THE EFFECTS OF BODY SIZE AND PARTICLE SIZE ON FEEDING RATES AND MORPHOLOGY OF THE LARVAE OF THREE CONGENERIC BARNACLES (CLASS CIRRIPEDIA: GENUS *BALANUS*)

by

TRACEY IRENE SMART

A THESIS

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_____

Dr. Richard B. Emlet, Chair of the Examining Committee

_____

Date

Committee in Charge: Dr. Richard B. Emlet, Chair
Dr. Alan L. Shanks
Dr. Stephen S. Rumrill

Accepted by:

_____

Dean of the Graduate School

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Approved: _____
Dr. Richard B. Emlet

Filtering rates of planktonic animals can be affected by body size, morphology, and particle size. Larvae of *Balanus crenatus*, *B. glandula*, and *B. improvisus* have similar morphologies but different sizes. At 15°C, feeding rates of *B. glandula* and *B. improvisus* increase with larval stage, while *B. crenatus* showed no increase in feeding rate. Differences in feeding rates may be the result of temperature, body size, or competitive ability. All three species consumed the diatom *Skeletonema costatum* at higher rates than other larger and smaller diatoms, suggesting that these species may not partition food resources based on larval size. The sizes of diatoms offered to sibling larvae of *B. glandula* did not induce phenotypic changes in intersetule distances or areas of the second antennae by Stage VI. These results imply that morphology of feeding appendages cannot be used to compensate for different food regimes.

CURRICULUM VITAE

NAME OF AUTHOR: Tracey Irene Smart

PLACE OF BIRTH: Marion, Ohio

DATE OF BIRTH: November 18, 1978

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon
University of Arizona

DEGREES AWARDED:

Master of Science in Biology, 2003, University of Oregon
Bachelor of Science in Ecology and Evolutionary Biology, 2001, University of Arizona

AREAS OF SPECIAL INTEREST:

Larval Ecology
Reproductive Biology, Development and Ecology of Cirripedes

PROFESSIONAL EXPERIENCE:

Graduate Teaching Fellow, Oregon Institute of Marine Biology (University of Oregon), Charleston, 2001-2003.

Research Assistant, South Slough National Estuarine Research Reserve, Charleston, OR, 2002.

After-school Marine Science Program Coordinator, Charleston Elementary School, Charleston, April-June 2002.

Research Assistant, Environmental Research Laboratory, University of Arizona, Tucson, AZ, 2000-2001

Research Assistant, Desert Plant Ecology Laboratory, University of Arizona, Tucson, AZ, 2000.

GRANTS, AWARDS AND HONORS:

Neil Richmond Memorial Fellowship, 2002

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CHAPTER I

GENERAL INTRODUCTION

The planktotrophic larval stage of marine benthic invertebrates can be considered as a mechanism for turning a small egg into a large juvenile through the accumulation of energy during planktonic feeding (Strathmann RR, 1987). This decreases the investment of the adult, but puts each cohort at great risk of mortality in the plankton from predation, starvation, and advection from the adult habitat (Morgan, 1995). Adaptations, including anti-predator defenses, efficient feeding mechanisms, and swimming behaviors that retain larvae near the adult habitat, may therefore have been strongly selected for over time.

The diversity of feeding structures among marine invertebrate larvae has stimulated interest in the performance of the different types of structures relative to one another and the rates at which each structure allows particle capture. High rates of particle capture mean high rates of energy accumulation and tissue growth, which may reduce mortality in a cohort by reducing the time required for the development of tissues needed for metamorphosis (Strathmann, 1987). The results of high feeding rates and the resultant growth may therefore be very important for the recruitment of marine invertebrate larvae and could have wide-ranging impacts for the ecology of adults, competition, dispersal, and life history evolution.

However, the study of feeding rates is a relatively small field and so a full understanding of the different feeding structures and their performance requires that a wide-range of species and larval types are represented in the literature. The best represented groups, at this time, are the pluteus larvae of echinoids and a few other ciliated larvae, such as veligers and bipinnaria. Relatively few studies have been reported for crustacean larvae, despite the fact that this group is considerably speciose and that there are several larval forms represented in this group.

In response to this, I report here the feeding rates for the larvae of one particularly underrepresented crustacean group, the Subclass Cirripedia. Chapter II of this thesis describes the feeding rates of the larvae of three species of barnacle (*Balanus crenatus, B. glandula,* and *B. improvisus*). Chapter III of this thesis compares the feeding rates of these animals on three foods of different sizes. Chapter IV of this thesis describes the effects of foods of different sizes on the morphology of the feeding appendages employed by barnacle larvae. Effectively, this thesis explores the feeding performance of barnacle larvae in the hopes that this information may lead to a greater understanding of the evolutionary pressures that have set the morphology of this larval type and the ecological consequences of possessing this type of larva.

CHAPTER II

FEEDING RATES AMONG THE LARVAE OF THREE CONGENERIC BARNACLES (*Balanus crenatus*, *Balanus glandula*, AND *Balanus improvisus*)

Introduction

In an overview of clearance rates of invertebrate larvae from several taxa, R Strathmann (1987) suggested that maximum feeding rate may be constrained by the morphology of the feeding structures, and that differences among taxa may be closely related to larval size. He suggested that the ability of larvae to collect food may impose limits on maximum larval size and fitness because of the risk of mortality with longer planktonic periods. Estimates for daily mortality from advection, predation, and starvation of planktotrophic larvae are very high (Morgan, 1995), however, efficient feeding and growth can reduce the time in the plankton and therefore loss at the larval stage. Some structures may be more efficient than others at collecting food, thus favored for maximizing nutritional intake, thereby reducing the time required to reach metamorphic competence and ultimately larval mortality in the plankton (Fenaux et al., 1994). Juvenile size at the end of the larval feeding phase may also affect the success of a species in recruiting into the adult habitat (Emlet, 1986; McConaugha, 1985; Vigliola

and Meekan, 2002) by giving new settlers an immediate size advantage. If planktotrophic larvae evolved as a mechanism for turning small eggs into larger juveniles as fast as possible in order to reduce mortality in the larval and juvenile stages (Strathmann, 1995), then there may be strong selective pressure to maximize feeding rates and make feeding structures highly efficient.

Data from a wide range of species with similar feeding mechanisms can therefore assist in evaluations of larval design and success (Strathmann, 1995). Among larvae that have been studied, feeding rates appear to group according to the structure used to capture particles and with body size. There are only a handful of studies, however, addressing feeding rates in larvae with similar forms, and so, grouping by feeding rate may actually be a function of the species tested or body size alone, not of constraints imposed by feeding structures.

Most feeding rate measurements have been done with larvae that capture particles via ciliated bands (e.g., plutei and veligers). There is a need, then, for equivalent measurements to be made for groups with other types of feeding structures. Reports of feeding rates by crustacean larvae, in particular, are few, especially for such a speciose group and despite an abundance of feeding rate measurements available for their filter-feeding adult counterparts (e.g., copepods). Anyone who has ever taken a plankton tow knows how abundant crustacean larvae can be, and they may be key players in determining standing stocks of phytoplankton and phytoplankton community composition (Porter, 1973; Kerfoot, 1987).

The nauplius larva in many crustacean groups has been retained since the Cambrian radiation (Muller and Walossek, 1986). This form, distinguished by three pairs of head appendages, a tissue extension over the mouth (the labrum), and a median eye (Sanders, 1963), has even been suggested as a defining characteristic of crustaceans (Walossek and Muller, 1990). Williams (1994) proposed that the nauplius is retained as a phylotypic stage because of functional plasticity that allows for diversification in later stages. Within the groups that possess a nauplius, the basic body plan has remained the same, with the second antennae used primarily for swimming and particle capture (Moyse, 1984; Paffenhofer and Lewis, 1989). Variations in size and allometry have appeared, which may lead to changes in rates of swimming and possibly feeding. Comparisons of feeding rates of nauplii from several different groups may therefore lead to a greater understanding of whether or not structure constrains feeding rate or if size alone constrains feeding rates. The present study examines the feeding rates and growth of three species of barnacle nauplii.

Several species of barnacle (genus *Balanus*) are abundant along the Oregon Coast. *Balanus crenatus* is a subtidal species found along open coasts and lower regions of estuaries, and can be found in the North Atlantic, northern Japan, and from Alaska to Santa Barbara (Morris et al., 1980). *B. glandula* is a mid to high intertidal barnacle, ranging from the Aleutian Islands (Alaska) to Bahía de San Quintín (Baja California) (Morris et al., 1980). It is found on the open coast and throughout the estuary, occurring even in relatively low salinities. *B. improvisus* is an obligate estuarine species and occurs in the upper Coos Estuary in a zone lower than *B. glandula*. Its range is from the

Northern Atlantic to the Northern Pacific and is believed to have been introduced to the Oregon Coast along with the eastern oyster *Crassostrea virginica* (Morris et al., 1980).

The nauplii and cyprid larvae of these three species have a considerable range of sizes and there are very close morphological resemblances between species at each naupliar stage (Table 1). Throughout stages, *B. improvisus* has the smallest larvae, with stage II averaging 323 μm and cyprids 523 μm in carapace length (Jones and Crisp, 1954). *B. crenatus* and *B. glandula* start with nauplii that are very similar in size, stage II nauplii are both approximately 430μm, but length diverges in later stages (Pyefinch, 1948; Brown and Roughgarden, 1985). *B. glandula* cyprids average 734 μm in length from field caught specimens, while *B. crenatus* is much larger with an 850 μm cyprid.

This study addresses the following questions: 1) What are the feeding rates of nauplii of three species within the genus *Balanus* (*B. crenatus, B. glandula, and B. improvisus*)? 2) Is feeding rate correlated with body size within three species within the genus *Balanus*? 3) How do feeding rates compare between these species within the genus *Balanus* and with other larvae and other nauplii? This investigation is the first to examine the feeding rates of nauplii within this genus and to directly compare feeding rates in several species with nauplius larvae under identical experimental conditions.

Materials and Methods

Larval Culture

Adults of *B. crenatus*, *B. glandula*, and *B. improvisus* with dark brown lamellae were collected from Coos Bay, Oregon, between September, 2002 and August, 2003.

Table I. Published lengths of the naupliar stages of *Balanus crenatus*, *B. glandula*, and *B. improvisus*

Stage	*B. crenatus* (μm)	*B. glandula* (μm)	*B. improvisus* (μm)
I	280	271	195
II	440	426	323
III	570	525	367
IV	730	567	402
V	840	649	496
VI	910	745	624
Source	Pyefinch (1948)	Brown and Roughgarden (1985)	Jones and Crisp (1954)

Adults of *B. crenatus* were collected from floating docks in the boat basin in Charleston, OR. *B. glandula* was collected from pylons of the Charleston Bridge and on rocks found near the mouth of the estuary. *B. improvisus* was collected from wooden pilings in Catching and Shinglehouse Sloughs. Ripe lamellae from individual adults were brought back to the Oregon Institute of Marine Biology and placed in separate beakers filled with 0.5 μm filtered seawater (FSW). When bright light was shone on the lamellae, nauplii began to hatch from egg cases and swam toward the light, where they were collected and placed in graduated cylinders. Nauplii were then counted by anesthetizing six 1mL subsamples with 7.5% MgCl in Bogorov trays. Nauplii were added to glass, culture jars containing 3L FSW to obtain a final concentration of 1 nauplius per 2 mL FSW. Jars were placed in a sea table with running water and stirred at 12 rpm with plexiglass paddles (Strathmann MF, 1987). Offspring from four parents were cultured for each species. Six culture jars were maintained for each parent in order to insure that there would be enough animals for feeding rate measurements and size determinations by the last larval stage. The offspring from these parents were maintained in separate cultures in order to look at differences between cohorts and because one parent from each species was cultured at a different time than others, due to constraints of reproductive seasonality and availability of mature broods.

Cultures were given fresh seawater and the centric diatom *Skeletonema costatum* (1×10^5 cells/mL) every other day (Brown and Roughgarden, 1985; Hentschel and Emlet, 2000). Jars were cleaned by pouring the contents through a 110 μm or 202 μm mesh sieve submerged in FSW. The contents were then rinsed by spraying clean FSW onto the

mesh with a turkey baster in order to break up algal clumps and exuviae, which then passed through the mesh. Nauplii were transferred back into rinsed culture jars filled with the appropriate volume of FSW.

Algae for larval cultures and feeding experiments were grown at 16ºC in an incubator with a 16L:8D cycle. Culture stocks and f/2 medium (Guillard, 1983) were added to autoclaved 2L of 0.45 μm filtered seawater in 2.8L Fernbach flasks equipped with rubber stoppers and bubbler tubes. Six-day-old algal cultures were centrifuged at 3000 rpm for 25 minutes, enumerated with a hemacytometer, and added to culture or feeding rate jars at the appropriate concentrations.

For each larval stage, feeding rates, carapace dimensions, and ash free dry masses were determined for three randomly selected replicate jars from each parent. Three replicate jars were used instead of all six for each parent because of space limitations in feeding rate experiments and because of loss throughout naupliar stages from mortality and sampling. When approximately 100% of the animals molted to the stage of interest, larvae from each jar were concentrated in graduated cylinders, counted, and subsampled for full body length, width and mass determinations. Nauplii and cyprids preserved in 4% seawater buffered formalin were measured with a dissecting microscope equipped with an ocular micrometer. Carapace length was measured from the anterior carapace edge to the tip of the caudal spine, and width was measured at the widest point of the carapace behind the frontolateral horns (Barnes and Barnes, 1959; Brown and Roughgarden, 1985). Ten animals from each of the three replicate jars of each parent and stage were measured in this way.

Ash free dry mass was determined for all naupliar stages and the cyprid stage by drying samples in pre-ashed aluminum pans in a 60˚C oven for 5-10 days until the weight remained unchanged for 3 consecutive days. Samples were weighed to the nearest 0.0001g on an Ainsworth Type 12 analytical balance and then placed in a muffle furnace at 500˚C for 6 hours (Bamstedt et al., 2000). Trays were placed in a dessicator, allowed to cool to room temperature, and weighed again.

One parent of each species was cultured at a separate time than the others. Parent CC of *B. crenatus* was cultured in winter, 2002. Parent NN of *B. glandula* was cultured in early spring, 2003. Parent O of *B. improvisus* was cultured in fall, 2002. All other parents were cultured in summer, 2003. The parent not cultured in summer, 2003, was subjected to the same procedures and sample sizes as all others, except larvae from all culture jars were pooled at each stage before subsampling for length, mass, and feeding rate measurements.

