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The relative effects of diet and irradiance on the growth and survival of post-larval red abalone, *Haliotis rufescens*

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San Jose State University, 1990

THE RELATIVE EFFECTS OF DIET AND
IRRADIANCE ON THE GROWTH AND
SURVIVAL OF POST-LARVAL RED
ABALONE, *HALIOTIS RUFESCENS*

a Thesis

presented to

The Faculty of the Department of Biology

San Jose State University

In Partial Fulfillment

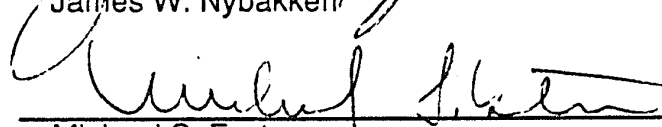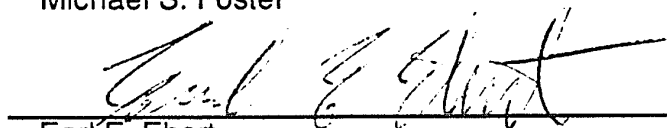of the Requirements for the Degree

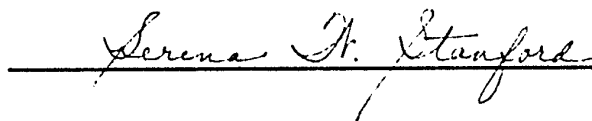Master of Science

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ABSTRACT

THE RELATIVE EFFECTS OF DIET AND IRRADIANCE ON THE GROWTH AND SURVIVAL OF POST-LARVAL RED ABALONE, *HALIOTIS RUFESCENS*

by Susan C. McBride

A factorial experiment was conducted to determine the effect of unialgal diatom diets and irradiance levels on the growth and survival of post-larval red abalone, *Haliotis rufescens*. Competent larvae were introduced to test containers previously inoculated with unialgal diatom cultures. Three diatoms of similiar size were used, *Navicula zostereti, Nitzschia bilobata*, and *Nitzschia closterium*. Diatoms were supplied to the post-larvae in excess. The irradiance levels were 15 to 25 μE and 50 to 60 μE. The photoperiod was set to simulate natural daylength.

All orthogonal factors and interactions tested resulted in significant differences between treatments. Shell length (growth) and proportional survival were greater in the high irradiance treatment. The rank order of diatoms showing the greatest to the least growth are *N. bilobata, N. zostereti,* and *N. closterium*. Significant interactive effects between main factors warned of inconsistent relationships between these factors.

Acknowledgements

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Introduction

Growth and survival of post-larval *Haliotis rufescens* are potentially influenced by behavior, development, and diet. Entry into the post-larval life stage is complicated by a rapid and dramatic behavioral metamorphosis from a swimming larva to a benthic, creeping post-larva. Complex interactions of the physical and chemical characteristics of the substrate regulate this behavioral metamorphosis (Morse, 1984; Hadfield, 1984). The post-larval period begins at metamorphosis with the secretion of the adult shell form. It is well established that many marine invertebrate larvae complete metamorphosis without feeding (Crisp, 1974). However, recent research has shown that lecithotrophic "non-feeding" larvae of *Haliotis rufescens* uptake amino acids in seawater (Jaeckle and Manahan, 1989a). These "non-feeding" larvae cannot feed on particulate matter but may acquire energy from dissolved organic material in seawater (Jaeckle and Manahan, 1989b). Larval rearing of *Haliotis* spp. is usually conducted in one micron, ultra-violet irradiated seawater. This highly treated seawater is known to contain significantly less dissolved organic material than unfiltered seawater (Manahan and Stephens, 1983). Thus at the time of settlement the amount of yolk material required for early post-larval development may be depleted due to the larval rearing conditions. This lower energy store may increase the importance of the first particulate diet available to the post-larval abalone. When the larvae have settled and metamorphosed into benthic, crawling post-larvae, *H. rufescens* larvae begin feeding (Hooker and Morse, 1985). Concurrent with the first, benthic, particulate feeding, the complete physical development of the internal anatomy continues until the formation of the first respiratory pore approximately two months later. Completion of this pore indicates the end of the post-larval life stage (Crofts,

1937; Leighton, 1972). Exact nutritional requirements during the post-larval period are generally unknown.

