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## Nutritional role of microalgae in the diet of first stage brachyuran crab larvae

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**NUTRITIONAL ROLE OF MICROALGAE IN THE DIET OF FIRST STAGE  
BRACHYURAN CRAB LARVAE**

By

Nicole Janette Casper

Accepted in Partial Completion  
Of the Requirements for the Degree  
Master of Science

Kathleen L. Kitto, Dean of the Graduate School

**ADVISORY COMMITTEE**

Chair, Dr. Stephen D. Sulkin

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## **MASTER'S THESIS**

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Nicole Janette Casper

March 8<sup>th</sup> 2013

**NUTRITIONAL ROLE OF MICROALGAE IN THE DIET OF FIRST  
STAGE BRACHYURAN CRAB LARVAE**

A Thesis  
Presented to  
The Faculty of  
Western Washington University

In Partial Fulfillment  
Of the Requirements for the Degree  
Master of Science

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March 2013

## ABSTRACT

The survival and distribution of benthic invertebrate larvae have important implications for the population dynamics of the species and, due to predator-prey interactions, can have important consequences to the communities in which they live. Nutrition and food availability are among the primary determinants of larval success. The feeding strategy of brachyuran larval crabs is a complex combination of omnivory and selection. Although ingestion of microalgae by larvae has been observed, their role in satisfying larval nutritional needs is unclear. This study examined the role ingesting phytoplankton plays in the nutrition of larval crabs by simulating conditions in which algal prey might increase survival or accelerate development. The alga, *Isochrysis galbana*, and zooplankters *Artemia* sp. nauplii and rotifer *Brachionis plicatilis*, were used as prey in experiments with larvae of three brachyuran crab species representing different families and hatching seasons: *Lophopanopeus bellus*, *Metacarcinus (Cancer) magister*, and *Hemigrapsus nudus*. Experimental conditions included exposure of larvae to algae alone immediately upon hatching, interspersed periods of algal and zooplankton prey and mixed algae-zooplankton prey. In post-hatching feeding experiments with *L. bellus* and *M. magister*, survival decreased and development was delayed as the initial period of starvation or algal feeding prior to zooplankton feeding was extended, with no differences between the unfed and algal-fed diets. Mean Point-of-No-Return (PNR) values for unfed and algal-fed treatments were not significantly different from one another and *post-hoc* contrasts showed no difference between the two experimental treatments, except in a few instances where stage duration of *L. bellus* larvae fed algae

for one day was shortened by just under a half-day, and for *M. magister* larvae where larvae fed algae for three days had 16.7% survival while all corresponding unfed larvae died. In patchy prey experiments *L. bellus* survival and *H. nudus* survival and development rate were unaffected by exposure to algae; however, presence of algae accelerated *L. bellus* larval development by 5% (0.77 days). Results from mixed prey diet experiments showed no difference in *L. bellus* survival and development rate when algae supplemented zooplankton. Results indicate that, overall, *I. galbana* does not significantly contribute to larval survival or development rate. Benefits of an opportunistic feeding strategy in which widespread, seemingly inefficient feeding on algae occurs might be contingent upon particular predator-prey species interactions (i.e. a more nutrient-laden alga), or may only pay off in particular conditions (i.e. a sparse prey field).

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## INTRODUCTION

Many benthic invertebrates have a planktonic larval form that faces unique challenges associated with development and survival. In contrast to the benthic adults, larvae are typically pelagic and their nutritional needs differ from those of the adult, whose primary function is reproduction, including gamete production, and enhancing contributions to fitness of its offspring. Benthic invertebrate larvae are ecologically significant because their survival and distribution have important implications for the population dynamics of the species. Variation in larval survival can impact juvenile recruitment success, affecting subsequent adult distribution and population size. As a result, in commercially important species like Dungeness and blue crabs, larval survival can even take on economic importance.

Both larval survival and distribution have fundamental implications for plankton community dynamics as well. At certain times and locations, crab larvae can dominate the meso-zooplankton (Coyle and Paul 1990; Schwamborn et al. 1999; Sulkin, pers. comm.) and can affect community trophic dynamics by putting grazing pressure on prey items (top-down regulation) while affecting population abundance of predators (bottom-up regulation). Crab zoeae feed on small holoplanktonic prey, larvae of other invertebrates, and microzooplankton (Sulkin 1975; Epifanio et al. 1991). Crab zoeae are also important prey for fish world-wide, contributing considerably to the diet of such pelagic species as yellowfin tuna in the Indian Ocean (Potier et al. 2007), anchovies, silversides and other zooplanktivorous fish in Australian (Mazumder et al. 2006) and south-east American saltmarshes (Allen et al. 1995), juvenile pink and chum salmon in

the Pacific (Murphy et al. 1988), and juvenile Chinook salmon in Puget Sound, WA (Duffy et al. 2010). Jellyfish have also been observed to prey heavily on larval crabs (Sulkin, pers. comm.)

The life cycle of brachyuran crabs is a multi-stage process in which larval crabs (zoeae) pass through several stages before entering a post-larval (megalopa) phase, followed by the juvenile. Mortality is high during larval development with most zoeae not surviving to become juveniles. Sources of larval loss from an area include dispersal into unsuitable habitats and mortality due to predation, water quality and insufficient nutrition (Thorson 1946; Paul et al. 1979; Anger et al. 1981; Peachey 2005; Potier et al. 2007).

Nutrition has been recognized as an important factor affecting the development and survival of larvae (Anger and Dawirs 1981; Olson and Olson 1989; Staton and Sulkin 1991; Fenaux et al. 1994). The larval stages of most brachyuran crabs are planktotrophic, requiring a source of particulate organic carbon soon after hatching to support development through the zoeal stages. Prey types that support development in the laboratory include micro- and small meso-zooplankton, especially the early larval stages of such invertebrates as polychaetes, sea urchins, and brine shrimp (Sulkin 1975; Bigford 1978). Presumably these larval prey support crab larval development because they still contain some lipid provided from their eggs that sustain development. For example, the lipid fraction of brine shrimp nauplii contains essential long chain polyunsaturated fatty acids (PUFA) that larvae of many crab species require to complete zoeal development (Levine and Sulkin 1984; Brown et al. 1997).

Small holoplanktonic species can also support larval crab development (Sulkin 1975; Sulkin and McKeen 1999). These heterotrophic species feed in the plankton themselves and their nutritional value to larval crabs can depend upon their diets which usually consist of microalgae. For example, rotifers cultured on an alga high in PUFA support higher larval crab survival than the same strain of rotifer fed an alga low in PUFA (Sulkin and McKeen 1999).

Research on the nutritional significance of direct consumption of microalgae in the diet of larval crabs has been inconclusive. Although gut analyses of zoeae collected from the field have revealed microalgae in the diet (Paul et al. 1979; Paul and Paul 1980), and laboratory studies have confirmed that brachyuran zoeae will ingest a variety of microalgae (Lehto et al. 1998; Sulkin et al. 1998b; Hinz et al. 2001; Perez and Sulkin 2005), diets consisting solely of microalgae are generally not sufficient to sustain development under laboratory conditions (Sulkin 1975; Incze and Paul 1983; Lehto et al. 1998; Sulkin et al. 1998b). Although ingestion of algae by newly-hatched larvae may delay mortality when compared to unfed treatments for some crab species (Sulkin 1975; Sulkin et al. 1998b; Garcia et al. 2011), the nutritional value of algae to developing zoeae has not been rigorously tested or clearly established. A study on *Metacarcinus magister* showed variable results among algal species. Larval feeding on two dinoflagellate species delayed larval mortality; however, feeding on the green alga *Dunaliella tertiolecta* actually reduced larval survival compared to an unfed control (Sulkin et al. 1998b).

The confusion on the nutritional role of microalgae in the diet of larval crabs may be due, in part, to the variety of outcomes one might expect. It is possible that as an adaptation to an uncertain prey environment, crab larvae do not select prey based on

nutritional value, but ingest virtually any prey they encounter. When algae are ingested purely by virtue of opportunistic encounter feeding (Perez and Sulkin 2005), there may be neither benefit nor detriment to the larva. It is also possible that ingested algae may be competing with more nutritious prey, either by taking up space in the larval digestive tract, or by competing for the energy expended and time spent for ingestion of more favorable prey, thereby having a net negative effect. Alternatively, ingested algae may sustain larvae immediately after hatching by providing energy until they encounter more nutritionally favorable zooplankton prey, or provide a source of energy between encounters with more nutritious prey. The former could be particularly important because after hatching, zoeae may require energy to swim higher in the water column to encounter the microzooplankton prey that will sustain their development (Sulkin 1984). Finally, there is the possibility that a mixed diet of algae combined with zooplankton will provide an advantage that a pure zooplankton diet does not, by providing additional nutrients that result in increased larval survival and/or more rapid development (Epifanio et al. 1991).

The present experiments were designed to clarify the role that ingesting phytoplankton plays in the nutrition of larval crabs by simulating conditions in which algal prey might alter survival or rate of development. The scenarios include the following: 1) conditions in which larval crabs do not encounter favored zooplankton prey immediately upon hatching but do access phytoplankton; 2) conditions in which larval crabs encounter fields of zooplankton prey interspersed with periods of either no prey or encounters with microalgae only; and 3) conditions in which larval crabs encounter mixed prey fields composed of both microalgae and zooplankton. The experiments were

conducted with larvae of several crab species that differed in season of spawning, larval size and taxonomic association. Model zooplankton and microalgal species that have been used successfully in invertebrate larval culture were used as prey.

## METHODS

### Experimental Approach

Experiments were designed to determine the role played by algal prey in the nutrition of larval crabs. Three conditions in which larval crabs were likely to encounter algae were simulated and the effects on survival and developmental rate were determined. First, initial algal feeding experiments were designed to determine the contribution of algae when larvae do not encounter zooplankton prey immediately upon hatching. Second, experiments determined the contribution of algae when larvae encounter patchy prey fields of zooplankton, in which periodic encounters with zooplankton prey are separated by periods of no access to zooplankton prey. Third, mixed prey experiments were designed to determine the contribution of algae when larvae encounter fields composed of both algae and zooplankton.

In the present study, a control zooplankton diet consisted of either *Artemia franciscana* Kellogg nauplii or the rotifer *Brachionis plicatilis* Muller, both of which have been used as prey in the laboratory to support larval crab development (Levine and Sulkin 1984). The algal diet consisted of *Isochrysis galbana* Parke, a brown alga containing high levels of long-chain polyunsaturated fatty acids (PUFA) (Volkman et al. 1989).

## Experimental organisms

### Ovigerous and larval crab collection and care

*Hemigrapsus nudus* Dana ovigers were collected from Shannon Point Beach in Anacortes, WA by hand in April 2011 (Figure 1). Ovigers were held in flow-through seawater tables at the Shannon Point Marine Center under ambient conditions (7-9°C; 28-30 psu). Their eggs were routinely monitored for development and, when heartbeats were visible, ovigers were transferred to individual 20cm diameter glass bowls containing 5µm filtered seawater (FSW) and held in an incubator at 15°C with a light:dark cycle of 12:12h. Immediately upon hatching, zoeae were collected with a pipette to ensure that Day 1 zoeae were used in the experiments.

*Lophopanopeus bellus* Stimpson ovigers were collected from Shannon Point Beach in Anacortes, WA (Figure 1) by hand in June and July 2010 and June through August 2011. Ovigers were held in flow-through seawater tables at the Shannon Point Marine Center under ambient conditions (10-14°C; 28-30psu). The ovigers were treated as described above until larvae were obtained.

Ovigers of *Metacarcinus (Cancer) magister* Dana were collected by SCUBA from Ship Harbor in Anacortes, WA (Figure 1) in February and March 2011. Each oviger was transferred to Shannon Point Marine Center and held in a separate 10L plexiglass flow-through tank (dimensions 0.55x 0.33 x 0.56 m) under ambient conditions (7-8°C; 30-32psu). Tanks were checked daily until hatching began. The tank was then drained, rinsed and refilled, assuring that larvae collected for experiments were less

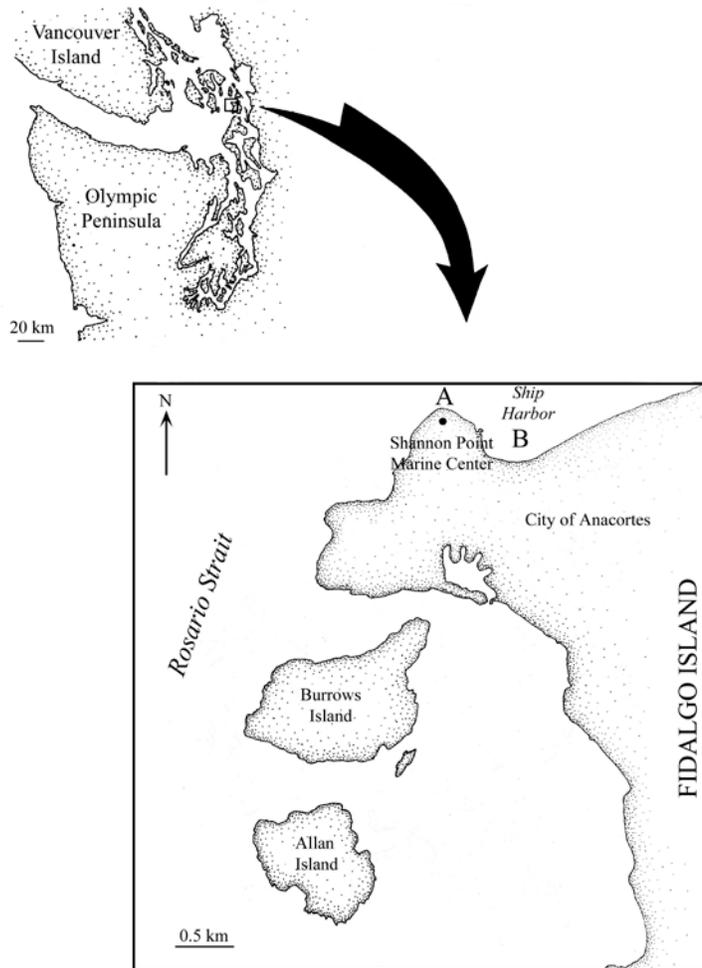


Figure 1. Map of crab oviger collection sites in Anacortes, WA. A- Beach collection site of *Hemigrapsus nudus* and *Lophopanopeus bellus*. B- SCUBA collection site of *Metacarcinus magister*.

than 24 hours old.