Feeding Rates

Animals were placed in 1 L glass jars to minimize edge effects (O'Brien, 1987) with 900mL FSW and 5.0 x 10^4 cells/mL *S. costatum* for 24 hours. At the beginning of each experiment, a 10 mL subsample of water was removed from control jars without larvae and vacuum filtered onto a 1.2 μm glass fiber filter, which was then placed in 90% acetone for chlorophyll extraction (Parsons et al., 1984). Jars were placed in a 15˚C cold room on a "roller table" (Larson and Shanks, 1996; Johnson, 1998). This mechanism simulates the formation of marine snow (Shanks and Edmundson, 1990), which occurs frequently in near-shore environments, as well as keeps animals and algae in suspension.

The roller table consisted of 3 levels of paired, foam-covered steel shafts connected to a motor that rotated at 1.6 rpm. Each level was equipped with identical fluorescent lights on 12L:12D cycle. Three replicate jars for each parent, with nauplii from three separate culture jars were placed at random on the roller table, one on each level. Each level therefore had three jars with larvae (one from each parent) and one control jar without larvae throughout the incubation.

Incubations lasted 24 hours to give animals time to acclimate to their new surroundings and to account for any circadian feeding patterns (Gauld, 1951). At the end of each experiment, animals were removed from jars by pouring the contents through a 110 μm mesh sieve. Ten mL subsamples from each jar with or without larvae were then filtered onto glass fiber filters. Chlorophyll a was extracted from these samples with 90% acetone for at least 24 hours (Parsons et al., 1984) while samples were stored at -20ºC. The chlorophyll content of these samples was determined with a Turner Model TD 700 fluorometer (Turner Designs, Sunnyvale, CA). Measurements were recorded before and after conversion to phaeopigments by the addition of 5% HCl. Pilot studies were used to calibrate the chlorophyll concentration with the algal cell concentration in experimental jars.

Clearance rate (the volume of water cleared of particles) and ingestion rate (the number of cells consumed) per animal over the 24-hour time interval were calculated from equations in Frost (1972). Analysis of control jars yielded an algal growth constant, *k*, from:

$$C_2 = C_1 e^{k(t_2 - t_1)} \qquad (1)$$

where C_2 and C_1 were cell concentrations in the control jar at times t_1 and t_2. A grazing coefficient *g* for each jar with nauplii was calculated from:

$$C_2^* = C_1^* e^{(k-g)(t_2-t_1)} \quad (2)$$

where C2* and C1* were the cell concentrations of the grazing jar at times t_1 and t_2. The average cell concentration, [C], over the 24-hour time interval was calculated with:

$$[C] = \frac{C_1^* \{ e^{(k-g)(t_2-t_1)} - 1\}}{(t_2-t_1)(k-g)} . \quad (3)$$

The clearance rate per animal over the 24-hour interval, *F*, was then calculated by:

$$F = Vg/N \quad (4)$$

where V was the volume of the jar and N is the number of nauplii in the jar. If animals are assumed to be 100% efficient at removing particles from the water passing through their filtering appendages, then F can also be referred to as the filtering rate. We can then use this to calculate the Ingestion Rate, *I*, by:

$$I = [C]*F. \quad (5)$$

The diameter and height of 30 cells of *S. costatum* were measured with a compound microscope and cell volume calculated based on the equation for a cylinder (Harrison et al., 1977). Clearance and ingestion rates are reported here because most feeding rates reported for larvae are clearance and most feeding rates reported for crustaceans are generally ingestion. Ingestion rates were converted to volume of algae consumed by multiplying the result of equation 5 by the cell volume of *S. costatum* measured above.

Feeding rates of naupliar stages II through VI were determined in this way. Stage I was not tested because it is very short, lasting from minutes to hours and is believed to

be non-feeding (Brown and Roughgarden, 1985). The cyprid larva is also non-feeding and consequently was not tested.

The development of nauplii was monitored by randomly subsampling culture jars, anesthetizing nauplii with 7.5% MgCl, and identifying stages based on morphology and size. When approximately 50% of the nauplii reached the stage of interest, this stage was removed for feeding experiments. This ensured that nauplii would not molt during feeding rate incubations.

Since only one other study on the feeding rates of cirripede nauplii has been done (Harms, 1987), very little is known about the appropriate algal concentrations and naupliar densities. Work with copepods and other crustaceans have shown these variables to have tremendous impacts on feeding rate determinations (for an overview, see Bamstedt et al., 2000). Mayzaud and Poulet (1978) and Hassett and Landry (1983) suggest the use of algal concentrations comparable to those at which animals are reared on since animals have been shown in the past to become acclimated to high food levels and to subsequently cease feeding when given lower algal concentrations. Preliminary experiments with barnacle nauplii also suggested the use of culture algal concentrations because animals appeared to cease feeding when given algal concentrations lower than those in culture jars. Feeding rates jars were, therefore, given half the algal ration for a two day period, 5.0×10^4 cells/mL for the 24 hour incubation.

Further preliminary work with different naupliar densities at experimental food concentrations led to the choice of 25 animals per 1 L jar. Above this density, individual feeding rates decreased in many stages, possibly due to interference. Below this density,

there was high variation in individual feeding rates in smaller stages, perhaps due to the low percentage of algae removed by so few animals and limitations in the sensitivity of the chlorophyll extraction.

Statistical Analysis

Data from feeding rates, carapace length, and ash free dry mass were subjected to two types of statistical tests. Nested ANOVA (with parent nested within species) and post hoc Bonferroni multiple comparisons were used to test for differences among feeding rates of species at selected naupliar stages (Zar, 1984; Systat 9). Species was considered a fixed factor while parent was considered a random factor (Underwood, 1997).

Results

Since ingestion rate is a function of clearance rate, and is reported most often for crustaceans, the statistical analyses of ingestion rate only are presented here. Ingestion rates increased between stages II and VI in *B. glandula* ($P=0.009$) and *B. improvisus* ($P<0.001$, see Table 2B-C and Figure 1). *B. crenatus*, on the other hand, showed no statistically significant increase in ingestion rate with larval growth from stage II to stage VI ($P=0.937$, see Table 2A and Figure 1).

B. glandula had the highest mean feeding rates of all three species, consuming *S. costatum* at almost twice the rate at which it was consumed by *B. crenatus* and *B. improvisus* during stage VI (Figure 2). Separate nested ANOVAs indicated that ingestion rates of both stage V and VI nauplii of all three species were significantly

different (Table 3 and Figure 2). Post hoc Bonferroni tests revealed that the ingestion rates of stage V *B. glandula* were significantly different from those of either *B. crenatus* (P=0.002) or *B. improvisus* (P=0.001). Ingestion rates of stage VI *B. glandula* were also significantly different from those of *B. crenatus* (P=0.001), but not stage VI *B. improvisus* (P=0.093). The mean ingestion rates of stage V *B. crenatus* were not significantly different from stage V *B. improvisus* (P=1.000). Ingestion rates of stage VI *B. crenatus* were also not significantly different from stage VI *B. improvisus* (P=0.337).

In *B. glandula*, there is little change in ingestion rate between stages II-IV, but there is a large increase in ingestion rate between stages IV and V (Figure 2). There is little change in the ingestion rate between stages III-V of *B. improvisus* (Figure 2). There are, however, larger increases in ingestion rate between stages II and III and stages V and VI. *B. crenatus*, on the other hand, shows no discernible differences in rate of increase in ingestion rate between stages, but rather there is a gentle positive slope between stages (Figure 2).

By stage VI, mean carapace lengths of each species were significantly different from one another (Table 3C and Figure 3). Pair-wise comparisons determined that the length of *B. crenatus* nauplii was significantly different from either *B. glandula* or *B. improvisus* (P<0.001 and P<0.001, respectively). The length of *B. glandula* nauplii was also significantly different from *B. improvisus* (P<0.001).

Mean ash free dry mass of stage VI nauplii also differed among species (Table 3D and Figure 4). Ash free dry mass of *B. improvisus* was significantly different from either

B. crenatus or *B. glandula* ($P<0.001$ and $P<0.001$, respectively), but *B. crenatus* and *B. glandula* were not significantly different from one another ($P=0.207$).

Discussion

Many studies have reported that feeding rates are related to body size in a wide variety of zooplankton species (e.g. Egloff and Palmer, 1971; Emmerson, 1980; Epp and Lewis, 1980; Frost, 1972; Gibson and Paffenhofer, 2000; Paffenhofer, 1984; Paffenhofer and Knowles, 1978; Rigler, 1961; Rosas et al., 1995; Sharma and Pant, 1984). Feeding rates often increase either linearly or exponentially with size. This trend is readily explained by metabolic demands imposed on animals: larger animals must consume more food to meet the energy requirements of larger and often more complex body systems (Schmidt-Nielson, 1997). For larvae, feeding rate increases with larger size is particularly important as animals start producing juvenile tissues and gathering energy reserves for metamorphosis.

The ingestion rates of nauplii of *Balanus glandula* and *B. improvisus* increased as the larvae grew between successive stages and this increase was statistically significant by stage VI. The rate of increase, however, was not consistent between stages. Emmerson (1980) reported that *Panaeus indicus* larvae increase their ingestion rates with each subsequent stage, but increases were not scaled equally between stages. Harms (1987) also found that the ingestion rates of *Elminius modestus* increase as nauplii grow, but the magnitude of the increase was highly variable between stages. This may be explained by changes that occur when animals molt, as each stage may have behavioral

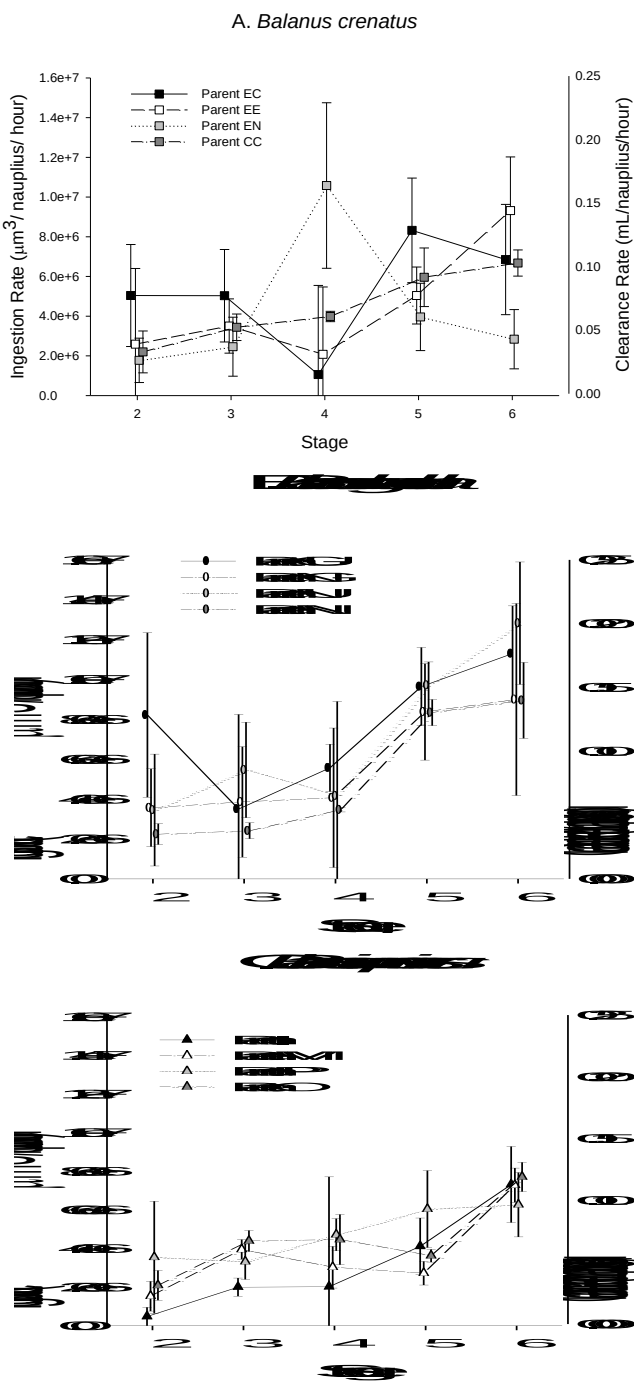


Figure 1. Ingestion rates and clearance rates of larvae from different parents of each of three barnacle species. A. *Balanus crenatus*, B. *Balanus glandula*, and C. *Balanus improvisus* (mean ± SE, n=3). See legends in graphs. Data points have been staggered where error bars overlap.

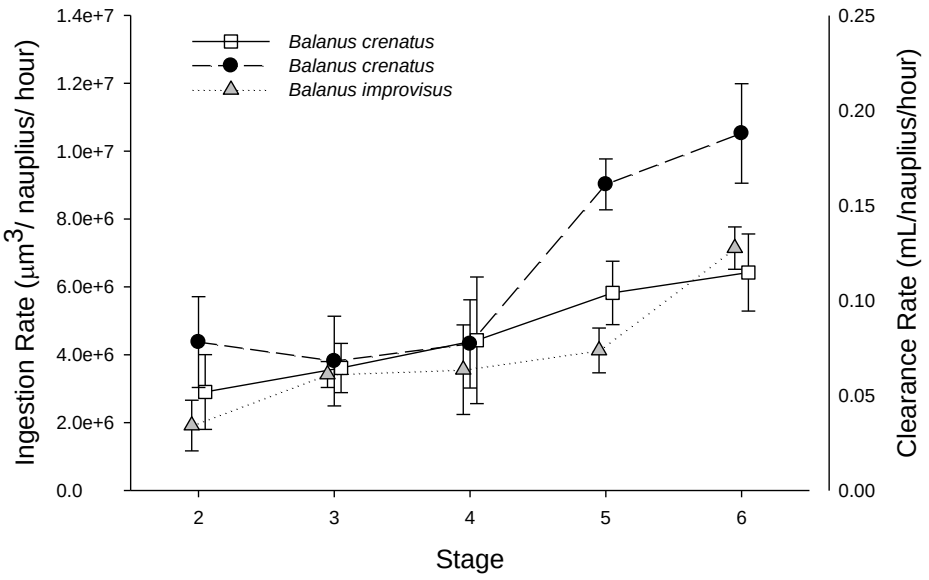


Figure 2. Mean ingestion rates and clearance rates of the naupliar stages of *B. crenatus*, *B. glandula*, and *B. improvisus*. (Mean ± SE, n=4). See legends in graphs. Data points have been staggered where error bars overlap.