When the digestive tract of *H. rufescens*, two to six days post-metamorphosis, were examined, diatoms of the Order Pennales were found (Norman-Boudreau et al, 1986). All diatoms were less than 10 μm in width. The largest, *Synedra* sp., was 30 μm in length and 5 μm in width. Post-larval abalone in most studies are commonly fed benthic diatoms that adventitiously settle on surfaces of rearing containers. These mixed populations of benthic diatoms have been successfully used to settle and rear post-larval *Haliotis* spp. in California, South Africa and Japan (Ebert and Houk, 1984; Genade et al, 1986; Oba, 1964; Seki, 1980). In France and Mexico a green alga , *Tetraselmis suecia,* was used in addition to benthic diatoms for post-larval forage (Flassch and Woitellier, 1977; Searcy-Bernal et al, 1989).

Typical diatom genera used in these studies are *Amphora, Cocconeis, Grammotophora, Navicula , Nitzschia* and *Synedra.* Typical survival of post-larval abalone until two months ranges from one to twelve percent (Ebert and Houk, 1984). Their growth is slow but more or less continuous (Leighton, 1974). Some of these differences may be attributed to the specific diatoms available to the young abalone. In this study, three benthic, pennate diatom cultures were developed to examine their effects on post-larval *H. rufescens* growth and survival.

Most researchers agree the initial diet is important in determining survival and growth in post-larval Haliotids (Ebert and Houk, 1984; Leighton, 1977; Kan-ho,1975). Paradoxically, the light required to culture sufficient diatoms in post-larval abalone rearing tanks may inhibit their growth. Feeding behavior in juvenile abalone is suppressed by high irradiances. Because of this, growth

rates are enhanced by subdued light or total darkness (Shibui, 1971; Uki, 1981). The effect of light irradiance on post-larval abalone growth and survival has not been directly addressed. The influence of light irradiance in the range of 60 to 80 μE was noticed to coincide with the highest growth and survival rates of *H. tuberculata* during the first fifteen days of the post-larval period (Flassch and Woitellier, 1977). To determine the influence of diatom species and irradiance on post-larval *H. rufescens*, the effects of three diatom diets and two irradiance levels on their growth and survival were examined.

Materials and Methods

This work was done at the California Department of Fish and Game Marine Resources Laboratory located 20 kilometers south of the Monterey Peninsula (N. lat. 36°26', W. long. 121°55'). The laboratory is continuously supplied with seawater pumped directly from the ocean (Ebert et al, 1974). This work was done over a ten week period from June to August 1989. Seawater temperature was maintained at 12 to 15°C and the salinity ranged from 33 to 34 ppt.

Changes in growth (shell length) were determined relative to three orthogonal factors, diet (D_i), light (L_j), and time (T_k) and one nested factor, containers, [$C(DLT)_{l(ijk)}$]. Groups of post-larvae were kept at each of the six combinations of diet and light. Three replicate containers of each treatment were destructivley sampled and preserved every two weeks .

The linear ANOVA model for this design is: $x_{ijklm} = \mu + D_i + L_j + DL_{ij} + T_k + DT_{ik} + LT_{jk} + DLT_{ijk} + C(DLT)_{l(ijk)} + e_{m(ijkl)}$ where μ is the grand mean and e is the error term. The estimation of mean square values for each source of variation (Underwood, 1981) is shown in Appendix 1.

The proportional abalone survival data were analyzed by a Three Way Model 1 ANOVA (Sokal and Rohlf, 1981). For both the abalone shell length data and the proportional abalone survival data, Newman-Kuels test (Winer, 1971) was used to find significant differences between sample means. The test for importance (Weldon and Slauson, 1986; Sokal and Rohlf,1981) was used to determine the relative contribution of each factor or interaction to the total variation. The test for importance relies on the fact that the explained sums of squares must be smaller than the total sums of squares. A measure of

importance is the ratio of the factor sums of squares over the total sums of squares. The importance of uncontrolled factors is represented by the error sums of squares over the total sum of squares.

A total of 162 test containers were randomly interspersed on the experimental water table, 24 for each of the six diet-light combinations and 18 as diatom controls (Figure 1). At two week intervals, post-larvae from three replicates of each treatment were collected on a 90 μm sieve. For an estimation of growth, the first five, live abalone with undamaged shell edges were measured to the nearest 0.005 mm with an ocular micrometer at 40X. All live and dead animals were counted and the sample preserved.

The shell lengths of the post-larval red abalone from two miscellaneous spawnings conducted at the Marine Resources Laboratory during 1988 were used in qualitative comparison to the data collected during this experiment. Growth rates were calculated from both sets of data.

