EFFECTS OF NATURAL FOOD AVAILABILITY ON LARVAL CONDITION OF *BALANUS GLANDULA* IN THE LABORATORY AND THE FIELD

by

JULE JACOB SCHULTZ

A THESIS

Presented to the Department of Biology
and the Graduate School of the University of Oregon
in partial fulfillment of the requirements
for the degree of
Master of Science

June 2005

"Effects of natural food availability on larval condition of *Balanus glandula* in the laboratory and the field," a thesis prepared by Jule Jacob Schultz in partial fulfillment of the requirements for the Master of Science degree in the Department of Biology. This thesis has been approved and accepted by:

_____

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

_____

Date

Committee in Charge: Dr. Richard B. Emlet, Chair
Dr. Alan L. Shanks
Dr. Craig M. Young

Accepted by:

_____

Dean of the Graduate School

An Abstract of the Thesis of

Jule Jacob Schultz for the degree of Master of Science

in the Department of Biology to be taken June 2005

TITLE: EFFECTS OF NATURAL FOOD AVAILABILITY ON LARVAL CONDITION OF *BALANUS GLANDULA* IN THE LABORATORY AND THE FIELD

Approved: _____
Dr. Richard B. Emlet

Food availability in the coastal oceanic environment off Oregon was shown to limit energy reserves and developmental rates of barnacle nauplii in some experiments but not others. In laboratory studies, when naupliar feeding rates measured by gut contents and fecal pellet production rates were similar for larvae raised on natural and supplemented rations, larval lipid content and development were similar between these rations (2 trials). When feeding rates of nauplii differed between food treatments so did lipid content and development (2 trials). A parallel study sampled lipid content and size of naturally occurring larvae and chlorophyll-*a* in the water column. While chlorophyll-*a* was an accurate measure of food availability measured by fecal pellet production, it explained little (2-4%) of the variation in cyprid quality. Efforts to understand variation in quality of natural populations of larvae will need to combine the effects of genetic variation, feeding history, and food quality.

CURRICULUM VITAE

NAME OF AUTHOR: Jule Jacob Schultz

PLACE OF BIRTH: Norman, OK

DATE OF BIRTH: December 20, 1977

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon
Lewis and Clark College

DEGREES AWARDED:

Master of Science in Biology, 2005, University of Oregon
Bachelor of Science in Biology, 2001, Lewis and Clark College

AREAS OF SPECIAL INTEREST:

Larval Ecology
Reproductive Ecology of Marine Invertebrates

PROFESSIONAL EXPERIENCE:

Grades Kindergarten through 12 Fellow, Oregon Institute of Marine Biology, (University of Oregon), 2004-2005.

Graduate Teaching Fellow, Oregon Institute of Marine Biology, (University of Oregon), 2003-2004.

Research Assistant, Oregon Institute of Marine Biology, (University of Oregon), 2001-2002.

GRANTS, AWARDS AND HONORS:

Neil Richmond Memorial Fellowship, 2003

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Richard Emlet, for his help. Dr. Emlet presented me with many opportunities to grow professionally and personally during my four years at OIMB. He has mentored me through a time of great growth and I thank him for it. I would also like to thank my committee, Dr. Emlet, Dr. Alan Shanks, and Dr. Craig Young for their numerous contributions to my thesis and project. I would like to extend a heartfelt thank you to my committee and Shawn Arellano for helping me with the writing of this thesis. Barb Butler proved herself unmatched in her willingness and ability to help me track down references and resources. I would also like to thank the community of OIMB for their friendship and support throughout my graduate degree.

I had many graduate and undergraduate students accompany me on my bi-weekly boat trips on the bay. Specifically, I would like to thank John Young for his help as a colleague and friend. Tracey Smart, Mike Berger, Ali Helms, Chistina Geierman, and Jim Trainer of the Emlet lab helped me with field and laboratory work.

Partial support of this work was provided by NSF grant OCE-9911682 to R. Emlet. Other financial support was provided by the NSF GK-12 Fellowship, a job as a hazardous waste technician, many GTF positions, and a job as director of OMSA.

Finally I would like to thank my parents, Margaret Studer and Robin Schultz. My mother gave me the drive to succeed and my father instilled in me the spirit of endless curiosity.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

GENERAL INTRODUCTION

Many marine invertebrates reproduce via free-swimming offspring that are ecologically distinct from their parents. These larvae function, in part, for dispersal (Pechenik 1999) and acquisition of energy (Strathmann 1987). Planktotrophic larvae accumulate energy and grow by feeding upon the phytoplankton in the water-column, thereby releasing their parents from investing large amounts of energy into individual offspring. However, larvae are at risk of mortality due to predation, transport away from suitable habitat, extreme environmental conditions, and for feeding larvae, starvation (reviewed by Thorson 1950, Morgan 1985). Laboratory manipulations of food availability and temperature affect the size, lipid content, and developmental rate of larvae (e.g. Emlet and Sadro submitted manuscript). Given that food availability and seawater temperature vary spatially and temporally in the field, the larvae of marine invertebrates that reproduce throughout the year will be exposed to varying environmental conditions. Studies of barnacle larvae from the field (Jarrett 2003) show that variations exist in larval size and lipid content over time and space, although there are few attempts to explain the causes of these variations. A growing body of evidence

suggests that larval size and lipid content affects the performance and survivorship of juvenile marine invertebrates (reviewed by Pechenik et al. 1998).

This study describes effects of food variation on the size, lipid content, and development of larvae of the intertidal barnacle *Balanus glandula*. Chapter II of this thesis compares the lipid content, stage duration, gut contents, and fecal pellet production between nauplii of *Balanus glandula* reared with natural rations of phytoplankton and nauplii reared with natural rations supplemented with laboratory grown phytoplankton. Chapter III of this thesis correlates the size and lipid content of field sampled cyprids of *Balanus glandula* with seawater chlorophyll *a* concentration over a one year period. Furthermore, seawater chlorophyll *a* concentration is investigated as a possible proxy for food availability in the field. This thesis investigates the effect of variations in natural food availability on larval size, lipid content, and development.

CHAPTER II

FOOD LIMITATION IN LARVAE OF THE BARNACLE *Balanus glandula* REARED WITH NATURAL RATIONS

Introduction

The life cycles of many marine invertebrates contain a free-swimming larval stage that functions, in part, for dispersal (Pechenik 1999). During this pelagic period, larvae must avoid predators, find suitable settlement substrata, and, for planktotrophic larvae, find and consume food. Herbivorous planktotrophic marine larvae feed upon the phytoplankton available in the water column, in part to turn small larvae or eggs into larger larvae suitable for settlement and recruitment into the adult habitat (R. Strathmann 1987). Phytoplankton is patchy, both temporally (e.g. Hutchinson 1944, Cowles et al. 1993, Cowlishaw 2004) and spatially (Lorenzen, 1971). Differences in phytoplankton abundance may be driven by cycles of upwelling, seasonal shifts in day length, temperature, and turbidity (Mackas et al. 1985), leading to variations in larval food availability in the water column.

Laboratory experiments have shown that decreases in phytoplankton rations can lead to decreases in larval survivorship (Olson and Olson 1989), size (Hentschel and Emlet, 2000), and lipid content (Gallager and Mann 1981, Hentschel and Emlet 2000) as well as increases in larval developmental period (Meidel et al. 1999). Larval mortality in the plankton is high (Rumrill 1990, Young and Chia 1987, but see Johnson and Shanks, 2003), and any increase in developmental rate will reduce the number of larvae available for settlement from a cohort (Thorson 1950, Morgan 1995). Larvae that do survive to become juveniles may be affected by their feeding history. When reared with low food rations as larvae, juveniles show reduced growth and survivorship, delayed settlement, or are smaller at metamorphosis (Pechenik et al. 1998, Miller and Emlet 1999, Pechenik et *al.* 2002, Emlet and Sadro submitted manuscript).

Many experiments on larval food limitation have manipulated concentrations of laboratory-reared algae, typically fed in a monoculture, while few experiments have used foods at natural concentrations. Rearing larvae with natural rations may more accurately reflect the nutritional conditions found in the field, allowing for investigations of how variations in natural food affect larval energy stores, size, and developmental rate. In echinoderm plutei, significant differences in developmental rate, growth, and structure occur between larvae reared with natural rations and larvae reared with natural rations supplemented with lab-grown phytoplankton (e.g. Paulay et al. 1985). Results suggest that natural concentrations of food limit the development and growth of echinoid and ophiuroid larvae in the field. These studies have rarely been repeated over time (however, see Fenaux et al. 1994), limiting our knowledge of how the variability of food

in the natural environment affects larval characteristics. No studies have investigated the effect of natural rations on herbivorous larvae of benthic crustaceans, although many studies suggest natural food limitation in adult copepods (Durbin et al. 1983, Checkley 1980). Numerous studies (reviewed by Olson and Olson 1989) propose that starvation may be an important factor influencing the recruitment of crustacean species.

The nutritional state of marine invertebrate larvae can be evaluated by measuring their lipid content (Holland and Walker 1975, Gallager and Mann 1981). Many marine invertebrate larvae contain neutral lipid droplets that are either remainders of the yolk or converted from the phytoplankton the larvae consume. In barnacle nauplii, the 1st and 2nd naupliar stages contain remnants of lipid from the yolk, while the 2nd through 6th stages feed actively in the plankton, accumulating lipid droplets in the form of multinucleate oil cells stored dorsal to the midgut (Holland 1987). These cells function as energy stores for the non-feeding cyprid stage. Cyprid lipid content positively correlates with the energy stores accumulated during the naupliar stages, time cyprids can spend searching for suitable settlement substrata (Gosselin and Qian 1996), and with the percent of cyprids metamorphosing to juveniles (Thiyagarajan et al., 2002). Although the effects of feeding in the natural environment on lipid content are not known in barnacle larvae, the lipid content of marine copepods increases with seawater chlorophyll *a* concentration, suggesting that recent feeding condition influences lipid content (Hakanson 1987). In the laboratory, Gallager and Mann (1981) used Nile Red stain to estimate lipid content and showed that it was a useful measure of nutritional condition in bivalve larvae. Well-fed larvae contained high amounts of lipid that were depleted during times of starvation.

When larvae are exposed to Nile Red, a hydrophobic lipid stain, their neutral lipids shine bright yellow and their polar lipids shine dull red under epifluorescent light. This allows the quantification of lipid, measured as area, using digital imaging software (Hentschel and Emlet 2000). Hentschel and Emlet (2000) found that barnacle cyprids reared from nauplii fed $1x10^4$ cells/mL of the chain-forming diatom *Skeletonema costatum* in the laboratory contained less lipid (measured as area) and were of a smaller size than cyprids from nauplii fed $1x10^5$ cells/mL of *S. costatum*.

Studies rearing larvae with natural rations have used chlorophyll *a* concentration and the abundance of phytoplankton of different sizes to assess food availability (Paulay et al. 1985, Fenaux 1994, Eckert 1995, Reitzel et al. 2005). These methods do not actually measure food intake, but rather serve as a measure of food availability. Some investigations of the feeding rates of marine larvae measure the number of particles cleared from the water by a larva over a period of time to determine the ingestion rate (reviewed by Hart and R. Strathmann 1995). This method has the advantage of being widely reported in the literature, but uses a large number of larvae and may not be accurate at low food concentrations (Bamstedt et al. 1999, Smart 2004). Other methods of comparing relative ingestion rates may be more suitable for studies using natural rations, which include limited numbers of animals and low food concentrations. Gut fluorescence analysis and fecal pellet production rates are both alternatives that have been used to accurately determine the feeding condition of crustacean zooplankton (Mackas 1976, Butler and Dam 1994). Gut fluorescence analysis uses fluorometry to measure the amount of fluorescent pigments within the body of a larva. This technique has the

advantage of using few larvae and that the larvae can be preserved by freezing. Unfortunately, gut fluorometry does not account for differences in the gut passage time of ingested particles. This method will not distinguish feeding conditions between larvae that have full guts but may have different ingestion rates. The analysis of fecal pellet production rate is an accurate proxy for ingestion rate in marine copepods (Besiktepe and Dam 2002). In the present study, I measured fecal pellet production rates and gut fluorescence of lab-reared barnacle nauplii to determine differences in feeding rates between food rations.

Balanus glandula Darwin is a common intertidal barnacle in the northeast Pacific and ranges from Baja California to Alaska. Locally, *B. glandula is* abundant on the Oregon Coast. In the Coos Estuary, it is reproductive for approximately 10 months of the year (Berger 2004). Its larvae may therefore be subjected to widely differing food availability upon release. Larvae of *B. glandula* are easily grown in the lab with a diet of the chain-forming diatom *S. costatum* (Brown and Roughgarden, 1985). As in most barnacles, *B. glandula* develops through six naupliar stages with 2^{nd}-6^{th} feeding, and metamorphoses into a non-feeding cyprid, prior to settlement.

The purpose of this study was to investigate the effects of natural food limitation on larvae of *B. glandula.* Specifically, this study determined how natural rations affected naupliar lipid accumulation, stage duration, fecal pellet production, and gut fluorescence throughout the year and compared these measures to those of nauplii reared with supplemented and diluted rations. Naupliar and cyprid lipid contents from the natural

food treatment were also correlated with chlorophyll *a* concentration of the natural seawater.

Materials and Methods

Larval culture

Adult *Balanus glandula* with mature, dark brown lamellae, were collected from beneath the west side of the Charleston bridge (Charleston, Oregon, 43° 20.4' N, 124° 19.4' W) in September 2003 through August 2004. The barnacles were transported to the Oregon Institute of Marine Biology (OIMB) in separate 50mL centrifuge tubes containing 0.45μm-filtered seawater (FSW). Pairs of lamellae from each adult were dissected out, placed in an individual 1-L jar containing FSW, and exposed to a fiber optic light to induce hatching of the nauplii (Brown and Roughgarden 1985). Hatched larvae from each parent were pipetted into a 100-mL graduated cylinder with FSW for counting, and six 1mL samples of nauplii from each graduated cylinder were counted on a Bogorov tray containing 7.5 % MgCl. Equal numbers of nauplii from six parents were placed in jars containing 3 L of seawater (see below for seawater treatments) at a concentration of 1 nauplius / 10 mL seawater. Jars containing nauplii were stirred with plexiglass paddles at 12 beats/minute (M. Strathmann 1987) and maintained in a sea-table. All trials were maintained at 12°C with a heat exchanger, except the trial in September 2003, in which the ambient temperature of the seawater in the sea table was estimated to be 12°C.

Larval culture water was replaced every 2 days by pouring the contents of the jar onto a submerged 130μm filter and rinsing with FSW. The larvae were retained on the filter and placed back into their respective jars containing seawater collected from the bay. Seawater was collected from the mouth of the Coos estuary within an hour of the daytime high tide with a hand operated bilge pump. Potential predators and competitors for food were excluded by placing 53μm mesh over the intake of the bilge pump. The 53μm filtered seawater was then placed in the cleaned jars at one of three food rations designed to emulate conditions of 1) starvation (diluted ration), 2) natural ration, and 3) satiation (supplemented ration). 1) For the diluted ration seawater was diluted 1:4 with .45μm-filtered seawater. 2) For the natural ration the 53μm-filtered seawater was placed directly into the jars. 3) For the supplemented ration, the 53μm-filtered seawater was supplemented with the laboratory reared centric diatom *Skeletonema costatum* to a final concentration of $1x10^5$ cells/mL (Hentschel and Emlet, 2000). Each food treatment included 4 replicate jars for a total of 12 jars per trial. A total of five replicate trials were conducted throughout the year: September 2003, January 2004, March 2004, May 2004, and August 2004.

To determine the chlorophyll *a* concentration of the natural rations, one sample of the 53μm-filtered seawater was taken before the seawater was placed in the culture jars. This 200-mL sample was filtered onto a Whatman glass microfiber filter, placed into a 90% acetone solution (Parsons et al. 1984), and held at –20°C in the dark for over 24 hours. Fluorescence of samples were read on a Turner Model TD 700 fluorometer (Turner Designs, Sunnyvale, CA) before and after the addition of 5% HCl to extract

phaeopigments. Chlorophyll *a* concentration (μg/L) was calculated according the equations of Parsons et al. (1984). Mean chlorophyll *a* concentration of the natural ration in each trial was determined by averaging the chlorophyll *a* of all the samples where nauplii were present.