Table 2. Results of nested ANOVAs to test for differences in mean ingestion rates of Stage II and Stage VI of A) *Balanus crenatus*, B) *B. glandula*, and C) *B. improvisus*.

A. Mean Ingestion Rates of Stage II and Stage VI *B. crenatus*

Source	Sum-of-squares	df	Mean-square	F-ratio	P
Species	8.74307E+10	1	8.74307E+10	0.007	0.937
Parent(Species)	7.85108E+13	6	1.30851E+13	1.333	0.299
Error	1.57023E+14	16	9.81395E+12		

B. Mean Ingestion Rates of Stage II and Stage VI *B. glandula*

Source	Sum-of-squares	df	Mean-square	F-ratio	P
Species	2.26864E+14	1	2.26864E+14	14.395	0.009
Parent(Species)	9.45448E+13	6	1.57575E+13	0.593	0.732
Error	4.25356E+14	16	2.65848E+13		

C. Mean Ingestion Rates of Stage II and Stage VI *B. improvisus*

Source	Sum-of-squares	df	Mean-square	F-ratio	P
Species	1.64129E+14	1	1.64129E+14	54.455	<0.001
Parent(Species)	1.80812E+13	6	3.01354E+12	0.450	0.835
Error	1.07185E+14	16	6.69906E+12		

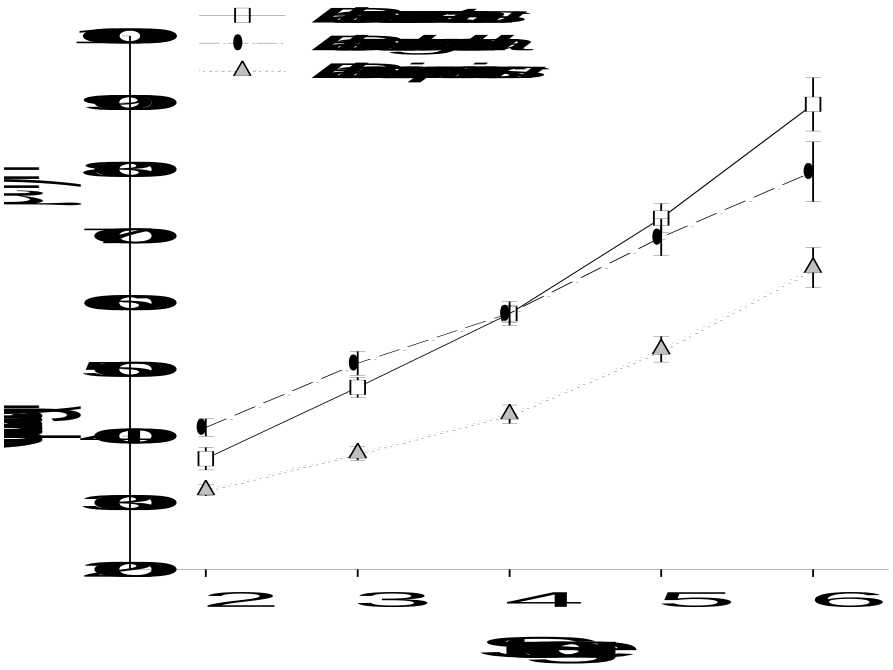


Figure 3. Mean carapace length of nauplii of *B. crenatus*, *B. glandula*, and *B. improvisus* (n=120, ± SD). See legend in graph.

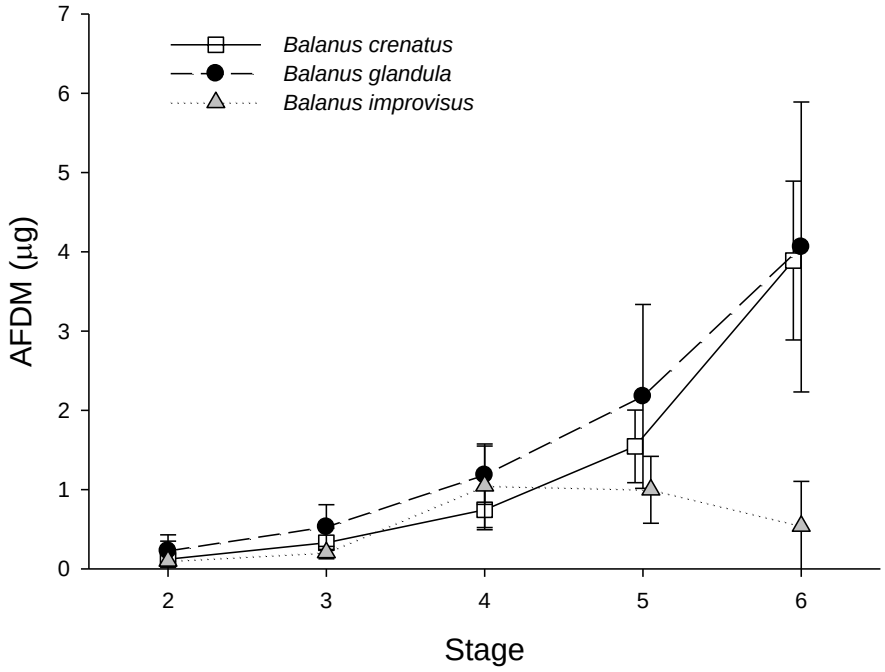


Figure 4. Mean ash free dry mass of nauplii of *B. crenatus*, *B. glandula*, and *B. improvisus* (n=12, ± SD). See legend in graph. Where error bars overlap, data points have been staggered.

Table 3. Results of nested ANOVAs to test for differences A) mean ingestion rates of Stage V, B) mean ingestion rates of Stage VI, C) mean carapace length of Stage VI, and D) mean ash free dry mass of the nauplii of *Balanus crenatus*, *B. glandula*, and *B. improvisus*.

A. Mean Ingestion Rates of Stage V

Source	Sum-of-squares	df	Mean-square	F-ratio	P
Species	1.66639E+14	2	8.33193E+13	20.125	<0.001
Parent(Species)	3.72353E+13	9	4.13726E+12	0.485	0.870
Error	2.04791E+14	24	8.53296E+12		

B. Mean Ingestion Rates of Stage VI

Source	Sum-of-squares	df	Mean-square	F-ratio	P
Species	3.38474E+14	2	1.69237E+14	16.087	0.001
Parent(Species)	9.46482E+13	9	1.05165E+13	0.799	0.620
Error	3.15719E+14	24	1.31549E+13		

C. Mean Carapace Length of Stage VI

Source	Sum-of-squares	df	Mean-square	F-ratio	P
Species	382741.625	2	191370.813	339.527	<0.001
Parent(Species)	5072.752	9	563.639	1.395	0.245
Error	9698.370	24	404.099		

D. Mean Ash Free Dry Masses of Stage VI

Source	Sum-of-squares	df	Mean-square	F-ratio	P
Species	59.893	2	29.946	59.066	<0.001
Parent(Species)	4.563	9	0.507	0.908	0.535
Error	13.397	24	0.558		

or metabolic differences that change their acquisition of food or their nutritional requirements. The appendages responsible for capturing food in barnacle nauplii, the second antennae, increase in area at a faster rate in later stages, suggesting that allometric relationships could also affect feeding rates differently in each stage. Larger antennae could potentially increase feeding rates, just as using a larger utensil would be able to deliver a larger volume of food to the human mouth, thereby leading to the large increases in feeding rates seen at stage V in *B. glandula* and stage VI in *B. improvisus*.

In contrast, larvae of *B. crenatus* did not show a significant increase in ingestion rates with larval growth. The present experiments were conducted at a relatively warm temperature (15ºC) because the temperature used needed to be one at which all three species were active. In initial experiments at lower temperatures, nauplii of *B. improvisus* were somewhat lethargic. Raising the temperature should have translated into higher feeding rates across species, assuming that this temperature was within the species' thermal limits (Schmidt-Nielson, 1997). *B. crenatus* on the Pacific Coast of North America has a more northerly distribution than *B. glandula* and more oceanic distribution than *B. improvisus* (Morris et al., 1980). The average temperatures experienced by nauplii of *B. crenatus* within this range are probably lower than those experienced by nauplii of either *B. glandula* or *B. improvisus* (Lalli and Parsons, 1997). Although *B. crenatus* has multiple broods throughout the year, a reproductive peak seems to occur at colder times of the year in winter and early spring (e.g. Narragansett Bay, RI: Lang and Ackenhusen-Johns, 1981; Millport, UK: Pyefinch, 1948; Coos Bay, OR: personal observation). As a result of distribution and reproductive timing, nauplii of *B.*

crenatus may be less adapted to higher temperatures than the other two species. As a consequence, their metabolic activity and feeding rate may not increase when exposed to temperatures such as those used here. Harms (1987) found that stage VI *Elminius modestus* ingestion rates were lower at 24°C than at 18°C. He attributed this decrease to 24°C being above the optimal temperature range for this species.

The data presented in Figure 5 indicates that *B. glandula* converts the volume of food consumed by each stage into a larger cyprid compared to either *B. crenatus* or *B. improvisus*. For *B. glandula* this means that their final larval stage has a larger reserve of energy to draw upon during its search for an appropriate site for settlement (Lucas et al., 1979). Larger energy reserves could translate into increased survival at settlement and increased chances of recruiting into the adult habitat (Gosselin and Qian, 1996; Highsmith and Emlet, 1986; Jarrett and Pechenik, 1997; Moran, 1997). *B. improvisus* does not increase in mass after stage IV, despite an increase in body length (Figures 3 and 4). This species consumes a large volume of food in later stages (comparable to that consumed by *B. crenatus*), but without the corresponding mass increase. Nauplii of *B. improvisus* may be very inefficient at extracting the nutrients from food needed to build up energy reserves for metamorphosis, using the food consumed only to build the tissues required for each molt and metamorphosis. The lack of energy reserves or low body mass at the cyprid stage may indicate reduced chances of recruiting into the adult habitat, especially compared to *B. crenatus* and *B. glandula*.

Ingestion rates reported for nauplii of *E. modestus* are comparable to those found in the current study (Harms, 1987). At 18°C, stage VI *E. modestus* larvae consumed

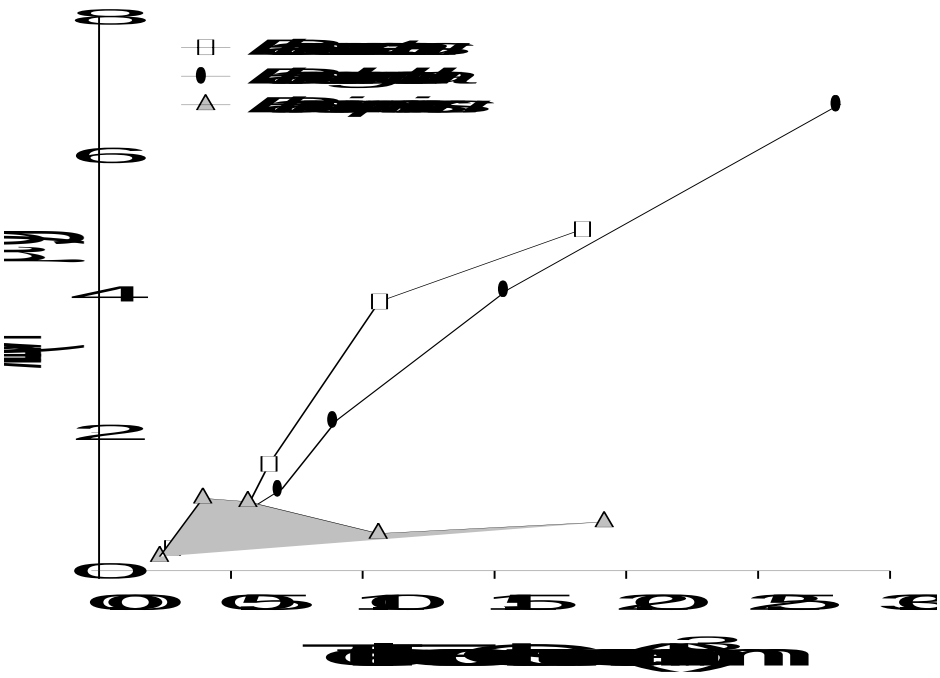


Figure 5. Estimated total volume of food consumed by each of three species over the duration of each naupliar stage relative to cumulative mass estimated by ash free dry mass measurements for the next stage (n=4). Food volume was estimated from the ingestion rate of each naupliar stage and the duration of that stage. Data points therefore represent AFDM of nauplius stage III through cyprid. See legend in graph.

Skeletonema costatum at a rate approximately equal to that of stage VI *B. crenatus* and *B. improvisus*, but lower than the rate of *B. glandula*. *E. modestus, B. crenatus*, and *B. improvisus* are all found in Northern Europe as well as other oceans, while *B. glandula* is restricted to the Pacific coast of North America. Perhaps the evolutionary pressures on *B. glandula* on this coast have selected for higher feeding rates, whereas higher feeding rates are not necessary for the other three species in all locations along their range.

On the whole, clearance rates of nauplii in the three *Balanus* species tested overlap with published values for other filter-feeding larvae (Strathmann RR, 1987), but they are distinctive (Figure 6). The data points for barnacle larvae form a line with a higher y-intercept but a lower slope than data points for all the other taxa (Figure 6). At smaller masses, clearance rates of barnacle nauplii exceed those of veligers of similar mass (Figure 6). But at greater body masses, clearance rates of barnacles are substantially lower than those of *Calanus pacificus* nauplii of similar mass. The aim of this study was not to maximize clearance rates for barnacle nauplii, but rather to maximize ingestion rates by using a high concentration of algae (Strathmann RR, 1987) and so clearance rates measured for these barnacles may actually be underestimated. If clearance rates are not underestimated, these data suggest that these three species of barnacle nauplii have a different relationship between body mass and clearance rate than do other larvae.