### **Algal cultures**

The haptophyte alga *Isochrysis galbana* was purchased from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (Boothbay Harbor, ME). Cultures were maintained and aerated at room temperature, using f/2-Si medium in a 20-L glass carboy under fluorescent light 24 hours/day. Cells were observed regularly under the microscope and counted using a hemocytometer. Algal culture density fluctuated between  $1 \times 10^6$  and  $3 \times 10^6$  cells  $\text{ml}^{-1}$ . When the culture became too dense, it was diluted with FSW and fresh f/2-Si medium was added. Cultures were diluted with 0.2 $\mu\text{m}$  FSW in 500ml glass beakers to attain the proper densities for each experiment. Although algal cultures were not axenic, they were examined regularly to control for contaminants.

### ***Artemia* and rotifer cultures**

Control diets consisted of zooplankton in excess. *Hemigrapsus nudus*, the smallest of the crab larvae, were fed rotifers for all experiments; *Lophopanopeus bellus* were fed freshly hatched *Artemia franciscana* nauplii for experiments when the nauplii hatched out small enough for larvae to ingest, and were fed rotifers when nauplii were too large to be ingested (Table 1). This was determined by inspections of larvae handling nauplii combined with macroscopic observation of nauplius size. *Metacarcinus magister* were fed *A. franciscana* nauplii for all experiments. Within any experimental run, all treatments received the same zooplankton prey.

Table 1. Number of broods and type of zooplankton used in each experiment. Where more than one brood is indicated, larvae from all broods hatching on the same day were pooled.

Experiment	Crab Species	Run	# of Broods	Zooplankton prey
Initial feeding on Microalgae	<i>Lophopanopeus</i>	1	2	<i>B. plicatilis</i>
	<i>bellus</i>	2	2	<i>B. plicatilis</i>
		3	1	<i>B. plicatilis</i>
		<hr/>		
	<i>Metacarcinus</i>	1	3	<i>A. franciscana</i>
	<i>magister</i>	2	5	<i>A. franciscana</i>
3		3	<i>A. franciscana</i>	
Patchy prey experiments	<i>Lophopanopeus</i>	1	1	<i>B. plicatilis</i>
	<i>bellus</i>	2	1	<i>B. plicatilis</i>
		3	2	<i>B. plicatilis</i>
		<hr/>		
	<i>Hemigrapsus</i>	1	1	<i>B. plicatilis</i>
	<i>nudus</i>	2	1	<i>B. plicatilis</i>
3		1	<i>B. plicatilis</i>	
Mixed prey experiments	<i>Lophopanopeus</i>	1	1	<i>A. franciscana</i>
	<i>bellus</i>	2	2	<i>A. franciscana</i>
		3	1	<i>A. franciscana</i>

*Artemia franciscana* cysts were obtained from Argent Chemical Laboratories (Redmond, WA) and were stored in a 15°C incubator. To set nauplii to hatch, cysts were added to an Imhoff cone containing 0.2µm FSW under constant light and aeration. Within 24 hours, hatched nauplii were collected using a 100µm filter and poured into a glass beaker as a thick slurry. Only non-feeding, freshly hatched nauplii were used in experiments. *A. franciscana* nauplii were hatched daily throughout the duration of experiments. Care was taken to add approximately 15-20 nauplii per ml to each tray well with a Pasteur pipette, approximately 1-2 drops of the slurry.

Rotifers were ordered from Reed Mariculture Inc. (Campbell, CA), and kept in a 20°C incubator to adapt to the temperature. Cultures of rotifers were kept in 500ml beakers with 0.2µm FSW in a 20°C incubator and fed high density ( $1 - 3 \times 10^6$  cells ml<sup>-1</sup>) *Isochrysis galbana*. Cultures were regularly harvested and transferred to new beakers with fresh algae in order to maintain them in an asexual growth phase. To prepare them for experiments, rotifer cultures were poured through an 80µm filter and rinsed thoroughly with 0.2µm FSW to remove *I. galbana* cells. Filtered rotifers and 0.2µm FSW were added to a 150ml beaker to create a dense rotifer suspension. Pasteur pipettes were used to dispense rotifers in excess to each tray well. Care was taken to ensure rotifer amount was consistent across the tray well experimental unit.

### **General experimental methods**

Twelve-well trays were used for all larval rearing experiments, with one zoea and 3ml of 0.2µm FSW containing the proper prey suspension in each well for each

experiment. Sibling larvae were distributed among all treatments in each experiment and, when possible, larvae from at least two broods hatching on the same day were pooled and the resulting mixture distributed haphazardly among treatments (Table 1). Trays were kept in a 15°C incubator. Larvae were monitored daily until they either died or molted to stage two, thereby exiting the experiment. Molts were determined by the presence in the well of a cast-off exoskeleton. Larvae were considered dead when they did not respond to any stimuli. Larval condition (molted; dead) was confirmed by observing them under a dissecting microscope. Each day, stage one larvae were carefully moved with a Pasteur pipette to a new tray with clean FSW and appropriate diet treatment. Two endpoints were measured: stage survival (based on % survival of each tray) and stage duration (mean day of first molt).

An algal density of 4,000 cells ml<sup>-1</sup> was used in all experiments except the mixed diet experiment which used three treatments of 50, 200 and 1000 cells ml<sup>-1</sup>, and in select preliminary experiments described below. Each day that algae were needed, a fresh batch of the appropriate concentration of algae was prepared from the stock culture.

Each of the three experiments described in *Experimental Approach* was run three times for each crab species tested (referred to hereafter as ‘Runs’) using different sets of sibling larvae. For each experimental Run, all treatments consisted of three replicate trays.

## Algal ingestion tests

Preliminary experiments were run to confirm that algae were being ingested by larvae when they were the only prey available, when larvae were fed algae after having been fed zooplankton for a period of time and when larvae were fed algae and zooplankton simultaneously (requiring visibility of algal cells in the gut when guts also contained zooplankton biomass).

Ingestion of algae was confirmed by the presence of chl *a* in the larval gut using epifluorescence microscopy to document its natural fluorescence. Selected larvae were removed from their treatments, rinsed thoroughly with 0.2 $\mu$ m FSW, mounted on slides, and examined for fluorescence using a Leica Leitz DMRB epifluorescence microscope. Larvae were exposed to blue light excitation (450-490 nm); any algal cells in the larval gut absorbed and re-emitted the light which passed through an emission filter (676 nm), appearing red. Larvae examined under epifluorescence were removed from the experiment. Photographs were taken using a Roper Scientific Photometrics CoolSnap cf camera and RS Image software (version 1.9.2) to document the results.

These preliminary tests confirmed that it was possible to see evidence of algal ingestion in the guts even when zooplankton biomass was present and confirmed that ingestion of algae occurred under all three experimental conditions. In these tests, relatively low algal densities were used (150-300 cells ml<sup>-1</sup>) to test possible threshold effects. Under these conditions, incidence of ingestion varied among tests, but typically ranged from 38%-60% of larvae tested. These results confirmed that larvae could ingest algae under the three experimental conditions and permitted analysis of their possible

nutritional contributions to larval developmental parameters such as survival and development rate.

Similar algal ingestion tests were conducted simultaneously with several of the main experiments outlined below to confirm algal ingestion was occurring under those particular experimental conditions. Algal densities ranged from 50-4,000 cells ml<sup>-1</sup>. Examples of fluorescence in the gut confirming ingestion of algae are shown for several treatments (Fig. 2).

## **Main Experiments**

### **Post-hatching feeding experiments**

Experiments were conducted on *Lophopanopeus bellus* and *Metacarcinus magister* to determine if immediate post-hatching feeding on only algae contributes nutritionally to newly-hatched larval crabs. The treatment set-up is shown diagrammatically in Figure 3 with half of the treatments receiving algae instead of the unfed periods. All initial periods of starvation (or algae) were followed by continuous zooplankton feeding in excess. Treatments incremented by one initial day of starvation or algal-feeding.

For each crab species tested, there were 12 treatments, consisting of a continuously fed zooplankton control (*Artemia franciscana* or *Brachionis plicatilis*), a continuously unfed control, five unfed treatments (unfed one day, unfed two days, unfed three days, unfed four days, unfed five days), and five algal-fed treatments (algal-fed one day, algal-fed two days, algal-fed three days, algal-fed four days, algal-fed five days).

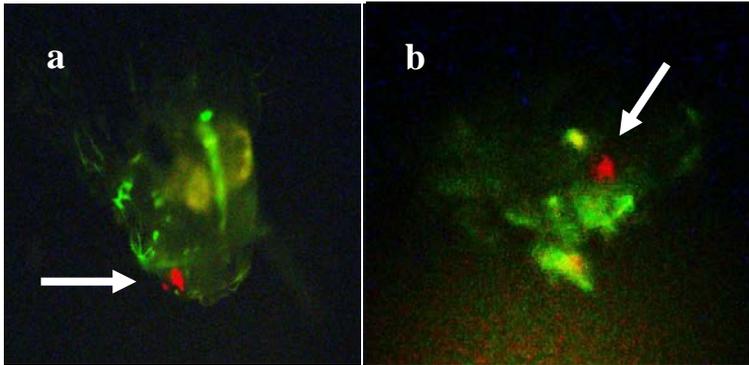


Figure 2. Epifluorescence images of stage I *Lophopanopeus bellus* larvae during mixed prey experiments in Run 2, in which they were fed a) zooplankton with 50 algal cells ml<sup>-1</sup> and b) zooplankton with 200 algal cells ml<sup>-1</sup>. Red spots indicated by arrows show presence of algal cells in larval gut.

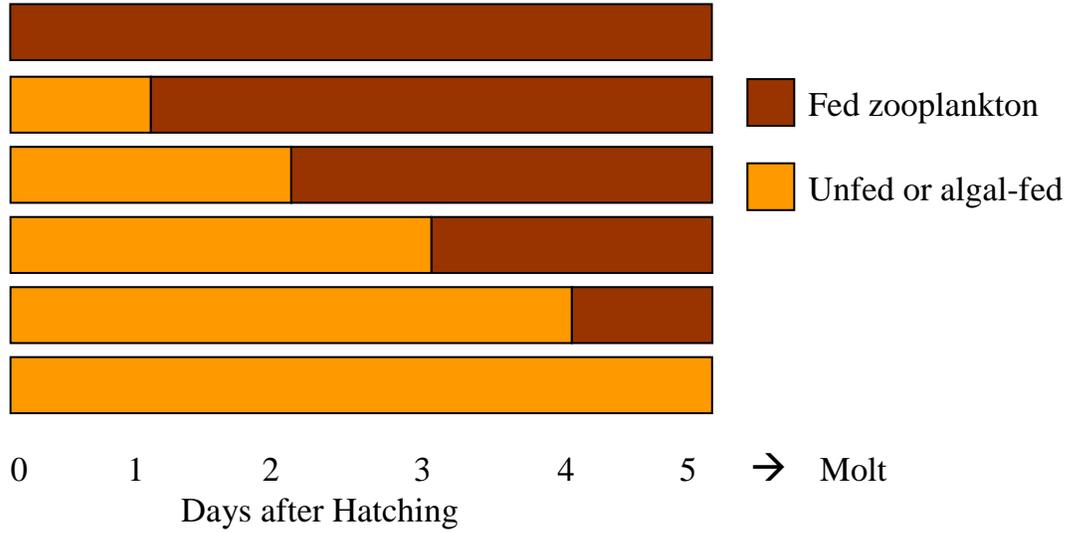


Figure 3. Diagrammatic representation of consecutive initial starvation treatments showing relative periods of starvation followed by feeding on zooplankton or feeding on algae only followed by zooplankton feeding. Days to molt vary and are shown here as an example only.

Each experiment was run three times with different brood groups, hereafter referred to as 'Runs.' For each Run, each treatment consisted of three replicate trays with 12 larvae per tray. A full Run therefore consisted of 36 trays and 432 larvae.

The progression of treatments throughout the experiment went as follows: On day one of the experiment, the fed control was the only treatment to receive zooplankton. All other trays were either unfed or fed algae. On day two of the experiment, the fed control, the unfed one day treatment and the algal-fed one day treatment received zooplankton, all other trays were either unfed or fed algae. The experiment continued this way until day six after which all larvae were fed zooplankton and were maintained until all larvae had molted to zoeal stage two or died.

Algal-fed treatments were fed *Isochrysis galbana* at a concentration of 4,000 cells  $\text{ml}^{-1}$ . Each day a beaker of *I. galbana* was prepared at a concentration of 12,000 cells  $\text{ml}^{-1}$ . Using a repeater pipette, 1ml of 12,000 cells  $\text{ml}^{-1}$  algal suspension was added to 2ml of 0.2 $\mu\text{m}$  FSW in each tray well to achieve a concentration of 4000 cells  $\text{ml}^{-1}$ . The algal suspension was agitated before being dispensed to ensure homogeneous distribution of algal cells. Larvae were checked daily for evidence of mortality and molting and each day living larvae were moved with a Pasteur pipette to a new tray with FSW and fed the appropriate diet.

Two types of analyses were conducted to assess the impact of early feeding on larval condition. Stage survival and stage duration were compared among treatments initially using a Two-way ANOVA with treatment as a fixed factor and Run as a random factor. Where Levene's Test of Equality of Variances showed variances to be non-homogenous,  $\alpha$  was adjusted to 0.025 to compensate (Gamst et al. 2008). Where

there was no significant treatment by Run interaction, data from all three Runs were further analyzed using special contrasts to compare treatments of increasing initial unfed periods against respective treatments of increasing initial algal-fed periods. For example, the unfed one day treatment was compared to the algal-fed one day treatment, the unfed two day treatment was compared to the algal-fed two day treatment, and so on for all pre-zooplankton feeding periods. Where the interaction term was significant, data from each Run were analyzed separately using simple main effects comparing unfed and algal-fed treatments as described above.

Extensive previous research (Chomiczewski 2009; Sulkin, pers. comm.) has indicated that trays do not produce effect as long as the experimental design assures that larvae from pooled broods are randomly assigned to trays, and trays are randomly assigned to treatments. Those precautions were followed in these experiments at all times so no tray term was included in the analysis.

To further assess the effects of initial periods of algal feeding on stage survival and duration, a modification of the Point of No Return (PNR) approach (Anger et al. 1980; Staton and Sulkin 1991) was employed. The unfed-PNR compared each sequential unfed treatment to the continuously fed control with a series of t-tests. When the first significant result for either reduction in stage survival or increase in stage duration of the unfed treatment as compared to the control was found, that 'day' (eg., the number of initial unfed days) was identified as the unfed-PNR. In these experiments, a total of three individual PNRs was determined for different brood sets ('Runs'). The same analysis was then repeated for sequential algal fed treatments, providing an algal-fed PNR (n=3). The two PNRs were then compared by a t-test to determine if feeding on algae delayed the

time of significantly reduced survival or resulted in a difference between the two treatments in reducing the stage duration (e.g. accelerated development). If either parameter was affected, it indicated a nutritional benefit provided by the algae.

