life cycle with many hypothetical stages and transformations (Rousseau et al. 1994, Peperzak et al. 2000). Another hypothesis for our between-experiment variability is that our culture media varies temporally because we make our media using natural seawater collected at different times of the year. During these times, ambient grazer densities and activities vary so the concentration of grazer cues could also vary. If these cues are stable, they may have persisted in our media and affected *Phaeocystis* colony formation. As a preliminary test of the hypothesis that media made from natural seawater can sometimes suppress colony formation, we recently measured *Phaeocystis* colony formation in identical conditions using media made from either artificial seawater or natural seawater. The percentage of cells within colonies was more than 10x greater in artificial seawater media (X ± SE=88 ± 4%) than in natural seawater media (X ± SE=8 ± 2%). Obviously, we are only beginning to understand the complexity associated with *Phaeocystis* growth and life-cycle transformations.

Consumer-prey interactions involving blooming phytoplankton offer an unusual opportunity to examine how trait-mediated responses scale up to affect ecosystem function (Hay and Kubanek 2002). However, demonstrating that the trait-mediated

responses of phytoplankton to their grazers affect natural communities and ecosystems remains a significant challenge. Although manipulating planktonic systems to test these hypotheses is difficult, especially due to the small size of some cells, 2 alternative strategies may shed light on the importance of grazer-associated cues in *Phaeocystis*-dominated systems. One strategy involves correlating grazer abundance with *Phaeocystis* concentrations. If mesozooplankton cues suppress colony formation, and if colony formation is critical for bloom initiation (Peperzak et al. 2000), then the onset of blooms should occur during periods of low mesozooplankton abundances. This pattern is expected for a threat-dependent response where the probability of grazer encounter increases with grazer density (Lubchenco and Gaines 1981). Indeed, *Phaeocystis* blooms are often associated with decreasing or low mesozooplankton abundance in the North Sea and surrounding areas (Martens 1981, Weisse 1983, Hansen and van Boekel 1991, van Boekel et al. 1992, Rousseau et al. 2000).

An alternative strategy involves measuring natural concentrations of these cues during *Phaeocystis* blooms. This requires knowing the chemical structure of these cues, which have yet to be described. One of the biggest obstacles to isolating water-borne chemical cues is extracting and concentrating these cues from dilute silutions. Although few studies have successfully isolated and identified such cues, we are optimistic that future studies will increase our understanding of grazer cues affecting *Phaeocystis* colony formation given our ability to extract the *Acartia* cue from filtrates. Until we discover the identity and natural concentrations of planktonic infochemicals, our understanding of their ecology and evolution remains hindered.

CHAPTER 4

ALLELOPATHY OF A BLOOM FORMING MARINE PHYTOPLANKTON: *PHAEOCYSTIS* SPP. IN BOTH MESOCOSM BLOOMS AND LABORATORY CULTURES

Abstract

We assessed the allelopathic effects of cell-free filtrates of a bloom of *Phaeocystis pouchetii* from an outdoor mesocosm against the Cryptophyte *Rhodomonas baltica.* The suppressive activity of filtrates varied with successionary state of the mesocosm. Mesocosm filtrates during an early diatom bloom did not affect *R. baltica* growth rates, but filtrates during the *P. pouchetii* bloom significantly suppressed *R. baltica* growth rates by as much as 100%. To test if other species of *Phaeocystis* also produced allelopathic effects, we compared the growth of *R. baltica* when exposed to cell-free filtrates of *Phaeocystis globosa* cultures versus media controls. We also assessed the effects of *R. baltica* filtrates on *R. baltica* growth to examine intraspecific allelopathy. *P. globosa* filtrates always suppressed *R. baltica* growth rates, while effects of *R. baltica* filtrates were variable. *P. globosa* filtrates were significantly more suppressive than *R. baltica* filtrates in 2 of 3 experiments, and did not differ in the remaining experiment. The inhibitory effects associated with a mesocosm bloom of *P. pouchetii* and a culture of *P. globosa* could not be explained by pH or nutrients alone. Our results suggest that both species of *Phaeocystis* release compounds that suppress growth of competitors. These chemically-mediated interactions could contribute to the formation and maintenance of *Phaeocystis* spp. blooms.

Introduction

Numerous primary producers, including vascular plants, seaweeds, and phytoplankton produce chemicals that inhibit the growth of competing organisms (e.g.,

Keating 1977, De Nys et al. 1991, Inderjit et al. 1995, Fistarol et al. 2004). These chemicals can inhibit the growth and survivorship of both intra- and interspecific competitors (Myklestad et al. 1995, Kearns and Hunter 2002), leading to strong effects on productivity (Fistarol et al. 2004), species succession (Keating 1977, 1978), and community structure (Whittaker and Feeny 1971, Fistarol et al. 2004). Allelopathic interactions could be especially important in planktonic communities where competition is intense and many phytoplankton compete for the same resources (Hutchinson 1944, 1961). Furthermore, allelopathy may be an important mechanism that allows certain phytoplankton to form nearly monospecific blooms (Smayda 1997).

While there is little debate that cultured phytoplankton can produce allelopathic chemicals (Pratt and Fong 1940, Houdan et al. 2004); questions remain about whether they actually do produce these metabolites in the presence of competitors, consumers, and under natural physical conditions, and about whether these chemicals are actually encountered by neighboring phytoplankton (Keating 1977, 1978, Maestrini and Bonin 1981, Lewis 1986). Critical tests should examine the allelopathic activity of filtrates from natural waters on phytoplankton growth. For example, Keating (1977, 1978) showed that cell-free filtrates from natural pond water can strongly affect the growth of various phytoplankton species. Only a few other studies examined the allelopathic activity of natural bloom samples but these studies relied on ecologically, unrealistic bioassays, including testing the allelopathic activity of internal cellular extracts rather than the activity of metabolites naturally released into the environment (Stabell et al. 1999, Reifel et al. 2001, Rengefors and Legrand 2001). Testing cellular extracts is unrealistic because competing phytoplankton are unlikely to encounter intracellular components of neighboring phytoplankton; however, encountering exudates from neighbors could be common. Thus, more realistic bioassays would examine the affects of exudates or filtrates on phytoplankton populations and communities.

To examine allelopathy of a planktonic community dominated by a blooming phytoplankton, rather than allelopathy from a more artificial phytoplankton culture, we measured growth rates of *Rhodomonas baltica* exposed to filtrates collected from a bloom of *Phaeocystis pouchetii* that occurred in a semi-natural mesocosm floating in a Norwegian fjord. *P. pouchetii* forms massive blooms that structure pelagic food webs and play a significant role in high latitude carbon cycles (Smith et al. 1991, Arrigo et al. 1999). As with many bloom-forming phytoplankton, the release of allelopathic substances by *Phaeocystis* spp. is widely assumed but rarely tested using ecologically realistic assays (Aanesen et al. 1998, Stabell et al. 1999, Hansen et al. 2003). Four studies found evidence for toxicity in *P. pouchetii* towards either sea urchin embryos, cod larvae, *Artemia*, blowflies, yeast, or mammalian blood cells (Aanesen et al. 1998, Stabell et al. 1999, Hansen et al. 2003, Hansen et al. 2004); however, tests of filtrates from *Phaeocystis* spp. on phytoplankton are lacking despite the need for ecologically relevant bioassays (Hay et al. 1998) and the observed inverse relationship between *Phaeocystis* spp. and other phytoplankton (Jacobsen et al. 1995, Peperzak et al. 1998). We also tested whether filtrates from a related *Phaeocystis* spp., grown under different physical conditions, might display similar effects as filtrates from the bloom of *P. pouchetii* that occurred in this mesocosm.

Materials and Methods

Mesocosm Experiment - An inability to predict the timing and locations of natural blooms impedes the mechanistic study of most bloom-forming phytoplankton in the field. However, blooms of *P. pouchetii* can be created reliably at the mesocosms of the University of Bergen's Marine Biological Station (61°16'N, 05°14'E; Egge and Heimdal 1994). We conducted a mesocosm experiment from 4-26 March 2002 to generate a bloom of *Phaeocystis pouchetii*. The mesocosm consisted of a polyethylene bag (4.5 m

deep, 2 m diameter, 11 m^3) mounted on a floating frame moored to a raft. The mesocosm allowed a 90% light penetration (PAR, photosynthetically active radiation) and was open to the air. Mesocosms were filled on 3 March with nutrient poor fjord water from 5 m depth. To homogenize conditions within the mesocosm and to prevent a rise in pH associated with mesocosm phytoplankton bloom (Jacobsen et al. 1995), an airlift-system pumped seawater within the mesocosm at a rate of 40 L min^{-1}. To increase the probability of a *P. pouchetii* bloom, we added an axenic culture of *P. pouchetii* (isolated from the same fjord the previous year) to the mesocosm to achieve a final concentration of 300 cells ml^{-1} at the start of the experiment. On the first experimental day (4 March), nitrate and phosphate were added to the mesocosm for a final concentration of 16 µM and 1 µM, respectively. Adjacent fjord seawater was continuously delivered into the mesocosm at a rate of 10% day^{-1} to prevent the pH increase associated with phytoplankton blooms. This seawater also served as a source for natural complements of organisms, in case they were absent during the initial filling of the mesocosm. To compensate for loss of nutrients due to this influx of water, 10% of the nutrient amount initially added was added daily for the entire experimental period. Based on low nutrient concentrations determined on 12 March, the mesocosm was given an additional nutrient spike on that day corresponding to 8 µM nitrate and 0.5 µM phosphate. Throughout the mesocosm experiment, the mesocosm temperature varied between 4.7 and 5.6^{o}C and maximum daily light levels within the mesocosm ranged from 107 to 1200 µM photons cm^{-2} sec^{-2}.