Most published data for feeding rates of nauplii, and crustaceans in general, are ingestion rates rather than clearance rates, and so comparisons across studies for nauplii are easier using ingestion rates. Figure 7 indicates that ingestion rates of barnacle nauplii

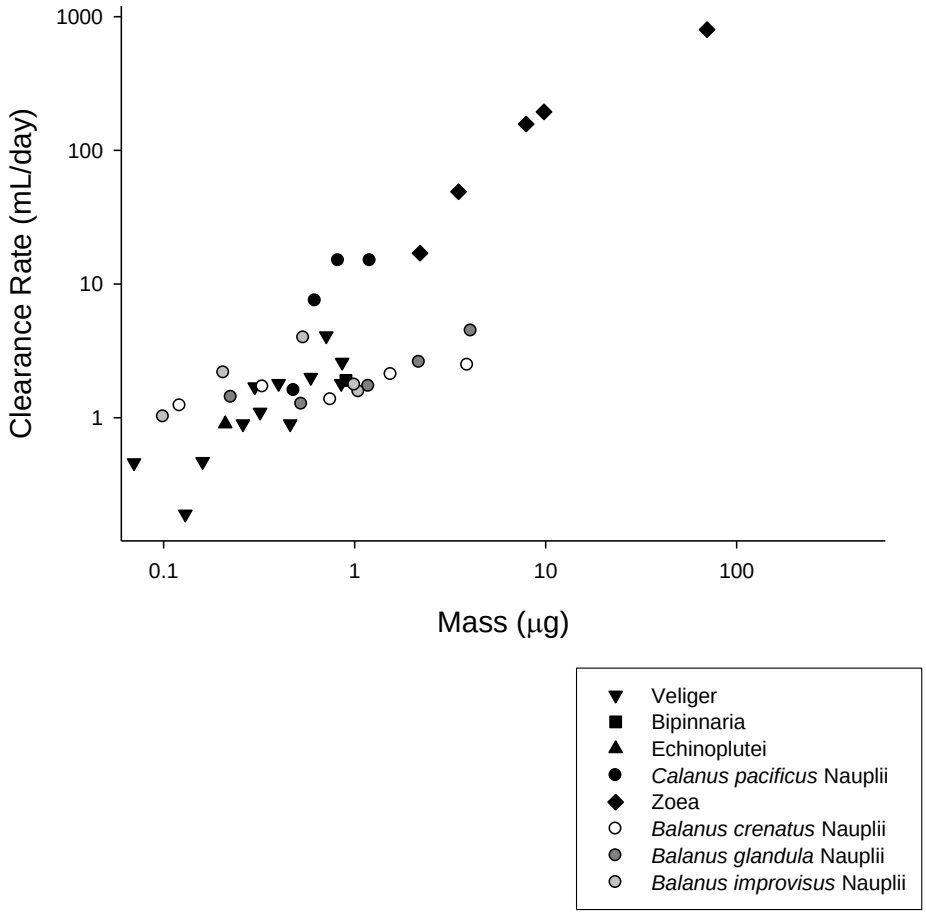


Figure 6. Comparison of published values for clearance rates of larvae relative to mass and clearance rates measured in this study. Some masses were converted from whole dry weight or carbon. See Strathmann RR (1987) for details.

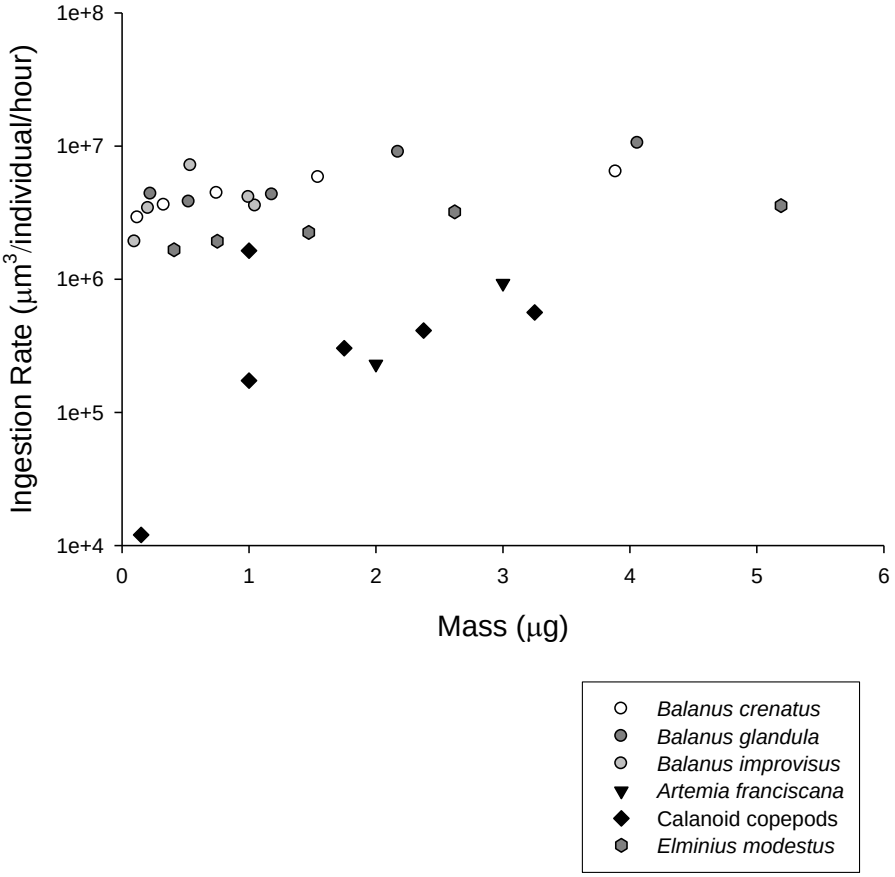


Figure 7. Comparison of published ingestion rates of nauplii relative to mass. Some ingestion rates and masses were converted from carbon. Ingestion rates are reported on a log-scale. See Table 4 for details.

Table 4. Ingestion rates of taxa possessing nauplius larvae. The different measures of ingestion were adjusted to approximate cell volume (μm^3). The different measures of mass were adjusted to approximate mass of organic material.

Species	Stage	Mass (μg)	Clearance Rate (mL/hr)	Ingestion Rate (μm^3/ind./hr)	Food Conc. (cells/mL)
Calanus pacificus	N3	0.48	0.067	1.73 e5	
	N4	0.62	0.313	3.03 e5	
	N5	0.82	0.625	4.11 e5	
	N6	1.2	0.625	5.63 e5	
Eucalanus pileatus	late N	1.0		4.3 e5	1.0 e4
Paracalanus spp.	late N	0.38		1.2 e4	
Artemia fransiscana	metanauplius	2.0		2.3 e5	2.0 e6
	metanauplius	3.0		9.3 e5	
Elminius modestus	N2	0.41		1.66 e6	1.0 e5
	N3	0.75		1.93 e6	
	N4	1.47		2.25 e6	
	N5	2.62		3.20 e6	
	N6	5.19		3.57 e6	
Balanus crenatus	N2	0.12	0.051	2.90 e6	5.0 e4
	N3	0.33	0.071	3.61 e6	
	N4	0.75	0.057	4.43 e6	
	N5	1.54	0.088	5.82 e6	
	N6	3.89	0.103	6.42 e6	
Balanus glandula	N2	0.23	0.059	4.37 e6	5.0 e4
	N3	0.53	0.053	3.81 e6	
	N4	1.18	0.072	4.32 e6	
	N5	2.17	0.108	9.02 e6	
	N6	4.06	0.186	1.05 e7	
Balanus improvisus	N2	0.10	0.042	1.91 e6	5.0 e4
	N3	0.21	0.090	3.42 e6	
	N4	1.05	0.065	3.56 e6	
	N5	1.00	0.073	4.13 e6	
	N6	0.54	0.156	7.14 e6	

Sources: Fernandez, 1979; Paffenhofer and Knowles (1978); Paffenhofer (1984); Evjemo and Olsen (1999); Evjemo et al. (2000); Harms (1987).

are much higher than rates of either calanoid copepod nauplii or nauplii of *Artemia franciscana*. Experiments with copepod nauplii used low concentrations of algae, thus the maximum ingestion rates of these species may not have been reported, which could explain why barnacle ingestion rates are so much higher. For example, Paffenhofer and Knowles (1978) used a mixture of foods at a concentration of 1.0×10^4 cells/mL for the ingestion rates of *Paracalanus* spp. For *Balanus* and *E. modestus* nauplii, however, the high concentration of algae probably means that reported ingestion rates are the maximum possible for these species (Strathmann RR, 1987), making the barnacle nauplii appear to ingest food at a very high rate compared to the other nauplii. Another possibility for the relatively lower ingestion rates of other nauplii compared to those of barnacle nauplii is the density of animals used in experimental chambers. Studies have shown that individual feeding rates decrease when animals are kept at high densities, probably due to interference in feeding currents or movements (see Bamstedt et al., 2000, for an overview). Evjemo and Olsen (1999) measured ingestion rates with a density of up to 6.3 animals/mL. Paffenhofer and Knowles (1978) measured ingestion rates with a density of up to 1.5 animals/mL. The current study maintained animals for ingestion rate measurements at less than 0.02 animals/mL, a much lower density than that used for copepods and anostracans. Ingestion rates of copepod and anostracan nauplii may therefore be underestimated, which would account for the large differences in feeding rates between these nauplii and those of the three *Balanus* species and *E. modestus*.

Data for ingestion rates of nauplii is also relatively patchy since rates for all naupliar stages were measured in only one other species, *Calanus pacificus*, that was not

a barnacle (Fernandez, 1979). Ingestion rates of *Eucalanus pileatus*, *Paracalanus* spp., and *Artemia franciscana* were not reported for all naupliar stages, and clearance rates were not reported for any naupliar stages in these species (Evjemo and Olsen, 1999; Evjemo et al., 2000; Paffenhofer, 1984; Paffenhofer and Knowles, 1978). Because of this, we cannot say for sure if the pattern of taxon-specific ingestion rates is real or if it is a function of the paucity of data.

The species of barnacle presented here seem to increase ingestion rate with body mass in a similar manner. These species of barnacle appear to require a large volume of food in order to obtain similar body masses as copepod and anostracan nauplii. This pattern may indicate behavioral differences for these species of nauplii that increase nutritional requirements or lower efficiency at extracting nutrients from a given volume of food. For instance, nauplii of *B. improvisus* consume a large amount of food without any increase in mass after stage IV (Figure 5). Perhaps swimming behavior in these species uses a large amount of energy compared to the type of swimming employed by other nauplii. Or digestive enzymes in these species have a lower activity level than other nauplii. Another possibility is an artifact caused by the food given to barnacle nauplii. All ingestion rates for *Balanus* spp. and *E. modestus* were measured using *S. costatum*, whereas the other studies used species like *Thalassiosira weisflogii* and *Isochrysis galbana*. Perhaps there are physical differences (chain-formation, frustrule texture, or taste) between these foods that either make affect a nauplius' ability to capture them or the desirability of the food. Overall, though, results indicate that feeding rates within the four barnacle species are very similar and that there may be mechanical,

behavioral, or physiological differences between these species and other taxa that impose constraints on feeding rates and nutritional requirements.

BRIDGE I

As demonstrated in Chapter II, the feeding rates of nauplii of *B. crenatus*, *B. glandula*, and *B. improvisus* appear to be distinctive when compared to other taxa. This may be caused by behavioral, physiological, or morphological differences between these temperate cirripede species and the other larvae for which feeding rates have been reported. One caveat for these comparisons, though, is that the foods upon which feeding rates were measured were not held constant between experiments. Larvae may prefer the taste of some foods over others, thus reducing or raising feeding rates. But perhaps more importantly, different species and larval types may be better equipped to capture some foods over others based on their feeding structure and size, which would then also affect feeding rates.

It can be hypothesized that the type and size of food upon which measurements are made may affect feeding rates within a species as well as between taxa, especially when larvae are of different sizes. Chapter III, therefore, addresses the effects of food size on the feeding rates of *B. crenatus*, *B. glandula*, and *B. improvisus* by measuring rates on centric diatoms of different individual cell sizes.

CHAPTER III

FOOD SIZE AND RATES OF CONSUMPTION BY THE NAUPLII OF THREE BARNACLE SPECIES (*Balanus crenatus*, *Balanus glandula*, AND *Balanus improvisus*)

Introduction

Since the late 1960's, zooplankton feeding rates have been used to assess grazing impacts on standing stocks of algae. Feeding rates have also become extremely useful for testing hypotheses on processes that shape zooplankton community structure, such as resource partitioning, ecological niches, and resource competition (Tokeshi, 1999). In many cases, investigators have found a strong correlation between the body size of the animal and the size of the food that it consumes at the highest rate. These include panaeid shrimp, calanoid copepods, and cladocerans (Rosas et al., 1995; Paffenhofer and Knowles, 1978; Burns, 1968). Some researchers believe that the correlation between body size and the size of the food consumed allows for the coexistence of many zooplankton species by partitioning available food resources along this size gradient (Lynch, 1977; Tilman, 1981).

Barnacle nauplii of different species are abundant members of zooplankton communities that are generally dominated by holoplanktonic organisms (Blanner, 1982;

Korn, 1999). The abundant grazers in these communities, such as copepods and their nauplii (Sprung, 1994), may drive competition for food resources between holoplanktonic species and meroplanktonic larvae (for review, see Strathmann, 1996). Larvae are therefore likely to be subject to the consequences of competition, such as resource partitioning based on size. Several *Balanus* species that occur on the Northeastern Pacific Coast have overlapping reproductive seasons and the nauplii of these species have relatively different body sizes (Barnes and Barnes, 1959; Brown and Roughgarden, 1985; Jones and Crisp, 1954; Pyefinch, 1948). Edible particles (diatoms, flagellates, etc.) of different sizes are available to these larvae within their reproductive seasons (Hughes, 1997). It is therefore possible that partitioning of food particles by size may take place between co-occurring balanid species.

Although the feeding rates of barnacle nauplii have not been reported to date, there is some evidence to support that planktotrophic cirripede larvae can be expected to exhibit a correlation between body size and particle size consumed at the highest rate. Cultured nauplii from temperate regions are known to grow particularly well when fed centric diatoms, while those from tropical regions develop better when fed flagellates (Moyse, 1963; Stone, 1989). Within the temperate species, those with larger nauplii also developed better in culture and experienced lower mortality when given larger centric diatoms (Stone, 1988; 1989). The relationship between the size of the particle captured and the size of the nauplius is probably due to a correlation between the size of the nauplius and the setular spacing on feeding appendages (the second antennae) (Stone, 1989).

Filter feeders are predisposed to capturing some particles at higher rates than others because of the morphology of their feeding appendages (Koehl, 1993). The filtering appendages in most planktotrophic organisms are thought to function as sieves that retain particles larger than the openings through which water passes on those appendages (i.e. setular spaces). Because the body size of cirripede nauplii is positively correlated with the size of the openings in their filtering appendages (Stone, 1989), large nauplii (either later stages or larger species) should capture and consume large particles at a higher rate than small particles. Conversely, small nauplii should capture and consume small particles at a higher rate than large particles. Particle size is important for determining its value as food due to the costs of capture and digestion (Stone, 1989), and so, the particle that a nauplius captures at the highest rate should also be the one that proves to be the best for growth in culture.