Naupliar lipid content, stage duration, and feeding condition

Cultures were cleaned every 2 days, and at each changing, approximately five larvae were taken from each jar with a turkey baster, placed in 1.7mL microcentrifuge tubes, frozen with liquid nitrogen, and stored at –80°C (Ohman, 1996) for later determination of stage and lipid content. Thawed larvae were stained with Nile Red for one hour using a 1:4 dilution of Nile Red Stock (2.5mg/100mL acetone) to FSW (Hentschel and Emlet 2000). Larvae were rinsed with FSW and photographed in color under blue epifluorescent light, using a CCD video camera with the gain set to "off". Images were captured using Optimas 5.2 software (Silver Spring, Maryland, USA).

Lipid content, measured as projected lipid area, was quantified from digital pictures with Optimas 5.2 software that recognized and quantified the area of yellow stained neutral lipid based on predefined color thresholds. Thresholds were created by visually examining stained lipid area of approximately 10 larvae and manually selecting the shade of yellow emitted by their lipid for use in the threshold. Separate thresholds were created for nauplii and cyprids because lipid color differs between stages, due in part to the green tint of the cyprid carapace.

The duration a stage was present in a ration was measured from nauplii used in lipid analyses. The day a naupliar stage first appeared was averaged for all four jars of a food ration, as was the last day a naupliar stage was found in a jar. Stage duration was determined by plotting the average first and the average last day a naupliar stage was present in a ration.

Naupliar gut fluorescence and fecal pellet production were used to measure larval feeding in the natural ration trials. Chlorophyll *a* and phaeopigment, recorded as gut fluorescence, from the naupliar gut were extracted by rinsing five nauplii from each jar with FSW, placing them in 15mL Falcon tubes with 4.5mL of 90% acetone, and storing them at –20°C in the dark for over 24 hours. The tubes were centrifuged for 10 minutes at 3000 rpm and allowed to warm to room temperature. The fluorescence of each solution was read before and after acidification with 2 drops of 5% HCl on a fluorometer fitted with chlorophyll *a* filters.

Fecal pellet production for each food treatment was determined by incubating five nauplii from each jar in a 50mL centrifuge tube with its respective food treatment. Six tubes were placed in 1-L jars (x 2 jars for each trial) and rotated on a roller table at 12°C for approximately 24 hours (as described by Larson and Shanks, 1996). The volume of each tube was filtered down to 5mL using a turkey baster fitted with 20μm mesh over the intake. The remaining nauplii, fecal pellets, and seawater were preserved with 0.5 mL 4% buffered formalin in 20mL scintillation vials. Two 1-mL samples from each vial of 5 nauplii were placed on a Bogorov tray, fecal pellets were counted, and naupliar stage was

noted. The average of the two fecal pellet counts per jar were used to obtain the mean of 4 jars per food treatment.

Statistical Analysis

To meet the assumption of homogeneity of variances, individual measurements of lipid area for each trial were square-root transformed except the March ‘04 data, which were fourth root transformed. Raw data on gut fluorescence for the January trial were analyzed, but all other data were square root transformed to meet the assumption of homoscedasticity. To meet the assumptions of homoscedasticity and normality, fecal pellet production data from the August ‘04 and May ‘04 trials were square root transformed and data from the March ‘04 trial were fourth root transformed.

Least square linear regressions of 6^{th} stage and cyprid lipid versus average seawater chlorophyll *a* concentration were performed because of a trend of increasing seawater chlorophyll *a* concentration and naupliar lipid content from the natural rations. Natural seawater chlorophyll *a* was averaged to represent the entire feeding experience of the larvae. Average chlorophyll *a* concentration of the natural rations were corrected for a left skew by taking the reciprocal of the average chlorophyll content to meet the assumptions of normality and homoscedasticity.

All transformed lipid data met the assumptions of homoscedasticity according to Cochran’s test. All lipid data sets were normally distributed, except that of March ‘04, according the Komogorov-Smirov test with a Lilliefors option. ANOVA’s are robust to departures from normality (Underwood 1997) so the March ‘04 data set was interpreted

using parametric statistics. Data from the May ‘04 and August ‘04 fecal pellet trials were not homoscedastic for the treatments of food and stage, respectively. Data from the average chlorophyll *a* concentration of the natural rations violated the assumption of homoscedasticity as well. Nevertheless, these data were analyzed with parametric statistics because ANOVA’s are robust to violations of homoscedasticity (Underwood, 1997).

To test for differences in dependent variables (lipid content, gut fluorescence, or fecal pellet production) within each trial set, two-way factorial ANOVA’s were performed with stage and food treatment considered as fixed factors. Gut fluorescence data of 2^{nd} stage nauplii from the January ’04 and March ’04 trials were analyzed with one-way ANOVA’s with food considered a fixed factor. One-way ANOVA’s were used in these trials to determine if the gut fluorescence of 2^{nd} stage nauplii differed among the three food rations. However, in other cases, nauplii were not present in the diluted ration and were thus not included in the analysis. Lipid content of 6^{th} stage nauplii was analyzed across trial using a one-way ANOVA with trial as a random factor. Lipid content and size of cyprid larvae were analyzed with two-way factorial mixed-model ANOVA’s (restricted version as recommended by Quinn and Keough 2002) with trial as a random factor and food as a fixed factor. A one-way ANOVA examined whether average chlorophyll *a* concentration of the natural food rations varied among trials.

Results

Naupliar lipid content, cyprid lipid content, and cyprid size from natural ration trials

In two trials (September ’03 and August ’04) nauplii contained similar amounts of lipid in supplemented and natural food treatments (Figure 1, Table 1). In two other trials (March ’04 and May ’04) nauplii from the supplemented ration contained more lipid than nauplii from the natural ration (Figure 1, Table 1). In one trial (January ‘04) lack of late stage nauplii from the natural ration prevented the assessment of lipid content. In all trials nauplii from the diluted ration contained little or no lipid. No significant effect of food concentration or trial was detected on and lipid content (Table 2), while trial had a significant affect on cyprid size (Table 3).

Only lipid data from 5^{th} and 6^{th} stage nauplii and cyprids from the supplemented and natural rations were included in the statistical analysis. The rarity of 5^{th} and 6^{th} stage nauplii in the diluted treatments and their lack of lipid in the earlier stages prevented their inclusion in the statistical analysis. Furthermore, January ’04 data could not be analyzed due to lack of 5^{th} and 6^{th} stage nauplii in the natural ration.

The null hypothesis is that the addition of phytoplankton food to the natural ration does not increase the lipid content of 5^{th} and 6^{th} stage nauplii relative to nauplii reared on solely a natural food ration. Lipid content of 5^{th} and 6^{th} stage nauplii differed significantly between supplemented and natural rations in trials run in September ’03, March ’04, and May ’04, but not August ’04 (Figure 1, Table 1). In the September ‘03 and August ’04 trials, multiple comparisons showed no significant differences in lipid content between supplemented or natural rations for 5^{th} stage nauplii alone or 6^{th} stage

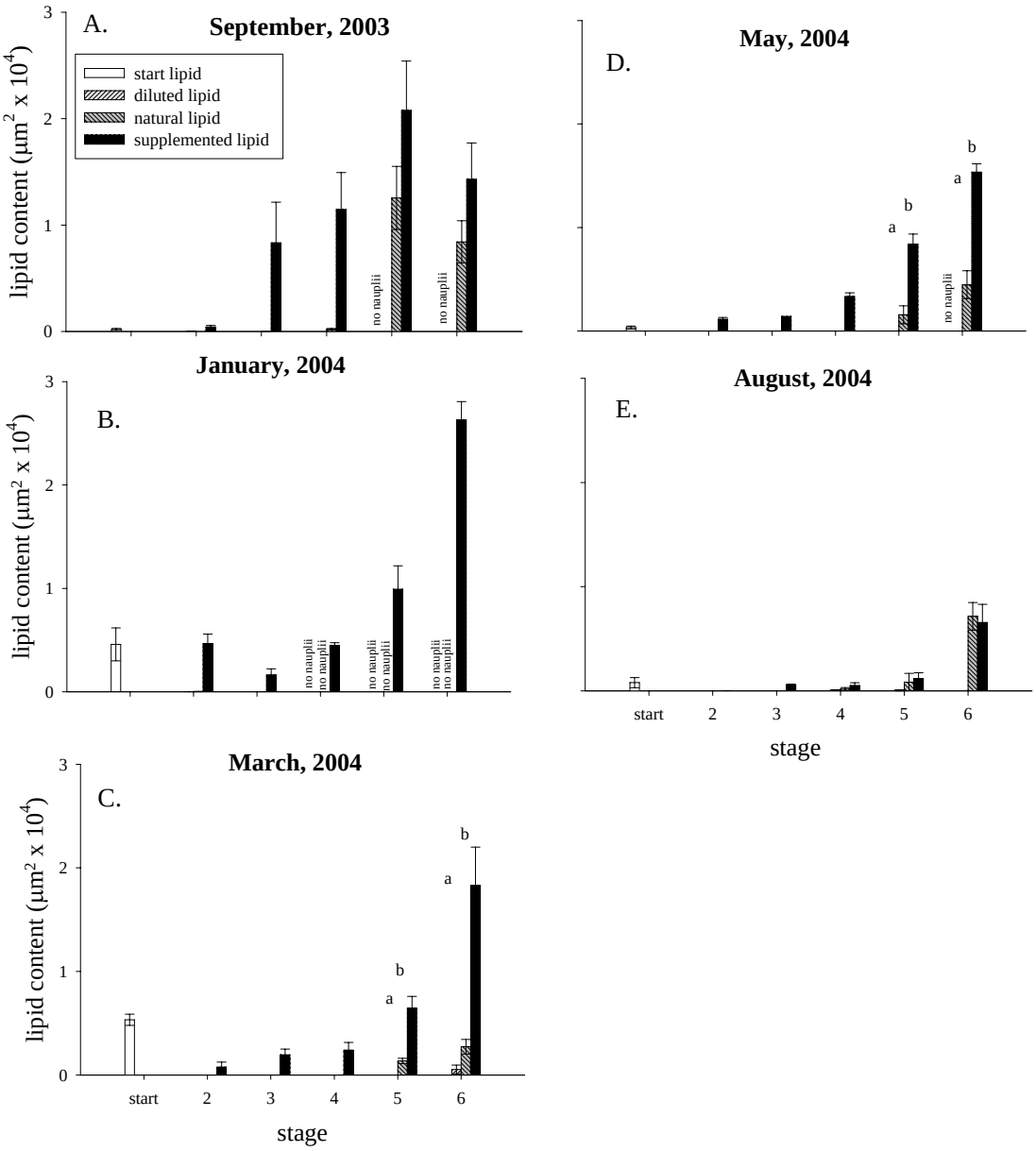

Figure 1 A-E. Mean lipid content ±1 std. error, measured as projected lipid area, of nauplii of *Balanus glandula* reared on natural rations throughout the year (n=4 jars per treatment). Bars above which different letters appear are statistically different among a given stage (Tukey HSD $p<0.05$). Start represents naupliar lipid content at hatching. The diluted ration contained a 1:4 dilution of 53μm-filtered seawater from the bay to 0.45mm filtered seawater from the laboratory, the natural ration contained only 53μm-filtered seawater, and the supplemented ration contained 53μm-filtered seawater plus $1x10^5$ cells/mL of the chain forming diatom *Skeletonema costatum*. Nauplii fed the diluted ration did not contain stained neutral lipids. Absence of nauplii from rations is noted in the figures.

Table 1. Two-way factorial ANOVAs on the effect of food treatment (supplemented and natural) and naupliar stage (5th and 6th) on lipid content in nauplii of *Balanus glandula* for 4 trials.

Trial	ss	df	Mean-square	F-ratio	*P*
September '03					
food	6647.00	1	6647.00	7.36	**0.019**
stage	4361.00	1	4361.00	4.83	**0.048**
food * stage	666.00	1	666.00	0.74	0.407
error	10832.04	12	902.67		
March '04					
food	1.65	1	1.65	55.13	**<0.001**
stage	0.37	1	0.37	12.17	**<0.01**
food * stage	0.05	1	0.05	1.65	0.223
error	0.36	12	0.03		
May '04					
food	35000.00	1	35000.00	106.00	**<0.001**
stage	17900.00	1	17900.00	54.10	**<0.001**
food * stage	5242.00	1	5242.00	15.90	**<0.01**
error	3966.36	12	330.53		
August '04					
food	103.00	1	103.00	0.24	0.634
stage	13300.00	1	13300.00	31.04	**<0.001**
food * stage	353.00	1	353.00	0.82	0.383
error	5154.71	12	429.56		

nauplii alone (Figure 1A&E, Tukey HSD, $p>0.05$). However, in March '04 and May '04 trials, nauplii reared with the supplemented ration contained greater lipid content than those of the same stages reared with the natural food ration (Figure 1C&D, Tukey HSD, $p<0.05$).

The null hypothesis is that naupliar stages do not differ in their lipid content. In all four trials where 5^{th} and 6^{th} stage nauplii were present (excludes January '04 trial), lipid content was significantly different between stages (Figure 1A, C-E; Table 1). Sixth stage nauplii contained more lipid than 5^{th} stage nauplii, except for the September '03 trial where 5^{th} stage nauplii contained more lipid than 6^{th} stage nauplii.

The null hypothesis is that food ration and trial do not affect naupliar lipid content and cyprid size and lipid content. In comparisons across trials, lipid content of 6^{th} stage nauplii reared with natural rations did not differ (Figure 2, ANOVA $F_{3,12} = 3.31$, $p=0.057$). Cyprid lipid content and size did not differ significantly among the natural and supplemented food treatments (Figure 3A&B, Tables 2 & 3). Trial and the interaction of trial and food significantly affected cyprid size (Figure 3B, Table 3). Cyprids from the August '04 trial were smaller than those from all other trials while cyprids from the September '03 trial were larger than those from the August '04 trial or May '04 trial (Tukey HSD, $p<0.05$). Cyprids ranged in size from approximately $1.1x10^5 \mu m^2$ to $2.0 x10^5 \mu m^2$.

The null hypothesis is that the mean seawater chlorophyll *a* concentration does not differ among trials. The mean seawater chlorophyll *a* concentration of the natural rations differed significantly among trials (Figure 4, $F=4.295$, $p=0.006$). A Tukey HSD

test revealed a significant difference only between September ‘03 and January ‘04 trials (Figure 4, Tukey HSD, $p<0.05$). The average chlorophyll *a* concentrations of the natural rations in the September ‘03 and August ‘04 trials were approximately 10μg/L, although daily amounts varied most highly in August ’04. During the January ‘04, March ‘04, and May ‘04 trials, average chlorophyll *a* was under 5μg/L in the natural rations.

The null hypothesis is that the average chlorophyll *a* of the natural rations does not correlate with the naupliar and cyprid lipid content. The lipid content of 6th stage

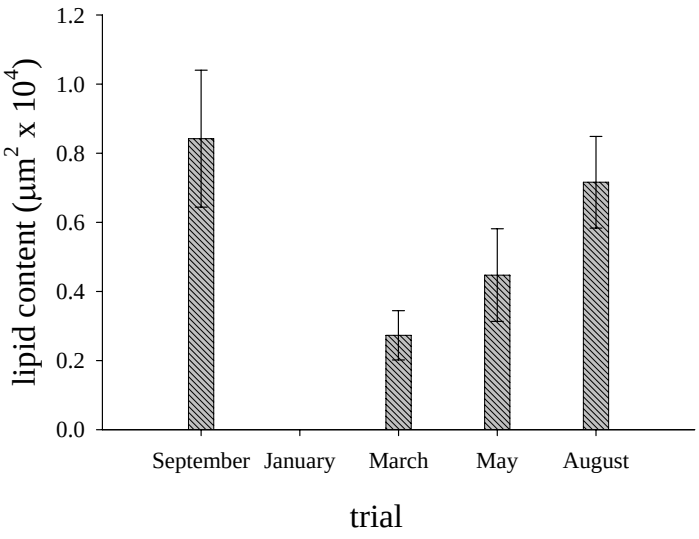

Figure 2. Lipid content of 6th stage nauplii of *Balanus glandula* reared on natural rations throughout the year. Bars represent means ±1 std. error for 4 jars within each experimental trial.