### **Patchy prey diet experiments**

Experiments were conducted on *Lophopanopeus bellus* and *Hemigrapsus nudus* to determine whether algal prey contributed nutritionally under conditions when encounters with favored zooplankton prey are intermittent. To simulate this under laboratory conditions, the following diet treatments were set up: larvae alternated between being fed *Brachionis plicatilis* for one day followed by being unfed for three days or being fed *B. plicatilis* for one day followed by being fed *Isochrysis galbana* for three days, after which the feeding pattern was repeated (Figure 4). The periods of zooplankton-feeding were chosen to be short so as to produce sub-optimal feeding conditions (Lehto et al. 1998; Sulkin et al. 1998a); that is, to support development, but at either reduced survival or slower rate of development as compared to an optimal diet treatment. Sub-optimal diets use a prey source known to sustain larval development, but provide it in insufficient amounts or for insufficient duration, thus producing delayed development or increased mortality. This allows for an assessment of algal nutritional contribution by substituting algae as prey for the unfed periods. Stage survival and stage duration were compared for treatments of alternating zooplankton/unfed periods against treatments of alternating zooplankton/algal-fed periods.

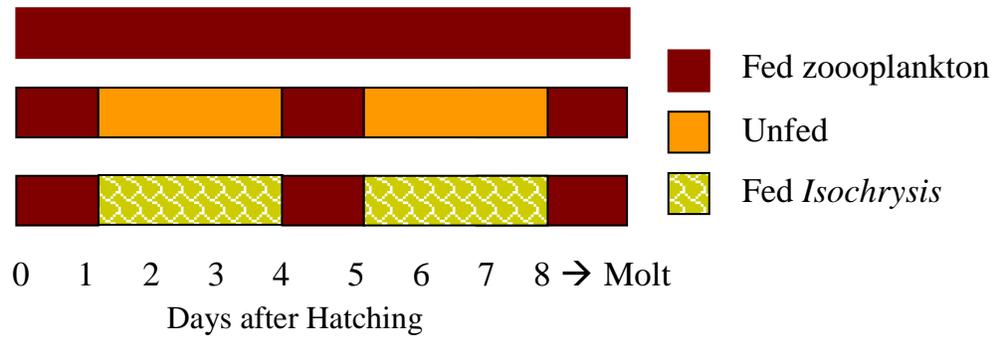


Figure 4. Diagrammatic representation of three treatments showing periods of zooplankton feeding alternating with periods of starvation and algae feeding.

Each treatment consisted of three replicate trays. There were nine trays total consisting of three fed control trays, three alternating fed zooplankton/unfed trays and three alternating fed zooplankton/fed algae trays (Figure 4). The progression of treatments throughout the experiment went as follows:

On day one, all three treatments were fed zooplankton. On day two, the fed control received zooplankton, the fed zooplankton/unfed treatment was not fed (but was still transferred to a new well with clean FSW), and the fed zooplankton/fed algae treatment was fed *Isochrysis galbana*. Days three and four repeated the procedure for day two. On day five, all three treatments were fed zooplankton for one day. This whole procedure was repeated again until day nine and beyond, when all treatments were fed zooplankton for the duration of the experiment until the larvae died or molted to stage two (up to 25 days for *Lophopanopeus bellus* and up to 18 days for *Hemigrapsus nudus*), thereby exiting the experiment. The experiment was run three times with different brood sets identified hereafter as ‘Runs.’

Two-way Analysis of Variance, with Run as a random factor and diet treatment as a fixed factor, was used to determine significant differences among diet treatments for each endpoint (stage survival; stage duration), followed by Tukey’s HSD *post-hoc* tests to identify where differences existed among treatments when the interaction between Run and treatment was not significant. In cases where the interaction between Run and treatment was significant, simple main effects contrasts were used to determine treatment differences within each Run. Where Levene’s test of equality of variances showed variances to be non-homogenous, *alpha* was adjusted to 0.025 (Gamst et al. 2008). Larvae were checked daily for evidence of mortality and molting and each day larvae

were moved with a Pasteur pipette to a new tray with fresh water and appropriate diet treatment.

### **Mixed prey diet experiments**

Experiments were conducted using larvae of *Lophopanopeus bellus* to determine whether consumption of algae occurs and contributes nutritionally when provided in combination with zooplankton. Diets of *Artemia franciscana* nauplii were compared to diets of *A. franciscana* mixed with *Isochrysis galbana* prey at varying densities.

Treatments consisted of *A. franciscana* only, *A. franciscana* mixed with *I. galbana* at 50 cells ml<sup>-1</sup>, *A. franciscana* mixed with *I. galbana* at 200 cells ml<sup>-1</sup> and *A. franciscana* mixed with *I. galbana* at 1000 cells ml<sup>-1</sup>. An estimated 15-20 nauplii ml<sup>-1</sup> was added to each tray well. All treatments were applied continuously from day of hatching.

Each treatment consisted of three replicate trays. There were 12 trays total consisting of three *Artemia franciscana* trays, three mixed 50 cells ml<sup>-1</sup> trays, three mixed 200 cells ml<sup>-1</sup> trays, and three mixed 1000 cells ml<sup>-1</sup> trays. The experiment was run three times with different brood-sets. The first experimental Run did not include the lowest density treatment (*A. franciscana* mixed with *Isochrysis galbana* at 50 cells ml<sup>-1</sup>).

Each day three beakers were prepared using dilutions from the stock *Isochrysis galbana* culture to provide concentrations of 150 cells ml<sup>-1</sup>, 600 cells ml<sup>-1</sup> and 3,000 cells ml<sup>-1</sup>. Using a repeater pipette, 1ml of each algal suspension was added to 2ml of 0.2µm FSW to each tray well to achieve the desired concentrations of 50 cells ml<sup>-1</sup>, 200 cells

ml<sup>-1</sup>, and 1000 cells ml<sup>-1</sup>. The algal suspensions were agitated before being dispensed to ensure equal distribution of algal cells.

*Artemia franciscana* nauplii were added to each of the mixed treatment tray wells. Care was taken to add an estimated 15-20 nauplii ml<sup>-1</sup> to each tray well with a Pasteur pipette, approximately 1-2 drops. *Artemia franciscana*-only treatments received 3ml of 0.2µm FSW and 1-2 drops of *A. franciscana* in their wells. Mixed 50 cell ml<sup>-1</sup> treatments received 2ml of 0.2µm FSW, 1ml *I. galbana* at 150 cells ml<sup>-1</sup>, and 1-2 drops *A. franciscana*. Mixed 200 treatments received 2ml of 0.2µm FSW, 1ml *I. galbana* at 600 cells ml<sup>-1</sup>, and 1-2 drops *A. franciscana*.

Two-way ANOVA, with Run as a random factor and treatment as a fixed factor, was used to determine significant differences among diet treatments for each endpoint (stage survival; stage duration), followed by Tukey's HSD *post-hoc* tests to identify where differences exist among treatments. Because the first experimental Run did not include the lowest density treatment (*A. franciscana* mixed with *Isochrysis galbana* at 50 cells ml<sup>-1</sup>), the Type IV Sum of Squares was specified when running the ANOVA to account for the missing data. In cases where the interaction between Run and treatment was significant, simple main effects contrasts were used to determine treatment differences within each Run. Where Levene's test of equality of variances showed variances to be non-homogenous, *alpha* was adjusted to 0.025 (Gamst et al. 2008).

## RESULTS

### Post-hatching feeding experiments

#### *Lophopanopeus bellus*

Stage survival of larvae subjected to various initial periods of either no food or feeding on microalgae prior to continuous zooplankton feeding is shown in Figure 5. There was an apparent decrease in survival for both experimental diets as the initial period prior to zooplankton feeding was extended. Data were analyzed by a Two-way ANOVA with treatment and Run as factors (Table 2). The interaction term was non-significant, and tests of the main effects indicated significant differences among treatments ( $p < 0.025$ ).

In order to determine possible differences between each unfed and algal-fed treatment with the same pre-zooplankton feeding period, data were subjected to special contrasts. For example, the unfed one day treatment was compared to the algal-fed one day treatment, the unfed two day treatment was compared to the algal-fed two day treatment, and so on for all pre-zooplankton feeding periods (Table 3). All paired comparisons were found to be non-significant ( $p > 0.025$ ) indicating that there was no significant difference in stage survival due to presence or absence of algal prey during any of the initial pre-zooplankton feeding periods.

Stage duration of larvae subjected to various initial periods of either being unfed or being fed microalgae prior to continuous zooplankton feeding are shown in Fig. 6. An increase in stage duration is apparent for both experimental diets as the initial period

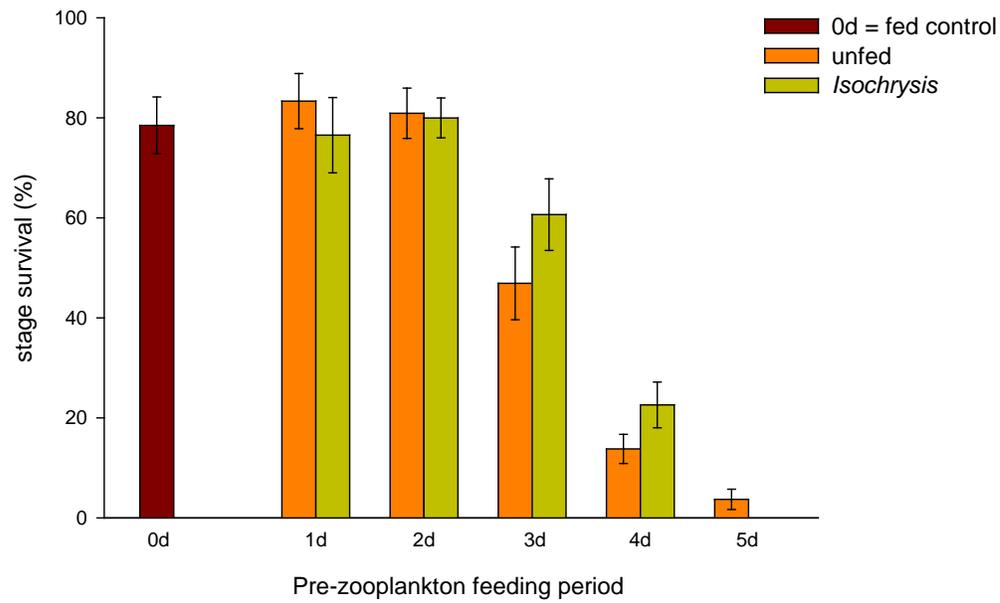


Figure 5. Effects on Stage I survival ( $\pm$  one standard error) of *Lophopanopeus bellus* larvae as initial period of experimental diet increases. Three Runs are combined. N=9 in all treatments except for *Isochrysis*-fed 3d where n=8. *Isochrysis*-fed larvae for 5 days had zero survival.

Table 2. Two-way ANOVA results for stage survival and stage duration of *Lophopanopeus bellus* larvae when exposed to various diet treatments for increasing durations (1-5 days) ( $\alpha= 0.025$ ). Stage survival data were arcsine square root transformed.

Source	SS	df	MS	F	P
Stage Survival					
Treatment	17.769	10	1.777	31.193	<0.001
Run	1.079	2	0.540	9.479	0.001
Treatment*Run	1.083	19	0.057	1.748	0.051
Error	2.144	65	0.033		
Stage duration					
Treatment	2898.547	8	362.318	45.913	<0.001
Run	85.867	2	42.934	7.585	0.004
Treatment*Run	129.535	16	8.096	10.089	<0.001
Error	424.346	525	0.808		

Table 3. Statistical results of special contrasts comparing stage survival of *Lophopanopeus bellus* larvae given experimental diet treatments (unfed vs. algal-fed) at varying pre-zooplankton feeding periods. There were no significant differences ( $\alpha = 0.025$ ). Runs are combined (n=9).

Pre-zooplankton feeding period	Unfed vs. Algal-fed contrast p-value
1	0.130
2	0.293
3	0.197
4	0.435
5	0.641

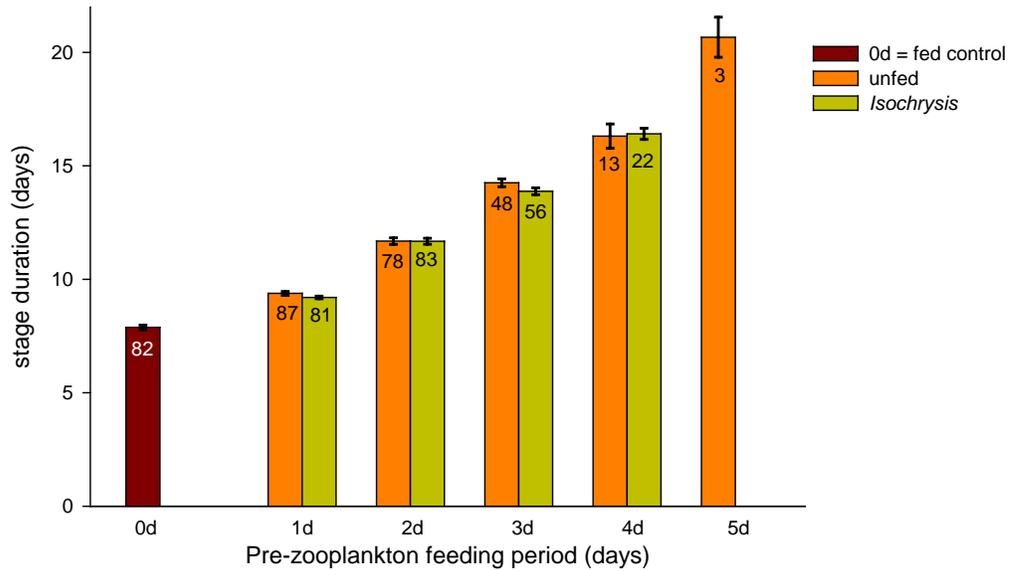


Figure 6. Effects on Stage I duration ( $\pm$  one standard error) of *Lophopanopeus bellus* larvae as initial period of experimental diet increases. Three Runs are combined. Sample sizes vary per treatment and are shown in each column. *Isochrysis*-fed larvae for 5 days had zero survival.

before zooplankton feeding was extended. A Two-way ANOVA analysis of the data showed a significant interaction between treatment and Run ( $p < 0.025$ ) (Table 2). Data were therefore subjected to simple main effects comparing unfed and algal-fed treatments within each Run to indicate possible differences between each treatment with the same pre-zooplankton feeding period. The only significant difference was between the unfed one day treatment and the algal-fed one day treatment for Run 2 (Table 4). In that Run, algal-fed larvae had a shorter stage duration than unfed larvae by just under a half-day (by 7%) (Fig. 7). All other paired comparisons were found to be non-significant indicating that overall there was no significant difference in stage duration due to presence or absence of algal prey during initial pre-zooplankton feeding periods.