At least four species in the genus *Balanus* (Crustacea: Cirripedia) can be found in close proximity to one another within the Coos Bay Estuary. Measurements done with cultured and field-collected specimens show that the larvae of *Balanus crenatus* are considerably larger than *B. glandula*, which are considerably larger than *B. improvisus* (Brown and Roughgarden, 1985; Jones and Crisp, 1954; Pyefinch, 1948; see Table 1). The range of the larval size in these three species permits their use in experiments to address whether the size of the food consumed at the highest rate is correlated to the size of a cirripede nauplius. These three species also have overlapping reproductive seasons (Puls, 2002; personal observation), thus increasing the possibility that they will display

resource partitioning in order to decrease the effects of competition (e.g. decreased growth rate or survivorship).

Specifically, I predict that the nauplii of *B. crenatus* will develop faster and obtain a larger size when given large food particles and would consume these large particles at a higher rate than would the smaller barnacle species or naupliar stages. I also predict that *B. crenatus* will perform better on and consume the large food at a higher rate than they will consume a smaller food. In keeping with this, *B. glandula* should consume an intermediate sized particle at the highest rate, and also develop best on this food. *B. improvisus* should consume the smallest particle at the highest rate and grow best on that same food.

Materials and Methods

Ripe lamellae of the three balanid species were collected from Coos Bay, Oregon, (Smart, Chapter 1 of this thesis). Adult *B. improvisus* were collected from wood pilings in Catching Slough in September, 2002. Adult *B. crenatus* were collected from mussels attached to the Charleston Docks in November, 2002. Adult *B. glandula* were taken from the Charleston Bridge pilings in February, 2003. Nauplii from a single parent of each of the species were raised in monoculture on at least two centric diatoms. Once nauplii hatched, they were concentrated and placed at a density of 1 nauplius per 2 mL into culture jars containing filtered seawater (FSW). Six jars, beginning with 3 liters of filtered seawater, were raised for each food treatment to ensure that there would be enough late stage nauplii for all measurements.

Nauplii of temperate species have been shown to grow well in culture on centric diatoms (Moyse, 1963; Stone, 1989). Many species of different cell size, including *Chaetocerous, Skeletonema*, and *Thalassiosira* species, are abundant in the coastal waters of Oregon (Cupp, 1943; Hasle and Syvertsen, 1996), thereby making them available to the nauplii of *B. crenatus*, *B. glandula*, and *B. improvisus*. Six jars from each parent and each of the three species were fed *Skeletonema costatum*. The six additional jars of *B. crenatus* were fed *Thalassiosira weisflogii*, while *Chaetocerous gracile* was fed to the six additional jars of *B. improvisus*. An additional six jars of *B. glandula* were fed *T. weisflogii* and six more jars were fed *C. gracile* because of this species' intermediate size. For a summary, see Table 5.

C. gracile is a small chain forming diatom in the plankton, but occured as single cells in culture (personal observation). Frustrules are equipped with four spines, two per theca. Each spine is commonly longer than the diameter of the cell (5.1μm). The intermediately sized *S. costatum* (diameter 11.7μm) is considered cosmopolitan and forms chains of two to twelve cells on average in the field and in culture (Hasle and Syvertsen, 1996; personal observation). *T. weisflogii* (cell diameter 13.0μm) also forms chains in the plankton, consisting of several large cells connected by a thin filament. In culture, however, chains did not form (personal observation).

Each diatom was grown in F/2 medium (Guillard, 1983) in a 15°C incubator on a 18:6 hour light:dark cycle. Every other day, cultured nauplii were fed six-day old diatom cultures. The same six-day old cultures were used in feeding rate experiments, cell volume determinations, and calibrations of chlorophyll/cell concentration. Cell volumes

Table 5. Summary of Experimental Design.

Species	Food	Number of Culture Jars
Balanus crenatus	*Thalassiosira weisflogii*	6
	Skeletonema costatum	6
Balanus glandula	*Thalassiosira weisflogii*	6
	Skeletonema costatum	6
	Chaetocerous gracile	6
Balanus improvisus	*Skeletonema costatum*	6
	Chaetocerous gracile	6

of each species were determined with a compound microscope. The diameter and height of thirty frustrules from each species were measured on three separate occasions. The cell volume for *C. gracile* was estimated by considering cells to approximate ellipsoids (Harrison et al., 1977). Volumes for cells of *S. costatum* and *T. weisflogii* were both calculated by assuming they were short cylinders. Larvae were fed equivalent cell volumes in cultures and in feeding rate studies.

Cultures were cleaned every other day by pouring the contents of each jar through a 100 or 202 μm mesh immersed in FSW, which allowed the diatoms to pass through but retained the nauplii. Nauplii were then transferred back into the jars filled with fresh FSW to which the correct volume of food was added (4.1×10^7 μm^3/mL FSW). Jars were kept in a table with running seawater and each was stirred with a plexiglass paddle at 12 rpm (Strathmann, 1987).

The clearance and ingestion rates were determined for naupliar stages II through VI of the three balanid species on the different sized foods. Because feeding rates may be strongly affected by preconditioning (Paffenhofer, 1984), the ingestion rates were measured only with the same food that nauplii were given in culture jars and at a comparable algal concentration. Feeding rates on alternate foods were measured by following protocols in Frost (1972) (see Chapter II of this thesis).

When all nauplii in culture jars molted into the next stage, jars were pooled and then subsampled for feeding rates, carapace dimensions, and ash free dry masses. Seventy-five nauplii from each food treatment were moved into three replicate 1 L jars for feeding rate measurements. Thirty nauplii from each food treatment were preserved

in 4% seawater buffered formalin for carapace measurements. Three samples of between 500 and 100 nauplii from each food treatment (depending on stage) were frozen for ash free dry mass. Afterwards, nauplii were redistributed into culture jars at the same density as before (2 per mL). Carapace dimensions (full length and width) of each stage for each food treatment were measured with a dissecting microscope. For ash free dry mass samples, animals were placed in pre-weighed aluminum pans (n=3 for each species, stage, and food treatment), dried for 5-10 days at 60°C, and then ashed at 500°C for 6 hours and weighed again (Bamstedt et al., 2000). Ingestion rate, carapace length, and ash free dry mass data were subjected to two sample t-tests when appropriate.

Results

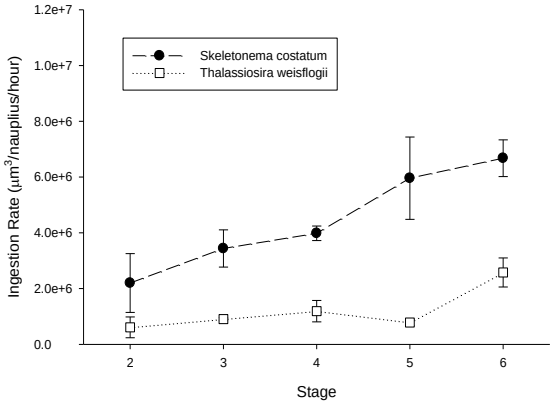
Figure 8 shows the mean ingestion rates for each of the three species and all food treatments. All three *Balanus* species consumed the diatom *Skeletonema costatum* at higher rates than other algal species.

B. crenatus consumed 3.6 times more *S. costatum* per hour than *T. weisflogii* in Stage II and 2.6 times more in Stage VI (Figure 8A), which is statistically significant ($t=4.886$, $df=4$, $P=0.009$). Carapace length and ash free dry mass of *B. crenatus* were also larger in the final nauplius stage on *S. costatum*, and differences were significant ($t=9.398$, $df=51$, $P=0.000$ and $t=4.174$, $df=7.3$, $P=0.004$, respectively). Stage VI nauplii averaged 904 μm in length and 4.0 μg on *S. costatum*, but only 795 μm and 1.5 μg on *T. weisflogii* (Figures 9A and 10A). Nauplii also grew faster on *S. costatum*, reaching the cyprid stage 3 days before those nauplii fed *T. weisflogii* (Table 6).

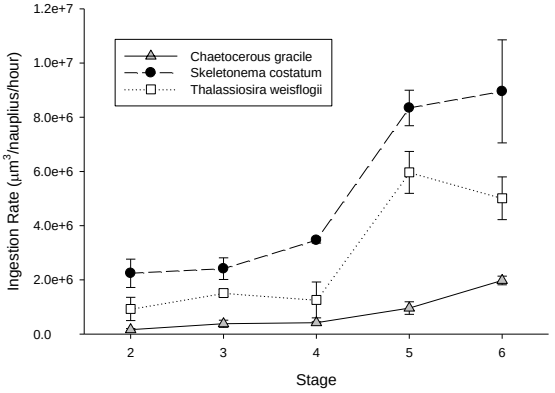
Nauplius stage II of *B. glandula* consumed 2.4 times more *S. costatum* per hour than *T. weisflogii* and 14 times more *C. gracile* (Figure 8B). Stage VI *B. glandula* consumed 1.8 times more *S. costatum* than *T. weisflogii* per hour and 4.5 times more *C. gracile*. Ingestion rates, nonetheless, were not statistically different. Growth was also higher on *S. costatum* than on *T. weisflogii* with carapace length reaching 810 μm versus 788 μm at stage VI, respectively, but differences were not significant (Figure 9B). However, stage VI nauplii fed *S. costatum* were significantly larger than those fed *C. gracile* (t=9.423, df=51, P=0.000) with an average length of 714 μm (Figure 9B). The ash free dry masses of stage VI *B. glandula* were just slightly higher when fed *S. costatum* than *T. weisflogii* and *C. gracile*, 5.2, 4.2, and 4.5 μg, respectively, but these differences were not significant (Figure 10B). Nauplii fed *S. costatum* also grew faster than those fed *T. weisflogii*, reaching the cyprid stage 6 days earlier (Table 6). However, nauplii fed *C. gracile* had identical growth rates as those fed *S. costatum*.

Stage II *B. improvisus* consumed *S. costatum* at a significantly higher rate than *C. gracile*, (Figure 8C, t=2.673, df =4, P=0.049). Stage VI *B. improvisus* also consumed *S. costatum* at a significantly faster rate (t=9.317, df=4, P=0.01). Stage VI *B. improvisus* reached a higher carapace length when fed *C. gracile*, 610 μm versus 535 μm with *S. costatum*, although this difference was not significant (Figure 9C). The ash free dry mass at Stage VI was higher on *S. costatum* than *C. gracile* (Figure 10C), but this difference was not statistically significant either. Nauplii fed *C. gracile* also developed into cyprids one day faster than nauplii fed *S. costatum* (Table 6).

A. *Balanus crenatus*



B. *Balanus glandula*



C. *Balanus improvisus*

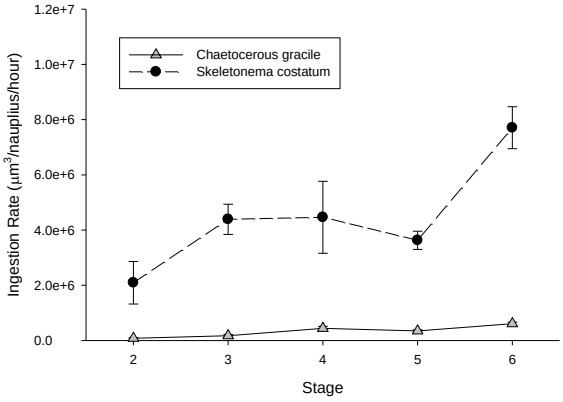
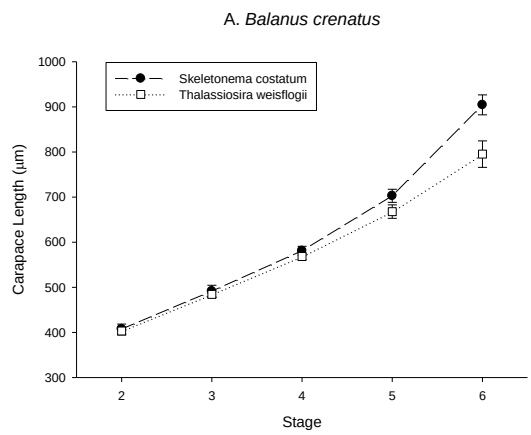
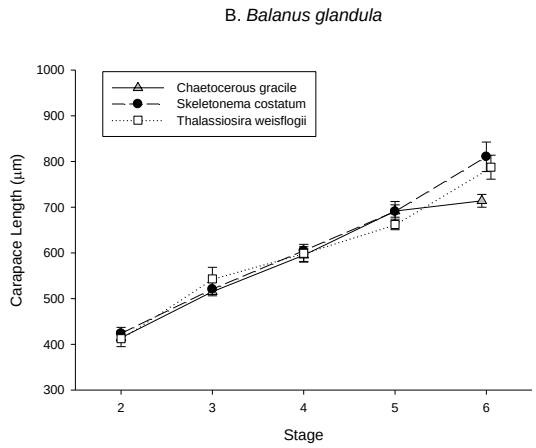
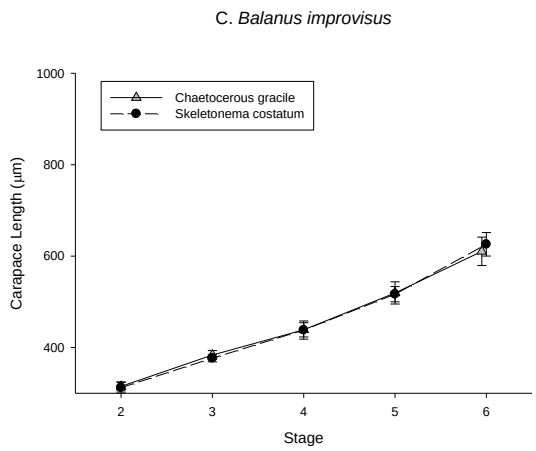


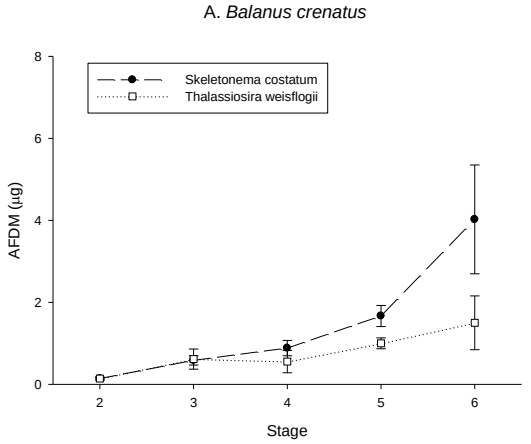
Figure 8 A-C. Volume corrected ingestion rates after 24 hours with 25 nauplii per replicate jar. (n=3, Mean ± 1 Standard Error).