To measure the intensity and timing of the phytoplankton bloom, we collected triplicate samples daily from 1 m depth in the mesocosm and analyzed these for chlorophyll *a* concentrations. Samples were filtered onto 0.45 µm cellulose acetate or GF/F filters, chlorophyll *a* was measured using the fluorometric method of Parsons et al. (1984), and the triplicate samples were averaged for each date.

The mesocosm developed an intense bloom of *Phaeocystis pouchetii*. To evaluate potential allelopathic effects of the bloom, we measured the growth rates of the phytoplankton *Rhodomonas baltica* when growing with mesocosm filtrates from various bloom stages and compared these to *R. baltica* growth rates in control filtrates. We chose *R. baltica* as a test species because *Rhodomonas* spp. may co-occur with *Phaeocystis* spp. (CCMP website, www.ccmp.bigelow.org) and because a culture was available at the Marine Biological Station. Thus, this assay served as an initial assessment of whether inhibitory effects on phytoplankton might be associated with *P. pouchetii* blooms. The stock *R. baltica* culture was grown at 7^{o}C under a light level of 80 μE m^{-2} sec^{-1} and it was periodically diluted with fresh medium to maintain exponential growth.

Starting on 12 March and every 3 days afterwards until 24 March, we measured the growth rates of *Rhodomonas baltica* cultured in filtrates either from the mesocosm (treatments) or from Raunefjorden, which held the mesocosm (controls). For each filtrate experiment, 10 L of mesocosm water was collected, brought to a cold room (5^{o}C), and gravity filtered through 2 capsule filters; first a 3.0 μm and then a 0.2 μm filter. This process removed cells so we could assess the effects of waterborne chemicals on algal growth independent of any contact inhibition cues associated with blooming organisms. Control seawater was filtered through several filters including a 0.2 μm filter. We added 1000 cells ml^{-1} *R. baltica* to both treatment and control filtrates. At the start of each filtrate experiment, all solutions were amended with 4 μM NH_4 from 15 March onwards and with 0.25 μM PO_4 from 18 March onwards to enhance growth during the assay and to minimize the potential for nutrient limitation to affect *R. baltica* growth. Two initial, 20 ml subsamples were collected from both solutions and counted with a ZM Coulter Counter. The filtrate solution containing *R. baltica* was divided among several 560 ml acid-washed, plastic bottles (N=3, 12 March; N=7, 15 March; N=5, 18 March; N=15, 21

March; and N=10, 24 March). Each bottle was completely filled and covered with a piece of plastic wrap before capping to minimize the addition of air bubbles. Each bottle was attached to a floating frame adjacent to the mesocosms with string so that the bottles hung approximately 1.5 m below the sea surface. Thus, *R. baltica* experienced similar physical conditions to the phytoplankton within the mesocosm.

Bottles were collected 20 h later, the contents were well-mixed, and 2-20 ml subsamples were collected for final *Rhodomonas baltica* counts using a coulter counter. For each replicate, the 2 final *R. baltica* concentrations were averaged. Algal growth rates were calculated as μ (Equation 4.1; day^{-1}):

$$\mu = (\ln C_t - \ln C_o) \times 24/(t-t_o) \qquad (4.1)$$

where C_t is the concentration (cells ml^{-1}) at the end of the experiment (t, in hours) and C_o is the concentration of cells at the start of the experiment (t_o, equation from Hansen and Hjorth [2002]).

<u>*Laboratory Experiment*</u> – Because the mesocosm experiment indicated an inhibitory effect of mesocosm filtrates on *Rhodomonas baltica*, we conducted a laboratory experiment to determine if other species of *Phaeocystis* also inhibited growth of *R. baltica*. We compared growth rates when exposed to filtrates from exponentially growing cultures of *Phaeocystis globosa*, to filtrates from another *R. baltica* culture, or to filtrates of media alone. We included a test of the effects of filtrates from *R. baltica* cultures on *R. baltica* growth to assess the relative suppression of *Phaeocystis* filtrates versus the intraspecific effects of filtrates from *R. baltica*. *Phaeocystis globosa* (CCMP 627) and *Rhodomonas baltica* were grown xenically in L1-Si medium (CCMP recipe) at 20°C

under a light:dark cycle of 14:10 h. Illumination was provided by a combination of cool white and daylight fluorescent bulbs at ~100-150 μE m^{-2} sec^{-1}.

On day 0, we inoculated 2200 ml cultures with either *Phaeocystis globosa* or *Rhodomonas baltica* at 20,000 cells ml^{-1} and 15,000 cells ml^{-1}, respectively. This represents high bloom concentrations of *Phaeocystis* spp. (Claustre et al. 1990, Hansen and van Boekel 1991). In addition, we incubated the same volume of L-Si media, but without phytoplankton, adjacent to these cultures. Liquid from these flasks served as the source for filtrates for assessing effects on algal growth. Every 2 days, we subsampled 10 ml from the cultures to monitor algal growth. *R. baltica* were counted with a Z2 Coulter Counter and *P. globosa* were counted with Palmer-Maloney chambers after preserving samples in Lugol's solution. A second *R. baltica* culture was inoculated and kept in exponential phase via frequent dilutions with media. This *R. baltica* culture served as the source of algae for the growth rate experiments.

Starting on day 0 and continuing every 2 days through day 6, we removed 260 ml from each culture as well as 260 ml media and filtered these volumes separately through GF/F filters to remove cells. We measured filtrate pH with a pH meter because pH can significantly affect phytoplankton growth (Hansen 2002). Then, we added *Rhodomonas baltica* to these filtrates at 5000 cells ml^{-1}. Each filtrate was spiked with L1-Si concentrations of nutrients and vitamins to prevent nutrient limitation of algal growth. Next, we subsampled 10 ml from each filtrate solution and determined the initial *R. baltica* concentrations using a Z2 Coulter Counter. Each solution was then divided among 9 replicate, 25 ml scintillation vials. Vials were rotated on a plankton wheel at 0.5 r.p.m. in a 20^{o}C incubator and at a light level of ~100-150 μE m^{-2} sec^{-1}. All vials were collected the following day and final *R. baltica* concentrations were measured with a Coulter Counter. Growth rates (μ) were determined using Equation 4.1.

Data Analysis - In both the mesocosm and laboratory experiments, growth rates were compared for all treatments on a given date using ANOVA. For the laboratory experiment, follow-up comparisons for each day were made using a Tukey test.

Results

Mesocosm Experiment - Chlorophyll *a* measurements within the mesocosm fell within the range of natural concentrations during *Phaeocystis* spp. blooms (Hansen and van Boekel 1991, Bautista et al. 1992, Gasparini et al. 2000) which can be as high as 55 µg l^{-1} (Hansen and van Boekel 1991). Chlorophyll *a* was strongly correlated with the concentration of *Phaeocystis pouchetii* cells and colonies (Nejstgaard et al. in preparation), so we report chlorophyll *a* as an indication of *P. pouchetii* concentrations during the bloom (Figure 4.1).

The inhibitory effects of mesocosm filtrates were associated with a *Phaeocystis pouchetii* bloom but not a diatom bloom that occurred earlier in the experiment. On day 9, when diatom concentrations peaked at 8610 cells ml^{-1} (Nejstgaard et al. in preparation), mesocosm filtrates did not significantly suppress *Rhodomonas baltica* growth compared to control filtrates (Figure 4.1, P = 0.530). The dominant diatoms were *Skeletonema costatum*, *Leptocylindrus* sp., *Thalassiosira* sp., and *Chaetoceros socialis*. In contrast, mesocosm filtrates from the start of the *P. pouchetii* bloom (day 12) suppressed *R. baltica* growth during the second experiment by 32% compared to controls (Figure 4.1, P = 0.023) and continued to suppress *R. baltica* growth for the 2 following experiments (Figure 4.1; day 15 [>100% suppression, P < 0.001] and day 18 [49% suppression, P = 0.046]). Mesocosm filtrates no longer inhibited *R. baltica* growth on day 21 during the observed *P. pouchetii* bloom peak (Figure 4.1, P = 0.858). However, *R. baltica* growth in both the treatment and control became extremely low during the course of these experiments. Given that our control water came from the

adjacent fjord and that a natural *P. pouchetii* bloom developed in the fjord toward the end of our experimental period (J. Long personal observation), allelopathic chemicals in the fjord bloom may have also occurred in the controls (thus confounding our "controls").