In order to further assess the potential effects of initial feeding by larvae on microalgae, the same data were subjected to a Point of No Return (PNR) type analysis (*sensu* Anger et al. 1981; Staton and Sulkin 1991) for stage survival and duration. Percent stage survival for each Run is shown in Table 5. Each treatment is compared to the continuously fed control in sequence with the first evidence of statistical significance using an Independent samples t-test indicating the PNR for that experiment. PNRs for unfed treatments in the three Runs were then compared to those for algal-fed treatments to determine if feeding on algae resulted in a delay of the first evidence of reduced survival. The mean PNRs for unfed and algal-fed treatments were 3.67 days and 4 days, respectively. An Independent samples t-test comparing the PNR means indicated no significant difference ( $p > 0.05$ ).

Mean stage duration results are shown in Table 6. A t-test comparing the mean PNRs between unfed and algal fed treatments (1.67 days and 1 day, respectively)

Table 4. Statistical results of a simple main effects test contrasting stage duration of *Lophopanopeus bellus* larvae given experimental diet treatments (unfed vs. algal-fed) at varying pre-zooplankton feeding periods. An asterisk indicates significant differences ( $\alpha = 0.025$ ).

Run	Pre-zooplankton feeding period	Unfed vs. Algal-fed contrast p-value
1	1d	0.632
	2d	0.541
	3d	0.340
	4d	0.360
2	1d	0.004*
	2d	0.032
	3d	0.183
	4d	0.940
3	1d	0.972
	2d	0.458
	3d	0.372
	4d	0.249

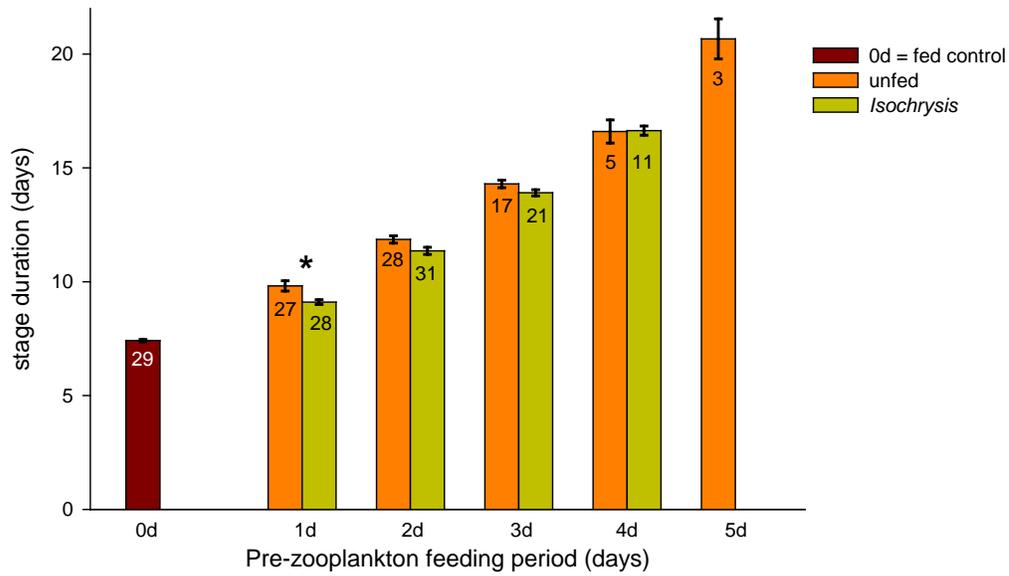


Figure 7. Effects on Stage I duration ( $\pm$  one standard error) of *Lophopanopeus bellus* larvae in Run 2 as initial period of experimental diet increases. Sample sizes vary per treatment and are shown in each column. *Isochrysis*-fed larvae for 5 days had zero survival. Asterisk denotes the paired bars that are significantly different from one another ( $\alpha= 0.025$ ).

Table 5. Percent survival for each PNR treatment for *Lophopanopeus bellus* larvae. First treatment for which percent survival is significantly different from fed control is indicated by an asterisk (t-test,  $\alpha=0.05$ ) for each of the three Runs.

Pre-zooplankton feeding period (days)	Run					
	1		2		3	
	Unfed	Algal-fed	Unfed	Algal-fed	Unfed	Algal-fed
0 (fed)	68.8	68.8	80.6	80.6	86.1	86.1
1	73.2	52.8	76.8	79.5	100.0	97.2
2	71.0	68.2	80.1	88.4	91.7	83.3
3	23.7*	45.8	49.0	58.3	67.9	72.8
4	11.9	15.0*	18.1*	33.3*	11.4*	19.4*
5	2.8	0.0	8.3	0.0	0.0	0.0
Unfed	0.0	-	0.0	-	0.0	-

Table 6. Stage duration (days) for each PNR treatment for *Lophopanopeus bellus* larvae. First treatment for which percent survival is significantly different from fed control is indicated by an asterisk (t-test,  $\alpha=0.05$ ) for each of the three Runs.

Pre- zooplankton feeding period (days)	Run					
	1		2		3	
	Unfed	Algal-fed	Unfed	Algal-fed	Unfed	Algal-fed
0 (fed)	8.8	8.8	7.4	7.4	7.7	7.7
1	9.1	9.2*	9.8	9.1*	9.3*	9.3*
2	10.9*	11.0	11.9*	11.4	12.6	12.5
3	13.2	12.8	14.3	13.9	14.6	14.4
4	14.2	14.8	16.6	16.6	18.0	17.8
5	-	-	20.7	-	-	-
Unfed	-	-	-	-	-	-

indicated no significant difference between the two ( $p > 0.05$ ).

Results for *Lophopanopeus bellus* indicate that initial feeding on microalgae neither increased survival nor reduced stage duration significantly in stage one larvae when compared to larvae that were not being fed during those initial periods.

### ***Metacarcinus magister***

*Metacarcinus magister* larvae were subjected to various initial periods of either no food or feeding on microalgae prior to continuous zooplankton feeding. Stage survival data are shown in Fig. 8. There was an apparent decrease in survival for both experimental diets as the initial period before zooplankton feeding was extended. A Two-way ANOVA analysis of the data showed a significant interaction between treatment and Run ( $p < 0.025$ ) (Table 7). Data were therefore subjected to simple main effects within each Run to indicate possible differences between each treatment with the same pre-zooplankton feeding period using an *alpha* of 0.025 (Table 8). The only significant difference indicated by these tests was between the unfed three-day treatment and the algal-fed three day treatment for Run one. In that Run, algal-fed larvae had a stage survival of 16.7% ( $\pm$  SE) while no unfed larvae survived (Fig. 9). All other paired comparisons were found to be non-significant indicating that overall there was no significant difference in stage survival due to presence or absence of algal prey during initial pre-zooplankton feeding periods.

Stage duration results of *Metacarcinus magister* larvae subjected to various initial periods of either being unfed or being fed microalgae prior to continuous zooplankton

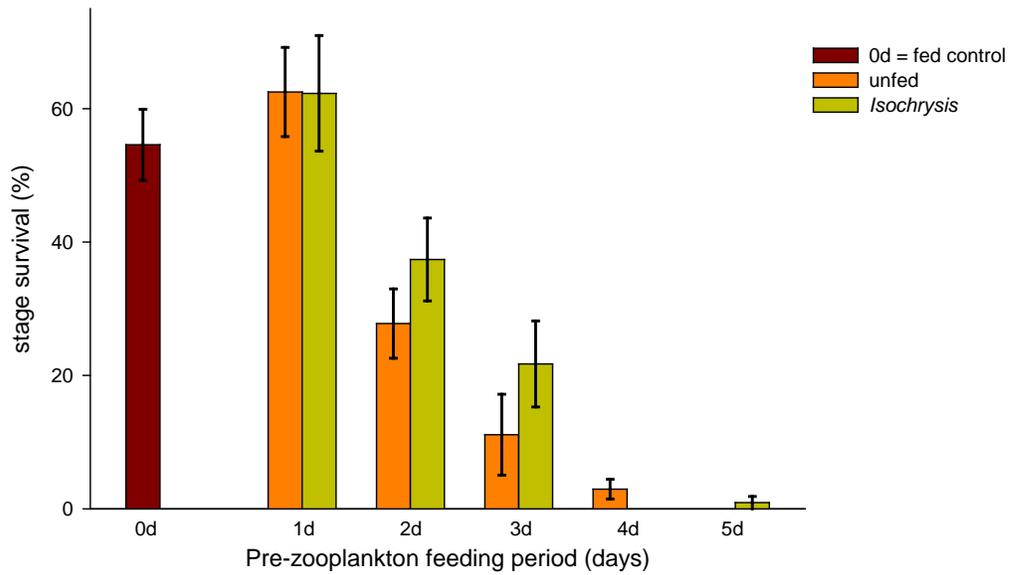


Figure 8. Effects on Stage I survival ( $\pm$  one standard error) of *Metacarcinus magister* larvae as initial period of experimental diet increases. Three Runs are combined (n=9). Algal-fed larvae for four days and unfed larvae for five days had zero survival.

Table 7. Two-way ANOVA results for stage survival and stage duration of *Metacarcinus magister* larvae when exposed to various diet treatments (unfed, algae) for increasing durations (1-5 days) ( $\alpha= 0.025$ ). Stage survival data was arcsine square root transformed.

Source	SS	df	MS	F	P
<b>Stage Survival</b>					
Treatment	12.170	10	1.217	19.008	<0.001
Run	1.325	2	0.663	10.363	0.001
Treatment*Run	1.282	20	0.064	3.484	<0.001
Error	1.190	65	0.018		
<b>Stage duration</b>					
Treatment	636.883	7	90.983	49.067	<0.001
Run	8.558	2	4.279	2.475	0.110
Treatment*Run	21.483	11	1.953	1.525	0.122
Error	346.947	271	1.280		

Table 8. Statistical results of a simple main effects test contrasting stage survival of *Metacarcinus magister* larvae given experimental diet treatments (unfed vs. algal-fed) at varying pre-zooplankton feeding periods. An asterisk indicates a significant difference ( $\alpha = 0.025$ ).

Run	Pre-zooplankton feeding period	Unfed vs. Algal-fed contrast p-value
1	1d	0.989
	2d	0.200
	3d	0.001*
	4d	1.000
	5d	0.386
2	1d	0.491
	2d	0.570
	3d	0.339
	4d	0.076
	5d	1.000
3	1d	0.296
	2d	0.354
	3d	0.076
	4d	0.354
	5d	1.000

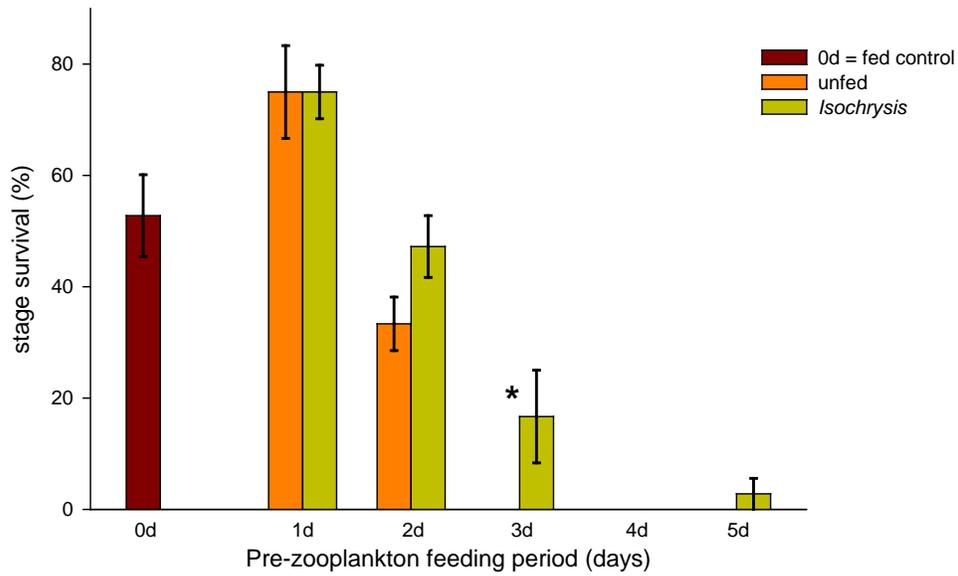


Figure 9. Effects on Stage I survival ( $\pm$  one standard error) of *Metacarcinus magister* larvae in Run 1 as initial period of experimental diet increases. N=3 in all treatments except for Unfed 1d where n=2. Unfed larvae for 3, 4 and 5 days had zero survival, as did algal-fed larvae for 4 days. Asterisk denotes the paired bars that are significantly different from one another ( $\alpha= 0.025$ ).

feeding are shown in Fig. 10. Stage duration increased for both experimental diets as the period before zooplankton feeding was extended. The interaction term in a Two-way ANOVA was non-significant (Table 7). Testing of the main effects showed a significant difference among treatments ( $p < 0.025$ ).

Data were then subjected to special contrasts to indicate possible differences between each treatment. The unfed one day treatment was compared to the algal-fed one day treatment and the unfed two day treatment was compared to the algal-fed two day treatment. Pre-zooplankton feeding periods of three, four and five days were omitted from contrasts because not all Runs had stage duration data at these periods due to zero survival. All paired comparisons were found to be non-significant indicating that there was no significant difference in stage duration due to presence or absence of algal prey during initial pre-zooplankton feeding periods (Table 9).

Data were further analyzed using the Point of No Return (PNR) analysis. Percent stage survival is shown in Table 10. Results showed no significant difference between unfed and algal-fed PNRs (2.67 days and 3.33 days, respectively) (t-test;  $p > 0.05$ ). Mean stage duration results are shown in Table 11. A t-test comparing the PNRs between unfed and algal-fed treatments (1.67 days and 1.33 days, respectively) indicated no significant difference between them ( $p > 0.05$ ).

Results for *Metacarcinus magister* stage one larvae indicate that the presence of microalgae during initial feeding neither increased survival nor reduced stage duration significantly when compared to larvae that were not being fed during those initial periods.