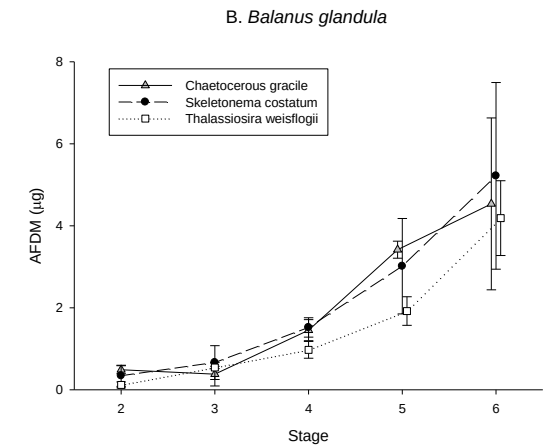


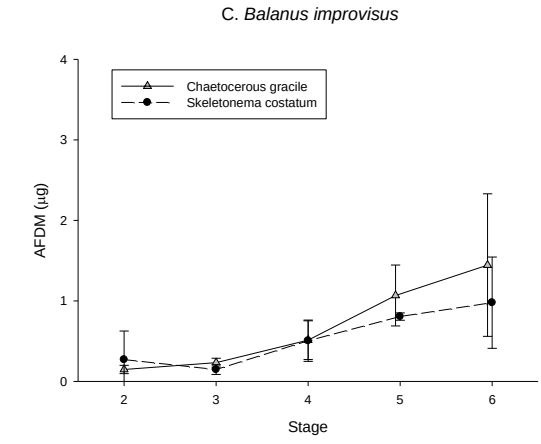




Figures 9 A-C. Total carapace length of cultured nauplii, measured from mid-way between the frontolateral horns to the tip of the caudal spine. (n=30, Mean ± 1 Standard Deviation). Where error bars overlap in Stage VI, data points have been staggered.







Figures 10 A-C. Ash-free dry mass of cultured nauplii. (n=3, Mean ± 1 Standard Deviation). Where error bars overlap in Stages V and VI, data points have been staggered.

Table 6. Development Time of Cultured Nauplii. Numbers represent days when an estimated 100% of nauplii had molted into each subsequent stage.

Stage	*Balanus crenatus*		*Balanus glandula*			*Balanus improvisus*	
	Skele.	Thal.	Chaet.	Skele.	Thal.	Chaet.	Skele.
II	0	0	0	0	0	0	0
III	4	4	4	4	4	5	5
IV	6	7	7	7	7	7	7
V	8	9	9	9	10	10	9
VI	11	14	12	12	16	12	14
Cyp	16	19	17	17	23	18	19

Altogether, the three species failed to demonstrate the predicted patterns in their consumption rates and growth. *B. glandula* followed the predicted pattern in its overall behavior, but probably not for the reasons outlined above.

Discussion

The rates at which filter feeders can capture particles are based on morphology and behavior (Koehl, 1993). Because of this, they should be suited to capturing some particles at higher rates than others, which is often positively correlated to body size (Rosas et al., 1995; Paffenhofer and Knowles, 1978). Despite previous work that suggested otherwise (Moyse, 1963; Stone, 1989), barnacle nauplii do not follow such a clear-cut pattern. The largest species (*B. crenatus*) and largest stage (VI) of all species did not consume the food with the largest cell size at the highest rate. Instead, each of the naupliar stages of *B. crenatus*, *B. glandula*, and *B. improvisus* consumed the diatom with the intermediate cell size, *S. costatum*, at a relatively higher rate than either of the other two diatoms offered. The intermediate-sized species, *B. glandula*, did consume the intermediate-sized food at the highest rate, as predicted. However, I do not believe this supports the original hypothesis. An alternative and more likely explanation is that *S. costatum* is consumed at the highest rate by *B. glandula* because all three species tested, regardless of stage, captured this food at the highest rate.

The discrepancy between these results and evidence for size selectivity in the literature of other species may be attributable to the different filtering mechanisms used by barnacles. Perhaps these barnacle species are simply not capable of efficiently

capturing particles larger or smaller than *S. costatum*. However, nauplii on the Oregon coast see variation in the size of particles available to them throughout their reproductive seasons, which corresponds to the range offered here (Hughes, 1997). In the Coos Bay Estuary, for instance, chlorophyll-dominant eukaryotes less than 3 μm diameter are most abundant in the fall and winter, while chlorophyll-dominant eukaryotes greater than 3 μm diameter are most abundant in the summer (Hughes, 1997). Although cultures tended toward smaller sizes when fed *C. gracile* or *T. weisflogii* and developed more slowly when fed *T. weisflogii* (Figures 9 and 10, Table 6), nauplii still reached the final larval stage, the cypris. *T. weisflogii* and *C. gracile* are therefore probably within of the range of particles that these species can and will ingest.

The nutritional value of a food has been shown to substantially affect an animal's growth rate and survivorship (Emmerson, 1980; Pedrotti and Fenaux, 1993). In a comparison of organic content corrected for cell volume, *T. weisflogii* had the lowest, *S. costatum* intermediate, and *C. gracile* the highest carbon content (Harrison et al., 1977). Thus, *T. weisflogii* may be a low quality food for nauplii. The low organic content could account for the diminished ingestion rates in monoculture experiments because a longer residence time may be required for the absorption of nutrients from a given volume of *T. weisflogii* cells. Low carbon content and low ingestion rates of *T. weisflogii* may also explain why both *B. crenatus* and *B. glandula* were smaller in size and mass when fed *T. weisflogii* than those fed *S. costatum* (Figures 9 and 10). By stage V, cultures fed *T. weisflogii* of both species were also a day behind in their development compared to those fed *S. costatum*, and this lag-time only increased in subsequent stages (Table 6).

Although the organic content of *C. gracile* suggests that it is a high quality food (Harrison et al., 1977), it was consumed at very low rates, even by *B. improvisus* and *B. glandula*. The presence of extracellular spines increased the cell diameter of *C. gracile* more than three-fold and could have contributed to low feeding rates by deterring animals from ingesting these cells. However, *B. improvisus* fed *C. gracile* tended to obtain higher masses than those fed *S. costatum* (Figure 10C) and carapace length was nearly identical on both foods (Figure 9C), suggesting that *C. gracile* was adequate nutrition and ingested at a high enough rate to allow growth. Hughes (1997) found that Coos Bay experienced relatively high abundances of phytoplankton in the 5 μm diameter range (the same diameter as *C. gracile*) during the summers of 1995 and 1996, corresponding to the reproductive season of *B. improvisus*. Because of this, it is possible that the Coos Bay population of *B. improvisus* nauplii are adept at capturing particles of this size and extracting nutrients from them. Coupled with the fact that *C. gracile* is thought to have a high organic content, nauplii may not require large amounts of this food in order to extract enough nutrients for survival and growth.

On the other hand, the morphology of *S. costatum* could very well be responsible for the considerably higher feeding rates demonstrated by all three species. In healthy cultures, *S. costatum* predominantly forms chains, generally ranging from 2 to 12 cells (Hasle and Syvertsen, 1996; personal observation). Breakage of longer chains during feeding can cause overestimates of feeding rates, the magnitude of which depends on the distribution of chain size as well as the animal's behavior (Deason, 1980). Clearance and ingestion rate determinations, such as those measured here, are based on the apparent

change in algal concentration in grazing chambers, assuming that the difference is due to the ingestion of all particles by grazers rather than cell damage caused by grazers (Frost, 1972; Saiz, 1993). Nauplii may have appeared to consume *S. costatum* at a higher rate than *C. gracile* or *T. weisflogii* because they broke some cells within the chain while ingesting others. The contents of broken cells would not contribute to chlorophyll samples and would appear to have been ingested.

High-speed films of particle capture in calanoid copepods have demonstrated that larger particles are detected by filter feeders from much farther away than smaller particles (Price et al., 1983). Large cells appear to be swept into the filter, handled, and retained more easily than small cells, thereby making them preferable over small particles. In fact, feeding rates gradually increase in *Calanus pacificus* as cell size increases (Frost, 1977). Chain formation essentially turns many small particles into few large ones. Subsequently, chain formation in diatoms might increase a nauplius' ability to detect, capture, and ingest these foods as well as reduce the cost of foraging. The "effective cell size" of *S. costatum* was therefore much larger than originally assumed, possibly easing detection and capture by barnacle nauplii, thus leading to higher ingestion rates of this food in all species and stages. Direct observation of particle capture when nauplii are offered single cells of various sizes and chains is required to evaluate this possibility.

If we consider the digestive tract of a nauplius to approximate a right circular cone and we know the digestive rate, we can estimate the volume of food that can be consumed by the nauplius in a given time. Cirripede nauplii seem to release fecal pellets

at a fairly high rate after removal from culture jars, with pellets appearing within five minutes of removal (personal observation). In the most extreme example, fecal pellets can be assumed to consist of 100% of the contents of the gut. Nauplii could then process 12 times the volume of their gut every hour. Stage VI nauplii of *B. crenatus* would process 3.8×10^7 μm^3 of algae in one hour, *B. glandula* would process 1.9×10^7 μm^3, and *B. improvisus* 2.6×10^7 μm^3. Consequently, stage VI nauplii could easily process the volume of *S. costatum* indicated by the ingestion rates presented here (Figure 8A-C). On the other hand, the digestive tracts stage II nauplii, as estimated above, are not large enough to process the volumes of algae suggested by the ingestion rates. Stage II of *B. crenatus* can only process 2.3×10^6 μm^3 of algae every hour, while stage II *B. glandula* and *B. improvisus* can only process 2.0×10^6 μm^3 and 9.4×10^5 μm^3, respectively. Perhaps smaller nauplii only ingest individual cells of *S. costatum* by breaking chains, while larger nauplii can assimilate entire chains. Admittedly, these gut capacity estimates may very well be overestimates because I assumed that the gut approximates a cone, the fecal pellet production rate is typical and not brought on by stress, and that fecal pellets contain the entire gut content, not just that of the hindgut. Violation of any of these assumptions probably means that any nauplius stage could not consume *S. costatum* at the rates measured here and these rates are likely to be inflated due to chain-formation and the mechanics of feeding.

In contrast to predictions, the largest species in this study, *B. crenatus*, did not consume the particle with the largest individual cell volume, *T. weisflogii*, faster than it consumed a smaller particle. The smallest species, *B. improvisus*, did not consume the

particle with the smallest individual cell volume, *C. gracile*, faster than it consumed a larger particle. Each of the three species consumed *S. costatum* at the highest rate, which was assumed to be an intermediate individual cell volume, but was actually the largest particle offered due to chain formation. This may tentatively indicate that these species have high dietary overlap and do not partition food resources based on body size, and possibly prefer chain-forming diatoms. It may also indicate that chain formation may have masked any correlation with body size. Correction for algal morphology, preferably by using only chain-forming or single-cell cultured diatoms, might eliminate some of the problems with food comparisons. The use of artificial beads might also assist in separating the effects of size on feeding rates from algal morphology and even taste. The average cell size offered to nauplii in these experiments may have been too small or underestimated, considering only individual cells were measured rather than entire chains. Further investigations into this subject should include food preference studies, where multiple food species are offered concurrently and consumption rates on each food type are measured. An alternative to laboratory studies would be to examine the gut contents of field collected nauplii because diatom frustules may not be immediately broken up during digestion.

BRIDGE II

As demonstrated in Chapter III, the size of the food affects the feeding rates of nauplii of *B. crenatus*, *B. glandula*, and *B. improvisus*. Specifically, feeding rates are significantly higher on the chain-forming centric diatom *Skeletonema costatum*, which has an intermediate individual cell size. It is likely that the morphology of the food, not only size, determines that rate at which the food can be captured. Although feeding rates were different between the types of foods, growth was not necessarily related to the size of particle offered.

From year to year and season to season, there is natural variation in the types and sizes of particles available in the plankton. Species that reproduce across several seasons or throughout the year may therefore be expected to adjust to these variations so as not to increase the duration of the larval period or reduce their size at the end of the larval period. Some marine invertebrate larvae have been shown to cope with low food concentrations by changing the morphology of their feeding structures in order to increase feeding rates. *B. glandula* is reproductive throughout the year, and so may react to variations in feeding regimes by adjusting the morphology of their feeding appendages to ensure adequate particle capture and feeding rates for growth to metamorphic competence. Chapter IV addresses the response of the feeding appendages of nauplii of *B. glandula* to different types of food.

CHAPTER IV

PHENOTYPIC PLASTICITY IN THE FEEDING APPENDAGES OF NAUPLII OF *Balanus glandula*

Introduction

Phenotypic plasticity is environmentally induced morphological variation among siblings expressed during development (Hadfield and Strathmann, 1996), and is recognized as an adaptation found in populations or species that live in temporally fluctuating environments (Bradshaw, 1965; Caswell, 1983; Stearns, 1989). Zooplankton live in these sorts of environments because they may experience variation in available food during their lifespan (Lampert, 1994). Phytoplankton types, sizes, and relative abundances may change across days, seasons, and years (e.g., Hughes, 1997; Tamigeneaux et al., 1999; Valiela, 1995). On some occasions, these variations may cause food limitation for holoplankton and meroplankton because phytoplankton abundance is low, available species require special mechanisms for capture, or phytoplankton species are inappropriate for consumption (Anil and Kurian, 1996; Benndorf and Horn, 1985; Fenaux et al., 1994; Huntley and Boyd, 1984; Olson and Olson, 1989; Qui and Qian, 1997). For instance, natural food limitation has been found

in *Daphnia* species (Brendelberger and Geller, 1985) and *Paracentrotus lividus* larvae (Fenaux et al., 1994).