Laboratory Experiment - Filtrates from laboratory cultures of *Phaeocystis globosa* suppressed growth of *Rhodomonas baltica* following inoculation of the *P. globosa* culture and for at least 6 days thereafter (Figure 4.2, $P < 0.05$). Filtrates from *R. baltica* suppressed *R. baltica* growth on day 0 (Figure 4.2, $P < 0.001$) and day 4 (Figure 4.2, $P < 0.001$) but not on day 2 (Figure 4.2, $P = 0.367$). On day 6, no intact *R. baltica* cells remained after exposure to *R. baltica* filtrates suggesting that cells exposed to this treatment were lysed. This effect occurred independent of pH; pH of the *R. baltica* culture from which the filtrates were collected on day 6 (pH = 9.24), when lysing occurred, was similar to pH on day 4 (pH = 9.30), when significant growth occurred in the *R. baltic*a filtrates (Figure 4.2, Table 4.1). The suppression of *R. baltica* growth was species-specific. *P. globosa* filtrates suppressed growth significantly more than *R. baltica* filtrates on days 2 and 4 despite the fact that *R. baltica* cultures were more concentrated on these days (Figure 4.2).

Although pH was always greater in the filtrates from phytoplankton cultures than filtrates from the media control, 2 observations suggest that pH alone fails to account for the inhibitory effects seen in the culture filtrates. First, the pH of the *Phaeocystis globosa* filtrates that suppressed growth on days 0 and 2 never had a pH greater than 8.27 yet a pH as high as 8.35 (Table 4.1, *Rhodomonas baltica* filtrates, day 2) did not significantly suppress *R. baltica* growth compared to controls. Second, on days 2 and 4, the pH was higher in the *R. baltica* filtrates than in the *P. globosa* filtrates but *P. globosa* filtrates displayed a stronger inhibitory effect (Table 4.1).

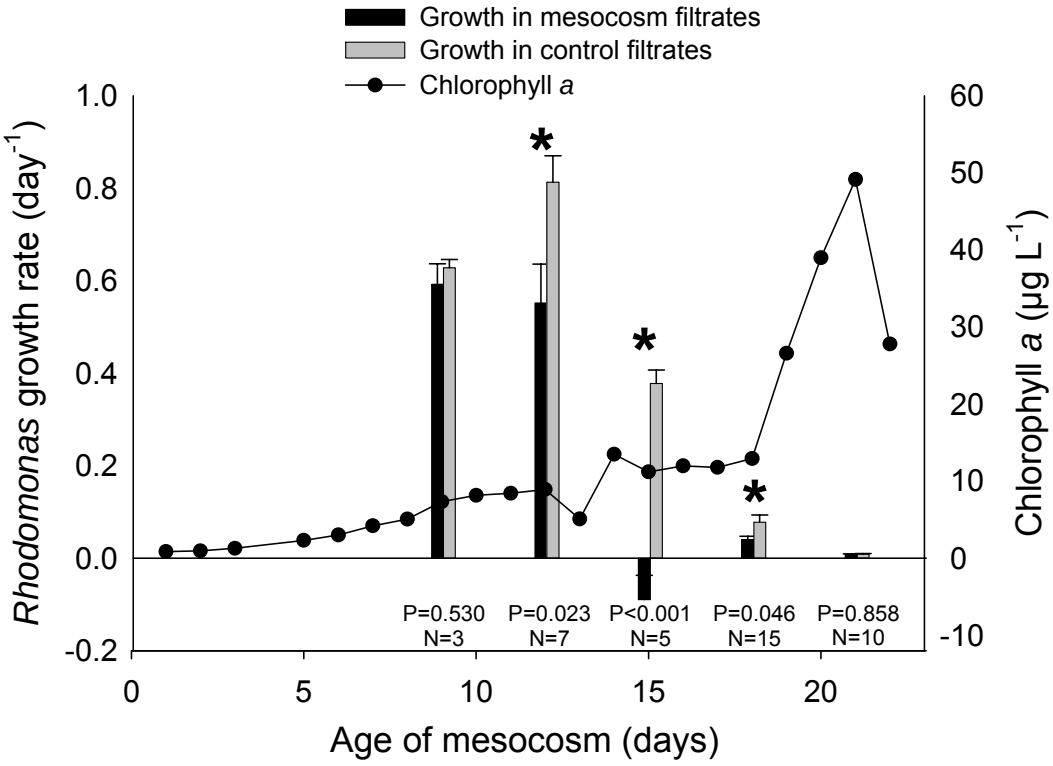

Figure 4.1. Growth rates (μ) of *Rhodomonas baltica* in cell-free filtrates from a mesocosm bloom dominated by *Phaeocystis pouchetii* (treatment bars) or in filtrates from fjord seawater (control bars). Line graph shows chlorophyll *a* concentrations within the mesocosm during the bloom. Chlorophyll *a* concentration was strongly related to *P. pouchetii* concentration. Asterisks denote significant differences ($P < 0.05$, ANOVA) between treatments and controls for each date. Growth rates are means + 1 SEM.

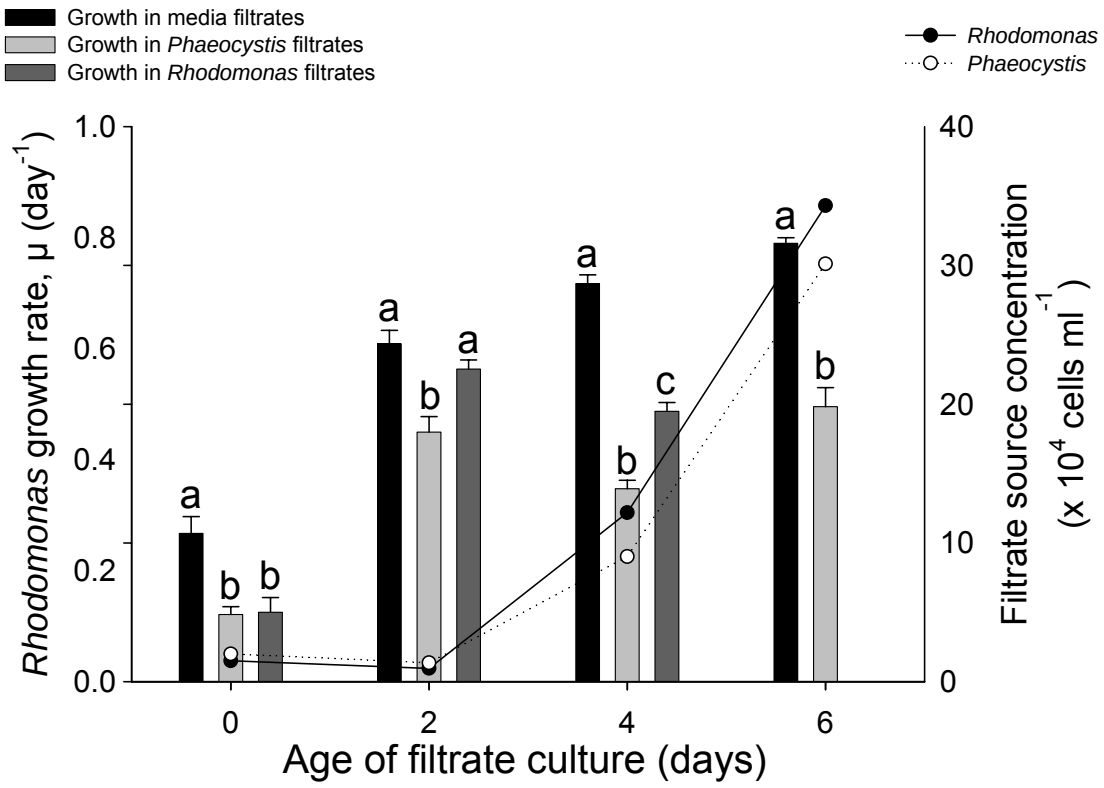

Figure 4.2. Growth rates (μ) of *Rhodomonas baltica* in cell-free filtrates from media, *R. baltica* cultures, or *Phaeocystis globosa* cultures. Line graph shows chlorophyll *a* concentrations in the cultures used as sources for the filtrates. All cells lysed in the *R. baltica* filtrates on day 6. Letters above bars indicate significant ($P < 0.05$, Tukey) among-treatment differences in growth rates for that day. Growth rates are means + 1 SEM.

Table 4.1. Filtrate pH during the laboratory experiment.

