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**THE USE OF PROTISTS AND DETRITUS AS A DIET FOR  
THE FIRST ZOEAL STAGE OF THE BRACHYURAN CRABS  
*CANCER MAGISTER* AND *HEMIGRAPSUS OREGONENSIS***

**Jason A. Lehto**

**August 1997**

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THE USE OF PROTISTS AND DETRITUS AS A DIET FOR THE FIRST  
ZOEAL STAGE OF THE BRACHYURAN CRABS *CANCER MAGISTER*  
AND *HEMIGRAPSUS OREGONENSIS*

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Jason A. Lehto

August 1997

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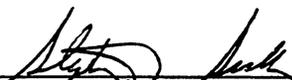
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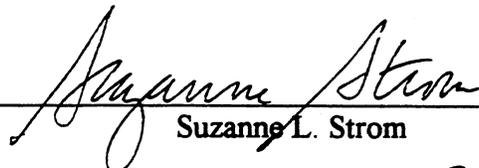
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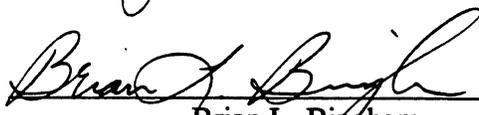
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MASTER'S THESIS

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by  
**Jason A. Lehto**

**ABSTRACT.** - To determine whether protists contribute to the diet of larval crabs, first stage larvae from the brachyuran crabs *Cancer magister* and *Hemigrapsus oregonensis* were fed diets that included *Noctiluca milaris*, *Prorocentrum micans* and *Dunaliella tertiolecta*, alone and in combination with a sub-optimal diet of *Artemia sp.* nauplii.

*Cancer magister* larvae fed diets of *Prorocentrum micans* or *Noctiluca milaris* alone, had a delay in mortality compared to the unfed control. When the sub-optimal diet was supplemented with *N. milaris* or *P. micans*, survival increased and was comparable to the *Artemia sp.* control diet although stage duration increased in the protist fed treatments. Larvae fed a sub-optimal diet plus *Dunaliella tertiolecta* showed lower survival compared to larvae fed the sub-optimal diet alone.

*Hemigrapsus oregonensis* larvae fed diets of *Prorocentrum micans* and *Noctiluca milaris* alone showed survival to stage II of 81.8% and 34.7% respectively. The survival for larvae raised on a diet of *P. micans* was comparable to the *Artemia sp.* control, although the development time was longer on the *P. micans* diet. A few larvae survived to stage II when fed a diet of *Dunaliella tertiolecta*. Larvae fed a combination of protists and a sub-optimal diet of *Artemia sp.* followed the same trends as the protist diets alone.

To determine whether bacteria and detritus contribute to the diet of larval crabs, first stage larvae from the intertidal crab *Hemigrapsus oregonensis* were fed diets that

included microbially colonized and uncolonized detritus alone and in combination with a sub-optimal diet of *Artemia sp.* nauplii.

*Hemigrapsus oregonensis* larvae fed diets of microbially colonized and uncolonized detritus alone, had a delay in mortality compared to the unfed control. When the sub-optimal diet was supplemented with colonized and uncolonized detritus survival increased over the sub-optimal diet alone. The stage duration was similar among larvae fed the sub-optimal diet alone and those fed the sub-optimal diet plus colonized and uncolonized detritus. Larval weights were highest in the *Artemia sp.* fed control and lowest in larvae fed the sub-optimal diet plus uncolonized detritus.

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## INTRODUCTION

Early models of coastal food webs focused on diatoms as the base of a linear food chain. In this traditional view, diatoms were grazed by copepods that were fed on by small fish, that were in turn preyed upon by larger fish. This food chain came under scrutiny in the 1960's and 70's because advancements in technology helped to show that such net plankton accounted for only a small fraction of the ocean's total primary production. Pomeroy (1974) described a new paradigm that suggested trophic pathways that included planktonic cells less than 5 microns such as bacteria and protists, as well as dissolved and particulate organic matter. This new model described how bacteria consume non-living organic matter and are themselves preyed upon by bacterivorous protists (Pomeroy, 1974). In 1983, Azam et al. added to Pomeroy's model by showing that there were many trophic levels within the microbial food web, and its products were utilized very efficiently by its members. Azam et al. (1983) described the cycling of energy within the microbial food web and named it the "microbial loop". This model implied that the microbial loop was a closed system, with very little carbon escaping to higher trophic levels (Ducklow et al., 1986). This was a conundrum for researchers because the microbial food web constituted a potentially large source of energy for higher organisms.

Much of the carbon production in the marine environment is by cells too small to be efficiently grazed by copepods (Sherr and Sherr, 1991). However, phagotrophic and mixotrophic protists have been identified as the major grazers of these microbes (Sanders

and Wickham, 1993). These protists graze on microbial cells and can repackaging their prey into biomass that is accessible to larger metazoans (Gifford, 1991). Recent advancements in technology have shown that protists are abundant in coastal waters (Stoecker and Capuzzo, 1990) and may be an important trophic linkage between bacterial communities and higher organisms (Pomeroy, 1992).

Many marine invertebrates begin their lives in a planktonic larval stage and must feed on other planktonic organisms to survive (Thorson, 1950). Despite the abundance of protists within the coastal zone, few studies have focused on the possible trophic pathway between protists and invertebrate larvae. This is probably because protists are not easily detectable in gut contents (Stoecker and Govoni, 1984). However, protists are likely candidates for larval predation for many reasons. Protists may provide essential nutrients to larval diets, particularly fatty acids and sterols (Stoecker and Capuzzo, 1990). Protists may also be an important prey item for invertebrate larvae, because they are of the particle-size ranges efficiently utilized by these groups (Stoecker and Capuzzo, 1990).

Most brachyuran crabs produce planktotrophic larvae that must feed in the plankton to support their development (Staton and Sulkin, 1991). Nutritional requirements vary among species, and can even change during the course of development within individual species (Sulkin and Van Heukelem, 1980; Levine and Sulkin, 1984b). Some brachyuran larvae may benefit from variability in their diets. Bigford (1978) showed that larvae fed a combination of prey items developed faster than diets consisting of one food item. Many larval crabs also require specific long-chain polyunsaturated fatty acids

and diets high in lipid content to develop fully (Levine and Sulkin, 1984b). Protists may provide the nutritional variability that crab larvae require.

The laboratory culture of larval crabs typically has depended on the use of artificial diets. Typical laboratory diets have included freshly-hatched nauplii of the brine shrimp *Artemia sp.* and the rotifer *Brachionus plicatilis* (Poole, 1964; Sulkin, 1975; Sulkin and Epifanio, 1975). Although these diets perform well in the laboratory and have provided valuable insights into crab larval nutrition, they would never be encountered by larval crabs living in the plankton. Because crab larvae have large eyes and are energetic swimmers, it has generally been assumed that they are active hunters, selectively choosing live motile prey. This idea was reinforced by the success of laboratory diets composed of motile zooplankters (Sulkin, 1975), and the discovery of barnacle nauplii and crab larvae within the gut contents of captured zoeae (Bigford, 1978). It was therefore theorized that larvae consumed mainly microcrustaceans such as copepod and barnacle nauplii.

However, Paul et al. (1989) reported that barnacle and copepod nauplii were often not present in nature in the densities that crab larvae required for development in the laboratory. In addressing the question of natural sources of nutrition for brachyuran larvae, Paul et al. (1989) and Incze and Paul (1983) suggested protists as a possible food source for larval crabs. Because of their high abundance and diversity, members of the microbial community could provide many of the dietary needs of brachyuran larvae.

Detrital material from near-shore habitats may be another potential source of nutrition for larval crabs. High biological productivity associated with seagrass ecosystems contributes an abundance of food to adult crab diets (Dumbauld et al., 1993).

A prominent role of eelgrass lies in its formation of particulate matter (Phillips, 1981). Eelgrass blades decay into particles that are colonized by bacteria and fungi, which can then nourish other organisms (Phillips, 1981). Seagrass detritus and the microflora associated with it provides a large and predictable source of carbon to sustain the adult crab populations living in estuarine habitats (Phillips and McRoy, 1980). The detrital production generated by these ecosystems is considerable (McRoy, 1966). However, little is known of the role of detritus in crab larval ecology. This organic matter could serve as a stable and predictable source of nutrition to larval crabs, especially for those species that release their larvae in eelgrass habitats. Levine and Sulkin (1984a) demonstrated that crab larvae are able to ingest calcium alginate microcapsules, indicating that brachyuran larvae are capable of capturing and ingesting non-living, non-motile prey. Detritus and its associated microfauna provided food resources sufficient for development of the larvae of the grapsid crab *Armases miersii* (Schuh and Diesel, 1995).

The purpose of the present study was to determine if crab larvae can use protists and eelgrass detritus as a source of nutrition during the first zoeal stage. Larvae from the cancrid crab *Cancer magister* were fed diets comprised of naturally-occurring protist species, and larvae from the grapsid crab *Hemigrapsus oregonensis* were fed diets comprised of either selected protists or eelgrass detrital particles and associated epiflora. Experiments were designed to determine if and how well the crab zoeae utilize marine protists and bacteria as a food source.

## MATERIALS AND METHODS

### Experimental Animals

Larvae of two species of brachyuran crabs, *Cancer magister* and *Hemigrapsus oregonensis*, were used in these experiments. These crab species were chosen because their larvae may exhibit different nutritional requirements and they spawn during different seasons. *Cancer magister*, commonly called the Dungeness crab, is a subtidal commercially important species, ranging in habitat from central California to Alaska (Lough, 1976). In Puget Sound, it usually inhabits subtidal eelgrass beds, which it uses for cover from predators, and where it mates and broods its young (Dinnel et al., 1986; McMillan, et al, 1995). The Dungeness crabs that inhabit Puget Sound are winter brooders, normally producing one brood of young per year. The larvae are released in the winter months, starting in the middle of February and ending in early April (Knudsen, 1964). The first stage zoeae are approximately 500 to 700  $\mu\text{m}$  from the tip of their rostrum to the end of their tail (Poole, 1966). While in the plankton, they molt through five zoeal stages and then metamorphose to the megalopa stage (Poole, 1966; Lough, 1976). Eventually the megalopae settle out of the water column and metamorphose into true crabs. The total process can take over 100 days to complete (Poole, 1966).

*Hemigrapsus oregonensis*, commonly referred to as the green shore crab, is found mainly in the low to mid-intertidal where it inhabits areas of mixed mud, sand and silt (Batie, 1982; Knudsen, 1964; Pittman, 1983). The green shore crab is a summer brooder, producing two broods of young per year, the first in May and the second in July and

August (Knudsen, 1964). Green shore crabs produce fewer larvae than the Dungeness crabs (Knudsen, 1964). *Hemigrapsus oregonensis* larvae are also smaller than the Dungeness larvae, ranging between 400 and 600  $\mu\text{m}$ . *Hemigrapsus oregonensis* larvae reside in the plankton through five zoeal stages and one megalopa stage before settling out of the water column and metamorphosing into a true crab (Batie, 1982).

### **Protist Feeding Experiments with *Cancer magister***

#### **Collection and Daily Procedures**

*Cancer magister* larvae were obtained from ovigerous females collected by SCUBA from a seagrass bed in Ship Harbor, Anacortes, Washington USA (Figure 1) and the female crabs were transported back to the Shannon Point Marine Center in buckets of seawater. The crabs were transferred to sea tables with running sea water, where they were held until the larvae hatched. The crabs were observed every other day for sign of hatching. All female crabs used in the individual experiments were measured across the width of the carapace using a Vernier caliper (Table 1). At the first sign that larvae were being released from their egg masses, the sea tables were drained, rinsed, and refilled, so that newly-hatched larvae could be obtained for the experiment. The following day, the larvae that had hatched overnight were collected from the surface of the tank with a large glass bowl. Larvae were gently agitated to achieve a homogeneous distribution within the bowl. Larvae were removed haphazardly from the bowl using a small Pasteur pipette, and were placed individually into cell wells in a 12-cell tray. For each brood, sufficient