Food limitation may result in longer planktonic periods because larvae require more time to gather the food with which they grow new larval tissues as well as juvenile tissues (Fenaux et al, 1994; Hadfield and Strathmann, 1996). Longer planktonic periods may increase the chances of advection from the adult habitat and predation risk. Food limitation could also increase the risk of mortality during and after settlement because of small size or reduced energy reserves of juveniles from a lack of nutritional input (Gosselin and Qian, 1996; Highsmith and Emlet, 1986; Jarrett and Pechenik, 1997; Moran, 1997). Selection may therefore favor larvae that can either adjust the morphology of feeding structures to efficiently capture the particles present or to capture a wide range of particles.

Indeed, many species of planktotrophic larvae show plastic responses to variation in phytoplankton supply by changing morphology of their feeding apparatus (Strathmann, 1996). Several taxa of ciliated larvae display phenotypic plasticity when exposed to low concentrations of algae. Plutei of *Dendraster excentricus*, *Paracentrotus lividus*, and *Strongylocentrotus droebachiensis* and bipinnariae of *Pisaster ochraceus* are some of the echinoderms that increase arm length and therefore ciliated band length in response to nutritional stress (Fenaux et al., 1994; George, 1999; Hadfield and Strathmann, 1996; Hart and Strathmann, 1994). Veligers of the oyster *Crassostrea virginica* also increase the relative size of the velum when reared at low food levels (Strathmann et al., 1993). In each of these examples, larger ciliated bands increase feeding capacity. Higher feeding

rates may in turn mitigate the effects of low food concentrations by decreasing the time required to accumulate energy reserves and develop tissues needed for metamorphosis.

Phenotypic plasticity in feeding structures has also been shown in non-ciliated filter feeding animals when reared under low food conditions. The thoracic appendages of planktontic cladocerans are equipped with long setae that bear many small setules, making up a mesh through which particle-laden water is filtered. Many species of *Daphnia* develop larger thoracic appendages in the presence of low food concentrations (Ghadouani and Pinel-Alloul, 2002; Gliwicz and Lampert, 1993; Lampert, 1994; Lampert and Brendelberger, 1996; Repka et al., 1999). The size of the mesh has also been shown to decrease at low food levels in several *Daphnia* species, which may aid in the capture of smaller bacteria (Brendelberger, 1991). The number of fan rays that constitute the mesh used by black fly larvae to filter particles is also dependent on food concentration (Lucas and Hunter, 1999).

Other studies have shown that some species also display plastic responses to the type of food upon which they are reared. McGregor (1963) found that sibling larvae of *Opifex fuscus* (Diptera: Culicidae) reared on two types of food developed mouth brushes with mesh sizes proportional to the size of food offered to them. Ghadouani and Pinel-Alloul (2002) documented that the mesh size of *D. pulicaria* increased when cultures were given inedible filamentous algae compared to those given healthy, single-celled and small-chain algae.

This study examines barnacle larvae that feed in the plankton for days to weeks prior to settlement and metamorphosis. Barnacle nauplii are believed to capture particles

by sieving water through a mesh created by their second antennae and then (possibly) their mandibles (Lang, 1979; Gauld, 1959; Moyse, 1984; Norris and Crisp, 1953; Rainbow and Walker, 1976; Strathmann RR, 1987; Walker et al., 1987). Because of the viscous flows that occur at this scale, the particle sizes that nauplii can capture may also depend on the mesh size of the second antenna (Koehl, 1993; Stone, 1989).

The barnacle *Balanus glandula* (Crustacea: Cirripedia) is reproductive year-round (Brown and Roughgarden, 1985), and larvae may be exposed to seasonal variation in phytoplankton standing stock and particle size (Hughes, 1997; Tamigeneaux et al., 1999; Valiela, 1995). Many diatom species of varying cell size, including *Chaetocerous*, *Skeletonema*, and *Thalassiosira* species, are abundant in the coastal waters of Oregon (Cupp, 1943; Hasle and Syvertson, 1996), making them available to the nauplii *of B. glandula*. Nauplii of this species may, therefore, have to contend with variable food availability, and they may be able to modify feeding appendages to account for these variations in food supply.

I tested whether or not phenotypic plasticity is induced in nauplii of *B. glandula* by the type of food offered in culture. Specifically, does the area of the second antennae, which are believed to be the primary feeding appendages (Norris and Crisp, 1953; Moyse, 1963; Walker et al., 1987), and the intersetular distances that make up the mesh of these appendages change in response to the size of food particle offered in culture? Nauplii of *B. glandula* were exposed to monocultures of phytoplankton of three different sizes to see if they would display morphological plasticity. Antennal area is predicted to change in proportion to the size of food offered. Intersetular distance is also predicted to

change in proportion to the size of particle offered: small particles will result in shorter distances between setules and large particles will result in longer distances between setules. If plasticity in feeding appendages exist, it may help in evaluating the sizes of food particles available for individuals and assist researchers in evaluating factors that influence the success of this species.

Materials and Methods

Ripe lamellae of *B. glandula* were collected from Charleston Bridge pilings (Coos Bay, OR) in February, 2003 (Smart, Chapter 1 of this thesis). When light was shone on lamellae, nauplii began to break out of egg cases and swim towards the light, where they were collected by pipette and concentrated in graduated cylinders. Numbers and density of larvae from each parent were determined by placing six 1mL samples in a Bogorov tray with 7.5% MgCl and counting with the aid of a dissecting microscope. Nauplii, from a single parent, were added into culture jars containing filtered seawater (FSW) at a density of 1 nauplius per 2 mL. Six jars, beginning with 3 liters of FSW, were established for each of three food treatments to ensure that there would be enough late stage nauplii for all measurements.

Three diatom species in the genera *Chaetocerous*, *Skeletonema*, and *Thalassiosira* were used in the present study. *C. gracile* is a small chain forming diatom (cell diameter 5.1 μm) that occurs as single cells in culture (personal observation). The intermediately sized *S. costatum* (cell diameter 11.7μm) typically forms chains of two to twelve cells in culture (Hasle and Syvertsen, 1996; personal observation). *T. weisflogii* (cell diameter

13.0 μm) also occurs as single cells in culture. Each diatom species was grown in F/2 medium (Guillard, 1983) in a 15°C incubator on a 18:6 hour light:dark cycle. Cell volumes of each species were determined with a compound microscope. The diameter and height of thirty cells from each species were measured on three separate occasions. The cell volume for *C. gracile* was estimated by assuming that cells were ellipsoidal (Harrison et al., 1977). Volumes for cells of *S. costatum* and *T. weisflogii* were both calculated by assuming that cells were short cylinders. Larvae were fed equivalent cell volumes of six-day old algae in culture jars (4.1×10^7 μm^3/mL FSW), which corresponded to 5.9×10^6, 1.0×10^5, and 2.6×10^5 cells/mL *C. gracile*, *S. costatum*, and *T. weisflogii*, respectively.

Cultures were cleaned every other day by pouring the contents of each jar through a 100 or 202 μm mesh sieve immersed in FSW, which allowed the diatoms and feces to pass through but retained the larvae. Nauplii were then transferred back into the jars filled with fresh FSW to which the correct volume of food was added. Jars were kept in a table with running seawater and each was stirred with a plexiglass paddle at 12 rpm (Strathmann MF, 1987).

Jars were pooled and then subsampled for each nauplius stage. Thirty nauplii from each food treatment were preserved in 4% seawater buffered formalin. Afterwards, nauplii were redistributed into culture jars at the same density as before (2 per mL). For each food treatment, carapace dimensions (length and width) of five to ten nauplii from stage II and stage VI were determined with a dissecting microscope. Length was measured from the carapace margin halfway between the frontolateral horns to the tip of

the caudal spine, and width was measured at the widest point anterior to the frontolateral horns. From these animals, the second antenna on the right side was removed using two, fine insect pins. The antenna was then mounted on a slide in 4% seawater buffered formalin and photographed on a compound microscope.

The area encompassing both the exopodite and endopodite was then traced and calculated from photographs with imaging software (Optimas 6.0). The spacings between the four most proximal setules and between the four most distal setules on each seta of the exopodite were measured from photographs using imaging software (Optimas 6.0, see Figure 11). Exopodite setae were selected for these measurements because they are believed to be the primary sites of phytoplankton capture (Moyse, 1984). The spacing between the setules changes along the length of a seta (Stone, 1989; personal observation) so proximal and distal measurements were made to encompass a broad range of "mesh" sizes. Setae 15 and 16 of stage II and setae 19-21 of stage VI were not used in the analysis because of the low number of setules on these setae (Figure 11). Stage II were measured to check for variation between food treatments prior to the animals having the opportunity to make morphological changes in response to food size, since stage II is the first feeding stage. Stage VI was the only other stage measured because if morphological change had occurred, it would most likely have appeared by this stage. Nauplii in earlier stages may not have had enough time to make morphological adjustments to food size.

Data for antennal area were then compared using one-factor analysis of variance (ANOVA) with food as a fixed factor. Data for setular spacing of proximal and distal

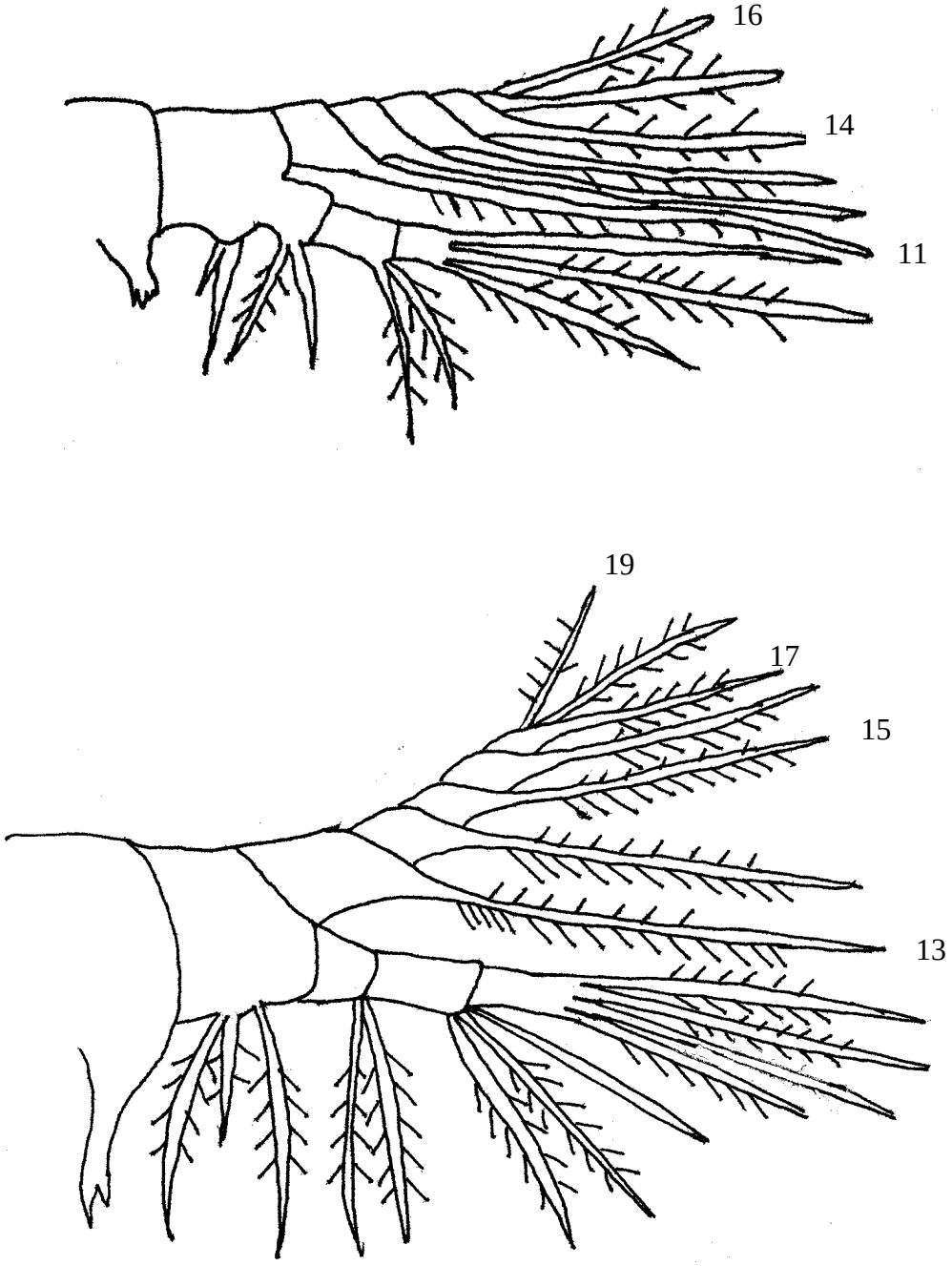


Figure 11. Second antenna showing both exopodite and endopodite from stage II (top) and stage VI (bottom) nauplii of *B. glandula*. Setae are numbered on the right.

regions were compared by multivariate analysis of variance (MANOVA, Quinn and Keough, 2002).

Results

The area of second antennae of nauplii of *B. glandula* showed no apparent change in response to food size by stage VI (Figure 12). Differences in antennal areas between food treatments were not significantly different in stage II or stage VI (Table 7).

Proximal setular spacings were not statistically different between food treatments at stage II or stage VI (Figure 13, Table 8A). For distal setules, spacing was not statistically different at stage II or stage VI (Figure 14, Table 8B).