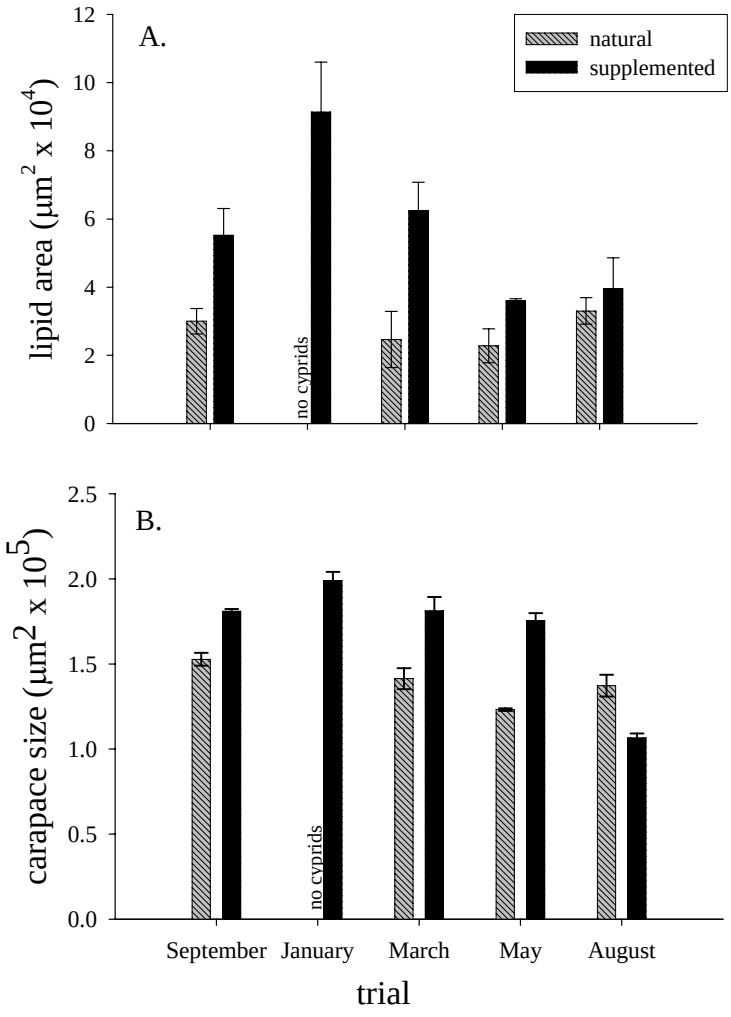

Figure 3 A&B. Cyprid lipid content (A) and size (B) of *Balanus glandula* reared with natural or supplemented rations. Bars represent means ±1 std. error for 4 jars within a ration.

Table 2. Two-way factorial way ANOVA on the effect of trial and food (supplmented and natural) on the lipid content of 3 day old cyprids.

Effect	ss	df	Mean-square	F-ratio	*P*
food	3.20E+09	1	3.20E+09	9.53	0.054
trial	7.65E+08	3	2.55E+08	1.88	0.161
trial * food	1.01E+09	3	3.35E+08	2.48	0.087
error	3.11E+09	23	1.35E+08		

Table 3. Two-way factorial ANOVA on the effect of trial and food (supplemented and natural) on cyprid size.

Effect	ss	df	Mean-square	F-ratio	*P*
food	3.47E+09	1	3.47E+09	1.50	0.308
trial	8.23E+09	3	2.74E+09	29.48	**<0.0001**
trial * food	6.94E+09	3	2.31E+09	24.86	**<0.0001**
error	1.86E+09	20	9.30E+07		

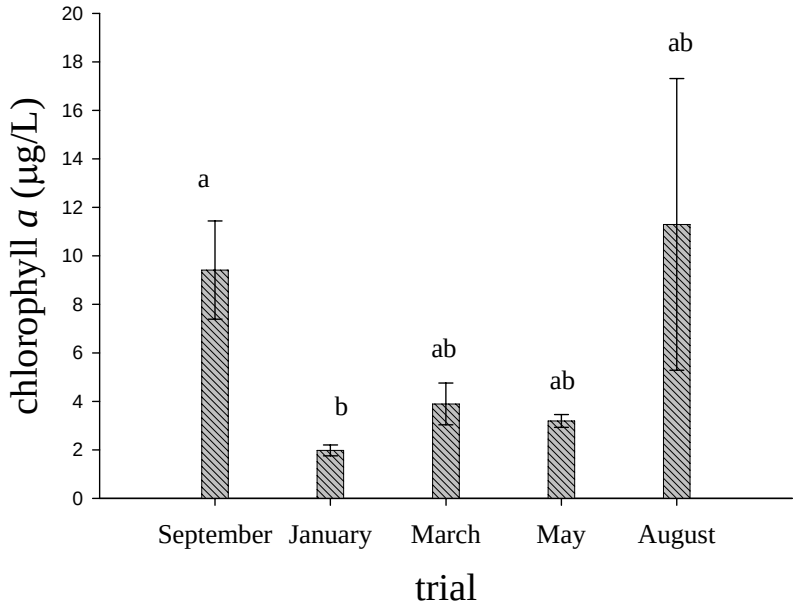

Figure 4. Seawater chlorophyll *a* concentration of the natural rations used in the experimental trials. Bars represent means ±1 std. error of chlorophyll *a* in the natural rations over the duration of a trial (the number of days chlorophyll *a* was measured varies based on length of trial). Bars above which the same letter appears are not statistically different (Tukey HSD, $p<0.05$).

nauplii and cyprids reared with natural rations increased with the average chlorophyll *a* concentration of the natural rations. Lipid content of 6th stage nauplii did not show a statistically significant correlation with the chlorophyll *a* of the natural ration though, most likely due to low replication (Figure 5, Linear regression t=2.48, p=0.13). However, lipid content of three day-old cyprids reared with natural seawater increased significantly with chlorophyll *a* concentration of the natural rations (Figure 6, Linear regression t=5.44, p=0.032).

Naupliar stage duration in natural rations trials

Just as trials differed in how the larvae in natural and supplemented treatments accumulated lipid, there was also variation among trials and food treatments in the duration of developmental stages. The average day a naupliar stage first appeared and was last found in a food ration did not differ between supplemented and natural rations in the September '03 or August '04 trials but did in the January '04, March '04, and May '04 trials (Figure 7A-E). In the September '03 trial, nauplii fed natural or supplemented rations developed to the 6th naupliar stage in 8 and to cyprid stage in 11 days. In the September '03 trial, naupliar stages in the diluted ration appeared later and remained longer than those in the natural and supplemented rations. In the August '04 trial, 6th stage nauplii first appeared at 8 days in the natural food treatment and at 10 days in the supplemented food treatment, while cyprids first appeared at 15 days in both food treatments (Figure 7E). No difference in stage duration between any rations was detected in the August '04 trial. In the January '04 trial nauplii receiving

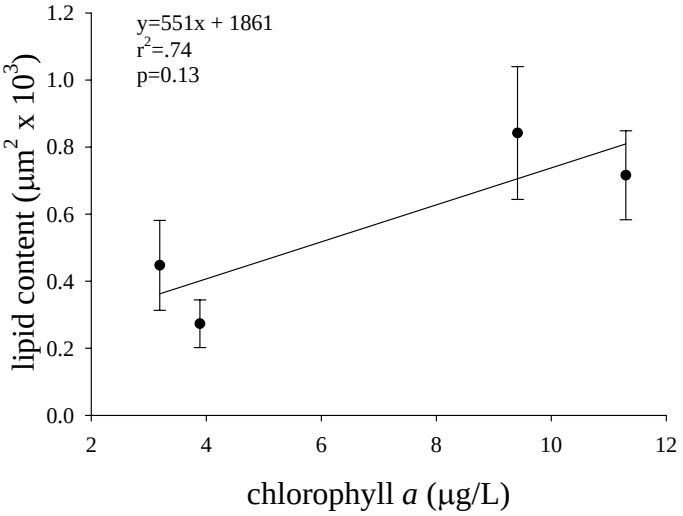

Figure 5. Mean lipid content (±1 std. Error) of 6th stage nauplii of *Balanus glandula* fed natural rations vs mean chlorophyll *a* concentration of 53-μm filtered seawater. Lipid content is the mean of 4 jars for each trial, chlorophyll *a* is the same as in figure 4. Trials conducted in September '04, March '04, May '04, and August '04 are shown. In the January '04 trial, no 6th stage nauplii were present in the natural ration. Line represents a least square linear regression through all data points.

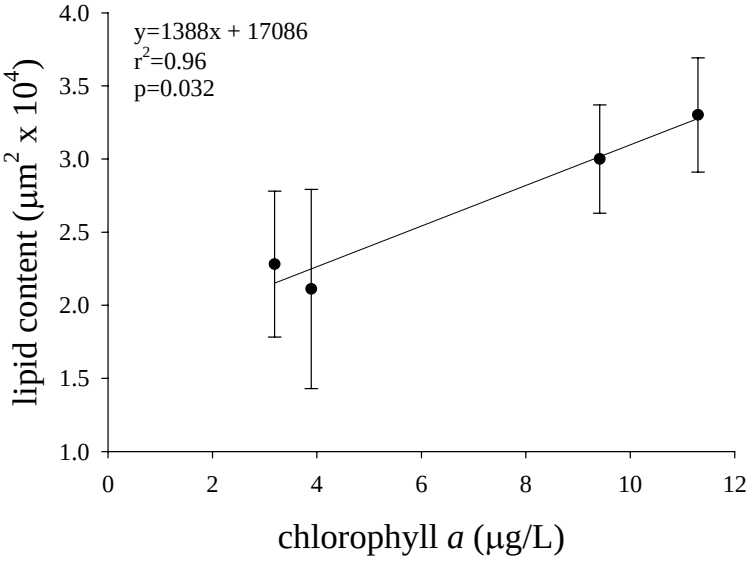

Figure 6. Mean lipid content (±1 std. Error) of 3-day old cyprids of *Balanus glandula* fed natural rations vs mean chlorophyll *a* concentration of 53-mm filtered seawater. Lipid content is the mean of 4 jars for each trial. Chlorophyll *a* is the same as in figure 4. Trials conducted in September '04, March '04, May '04, and August '04 are shown. In the January '04 trial, no cyprids were present in the natural ration. Line represents a least square linear regression through all data points.

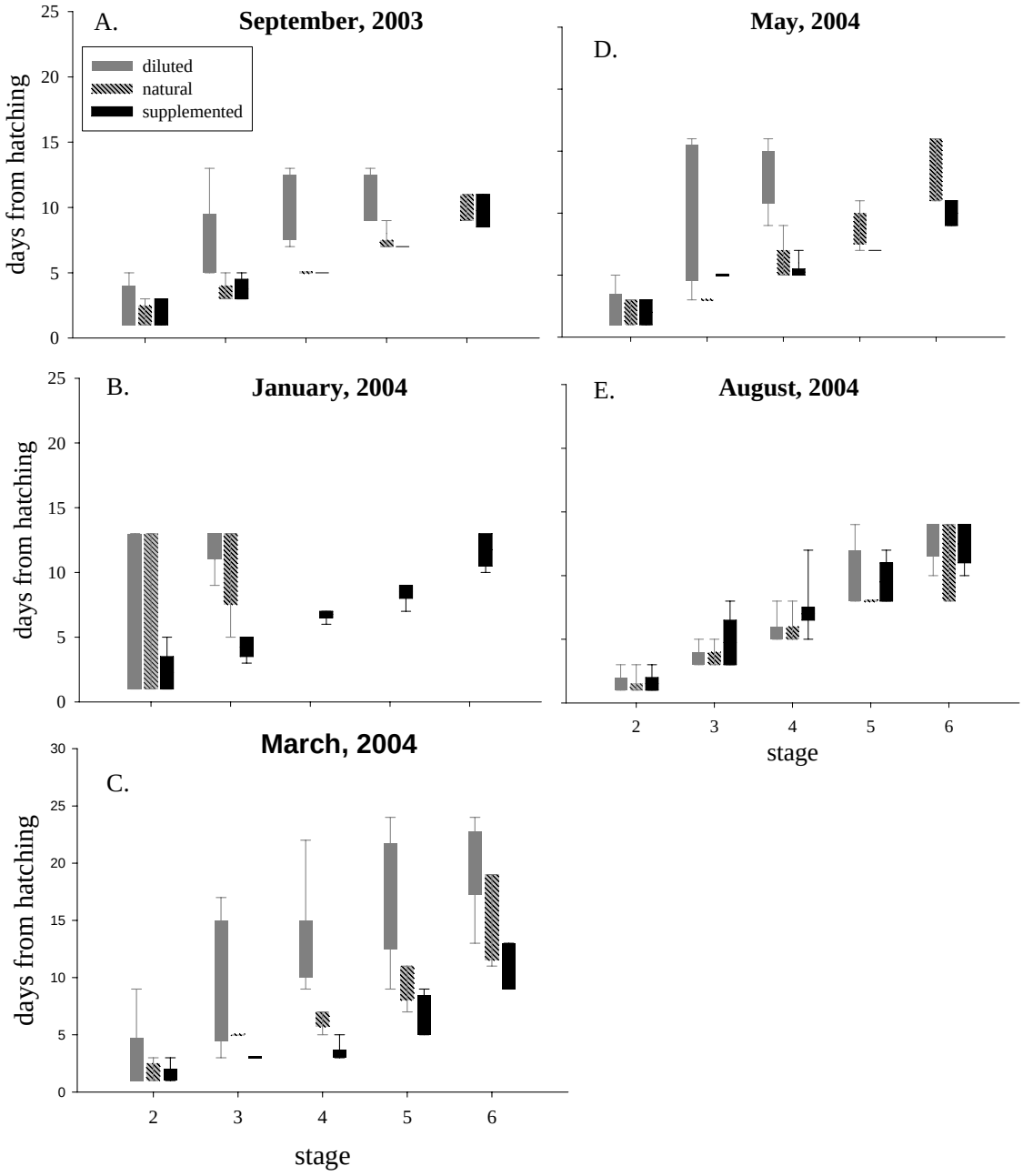

Figure 7A-E. Duration in stage of nauplii of *Balanus* glandula reared with natural rations of phytoplankton. The vertical height of the bars represent mean (n=4 jars/food ration) days naupliar stage was present in a ration, with the lower end representing mean time after hatching that stage occurred in a ration and the upper end representing the mean time after hatching the stage last occurred in a ration. Lower error bars represent first appearance of a naupliar stage in a ration. Upper error bars represent the last day a naupliar stage was occurred in a ration.

the natural or the diluted ration did not develop past stage 3 by day 13 when the trial was terminated (Figure 7B). In the January ’04 trial, nauplii from the natural ration metamorphosed from 2nd to 3rd stage later than those in the supplemented ration but before those in the diluted ration. Naupliar stages from natural rations in the March ‘04 trial appeared later and remained longer than naupliar stages from the supplemented ration, but appeared sooner and developed more rapidly than those from the diluted ration (Figure 7C). In the March ’04 trial, nauplii from the natural ration developed to the 6th naupliar stage by day 12 and to the cyprid stage by day 18. In the May ’04 trial, 2nd through 5th stage nauplii from natural ration appeared at similar times but remained in their respective stages longer than nauplii of equivalent stages from the supplemented ration (Figure 7D). In the May ’04 trial 6th stage nauplii from the natural ration appeared later and remained longer than 6th stage nauplii from supplemented ration (Figure 7D). In the May ’04 trial nauplii in the natural ration developed to the 6th naupliar stage and to the cyprid stage at days 12 and 15, respectively, while nauplii in the supplemented ration developed to the 6th naupliar stage and to the cyprid stage at days 9 and 11, respectively (Figure 7D).

Naupliar gut fluorescence and fecal pellet production from natural ration trials

Gut fluorescence in the various naupliar stages varied among trials. Every naupliar stage was not assessed for gut fluorescence from each trial. Early larval stages in the diluted ration treatment had fluorescent pigments in their guts in September ’03, March ’04, May ’04, and August ’04 and may have in January ’04, but these stages were

not in samples collected. Similarly, samples of late stage nauplii were not collected for gut fluorescence from the supplemented ration in the August ’04 trial and the March ’04 trial. In other cases (e.g. January ’04 trial, 4-6th stage nauplii from natural and diluted rations) nauplii were not present in sufficient quantities or at all for assessment of gut fluorescence.