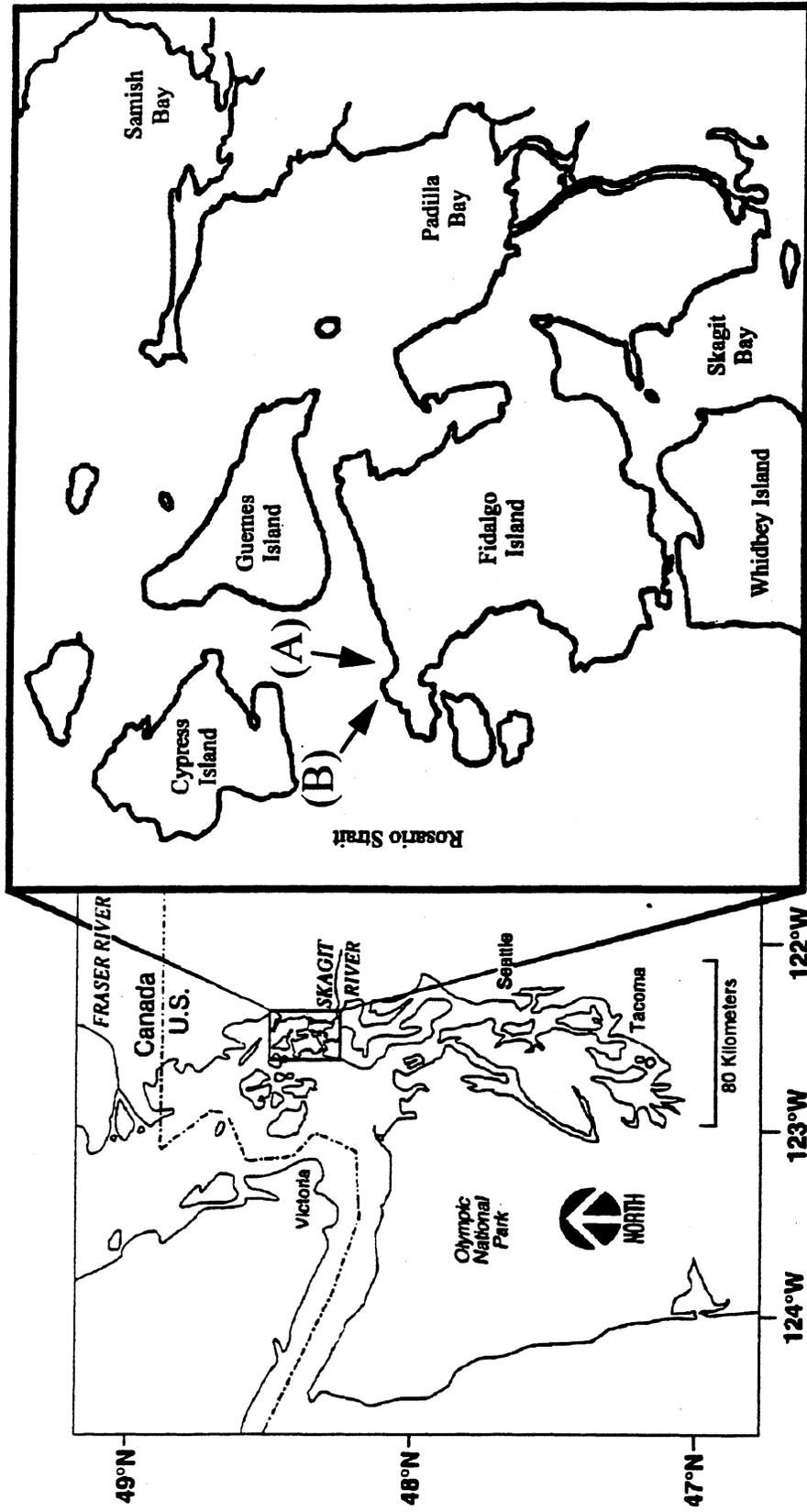


Figure 1. Locations of sites where *Cancer magister* and *Hemigrapsus oregonensis* ovigers were collected. *C. magister* ovigers were collected from a sub-tidal eelgrass bed in Ships Harbor (A), *H. oregonensis* ovigers were collected from the intertidal cobble beach at the Shannon Point Marine Center (B).

Table 1. Carapace width for *Cancer magister* females whose larvae were used in experiments A-D.

<i>C. magister</i> Experiment	Carapace Width (mm)
A	115
B	119
C	133
D	105

numbers of trays were set up so that three trays (36 larvae) could be randomly distributed among all diet treatments to be tested in a given experiment.

In experiment A, a total of 18 trays were set up with larvae from a single brood. The trays were then randomly assigned among six diet treatments (Table 2). A similar approach was used in each of the other three experiments with the number of trays initially set up in each case sufficient to permit assignment of three to each diet treatment tested (Tables 2 and 3).

### Diet Treatments

A total of four experiments were conducted to test the effects of selected diet treatments on the survival, development time and growth of the first zoeal stage. The diet treatments tested simultaneously in each of the four experiment are shown in Tables 2 and 3. Larvae were fed diets of *Noctiluca milaris*, *Prorocentrum micans*, or *Dunaliella tertiolecta* alone or in combination with a sub-optimal diet of freshly-hatched nauplii of the brine shrimp *Artemia sp.* The first two species are dinoflagellates, chosen because they are present in local waters and could be encountered by larval crabs. Choosing dinoflagellates as a food item had other practical value. Dinoflagellates are second only to diatoms as primary producers in coastal waters, and many species have a higher caloric content per cell than diatoms (Taylor, 1990). Some dinoflagellate species have also been used successfully in the aquaculture of larval fish (Taylor, 1990). These two dinoflagellates were also chosen because of their relative differences in size, swimming speed and feeding habits.

Table 2. Diet treatments administered in experiments A-C for *Cancer magister* larvae. Sub-optimal diet consisted of larvae fed *Artemia* nauplii 24h and then starved 48 h. Protists were substituted for the 48 hr starved intervals

Experiment	<i>Artemia</i>	Unfed	Sub-optimal	<i>P. micans</i>		<i>N. milaris</i>		<i>D. tertiolecta</i>	
				Sub-optimal	Sub-optimal	Sub-optimal	Sub-optimal	Sub-optimal	Sub-optimal
A	X	X	X	X		X		X	
B	X	X	X			X*		X	X
C	X	X	X					X	X

\* fed *Artemia* sp. nauplii on day five of experiment B

Table 3. Diet treatments administered in experiment D for *Cancer magister* larvae. Sub-optimal diet consisted of larvae fed *Artemia* nauplii 24h and then starved 72 h. Protists were substituted during the 72 hr starved.

Experiment	<i>Artemia</i>	Unfed	Sub-optimal	<i>P. micans</i>	<i>N. milaris</i>	
					<i>N. milaris</i>	Sub-optimal
D	X	X	X	X	X	X

*Noctiluca milaris* is a heterotrophic dinoflagellate found throughout coastal waters. Because of its large size (300–400  $\mu\text{m}$ ), it was one of the first dinoflagellates identified and studied. *Noctiluca milaris* uses its oral arm to ingest living or nonliving particles by phagocytosis and has a relatively low division rate, generally 0.5 cell divisions per day (Taylor, 1990). *Noctiluca milaris* was chosen as a target diet treatment for larval crabs because of its large size and slow movement.

*Prorocentrum micans* was selected as a prey treatment because it is smaller (50–70  $\mu\text{m}$ ) and swims more rapidly than *Noctiluca milaris* and because it was used to feed the *N. milaris* and must, therefore, be accounted for in the results. *Prorocentrum micans* is a common, planktonic, autotrophic dinoflagellate. The protists will normally exist in bands where they can optimize photosynthesis but not be bleached by high light levels (Sebastian et al., 1994). Because *P. micans* exists in these bands, they may provide larval crabs with patches of high density prey.

*Dunaliella tertiolecta* is approximately 10  $\mu\text{m}$  in diameter. This autotrophic species is also found throughout Puget Sound and is a member of the division Chlorophyta. *Dunaliella tertiolecta* was used in these experiments as an example of a poor protist diet because studies have shown that the survival of *Hemigrapsus oregonensis* larvae was lower when fed a diet of *D. tertiolecta* fed rotifers versus *Isochrysis galbana* fed rotifers (Hartman, 1994).

Sub-optimal diets were used in these experiments because preliminary studies suggested that while some protists were being eaten, they could not support larval development alone. The use of “sub-optimal” diets provides a basis for studying the

nutritional value of prey that cannot by themselves sustain larval development (Levine & Sulkin, 1984a). A “sub-optimal” diet is one in which a prey that can sustain development is manipulated such that larval survival is reduced or development is delayed as compared to its use in an optimal condition. The target prey can then be added to the sub-optimal diet to determine whether it can make a nutritional contribution. A sub-optimal diet for *Cancer magister* larvae was described by Blanco (1996) and consists of a cycle of 24 hours of *Artemia sp.* nauplii followed by 48 hours unfed. When the protist diets were fed in combination with the sub-optimal diet, crab larvae were fed *Artemia sp.* nauplii for 24 hours, followed by 48 hours of exposure to the selected protist diet. This cycle was repeated until the experiment was terminated. All diets were tested against fed (continuous diet of *Artemia sp.* nauplii) and unfed controls. *Artemia sp.* nauplii have been used frequently for culturing larval crabs, and are often considered an optimal diet. (Costlow et al., 1959; Bookhout & Costlow, 1970; Goy & Costlow, 1980; Anger et al., 1981; Harms & Seeger, 1989; Sulkin & McKeen, 1989).

#### Maintenance of Protists in Culture

*Noctiluca milaris* was cultured in four-liter polycarbonate bottles containing 0.2 µm filtered and autoclaved seawater. The *N. milaris* were fed approximately 300 ml of the autotrophic dinoflagellate *Prorocentrum micans* culture per week. *Noctiluca milaris* were transferred bi-monthly to fresh media to ensure logarithmic growth. The *N. milaris* were incubated at 12° C and kept approximately one meter away from any light sources. Keeping the cultures away from light ensures that the *P. micans* being used as prey will

not overgrow the *N. milaris*; and that the *P. micans* cells will be dispersed throughout the culture rather than concentrating toward the light (Taylor, 1990). *Noctiluca milaris* were concentrated before use in diet treatments by reverse filtration with a 80  $\mu\text{m}$  filter. The culture was gently agitated, and approximately two liters of the culture was poured carefully into a beaker. Latex tubing was placed into the 80  $\mu\text{m}$  sieve and a siphon was initiated. The siphon gently pulled the liquid from the pitcher, thus concentrating the *N. milaris*. Two liters of culture were reduced to one liter to double the density of *N. milaris*. Concentrating the culture was done to ensure high numbers of prey for the larvae. Density counts of *N. milaris* were conducted daily in the following manner. Approximately 10 ml of concentrated culture was killed using acid Lugol's. The dead sample was placed into a settling chamber, and was allowed to settle for at least one hour. The settling chamber was then placed on an inverted light microscope and the number of cells was counted. Table 4 shows the average densities of *N. milaris* fed to the larvae for each of the four experiments.

*Prorocentrum micans* and *Dunaliella tertiolecta* were cultured separately in two one-liter polycarbonate bottles. Two liters of seawater were filtered through a 0.2  $\mu\text{m}$  pore size cartridge filter and placed into the polycarbonate bottles. The bottles were then autoclaved for 40 minutes, removed from the autoclave and allowed to cool to room temperature. These bottles were inoculated with approximately 4 ml of concentrated nutrient solution to yield f/2 medium. The bottles were placed in the incubator to cool, and then inoculated with either a *P. micans* or *D. tertiolecta* culture. The cultures were maintained in an incubator with an ambient temperature of 22° C and a 12 hour light:dark

Table 4. Average *Noctiluca milaris* cell counts for *Cancer magister* experiments A-D.

<i>C. magister</i> Experiment	Average <i>N. milaris</i> /ml
A	18
B	17
C	17
D	7

photoperiod; dinoflagellates prefer periodic light cycles to continuous illumination (Taylor, 1990). Approximately one-third of the culture was transferred to fresh nutrient medium every other week to keep the cultures at their maximum growth potential. Previous studies have shown that algal cells reach their highest densities after 2 weeks, and then enter the stationary phase (Sebastian et al., 1994). Cultures were also observed weekly for contamination and general health. There were approximately 6 two-liter bottles of each protist species being cultured throughout the experiments. One bottle was used per day for crab larval feeding. The bottle was mixed gently and poured into a beaker that was used to fill the protist diet trays. After the feeding, the culture bottle would be refilled with f/2 nutrient medium, and then not used for five days to allow the culture to recover.

*Artemia sp.* nauplii have been used repeatedly as an optimal food source for crab larvae (Costlow et al., 1959; Bookhout & Costlow, 1970; Goy & Costlow, 1980; Anger et al., 1981; Harms & Seeger, 1989; Sulkin & McKeen, 1989). The brine shrimp nauplii are easily and quickly hatched in the laboratory and are small enough for larvae of most species to consume (< 500  $\mu\text{m}$ ). The lipid and fatty acid content of the *Artemia sp.* nauplii are beneficial to many larval crabs (Levine & Sulkin, 1984b). *Artemia sp.* nauplii cultures were prepared daily by adding a teaspoon of cysts to approximately one liter of 5  $\mu\text{m}$  filtered seawater. The culture flask was aerated and kept under constant high intensity light. Cultures were harvested approximately every 20 hours. Harvesting the brine shrimp involved pulling out the air stone and waiting a few minutes while the live *Artemia sp.* nauplii separated from the cysts. A stop cock was released and the live *Artemia sp.* nauplii were concentrated into a sieve. The stop cock was closed before the cysts could

pour into the sieve. The nauplii were rinsed with filtered seawater into a slurry at the bottom of the sieve. An Eppendorf repeater pipette was used to pull approximately 20  $\mu$ l of slurry out of the sieve and subsequently injected into the cell wells. The concentration of nauplii in the cell wells was approximately 30 nauplii per ml.