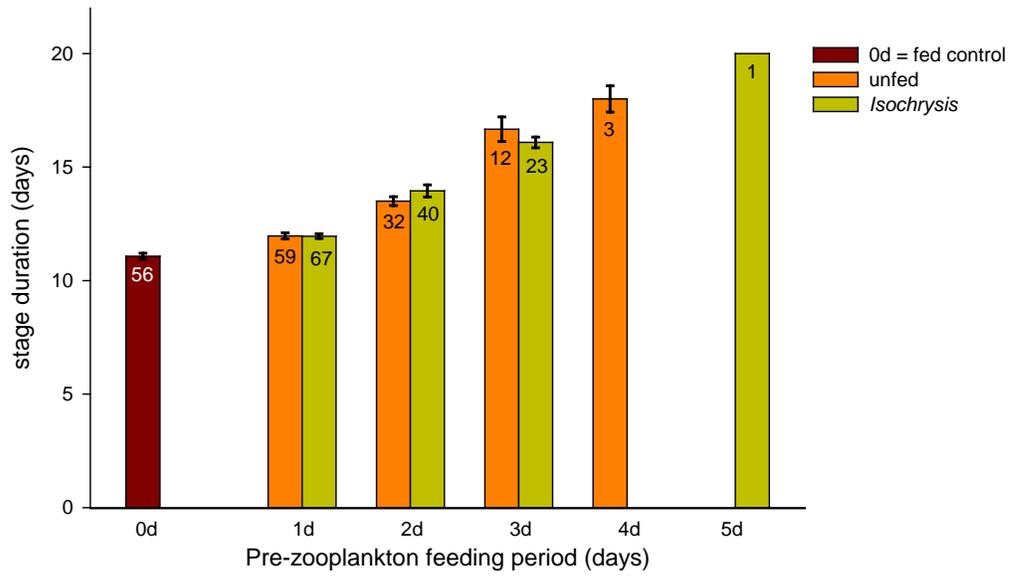


Figure 10. Effects on Stage I duration ( $\pm$  one standard error) of *Metacarcinus magister* larvae as initial period of experimental diet increases. Three Runs are combined. Sample sizes vary per treatment and are shown in each column. Algal-fed larvae for four days had zero survival, as did unfed larvae for five days.

Table 9. Statistical results of special contrasts comparing stage duration of *Metacarcinus magister* larvae given experimental diet treatments (unfed vs. algal-fed) at varying pre-zooplankton feeding periods. There were no significant differences ( $\alpha = 0.025$ ). Runs are combined (n=108). Pre-zooplankton feeding periods of three, four and five days were omitted from contrasts due to zero survival in some Runs.

Pre-zooplankton feeding period	Unfed vs. Algal-fed contrast p-value
1	0.857
2	0.218

Table 10. Percent survival for each PNR treatment for *Metacarcinus magister* larvae. First treatment for which percent survival is significantly different from fed control is indicated by an asterisk (t-test,  $\alpha=0.05$ ).

Pre-zooplankton feeding period (days)	Run					
	1		2		3	
	Unfed	Algal-fed	Unfed	Algal-fed	Unfed	Algal-fed
0 (fed)	52.8	52.8	66.5	66.5	44.4	44.4
1	75.0	75.0	75.0	80.6	41.7	31.3
2	33.3	47.2	38.9*	45.5	11.1	19.4
3	0.0*	16.7*	33.3	42.7	0.0*	5.8*
4	0.0	0.0	5.8	0.0*	3.0	0.0
5	0.0	3.0	0.0	0.0	0.0	0.0
Unfed	0.0	-	0.0	-	0.0	-

Table 11. Stage duration (days) for each PNR treatment for *Metacarcinus magister* larvae. First treatment for which percent survival is significantly different from fed control is indicated by an asterisk (t-test,  $\alpha=0.05$ ).

Pre-zooplankton feeding period (days)	Run					
	1		2		3	
	Unfed	Algal-fed	Unfed	Algal-fed	Unfed	Algal-fed
0 (fed)	11.5	11.5	10.9	10.9	10.8	10.8
1	8.2	11.8	12.1*	12.0*	11.7	12.1*
2	14.1*	14.5*	13.1	13.3	9.2*	9.5
3	-	15.9	16.8	16.0	-	11.0
4	-	-	18.5	-	5.7	-
5	-	-	-	-	-	-
Unfed	-	-	-	-	-	-

## Patchy prey diet experiments

### *Lophopanopeus bellus*

Daily survival of larvae subjected to intermittent periods of no prey or algal prey alternating with zooplankton prey are shown in Figure 11. Stage survival data were analyzed by a Two-way ANOVA with treatment and Run as factors (Table 12). The interaction term was non-significant ( $p > 0.05$ ), as were tests of the main effects. Because the survival of both experimental diets appears to be quite different from the control (Fig. 11), and lack of significance does not definitively rule out an effect, effect size was estimated in SPSS using Partial  $\eta^2$ . Effect size is the magnitude of the observed effect, and Partial  $\eta^2$  gives the proportion of the effect and error variance explained by the effect (treatment). Although the ANOVA showed that the means were not significantly different, the effect size was large. The  $\eta^2$  was 0.62, indicating that treatment, by itself, accounted for 62% of the overall ( $SS_{\text{treatment}} + SS_{\text{error}}$ ) variance. To examine treatment more closely, differences between the fed control and the intermittent periods of zooplankton feeding alternating with either algae or no food were tested using *post-hoc* Tukey's HSD contrasts (Fig. 12). The fed control was significantly different from the experimental diet treatments. The two experimental diet treatments were not significantly different from one another. Looking at daily survival, approximately half of the mortality that occurs in the two experimental treatments occurred within the first five days (Fig. 11). Both the fed/unfed treatment and the fed/algal-fed treatment follow roughly the same slope throughout. The experimental treatment that included three days of no access to prey interspersed with one day of feeding on zooplankton produced a sub-

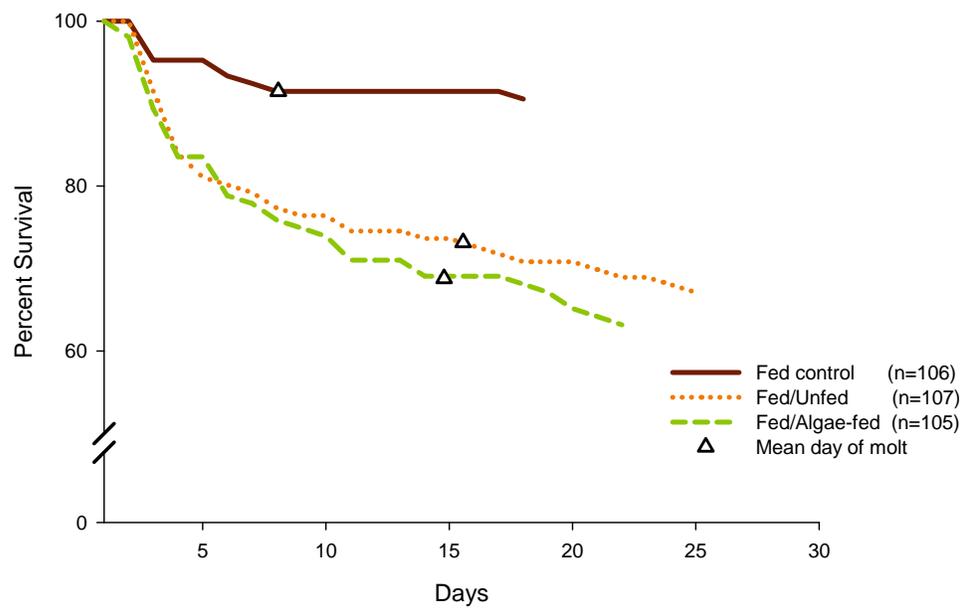


Figure 11. Daily survival of *Lophopanopeus bellus* larvae as intermittent diet treatments vary. Three Runs are combined. Sample sizes vary per treatment and are shown in the legend. Mean day of molt is shown for each treatment. Length of each treatment line corresponds to the day on which all larvae in that treatment had either molted or died.

Table 12. Two-way ANOVA results for stage survival and stage duration of *Lophopanopeus bellus* larvae when exposed to various patchy diet treatments. Stage duration  $\alpha = 0.025$ . Stage survival data was arcsine square root transformed.

Source	SS	df	MS	F	P
<b>Stage Survival</b>					
Treatment	0.664	2	0.332	3.324	0.141
Run	0.530	2	0.265	2.656	0.185
Treatment*Run	0.399	4	0.100	2.369	0.091
Error	0.758	18	0.042		
<b>Stage duration</b>					
Treatment	2746.419	2	1373.209	298.154	<0.001
Run	0.997	2	0.498	0.107	0.901
Treatment*Run	18.835	4	4.709	2.100	0.082
Error	504.432	225	2.242		

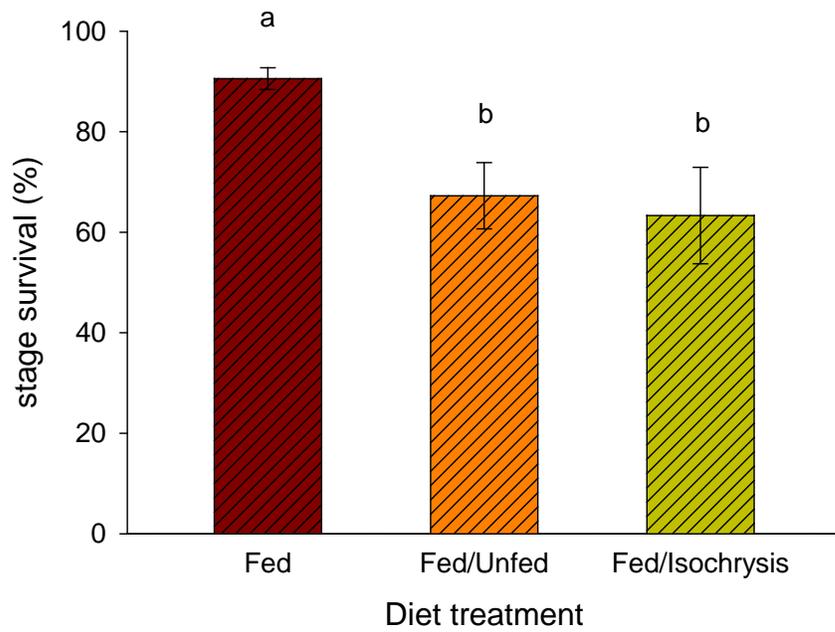


Figure 12. Effects on stage survival ( $\pm$  one standard error) of *Lophopanopeus bellus* larvae as intermittent diet treatments vary. Three Runs are combined (n=9). Shared letters indicate no significant differences between treatments (Tukey's HSD *post hoc* contrasts,  $\alpha = 0.05$ ).

optimal result; namely, reduced survival and delayed development as compared to a continuously fed control. Adding algal prey to the sub-optimal diet, however, did not produce increased survival as compared to the sub-optimal diet.

Stage duration of larvae subjected to intermittent periods of no prey or algal prey alternating with zooplankton prey are shown in Figure 13. Data were analyzed by a Two-way ANOVA (Table 12). The interaction term was non-significant ( $p > 0.025$ ). Tests of the main effects showed treatment to be significant. Differences between the fed control and the intermittent periods of zooplankton feeding alternating with either algae or no food were tested using *post-hoc* Tukey's HSD contrasts. The fed control was significantly different from both experimental diet treatments ( $p < 0.025$ ), and the experimental diet treatments were significantly different from one another (Fig. 13). Larvae in the fed control had the shortest mean stage duration (8 days), with accelerated development compared to the fed/unfed treatment of 7.5 days or 48% and to the fed/algal-fed treatment by 6.7 days or 45%. In contrast to the survival results, addition of algae to the sub-optimal diet did show a statistically significant impact on stage duration, producing an acceleration of development by 5%. Results thus indicate a significant but small degree of accelerated development in *Lophopanopeus bellus* larvae due to the ingestion of algal prey in patchy prey environments.

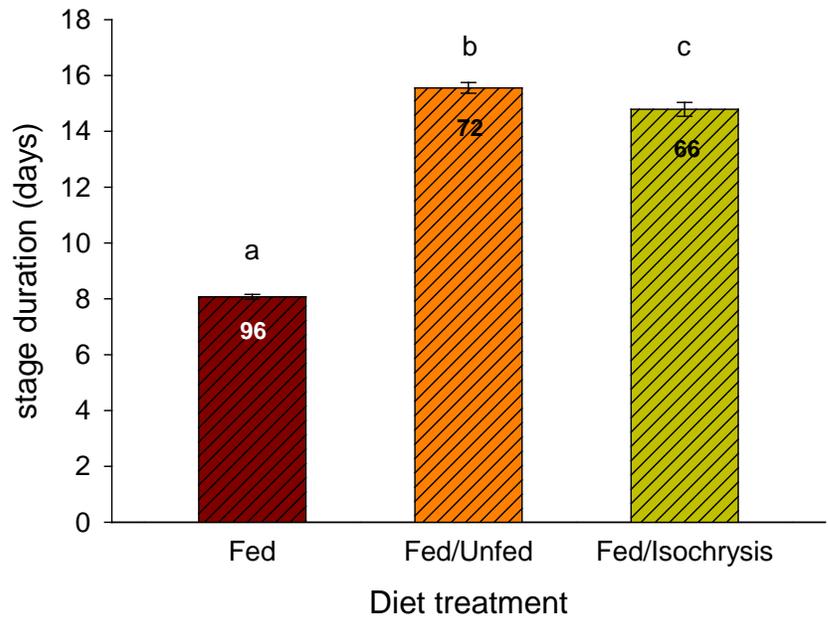


Figure 13. Effects on stage duration ( $\pm$  one standard error) of *Lophopanopeus bellus* larvae as intermittent diet treatments vary. Three Runs are combined. Sample sizes vary per treatment and are shown in each column. Different letters indicate significant differences between treatments (Tukey's HSD *post hoc* contrasts,  $\alpha = 0.025$ ).

## *Hemigrapsus nudus*

Daily survival of larvae subjected to intermittent periods of no prey or algal prey alternating with zooplankton prey are shown in Figure 14. Stage survival data were analyzed by a Two-way ANOVA with treatment and Run as factors (Table 13). The interaction term was non-significant ( $p > 0.05$ ). Tests of the main effects showed treatment to be significant. Differences between the fed control and the intermittent periods of zooplankton feeding alternating with either algae or no food were tested using *post-hoc* Tukey's HSD contrasts. The fed control had significantly higher survival from the experimental diet treatments ( $p < 0.05$ ) by at least 63% (Fig. 15). The two experimental diet treatments were not significantly different from one another. Looking at daily survival, the two experimental diet treatments follow the same slope as the fed control until day six, after which they sharply drop away: approximately 60% mortality occurring over the next ten to twelve days (Fig. 14). Both fed/unfed and fed/algal-fed treatments follow roughly the same pattern, as is the case with *Lophopanopeus bellus*. Results suggest that ingestion of algae does not increase stage survival of *Hemigrapsus nudus* larvae as compared to the unfed sub-optimal diet.