	Filtrate Source		
Day	Media	*R. baltica*	*P. globosa*
0	8.11	8.23	8.23
2	8.10	8.35	8.27
4	8.29	9.30	8.53
6	8.07	9.24	8.55

Discussion

Cell-free filtrates from a mesocosm bloom of *Phaeocystis pouchetii* of similar magnitude to natural blooms suppressed *Rhodomonas baltica* growth rates by 32-100%. *P. pouchetii* dominated the phytoplankton during the mesocosm bloom suggesting that it was the most likely cause of these effects. To see if this was a trait of this particular species of *Phaeocystis*, or something dependent on specific conditions of the mesocosm environment, we evaluated the allelopathic effects of a different *Phaeocystis* species grown under different physical conditions (e.g., laboratory setting at warmer temperatures). Despite using different species and different environmental conditions, we documented similar allelopathic effects. Cell-free filtrates from a laboratory culture of the single species *Phaeocystis globosa* consistently suppressed *R. baltica* growth by 26-54%. Like the coccolithophore genus *Prymnesium* (Houdan et al. 2004) and the dinoflagellate genus *Alexandrium* (Arzul et al. 1999), species of *Phaeocystis* may generally be producers of allelopathic agents. The effects of intraspecific allelopathy in *R. baltica* were variable; suppressing growth by 26-53% but these were sometimes not significantly different from controls.

Phytoplankton allelochemicals may inhibit competing phytoplankton via several pathways including inhibiting photosynthetic electron transport, inhibiting carbon fixation, causing paralysis, or lysing cell membranes (Maestrini and Bonin 1981, Sukenik et al. 2002, Gross 2003). Although the mode of action of allelopathic chemicals is unknown for *Phaeocystis* spp., our experiments suggest that these chemicals sometimes induced cell lysis in *Rhodomonas baltica*. The negative growth rates observed during the mesocosm filtrate experiment on day 15 indicates that after 20 hours, the *R. baltica* population was smaller than it was at the start of the experiment. This rapid loss of cells suggests that filtrates actually lysed *R. baltica* cells. Cell lysis of phytoplankton by allelopathic chemicals could be common (Rengefors and Legrand 2001, Fistarol et al.

2003). Rengefors and Legrand (2001) also observed blistering and lysing of a freshwater species of *Rhodomonas* due to filtrates from a dinoflagellate culture. However, *Rhodomonas* spp. are not necessarily more susceptible to allelopathic chemicals than other phytoplankton. Kubanek et al. (submitted) found that *Rhodomonas lens* was less susceptible to *Karenia brevis* allelochemicals than several other phytoplankton species.

In addition to allelopathic chemicals, we considered several alternative factors that could have affected *Rhodomonas baltica* growth in our experiments. Phytoplankton blooms and dense cultures can significantly deplete nutrient concentrations and this could decrease growth as phytoplankton become nutrient-limited. However, differences in inorganic nutrient concentrations do not account for the effects we observed because most of these filtrates were nutrient-rich due to our addition of nutrients prior to the experiment. High phytoplankton concentrations, such as those found during *Phaeocystis* spp. blooms or in dense *Phaeocystis* spp. cultures, can significantly decrease concentrations of inorganic carbon and increase the pH of their medium; both factors can significantly affect phytoplankton growth (Schmidt and Hansen 2001, Hansen 2002, Pedersen and Hansen 2003). However, pH fails to adequately account for the observed effects on *R. baltica* growth for 2 reasons. First, the airlift, pump system used in the mesocosm prevents significant pH changes during phytoplankton blooms (Jacobsen et al. 1995) yet we saw significant effects from bloom filtrates. Second, pH does not fully explain our results from the laboratory experiment because a higher pH in treatments versus controls did not always lead to a significant suppression of growth in treatments. Our studies agree with previous authors that a waterborne chemical associated with some phytoplankton, independent of pH and nutrients, can suppress the growth of other phytoplankton (Pratt and Fong 1940, Keating 1977).

Phytoplankton can produce significant chemical effects on the water mass around them, such as when they produce easily detected levels of dimethyl sulfide (Gibson et al. 1990, Crocker et al. 1995, Osinga et al. 1996) and acrylate (Noordkamp et al. 1998), or when their metabolic activities alter water column pH (Hansen 2002). Thus, it is not unreasonable to expect that they could release other metabolites at levels that affect co-occurring competitors. Localized effects of released allelopathic metabolites could be especially effective when species bloom during periods of stratification, reduced turbulence, or calm weather conditions (Hulot and Huisman 2004). For example, *Phaeocystis* spp. frequently bloom in fjords and embayments where mixing is relatively low (Hansen 2002). Unfortunately, the previous emphasis on assays of laboratory, phytoplankton cultures or assays of cellular extracts, instead of exudates, prevents a complete understanding of the role of exuded chemicals in more realistic settings. Our study of a marine phytoplankton bloom and the studies of natural, freshwater samples (Keating 1977, 1978) indicate that phytoplankton grown under more natural conditions can suppress the growth of competitors and affect succession in phytoplankton communities.

REFERENCES

Aanesen, R. T., H. C. Eilertsen, and O. B. Stabell. 1998. Light-induced toxic properties of the marine alga *Phaeocystis pouchetii* towards cod larvae. Aquatic Toxicology **40**:109-121.

Agrawal, A. A. 2000. Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. Oikos **89**:493-500.

Arrigo, K. R., D. H. Robinson, D. L. Worthen, R. B. Dunbar, G. R. DiTullio, M. VanWoert, and M. P. Lizotte. 1999. Phytoplankton community structure and the drawdown of nutrients and CO_2 in the Southern Ocean. Science **283**:365-367.

Arzul, G., M. Seguel, L. Guzman, and E. Erard-Le Denn. 1999. Comparison of allelopathic properties in three toxic *Alexandrium* species. Journal of Experimental Marine Biology and Ecology **232**:285-295.

Avila, C. and V. J. Paul. 1997. Chemical ecology of the nudibranch *Glossodoris pallida*: Is the location of diet-derived metabolites important for defense? Marine Ecology-Progress Series **150**:171-180.

Bailey, J. K., J. A. Schweitzer, B. J. Rehill, R. L. Lindroth, G. D. Martinsen, and T. G. Whitham. 2004. Beavers as molecular geneticists: A genetic basis to the foraging of an ecosystem engineer. Ecology **85**:603-608.

Barrett, R. D. H. and A. A. Agrawal. 2004. Interactive effects of genotype, environment, and ontogeny on resistance of cucumber (*Cucumis sativus*) to the generalist herbivore, *Spodoptera exigua*. Journal of Chemical Ecology **30**:37-51.

Bartram, W. C. 1980. Experimental development of a model for the feeding of neritic copepods on phytoplankton. Journal of Plankton Research **3**:25-51.

Bautista, B., R. P. Harris, P. R. G. Tranter, and D. Harbour. 1992. *In situ* copepod feeding and grazing rates during a spring bloom dominated by *Phaeocystis* sp. in the English Channel. Journal of Plankton Research **14**:691-703.

Berenbaum, M. R. and E. Miliczky. 1984. Mantids and milkweed bugs - efficacy of aposematic coloration against invertebrate predators. American Midland Naturalist **111**:64-68.

Berggreen, U., B. Hansen, and T. Kiorboe. 1988. Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development - implications for determination of copepod production. Marine Biology **99**:341-352.

Bernays, E. A. and R. F. Chapman. 2000. Plant secondary compounds and grasshoppers: beyond plant defenses. Journal of Chemical Ecology **26**:1773-1794.

Bernays, E. A. and J. C. Lee. 1988. Food aversion learning in the polyphagous grasshopper *Schistocerca americana*. Physiological Entomology **13**:131-137.

Bernstein, C., A. Kacelnik, and J. R. Krebs. 1988. Individual Decisions and the Distribution of Predators in a Patchy Environment. Journal of Animal Ecology **57**:1007-1026.

Bernstein, I. L. 1978. Learned taste aversions in children receiving chemotherapy. Science **200**:1302-1303.

Besiktepe, S. and H. G. Dam. 2002. Coupling of ingestion and defecation as a function of diet in the calanoid copepod *Acartia tonsa*. Marine Ecology-Progress Series **229**:151-164.

Biddanda, B. and R. Benner. 1997. Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. Limnology and Oceanography **42**:506-518.

Bolser, R. C. and M. E. Hay. 1996. Are tropical plants better defended? Palatability and defenses of temperate vs. tropical seaweeds. Ecology **77**:2269-2286.

Burghardt, G. M., H. C. Wilcoxon, and J. A. Czaplick. 1973. Conditioning in garter snakes - aversion to palatable prey induced by delayed illness. Animal Learning & Behavior **1**:317-320.

Butler, N. M., C. A. Suttle, and W. E. Neill. 1989. Discrimination by freshwater zooplankton between single algal cells differing in nutritional status. Oecologia **78**:368-372.

Cariou, V., R. Casotti, J. L. Birrien, and D. Vaulot. 1994. The initiation of *Phaeocystis* colonies. Journal of Plankton Research **16**:457-470.

Cates, R. O. and G. H. Orians. 1975. Successional status and the palatability of plants to generalized herbivores. Ecology **68**:1863-1876.

Cimino, G., S. Derosa, S. Destefano, R. Morrone, and G. Sodano. 1985. The chemical defense of nudibranch mollusks - structure, biosynthetic origin and defensive properties of terpenoids from the Dorid nudibranch *Dendrodoris grandiflora*. Tetrahedron **41**:1093-1100.

Cimino, G., S. Derosa, S. Destefano, and G. Sodano. 1982. The chemical defense of 4 Mediterranean nudibranchs. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology **73**:471-474.