### Larval Crab Cultures

Feeding studies were conducted using tissue culture trays, each containing 12 cell wells. Each tray contained a single diet treatment with approximately 5 ml of medium per cell. For each diet treatment in each experiment, three trays (36 larvae) were tested. Trays were incubated at 12° C on a 10:14 hour light:dark photoperiod which corresponded to natural conditions at the time. Larvae were transferred by pipette daily to clean culture medium and fed the appropriate diet. Separate pipettes were used for each diet treatment to reduce the chance of cross-contamination of diets. During the daily transfer, incidence of molting or death was observed and noted on data sheets. A larva was considered dead when no movement could be detected and the individual was opaque (Dawirs, 1982; Holzer, 1988). Molted larvae were apparent from a translucent exoskeleton at the bottom of the cell well (Holzer, 1988). Trays were rotated within the incubator after the larvae were transferred to reduce the chance of location effects within the incubator. Trays were maintained in culture until all larvae had died or molted to zoeal stage II. Data from these experiments were used to determine survival through the first zoeal stage and stage duration.

### Procedures for Weighing

Larval weight was also used as an indicator of nutritional value for each diet treatment. Aluminum foil boats (2x2 cm) were cut out and numbered. The boats were placed in glass finger bowls and baked at 100° C for 24 hours. After cooling in a desiccator, the boats were weighed on a Mettler M3 analytical balance, and placed back into the desiccator until needed. Larvae molting to the second zoeal stage were pipetted onto a paper towel and rinsed with RO water from a syringe. The syringe was then used to lift the larva off the paper towel using surface tension, and gently place it onto the weigh boat. The larva was then baked in a drying oven for 24 hours at 100° C, and cooled in a desiccator until weighing. Five larvae from each treatment that molted to stage two were dried and weighed. Three weigh boat blanks were also weighed and the average difference in their weight between the first and second weighing was subtracted from each of the larval weights.

### **Protist Feeding Experiments with *Hemigrapsus oregonensis***

#### Collection and Daily Procedures

*Hemigrapsus oregonensis* larvae were obtained from ovigerous females collected from the intertidal zone of the cobble beach at Shannon Point Marine Center in Anacortes, WA, USA (Figure 1). Females were held in glass finger bowls filled with filtered seawater. All bowls were placed into an incubator at 15° C and a 12 hour light:dark photoperiod, which corresponded to natural conditions at the time (Pittman, 1983; Sulkin, 1986). Ovigerous females were observed daily for hatching, and were placed into fresh,

filtered seawater. Females remained in bowls no longer than one week before use in experiments. The two crabs used for experimentation were measured across their carapace using a Vernier caliper. The female crab used in experiment A had a carapace width of 15 mm, while the crab used in experiment B had a carapace width of 12 mm. When larvae hatched they were assigned to diet treatments as described above for the *Cancer magister* larvae.

### Diet Treatments

Two experiments were conducted to test the effects of specific diet treatments on larval survival, weight, development time, or time to death. The diet treatments for these experiments are shown in Table 5. Feeding studies were conducted in a similar manner to those described for *Cancer magister* larvae. For each brood, sufficient numbers of trays were set up so that three trays (36 larvae) could be randomly distributed among all diet treatments to be tested in a given experiment. In experiment A for *Hemigrapsus oregonensis*, a total of 24 trays were set up with larvae from a single brood. The trays were then randomly assigned to the eight diet treatments. Larvae were fed diets of *Noctiluca milaris*, *Prorocentrum micans*, or *Dunaliella tertiolecta* alone or in combination with a sub-optimal diet of freshly-hatched nauplii of the brine shrimp *Artemia sp.* As in the experiments for *C. magister*, cell counts of *N. milaris* were taken daily. The average daily cell counts of *N. milaris* for experiments A and B were 14 *N. milaris* per ml. The sub-optimal diet for *H. oregonensis* was described by Hartman (1994) and consisted of larvae unfed for the first three days after hatching and fed *Artemia sp.* nauplii every day

Table 5. Diet treatments administered in experiments A & B for *Hemigrapsus oregonensis* larvae. The sub-optimal diet consisted of larvae unfed for the first 3 days after hatching and then fed *Artemia* nauplii everyday thereafter

Experiment	<i>Artemia</i>	Unfed	Sub-optimal	<i>P. micans</i>		<i>N. milaris</i>		<i>D. tertiolecta</i>	
				Sub-optimal	Sub-optimal	Sub-optimal	Sub-optimal	Sub-optimal	Sub-optimal
A	X	X	X	X	X	X	X	X	X
B	X	X	X	X	X	X	X	X	X

after. When the protist diets were fed in combination with the sub-optimal diet, they were fed a protist diet for the first three days after hatching and then transferred to a diet of *Artemia sp.* nauplii every day after. All diets were tested against fed and unfed controls. All cultures were maintained in the same manner as described above in the experiments for *C. magister*. Five larvae from each treatment surviving to zoeal stage II were weighed using the same method described above in the *C. magister* experiments.

### **Detrital Feeding Experiments with *Hemigrapsus oregonensis***

#### **Method of Producing Detritus in the Laboratory**

Fresh eelgrass (*Zostera marina*) was gathered from Padilla Bay, WA (Figure 1). The eelgrass was rinsed several times in a sea table, and then placed into a blender containing approximately 400 ml of filtered seawater. This mixture was pureed for five minutes, poured into a large glass bowl, and baked in a drying oven for 48 hours at 80° C. The dried mixture was scraped into a pestle, pulverized for ten minutes, and sifted through a 363 µm sieve onto wax paper. The smaller than 363 µm fraction was placed into a beaker, sealed with parafilm, and kept in a desiccator for further use in detritus treatments. Approximately 0.4 grams of "detritus" were placed in several two-liter polycarbonate bottles filled with 0.2 µm filtered seawater. From these stock detritus bottles, two treatments were established, one in which the detritus was conditioned to develop an associated microbial film "colonized" and one where microbial activity was kept to a minimum "uncolonized". The set of bottles that would be identified as "uncolonized" were autoclaved for 40 minutes. After autoclaving, the bottles were cooled to room

temperature, sealed and then placed into an incubator at 22° C. The bottles that contained detrital material to be colonized by microbial films were sealed with a foam stopper, pierced with a glass bubbling rod and aerated continually throughout the experiment. All of the bottles were agitated daily, and were allowed to incubate for a week prior to the start of the experiment. After ten days, detritus from each of the treatment bottles was tested for the presence or absence of bacteria using DAPI staining techniques and epifluorescent microscopy. Three slides were prepared from each detrital treatment and the slides containing the “colonized” and “uncolonized” detritus were observed under the epifluorescent microscope. The detritus in the “colonized” slides was covered with blue fluorescently labeled microbes, while the detritus in the “uncolonized” slides contained comparatively few microbes.

### Daily Procedures

*Hemigrapsus oregonensis* larvae were obtained from ovigerous females collected from the intertidal zone of the cobble beach at Shannon Point Marine Center in Anacortes, WA (Figure 1). Females were maintained in the same manner as described above in protist feeding experiments for *H. oregonensis*. When three broods of larvae hatched on a single day, the experiment was started. The three broods were pooled within a single bowl and larvae were randomly assigned to diet treatments. Ten larvae were placed into each of 28 small (8 cm diameter) glass finger bowls. The bowls were randomly assigned to the seven diet treatments. All of the bowls were placed in an incubator on a shaker table in order to keep detrital particles in suspension and available to the larvae. The

incubator was set at 15° C and a 12 hour light:dark photoperiod, corresponding to natural conditions at the time (Pittman, 1983; Sulkin, 1986).

### Diet Treatments

The diet treatments consisted of microbially-colonized detritus and uncolonized detrital material, alone and in combination with a sub-optimal diet of *Artemia sp.* nauplii. The sub-optimal diet used in this experiment was the same as used above in the *Hemigrapsus oregonensis* protist diet experiments. These diets were tested against sub-optimal, fed and unfed controls. The larvae were observed daily for molting or death, and then transferred to fresh diet treatments. The small glass bowls were rotated within their stack as well as on the shaker table to minimize the risk of an incubator effect. Molted stage two larvae were weighed using the same procedure as described above for *Cancer magister* and *H. oregonensis* larvae.

### **Data Analysis**

The percent survival to stage II of each diet treatment was determined, and data were arcsine transformed and analyzed by ANOVA. Only treatments in which some larvae survived to stage II were included in the analysis. If there were significant differences ( $P < 0.05$ ) between treatments, Tukey's HSD ( $P = 0.05$ ) was used to determine which diets had different survival (Zar, 1984).

Mean day of molt was also used as an indicator of nutritional value. The collection of these data often led to severely unbalanced design which caused non-homogeneous

variance. The nonparametric Kruskal-Wallis ANOVA was used for data that had heterogeneous variance. If there were significant differences ( $P < 0.05$ ) among treatments, a comparison of mean ranks ( $P = 0.05$ ) was used to determine which diets had differed in mean day of molt (Siegel, 1992).

If a diet treatment had zero percent survival and consequently no molting to stage II, the mean day of death was compared to other diets with zero survival. A standard one way ANOVA ( $P < 0.05$ ) was used to analyze the data, and Tukey's HSD ( $P = 0.05$ ) compared treatment means if there were significant differences (Zar, 1984).

Weight data was analyzed by ANOVA. If significant differences ( $P < 0.05$ ) were found between diet treatments means were compared by Tukey's HSD ( $P = 0.05$ ) (Zar, 1984).

## RESULTS

### *Cancer magister* Experiment A

#### Survival

Daily survival of *Cancer magister* larvae raised on the six diet treatments tested in experiment A is shown in Figure 2 (See Table 2 for diet treatments). The *Artemia sp.* fed control sustained survival to zoeal stage II of 86.0% (Table 6). While no larvae survived to zoeal stage II in the unfed controls or when fed diets consisting solely of either *Noctiluca milaris* or *Prorocentrum micans*, it is apparent from Figure 2 that mortality was delayed in larvae fed the latter two diets as compared to the unfed control. This is confirmed by comparing mean day of death among these three diet treatments (Table 7). Larvae that were left unfed died earliest, while larvae fed only *Prorocentrum micans* or *Noctiluca milaris* lived significantly longer than those that were unfed. This result implies that the protist-fed larvae were consuming and deriving some level of nutritional benefit from their prey.

In experiment A, the sub-optimal *Artemia sp.* diet sustained 36.3% survival to stage II (Table 6). This was significantly lower than the *Artemia sp.* diet alone. However, when the sub-optimal *Artemia sp.* diet was supplemented as described in the methods with *Noctiluca milaris*, survival increased to 83.3%, equal to that of the *Artemia sp.* control. These results indicate that *Cancer magister* larvae are consuming *N. milaris*, with substantial nutritional benefit.

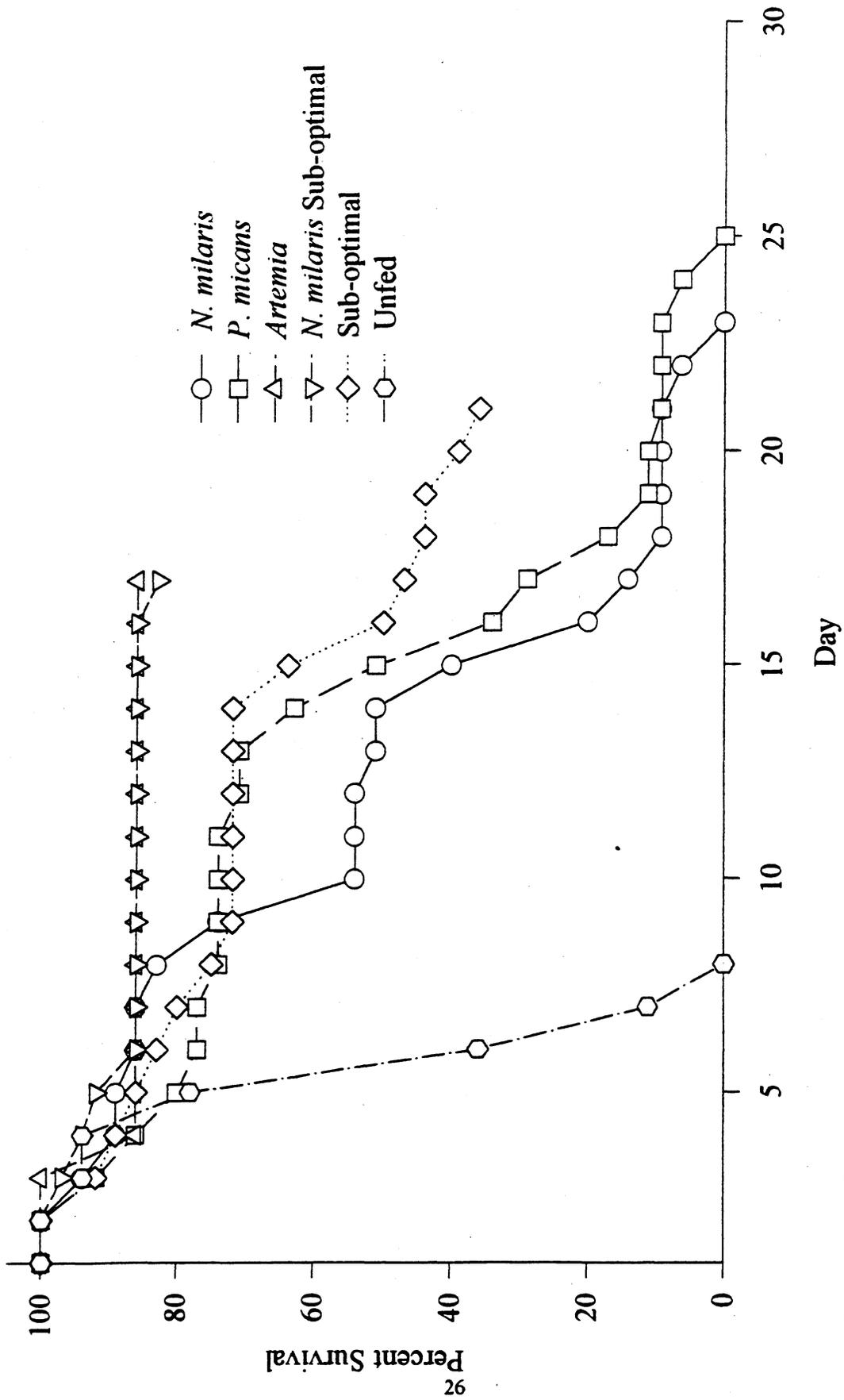


Figure 2. Daily percent survival for *Cancer magister* larvae fed the indicated diets from experiment A