Stage duration of larvae subjected to intermittent periods of no prey or algal prey alternating with zooplankton prey are shown in Figure 16. Data were analyzed by a Two-way ANOVA (Table 13). The interaction term was significant ( $p < 0.025$ ), and data were therefore subjected to simple main effects contrasts to assess differences between treatments within each Run. For all Runs, the fed control had a significantly shorter stage duration as compared to the two experimental treatments ( $p < 0.025$ ), ranging from 2.5 days (25%) in Run three, to 5.3 days (42%) in Run one (Fig. 16). For Runs one and two,

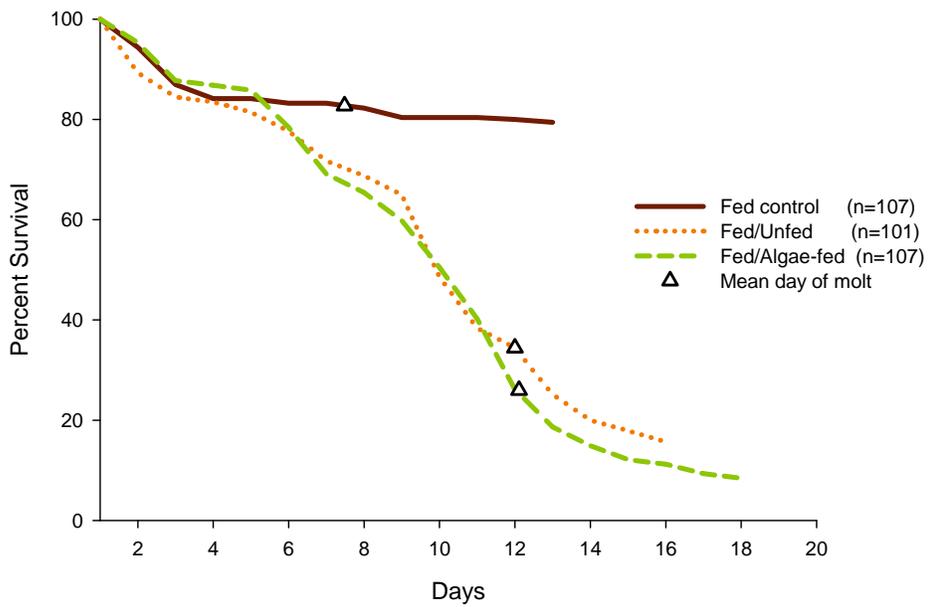


Figure 14. Daily survival of *Hemigrapsus nudus* larvae as intermittent diet treatments vary. Three Runs are combined. Sample sizes vary per treatment and are shown in the legend. Mean day of molt is shown for each treatment. Length of each treatment line corresponds to the day on which all larvae in that treatment had either molted or died.

Table 13. Two-way ANOVA results for stage survival and stage duration of *Hemigrapsus nudus* larvae when exposed to various patchy diet treatments. Stage duration  $\alpha = 0.025$ . Stage survival data was arcsine square root transformed.

Source	SS	df	MS	F	P
<b>Stage Survival</b>					
Treatment	4.661	2	2.330	28.264	0.004
Run	0.160	2	0.080	0.971	0.453
Treatment*Run	0.330	4	0.082	1.428	0.265
Error	1.039	18	0.058		
<b>Stage duration</b>					
Treatment	236.716	2	118.358	34.017	0.003
Run	5.876	2	2.938	0.901	0.472
Treatment*Run	15.924	4	3.981	7.690	<0.001
Error	51.766	100	0.518		

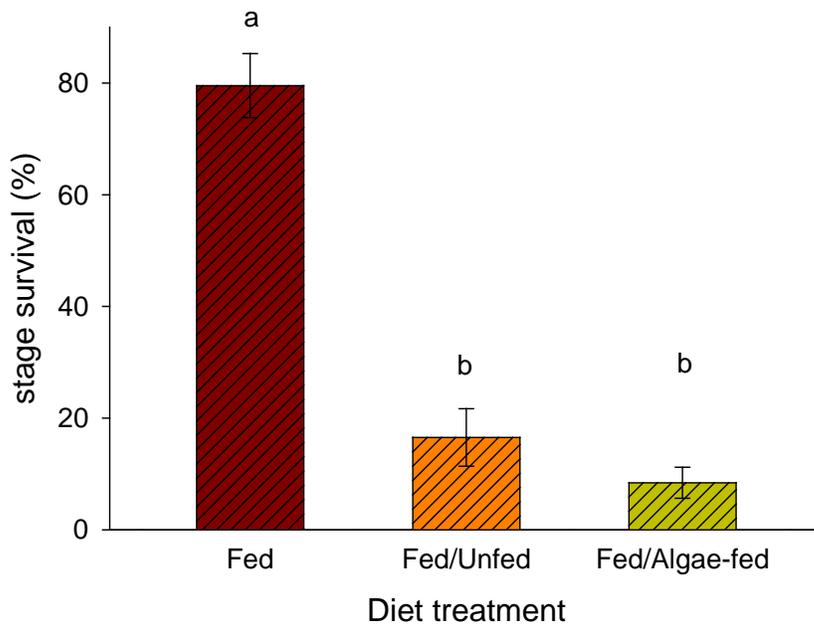


Figure 15. Effects on stage survival ( $\pm$  one standard error) of *Hemigrapsus nudus* larvae as intermittent diet treatments vary. Three Runs are combined (n=9). Shared letters indicate no significant differences between treatments (Tukey's HSD *post hoc* contrasts,  $\alpha = 0.05$ ).

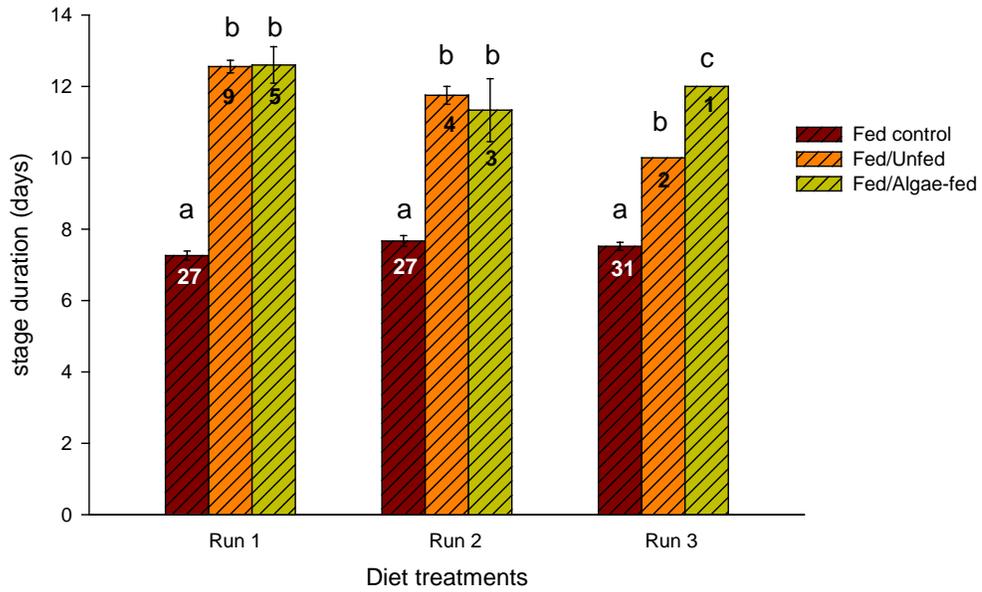


Figure 16. Effects on stage duration ( $\pm$  one standard error) of *Hemigrapsus nudus* larvae as intermittent diet treatments vary. Sample sizes vary per treatment and are shown in each column. Different letters indicate significant differences between treatments within each Run only, not between Runs (simple main effects contrasts,  $\alpha = 0.025$ ).

the fed/unfed treatment and the fed/algal-fed treatment were not significantly different from one another. Statistical tests between the two experimental treatments have little meaning for Run three due to small sample sizes ( $n = 2$  and  $n = 1$ ) as a result of high mortality. Overall results suggest no significant difference in stage duration of *Hemigrapsus nudus* larvae due to presence or absence of algal prey during patchy prey environments.

### **Mixed prey diet experiments**

#### ***Lophopanopeus bellus***

Stage survival of larvae subjected to varying concentrations of algal prey mixed with zooplankton prey is shown in Figure 17. All treatments showed relatively high survival. Data were analyzed by a Two-way ANOVA with treatment and Run as factors (Table 14). The interaction term was non-significant ( $p > 0.05$ ). Tests of the main effects showed treatment to be non-significant. Looking at daily survival, all four treatments follow roughly the same pattern, with approximately 30% mortality occurring within 20 days (Fig. 18). Overall results suggest no significant difference in stage survival when algae supplements zooplankton in the diet of newly hatched *Lophopanopeus bellus* larvae.

Stage duration of larvae subjected to varying concentrations of algal prey mixed with zooplankton prey are shown in Figure 19. Data were analyzed by a Two-way ANOVA with treatment and Run as factors (Table 14). The interaction term was non-significant ( $p > 0.05$ ). Tests of the main effects showed treatment to be non-significant.

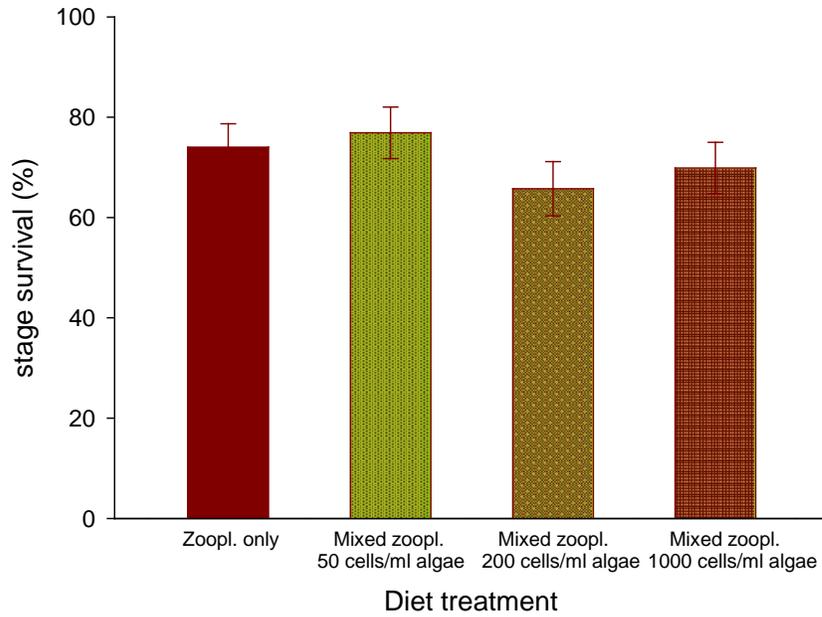


Figure 17. Effects on Stage I survival ( $\pm$  one standard error) of *Lophopanopeus bellus* larvae as mixed experimental diet varies. Zooplankton density (*Artemia franciscana*) remained consistent across all treatments. Three Runs are combined (n = 9).

Table 14. Two-way ANOVA results for stage survival and stage duration of *Lophopanopeus bellus* larvae when exposed to various mixed diet treatments of zooplankton and algal prey. Stage duration  $\alpha = 0.025$ . Sum of Squares Type IV was used.

Source	SS Type IV	df	MS	F	P
<b>Stage Survival</b>					
Treatment	0.035	3	0.012	1.521	0.318
Run	0.631	2	0.315	40.718	0.001
Treatment*Run	0.039	5	0.008	0.568	0.723
Error	0.300	22	0.014		
<b>Stage duration</b>					
Treatment	11.726	3	3.909	2.088	0.217
Run	51.721	2	25.860	13.805	0.008
Treatment*Run	9.294	5	1.859	0.626	0.680
Error	781.443	263	2.971		

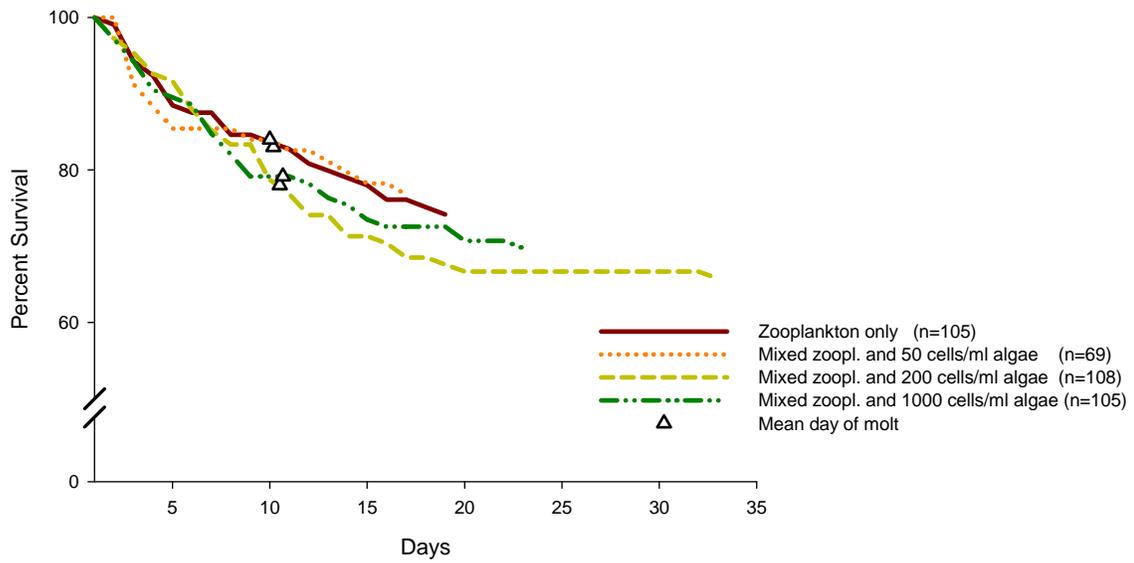


Figure 18. Daily survival of *Lophopanopeus bellus* larvae as mixed diet treatments vary. Three Runs are combined. Sample sizes vary per treatment and are shown in the legend. Mean day of molt is shown for each treatment. Length of each treatment line corresponds to the day on which all larvae in that treatment had either molted or died.