In the September ’03 trial, the amount of fluorescent pigment in the naupliar gut did not differ significantly between supplemented and natural rations, but did differ across 3rd-6th stages (Figure 8A, Table 4). In the August ’04 trial, gut fluorescence of 5th and 6th stage nauplii did not differ significantly between supplemented and natural rations or stages (Figure 8E; Table 4).

In contrast to the September ’03 and August ’04 trials, food treatment significantly affected the gut fluorescence of equivalently staged nauplii in the January ’04, March ’04, and May ’04 trials (Figure 8, Table 4). In the January ‘04 and March ‘04 trials, food treatment affected gut fluorescence in stage two nauplii (Figure 8B&C, ANOVA: January, $F_{2, 9} = 23.74$, $p<0.001$, March, $F_{1,9} = 56.95$, $p<0.001$) such that nauplii from the supplemented rations had significantly more gut fluorescence than nauplii from either the natural or diluted ration (Tukey HSD, $p<0.05$). Fifth stage nauplii fed supplemented rations in the March ‘04 trial contained more gut fluorescence than nauplii fed a natural ration (ANOVA $F_{1,6} = 225.30$, $p<0.001$, Tukey HSD, $p<0.05$). In the May ‘04 trial, gut fluorescence of 4th-6th stage nauplii differed significantly between supplemented and natural food treatments and stage, and also there was a statistically significant interaction of the factors (Figure 8D; Table 4). Fifth or sixth stage nauplii

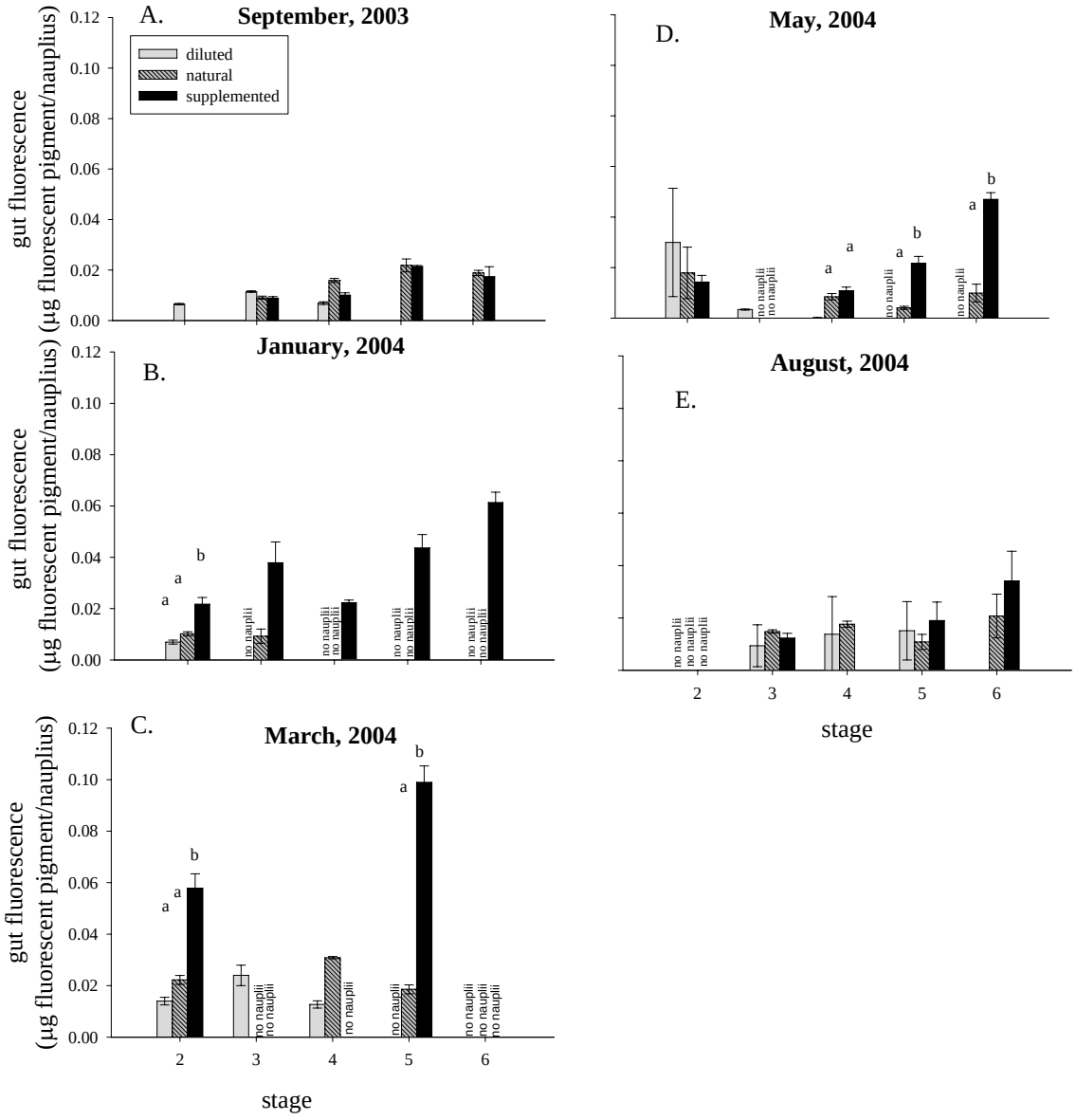

Figure 8 A-E. Gut fluorescence (µg/nauplius) of nauplii of *Balanus glandula* reared with natural rations of phytoplankton. Bars represent means ±1 std. error (n=4 jars/ration). Rations are as in figure 1. Bars above which different letters appear are statistically different among a given stage (Tukey HSD $p<0.05$). Absence of naupliar stages from assessment of gut fluorescence is noted.

Table 4. Two-way factorial ANOVA on the effect of food (supplemented and natural) and stage on gut fluorescence of nauplii of *Balanus glandula*. Naupliar stages analyzed are indicated.

Trial	ss	df	Mean-square	F-ratio	*P*
September '03					
stage (3-6)	0.012	3	0.004	19.350	**<0.0001**
food	0.001	1	0.001	2.970	0.099
food * stage	0.001	3	0.000	1.170	0.343
error	0.004	22	0.000		
May '04					
stage (4-6)	0.016	2	0.008	23.393	**<0.0001**
food	0.031	1	0.031	89.858	**<0.0001**
food * stage	0.012	2	0.006	17.542	**<0.0001**
error	0.006	18	0.000		
August '04					
stage (5-6)	0.006	1	0.006	2.544	0.139
food	0.005	1	0.005	1.940	0.191
food * stage	0.000	1	0.000	0.049	0.829
error	0.026	11	0.002		

reared with supplemented rations in May ’04 had higher gut fluorescence than their equivalent stages reared with natural rations (Tukey HSD, $p<0.05$).

Naupliar fecal pellet production was determined in the March ’04, May ’04, and August ’04 trials only and, the naupliar stages assessed for fecal pellet production varied between trials. Early larval stages from all rations in the May ’04 and August ’04 trials were present in culture jars but some food treatments were not sampled for fecal pellet production. Fecal pellet production was not assessed in late-stage nauplii from the diluted rations because of insufficient numbers or absence of these stages.

In the August ‘04 trial, food treatment, stage, and their interaction significantly affected fecal pellet production of 3^{rd}-5^{th} stage nauplii (Table 5). In the August ’04 trial, 4^{th} and 5^{th} stage nauplii from the natural ration produced significantly more fecal pellets than similarly staged nauplii from the supplemented ration (Figure 9C, Tukey HSD, $p<0.05$). In the March ’04 trial, food treatment, stage, and the interaction of these factors significantly affected fecal pellet production of 2^{nd}-4^{th} and 6^{th} stage nauplii (Table 5). Similarly, in the May ’04 trial, food treatment, stage, and their interaction significantly affected fecal pellet production of 2^{nd}, 4^{th} and 5^{th} stage nauplii (Table 4). Unlike the August ’04 trial, in the March ’04 and May ’04 trials, late stage nauplii from the supplemented ration produced more fecal pellets than similarly staged nauplii from the natural ration (Figure 9A & B, Tukey HSD, $p<0.05$).

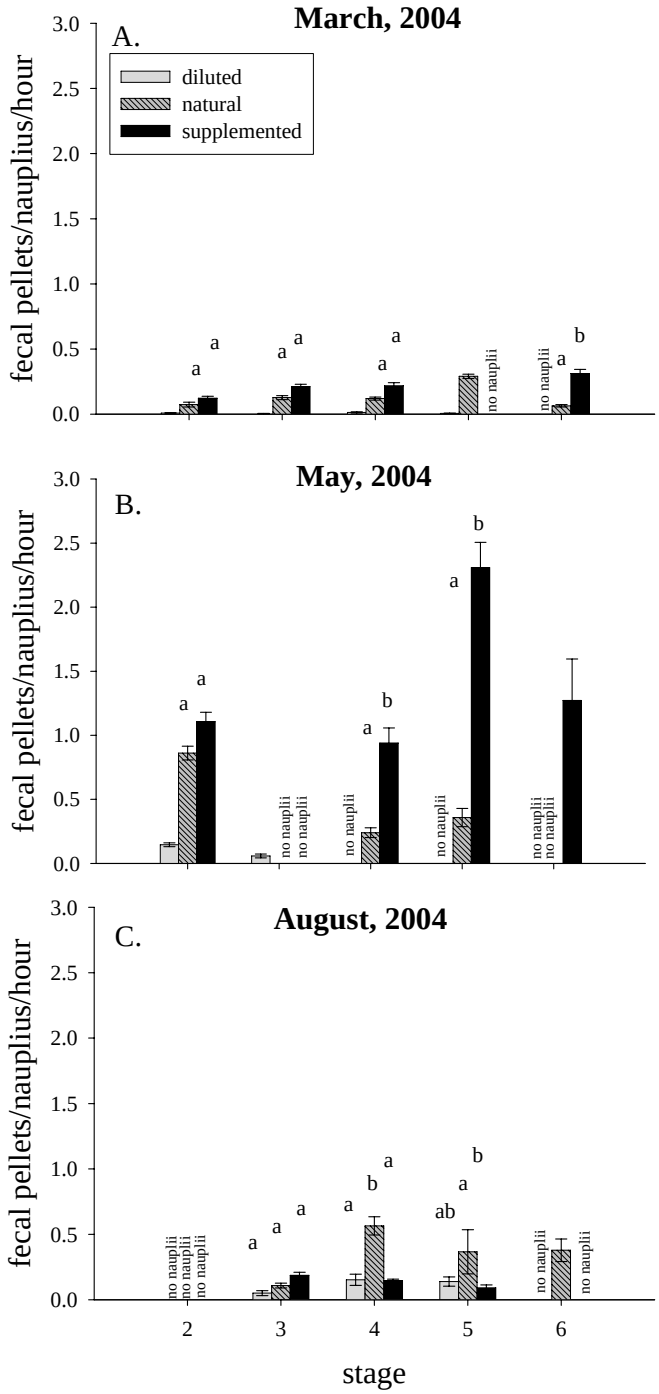

Figure 9 A-C. Fecal pellet production of nauplii of *Balanus glandula* reared with natural rations. Bars represent mean number ±1 std. error of fecal pellets produced per nauplius per hour (n=4 jars/treatment). Bars above which different letters appear are statistically different among a given stage (Tukey HSD $p<0.05$). Absence of naupliar stages from assessment of fecal pellet production is noted.

Table 5. Two-way factorial ANOVA for the effect of food (where D= diluted, N=natural, S=supplemented rations) and stage on fecal pellet production of nauplii of *Balanus glandula*. Stages tested are indicated.

Trial	ss	df	Mean-square	F-ratio	*P*
August '04					
food (S, N, D)	0.445	2	0.223	12.510	**<0.0001**
stage (3-5)	0.223	2	0.112	6.280	**0.005**
food * stage	0.281	4	0.070	3.960	**0.010**
error	0.605	34	0.018		
May '04					
food (S & N)	0.500	1	0.500	75.340	**<0.0001**
stage (2, 4-5)	0.132	2	0.066	9.950	**0.001**
food * stage	0.202	2	0.101	15.240	**<0.0001**
error	0.146	22	0.007		
March '04					
food (S & N)	0.056	3	0.019	2.910	**0.047**
stage (2-4, 6)	0.265	1	0.265	41.130	**<0.0001**
food * stage	0.089	3	0.030	4.610	**0.008**
error	0.232	36	0.006		

Discussion

This study compares lipid content, stage duration, gut fluorescence, and fecal pellet production of nauplii of *Balanus glandula* fed a natural diet with those fed a natural diet supplemented with lab grown phytoplankton or a natural diet diluted with filtered seawater in five trials over a one-year period. In general, nauplii in the diluted ration did not develop to the 5^{th} and 6^{th} stage in the trials and thus, assessment of lipid content and feeding indices were not performed. The lipid content, stage duration, gut fluorescence and fecal pellet production rates of nauplii given natural and supplemented food rations were similar in the trials conducted in September 2003 and August 2004, suggesting that lipid content and developmental rate of nauplii from the natural diet were not limited by food in these trials. The lipid content, stage duration, gut fluorescence, and fecal pellet production of nauplii given natural rations suggest that these larvae were food limited in the trials conducted in January, March, and May 2004. This pattern of limitation of lipid content and stage duration between trials is similar to that of gut fluorescence and fecal pellet production, suggesting that differences or similarities in naupliar feeding within the food treatments, and not another factor, may be responsible for the patterns of lipid content and development. The quantity of food available in the natural rations may not have maximized larval lipid content and development. However, food limitation may also have been caused by differences in food quality (Sterner and Hessen 1994) or by algae that are too large to be ingested.

Chlorophyll *a* concentrations through the year may explain differences in food limitation between trials. Chlorophyll *a* concentration is a proxy for phytoplankton abundance, an important food source for many marine larvae. The positive relationship between the lipid content of 6^{th} stage nauplii (Figure 5, $p=0.13$) and cyprids (Figure 6, $p=0.03$) with the average chlorophyll *a* concentration in seawater suggests that food concentration may limit the lipid content of larvae in the natural rations.

My data agree with other laboratory experiments that suggest natural rations limit the development, growth, and lipid content of planktotrophic marine larvae (Paulay et al. 1985, Fenaux et al 1994, Reitzel et al. 2004). Paulay et al. (1985) showed that the growth and development of echinoderms and mollusks were food limited when reared with natural rations, while Reitzel et al. (2004) suggested that larval sand dollar lipid content is limited by the food available in natural seawater. In subtropical waters in Florida, sand dollar larvae reared with natural rations showed reduced growth and longer development compared to larvae from a supplemented ration (Eckert 1995).

Lack of differences in lipid content and stage duration between natural and supplemented rations within September ’03 and August ’04 trials suggest, however, that nauplii of *Balanus glandula* are not food limited during some parts of the year. Other experiments using natural rations in mesocosms suggest that marine invertebrate zooplankton are not food limited in oligotrophic environments. Olson (1987a) showed maximal developmental rates of crown-of-thorns starfish larvae in Australia under normal phytoplankton conditions. Similarly, the Antarctic asteroid *Odontaster validus* develops normally under natural food conditions (Olson 1987b).

Studies suggest that crustacean larvae may be sensitive to fluctuations in food availability due to their trophic position (Olson and Olson 1989), but few studies have attempted to rear crustacean larvae in the laboratory under a natural food environment. Comparing lab-reared and field-sampled copepod developmental rate and size, Ohman (1985) suggested that *Pseudocalanus sp.* from Dabob Bay, WA was not food limited. However, at the same location, Frost (1985) showed that the egg production of *Pseudocalanus sp.* did not vary with chlorophyll *a* concentration, while the egg production of *Calanus pacificus* did, suggesting that food may affect species differently.

Hentschel and Emlet (2000), using similar techniques as in this study, found that cyprids of *Balanus glandula* reared in the laboratory with a higher food ration contained more lipid and obtained a larger size than cyprids reared with a lower food ration. The cyprid lipid content from the natural food treatments in this study falls between the values reported by Hentschel and Emlet (2000) for "high" and "low" food cyprids ($1x10^5$ and $1x10^4$ cells/mL of *Skeletonema costatum*, respectively) while cyprid lipid content from the supplemented rations in this study are similar to the cyprid lipid content of the high food treatment in Hentschel and Emlet (2000). Comparisons of the lipid content and size of cyprids I reared with natural rations to the results of Hentschel and Emlet (2000) suggest that the nutritional value of the natural ration is intermediary to "high" and "low" laboratory food rations. The lipid content of cyprids I reared with supplemented rations matches those of the constant high food treatment in Hentschel and Emlet (2000).