Table 6. Percent survival to zoeal stage II for *Cancer magister* larvae fed 6 diets in Experiment A. The sub-optimal diet consisted of larvae fed *Artemia sp.* 24 hrs and Unfed 48hrs. Results of a ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Percent Survival	n	Standard Error	Tukey's HSD
<i>Artemia</i>	86.0	3	3.0	a
<i>N. milaris</i> Sub-optimal	83.3	3	4.9	a
Sub-optimal	36.3	3	9.9	b

Table 7. Mean day of death for *Cancer magister* larvae fed the three diet treatments in Experiment A that did not sustain development to zoeal stage II Results of ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Mean Day of Death	n	Standard Error	Tukey's HSD
<i>N. milaris</i>	12.9	35	0.92	a
<i>P. micans</i>	14.1	35	1.12	a
Unfed	6.1	36	0.19	b

### Stage Duration

The mean day of molt (stage duration) was calculated for larvae cultured on diets that supported development to zoeal stage II (Table 8). Due to the nature of the experimental design, the replication for stage duration was severely unbalanced, leading to non-homogeneous variance. Therefore, the non-parametric Kruskal-Wallis ANOVA was used to determine whether there were differences in stage duration among diet treatments. Results of the Kruskal-Wallis analysis indicated a significant difference among the treatments ( $P < 0.05$ ). Larvae fed the *Artemia sp.* control diet had a significantly faster development rate than larvae fed other diets, with larvae fed the sub-optimal *Artemia sp.* diet taking longer to molt to zoeal stage II. Addition of *Noctiluca milaris* to the sub-optimal diet did not reduce stage duration over the sub-optimal diet alone.

### ***Cancer magister* Experiment B**

#### Survival

Daily survival for *Cancer magister* larvae for experiment B is shown in Figure 3 (See Table 2 for diet treatments). Only unfed larvae failed to develop to zoeal stage II, with some larvae from all other (sub-optimal) treatments surviving to stage II. The *Artemia sp.* fed control sustained survival to zoeal stage II of 86.0% (Table 9). In this experiment, larvae fed a diet of only *Noctiluca milaris* sustained 22.0% survival. However, the larvae raised on *N. milaris* were accidentally fed *Artemia sp.* on day five after hatching confounding this result. Therefore, this treatment has been omitted in Figure 3. The sub-optimal *Artemia sp.* diet sustained 37.0% survival to stage II,

Table 8. Mean day of molt to zoeal stage II for *Cancer magister* larvae fed the three diet treatments in Experiment A that sustained development to zoeal stage II. Results of a Kruskal-Wallis ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a comparison of mean rank ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Mean Day of Molt	n	Standard Error	Comparison of Means
<i>Artemia</i>	10.9	31	0.19	a
<i>N. milaris</i> Sub-optimal	14.9	30	0.58	b
Sub-optimal	17.1	13	0.65	b

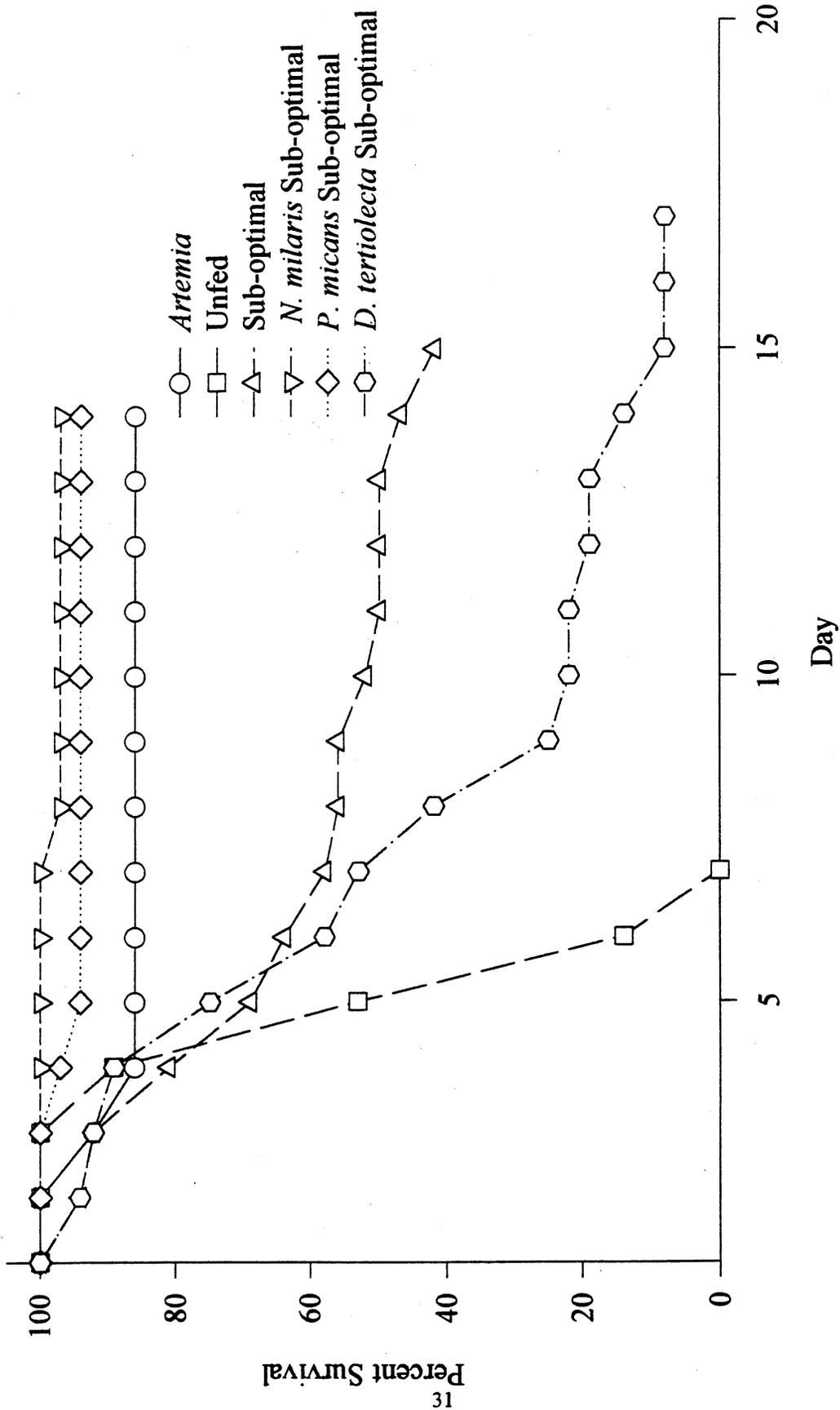


Figure 3. Daily percent survival for *Cancer magister* larvae fed the indicated diets from experiment B

Table 9. Percent survival to zoeal stage II for *Cancer magister* larvae fed 7 diets in Experiment B. The sub-optimal diet consisted of larvae fed *Artemia sp.* 24 hrs and Unfed 48 hrs. Results of ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Percent Survival	n	Standard Error	Tukey's HSD
<i>N. milaris</i> Sub-optimal	97.3	3	2.7	a
<i>P. micans</i> Sub-optimal	94.3	3	5.7	a
<i>Artemia</i>	86.0	3	3.0	a
Sub-optimal	37.0	3	6.0	b
<i>N. milaris</i> *	22.0	3	7.4	b
<i>D. tertiolecta</i> Sub-optimal	8.3	3	4.9	b

\* larvae were fed *Artemia sp.* on day 5 of experiment

significantly lower than the *Artemia sp.* diet alone. However, when the sub-optimal *Artemia sp.* was supplemented as described in the methods with either *Noctiluca milaris* or *Prorocentrum micans*, survival increased to 97.3% and 94.3% respectively, equal to that of the *Artemia sp.* control. Larvae fed the sub-optimal plus *Dunaliella tertiolecta* diet showed an 8.3% survival to stage II, not significantly different than for larvae fed only the sub-optimal diet of *Artemia sp.* nauplii. These results indicate that *C. magister* larvae are consuming and obtaining, substantial nutritional benefit from the *N. milaris* and *P. micans* diets. The results for *D. tertiolecta* are equivocal.

#### Stage Duration

Mean day of molt (stage duration) was calculated for larvae cultured on diets that supported development to zoeal stage II (Table 10). Larvae fed the *Artemia sp.* control diet had a significantly faster development rate than larvae fed other diets, with larvae fed the sub-optimal *Artemia sp.* diet taking almost twice as long to molt to zoeal stage II (Table 10). Larvae fed the sub-optimal diet plus *Prorocentrum micans* or *Noctiluca milaris* developed significantly faster than did larvae fed the sub-optimal diet alone. The sub-optimal diet plus *P. micans* or *N. milaris* did not differ significantly from one another or from the sub-optimal diet plus *Dunaliella tertiolecta*. Larvae fed the sub-optimal diet plus *D. tertiolecta* did not differ from the sub-optimal diet alone.

Table 10. Mean day of molt to zoeal stage II for *Cancer magister* larvae fed 5 diets in Experiment B. The sub-optimal diet consisted of larvae fed *Artemia sp.* 24 hrs and Unfed 48 hrs. Results of a Kruskal-Wallis ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a comparison of mean rank ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Mean Day of Molt	n	Standard Error	Comparison of Means
<i>Artemia</i>	9.6	31	0.26	a
<i>N. milaris</i> Sub-optimal	11.9	35	0.17	b
<i>P. micans</i> Sub-optimal	11.2	34	0.29	b
<i>D. tertiolecta</i> Sub-optimal	18.7	3	1.30	b c
Sub-optimal	18.8	12	0.22	c

## Weight

Data collected from larval weights were analyzed by ANOVA comparing weight of larvae among diet treatments (Table 11). There was a significant difference between the weight of larvae fed a sub-optimal diet plus *Noctiluca milaris* and a sub-optimal diet plus *Prorocentrum micans* treatments, with other treatments not distinguishable from one another.

## ***Cancer magister* Experiment C**

### Survival

Figure 4. shows daily survival for *Cancer magister* larvae in experiment C (See Table 2 for diet treatments). Only unfed larvae failed to survive to zoeal stage II. The *Artemia sp.* fed control sustained survival to zoeal stage II of 86.3% (Table 12). In experiment C, the sub-optimal *Artemia sp.* diet sustained 69.3% survival to stage II. This was not significantly different than the *Artemia sp.* diet alone. When the *Artemia sp.* was supplemented as described in the methods with *Noctiluca milaris* or *Prorocentrum micans*, survival increased to 96.0% and 86.3% respectively, equal to that of the *Artemia sp.* control but, not significantly different than the sub-optimal diet of *Artemia sp.* Larvae fed the sub-optimal plus *Dunaliella tertiolecta* diet showed an 14.0% survival to stage II, significantly lower than for larvae fed only the sub-optimal diet of *Artemia sp.* These results are consistent with those reported for experiment A and B with respect to *N. milaris* and *P. micans* and also suggests that the larvae are, indeed, consuming *D. tertiolecta*, with negative nutritional results.