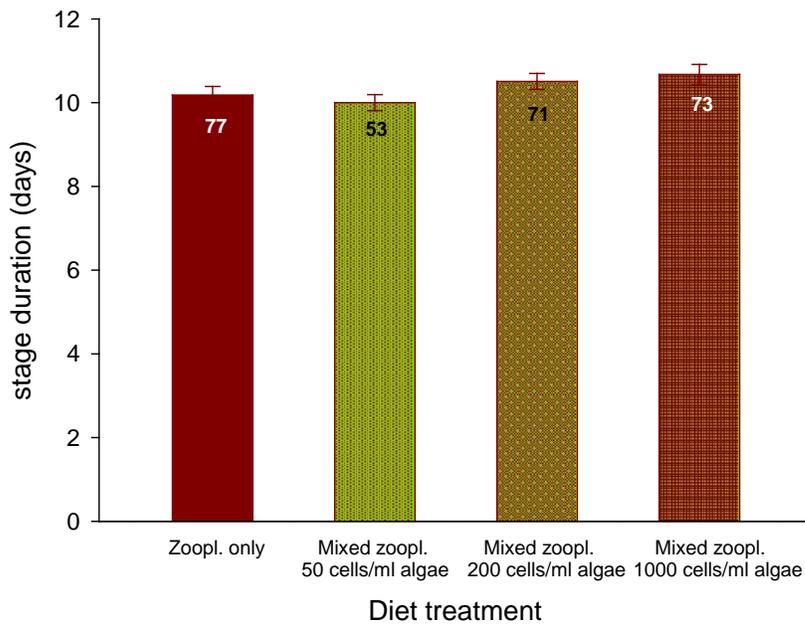


Figure 19. Effects on Stage I duration ( $\pm$  one standard error) of *Lophopanopeus bellus* larvae as mixed experimental diet varies. Zooplankton density (*Artemia franciscana*) remained consistent across all treatments. Three Runs are combined. Sample sizes vary per treatment and are shown in each column.

Overall results suggest no significant difference in stage duration when algae supplements zooplankton in the diet of newly hatched *Lophopanopeus bellus* larvae.

## DISCUSSION

Because of the unpredictable, and almost infinite, combinations of algal species larval crabs might encounter in nature, and the various algal densities that might be encountered, any attempt to simulate such natural assemblages for experimental purposes would be arbitrary. For the purposes of my experiments, therefore, and consistent with the intent to determine possible contributions of microalgae to the nutrition of larval crabs, I decided to use a monoculture of an alga of proven nutritional value in the mariculture of a variety of invertebrate larvae. *Isochrysis galbana* has been used extensively as a diet in bivalve larval culture in hatchery environments (Sulkin and Epifanio 1975; Harms and Seeger 1989; Brown et al. 1997). The value of this algal species as a food item for a variety of species is thought to be due to its relatively high content of long chain omega-3 polyunsaturated fatty acids (PUFA) (Volkman et al. 1989; Brown et al. 1997). Although this alga has not been shown to support the development of larval crabs directly, it increases the nutritional value of rotifers as a prey for larval crabs (Hartman and Sulkin 1999; Sulkin and McKeen 1999). This is consistent with the results of Levine and Sulkin (1978) showing that larval crabs require a dietary source high in such PUFA to develop normally. The use of *I. galbana*, therefore, is a possible 'best-case' scenario for assessing the role of microalgae in contributing to the nutrition of larval crabs.

A similar experimental challenge is presented in selecting a model zooplankter for use in diet studies. The choices of freshly-hatched nauplii of the brine shrimp *Artemia franciscana* and the rotifer *Brachionis plicatilis* are based on their extensive use in crab

larval culture (Sulkin and Epifanio 1975; Sulkin and Van Heukelem 1980; Anger and Dawirs 1981; Anger et al. 1981; Levine and Sulkin 1984; Hartman and Sulkin 1999).

I chose a high algal density for most of the experiments ( $4 \times 10^3$  cells  $\text{ml}^{-1}$ ) to ensure that algal density was not a factor limiting ingestion, and to increase chances of detecting any experimental effect that might occur. It has been shown that larval crabs will increase ingestion of prey cells or particles when presented in higher densities (Incze and Paul 1983; Schwamborn et al. 2006; Shaber and Sulkin 2007), and the chosen density is well within the range used for similar feeding studies with various protist prey (Harms and Seeger 1989; Paul et al. 1989; Harms et al. 1994; Sulkin et al. 2003). Moreover, in phytoplankton blooms off the California coast, various phytoplankton species' abundance can exceed  $1 \times 10^3$  cells  $\text{ml}^{-1}$  (Horner et al. 1997); and in the spring, at the chlorophyll maximum depth in the Gulf of Alaska, certain diatom concentrations can reach  $2 \times 10^5$  cells  $\text{ml}^{-1}$  (Paul et al. 1989). Algal quantity can be quantified by cell density or by carbon content. Zoeae ingest individual cells presumably based on encounters (Perez and Sulkin 2005), yet oceanographers commonly measure algal biomass in terms of carbon. Harms and Seeger (1989) used a carbon content of approximately  $1 \mu\text{g C ml}^{-1}$  based on naturally occurring maximum carbon concentrations for their larval crab diet studies. Algal carbon content in the present experiments is  $0.1 \mu\text{g C ml}^{-1}$  based on measurements of *Isochrysis galbana* made elsewhere (Berggreen et al. 1988), which is a full magnitude lower than that used by Harms and Seeger (1989). Because such maximum values are uncommon, algal densities in the present experiments fall within naturally occurring densities in the field, while maximizing the ability to detect an effect.

A comprehensive study looking at algal contribution in various scenarios that newly hatched crabs are likely to encounter has not been previously reported. Crab species in the present study represented a variety of families, hatching seasons, habitats and larval sizes. Previous studies and personal observations have shown Grapsidae larvae, Xanthid larvae and *Metacarcinus magister* (Cancridae) larvae to have different capacities to withstand nutritional stress (Staton and Sulkin 1991; Sulkin et al. 1998a; Sulkin, pers. comm.). Previous findings in these capacities among families were both supported and refuted in the following experiments.

### **Post hatching feeding experiments**

Post-hatching feeding experiments were conducted to pinpoint the period immediately after hatching when feeding is vital, but preferred zooplankton prey may not be available. Newly hatched larvae may face logistical challenges to obtain food. Because larvae hatch from a benthic adult, they must first swim up into the water column to access meso-zooplankton prey. It is during this period, when they must expend energy, but have depleted reserves, that an encounter with any prey type, including microalgae, may provide an essential source of nutrition. Results have shown that as the initial period of starvation after hatching is extended, the lower the larval survival and the more delayed the development will be, despite subsequent feeding (Staton and Sulkin 1991). Moreover, if sufficient resources are acquired within the first few days of life, subsequent food availability may not affect survival to the second stage (Anger and Dawirs 1981).

The present experiment examined how an algal diet would affect survival and development if made available to larvae during this critical early period.

Results from *Post-hatching feeding* experiments reported here confirm previous studies on brachyuran larvae that an early source of nutrition is necessary for continued survival and development (Sulkin 1975; Bigford 1978; Anger and Dawirs 1981; Anger et al. 1981; Staton and Sulkin 1991). As would be expected for planktotrophic larvae, as the initial period before zooplankton feeding increased, stage survival decreased and stage duration was extended (Figs. 5 and 6 for *Lophopanopeus bellus*, Figs. 8 and 9 for *Metacarcinus magister*). Provision of algae before subsequent zooplankton feeding did not significantly improve larval survival or accelerate development when compared to unfed treatments. Similarly, mean Point of No Return (PNR) analyses for both crab species showed that, although algae and unfed treatments both differed from the fed controls on comparable treatment days, there were no significant differences between them. Only a few exceptions occurred, with increased survival or accelerated development on an algal diet seen in a few individual Runs. Thus, the results indicate that availability of algae as prey in the period immediately following hatching does not contribute to the nutritional needs of the larval crabs in ways that are manifested in stage survival or duration. Previous studies on *Metacarcinus magister* and other brachyuran species have shown that these species ingest a wide variety of prey items, including toxic algae and chlorophytes (Perez and Sulkin 2005), autotrophic dinoflagellates (Hinz et al. 2001), heterotrophic dinoflagellates (Sulkin et al. 1998b) and diatoms (Hartman and Letterman 1978). However, the evidence as to the nutritional value of ingesting these prey has been inconclusive. Although larvae readily ingested the autotrophic

dinoflagellate *Prorocentrum micans* and the heterotrophic dinoflagellate *Noctiluca scintillans* (fed *P. micans*), neither was sufficient on its own to sustain development to the second stage (Sulkin et al. 1998b). However, some nutritional benefit was derived from the algal diets because mortality was delayed compared to the unfed control.

Furthermore, in experiments with several diatom species, Hartman and Letterman (1978) found in some cases that diatoms support *M. magister* development through the fourth and fifth larval stages. Experiments looking at the effect of the dinoflagellate *Prorocentrum micans* on survival and development of another brachyuran crab, *Hemigrapsus oregonensis*, have been equivocal (Lehto et al. 1998; Hinz et al. 2001). While *H. oregonensis* larvae did not survive to stage two on a diet of *P. micans* in one study (Hinz et al. 2001), survival to stage two in another study was equivalent to the zooplankton-fed control, although development was delayed (Lehto et al. 1998). In addition, two other species of algae (*Noctiluca milaris* and *Dunaliella tertiolecta*) supported low to medium survival to stage two, while all larvae in the unfed control died. These results generally contradict those shown in the present study, where algal-fed periods did not significantly alter stage survival or duration of *Lophopanopeus bellus* or *M. magister* larvae compared to unfed periods. Ambiguous results such as these have made the nutritional role of algae to newly-hatched larval crabs difficult to assess. Algal prey seem to have a beneficial effect on survival and development of newly-hatched larvae only in certain cases. Differences among literature reports, and with results reported here, may be due to the specific microalgal prey used, to the crab species, or to a combination of the two.

The use of Point of No Return analyses assessed not only the effects of early starvation, as was the case with previous studies (Anger and Dawirs 1981; Staton and Sulkin 1991), but took these experiments further by assessing what effect algae had on survival and development during these periods of starvation. Anger and Dawirs (1981) found with *Hyas araneus* that the PNR for 50% larval survival (PNR<sub>50</sub>) was reached after half of the maximum time an unfed larva could survive. According to their model, in the present experiment the PNR<sub>50</sub> threshold would have been at 5.5 days (unfed *L. bellus* and *M. magister* larvae both survived for a maximum of 11 days). In fact, the PNR<sub>50</sub> was reached roughly by day three for *Lophopanopeus bellus* and by day two for *Metacarcinus magister*, much earlier than for *Hyas araneus*, indicating differences in starvation resistance among crab species. Furthermore, Anger and Dawirs (1981) found that if more than 70% of the maximum unfed period had passed (11 days in the present experiment) no larva would recover (PNR<sub>100</sub>). In the present experiment, all *M. magister* larvae had indeed died well before the theoretical PNR<sub>100</sub> threshold (7.7 days), and only four *L. bellus* larvae out of the initial 108 were still alive after five days of starvation (approximately two days before the theoretical PNR<sub>100</sub> would be reached, but the longest unfed period in the scope of the experiment). It seems likely that, after another two days without food, the last four larvae would have been depleted of energy reserves, rendering them unable to molt, and yielding similar findings to those for *Hyas araneus*. The provision of algae during these unfed periods did not alter the mean PNRs significantly, having no effect on larval ability to molt or their time to molt.

In another study using PNR analysis with brachyuran crab larvae, Staton and Sulkin (1991) found that the PNR for significantly increased mortality and delayed

development for *Sesarma cinereum* was reached when initial starvation extended longer than one third of normal stage duration. In the present study, *Lophopanopeus bellus* larvae straddled this threshold – it took longer for starvation to affect their survival, but less time for it to affect their development rate; while for *Metacarcinus magister* larvae, both survival and development rate had been significantly affected well before one third of normal stage duration had elapsed. The present results are perhaps most comparable to those found in similar delayed feeding experiments with a commercially important brachyuran crab, *Chionoecetes opilio*; after three days of starvation, resumption of feeding was considered ‘useless’ for commercial rearing, given the amount of mortality that occurred (Kon 1979). The PNRs for survival in unfed treatments were 3.67 days for *L. bellus* and 2.67 days for *M. magister*. Again, the provision of algae did not have any evident effect during these critical periods.

Overall *Lophopanopeus bellus* larvae exhibited higher starvation resistance than did *Metacarcinus magister* larvae. This is consistent with previous observations of *M. magister* in response to nutritional stress (Sulkin et al. 1998a) and the relative hardiness of Xanthid family larvae (Staton and Sulkin 1991 and references therein). Such variation among species is not unusual. *Sesarma reticulatum*, a congener of *S. cinereum*, exhibited so much starvation resistance that 100% of the larvae in initial starvation treatments survived to the second zoeal stage, including those starved for five days (Staton and Sulkin 1991). Despite these differences in resilience among crab species, apparent even with congeners, in the present study these differences did not alter the effects of an algal diet on larval survival or development.

## Patchy prey diet experiments

Patchy prey diet experiments were designed to assess algal contribution in times when encounters with meso-zooplankton prey are intermittent. Studies assessing larval king crab prey abundance in southeastern Alaska have shown patchiness of zooplankton prey both temporally and spatially. Furthermore as larvae traverse the water column in diel migrations (Paul et al. 1989), they undoubtedly encounter alternatively dense and sparse aggregations of such prey (Mackas et al. 1985). Moreover, oceanographic phenomena create environmental conditions that support episodic nutrient enrichment resulting in seasonal phytoplankton blooms (Mackas et al. 1985; Horner et al. 1997), which, in turn, can lead to increased zooplankton abundance. Given the likelihood that larval crabs will be encountering patches of plentiful prey interspersed with sparse, if any prey, these experiments examined how an algal diet would affect larval survival and development if made available between encounters with zooplankton prey.