Cimino, G., S. Derosa, S. Destefano, G. Sodano, and G. Villani. 1983. Dorid nudibranch elaborates its own chemical defense. Science **219**:1237-1238.

Claustre, H., S. A. Poulet, R. Williams, J. C. Marty, S. Coombs, F. Benmlih, A. M. Hapette, and V. Martinjezequel. 1990. A biochemical investigation of a *Phaeocystis* sp. bloom in the Irish Sea. Journal of the Marine Biological Association of the United Kingdom **70**:197-207.

Coble, D. W., G. B. Farabee, and R. O. Anderson. 1985. Comparative learning ability of selected fishes. Canadian Journal of Fisheries and Aquatic Sciences **42**:791-796.

Coley, P. D. and T. M. Aide. 1991. Comparison of herbivory and plant defenses in temperate and tropical broad-eaved forests. Pages 25-49. In: Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions, edited by P. W. Price, T. M. Lewinsohn, G. W. Fernandes, W. W. Benson, Wiley & Sons, New York.

Crocker, K. M., M. E. Ondrusek, R. L. Petty, and R. C. Smith. 1995. Dimethylsulfide, algal pigments and light in an Antarctic *Phaeocystis* sp. bloom. Marine Biology **124**:335-340.

Cronin, G. and M. E. Hay. 1996. Susceptibility to herbivores depends on recent history of both the plant and animal. Ecology **77**:1531-1543.

Cronin, G., M. E. Hay, W. Fenical, and N. Lindquist. 1995. Distribution, density, and sequestration of host chemical defenses by the specialist nudibranch *Tritonia hamnerorum* found at high densities on the sea fan *Gorgonia ventalina*. Marine Ecology-Progress Series **119**:177-189.

Cronin, G., K. D. Wissing, and D. Lodge. 1998. Comparative feeding selectivity of herbivorous insects on water lilies: aquatic vs. semi-terrestrial insects and submersed vs. floating leaves. Freshwater Biology **39**:243-257.

Crossland, M. R. 2001. Ability of predatory native Australian fishes to learn to avoid toxic larvae of the introduced toad *Bufo marinus*. Journal of Fish Biology **59**:319-329.

Cruz-Rivera, E. and M. E. Hay. 2000. Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. Ecology **81**:201-219.

Cruz-Rivera, E. and M. E. Hay. 2003. Prey nutritional quality interacts with chemical defenses to affect consumer feeding and fitness. Ecological Monographs **73**:483-506.

Davies, A. G., I. Demadariaga, B. Bautista, F. Fernandez, D. S. Harbour, P. Serret, and P. R. G. Tranter. 1992. The ecology of a coastal *Phaeocystis* bloom in the north-western English Channel in 1990. Journal of the Marine Biological Association of the United Kingdom **72**:691-708.

De Moraes, C. M., W. J. Lewis, P. W. Pare, H. T. Alborn, and J. H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. Nature **393**:570-573.

De Nys, R., J. C. Coll, and I. R. Price. 1991. Chemically mediated interactions between the red alga *Plocamium hamatum* (Rhodophyta) and the octocoral *Sinularia cruciata* (Alcyonacea). Marine Biology **108**:315-320.

Denno, R. F. and M. S. McClure. 1983. Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York.

Dethier, V. G. and M. T. Yost. 1979. Oligophagy and absence of food aversion learning in tobacco hornworms, *Manduca sexta*. Physiological Entomology **4**:125-130.

Dodson, S. 1988. The ecological role of chemical stimuli for the zooplankton predator avoidance behavior in *Daphnia*. Limnology and Oceanography **33**:1431-1439.

Dodson, S. I. 1974. Adaptive change in plankton morphology in response to size-selective predation - new hypothesis of cyclomorphosis. Limnology and Oceanography **19**:721-729.

Egge, J. K. and B. R. Heimdal. 1994. Blooms of phytoplankton including *Emiliania huxleyi* (Haptophyta): effects of nutrient supply in different N:P ratios. Sarsia **79**:333-348.

Estep, K. W., J. C. Nejstgaard, H. R. Skjoldal, and F. Rey. 1990. Predation by copepods upon natural populations of *Phaeocystis pouchetii* as a function of the physiological state of the prey. Marine Ecology Progress Series **67**:235-249.

Eyster, L. S. and S. E. Stancyk. 1981. Reproduction, growth and trophic Interactions of *Doriopsilla pharpa* Marcus in South Carolina. Bulletin of Marine Science **31**:72-82.

Fernandez, D. and J. L. Acuna. 2003. Enhancement of marine phytoplankton blooms by appendicularian grazers. Limnology and Oceanography **48**:587-593.

Fistarol, G. O., C. Legrand, and E. Graneli. 2003. Allelopathic effect of *Prymnesium parvum* on a natural plankton community. Marine Ecology-Progress Series **255**:115-125.

Fistarol, G. O., C. Legrand, E. Selander, C. Hummert, W. Stolte, and E. Graneli. 2004. Allelopathy in *Alexandrium* spp.: effect on a natural plankton community and on algal monocultures. Aquatic Microbial Ecology **35**:45-56.

Gamble, J. C. 1978. Copepod grazing during a declining spring phytoplankton bloom in northern North Sea. Marine Biology **49**:303-315.

Garcia, J., W. G. Hankins, and K. W. Rusiniak. 1974. Behavioral regulation of milieu interne in man and rat. Science **185**:824-831.

Gasparini, S., M. H. Daro, E. Antajan, M. Tackx, V. Rousseau, J. Y. Parent, and C. Lancelot. 2000. Mesozooplankton grazing during the *Phaeocystis globosa* bloom in the southern bight of the North Sea. Journal of Sea Research **43**:345-356.

Gaudy, R. 1974. Feeding four species of pelagic copepods under experimental conditions. Marine Biology **25**:125-141.

Gavagnin, M., E. Mollo, G. Calado, S. Fahey, M. Ghiselin, J. Ortea, and G. Cimino. 2001a. Chemical studies of porostome nudibranchs: comparative and ecological aspects. Chemoecology **11**:131-136.

Gavagnin, M., E. Mollo, F. Castelluccio, M. T. Ghiselin, G. Calado, and G. Cimino. 2001b. Can molluscs biosynthesize typical sponge metabolites? The case of the nudibranch *Doriopsilla areolata*. Tetrahedron **57**:8913-8916.

Gelperin, A. 1975. Rapid food aversion learning by a terrestrial mollusk. Science **189**:567-570.

Gerhart, D. J. 1984. Prostaglandin A2 - an agent of chemical defense in the Caribbean gorgonian *Plexaura homomalla*. Marine Ecology-Progress Series **19**:181-187.

Gerhart, D. J. 1991. Emesis, learned aversion, and chemical defense in octocorals - a central role for prostaglandins. American Journal of Physiology **260**:R839-R843.

Gerhart, D. J. and J. C. Coll. 1993. Pukalide, a widely distributed octocoral diterpenoid, induces vomiting in fish. Journal of Chemical Ecology **19**:2697-2704.

Gibson, J. A. E., R. C. Garrick, H. R. Burton, and A. R. McTaggart. 1990. Dimethylsulfide and the alga *Phaeocystis pouchetii* in Antarctic coastal waters. Marine Biology **104**:339-346.

Gifford, D. J., L. M. Fessenden, P. R. Garrahan, and E. Martin. 1995. Grazing by microzooplankton and mesozooplankton in the high-latitude North Atlantic Ocean - spring versus summer dynamics. Journal of Geophysical Research-Oceans **100**:6665-6675.

Gross, E. M. 2003. Allelopathy of aquatic autotrophs. Critical Reviews in Plant Sciences **22**:313-339.

Guida, V. G. 1976. Sponge predation in oyster reef community as demonstrated with *Cliona celata* Grant. Journal of Experimental Marine Biology and Ecology **25**:109-122.

Guillard, R. R. L. and P. E. Hargraves. 1993. *Stichochrysis immobilis* is a diatom, not a Chyrsophyte. Phycologia **32**:234-236.

Hamm, C., M. Reigstad, C. W. Riser, A. Muhlebach, and P. Wassmann. 2001. On the trophic fate of *Phaeocystis pouchetii*. VII. Sterols and fatty acids reveal sedimentation of *P. pouchetii*-derived organic matter via krill fecal strings. Marine Ecology-Progress Series **209**:55-69.

Hamm, C. E. 2000. Architecture, ecology and biogeochemistry of *Phaeocystis* colonies. Journal of Sea Research **43**:307-315.

Hansen, B., P. K. Bjornsen, and P. J. Hansen. 1994a. The size ratio between planktonic predators and their prey. Limnology and Oceanography **39**:395-403.

Hansen, B., P. Verity, T. Falkenhaug, K. S. Tande, and F. Norrbin. 1994b. On the trophic fate of *Phaeocystis pouchetti* (Harriot). V. Trophic relationships between *Phaeocystis* and zooplankton: an assessment of methods and size dependence. Journal of Plankton Research **16**:487-511.