Table 11. Day 1 zoal stage II weights (mg) for *Cancer magister* larvae fed 4 diets in Experiment B. The sub-optimal diet consisted of larvae fed *Artemia sp.* 24 hrs and Unfed 48 hrs. Results of ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Larval Weights (mg)	n	Standard Error	Tukey's HSD
<i>N.mylaris</i> Sub-optimal	0.053	5	0.0022	a
Sub-optimal	0.043	5	0.0032	a b
<i>Artemia</i>	0.038	5	0.0049	a b
<i>P. micans</i> Sub-optimal	0.033	5	0.0054	b

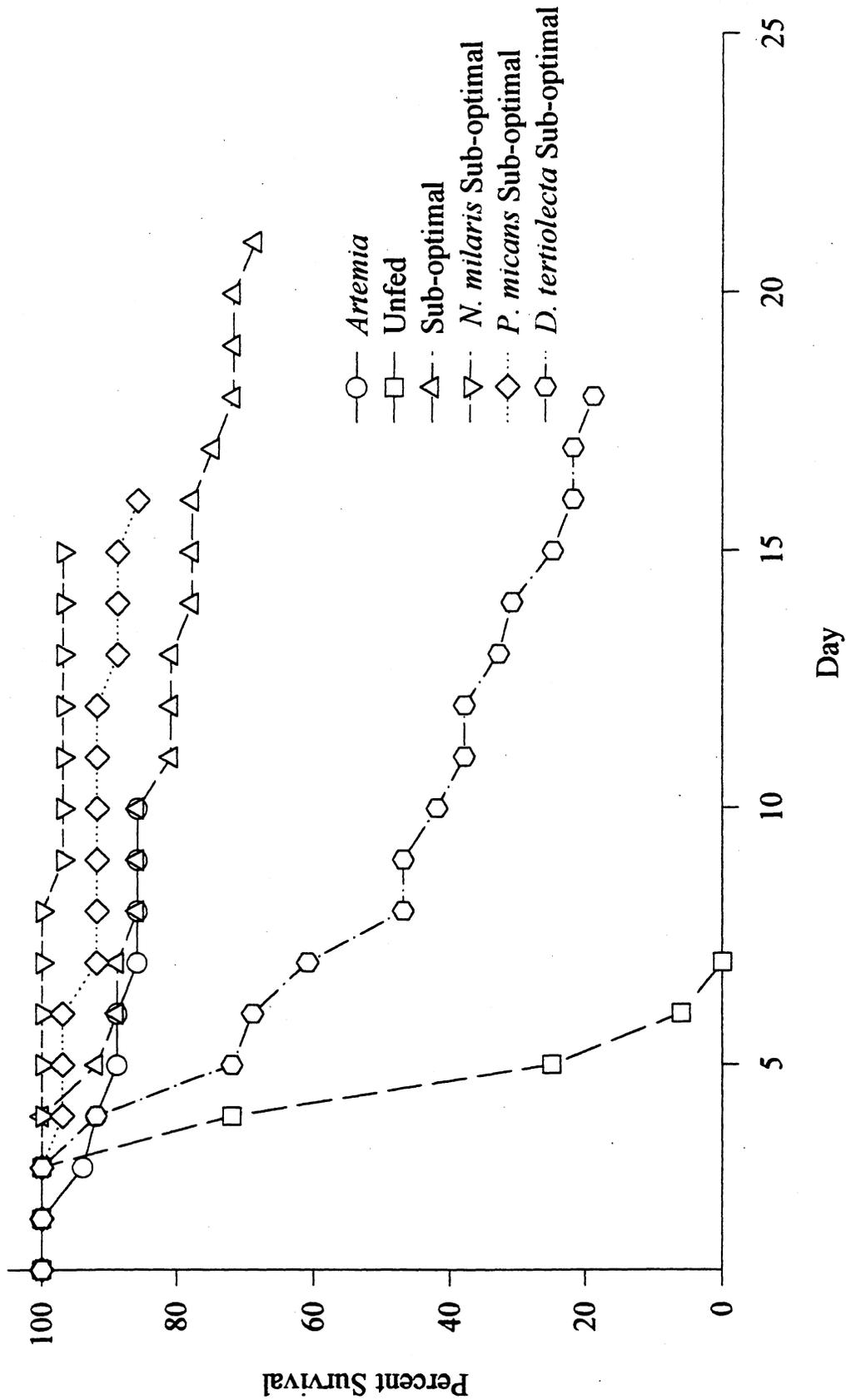


Figure 4. Daily percent survival for *Cancer magister* larvae fed the indicated diets from experiment C

Table 12. Percent survival to zoeal stage II for *Cancer magister* larvae fed 5 diets in Experiment C. The sub-optimal diet consisted of larvae fed *Artemia sp.* 24 hrs and Unfed 48 hrs. Results of ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Percent Survival	n	Standard Error	Tukey's HSD
<i>N. milaris</i> Sub-optimal	96.0	3	4.0	a
<i>Artemia</i>	86.3	3	5.7	a
<i>P. micans</i> Sub-optimal	86.3	3	5.7	a
Sub-optimal	69.3	3	9.9	a
<i>D. tertioleta</i> Sub-optimal	14.0	3	7.4	b

### Stage Duration

The mean day of molt (stage duration) was calculated for larvae cultured on diets that supported development to zoeal stage II (Table 13). Larvae fed the *Artemia sp.* control diet had a significantly faster development rate than larvae fed other diets, with larvae fed the sub-optimal *Artemia sp.* diet taking twice as long to molt to zoeal stage II. Larvae fed the sub-optimal diet plus *Prorocentrum micans* and *Noctiluca milaris* developed significantly faster than did larvae fed the sub-optimal diet. However, the sub-optimal diet plus *P. micans* or *N. milaris* did not differ significantly from one another. Larvae fed a diet of the sub-optimal plus *Dunaliella tertiolecta* did not differ from the sub-optimal diet alone, or larvae fed a sub-optimal plus *N. milaris* diet.

### Weight

Data collected from larval weights were analyzed by ANOVA comparing weight of larvae among diet treatments (Table 14). No significant differences were found ( $P>0.05$ ), therefore there was no statistical difference in day 1 stage II larval weights between diet treatments.

## ***Cancer magister* Experiment D**

### Survival

Experiment D involved a series of tests with larvae employing a different sub-optimal diet schedule. The sub-optimal diet extended the unfed (or protist) period for 72 hours (rather than 48 hours). Daily survival is shown in Figure 5 (See Table 3 for diet

Table 13. Mean day of molt to zoeal stage II for *Cancer magister* larvae fed 5 diets in Experiment C. The sub-optimal diet consisted of larvae fed *Artemia sp.* 24 hrs and Unfed 48 hrs. Results of a Kruskal-Wallis ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a comparison of mean rank ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Mean Day of Molt	n	Standard Error	Comparison of Means
<i>Artemia</i>	9.3	31	0.09	a
<i>P. micans</i> Sub-optimal	11.2	30	0.23	b
<i>N. milaris</i> Sub-optimal	12.3	23	0.25	b c
<i>D. tertiolecta</i> Sub-optimal	18.8	6	0.54	c d
Sub-optimal	18.8	25	0.35	d

Table 14. Day 1 zoeal stage II weights (mg) for *Cancer magister* larvae fed 4 diets in Experiment C. The sub-optimal diet consisted of larvae fed *Artemia sp.* 24 hrs and Unfed 48 hrs. Results of ANOVA ( $P>0.05$ ) indicated no significant differences. The sample sizes are represented by n.

Diet Treatment	Larval Weights (mg)	n	Standard Error
<i>Artemia</i>	0.054	5	0.0031
<i>P. micans</i> Sub-optimal	0.049	4	0.0043
<i>N. milaris</i> Sub-optimal	0.055	5	0.0013
Sub-optimal	0.045	5	0.0022

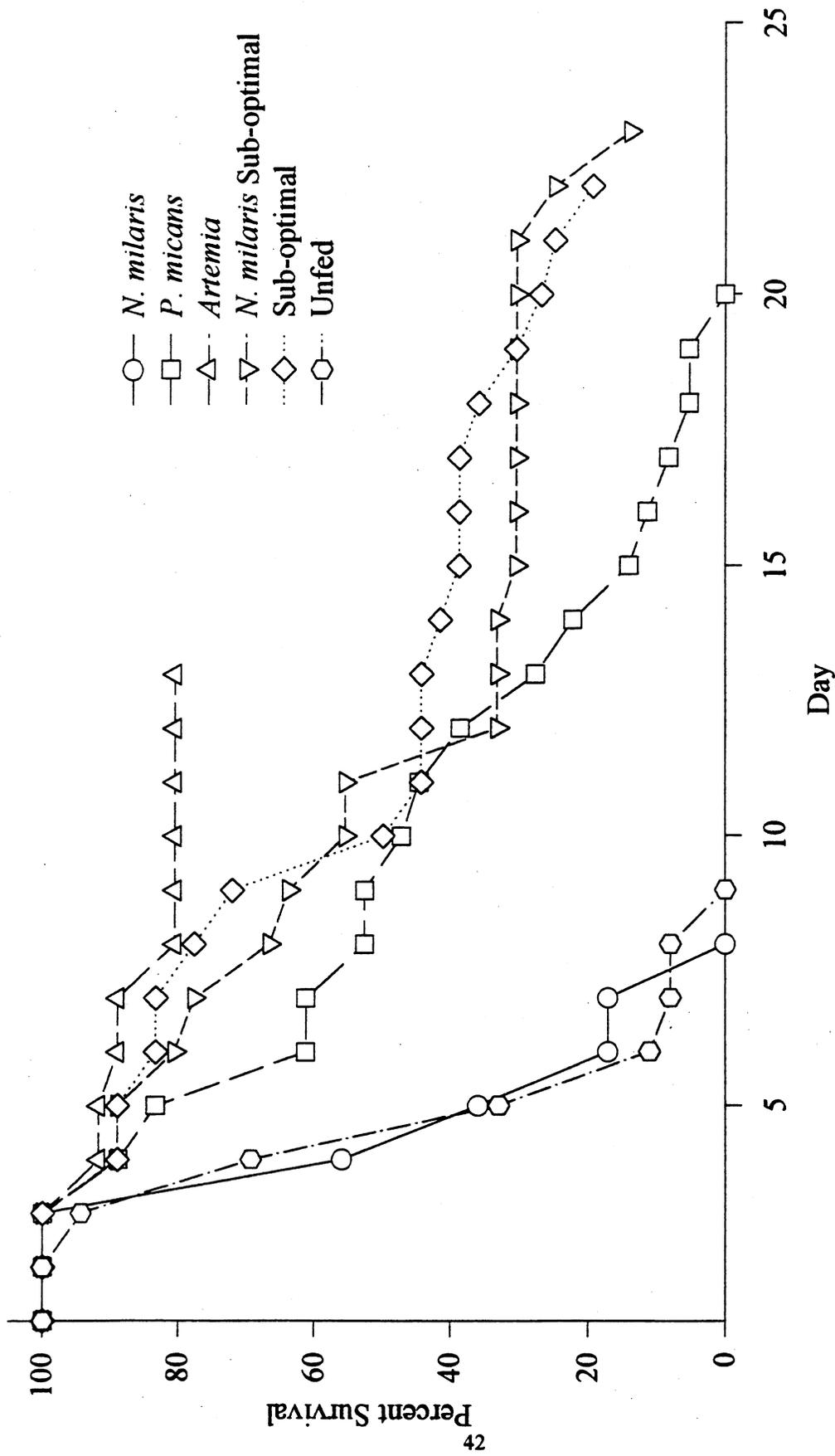


Figure 5. Daily percent survival for *Cancer magister* larvae fed the indicated diets from experiment D

treatments). In this experiment, no larvae survived to the second zoeal stage in the unfed control or when fed only *Noctiluca milaris* or *Prorocentrum micans* (Figure 5). The daily survival data however, suggest that mortality was delayed when larvae were fed a diet of *P. micans*. Mean day of death was used to compare diets that did not sustain survival to zoeal stage II (Table 15). A diet of only *P. micans* delayed mortality as compared to larvae in the unfed control or when fed only *N. milaris*. There was no statistical difference in mean day of death between larvae in the unfed control or *N. milaris*.

Percent survival to zoeal stage II is shown in Table 16. In this experiment, the sub-optimal diet of *Artemia sp.* plus unfed 72 hours sustained only 28% of the larvae to the second instar, significantly lower than the *Artemia sp.* diet alone (Table 16). When *Noctiluca milaris* was substituted for the 72 hour time interval, only 16.7% of the larvae survived, not significantly different from the sub-optimal diet alone

### Stage Duration

The mean day of molt was compared for diets that sustained development to the second instar (Table 17). The *Artemia sp.* fed larvae had significantly faster development than larvae fed other diets. Larvae fed the sub-optimal diet plus *Noctiluca milaris* did not molt significantly faster than the sub-optimal diet alone.