Few studies have linked differences in natural food availability over time with larval growth and development. Fenaux et al. (1994) suggested that the development and

growth of echinopluteus larvae of *Paracentrotus lividus* were limited in the spring and fall in the Mediterranean, although less so when the chlorophyll *a* concentration of the seawater was greater. Egg production of *Acartia tonsa* copepods has been linked with seawater chlorophyll *a* concentration throughout the year (Durbin et al. 1983). In the present study, naupliar lipid content and stage durations were food limited in trials with the lowest natural chlorophyll *a* concentrations, but naupliar food limitation was not detected during trials with higher chlorophyll *a* concentration. On the West Coast of North America, seasonal cycles of daylight, upwelling, and temperature affect the chlorophyll *a* concentration of the seawater such that phytoplankton blooms occur primarily in the early spring and throughout the summer (Small et al. 1972, Small and Menzies 1981, Pearcy and Keene 1974). Seasonal variation in phytoplankton standing stock, and presumably naupliar food availability, suggests there may be a seasonal pattern of food limitation in barnacle larvae. If the lipid content and size of cyprids of *Balanus glandula* are primarily influenced by food concentration, peaks in these parameters should occur around the months of the highest seawater chlorophyll *a* concentration (see chapter III). Phillips and Gaines (2002) found increases in lipid content, developmental rate, and size of field-sampled mussel larvae with increases of field chlorophyll *a* concentration. The size of mussel veligers settling in southern California decreases throughout the year, as does seawater chlorophyll *a* concentration, suggesting a coupling of larval size and food availability (Phillips and Gaines 2002).

My results suggest that during times of low food abundance, nauplii will take longer to develop, and thus remain in the plankton longer. Death rates of larvae are high

in the field (Morgan, 1995) and any increase in stage duration will impart an increased risk of mortality on an individual. Cyprid lipid stores positively affect metamorphic success to the juvenile stage (Thiyagarajan et al. 2002), rate of juvenile growth (Jarrett, 1997), and juvenile survivorship (Emlet and Sadro submitted manuscript). Barnacle cyprids with little energy stores have less time to search for settlement sites and may be less picky about settlement substrata than cyprids with greater energy stores (Pechenik et al. 1993), possibly leading to a less favorable adult habitat which may reduce adult fitness. Larval size (Marshall and Keough 2004) affects juvenile size and growth rate, two important life history traits that may affect competition for space in the intertidal (Connell, 1961). Larval pelagic duration may represent a tradeoff between risk of mortality in the plankton and ingesting enough energy to adequately metamophose to and perform as a juvenile.

Conclusions

This study investigated the effect of natural food availability on the feeding, lipid content, and developmental rate of larvae of *Balanus glandula* reared in the laboratory with seawater rations taken from the field. During periods when larvae were fed on natural rations (seawater rations taken from the field), larval lipid content, stage duration, naupliar gut fluorescence and fecal pellet prodcution match those from the supplemented ration (seawater plus lab reared phytoplankton) suggesting that larvae from the natural rations were not food limited. When naupliar gut fluorescence and fecal pellet production of larvae fed the natural ration were lower than those of the supplemented

ration, lipid content and stage duration were lower as well. The lipid content of 6th stage nauplii and cyprids were positively correlated with the chlorophyll *a* concentration of their natural rations, suggesting that food concentration may be an important factor limiting the energy stores and development of barnacle larvae. Furthermore, these results suggest that the natural food concentrations may limit the lipid content, size, and development of larvae of *Balanus glandula* in the field during some times of the year and not at other times of the year.

BRIDGE I

The results of Chapter II suggest that the lipid content and development of nauplii of *Balanus glandula* are at times food limited by natural phytoplankton rations. In trials conducted in September ’03 and August ’04, when chlorophyll *a* concentrations were highest, the lipid content and development of nauplii fed the natural phytoplankton ration matched that of a natural ration supplemented with laboratory grown phytoplankton. In trials conducted in January, March, and May, lipid content and stage duration of the natural ration were food limited compared to the supplemented ration.

The results of laboratory studies must be extrapolated to the field with caution, as larvae are exposed to a multitude of environmental factors in the field that cannot be reproduced in the laboratory. Although food fluctuates seasonally in the field, so do other factors such as temperature, turbulence, and upwelling. It is not known to what extent these parameters influence larval size and lipid content in the field. Chapter III measures the size and lipid content of *Balanus glandula* larvae from the field, along with chlorophyll *a* concentration to understand the effect of natural variations in food availability on larval condition in the field. In addition, seawater chlorophyll *a* was investigated as a possible proxy for food availability via correlations with fecal pellet production rates.

CHAPTER III

LINKING LARVAL CONDITION WITH FIELD FOOD AVAILABILITY AND SEAWATER TEMPERATURE

Introduction

Marine invertebrate life cycles often include a free-swimming, planktotrophic larval stage that feeds on phytoplankton while growing and developing in the water column (Pechenik 1999). Larvae may spend hours to months in their pelagic environment, where they may be exposed to temporal and spatial fluctuations in a host of physical and biological parameters. For planktotrophic larvae, exposure to variations in food availability may affect larval developmental rate (Eckert 1995, Meidel et al. 1999, Qiu and Qian 1997, Reitzel et al. 2004), size at metamorphosis (West and Costlow 1987, Paulay et al. 1985, Fenaux et al. 1994), energetic stores (Gallager and Mann 1981, Hentschel and Emlet 2000, Phillips 2002), and survivorship (Epifanio et al. 1991, Qiu and Qian 1997), while variations in temperature may affect larval size and developmental rate (Barnes 1953, Gallager and Mann 1981, Ouellet and Allard 2002, Emlet and Sadro submitted manuscript). The effects of larval feeding can persist through metamorphosis to the juvenile stage (reviewed by Pechenik et al. 1998). Larval energetic reserves

correlate positively with metamorphic success in barnacles (Thiyagarajan et al. 2002). Larger larval size leads to greater juvenile size in mussels (Phillips 2002), higher growth rates in barnacles (Jarrett and Pechenik 1997), and increased survivorship in barnacles (Emlet and Sadro submitted manuscript).

Many studies have reported that larvae sampled from the field vary in size and energy stores (Barnes 1953, Phillips and Gaines 2002, Ouellet and Allard 2002, Jarrett 2003). In one study, shell size of mussel veligers differed among spring, summer, and fall, as well as among sampling sites separated by approximately 50km (Phillips and Gaines 2002). In another study, barnacle cyprid organic content varied over the course of two months (Jarrett and Pechenik 1997). Although variations in larval size and energy stores between samples are commonly observed in the field, few studies have attempted to explain the reasons for these differences by measuring larval size and energy content and food availability simultaneously.

Several studies have pointed to indirect and direct effects of physical oceanic parameters to explain the variation in larval size and energy stores. Phillips and Gaines (2002) suggested that temporal and spatial differences in larval size were due to differences in food availability that was caused partially by seasonal cycles of upwelling. Larval food availability is closely linked with phytoplankton standing stock (Fenaux et al. 1994), which, in turn, is influenced by available nutrients, temperature day-length, and water stratification (Small et al. 1972, Small and Menzies 1981, Pearcy and Keene 1974, reviewed by Mackas et al. 1985).

In upwelling systems, such as the West Coast of North America, cold, nutrient rich water is brought to the surface by wind-driven offshore transport of the surface water in the spring and summer. Although upwelling may increase available food for invertebrate larvae, it may also transport them away from suitable settlement substrata, thereby delaying metamorphosis of those transported offshore (Roughgarden et al. 1988). For barnacle larvae, upwelling may serve to increase larval energy stores through naupliar feeding, but may also decrease energy reserves in non-feeding cyprids by delaying settlement. Jarrett (2003) and Miron et al. (1999) suggested that a delay in settlement and the corresponding use of energy stores caused the observed decline in the organic content of field caught cyprids. However, Ouellet and Allard (2002) suggested that seasonal differences in water temperature determined differences in size and energy reserves in lobster larvae in the field. Although evidence exists to support all of these explanations, these studies did not directly correlate variation in field environmental parameters with variation in condition of field caught larvae.

The common intertidal acorn barnacle *Balanus glandula* is a good model organism to study how changing environmental conditions affect larval size and lipid content. Found from Baja California to Alaska, *B. glandula* reproduces ten months of the year in some habitats of coastal Oregon (Berger 2004) and its offspring are, hence, exposed to a variety of environmental conditions. Larvae of *B. glandula* pass through six feeding naupliar instars and a non-feeding cyprid stage before they settle to benthic adult habitat. When reared under variable food environments, the sizes of the first five naupliar stages are fixed while the 6th naupliar stage and the cyprid vary in size (Emlet

unpublished). This matches patterns published for larvae of *Balanus eburneus* (West and Costlow 1987). During the naupliar stages the size and energy reserves of the larvae increase via feeding in the water column (Holland and Walker 1975). Lipids and proteins accumulated by nauplii are used as energy by the cyprids to search for suitable settlement substrata and for metamorphosis into juveniles (Lucas et al 1979, Holland 1987). The duration of the cyprid stage is limited by larval energy reserves accumulated as a nauplius and may last for up to four weeks in cyprids of *Semibalanus balanoides* (Lucas et al. 1979). At the time of capture, cyprid lipid stores may be a product of both the naupliar and cyprid pelagic experience, but cyprid size is affected the pelagic experience of the nauplius. Lipid reserves are easily visualized by staining nauplii with the lipid-specific stain Nile Red, which causes neutral lipids to fluoresce bright yellow and polar lipids to fluoresce dull red under epifluorescent light (e.g. Hentschel and Emlet 2000).

To examine the link between variation in the environment and larval condition, this study correlates the size and lipid content of field-caught cyprids and nauplii of the intertidal barnacle *Balanus glandula* with chlorophyll *a* concentration, temperature, and upwelling data over the course of one year in Coos Bay region (September 2003-August 2004). Furthermore, this study correlates the fecal pellet production of field-caught nauplii of *B. glandula* with seawater chlorophyll *a* concentration in order to determine if chlorophyll *a* concentration determines feeding rates.

Methods

Larval collection and preservation

Larvae of *Balanus glandula* were obtained from the field by plankton tows for analysis of size, lipid content, and feeding-rate. Plankton tows were made using a 130μm mesh plankton net towed for approximately 10 minutes at the mouth of the Coos Bay estuary within one hour of the day-time high tide (high-high or high-low tide). The resulting plankton sample was placed in 3 liters of 0.45μm-filtered seawater (FSW). Positively phototactic zooplankton were concentrated by shining a fiber optic light source at the edge of the jar. About 15mL of this concentrated plankton were collected using a turkey-baster, frozen in 15mL falcon tubes with liquid nitrogen, and placed in a -80°C freezer.

Later, the concentrated plankton samples were thawed and sorted to select nauplii and cyprids of *B. glandula* (Brown 1985, Standing 1980). Nauplii of *B. glandula* were identified based on their size, which is generally smaller for a given stage than that of other local nauplii and the unique 45-degree angle of their frontolateral horns compared to their naupliar shield (Arnsberg 2001). Cyprids of *Balanus glandula* are easily identified based upon their brown pigmentation and the pitted sculpturing of their carapace (Standing 1980). Larvae were placed in 1.7 mL microcentrifuge tubes with 1 mL of FSW, frozen with liquid nitrogen (Hentschel and Emlet 2000), and stored in –80°C freezer (Ohman, 1996) for later lipid and size determination. The effects of freezing, thawing, and refreezing larvae on lipid content is not known, although there is no indication that it changed the appearance or sizes of neutral lipid droplets from those

cyprids that were frozen once. Also freezing copepods once did not affect measures of lipid content (Ohman, 1996).

Assessing the condition of barnacle larvae

To assess larval condition of *B. glandula*, larval size and lipid content were measured. The number of larvae collected varied by sampling date, but did not exceed 20 individuals per sampling day. Larval lipids were stained by incubation in a 1:4 dilution of Nile Red Stock (2.5mg/100mL acetone) to FSW for one hour (Hentschel and Emlet 2000). Larvae were rinsed with FSW and photographed in color under epifluorescent light using a CCD video camera with the gain set to off. Images were captured and measurements were made using Optimas 5.2 software (Silver Spring, Maryland, USA).

Lipid content was estimated by quantifying the projected area of stained neutral lipid droplets from digital images of nauplii and cyprids. This two dimensional measure was determined from predetermined color thresholds in Optimas 5.2 that were created by manually selecting the neutral lipid color emitted from 10 stained larvae. Separate thresholds were created for nauplii and cyprids because the color of neutral lipid differs depending on whether it is shining through the cyprid carapace or naupliar shield (personal observation). The size of cyprids was quantified by manually tracing the outline of the carapace from the digital image used in lipid determination. Although the size of individual cyprids were not repeatedly measured to determine the accuracy of this

method, a similar method of determining cyprid size was used by Hentschel and Emlet (2000) and produced consistent results.

Determination of food availability by fecal pellet production rates

Food availability in natural seawater was estimated by fecal pellet production rates. Nauplii of *Balanus glandula* were sorted from live plankton samples and 1-5 nauplii were placed in 50mL centrifuge tubes containing 53μm-filtered seawater. Tubes were placed on a roller table for approximately 24 hours at 12°C. This roller table was created to study marine snow, but also keeps larvae and phytoplankton in suspension (Larson and Shanks 1996, Smart 2003). Tube contents were filtered down to 5mL with a turkey baster fitted with 20μm mesh and preserved with 0.5mL 4% buffered formalin. Fecal pellet production per nauplius was estimated by counting two 1-mL sub-samples from each tube on a Bogorov tray, averaging these counts, and dividing by the number of nauplii in the tube. The number of replicate tubes of each naupliar stage varied from 2-4 on any day, depending upon the number of nauplii obtained from the plankton sample.

Physical parameter data collection

Samples of seawater for the determination of chlorophyll *a* concentration were taken at the mouth of Coos Bay estuary when plankton samples were collected. In the lab, a portion of this seawater was filtered through a 53μm nylon filter for use in feeding experiments. Raw and 53μm-filtered seawater samples were filtered onto 3μm glass microfiber filters, placed directly into 10mL of 90% acetone, and stored in the dark at –

20C for at least 24 hours for chlorophyll *a* extraction. Fluorescence of these samples was read before and after acidification with 5% HCl on a Turner Model TD 700 fluorometer fitted with chlorophyll *a* specific excitation and emission filters. Chlorophyll *a* concentration in μg/L was calculated with the equations of Parsons et al. (1984). Data on seawater temperature was taken from buoy 46050 located off the Oregon Coast (http://www.ndbc.noaa.gov/Maps/Northwest.shtml). Buoy 46050 is located at 44°37'16"N 124°31'42" W, 32km northwest of Newport, Oregon. The mouth of Coos Bay, where plankton and water samples were collected, is located 160 km south of buoy 46050. Because buoys to the north (buoy # 46050) and the south (buoy # 46015) show similar trends in seawater temperature (personal observation), data from buoy 46050 are assumed to accurately represent the conditions offshore of Coos Bay, Oregon. Upwelling index is based on a model that uses differences in atmospheric pressure to estimate Ekman transport. Estimates of upwelling index at 42N 125W (the site closest to the buoy 46015) were taken from the Pacific Fisheries Environmental Laboratory website (http://www.pfeg.noaa.gov).

Statistical analysis

The effect of capture day on cyprid lipid content and size, as well as the effect of stage on fecal pellet production, were analyzed by Kruskal-Wallis tests, due to violations of normality. Data on fecal pellet production/nauplius/hour for separate stages were square root transformed to meet the assumptions of homoscedasticity and normality for parametric statistical analysis. Linear and curvilinear regressions were fitted to all data

points with SigmaPlot 6.0 for Windows. The curvilinear model used was $y=a(1-e^{-bx})$, where *a* is the asymptotic maximum value of Y (fecal pellet production/nauplius/day) and *b* is the rate of change of Y with respect to X (chlorophyll *a* concentration) (Besiktepe and Dam 2002). Least square linear regressions were fit to all data points using Statistica 6.0 for Windows.