Hansen, E., H. C. Eilertsen, A. Ernstsen, and A. M. Geneviere. 2003. Anti-mitotic activity towards sea urchin embryos in extracts from the marine haptophycean *Phaeocystis pouchetii* (Hariot) Lagerheim collected along the coast of northern Norway. Toxicon **41**:803-812.

Hansen, E., A. Ernstsen, and H. C. Eilertsen. 2004. Isolation and characterisation of a cytotoxic polyunsaturated aldehyde from the marine phytoplankter *Phaeocystis pouchetii* (Hariot) Lagerheim. Toxicology **199**:207-217.

Hansen, F. C., M. Reckermann, W. Breteler, and R. Riegman. 1993. *Phaeocystis* blooming enhanced by copepod predation on protozoa - evidence from incubation experiments. Marine Ecology-Progress Series **102**:51-57.

Hansen, F. C. and W. H. M. van Boekel. 1991. Grazing pressure of the calanoid copepod *Temora longicornis* on a *Phaeocystis* dominated spring bloom in a Dutch tidal inlet. Marine Ecology-Progress Series **78**:123-129.

Hansen, P. J. 2002. Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession. Aquatic Microbial Ecology **28**:279-288.

Hansen, P. J. and M. Hjorth. 2002. Growth and grazing responses of *Chrysochromulina ericina* (Prymnesiophyceae): the role of irradiance, prey concentration and pH. Marine Biology **141**:975-983.

Harvell, C. D. 1990. The ecology and evolution of inducible defenses. Quarterly Review of Biology **65**:323-340.

Havel, J. E. 1987. Predator-induced defenses: a review. Pages 263-278 *in* W. C. Kerfoot and A. Sih, editors. Predation: Direct and Indirect Impacts on Aquatic Communities. University Press of New England, Hanover.

Hay, M. E. 1992. The role of seaweed chemical defenses in the evolution of feeding specialization and in the mediation of complex interactions. Pages 93-118 *in* V. J. Paul, editor. Ecological Roles for Marine Natural Products. Comstock Publishing Associates, Ithaca, New York, USA.

Hay, M. E. 1996. Marine chemical ecology: what's known and what's next? Journal of Experimental Marine Biology and Ecology **200**:103-134.

Hay, M. E. 1997. The ecology and evolution of seaweed-herbivore interactions on coral reefs. Coral Reefs **16**:S67-S76.

Hay, M. E., Q. E. Kappel, and W. Fenical. 1994. Synergisms in plant defenses against herbivores: interactions of chemistry, calcification, and plant quality. Ecology **75**:1714-1726.

Hay, M. E. and J. Kubanek. 2002. Community and ecosystem level consequences of chemical cues in the plankton. Journal of Chemical Ecology **28**:2001-2016.

Hay, M. E., J. J. Stachowicz, E. Cruz-Rivera, S. Bullard, M. S. Deal, and N. Lindquist. 1998. Bioassays with marine and freshwater macroorganisms. Pages 39-141 *in* K. F. Haynes and J. G. Millar, editors. Methods in Chemical Ecology. Chapman and Hall, New York.

Hegarty, S. G. and T. A. Villareal. 1998. Effects of light level and N : P supply ratio on the competition between *Phaeocystis cf. pouchetii* (Hariot) Lagerheim (Prymnesiophyceae) and five diatom species. Journal of Experimental Marine Biology and Ecology **226**:241-258.

Hessen, D. O. and E. Van Donk. 1993. Morphological changes in *Scenedesmus* induced by substances released from *Daphnia*. Archiv Fur Hydrobiologie **127**:129-140.

Houdan, A., A. Bonnard, J. Fresnel, S. Fouchard, C. Billard, and I. Probert. 2004. Toxicity of coastal coccolithophores (Prymnesiophyceae, Haptophyta). Journal of Plankton Research **26**:875-883.

Hughes, R. N. 1979. Optimal diets under the energy maximization premise: effects of recognition time and learning. American Naturalist **113**:209-221.

Hulot, F. D. and J. Huisman. 2004. Allelopathic interactions between phytoplankton species: the roles of heterotrophic bacteria and mixing intensity. Limnology and Oceanography **49**:1424-1434.

Huntley, M., P. Sykes, S. Rohan, and V. Marin. 1986. Chemically-mediated rejection of dinoflagellate prey by the copepods *Calanus pacificus* and *Paracalanus parvus*: mechanism, occurrence and significance. Marine Ecology Progress Series **28**:105-120.

Huntley, M., K. Tande, and H. C. Eilertsen. 1987. On the trophic fate of *Phaeocystis pouchetii* (Hariot) .2. Grazing rates of *Calanus hyperboreus* (Kroyer) on diatoms and different size categories of *Phaeocysti pouchetii*. Journal of Experimental Marine Biology and Ecology **110**:197-212.

Hutchinson, G. E. 1944. Limnological studies in Connecticut. VI. A critical examination of the supposed relationship between phytoplankton periodicity and chemical changes in lake waters. Ecology **25**:3-26.

Hutchinson, G. E. 1961. The paradox of the plankton. American Naturalist **95**:137-145.

Inderjit, K. M. M. Dakshini, and F. A. Einhellig. 1995. Allelopathy : Organisms, Processes, and Applications. American Chemical Society, Washington, D.C.

Irigoien, X., J. Huisman, and R. P. Harris. 2004. Global biodiversity patterns of marine phytoplankton and zooplankton. Nature **429**:863-867.

Jacobsen, A., J. K. Egge, and B. R. Heimdal. 1995. Effects of increased concentration of nitrate and phosphate during a springbloom experiment in mesocosm. Journal of Experimental Marine Biology and Ecology **187**:239-251.

Jakobsen, H. H. and K. W. Tang. 2002. Effects of protozoan grazing on colony formation in *Phaeocystis globosa* (Prymnesiophyceae) and the potential costs and benefits. Aquatic Microbial Ecology **27**:261-273.

Janzen, D. H. 1979. New horizons in the biology of plant defenses. Pages 331-350 *in* G. A. Rosenthal and D. H. Janzen, editors. Herbivores: Their Interactions with Secondary Plant Metabolites. Academic Press, New York.

Johansson, N. and E. Graneli. 1999. Influence of different nutrient conditions on cell density, chemical composition and toxicity of *Prymnesium parvum* (Haptophyta) in semi-continuous cultures. Journal of Experimental Marine Biology and Ecology **239**:243-258.

Jones, D. A. 1962. Selective eating of acyanogenic form of plant *Lotus corniculatus* L. by various animals. Nature **193**:1109-1110.

Karban, R. 1992. Plant variation: its effects on populations of herbivorous insects. Pages 195-215 *in* R. S. Fritz and E. L. Simms, editors. Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics. University of Chicago Press, Chicago.

Karban, R. and I. T. Baldwin. 1997. Induced Responses to Herbivory. University of Chicago Press.

Karban, R., J. Maron, G. W. Felton, G. Ervin, and H. Eichenseer. 2003. Herbivore damage to sagebrush induces resistance in wild tobacco: evidence for eavesdropping between plants. Oikos **100**:325-332.

Karban, R. and J. H. Myers. 1989. Induced plant responses to herbivory. Annual Review of Ecology and Systematics **20**:331-348.

Kearns, K. D. and M. D. Hunter. 2002. Algal extracellular products suppress *Anabaena flos-aquae* heterocyst spacing. Microbial Ecology **43**:174-180.

Keating, K. I. 1977. Allelopathic influence on blue-green bloom sequence in a eutrophic lake. Science **196**:885-887.

Keating, K. I. 1978. Blue-green algal inhibition of diatom growth: transition from mesotrophic to eutrophic community structure. Science **199**:971-973.

Kimball, B. A., F. D. Provenza, and E. A. Burritt. 2002. Importance of alternative foods on the persistence of flavor aversions: implications for applied flavor avoidance learning. Applied Animal Behaviour Science **76**:249-258.

Koroleff, F. 1976. Determination of NH4-N. *in* K. Grasshoff, editor. Methods of Seawater Analysis. Verlag Chemie, Weinheim.

Koski, M., M. Rosenberg, M. Viitasalo, S. Tanskanen, and U. Sjolund. 1999. Is *Prymnesium patelliferum* toxic for copepods? Grazing, egg production, and

egestion of the calanoid copepod *Eurytemora affinis* in mixtures of "good" and "bad" food. Ices Journal of Marine Science **56**:131-139.

Kubo, I. and I. Ganjian. 1981. Insect antifeedant terpenes, hot-tasting to humans. Experientia **37**:1063-1064.

Lampert, W., K. O. Rothhaupt, and E. Vonelert. 1994. Chemical induction of colony formation in a green alga (*Scenedesmus acutus*) by grazers (*Daphnia*). Limnology and Oceanography **39**:1543-1550.

Lange, M., Y. Q. Chen, and L. K. Medlin. 2002. Molecular genetic delineation of *Phaeocystis* species (Prymnesiophyceae) using coding and non-coding regions of nuclear and plastid genomes. European Journal of Phycology **37**:77-92.