### ***Hemigrapsus oregonensis* Protist Experiments**

Because *Hemigrapsus oregonensis* protists experiments A and B had all of their diet treatments in common and were run simultaneously, data from both experiments were

Table 15. Mean day of death for *Cancer magister* larvae fed the three diet treatments in Experiment D that did not sustain development to zoeal stage II. The sub-optimal diet consisted of larvae fed *Artemia sp.* 24 hrs and Unfed 72 hrs. Results of ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Mean Day of Death	n	Standard Error	Tukey's HSD
<i>P. micans</i>	10.3	36	0.81	a
<i>N. milaris</i>	5.3	36	0.24	b
Unfed	5.3	36	0.27	b

Table 16. Percent survival to zoeal stage II for *Cancer magister* larvae fed 3 diets in Experiment D. The sub-optimal diet consisted of larvae fed *Artemia sp.* 24 hrs and Unfed 72 hrs. Results of ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Percent Survival	n	Standard Error	Tukey's HSD
<i>Artemia</i>	80.7	3	15.5	a
Sub-optimal	28.0	3	11.0	b
<i>N. milaris</i> Sub-optimal	16.7	3	8.3	b

Table 17. Mean day of molt to zoeal stage II for *Cancer magister* larvae fed 3 diets in Experiment D. The sub-optimal diet consisted of larvae fed *Artemia sp.* 24 hrs and Unfed 72 hrs. Results of a Kruskal-Wallis ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a comparison of mean rank ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Mean Day of Molt	n	Standard Error	Comparison of Means
<i>Artemia</i>	11.6	28	0.21	a
<i>N. milaris</i> Sub-optimal	17.0	10	1.49	b
Sub-optimal	18.7	7	1.15	b

pooled into one data set and analyzed as described previously for the *Cancer magister* data. Daily survival for *H. oregonensis* larvae raised on all nine diet treatments tested in Experiment A & B is shown in Figure 6. For purposes of clarity in presentation, the results of the sub-optimal diet alone and in combination with the protist diets are analyzed and presented separately from those of the protist diets alone because the protist diets alone supported the development of *H. oregonensis* larvae.

## **Protist Diet Treatments**

### **Survival**

A comparison of percent survival to zoeal stage II (Figure 6 and Table 18) showed that the *Artemia sp.* fed control sustained survival of 78.8%, while there was no survival to stage II in the unfed controls (Figure 6). The *Dunaliella tertiolecta*-fed larvae showed a 2.7% survival rate to zoeal stage II. Larvae fed only diets of *Prorocentrum micans* and *Noctiluca milaris* showed survival of 81.8% and 34.7% respectively, both significantly higher than the *D. tertiolecta* fed larvae. There was no difference in survival between larvae raised on a diet of *Prorocentrum micans* or *Artemia sp.*; however, both diets supported higher survival than larvae fed a diet of *N. milaris*.

Because *Prorocentrum micans* performed so well as a nutritional source for *Hemigrapsus oregonensis* larvae, the experiment was continued to determine how many instars the diet of *P. micans* could support. The control diet of *Artemia sp.* was also continued to determine how the two diets compared. Larvae fed a diet of *P. micans*

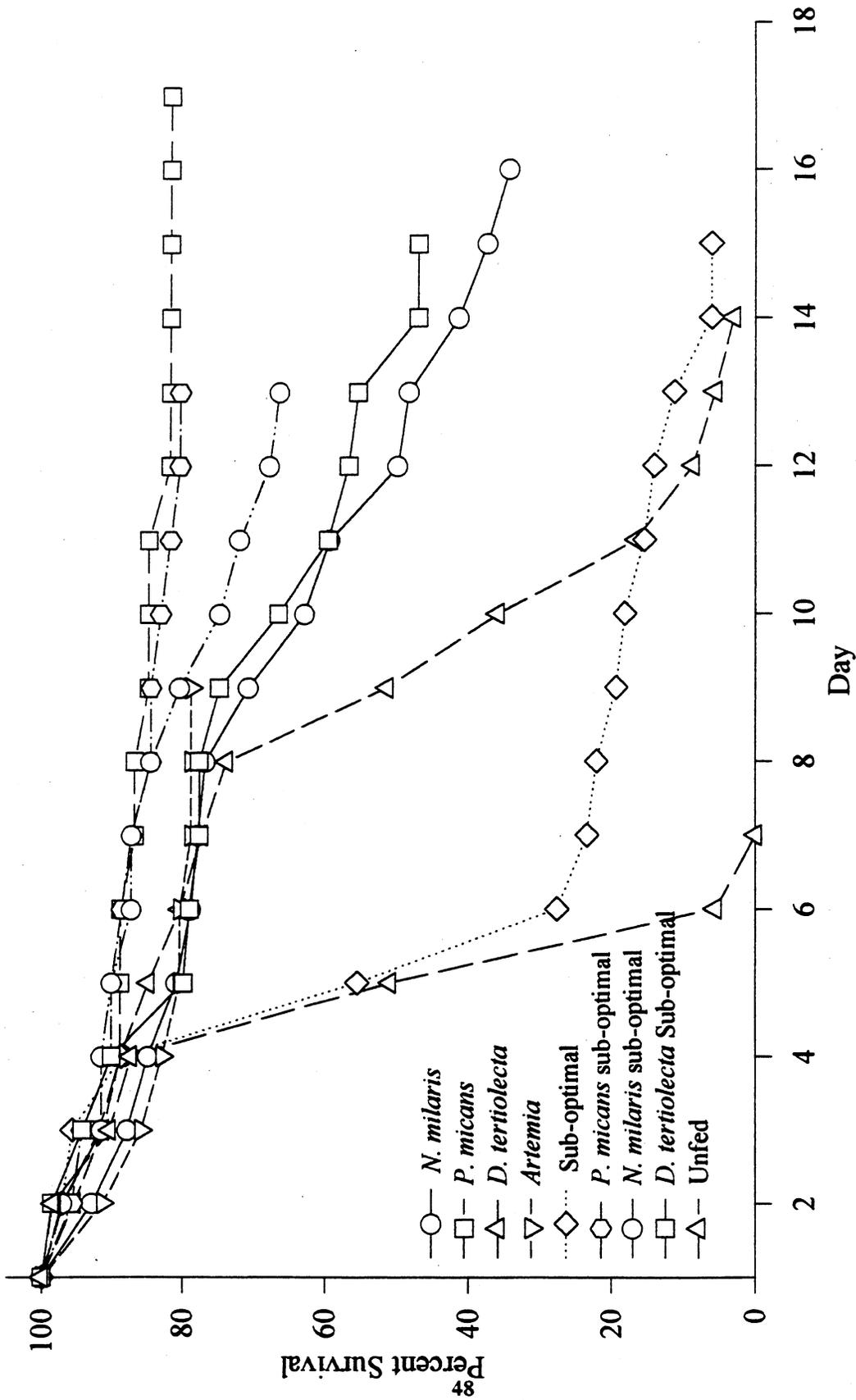


Figure 6. Daily percent survival for *Hemigrapsus oregonensis* larvae fed the indicated diets from experiments A & B.

Table 18. Percent survival to zoeal stage II for *Hemigrapsus oregonensis* larvae fed 4 diets in Experiments A & B. Results of ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Percent Survival	n	Standard Error	Tukey's HSD
<i>P. micans</i>	81.8	6	4.0	a
<i>Artemia</i>	78.8	6	6.3	a
<i>N. milaris</i>	34.7	6	6.7	b
<i>D. tertiolecta</i>	2.7	6	1.7	c

molted through three more instars, as many as the *Artemia sp.* control diet. However, no larvae metamorphosed to the megalopae stage.

### Stage Duration

The mean day of molt (stage duration) was calculated for diets that supported development to zoeal stage II (Table 19). Larvae fed the *Artemia sp.* control had the fastest development, significantly faster than any other diet. Although, larvae fed a diet of only *Noctiluca milaris* appear to molt approximately 1 day before larvae raised on *Prorocentrum micans*, there was no statistical difference in the mean day of molt between larvae raised on these two diets

### Weight

An analysis of data collected on larval weights is shown in Table 20. The *Artemia sp.* fed control had the highest mean day 1, stage II weights among diet treatments sustaining development to the second instar. Larvae fed only *Prorocentrum micans* had weights that were not statistically different than those fed the *Artemia sp.* control diet. The *Noctiluca milaris* diet showed larval weights lower than those fed *P. micans* alone or the *Artemia sp.* control diet, however, only significantly lower than the *Artemia sp.* diet.

### **Sub-optimal Diet Treatments**

The above results indicate that *Hemigrapsus oregonensis* larvae are consuming all three protists, with substantial nutritional benefit derived from the *Prorocentrum micans*

Table 19. Mean day of molt to zoeal stage II for *Hemigrapsus oregonensis* larvae fed diets in Experiments A & B. Results of a Kruskal-Wallis ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a comparison of mean rank ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Mean Day of Molt	n	Standard Error	Comparison of Mean Ranks
<i>Artemia</i>	7.7	55	0.11	a
<i>N. milaris</i>	8.7	25	0.35	b
<i>P. micans</i>	9.6	58	0.44	b
<i>D. tertiolecta</i>	10.5	2	0.50	-

Table 20. Day 1 zoeal stage II weights (mg) for *Hemigrapsus oregonensis* larvae fed 3 diets in Experiments A & B. Results of ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Larval Weight (mg)	n	Standard Error	Tukey's HSD
<i>Artemia</i>	0.027	5	0.003	a
<i>P. micans</i>	0.015	5	0.001	a b
<i>N. milaris</i>	0.006	5	0.002	b

and *Noctiluca milaris* diets without the addition of a sub-optimal diet of *Artemia sp.*

However, the results of the sub-optimal treatments are presented as confirmation.

### Survival

Percent survival through zoeal stage I were arcsine transformed and the survival as a function of diet was analyzed by ANOVA (Table 21). The sub-optimal *Artemia sp.* diet sustained only 5.7% survival to zoeal stage II (Table 21). This was significantly lower than the *Artemia sp.* diet alone. However, when the *Artemia sp.* sub-optimal diet was supplemented with either *Prorocentrum micans* or *Noctiluca milaris*, survival increased to 81.7% and 66.2% respectively. The survival of larvae raised on these diets did not differ significantly from larvae cultured on the *Artemia sp.* control diet. Larvae fed the sub-optimal diet of *Artemia sp.* plus *Dunaliella tertiolecta* diet showed 48.3% survival to zoeal stage II, significantly higher than the larvae fed only the sub-optimal diet of *Artemia sp.*, but less than the *Artemia sp.* fed control.

### Stage Duration

The mean day of molt (stage duration) was calculated for larvae raised on diets that supported development to zoeal stage II (Table 22). When larvae were fed a sub-optimal diet of *Artemia sp.* plus *Prorocentrum micans* the average stage duration was only 8.5 days, significantly longer than the *Artemia sp.* control diet, but significantly faster than the *Artemia sp.* sub-optimal diet alone. Larvae fed the sub-optimal *Artemia sp.* diet plus *Noctiluca milaris* developed significantly faster than the sub-optimal *Artemia sp.* diet plus

Table 21. Percent survival to zoeal stage II for *Hemigrapsus oregonensis* larvae fed 5 diets in Experiments A & B. The sub-optimal diet consisted of larvae starved for the first 3 days and fed *Artemia sp* every day after. Results of ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Percent Survival	n	Standard Error	Tukeys HSD
<i>P. micans</i> Sub-optimal	81.7	6	5.0	a
<i>Artemia</i>	78.8	6	6.3	a
<i>N. milaris</i> Sub-optimal	66.2	6	5.9	a b
<i>D. tertiolecta</i> Sub-optimal	48.3	6	5.0	b
Sub-optimal	5.7	6	2.8	c

Table 22. Mean day of molt to stage II for *Hemigrapsus oregonensis* larvae fed 5 diets in Experiments A & B. The sub-optimal diet consisted of larvae starved for the first 3 days and fed *Artemia sp* every day after. Results of a Kruskal-Wallis ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Comparison of Mean Rank ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Mean Day of Molt	n	Standard Error	Comparison of Mean Ranks
<i>Artemia</i>	7.7	55	0.11	a
<i>P. micans</i> Sub-optimal	8.5	57	0.16	b
<i>N. milaris</i> Sub-optimal	9.3	46	0.17	c
<i>D. tertiolecta</i> Sub-optimal	11.3	35	0.17	d
Sub-optimal	13.3	4	0.25	-

*Dunaliella tertiolecta*. Larvae cultured on the sub-optimal plus *D. tertiolecta* diet had the longest stage duration, significantly longer than on any other diet.

### Weight

Data collected from larval weights were analyzed by a Kruskal-Wallis ANOVA comparing weight of larvae among diet treatments (Table 23). No significant differences were found among the treatments.

## ***Hemigrapsus oregonensis* Detritus Experiments**

### Survival

Daily survival for *Hemigrapsus oregonensis* larvae raised on the seven diet treatments tested in the detritus experiment is shown in Figure 7. The *Artemia sp.* fed control sustained 97.5% survival to zoeal stage II (Table 24), while no larvae survived to stage II in the unfed controls or when fed either detrital diet alone. Mean day of death was used to compare the diets that did not sustain any development to zoeal stage II (Table 25). Unfed larvae died earliest with a mean day of death of 4.5 days. Larvae fed only uncolonized detritus lived significantly longer than those that were unfed, and larvae fed microbially-colonized detritus lived significantly longer than those fed uncolonized detritus. Therefore, it is apparent larvae are consuming both detrital diets and deriving some nutritional benefit from them.