In *Patchy prey* experiments, intermittent presence of prey significantly decreased larval survival and delayed stage duration compared to continuously fed controls. In addition, presence of algae compared to unfed periods did not significantly affect *Lophopanopeus bellus* or *Hemigrapsus nudus* survival, but did accelerate *L. bellus* development by 5% (Fig. 13). That there are survival differences between controls and experimental treatments is not surprising. Experimental survival rates may be more likely to approximate survival rates in nature than the fed controls do, which provide zooplankton in excess continuously. In looking at discrepancies between laboratory and

field tests, a study on rates of larval fish prey ingestion suggested that encounters with patchy prey fields are frequent and that plankton exist at much higher densities than some sampling techniques show (Mackenzie et al. 1990). Furthermore, prey, even if at low density overall, are distributed patchily, creating highly concentrated areas that larvae can exploit (MacKenzie et al. 1990; Welch and Epifanio 1995). Larvae in the field are therefore likely to be relatively well adapted to these intermittent periods without food. However, unlike results for *L. bellus*, neither experimental treatment sustained high survival in *H. nudus*; both were at or below 20% (Fig. 15). Although the Grapsid family has been deemed relatively ‘hardy’ with respect to dietary requirements (Staton and Sulkin 1991; Sulkin et al. 1998a), *H. nudus* larvae fared worse in the present experiments when encountering simulated patchy prey conditions than did *L. bellus* whose larvae had a survival rate of at least 60% (Fig. 12), even when encountering no food for 3-day periods. Larvae in the fed controls had high survival, indicating that the stress of the treatments, rather than a weak *H. nudus* brood, was the cause of high mortality. However, other studies have shown results similar to that of *L. bellus*. Sulkin et al. (1998a) found that *Metacarcinus magister* larvae also had considerable survival (44.6%) in a similar fed/unfed treatment, though they had been previously observed to be relatively susceptible to dietary stress. The specific timing of these unfed periods can make a difference in larval ability to successfully complete the molt cycle, and differences have been observed among various decapod species in how these effects are manifested (Anger 1987). This may, in part, explain the inconsistent results found within the present study and between this and other studies. Likewise, although there is evidence showing relative hardiness during intermittent feeding, the nutritional role of algae during

these periods is still relatively uncertain. In the present study, an algal diet only made a marginal difference in the development rate of one species. However, in a patchy prey experiment with *Metacarcinus magister*, two species of dinoflagellates, *Prorocentrum micans* and *Noctiluca scintillans*, supported higher *M. magister* survival and accelerated development than the fed/unfed treatment (Sulkin et al. 1998b). Furthermore, survival was equivalent to the zooplankton-fed (*Artemia* sp.) control. On the other hand, a third alga (*Dunaliella tertiolecta*) actually reduced survival compared to the fed/unfed treatment. While larvae in our experiments were presumably more nutritionally stressed than those in the above-mentioned study, (having three days between zooplankton feeding rather than two days), a condition that might be expected to further accentuate any marginal algal nutritional contribution, I found less of an effect than did Sulkin et al. (1998b). Overall the above findings illustrate that different algal species can vary in their nutritional roles in the larval crab diet. Perhaps not only quality, but quantity of algae makes a difference. Bioenergetic studies show that a large quantity of diatoms is required to sustain larvae of various species (Paul et al. 1989). Densities upwards of  $3 \times 10^3$  cells  $\text{ml}^{-1}$  of one diatom supported 82% of stage one king crab larvae to the next stage, while densities lower than  $15 \times 10^3$  cells  $\text{ml}^{-1}$  of another diatom supported only 15% survival. Although these densities have been documented in the field (Paul et al. 1989), they are on the upper end of the spectrum. Moreover, Incze and Paul (1983) found that quantities of a particularly large diatom necessary to support respiratory requirements of stage one tanner crab larvae are unlikely to be found co-existing with those larvae. Perhaps therefore, in a patchy prey environment, it is a delicate combination of quality and quantity of algal cells, in addition to timing relative to the molt cycle that makes the

difference between a marginal and a substantial effect on a larva's survival and development.

### **Mixed prey diet experiments**

Of all potential prey scenarios, it is perhaps most likely that larval crabs would be encountering both zooplankton and algal prey simultaneously. Despite the demonstrated ability of larvae to choose and reject certain prey types (Hinz et al. 2001; Perez and Sulkin 2005; Schwamborn et al. 2006), previous studies with mixed diets of zooplankton and algae, including preliminary tests of larvae under the present experimental conditions (Fig. 2), have indicated that larvae will ingest algae even when zooplankton are present (Paul et al. 1989; Schwamborn et al. 2006; Shaber and Sulkin 2007). Laboratory experiments with king crab larvae even show preferential ingestion of diatoms over copepod or cirripede nauplii (Paul et al. 1989), and studies of the crab *Aratus pisonii* demonstrate larval selection of large diatoms over copepods and other zooplankton (Schwamborn et al. 2006). It seems likely then that larvae in the field would ingest both phytoplankton and zooplankton, as evidenced by field collections of larvae that contain algae in their guts (Paul et al. 1979; Paul and Paul 1980; Paul et al. 1989; Harms et al. 1994). Moreover, it has been suggested that a mixed diet of algae and zooplankton might provide micronutrients that a pure zooplankton diet would not, resulting in increased survival or more rapid development (Incze and Paul 1983; Epifanio et al. 1991; Welch and Epifanio 1995). Mesocosm-raised larvae showed equal growth and faster development than laboratory raised larvae, despite zooplankton prey in enclosures having

likely had lower PUFA levels than the *Artemia* spp nauplii used in lab experiments, leading to the suggestion that unquantified algal communities might have led to the unexpected result (Welch and Epifanio 1995). Mixed prey diet experiments were conducted in the present study to test the hypothesis that algal prey might work synergistically with zooplankton to improve larval survival or accelerate development rate.

My results did not support that hypothesis, showing that for a variety of algal densities, neither larval survival nor development rate was affected compared to the zooplankton-fed control (Figs. 17 and 19). All treatments had high survival (above 60%) and moderately short stage durations. It may be possible that such well-fed larvae, receiving *Artemia* spp. nauplii in excess daily, were at the height of their developmental rate, and any effects of micronutrients provided by the algal portion of their diet were of too small a value to be assessed by the present study parameters. In contrast, the Atlantic mud crab larvae in mesocosm enclosures (Welch and Epifanio 1995) were not carefully provided with a PUFA-rich diet, perhaps allowing for a greater difference to be observed. To account for this, future mixed diet studies should include the combination of a sub-optimal zooplankton diet with an algal diet. However, it has been suggested that larvae behave differently with respect to low prey abundances in the laboratory than they do in the field (MacKenzie et al. 1990; Welch and Epifanio 1995), and this may hamper the ability to test for subtle nutritional differences in the laboratory.

## Comparative nutritional value of algae

Although *Isochrysis galbana* has been widely used to successfully rear larval invertebrates, and represented a 'best case scenario', it did not differ overall in its effect on survival and development compared to unfed treatments in the present study. As discussed earlier, *I. galbana* contains PUFAs essential to larval crab development and passes those on to heterotrophic prey capable of supporting higher crab larval survival (Sulkin and McKeen 1999), particularly when compared to similar treatments using the green alga *Dunaliella tertiolecta*. Two PUFAs (eicosapentaenoic acid-EPA and docosahexaenoic acid-DHA) have been identified as the most likely diet constituents to sustain survival and accelerate development, possibly because crab larvae either may not have the capacity to elongate their precursor, linolenic acid (Levine and Sulkin 1984), or if they do, must expend energy to do so (Pillsbury 1985). Studies on the biochemical composition of microalgae have shown that *I. galbana* can vary in its fatty acid content from containing only one of those fatty acids – DHA (Pillsbury 1985; Brown et al. 1997), to containing trace amounts (Volkman et al. 1989) or more of EPA (Wacker et al. 2002). In contrast, diatoms are considered richer sources of both fatty acids, and have more lipid than any other algal class tested including Prymnesiophytes (*I. galbana*) (Brown et al. 1997). It may not be surprising therefore, that diatoms have supported survival and late stage development in larval crabs in several studies (Hartman and Letterman 1978; Harms and Seeger 1989; Paul et al. 1989).

The dinoflagellate *Prorocentrum micans*, shown to increase *Metacarcinus magister* survival and accelerate development (Sulkin et al. 1998b), contains above

average lipid content and both essential PUFAs, (high levels of DHA though lower levels of EPA) (Laabir et al. 2001). Sulkin (1975) suggested that the increase in lipids found in larvae nearing molt may point to lipids as a dietary prerequisite for a timely molt, and found that two diets that sustained late stage *Callinectes sapidus* larvae contain 2-3 times as much lipid as a *D. tertiolecta*-fed rotifer diet. However, any discussion of the relative nutritional content of *I. galbana* also must take into account that both of these PUFAs may be nutritionally required only in older larvae for success through to the megalopa stage, and may not be as useful in explaining results for the first larval stage (Levine and Sulkin 1984). In addition, fatty acid composition can vary widely, particularly in *I. galbana*, possibly due to genetics of different strains or culture conditions (Volkman et al. 1989).

A study on vitamin content of several microalgae species used in mariculture showed each species had low concentrations of at least one vitamin (De Roeck-Holtzauer et al. 1991), in addition to results showing varying concentrations of other vital nutrients like fatty acids, leading several authors (Volkman et al. 1989; Brown et al. 1997) to surmise that perhaps for the optimum nutritional contribution for crustacean larvae, a mixed diet of algae would need to be ingested. In nature, larvae would rarely encounter only one species of alga, unless in the midst of a monospecific bloom; however, blooms tend to change composition temporally and spatially (Paul et al. 1989; Horner et al. 1997; Turner and Tester 1997). Since only one algal species was available to the larvae in the present study, perhaps future studies should attempt to simulate more realistic mixtures of algal types.

## Effects of hatching season and larval size

Because phytoplankton abundance and composition varies considerably based on season (Paul et al. 1989; Smith and Hobson 1994), it is reasonable to speculate that larvae hatching in one season might have a different nutritional relationship to algae than larvae hatching in another. *Hemigrapsus nudus* hatch in the spring when phytoplankton can reach their maximum abundance during spring blooms (Paul et al. 1989; Coyle and Paul 1990), while *Lophopanopeus bellus* hatch throughout the summer, after spring phytoplankton blooms have been grazed to lower levels but high levels of irradiance still support photosynthesis. In contrast, larvae of *Metacarcinus magister* hatch into the plankton in the winter months during a time of relatively low algal abundance (Thorson 1946; Smith and Hobson 1994; Horner et al. 1997). It would be reasonable to hypothesize that such a plentiful source of prey might be well utilized by *H. nudus* larvae encountering algal cells in a patchy prey environment, or *L. bellus* larvae needing to feed immediately after hatching. *Metacarcinus magister* adults can be found in subtidal eelgrass beds, and it has been suggested that this concentrated area of detrital material and productivity could make up for the generally low levels of primary productivity elsewhere in the water column (Sulkin et al. 1998b), particularly because larvae that are fed detrital particles, especially microbially enriched detritus, in various combinations show increased survival and more rapid development (Lehto et al. 1998). Studies on king crab larvae, a species that can hatch during spring blooms, also show some algal benefit (Paul et al. 1989). Despite this, season of hatching does not seem to affect the likelihood of larvae ingesting algal prey (Perez and Sulkin 2005), or benefiting from it

(present study), corroborating some of the earliest observations in larval ecology, that many planktotrophic larvae thrive regardless of phytoplankton abundance (Thorson 1950).

In addition to representing a variety of hatching seasons, the three crab species were all of varying larval sizes, *Hemigrapsus nudus* being the smallest and *Metacarcinus magister* the largest, with a difference of approximately 1mm (tip of dorsal spine to tip of rostral spine; Perez and Sulkin 2005). Although larval size could potentially affect the ability to effectively manipulate prey cells and thereby interfere with ingestion, the algal diet did not reveal any differential benefit among crab species. This is confirmed by an earlier comprehensive study of predator/prey size ratios involving *H. nudus* and *M. magister*, in which both species ingested cells larger and smaller than *Isochrysis galbana*, and no relationship between larval size and incidence of algal ingestion was found (Perez and Sulkin 2005).

### **Nutritional ecology**

Food limitation and larval resistance to starvation in times of nutritional stress are major factors affecting larval mortality and hence, population dynamics of adults. In a major review of invertebrate larval ecology and food limitation, food availability was identified as one of three major determinants of recruitment success (Olson and Olson 1989). Because larval crabs are an abundant and therefore key component in the ecosystem at particular times, the larger implications of their survival on the wider ecosystem should not be overlooked, not least because larval crabs play an important role

as prey for several commercially important fish species (Murphy et al. 1988; Mazumder et al. 2006; Potier et al. 2007; Duffy et al. 2010). In addition, regardless of any nutritional benefit of an algal diet to larval crabs, it has been widely reported, both in the present study and in previous work, that larvae do in fact ingest algal cells on a regular basis (Paul et al. 1979; Paul and Paul 1980; Incze and Paul 1983; Lehto et al. 1998; Sulkin et al. 1998b; Perez and Sulkin 2005). This feeding strategy may have an effect on the regulation of phytoplankton abundance on small temporal and spatial scales.

There is a tendency to presume that there must be a benefit to any trait an organism has evolved – that an adaptation will confer with it an increase in fitness. But it is equally reasonable to suppose that, in an environment constrained by resources, any beneficial morphology or behavior is likely to have a cost associated with it – a tradeoff. Examples of evolutionary tradeoffs exist from marine phytoplankton optimizing grazing defenses at the cost of nutrient acquisition (Sunda and Hardison 2010), to leopard frogs whose fast growth comes at the risk of high mortality (Schiesari et al. 2006), to Darwin’s famous finches whose beak morphologies are so highly specialized that it can affect their survival (Grant et al. 1976). In an environment so full of risks to the larval crab: predation, starvation, habitat suitability, among others, it would seem that any inefficiency would not persist - unless the benefits outweighed the costs. The larval crab’s feeding strategy may be loosely analogous to that of finches whose large beak size allows them to feed generally on both large and small seeds, but whose feeding efficiency on small seeds is far outweighed by that of small-beaked finches. The seemingly opportunistic feeding strategy of larval crabs, which includes time and energy spent handling and rejecting algal cells (Hinz et al. 2001), or spent ingesting algal cells with

questionable nutritional benefit (Perez and Sulkin 2005), may only pay off in prey assemblages where many beneficial cells occur, or in very sparse areas where opportunism is more likely to yield some sort of sustenance. Changing environmental conditions, such as a seasonal shift in fruiting trees for finches (Grant et al. 1976), a seasonal shift in phytoplankton abundance (Paul et al. 1989), or a spatially varying plankton population for larval crabs, as well as particular species-specific interactions, will likely be part of the web of complex interactions that determine overall success or failure of this feeding strategy. Given that larval mortality is high in nature, a win-win strategy is almost certainly rare. Overall, however, feeding efficiency may be of higher importance for the adult stage crab, than it is for the larval stage. The focus on efficiency (feeding efficiency in this particular argument) may be a result of its potential importance for adult organisms' fitness. In the case of the adult form, whose principal function is reproduction, maximizing nutritional efficiency provides for more energy to be devoted to that process, either in terms of increased fecundity and/or to increasing the fitness of offspring (Thorson 1950; Vance 1973). The understanding and study of larval forms has historically trailed behind that of adults. The primary function of a larva, quite different from that of an adult, is to acquire enough resources to reach the next stage. As a result, certain morphological features, present upon hatching, may not be utilized to their greatest potential during these early larval stages. Larval crab eyes are a chief example of this, given that larvae are not visual predators until later in maturity, yet their eyes are one of their most prominent physical features. Morphological characteristics of feeding appendages are mainly preserved from one larval stage to the next, as are behavioral characteristics, e.g. the tendency to ingest prey of marginal nutritional value (Shaber and

Sulkin 2007). Opportunistic feeding therefore, rather than feeding efficiency, may be the most successful strategy that larvae have in their unpredictable prey environment.

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