Larson, T. R. and T. A. V. Rees. 1996. Changes in cell composition and lipid metabolism mediated by sodium and nitrogen availability in the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). Journal of Phycology **32**:388-393.

Laska, M. and K. Metzker. 1998. Food avoidance learning in squirrel monkeys and common marmosets. Learning & Memory **5**:193-203.

Lass, S. and P. Spaak. 2003. Chemically induced anti-predator defences in plankton: a review. Hydrobiologia **491**:221-239.

Lawrence, R., B. M. Potts, and T. G. Whitham. 2003. Relative importance of plant ontogeny, host genetic variation, and leaf age for a common herbivore. Ecology **84**:1171-1178.

Lee, J. C. and E. A. Bernays. 1988. Declining acceptability of a food plant for the polyphagous grasshopper *Schistocerca americana*: the role of food aversion learning. Physiological Entomology **13**:291-301.

Lewis, W. M., Jr. 1986. Evolutionary interpretations of allelochemical interactions in phytoplankton algae. The American Naturalist **127**:184-194.

Lill, J. T. and R. J. Marquis. 2001. The effects of leaf quality on herbivore performance and attack from natural enemies. Oecologia **126**:418-428.

Lindquist, N. and M. E. Hay. 1995. Can small rare prey be chemically defended - the case for marine larvae. Ecology **76**:1347-1358.

Lubchenco, J. and S. D. Gaines. 1981. A unified approach to marine plant-herbivore Interactions .1. Populations and communities. Annual Review of Ecology and Systematics **12**:405-437.

Lurling, M. 1998. Effect of grazing-associated infochemicals on growth and morphological, development in *Scenedesmus acutus* (Chlorophyceae). Journal of Phycology **34**:578-586.

Lurling, M. 1999. Grazer-induced coenobial formation in clonal cultures of *Scenendesmus obliquus* (Chlorococcales, Chlorophyceae). Journal of Phycology **35**:19-23.

Lurling, M. and W. Beekman. 1999. Grazer-induced defenses in *Scenedesmus* (Chlorococcales; Chlorophyceae): coenobium and spine formation. Phycologia **38**:368-376.

Lurling, M. and E. Van Donk. 1996. Zooplankton-induced unicell-colony transformation in *Scenedesmus acutus* and its effect on growth of herbivore *Daphnia*. Oecologia **108**:432-437.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications **10(3)**:689-710.

Maestrini, S. Y. and D. J. Bonin. 1981. Allelopathic relationships between phytoplankton species. Canadian Bulletin of Fisheries and Aquatic Sciences:323-338.

Martens, P. 1981. On the *Acartia* species of the northern wadden sea of Sylt. Kieler Meeresforsch **5**:153-163.

Moon, D. C. and P. Stiling. 2002. Top-down, bottom-up, or side to side? Within-trophic-level interactions modify trophic dynamics of a salt marsh herbivore. Oikos **98**:480-490.

Myklestad, S. M., B. Ramlo, and S. Hestmann. 1995. Demonstration of strong interaction between the flagellate *Chrysochromulina polylepis* (Prymnesiophyceae) and a marine diatom. Pages 633-638 *in* P. Lassus, G. Arzul, E. Erard, P. Gentien, and C. Marcaillou, editors. Harmful Marine Algal Blooms. Lavoisier, Intercept Ltd., Hampshire.

Nicolaus, L. K., J. F. Cassel, R. B. Carlson, and C. R. Gustavson. 1983. Taste-aversion conditioning of crows to control predation on eggs. Science **220**:212-214.

Noordkamp, D. J. B., M. Schotten, W. W. C. Gieskes, L. J. Forney, J. C. Gottschal, and M. van Rijssel. 1998. High acrylate concentrations in the mucus of *Phaeocystis globosa* colonies. Aquatic Microbial Ecology **16**:45-52.

Ohsuka, A. 1962. J Chem Soc Japan **83**:757.

Okuda, R. K., P. J. Scheuer, J. E. Hochlowski, R. P. Walker, and D. J. Faulkner. 1983. Sesquiterpenoid Constituents of 8 Porostome Nudibranchs. Journal of Organic Chemistry **48**:1866-1869.

Osinga, R., R. L. J. Kwint, W. E. Lewis, G. W. Kraay, J. D. Lont, H. J. Lindeboom, and F. C. van Duyl. 1996. Production and fate of dimethylsulfide and dimethylsulfoniopropionate in pelagic mesocosms: The role of sedimentation. Marine Ecology-Progress Series **131**:275-286.

Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford.

Paul, V. J., Y. Seo, K. W. Cho, J. R. Rho, J. Shin, and P. R. Bergquist. 1997. Sesquiterpenoids of the drimane class from a sponge of the genus *Dysidea*. Journal of Natural Products **60**:1115-1120.

Pawlik, J. R., B. Chanas, R. J. Toonen, and W. Fenical. 1995. Defenses of Caribbean sponges against predatory reef fish .1. chemical deterrency. Marine Ecology-Progress Series **127**:183-194.

Pedersen, M. F. and P. J. Hansen. 2003. Effects of high pH on a natural marine planktonic community. Marine Ecology-Progress Series **260**:19-31.

Peperzak, L. 1993. Daily irradiane governs growth rate and colony formation of Phaeocystis (Prymnesiophyceae). Journal of Plankton Research **15**:809-821.

Peperzak, L., F. Colijn, W. W. C. Gieskes, and J. C. H. Peeters. 1998. Development of the diatom-*Phaeocystis* spring bloom in the Dutch coastal zone of the North Sea: the silicon depletion versus the daily irradiance threshold hypothesis. Journal of Plankton Research **20**:517-537.

Peperzak, L., R. N. M. Duin, F. Colijn, and W. W. C. Gieskes. 2000. Growth and mortality of flagellates and non-flagellate cells of *Phaeocystis globosa* (Prymnesiophyceae). Journal of Plankton Research **22**:107-119.

Pratt, R. and J. Fong. 1940. Studies on *Chlorella vulgaris* II. Further evidence that *Chlorella* cells form a growth-inhibiting substance. American Journal of Botany **27**:431-436.

Provenza, F. D. 1995. Tracking variable environments - there is more than one kind of memory. Journal of Chemical Ecology **21**:911-923.

Provenza, F. D., J. J. Lynch, and C. D. Cheney. 1995. Effects of a flavor and food restriction on the response of sheep to novel foods. Applied Animal Behaviour Science **43**:83-93.

Provenza, F. D., J. J. Villalba, L. E. Dziba, S. B. Atwood, and R. E. Banner. 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Research **49**:257-274.

Ratcliffe, J. M., M. B. Fenton, and B. G. Galef. 2003. An exception to the rule: common vampire bats do not learn taste aversions. Animal Behaviour **65**:385-389.

Reeve, M. R. and M. A. Walter. 1977. Observations on existence of lower threshold and upper critical food concentrations for copepod *Acartia tonsa* Dana. Journal of Experimental Marine Biology and Ecology **29**:211-221.

Reifel, K. M., M. P. McCoy, M. A. Tiffany, T. E. Rocke, C. C. Trees, S. B. Barlow, D. J. Faulkner, and S. H. Hurlbert. 2001. *Pleurochrysis pseudoroscoffensis*

(Prymnesiophyceae) blooms on the surface of the Salton Sea, California. Hydrobiologia **466**:177-185.

Rengefors, K. and C. Legrand. 2001. Toxicity in *Peridinium aciculiferum*: an adaptive strategy to outcompete other winter phytoplankton? Limnology and Oceanography **46**:1990-1997.

Riegman, R., A. A. M. Noordeloos, and G. C. Cadee. 1992. *Phaeocystis* blooms and eutrophication of the continental coastal zones of the North Sea. Marine Biology **112**:479-484.

Riessen, H. P. 1999. Predator-induced life history shifts in *Daphnia*: a synthesis of studies using meta-analysis. Canadian Journal of Fisheries and Aquatic Sciences **56**:2487-2494.

Rousseau, V., S. Becquevort, J. Y. Parent, S. Gasparini, M. H. Daro, M. Tackx, and C. Lancelot. 2000. Trophic efficiency of the planktonic food web in a coastal ecosystem dominated by *Phaeocystis* colonies. Journal of Sea Research **43**:357-372.

Rousseau, V., D. Vaulot, R. Casotti, V. Cariou, J. Lenz, J. Gunkel, and M. Baumann. 1994. The life cycle of *Phaeocystis* (Prymnesiophyceae) - evidence and hypotheses. Journal of Marine Systems **5**:23-39.

Ruel, J. and T. G. Whitham. 2002. Fast-growing juvenile pinyons suffer greater herbivory when mature. Ecology **83**:2691-2699.

Ruiz, G. M., P. W. Fofonoff, J. T. Carlton, M. J. Wonham, and A. H. Hines. 2000. Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. Annu. Rev. Ecol. Syst. **31**:481-531.

Schmidt, L. E. and P. J. Hansen. 2001. Allelopathy in the prymnesiophyte *Chrysochromulina polylepis*: effect of cell concentration, growth phase and pH. Marine Ecology-Progress Series **216**:67-81.