An analysis of percent to zoeal stage II is shown in Table 24. The sub-optimal *Artemia sp.* diet sustained 2.5% survival to stage II, significantly lower than the *Artemia*

Table 23. Day 1 zoéal stage II weights (mg) for *Hemigrapsus oregonensis* larvae fed 4 diets in Experiments A & B. The sub-optimal diet consisted of larvae starved for 3 days and fed *Artemia sp* every day after. Results of a Kruskal-Wallis ANOVA ( $P>0.05$ ) indicated no significant differences among samples. The sample sizes are represented by n.

Diet Treatment	Larval Weight (mg)	n	Standard Error
<i>P. micans</i> Sub-optimal	0.048	9	0.027
<i>Artemia</i>	0.027	10	0.003
<i>N. milaris</i> Sub-optimal	0.007	10	0.001
<i>D. tertiolecta</i> Sub-optimal	0.001	10	0.002

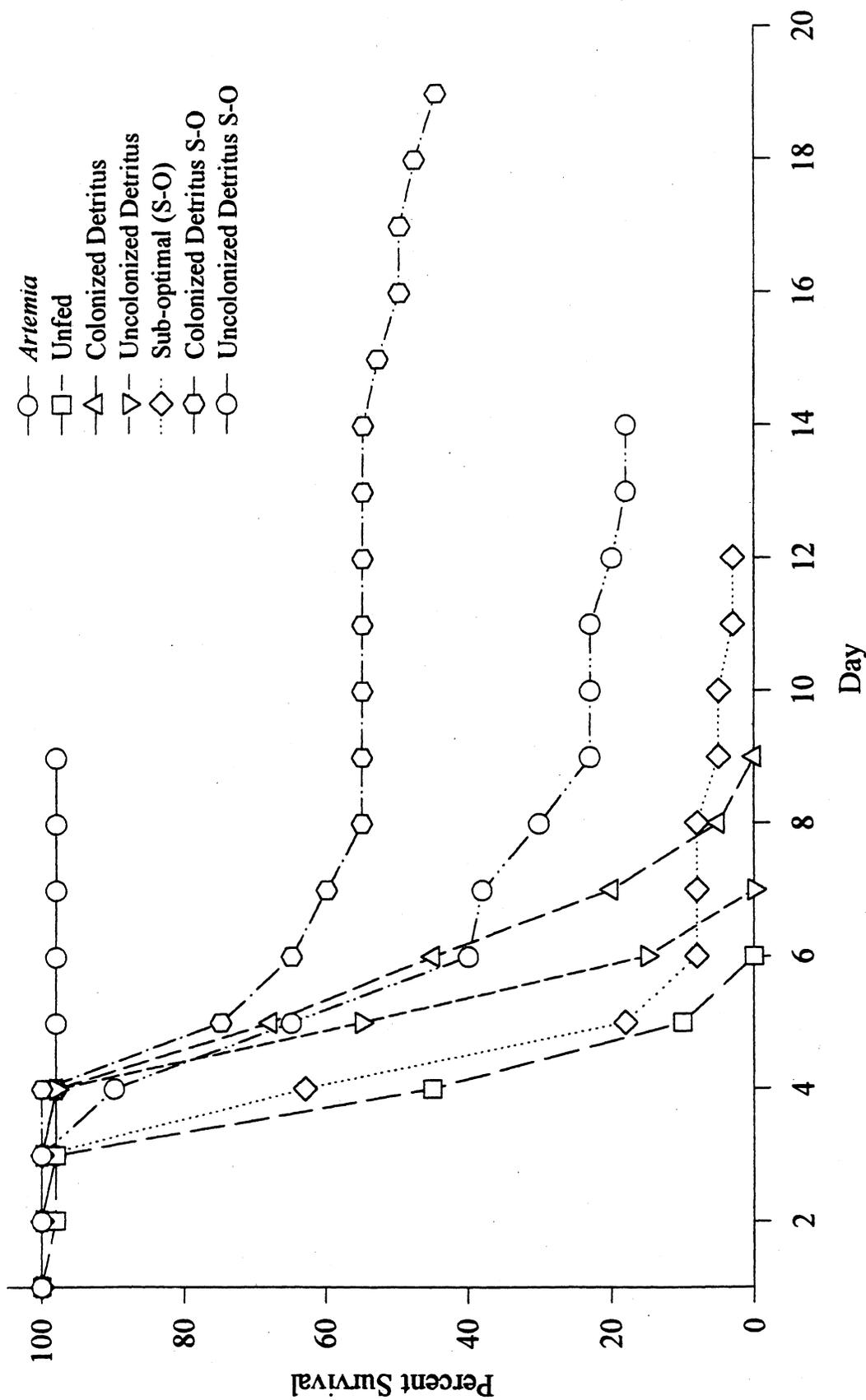


Figure 7. Daily percent survival for *Hemigrapsus oregonensis* larvae fed the indicated diets from the detritus experiment.

Table 24. Percent survival to zoeal stage II for *Hemigrapsus oregonensis* larvae fed 4 diets in the Detritus Experiment. The sub-optimal (S-O) diet consisted of larvae starved for the first 3 days and fed *Artemia sp* every day after. Results of a Kruskal-Wallis ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Comparison of Mean Ranks ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Percent Survival	n	Standard Error	Comparison of Mean Ranks
<i>Artemia</i>	97.5	4	2.5	a
Colonized Detritus S-O	45.0	4	18.5	a b
Uncolonized Detritus S-O	17.5	4	7.5	a b
Sub-optimal (S-O)	2.5	4	2.5	b

Table 25. Mean day of death for *Hemigrapsus oregonensis* larvae on the three diet treatments in the Detritus Experiment that did not sustain development to zoeal stage II. Results of ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Mean Day of Death	n	Standard Error	Tukeys HSD
Colonized Detritus	6.4	40	0.19	a
Uncolonized Detritus	5.7	40	0.12	b
Unfed	4.5	40	0.12	c

*sp.* diet alone. However, when the *Artemia sp.* sub-optimal diet was supplemented as described in the methods with detritus colonized with microbial films or detritus that was relatively uncolonized, survival increased to 45% and 17.5% respectively. The comparison of mean rank was not able to clearly distinguish among the treatments. However, it seems reasonable to conclude that the addition of detritus to the sub-optimal diet increased survival.

### Stage Duration

The mean day of molt (stage duration) was calculated for diets that supported larvae to the second instar (Table 26). Larvae fed the *Artemia sp.* control diet had significantly faster development rate than larvae fed other diets, with larvae fed the sub-optimal diet treatment taking almost twice as long to molt to zoeal stage II. Larvae fed the sub-optimal diet plus microbially-colonized detritus and uncolonized detritus developed at the same rate.

### Weight

Larvae fed the *Artemia sp.* control diet had the highest mean day 1, stage II weights among diet treatments sustaining development to the first molt (Table 27). Larvae fed a sub-optimal diet plus microbially colonized detritus were significantly lower in weight than the *Artemia sp.* control diet, although they did show significantly greater weight than did larvae fed a sub-optimal diet plus uncolonized detritus.

Table 26. Mean day of molt to stage II for *Hemigrapsus oregonensis* larvae fed 4 diets in the Detritus Experiment. The sub-optimal (S-O) diet consisted of larvae starved for the first 3 days and fed *Artemia sp* every day after. Results of a Kruskal-Wallis ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Comparison of Mean Ranks ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Mean Day of Molt	n	Standard Error	Comparison of Mean Ranks
<i>Artemia</i>	6.7	39	0.18	a
Uncolonized Detritus S-O	12.0	7	0.38	b
Colonized Detritus S-O	12.4	18	0.44	b
Sub-optimal (S-O)	12.0	1	-	-

Table 27. Day 1 zoeal stage II weights (mg) for *Hemigrapsus oregonensis* larvae fed 3 diets in the Detritus Experiment. The sub-optimal diet consisted of larvae starved for 3 days and fed *Artemia sp* every day after. Results of a ANOVA ( $P < 0.05$ ) indicated significant differences among samples, and results of a Tukey's HSD ( $P = 0.05$ ) are shown. Treatments assigned the same letter are not significantly different from one another. The sample sizes are represented by n.

Diet Treatment	Larval Weight (mg)	n	Standard Error	Tukeys HSD
<i>Artemia</i>	0.019	5	0.0016	a
Colonized Detritus S-O	0.006	5	0.0027	b
Uncolonized Detritus S-O	0.001	5	0.0041	c

## DISCUSSION

### Nutritional Value of Protist Diets to *Cancer magister*

The results for the four experiments (A-D) on the first zoeal stage of *Cancer magister* were generally consistent with one another and indicate that *C. magister* larvae are consuming the three protists and deriving significant nutritional benefit from the two dinoflagellates, *Noctiluca milaris* and *Prorocentrum micans*.

When larvae were fed either of the two dinoflagellates alone, neither was able to sustain development (Figures 2 and 5), but both diets resulted in delayed mortality as compared to unfed controls (Tables 7 and 15). In experiment A, both species caused a delay in mortality, while in experiment D, only *Prorocentrum micans* caused a delay. *Noctiluca milaris* may have not caused a delay in mortality in experiment D because the number of *N. milaris* cells fed to the larvae was greatly reduced compared to the other three experiments (Table 4). These results indicate that consumption of protists is occurring, but that these protist species are not sufficient either in quantitative or qualitative terms to sustain development by themselves. These experiments were not designed to pursue this matter further.

However, the use of sub-optimal diets of *Artemia sp.* nauplii, supplemented by protists, permitted a more thorough evaluation of their dietary contributions. The addition of *Noctiluca milaris* to the sub-optimal diet resulted in survival equal to that obtained on the *Artemia sp.* nauplii control in experiments A, B, and C (Tables 6, 9, and 12). This was not the case in experiment D in which a far more nutritionally stressful sub-optimal diet

was used (Table 16). The number of *N. milaris* cells fed to the larvae in the sub-optimal diet was also greatly reduced compared to experiments A, B, and C. Perhaps some threshold of nutritional status was not provided by this sub-optimal diet and the addition of *N. milaris* in this case was not sufficient to overcome that situation. Nevertheless, it is apparent that larvae are consuming and deriving some level of nutritional benefit from *N. milaris*. This is confirmed by the fact that addition of *N. milaris* to the sub-optimal diet resulted in an acceleration of development in experiments A and B (Tables 8 and 10), with the results equivocal in experiment C, probably due to the comparatively high survival of larvae on the sub-optimal diet in experiment C (Table 13).

The addition of the protist *Prorocentrum micans* to the sub-optimal diet resulted in survival equal to that of the *Artemia sp.* nauplii control in the two experiment in which they were tested (B and C) (Tables 9 and 12). In both cases, addition of *P. micans* to the sub-optimal diet accelerated development over the sub-optimal , but did not equal that of the *Artemia sp.* nauplii control (Tables 10 and 13).

Addition of the green alga *Dunaliella tertiolecta* to the sub-optimal control resulted in a substantial reduction in survival in both experiments B and C (Tables 9 and 12). Such reduction was statistically significant in experiment C in which the sub-optimal control resulted in substantial survival. Thus, it appears that the larvae are consuming *D. tertiolecta*, but that doing so has some deleterious effect. Perhaps, it is, in effect, diluting the value of more nutritious components of the diet by taking up space in the gut. It might also be competing in some way for active uptake sites in the gut. Pursuit of this matter might provide interesting information about the digestive process in crab larvae.

In the two experiment where *Noctiluca milaris* and *Prorocentrum micans* were compared directly (B and C), there was no significant difference in survival or stage duration for larvae fed the two diets (Tables 9, 10, 12, and 13). Thus both seem equally suitable as diet components. However, it is important to remember that *N. milaris* was itself being fed *P. micans*. While the experimental procedures made it unlikely that many *P. micans* cells were present in the *N. milaris* cultures fed to the crab larvae, their positive nutritional elements may have been passed through the *N. milaris* cultures. It would be instructive to repeat the *N. milaris* diet using a different prey species. Perhaps *N. milaris* can serve as a vehicle for assessing specific nutritional requirements of *Cancer magister* larvae by manipulating its biochemical constituency. *Prorocentrum micans* may have contributed indirectly to the *N. milaris* diet by serving as the source of essential fatty acids that are transmitted through *N. milaris*. Sulkin and McKeen (unpublished data) found that larvae of the crab *Cancer oregonensis* raised on a diet of rotifers fed *Dunaliella* were unable to survive to the megalopa stage, while larvae fed the same rotifer that had been raised on *Isochrysis* did survive to the megalopa stage. Volkman et al. (1989) has shown that *Isochrysis* contains high concentrations of  $\omega$ 3 fatty acids, while *Dunaliella* does not.

#### **Nutritional Value of Protist Diets to *Hemigrapsus oregonensis***

Stage I larvae of *Hemigrapsus oregonensis* are sustained on diets of both *Noctiluca milaris* and *Prorocentrum micans* when fed these two diets alone. Indeed, *P. micans*, by itself, sustained survival as well as did the *Artemia sp.* nauplii control, although development was prolonged (Tables 18 and 19). That this diet is able to sustain

development effectively is further illustrated by the fact that it was able to sustain development through the third zoeal stage. *Noctiluca milaris* was also able to sustain substantial development to zoeal stage II although survival was significantly reduced as compared to larvae raised on the *Artemia sp.* control (Table 18). This result is consistent with the hypothesis put forth earlier that *N. milaris* may serve as a vehicle for providing the important nutritional components provided by *P. micans* and that, in this case, there is value in not diluting its effect by including the "middle man" in the form of *N. milaris*.