Results

Seawater chlorophyll a concentration

Raw seawater chlorophyll *a* concentration varied during the year (Figure 1). From November through February chlorophyll *a* concentrations were approximately 0.3-2μg/L. From March through the beginning of April, chlorophyll *a* concentration increased to 6 μg/L, and on one day was 20 μg/L. Then it decreased to under 2 μg/L near the end of April. Seawater chlorophyll *a* concentration continued to fluctuate through the summer, but remained above about 5μg/L from mid June to mid August. There were three days in August where chlorophyll *a* concentrations were 19, 21, and 40 μg/L..

Cyprid size and lipid content

Cyprid size, measured as carapace area, and lipid content, measured as the projected area of stained neutral lipids, varied among sampling dates (Figure 2A, Kruskal-Wallis, size: $H=119.38$, d.f.=34, $p<0.001$, lipid: $H=95.36$, d.f.=34, $p<0.001$). Cyprids were largest in September through October of 2003 and April through early May of 2004, with mean sizes ranging from 1.8 to 2.2 x $10^5 \mu m^2$. Cyprid lipid content was

highest during these months and ranged from 4.5 to 9.4 x 10^4 μm^2. The smallest cyprids with the least lipid were collected in November and December of 2003 and May of 2004, with size and lipid distributions of 1.4-1.6 x 10^5 μm^2 and 1.5-3.3 x 10^4 μm^2, respectively. Cyprid size and lipid content do not follow a seasonal trend, although the smallest cyprids with the least lipid content occurred in late fall '03 and late spring '04.

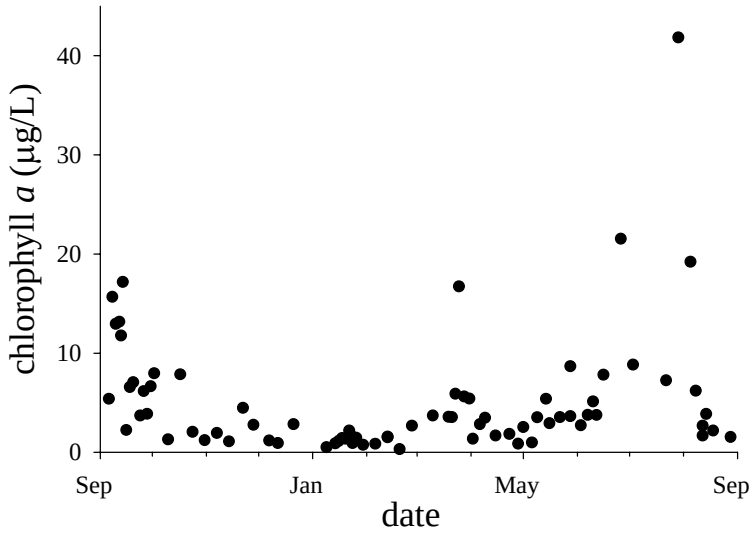

Figure 1. Chlorophyll *a* concentration of raw seawater samples taken in front of the OIMB boathouse in Charleston, OR. Points represent individual samples.

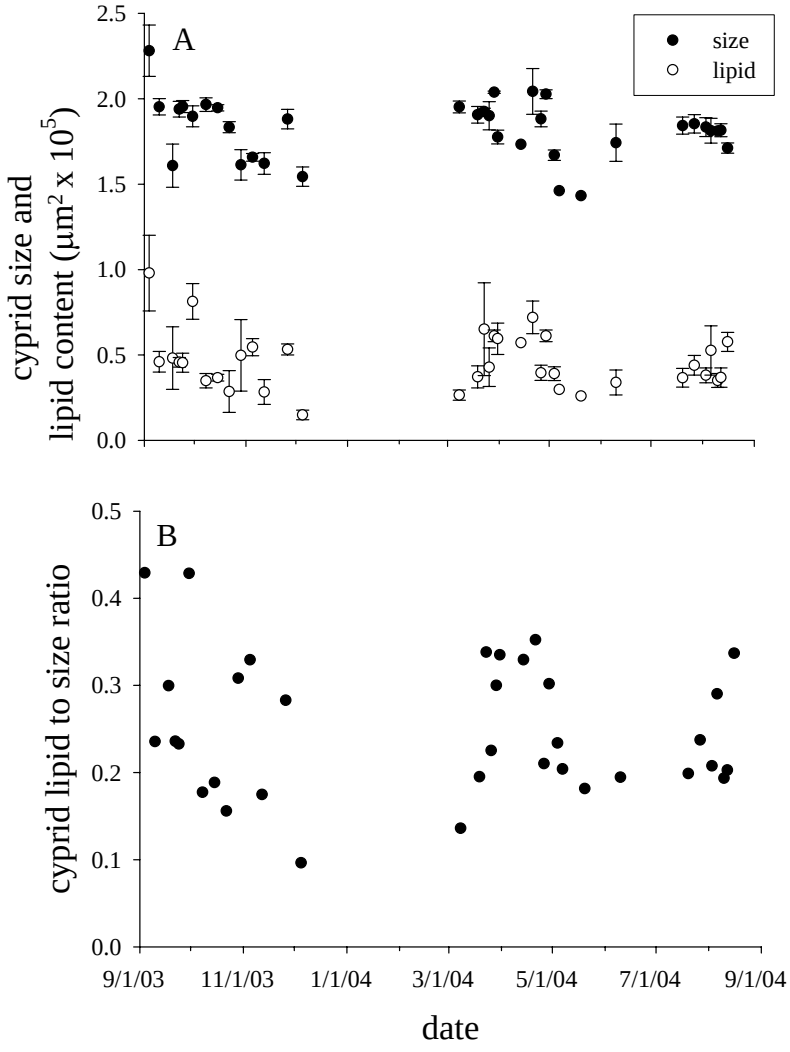

Figure 2. A) Cyprid size, measured as carapace area, and lipid content, measured as projected lipid area, of *Balanus glandula* sampled from the mouth of the Coos Bay estuary over a one year period. Points represent means $\pm$1 standard error. B) The ratio of lipid to size for cyprids. Points represent mean cyprid lipid content divided by mean cyprid size.

Cyprid size and lipid content increased with increasing seawater chlorophyll *a* concentration, although only the correlation between cyprid size and chlorophyll *a* concentration was significant (Figure 3A, Linear regression: size, t=2.077, p=0.04, lipid, t=0.99, p=0.32). Raw seawater chlrophyll *a* concentration explained 2% and 0.4% of the variation in cyprid size and lipid content, respectively. The ratio of cyprid lipid to size did not correlate significantly with seawater chlorophyll *a* (Figure 3B, Linear regression: t= 0.65, p=0.52).

Cyprid size and lipid content significantly decreased with increasing water temperature with approximately 2% of the variation in both parameters explained by water temperature (Figure 4A, Linear regression: lipid, t=-2.05, p=0.04, size, t=-2.38, p=0.039). Upwelling index did not correlate significantly with cyprid size and lipid content (Figure 4B, Linear regression: Lipid, t=-0.138, p=0.89; Size, t=0.0025, p=0.997). Cyprid lipid content increases with cyprid size (Figure 5, linear regression: t=3.77, p<0.001), with approximately 6% of the variation in cyprid lipid content explained by cyprid size.

Naupliar lipid content

Naupliar lipid content did not correlate significantly with raw seawater chlorophyll *a* concentration in any stage (Figure 6A-C, Linear regression 4^{th} stage, t=-.343, p=0.73, 5^{th} stage, t=0.454, p=0.66, 6^{th} stage, t=-2.03, p=0.051).

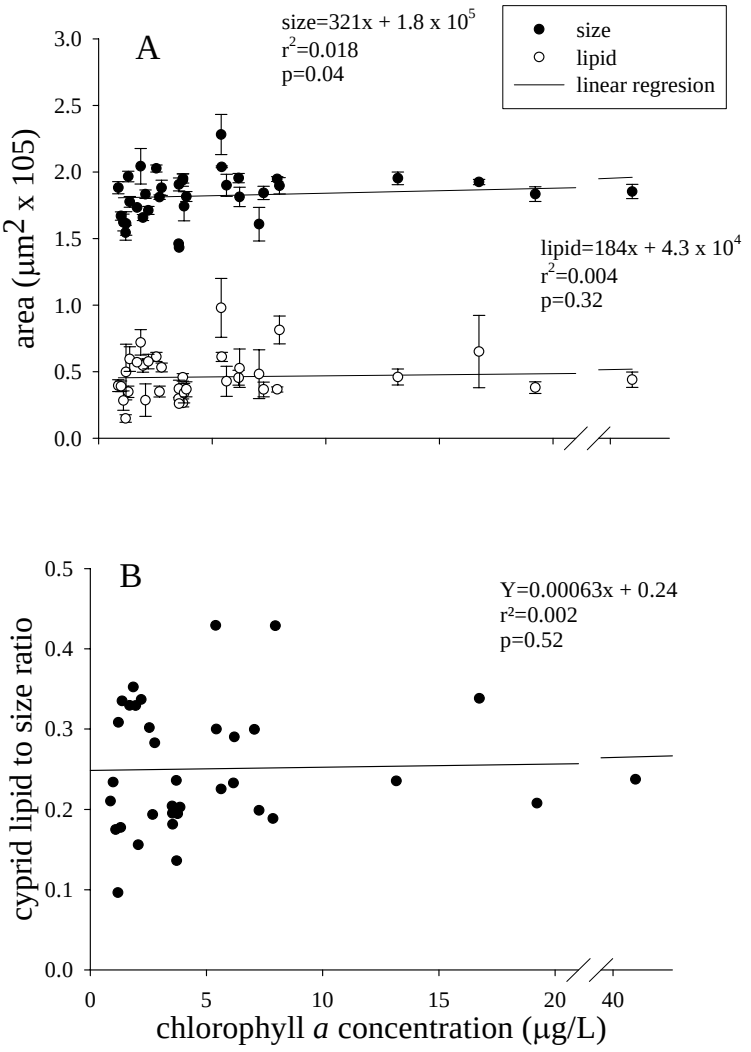

Figure 3. A) Relationship between cyprid size and lipid content of *Balanus glandula* and seawater chlorophyll *a* concentration. Points represent means ±1 standard error. B) Relationship the ratio of lipid to size for cyprids and seawater chlorophyll *a* concentration. Points represent mean cyprid lipid content divided by mean cyprid size. Lines in A and B represent least square linear regressions through all data points.

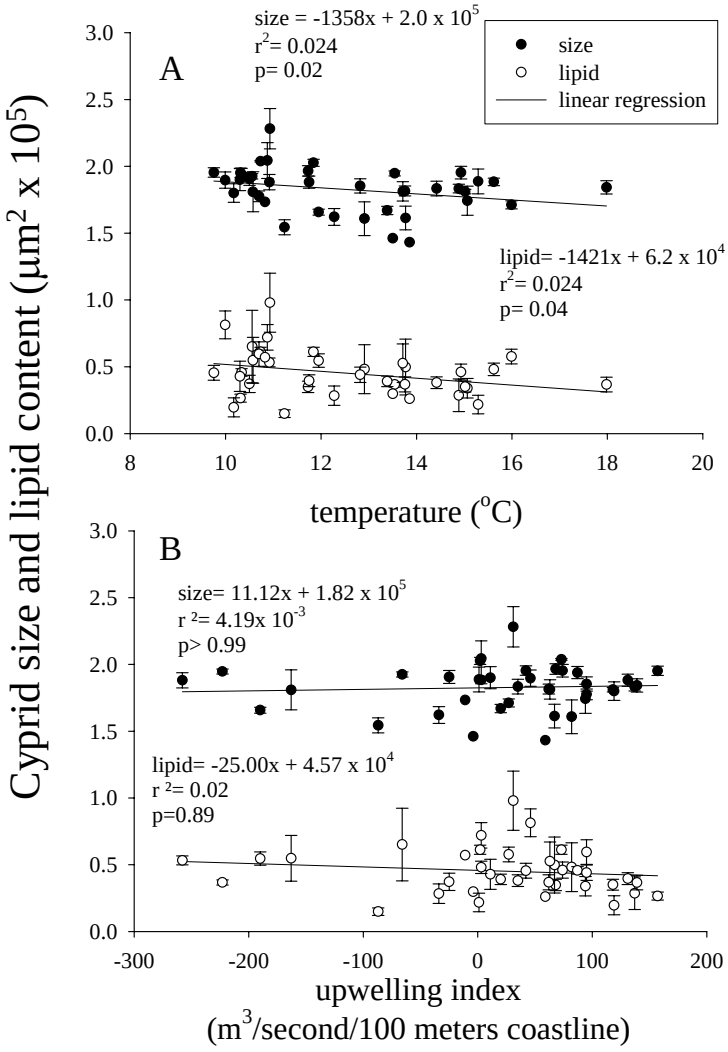

Figure 4. Relationship between cyprid size and lipid content of *Balanus glandula* and A) mean daily oceanic water temperature, and B) mean daily upwelling index. All points represent mean s ±1 standard error. Lines represent least square linear regressions through all data points.

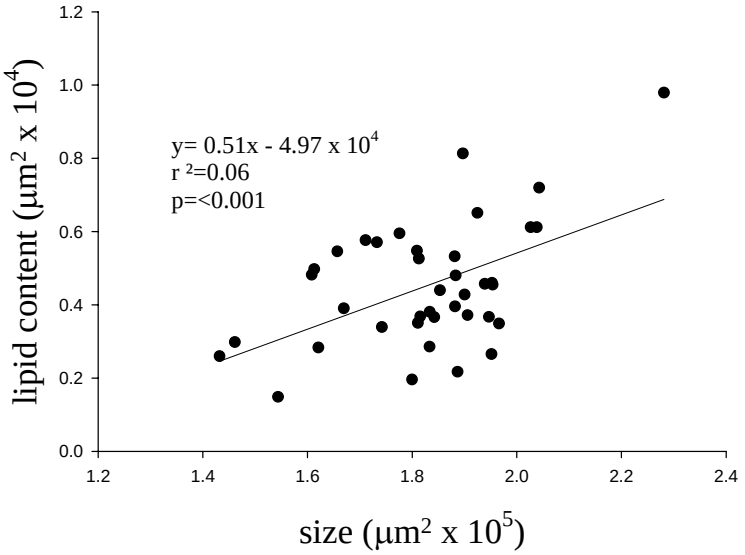

Figure 5. The relationship between cyprid lipid content and cyprid size of *Balanus glandula*. Points represent mean cyprid size and mean cyprid lipid content. Line represents a least square linear regression through all data points.

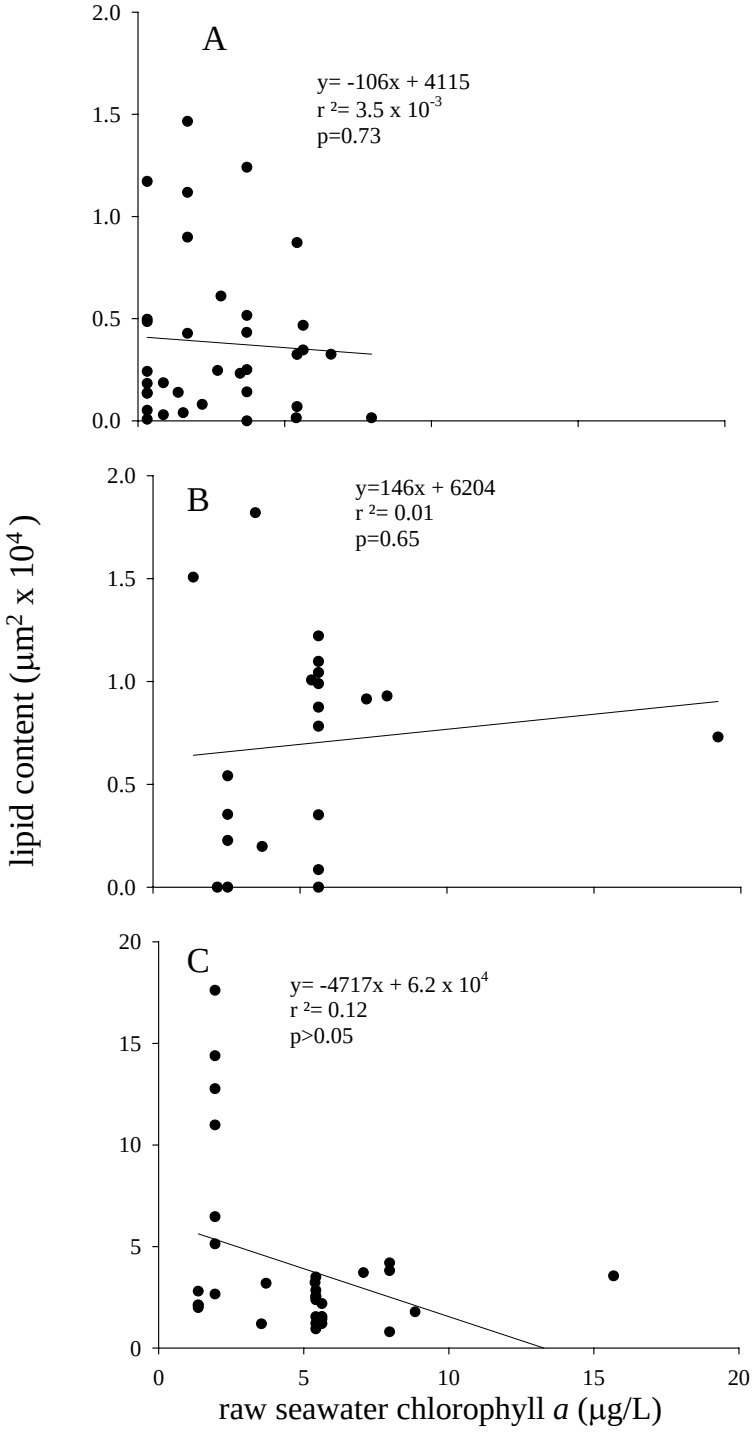

Figure 6. The relationship between raw seawater chlorophyll *a* concentration and the lipid content of A) 4th stage B) 5th stage C) 6th stage field caught nauplii of *Balanus glandula.* Points represent the lipid content of a single nauplius. Line represents a least square linear regression through all data points.