Sih, A. 1987. Predators and prey lifestyles: an evolutionary and ecological overview. Pages 203-224 *in* W. C. Kerfoot and A. Sih, editors. Predation: Direct and Indirect Impacts on Aquatic Communities. University Press of New England, Hanover.

Sinclair, A. R. E. and M. Norton-Griffiths. 1979. Serengeti: dynamics of an ecosystem. University of Chicago Press, Chicago, Illinois, USA.

Siska, E. L., S. C. Pennings, T. L. Buck, and M. D. Hanisak. 2002. Latitudinal variation in palatability of salt marsh plants: which traits are responsible? Ecology **83**:3369-3381.

Smayda, T. J. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnology and Oceanography **42**:1137-1153.

Smith, W. O., L. A. Codispoti, D. M. Nelson, T. Manley, E. J. Buskey, H. J. Niebauer, and G. F. Cota. 1991. Importance of *Phaeocystis* blooms in the high latitude ocean carbon cycle. Nature **352**:514-516.

Stabell, O. B., R. T. Aanesen, and H. C. Eilertsen. 1999. Toxic peculiarities of the marine alga *Phaeocystis pouchetii* detected by in vivo and in vitro bioassay methods. Aquatic Toxicology **44**:279-288.

Stelfox-Widdicombe, C. E., S. D. Archer, P. H. Burkill, and J. Stefels. 2004. Microzooplankton grazing in *Phaeocystis* and diatom-dominated waters in the southern North Sea in spring. Journal of Sea Research **51**:37-51.

Stibor, H. and J. Luning. 1994. Predator-induced phenotypic variation in the pattern of growth and reproduction in *Daphnia hyalina* (Crustacea, Cladocera). Functional Ecology **8**:97-101.

Stout, M. J., K. V. Workman, R. M. Bostock, and S. S. Duffey. 1998. Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. Oecologia **113**:74-81.

Strickland, J. D. H. and T. R. Parsons, editors. 1972. A Practical Handbook of Seawater Analysis, 2 edition. Fisheries Research Board of Canada, Ottawa.

Sukenik, A., R. Eshkol, A. Livne, O. Hadas, M. Rom, D. Tchernov, A. Vardi, and A. Kaplan. 2002. Inhibition of growth and photosynthesis of the dinoflagellate *Peridinium gatunense* by *Microcystis* sp. (cyanobacteria): A novel allelopathic mechanism. Limnology and Oceanography **47**:1656-1663.

Tande, K. S. and U. Bamstedt. 1987. On the trophic fate of *Phaeocystis pouchetii* .1. Copepod feeding rates on solitary cells and colonies of *Phaeocystis pouchetii*. Sarsia **72**:313-320.

Tang, K. W. 2003. Grazing and colony size development in *Phaeocystis globosa* (Prymnesiophyceae): the role of a chemical signal. Journal of Plankton Research **25**:831-842.

Tang, K. W., H. H. Jakobsen, and A. W. Visser. 2001. *Phaeocystis globosa* (Prymnesiophyceae) and the planktonic food web: Feeding, growth, and trophic interactions among grazers. Limnology and Oceanography **46**:1860-1870.

Taroncher-Oldenburg, G., D. M. Kulis, and D. M. Anderson. 1997. Toxin variability during the cell cycle of the dinoflagellate *Alexandrium fundyense*. Limnology and Oceanography **42**:1178-1188.

Taylor, R. B., N. Lindquist, J. Kubanek, and M. E. Hay. 2003. Intraspecific variation in palatability and defensive chemistry of brown seaweeds: effects on herbivore fitness. Oecologia **136**:412-423.

Teegarden, G. J. 1999. Copepod grazing selection and particle discrimination on the basis of PSP toxin content. Marine Ecology-Progress Series **181**:163-176.

Tester, P. A. and P. K. Fowler. 1990. Brevetoxin contamination of Mercenaria mercenaria and Crassostrea virginica: a management issue. *in* E. Graneli, B. Sundstron, L. Edler, and D. M. Anderson, editors. Toxic Marine Phytoplankton. Elsevier Scientific Publishers, New York.

Thacker, R. W., D. G. Nagle, and V. J. Paul. 1997. Effects of repeated exposures to marine cyanobacterial secondary metabolites on feeding by juvenile rabbitfish and parrotfish. Marine Ecology-Progress Series **147**:21-29.

Tollrian, R. and S. I. Dodson. 1999. Inducible defenses in cladoceran: constraints, costs, and multipredator environments. Pages 177-202 *in* R. Tollrian and C. D. Harvell, editors. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, New Jersey.

Trussell, G. C., P. J. Ewanchuk, and M. D. Bertness. 2002. Field evidence of trait-mediated indirect interactions in a rocky intertidal food web. Ecology Letters **5**:241-245.

Trussell, G. C., P. J. Ewanchuk, and M. D. Bertness. 2003. Trait-mediated effects in rocky intertidal food chains: Predator risk cues alter prey feeding rates. Ecology **84**:629-640.

Trussell, G. C., P. J. Ewanchuk, M. D. Bertness, and B. R. Silliman. 2004. Trophic cascades in rocky shore tide pools: distinguishing lethal and nonlethal effects. Oecologia **139**:427-432.

Turner, J. T., A. Ianora, F. Esposito, Y. Carotenuto, and A. Miralto. 2002. Zooplankton feeding ecology: does a diet of *Phaeocystis* support good copepod grazing, survival, egg production and egg hatching success? Journal of Plankton Research **24**:1185-1195.

van Boekel, W. H. M., F. C. Hansen, R. Riegman, and R. P. M. Bak. 1992. Lysis induced decline of a *Phaeocystis* spring bloom and coupling with the microbial foodweb. Marine Ecology-Progress Series **81**:269-276.

Van Zandt, P. A. and A. A. Agrawal. 2004. Specificity of induced plant responses to specialist herbivores of the common milkweed *Asclepias syriaca*. Oikos **104**:401-409.

Veldhuis, M. J. W. and W. Admiraal. 1987. Influence of phosphate depletion on the growth and colony formation of *Phaeocystis pouchetii*. Marine Biology **95**:47-54.

Verity, P. G. 2000. Grazing experiments and model simulations of the role of zooplankton in *Phaeocystis* food webs. Journal of Sea Research **43**:317-343.

Verity, P. G. and T. J. Smayda. 1989. Nutritional value of *Phaeocystis pouchetii* (Prymnesiophyceae) and other phytoplankton for *Acartia* Spp. (Copepoda) - ingestion, egg production, and growth of nauplii. Marine Biology **100**:161-171.

Verity, P. G., T. J. Smayda, and E. Sakshaug. 1991. Photosynthesis, excretion, and growth rates of *Phaeocystis* colonies and solitary cells. Polar Research **10**:117-128.

Verity, P. G., T. A. Villareal, and T. J. Smayda. 1988. Ecological investigations of blooms of colonial *Phaeocystis pouchetti* .1. Abundance, biochemical composition, and metabolic rates. Journal of Plankton Research **10**:219-248.

Walter, T. C. 1989. Review of the new-world species of *Pseudodiaptomus* (Copepoda, Calanoida), with a key to the species. Bulletin of Marine Science **45**:590-628.

Weisse, T. 1983. Feeding of calanoid copepods in relation to *Phaeocystis pouchetii* blooms in the German Wadden Sea area off Sylt. Marine Biology **74**:87-94.

Weisse, T. and U. Scheffelmoser. 1990. Growth and grazing loss rates in single celled *Phaeocystis* sp. (Prymnesiophyceae). Marine Biology **106**:153-158.

Weisse, T., K. Tande, P. Verity, F. Hansen, and W. Gieskes. 1994. The trophic significance of *Phaeocystis* blooms. Journal of Marine Systems **5**:67-79.

Whitham, T. G., W. P. Young, G. D. Martinsen, C. A. Gehring, J. A. Schweitzer, S. M. Shuster, G. M. Wimp, D. G. Fischer, J. K. Bailey, R. L. Lindroth, S. Woolbright, and C. R. Kuske. 2003. Community and ecosystem genetics: A consequence of the extended phenotype. Ecology **84**:559-573.

Whittaker, R. H. and P. P. Feeny. 1971. Allelochemics: chemical interactions between species. Science **171**:757-770.

Wilcoxon, H. C., W. B. Dragoin, and P. A. Kral. 1971. Illness induced aversions in rat and quail: relative salience of visual and gustatory cues. Science **171**:826-828.

Ylioja, T., H. Roininen, M. P. Ayres, M. Rousi, and P. W. Price. 1999. Host-driven population dynamics in an herbivorous insect. Proceedings of the National Academy of Sciences of the United States of America **96**:10735-10740.

Yoerg, S. I. 1991. Social feeding reverses learned flavor aversions in spotted hyenas (*Crocuta Crocuta*). Journal of Comparative Psychology **105**:185-189.

Zar, J. H. 1999. Biostatistical Analysis, 4th edition. Prentice Hall, Upper Saddle River, New Jersey.