The relative nutritional hardness of *Hemigrapsus oregonensis* is reflected in its ability to develop through zoeal stage I on a diet consisting solely of *Dunaliella tertiolecta* (Table 18). The nutritional contribution of *D. tertiolecta* is shown further when it is added to the sub-optimal diet, resulting in increased survival (Table 21). This also illustrates a considerable difference between *Cancer magister* and *H. oregonensis*, as do the results above for *Noctiluca milaris* and *Prorocentrum micans* when provided alone. As expected, when any of these protists are added to sub-optimal diets, survival is increased, further attesting to their nutritional contributions (Table 21).

#### **Use of Detritus by *Hemigrapsus oregonensis* larvae**

Results reported here clearly establish that *Hemigrapsus oregonensis* stage I larvae can consume detritus and derive some nutritional benefit from it. Although detritus will not sustain development by itself, larvae consuming it show a delay in mortality, particularly when the detritus has been colonized by microbial films (Figure 7). When combined with a sub-optimal diet, both detrital diets increased survival over the sub-

optimal control, with colonized detritus again the more effective treatment (Table 24). In neither case did survival increase to equal that of the *Artemia sp.* nauplii control and there was no acceleration of development as compared to the sub-optimal control.

This result has two important implications. Firstly, it is clear that *Hemigrapsus* larvae are omnivores and do not require that their diets consist solely of living motile prey. Other studies have shown that members of the grapsid family are able to utilize a wide variety of food items (McConnaugha, 1985; Johnson, 1995; Schuh and Diesel, 1995). Larvae of the grapsid crab *Armases miersii* are omnivorous scavengers (McConnaugha, 1985). *Hemigrapsus oregonensis* larvae showed an increase in weight and a significant delay in mortality when fed diets of detrital particles (Johnson, 1995). Detrital particles were an appropriate dietary source for the development of the larvae of the grapsid crab *Armases miersii* (Schuh and Diesel, 1995). Secondly, the role of detritus in providing carbon and micronutrients to crab larvae is a field that deserves further consideration.

### Comparison of Crab Species

*Hemigrapsus oregonensis* larvae seem better able to utilize protists as a source of nutrition than do *Cancer magister* larvae. *Hemigrapsus oregonensis* larvae were able to sustain development when fed the protist diets alone, while no *Cancer magister* larvae developed to zoeal stage II when fed the protist diets alone. Many brachyuran species differ in their dependence on obtaining specific nutrients via the diet (Sulkin, 1975; Sulkin & Van Heukelem, 1980; Levine & Sulkin, 1984b). Members of the Family Grapsidae have been defined as comparatively resistant to starvation and nutritionally flexible. Anger

(1995b) found that the grapsid crab *Sesarma curacaoense* had greater starvation resistance than most other planktotrophic marine decapod larvae. Anger (1995a) also demonstrated that larvae from the grapsid crab *Armases miersii* were much more independent from a dietary source of nutrition than were most other marine decapod larvae. Larvae of *Sesarma reticulatum*, another grapsid crab, also showed nutritional flexibility as well as lecithotrophic tendencies (Staton & Sulkin, 1991). The results from the current *H. oregonensis* experiments support the theory that the grapsid family is nutritionally flexible.

### Comparison of Diets

The results indicate that protists differ from one another in their nutritional value for brachyuran crab larvae. The dinoflagellate diets consistently supplied better nutrition for larval crabs than did the green alga *Dunaliella tertiolecta*, with *Prorocentrum micans* being of greatest value. The Chlorophyte *D. tertiolecta* was of little or no value to the *Cancer magister* larvae, but did contribute nutritionally to the *Hemigrapsus oregonensis* larvae. This is consistent with results for larvae of the queen conch, *Strombus gigas*, that showed that *D. tertiolecta* was not a suitable food while a diet of *Prorocentrum minimum* was able to support queen conch larval development (Pillsbury, 1985).

Prey organisms must meet criteria related to ease of capture and ingestion. These criteria include relationships between size and shape of the prey item (Sulkin, 1975). The size of prey items available for each larval stage may need to be within a restricted range for a larva to successfully capture and ingest them (Lough, 1974). As a general rule,

organisms can consume food that is roughly ten percent the size of their own bodies (Pomeroy, 1992). Sulkin and Epifanio (1975) found that the nauplii of *Artemia salina* were too large for the first stage zoeae of the crab *Callinectes sapidus*. Many particle feeders appear to be inefficient consumers, allowing undigested plankton to pass through their gut (Pomeroy and Wiebe, 1988). In this study, the smallest prey item, *Dunaliella tertiolecta*, was also the diet that performed the most poorly, while the larger prey organisms such as *Prorocentrum micans* and *Noctiluca milaris* were able to support development, or at least delay mortality. Larvae of the crab *Hyas araneus* could not consume the flagellate *Oxyrrhis sp.* because of its small size (Anger and Nair, 1979). However, when the *H. araneus* larvae were fed a larger ciliate, *Fabraea salina*, it was consumed, and was of some food value to the larvae (Anger and Nair, 1979). Stickney and Perkins (1981) reported that a large cell size increased the suitability of algae as food for the larvae of the Northern shrimp *Pandalus borealis*.

While particle size is important for prey capture, the biochemical composition of a prey item will determine its nutritional quality to larval crabs (Bigford, 1978). Studies have shown that larval crabs require specific dietary constituents that may change as development proceeds (Levine & Sulkin, 1984b; Sulkin & Van Heukelem, 1980). Diets high in long-chain polyunsaturated fatty acids are often needed for successful development (Levine & Sulkin, 1984b). Levine & Sulkin (1984b) reported that 20: 5 $\omega$ 3 and 22: 6 $\omega$ 3 polyunsaturated fatty acids were significant in promoting the development of the crab *Eurypanopeus depressus*. The dinoflagellate *Prorocentrum minimum* has high levels of 20: 5 $\omega$ 3 and 22: 6 $\omega$ 3 PUFA's (Pillsbury, 1985) while *Dunaliella tertiolecta* does not

contain high concentrations of these fatty acids (Volkman et al., 1989). The results of the current experiments are consistent with this analysis. The species of *Prorocentrum* used as a diet supported development of *Cancer magister* and *Hemigrapsus oregonensis* larvae much more effectively than did *D. tertiolecta*. The average stage duration was also abbreviated when larvae were fed diets of *P. micans* compared to larvae fed a diet of *D. tertiolecta*. Pillsbury (1985) speculated that faster growth was possible when polyunsaturated fatty acids are present in the diet because little energy is required for the conversion of linolenic acid to longer-chained fatty acids.

This study identified a qualitative difference in eelgrass detritus that has few microbes associated with it versus eelgrass that has been more heavily colonized by microbes. Although uncolonized eelgrass detritus is of some nutritional value to *Hemigrapsus oregonensis* larvae, the presence of the microbial layer produced better survival. Cummins (1974) referred to the microbial layer on detritus as the “peanut butter on the cracker”. Evidence suggests that there is a nutritional dependence by detrital consumers on the microbial flora associated with detritus rather than the substrate itself (Mackay & Kalff, 1973; Barlocher & Kendrick, 1973). Larvae may benefit nutritionally from the activities of the microbial community either through a biochemical modification of the leaf or from products of microbial metabolism (Lawson et al., 1984). The bacteria may also soften the eelgrass detritus, thus making it more palatable to the larvae.

## Implications for Crab Larval Ecology

These findings have important implications for crab larval ecology. Food availability is an important factor controlling the survival of planktotrophic larvae (Thorson, 1946; Lough, 1974; Sulkin, 1975). Many studies have shown that larval crabs are most susceptible to starvation during the early zoeal stages (Anger et al., 1981; Dawirs, 1984; Staton and Sulkin, 1991) and that food deprivation in these early stages can severely affect their chances of later survival (Anger et al., 1981). Therefore, a predictable and abundant source of food is essential to the larval crab's survival. Because protists are abundant within the marine environment (Lessard, 1991; Sherr and Sherr, 1991), they may provide an important first source of food for many zoeae. *Cancer magister* larvae can consume the heterotrophic protists, *Oxyrrhis marina* and *Strombidinopsis acuminatum*, and showed increased larval weights when fed these diets (Hutchinson, 1994). In a study by Sulkin (1975), the ciliated protist *Paraureonema virginianum* delayed the mortality of the first stage zoeae of the crab *Callinectes sapidus*. The ability to utilize phytoplankton even for a limited period of time can have considerable ecological importance (Harms and Seeger, 1989). Larvae raised in a field enclosure had higher survival than larvae raised in the laboratory on a mono-culture diet (Epifanio et al., 1991). Epifanio et al. (1991) suggested that the diversity of the field-enclosed diet may have led to the higher survival of the field-enclosed larvae. Phytoplankton thus may not only act as an additional food supply when zooplankton concentrations are low, but may also provide a better biochemical diversity for the nutritional requirements of the larvae (Harms and Seeger, 1989).

It has generally been assumed that decapod larvae are predatory, stalking and consuming such prey as copepod or barnacle nauplii (Harms and Seeger, 1989). This assumption is based upon the fact that many of the laboratory diets that were successful were motile animal prey such as *Artemia* nauplii and rotifers (Sulkin, 1975; Sulkin and Epifanio, 1975). There has also been speculation that the larva's large compound eyes and strong swimming ability were used in stalking and capturing prey. However, Paul et al. (1989) reported that copepod nauplii are difficult for zoeae to capture, and copepod abundance in the plankton is often too low to provide adequate nutrition. Paul et al. (1989) suggested that microplankton including dinoflagellates and other protists may play an important role in zoeal survival. Other studies have demonstrated that crab larvae can consume a variety of planktonic organisms, and in some cases detrital-like material (Paul et al., 1989; Levine and Sulkin, 1984a). These studies suggest that crab larvae are not obligate carnivores, but instead omnivores whose nutritional needs could be satisfied by a variety of prey types (Levine & Sulkin, 1984a). The results from experiments reported here support the omnivore model, and clearly show that crab larvae are able to consume not only protistan prey in a range of size and feeding habits, but also detrital material. Despite the general assumption that zooplankton are the primary prey for larvae of most species of crabs, it appears that a protists and perhaps detritus are also important sources of nutrition.

Many crab species appear to time their larval release during periods of high plankton productivity (Knudsen, 1964). The presence of phytoplankton in high concentrations is actually a cue for some invertebrates to initiate spawning (Starr, 1990).

This strategy is beneficial in that it assures ample nutrition for the planktotrophic larvae. However, some crab species, including several members of the Cancrid family, release their larvae during the winter months in Puget Sound, typically a time of low primary productivity (Strathmann, 1987). This circumstance raises the question of whether larvae of these species can and must exploit alternate sources of nutrition. Adult *Cancer magister* inhabit eelgrass beds and often use these areas as spawning grounds (Gunderson et al., 1990). The advantages typically attributed to such spawning habitats are the high biological productivity, warmer temperatures and refuge from predators that they provide to the egg bearing adults (Gunderson et al., 1990). Although all of these reasons are valid, the Dungeness crab may also be utilizing eelgrass beds as spawning grounds because the eelgrass provides an abundant and predictable food source for their larvae in the form of detritus. This alternate model suggests that in some crabs, reproductive cycles do not have to be co-incident with times of high primary production; instead, such species may release their larvae at time of the year when there are high levels of detrital material. In the Puget Sound basin, levels of detrital material are highest during the winter months when many plant and algae species are senescent and winter storms bring these dying plants into suspension. Although this theory was not directly tested in the present experiments, the results of the *Hemigrapsus oregonensis* detrital feeding experiment suggest the possibility that larvae of other brachyuran species are utilizing detrital material as a source of nutrition for first-feeding stages.

### **Implications for Microbial Ecology and Coastal Food Webs**

This study contributes to an assessment of the role of the microbial food web in serving as the base of metazoan food webs. Phagotrophic protozoa have been identified as the major grazers of picoplankton and nanoplankton (Sherr and Sherr, 1991). Protozoa may also be an important link between these microbes and larger metazoans. In general, metazoans cannot directly use the small, abundant planktonic cells as food, so another trophic link must connect the microbial producers to the metazoa (Sherr and Sherr, 1991). If larger metazoans like crab larvae can utilize protists as a source of nutrition, it supports the idea that protists are a possible link between bacteria and metazoa.

Knowledge of protozoan-metazoan interactions and their role in determining the fate of phytoplankton and bacterial production, is essential to understanding energy and material flow in aquatic food webs (Sanders and Wickham, 1993). Like Stoecker and Govoni's (1984) research, the experiments conducted in this thesis show that metazoans can consume and derive nutritional benefit from protists, providing additional evidence for a link between the microbial food web and larger metazoans. In addition, the present study demonstrated the linkages between particulate organic matter and metazoans on the part of larvae of *Hemigrapsus oregonensis* that fed on, and gained nutritional benefit from, detritus and its associated microflora.

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