Naupliar feeding rate

Feeding rate, measured by fecal pellet production, of nauplii of *Balanus glandula* differed between stages (Kruskal-Wallis test, H=26.3, n=4, $p<0.001$). In all stages fecal pellet production increased significantly with increasing seawater chlorophyll *a* concentration, with 28-65% of the variation in fecal pellet production explained by chlorophyll *a* concentration (Figure 7A-D, Table 1). The relationship between fecal pellet production and chlorophyll *a* concentration of naupliar stages 2 and 4 were best described by a curvilinear regression (r^2=0.28 and 0.44, respectively), while relationship of stages 5 and 6 were best fit by a linear model (r^2=.065 and .060, respectively).

Discussion

This study investigated how larval condition, measured by size and lipid content of *Balanus glandula* varies through the year and with fluctuations in food availability and temperature. Larval condition varied over time and was weakly correlated with seawater chlorophyll *a* and temperature. Furthermore, chlorophyll *a* concentration was found to be an accurate proxy for food availability. I suggest that food availability and seawater temperature along with other factors control the condition of larvae of *B. glandula* in the field.

Variations in cyprid size and lipid content may be explained in part by naupliar food availability because: 1. Cyprids were largest and contained the most lipid content

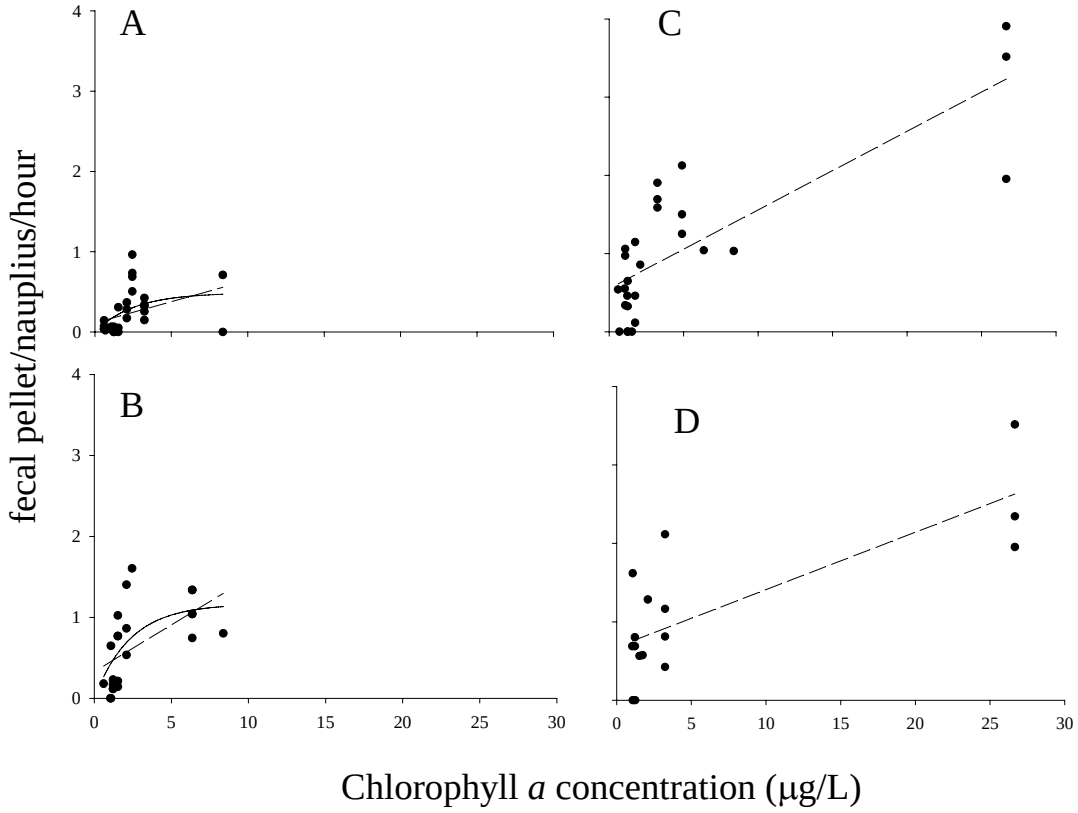

Figure 7A-D. Relationship between 53μm filtered seawater chlorophyll *a* concentration and A) 2nd stage B) 4th stage C) 5th stage D) 6th stage fecal pellet production of field collected *Balanus glandula* nauplii. Each point represents fecal pellet production for one larva. Lines represent the linear regressions (dashed lines) and curvilinear fits (solid lines) through all data points. Summary of regression statistics is given in Table 1.

Table 1. Summary of linear and curvilinear models fitted to the data on the relationship between seawater chlorophyll a concentration (μg/L) and fecal pellet production (fecal pellets/nauplius/hour) of Balanus glandula nauplii. For the linear models, a=intercept and b=slope. ns=not significant (p>0.05). The curvilinear model used was $Y=a(1-e^{-bX})$, where *a* is asymptotic maximum value of Y (fecal pellet production) and *b* is the rate of increase of Y with respect to X (chlorophyll concentration).

	2nd stage		4th stage		5th stage		6th stage	
regression	Linear	Curvilinear	Linear	Curvilinear	Linear	Curvilinear	Linear	Curvilinear
a	0.11	0.49	0.33	1.16	0.10	ns	0.07	ns
b	0.05	0.39	0.12	0.43	0.55	ns	0.68	ns
r^2	0.15	0.28	0.31	0.44	0.65	ns	0.60	ns
n	30	30	22	22	27	ns	16	ns
p	0.04	<0.01	<0.01	<0.01	<0.01	ns	<0.01	ns

during periods of high seawater chlorophyll *a* concentration and 2. Cyprid size is significantly, but weakly, correlated with seawater chlorophyll *a* concentration.

Seawater chlorophyll *a* concentration varied throughout the study, with the highest concentrations occurring in spring and late summer. Chlorophyll *a* concentrations were highest in September ’03, March ’04, and June-August ’04, and fell within the range of values reported for the Oregon Coast (Menge et al. 1997, Roegner and Shanks 2001). The size and lipid content of field-caught cyprids also varied between sampling dates. The largest cyprids occurred in early September 2003 and during March and April 2004 (Figure 2). Cyprid lipid content content peaked in early September 2003 and late-April 2004, concurrent with peaks in seawater chlorophyll *a* concentration. Chlorophyll *a* concentration is a measure of phytoplankton standing stock, which is an important food source for many invertebrate larvae (M. Strathmann 1987). Peaks in chlorophyll *a* concentration and simultaneous peaks in cyprid size as well as lipid content may facilitate a coupling between temporal variations in naupliar feeding history and cyprid condition.

Other studies have found substantial variation in larval size and energy stores from the field over various temporal scales (Barnes 1953, Jarrett 2003, Emlet and Sadro unpublished manuscript). Jarrett (2003) found a general decrease in cyprid organic content over the course of only two months. Emlet and Sadro (submitted manuscript) observed substantial variation in cyprid size of *B. glandula* from monthly samples taken from the Coos Bay estuary both between and within sampling dates, with a size range of approximately 1.5 to 2.4 $x10^5 \mu m^2$. The sizes of cyprids of *Balanus glandula* in my study

fell within the size distribution reported by Emlet and Sadro (submitted manuscript), although sizes are generally lower in my study.

Cyprid size varied positively with seawater chlorophyll *a* concentration however, the regression analysis explained less than 2% of the variation seen in cyprid size. Cyprid lipid content was not significantly correlated with seawater chlorophyll *a* concentration. Positive correlation between seawater chlorophyll *a*, which is a measure of phytoplankton standing stock, suggests food concentrations may be responsible for the observed patterns in cyprid size. In agreement with this study, Checkley (1985) found a positive correlation between seawater chlorophyll *a* concentration and cephalothoracic length and egg production of adult copepods.

The size and lipid content of cyprids of *B. glandula* from the field are equivalent to those reared in the laboratory with diet that maximized larval growth (J. Schultz, Chapter II, Figure 3) suggesting that larvae in the field are well fed. *Semibalanus balanoides* releases nauplii in response to phytoplankton blooms, probably to ensure adequate concentrations of food are available for larval growth and development (Barnes 1957, Starr et al. 1991). If larvae of *B. glandula* are released when larval food concentrations are high the chances of larval food limitation in the plankton may be reduced, and this may explain why field caught cyprids of *B. glandula* appear to be well fed.

Positive correlations between seawater chlorophyll *a* concentration and naupliar fecal pellet production, supports the hypothesis that food concentration affects larval condition. In agreement with the findings of this study Besiktepe and Dam (2002) and Butler and Dam (1994) found that an increase in ingestion rate or phytoplankton concentration

increases the fecal pellet production rate of adult copepods. However, no studies that I am aware of correlate natural seawater chlorophyll *a* concentration and fecal pellet production. Subsequent studies can use the less time consuming measure of natural chlorophyll *a* to measure food availability for barnacle nauplii.

Different regression models best explain the patterns of fecal pellet production across stage. A least square linear regression best explains the variation in the relationship between fecal pellet production of 5^{th} and 6^{th} stage nauplii and chlorophyll *a* concentration, but a curvilinear model explained more of the variation than a linear model in 2^{nd} and 4^{th} stage relationships. Correlations of fecal production rates for adult copepods and food availability are generally modeled as curvilinear (Butler and Dam 1994) because feeding rate plateaus at high food concentrations. However, Bamstedt et al. (1999) and Besiktepe and Dam (2002) suggest different regression models are needed to explain the variation in fecal pellet production for different food types for adult copepods. My results suggest a single regression model is not appropriate for every naupliar stage of *B. glandula* at chlorophyll *a* concentrations seen in this study.

Given that larval food availability explains small amounts of the variation in larval size and lipid content, other physical factors may influence larval condition as well. This study found a significant negative correlation between seawater temperature and the size and the lipid content of cyprids of *B. glandula*. Emlet and Sadro (submitted manuscript) also found decreased temperatures cause an increase in cyprid size. In many coastal areas, seawater temperature may be warmer in the summer and colder in the spring and winter. Checkley (1985) and Ouellet and Allard (2002) found negative correlations

between zooplankton size and seasonal increases in seawater temperature. However, cyprid condition in the present study does not follow a clear seasonal pattern that would be predicted from seasonal differences in seawater temperature, such that the largest cyprids occur in the coldest seasons. A possible explanation is that seasonal differences in seawater temperature are affected by intermittent periods of cold seawater brought to the surface by upwelling, which usually occurs in the warm summer months.

The results of this study are important to researchers using laboratory studies to understand why larval condition varies in the field. These results suggest that food availability and water temperature in the field affect the size and lipid content of cyprids of *Balanus glandula* in the direction that is expected based on laboratory experiments. While food availability significantly affects cyprid size and seawater temperature significantly affects cyprid size and lipid content in the field, the influence of food and temperature on the overall variation is small and thus, other factors may account for much of the variation in larval size and lipid content in the field.

Genetic variation among larvae may contribute to differences in larval size and lipid content. Nauplii of barnacles taken from parents living within feet of each other show differences in cyprid size when reared under the same food environment (Emlet and Sadro submitted manuscript) suggesting that genetic differences between larval sources in very close spatial proximity lead to differences in larval condition.

A correlation between aspects of the larval environment, such as food availability or seawater temperature, and cyprid size or lipid content may be obscured if larvae have previous experience in a different environment. Cyprids are non-feeding larvae and

measurements of food availability taken the day the cyprids are captured may not reflect the feeding environment of the nauplii. Short-term variations in food availability on the order of hours to days may not be detected in cyprid condition. In this case the difference between the naupliar nutritional environment and the measured nutritional environment of the cyprid may obscure a correlation between cyprid condition and measured field food availability.

Although consumption rates are implicated in many laboratory food limitation studies, food quality may influence the condition of zooplankton in the field as well. Lower quality food may reduce feeding rates (Butler and Dam 1994) or change the amount needed for organism growth (Sterner and Hessen 1994), both of which may affect the size and lipid content of zooplankton. Thiyagarajan et al. (2002) found a positive relationship between food quality during the nauplius stage and the lipid content of cyprids.

Conclusions

This study suggests that differences in the size and lipid content of cyprids may be affected by chlorophyll *a* concentration and seawater temperature. Seawater chlorophyll *a* concentration varied throughout the year, and was highest in spring and throughout summer. During times of high chlorophyll *a* concentration the largest cyprids containing the most lipid were collected. Chlorophyll *a* concentration and seawater temperature correlate significantly, but weakly, with cyprid size. Increased seawater chlorophyll *a* concentration was correlated positively with cyprid size whereas increased temperature

was negatively correlated with cyprid size and lipid content. However, little of the variation (2-4%) in cyprid size and lipid content was explained by seawater chlorophyll *a* concentration and temperature. Seawater chlorophyll *a* concentration may be used a proxy for food availability in barnacle nauplii because seawater chlorophyll *a* concentration correlated with naupliar feeding rate as measured by fecal pellet production.

BIBLIOGRAPHY

Chapter I

Emlet RB, Sadro SS (submitted manuscript) Linking stages of life history: How larval quality translates into juvenile performance for an intertidal barnacle (*Balanus glandula*).

Jarrett JN (2003) Seasonal variation in larval condition and postsettlement performance of the barnacle *Semibalanus balanoides*. Ecology 84:384-390

Morgan SG (1995) Life and Death in the Plankton: Larval Mortality and Adaptation. In: McEdward L (ed) Ecology of Marine Invertebrate Larvae. CRC, New York, p 279-322

Pechenik JA (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Marine Ecology Progress Series 177:269-277

Pechenik JA, Wendt DE, Jarrett JN (1998) Metamorphosis is not a new beginning: larval experience influences juvenile performance. BioScience 48:901-910

Strathmann RR (1987) Larval Feeding. In: Giese AC, Pearse JS, Pearse VB (eds) Reproduction of Marine Invertebrates, Vol IX: General Aspects: Seeking Unity in Diversity, pp. 465-550.

Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Biological Review 25:1-43

Chapter II

Bamstedt U, Nejstgaard JC, Solberg PT (1999) Utilization of small-sized food algae by *Calanus finmarchicus* (Copepoda, Calanoida) and the significance of feeding history. Sarsia 84:19-38

Berger MS (2004) Living along an estuarine gradient: Juvenile performance, reproductive patterns, and heat-shock protein expression in the barnacle *Balanus glandula*. Ph.D, University of Oregon

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

Brown SK, Roughgarden J (1985) Growth, morphology, and laboratory culture of larvae of *Balanus glandula* (Cirripedia: thoracica). Journal of crustacean biology 5:574-590

Butler M, Dam HG (1994) Production rates and characteristics of fecal pellets of the copepod *Acartia tonsa* under simulated phytoplankton bloom conditions: implications for vertical fluxes. Marine Ecology Progress Series 114:81-91

Checkley DM (1985) Nitrogen limitation of zooplankon production and its effect on the marine nitrogen cycle. In: Lampart W (ed) Food limitation and the structure of zooplankton communities. Stuttgart, Plon, W. Germany, p 103-113

Connell JH (1961) The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology 42:710-723

Cowles T, Desideiroi R, Neuer S (1993) *In situ* characterization of phytoplankton assemblages using fluorescence emission spectra. Marine Biology 115:217-222

Cowlishaw RJ (2004) Seasonal coupling between ciliate and phytoplankton standing stocks in the South Slough of Coos Bay, Oregon. Estuaries 27:539-550

Durbin EG, Durbin AG, Smayda TJ, Verity PG (1983) Food limitation of production by adult *Acartia tonsa* in Narragansett Bay, Rhode Island. Limnology and Oceanography 28:1199-1213

Eckert GL (1995) A novel larval feeding strategy of the tropical sand dollar, *Encope michelini*: Adaptation to food limitation and an evolutionary link between planktotrophy and lecithotrophy. Journal of Experimental Marine Biology and Ecology 187:103-128

Emlet RB, Sadro SS (unpublished manuscript) Linking stages of life history: How larval quality translates into juvenile performance for an intertidal barnacle (*Balanus glandula*).

Fenaux L, Strathmann MF, Strathmann RR (1994) Five tests of food-limited growth of larvae in coastal waters by comparisons of rates of development and form of echinopleutei. Limnology and Oceanography 39:84-96

Frost BW (1985) Food limitation of the planktonic marine copepods *Calanus pacificus* and *Pseudocalanus sp.* in a temperate fjord. In: Lampart W (ed) Advances in limnology. Stuttgart, Plon, W. Germany, p 1-13

Gallager SM, Man R (1981) Use of lipid-specific staining techniques for assaying condition in cultured bivalve larvae. Journal of Shellfish Research 1:69-73

Gosselin LA, Qian P-Y (1996) Early post-settlement mortality of an intertidal barnacle: a critical period for survival. Marine ecology progress series 135:69-75

Hakanson JL (1987) The feeding condition of *Calanus pacificus* and other zooplankton in relation to phytoplankton pigments in the California Current. Limnology and Oceanography 32:881-894

Hart MW, Strathmann RR (1995) Mechanisms and rates of suspension feeding. In: McEdward L (ed) Ecology of Marine Invertebrate Larvae. CRC Press, New York, NY, p 193-221

Hentschel BT, Emlet RB (2000) Metamorphosis of barnacle nauplii: effects of food variability and a comparison with amphibian models. Ecology 81:3495-3508

Holland DL (1987) Lipid biochemistry of barnacles. In: Southward AJ (ed) Crustacean issues, Vol 5, p 227-248

Holland DL, Walker G (1975) The biochemical composition of the cypris larva of the barnacle *Balanus balanoides* L. J. Cons. int. Explor. Mer 36:162-165

Hutchinson GE, (1944) Limnological studies in Connecticut. VII. A critical examination of the supposed relationship between phytoplankton periodicity and chemical changes in the water. Ecology 25: 3-26.

Hughes MP (1997) Temporal and spatial variability of phytoplankton in coastal and estuarine habitats in Coos Bay, Oregon. Masters, University of Oregon

Jarrett JN, Pechenik JA (1997) Temporal variation in cyprid quality and juvenile growth capacity for an intertidal barnacle. Ecology 78:1262-1265

Johnson KB, Shanks AL (2003) Low rates of predation on planktonic marine invertebrate larvae. Marine Ecology Progress Series 248:125-139

Lorenzen CJ (1971) Continuity in the distribution of surface chlorophyll. J. Cons. int. Explor. Mer 34:18-23

Mackas D, Bohrer R (1976) Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. Journal of experimental marine biology and ecology 25:77-85

Mackas DL, Denman K, L., Abbott MR (1985) Plankton patchiness: Biology in the physical vernacular. Bulletin of Marine Science 37:652-674

Marshall DJ, Keough MJ (2004) Variable effects of larval size on post-metamorphic performance in the field. Marine Ecology Progress Series 279:73-80

Meidel S, Scheibling R, Metaxas A (1999) Relative importance of parental and larval nutrition on larval development and metamorphosis of the sea urchin *Strongylocentrotus droebachiensis*. Journal of Experimental Marine Biology and Ecology 240:161-178

Menge BA, Daley BA, Wheeler PA, Strub TP (1997) Rocky intertidal oceanography: An association between community structure and nearshore phytoplankton concentration. Limnology and Oceanography 42:57-66

Miller BA, Emlet RB (1999) Development of newly metamorphosed juvenile sea urchins: morphology, the effects of temperature and larval food ration, and a method for determining age. Journal of Experimental Marine Biology and Ecology 235:67-90

Morgan SG (1995) Life and Death in the Plankton: Larval Mortality and Adaptation. In: McEdward L (ed) Ecology of Marine Invertebrate Larvae. CRC, New York, p 279-322

Ohman MD (1985) Resource-satiated population growth of the copepod *Pseudocalanus sp.* In: Lampart W (ed) Advances in limnology. Stuttgart, Plon, W. Germany

Ohman MD (1996) Freezing and storage of copepod samples for the analysis of lipids. Marine ecology progress series 130:298-298

Olson R, Bosch I, Pearse J (1987b) The hypothesis of Antarctic larval starvation examined for the asteroid *Odontaster validus*. Limnology and Oceanography 32:686-690

Olson RR (1987a) *In situ* culturing as a test of the larval starvation hypothesis for the crown-of-thrown starfish, *Acanthaster planci*. Limnology and Oceanography 32:895–904

Olson RR, Olson MH (1989) Food limitation of planktotrophic marine invertebrate larvae: Does it control recruitment success? Annual Review of Ecology and Systematics 20:225-247

Parsons TR, Lalli CM (1988) Comparative oceanic ecology of the plankton communities of the subarctic Atlantic and Pacific oceans. Oceanography and Marine Biology Annual Review 26:317-359

Parsons TR, Lalli CM, Maita Y (1984) A Manual of Chemical and Biological Methods for Seawater Analysis, Vol. Pergamon Press, New York, NY

Paulay G, Boring L, Strathmannn RR (1985) Food limited growth and development of larvae: experiments with natural sea water. Journal of experimental marine biology and ecology 93:1-10

Pearcy WG, Keene DF (1974) Remote sensing of water color and sea surface temperatures off the Oregon coast. Limnology and Oceanography 19:573-583

Pechenik JA (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Marine Ecology Progress Series 177:269-277

Pechenik JA, Jarrett JN, Rooney J (2002) Relationship between larval nutritional experience, larval growth rates, and juvenile growth rates in the prosobranch gastropod *Crepidula fornicata*. Journal of Experimental Marine Biology and Ecology 280:63-78

Pechenik JA, Rittschof D, Schmidt AR (1993) Influence of delayed metamorphosis on survival and growth of juvenile barnacles *Balanus amphitrite*. Marine Biology 115:287-294

Pechenik JA, Wendt DE, Jarrett JN (1998) Metamorphosis is not a new beginning: larval experience influences juvenile performance. BioScience 48:901-910

Phillips NE, Gaines SD (2002) Spatial and temporal variability in size at settlement of intertidal mytilid mussels from around Pt. Conception, California. Invertebrate Reproduction and Development 41:171-177

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists, Vol. Cambridge University Press, Cambridge

Reitzel AM, Webb J, Arellano S (2004) Growth, development and condition of *Dendraster excentricus* larvae reared on natural and laboratory diets. Journal of Plankton Research 26:901-908

Rumrill SS (1990) Natural mortality of marine invertebrate larvae. Ophelia 32:163-168

Small LF, Curl H, Glooschenko WA (1972) Effects of solar radiation and upwelling on daily primary production off Oregon. Journal of the Fisheries Reseach Board of Canada 29:1269-1275

Small LF, Menzies DW (1981) Patterns of primary productivity and biomass in a coastal upwelling region. Deep-Sea Research 28A:123-149

Smart TI (2004) The effects of body size and particle size on feeding rates and mophology of the larvae of three congeneric barnacles (Class Cirripedia: Genus *Balanus*). Master's, University of Oregon

Sterner RW, Hessen DO (1994) Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25:1-29

Strathmann MF (1987) Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast, Vol. University of Washington Press, Seattle, WA

Strathmann RR (1987) Larval Feeding. In: Giese AC, Pearse JS, Pearse VB (eds) Reproduction of Marine Invertebrates, Vol IX: General Aspects: Seeking Unity in Diversity

Thiyagarajan V, Harder T, Qian P-Y (2002) Relationship between cyprid energy reserves and metamorphosis in the barnacle *Balanus amphitrite* Darwin. Journal of Experimental Marine Biology and Ecology 280:79-93

Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Biological Review 25:1-43

Underwood AJ (1997) Experiments in Ecology, Vol. Cambridge University Press, Cambridge, United Kingdom

Young CM, Chia F-S (1987) Abundance and distribution of pelagic larvae as influenced by predation, behavior, and hydrographic factors. In: Giese AC, Pearse JS, Pearse VB (eds) Repreduction of marine invertebrates, Vol 9. Blackwell scientific publications, Palo Alto, Califonia, p 385-463

Chapter III

Arnsberg AL (2001) The Barnacles: Cirripedia. In: Shanks AL (ed) An identification guide to the larval marine invertebrates of the pacific northwest. Oregon State University Press, Corvallis, p 156-175

Bamstedt U, Nejstgaard JC, Solberg PT (1999) Utilization of small-sized food algae by Calanus finmarchicus (Copepoda, Calanoida) and the significance of feeding history. Sarsia 84:19-38

Barnes H (1953) Size variations in the cyprids of some common barnacles. Journal of the Marine Biological Association of the U.K. 32:297-304

Barnes H (1957) Process of restoration and synchronization in marine ecology. The spring diatom increase and the 'spawning' of the common barnacle, *Semibalanus balanoides* (L.). Ann. Biol. 33:67-85

Berger MS (2004) Living along an estuarine gradient: Juvenile performance, reproductive patterns, and heat-shock protein expression in the barnacle *Balanus glandula*. Ph.D, University of Oregon

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

Brown SK, Roughgarden J (1985) Growth, morphology, and laboratory culture of larvae of *Balanus glandula* (Cirripedia: thoracica). Journal of Crustacean biology 5:574-590

Butler M, Dam HG (1994) Production rates and characteristics of fecal pellets of the copepod *Acartia tonsa* under simulated phytoplankton bloom conditions: implications for vertical fluxes. Marine Ecology Progress Series 114:81-91

Checkley DM (1985) Nitrogen limitation of zooplankon production and its effect on the marine nitrogen cycle. In: Lampart W (ed) Food limitation and the structure of zooplankton communities. Stuttgart, Plon, W. Germany, p 103-113

Eckert GL (1995) A novel larval feeding strategy of the tropical sand dollar, *Encope michelini*: Adaptation to food limitation and an evolutionary link between planktotrophy and lecithotrophy. Journal of Experimental Marine Biology and Ecology 187:103-128

Emlet RB, Sadro SS (unpublished manuscript) Linking stages of life history: How larval quality translates into juvenile performance for an intertidal barnacle (*Balanus glandula*).

Epifanio CE, Cope JS, Rowe PM, Jenkins FM (1991) Comparison of rates of development of Atlantic mud crab larvae in the laboratory and in the field-deployed enclosures. Journal of Crustacean Biology 11:540-545

Fenaux L, Strathmann MF, Strathmann RR (1994) Five tests of food-limited growth of larvae in coastal waters by comparisions of rates of development and form of echinopleutei. Limnology and Oceanography 39:84-96

Gallager SM, Man R (1981) Use of lipid-specific staining techniques for assaying condition in cultured bivalve larvae. Journal of Shellfish Research 1:69-73

Hentschel BT, Emlet RB (2000) Metamorphosis of barnacle nauplii: effects of food variability and a comparison with amphibian models. Ecology 81:3495-3508

Holland DL (1987) Lipid biochemistry of barnacles. In: Southward AJ (ed) Crustacean issues, Vol 5, p 227-248

Holland DL, Walker G (1975) The biochemical composition of the cypris larva of the barnacle *Balanus balanoides* L. J. Cons. int. Explor. Mer 36:162-165

Hughes MP (1997) Temporal and spatial variability of phytoplankton in coastal and estuarine habitats in Coos Bay, Oregon. Masters, University of Oregon

Jarrett JN (2003) Seasonal variation in larval condition and postsettlement performance of the barnacle *Semibalanus balanoides*. Ecology 84:384-390

Jarrett JN, Pechenik JA (1997) Temporal variation in cyprid quality and juvenile growth capacity for an intertidal barnacle. Ecology 78:1262-1265

Lucas MI, Walker G, Holland DL, Crisp DJ (1979) An energy budget for the free-swimming and metamorphosing larvae of *Balanus balanoides* (Crustacea: Cirripedia). Marine biology 55:221-229

Mackas DL, Denman K, L., Abbott MR (1985) Plankton patchiness: Biology in the physical vernacular. Bulletin of Marine Science 37:652-674

Meidel S, Scheibling R, Metaxas A (1999) Relative importance of parental and larval nutrition on larval development and metamorphosis of the sea urchin *Strongylocentrotus droebachiensis*. Journal of Experimental Marine Biology and Ecology 240:161-178

Menge BA, Daley BA, Wheeler PA, Strub TP (1997) Rocky intertidal oceanography: An association between community structure and nearshore phytoplankton concentration. Limnology and Oceanography 42:57-66

Miron G, Boudreau B, Bourget E (1999) Intertidal barnacle distribution: a case study using multiple working hypotheses. Marine Ecology Progress Series 189:205-219

Ohman MD (1996) Freezing and storage of copepod samples for the analysis of lipids. Marine Ecology Progress Series 130:298-298

Ouellet P, Allard J-P (2002) Seasonal and interannual variability in larval lobster *Homarus americanus* size, growth, and condition in the Magdalen Islands, southern Gulf of St. Lawrence. Marine ecology progress series 230:241-251

Paulay G, Boring L, Strathmannn RR (1985) Food limited growth and development of larvae: experiments with natural sea water. Journal of experimental marine biology and ecology 93:1-10

Pearcy WG, Keene DF (1974) Remote sensing of water color and sea surface temperatures off the Oregon coast. Limnology and Oceanography 19:573-583

Pechenik JA (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Marine Ecology Progress Series 177:269-277

Pechenik JA, Wendt DE, Jarrett JN (1998) Metamorphosis is not a new beginning: larval experience influences juvenile performance. BioScience 48:901-910

Phillips NE (2002) Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. Ecology 83:2562-2574

Phillips NE, Gaines SD (2002) Spatial and temporal variability in size at settlement of intertidal mytilid mussels from around Pt. Conception, California. Invertebrate Reproduction and Development 41:171-177

Qiu J-W, Qian P-Y (1997) Effects of food availability, larval source and culture method on larval development of *Balanus amphitrite* Darwin: implications for experimental design. Journal of Experimental Marine Biology and Ecology 217:47-61

Reitzel AM, Webb J, Arellano S (2004) Growth, development and condition of *Dendraster excentricus* larvae reared on natural and laboratory diets. Journal of Plankton Research 26:901-908

Roughgarden J, Gaines SD, Possingham H (1988) Recruitment dynamics in complex life cycles. Science 241:1460-1466

Small LF, Curl H, Glooschenko WA (1972) Effects of solar radiation and upwelling on daily primary production off Oregon. Journal of the Fisheries Research Board of Canada 29:1269-1275

Small LF, Menzies DW (1981) Patterns of primary productivity and biomass in a coastal upwelling region. Deep-Sea Research 28A:123-149

Standing JD (1980) Common inshore barnacle cyprids of the Oregonian faunal province (Crustacea: Cirripedia). Proceedings of the Biological Society of Washington 93:1184-1203

Starr M, Himmelman JH, Therriault J-C (1991) Coupling of nauplii release in barnacle with phytoplankton blooms: a parallel strategy to that of spawning in urchins and mussels. Journal of Plankton Research 13:561-571

Thiyagarajan V, Harder T, Qian P-Y (2002) Relationship between cyprid energy reserves and metamorphosis in the barnacle *Balanus amphitrite* Darwin. Journal of Experimental Marine Biology and Ecology 280:79-93

West TL, Costlow JD (1987) Size regulation in larvae of the crustacean *Balanus eburneus* (Cirripedia: Thoracica). Marine biology 96:47-58