Discussion

Frequent exposure to food limitation may have dire consequences for planktonic filter feeders. For example, nutritional stress may result in starvation, decreased fecundity, and delayed reproductive maturity in the cladoceran, *Daphnia pulex* (Luning, 1992). For larvae of *Paracentrotus lividus*, food limitation may also result in starvation, but perhaps more importantly, it imposes a longer planktonic period before larvae obtain sufficient nutrition to become competent to metamorphose (Fenaux et al., 1994). Extension of the larval stage may result in greater losses due to transport from the adult habitat and predation, which, in turn, result in decreased recruitment (Hart and Strathmann, 1994). If food scarcity occurs often, plasticity in the development of feeding

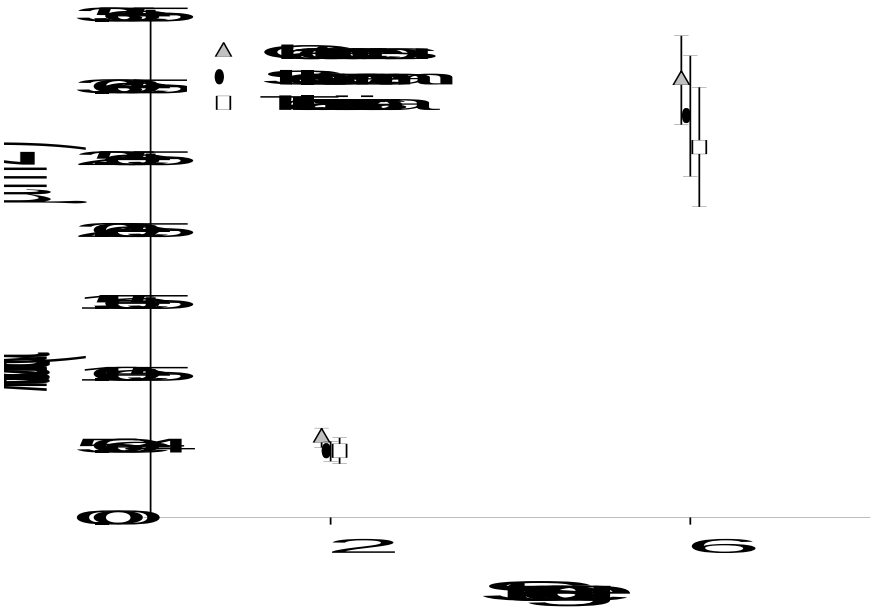


Figure 12. Antenna area of stage II and stage VI nauplii of *Balanus glandula* (n=10 ± SD for *S. costatum* and *T. weisflogii*; n=5 ± SD for *C. gracile*). Data points have been staggered where error bars overlap.

Table 7. Results of one-factor ANOVA for antennal area with food as a fixed factor.

A. Stage II

Source	SS	df	MS	F-ratio	P
FOOD	3.4277 E 08	2	1.7138 E 08	2.808	0.082
Error	1.3426 E 09	22	6.1027 E 07		

B. Stage VI

Source	SS	df	MS	F-ratio	P
FOOD	7.4285 E 09	2	3.7143 E 09	2.318	0.122
Error	3.5252 E 10	22	1.6024 E 09		

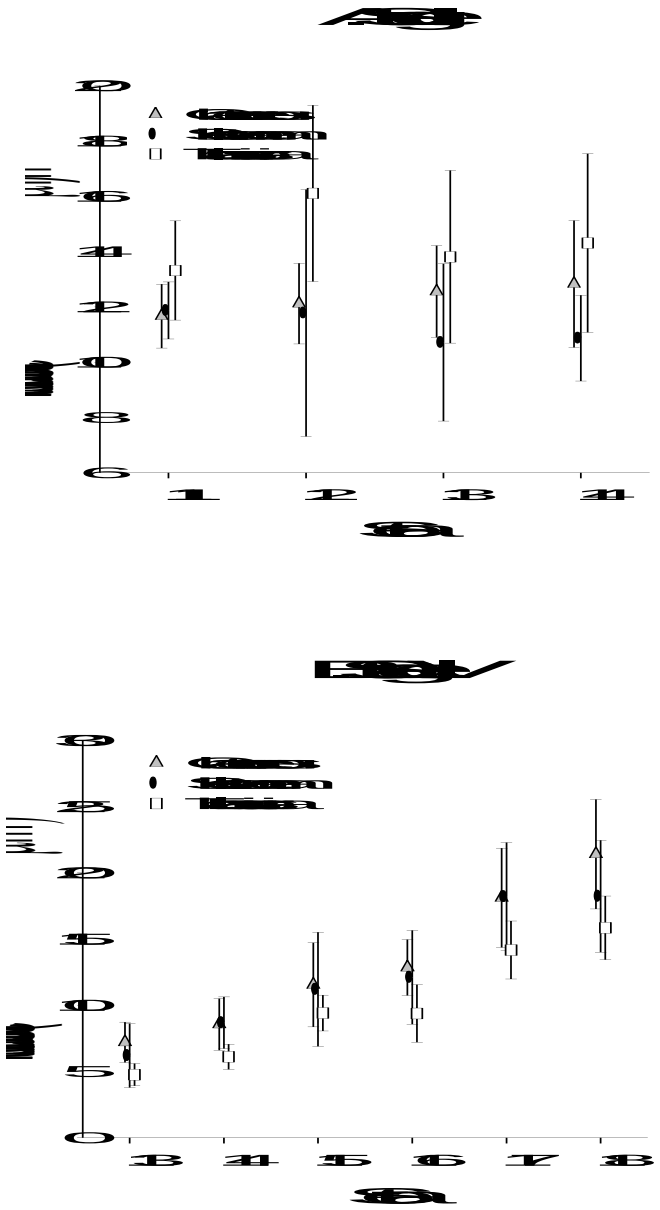


Figure 13. Proximal intersetular distances of stage II (A) and stage VI (B) nauplii of *B. glandula* (n=5 ± SD). Data points have been staggered where error bars overlap.

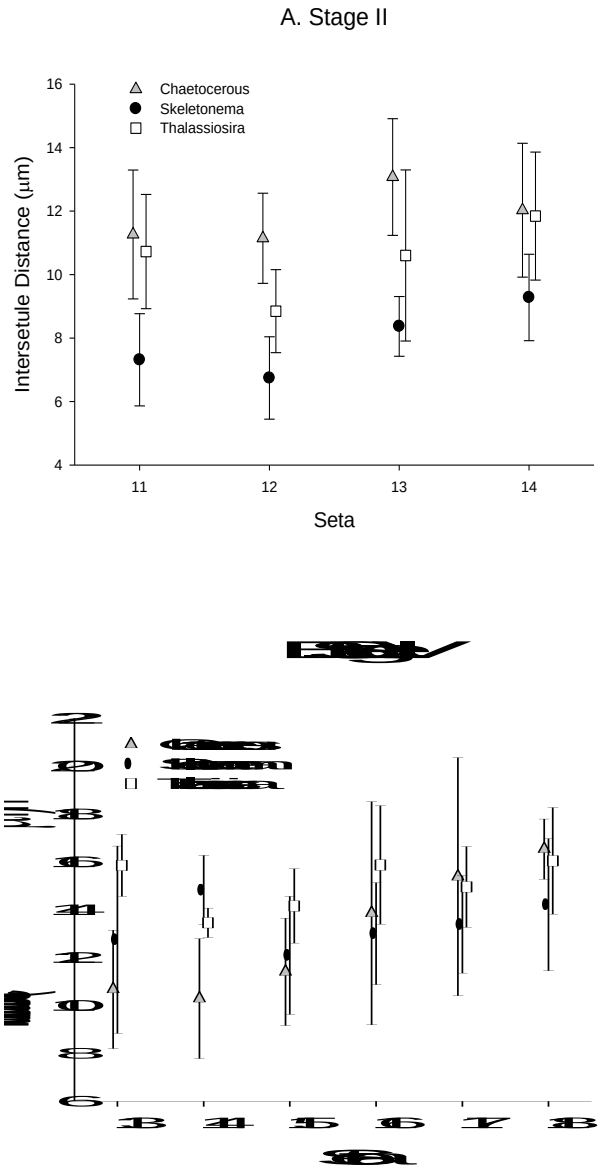


Figure 14. Distal intersetular distances of stage II (A) and stage VI (B) nauplii of *B. glandula* (n=5 ± SD). Data points have been staggered where error bars overlap.

Table 8. Pillai trace results of MANOVA for proximal and distal intersetular distances at stage II and VI.

A. Proximal

	F-statistic	df	F	P
Stage II	1.088	8, 20	0.607	0.411
Stage VI	0.820	12, 16	0.762	0.630

B. Distal

	F-statistic	df	F	P
Stage II	2.356	8, 14	1.148	0.077
Stage VI	1.172	12, 12	1.079	0.394

structures could alleviate nutritional stress by allowing for increased feeding rates, thus increased nutritional intake (Strathmann et al., 1993).

Variations in feeding regimes result in morphological changes in the feeding structures of a wide variety of planktonic suspension feeders. Plutei (Bertram and Strathmann, 1998; Boidron-Metairon, 1988; Fenaux et al., 1994; Hart and Strathmann, 1994;), bipinnaria (George, 1999), and veligers (Strathmann et al., 1993) increase the relative size of ciliated bands in response to low food concentrations. In cladocerans, low food concentrations result in an increase in the relative size of appendages used for feeding (Brendelberger and Geller, 1985; Lampert, 1994; Lampert and Brendelberger, 1996) and in the sizes of the mesh responsible for capturing particles (Ghadouani and Pinel-Alloul, 2002; Lampert and Bredelberger, 1996; Repka et al., 1999). Different types of food can also cause changes in the sizes of mesh in cladocerans (Lampert and Bredelberger, 1996; Repka et al., 1999) and in dipteran larvae (Lucas and Hunter, 1999; McGregor, 1963). Larger ciliated bands in echinoderms and molluscs and larger feeding appendages in arthropods allow higher feeding rates, and changes in mesh size increase the ability to capture the particle size present. These organisms are adapted to cope with variations in food conditions through morphological plasticity, which decreases time to competency or reproductive maturity.

Although the feeding appendages of nauplii of *B. glandula* were hypothesized to change to cope with particles of different sizes, the area of the second antenna was not, in fact, significantly different between food treatments. By stage VI, there was also almost no difference in intersetular distances between food treatments. There was a trend,

however, of increased antennal area as food size decreased. According to power tests, at least ten more individuals would need to be measured to provide a good chance at detecting a significant difference in antennal area at stage VI between food treatments. With the current sample size, this experimental design had only a 42% chance of detecting a difference in antennal area at stage VI. With this in mind, however, results suggest that nauplii of *B. glandula* do not adjust the morphology of their feeding appendages in response to variation in size of phytoplankton.

Particle capture in barnacle nauplii is poorly understood compared to mechanisms in copepods. Cinephotography of *Lepas pectinata* indicates that particles are captured during the swimming stroke by the extended exopodite and then moved ventrally along the smaller endopodite of the second antenna (Moyse, 1984). However, the debate continues as to whether algal cells become caught in the mesh of the exopodite, or if the exopodite acts like a paddle and sweeps particle laden water toward gnathobases where particles are transferred to the mouth (Lang, 1979; Gauld, 1959; Norris and Crisp, 1953; Rainbow and Walker, 1976; Walker et al., 1987). Reynolds numbers for copepod nauplii indicate that the second antennae act more like paddles than sieves, moving parcels of water containing particles, rather than straining particles out of the fluid (Cheer and Koehl, 1987). Changing the setular spacing would, therefore, have little effect on the function of the antenna (Cheer and Koehl, 1987), thus eliminating the need for morphological plasticity in this appendage.

Most studies that have demonstrated morphological plasticity used variations in food concentration that approximated food limitation, whereas this study tested the

effects of particle size on feeding appendages. Nauplii of *B. glandula* grew similarly on all three species of diatom offered (see Chapter III, Figure 10B), suggesting that none of the species of diatoms used here were of low enough quality to cause food limitation. Plasticity may only be selected for when costs associated with loss due to decreased nutritional intake outweigh metabolic costs of increasing drag and allocation to feeding structures while increasing feeding appendage size. The observed growth suggests that morphological plasticity would therefore not be needed by nauplii in this particular situation.

The second antenna of a nauplius is a jointed appendage that can change its conformation much like the human hand and may be capable of capturing particles of different sizes based on how the setae of this hand are positioned relative to water flow and particle size and shape. The ability to move setae together and apart may also mitigate the need for morphological plasticity. If nauplii of *B. glandula* are able to capture a wide range of particles without need of phenotypic plasticity, feeding ability might be one trait that allows this species to reproduce year-round. If all nauplii are able to capture a wide range of particles just through behavioral adjustments, this trait may also account for the conservation of this body form among crustacean groups (Williams, 1994).

The morphology of the second antennae of barnacle nauplii has been used in the past to predict feeding niches (Moyse, 1964; Moyse, 1984; Stone, 1989). Specifically, the intersetular distances are thought to be related to the particle size best captured by a nauplius. For instance, nauplii of *B. crenatus* have widely spaced setules compared to

those of *Chthamalus montagui* (Stone, 1989). Culture of *B. crenatus* is more successful when nauplii are given large diatoms, whereas culture of *C. montagui* is more successful when nauplii are give small diatoms or small flagellates. If setular spacing was a plastic trait, it could not be reliably used to predict a species' feeding niche. Rather, it could be used to predict the feeding history of a particular larva because the mesh size would be a record of the food size available to nauplii. The present results, however, do not indicate that antenna morphology can be used to predict the size of food available to larvae because nauplii of *B. glandula* do not show plasticity in the morphology of the second antenna in response to food size. If mesh sizes and antennal areas are non-plastic traits within a species, the morphology of the second antenna may therefore stand up as a good indicator of feeding niche.

CHAPTER V

CONCLUDING SUMMARY

The objective of this thesis was to add to the current understanding of feeding performance of marine invertebrate larvae. Since the larval stages may be exposed to high rates of mortality during the planktonic period, it can be expected that mechanisms for reaching metamorphic competence without sacrificing the ability to successfully recruit into the adult habitat may be strongly selected for. One possible mechanism is high feeding rates, which would provide the nutrients needed for growth of tissues required for the juvenile stage and the build-up of energy reserves required for the arduous process of metamorphosis.

Chapters II and III of this thesis have offered a comparative approach to feeding performance of barnacle nauplii. The feeding rates of temperate barnacle nauplii may be distinctive compared to other taxa, which provides a starting point for investigations as to whether mechanism, size, or behavior determines feeding rates. Results presented here indicate that behavior may determine feeding rates, as feeding rates of temperate barnacle nauplii are different from larvae of similar sizes and are different from other nauplii with similar feeding mechanisms. The conversion of ingested food to increase in size for the species tested (*B. crenatus*, *B. glandula*, and *B. improvisus*) appears to be quite different, and may indicate that the species with the largest final size may have better chances of

recruiting into the adult habitat. Results also suggest that these species have high dietary overlap, and capture chain-forming diatoms efficiently, which mirrors the abundance of chain-forming diatoms in temperate near-shore environments.

Chapter IV indicates that nauplii of *B. glandula* do not need morphological changes to capture and grow on a wide-range of particle sizes. This suggests one of two possible feeding mechanisms. First, that the second antennae (thought to be the primary feeding appendages) are used by nauplii as a paddle to sweep food toward the mouth, as has been suggested but never definitively shown for this group. Or second, that the complex musculature and jointed nature of the second antennae can be used by a nauplius to behaviorally modify the mesh on these appendages to capture particles of different sizes. The ability of a nauplius to capture a wide range of particles without morphological adjustments may be one factor that has led to the conservation of this larval form over eons of evolution.

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