Three aliquots of a diatom sample from each test container were counted on a Brite Line Improved Neubauer hemacytometer (Stein, 1973). A diatom sample of known volume was also collected on Millapore AA, 0.40 μm pore filter and analyzed for chlorophyll a content (Parsons et al, 1984). Chlorophyll a (chl a) content was calculated according to the equations of Humphrey (1979). After sampling, the test containers were replaced to their original position to maintain uniform conditions for the duration of the experiment.

Diatom control containers contained no abalone. They were inoculated with the same diatom cell density as the abalone treatment containers, but at each two week interval all diatoms were removed from the control containers and sampled as described above for cell density and chl a content. These data

were used qualitatively to assess the amount of each diatom diet available to the post-larvae in the test containers. In the abalone test containers, new diatom inocula were added every two weeks.

The diets supplied to the post-larval abalone were unialgal but not axenic cultures of Nitzschia bilobata (N. bilobata), Nitzschia closterium (N. closterium), and Navicula zostereti (N. zostereti). These three common species of sessile, benthic diatoms were isolated by capillary pipette (Stein, 1973) and grown in sterilized seawater (Keller et al, 1988). The established cultures were transferred to 11 liter polyethylene buckets with flowing seawater as media. The diatoms readily attached and grew on the sides of the buckets and test containers. The incoming seawater to both the test containers and the diatom cultures was treated by 1 μm cartridge filtration and UV irradiation.

The lighting system was two General Electric Chroma 50© flourescent bulbs placed 20 cm above the experimental water table. The spectra of light emitted from the Chroma 50 bulbs simulated natural light conditions penetrating coastal waters at 0 - 10 m depth (Jerlov, 1976). Light measurements were made at the water surface of the test containers every two weeks with a Licor LI-185 integrating quantum / radiometer / photometer. (Irradiance in the photosynthetically active region , 400 to 700nm, was measured in microeinsteins, photons $m^{-2}s^{-1}$).

The light treatment was segregated. A piece of plastic window screen suspended from the wooden frame supports at either end of the bulbs shaded the two far ends of the water table. This structure provided two light treatments, a central high irradiance of 50-60 μE and two low irradiance areas of 15-25 μE . The irradiance available to ten species of benthic, estuarine diatoms, including

Nitzschia spp. and *Navicula* spp., was in the range of 20 to 85 μE (Admiraal, 1977). These levels of irradiance produced light saturated growth in all ten species. The lights were controlled with an electric timer to simulate natural day length.

A manifold with an individual line and control valve for each test container supplied each test container with 33-37 ml/min of running seawater (Figure 1). The turnover time per container of 35 to 45 minutes was determined by dye tests.

The test containers were one liter polyethylene freezer storage boxes, 10.5 X 10.5 X 13.0 cm. The total surface area available to the abalone and diatoms was 580 cm^2. A 0.5 X 1.5 cm hole for drainage was made 0.5 cm below the rim and covered with 180 μm Nitex mesh. The volume of seawater in each test container was 920 ml. The containers were uncovered for the duration of the experiment.

The manifold tubing and valves were also made of polyethylene. All test containers and manifold supplies were washed in Alconox©, rinsed in freshwater, left submerged in running filtered seawater for one month and then washed again before use in the experiment.

Abalone larvae were obtained in May 1989 from an induced, synchronous, mass spawning of eight adult *H. rufescens*. The adult abalone were artificially conditioned first and second generation laboratory reared broodstock. Larvae were reared according to Ebert and Houk (1984). Competent, 0.262 mm, larvae were collected on sieves, counted, measured and distributed at 200-300 per test container. In several studies, this larval settling density had the greatest survival for the post-larval period (Leighton, 1985;

Ebert and Houk, 1984; Inoue, 1976; Shibui, 1972). Each test container and diatom control container were inoculated with one of the unialgal cultures 24 hours prior to introduction of abalone and every two weeks during the experiment (Table 1).

After completion of the experiment, one sample of each diatom species was tested for carbon, hydrogen and nitrogen (CHN analysis) using a Controlled Equipment 240XA elemental analyzer.

Results

For the remainder of this paper, time 1, 2, 3, 4, and 5 are synonomous with ages 3, 5, 7, 9, and 11 weeks respectively.

Abalone Shell Length

Results of Chi Square Goodness of Fit Test for Normality (Zar, 1984) of all shell length data showed that the size frequency distributions of the observed abalone shell lengths did not differ significantly from the expected frequencies (X^2 = 17.131, df=9, p=0.0467). Cochran's Test for Homogeneity of Variance (Winer, 1971) showed no significant differences between sample variances (C=0.7457, k=3, df=4, p=0.05). The results of these tests indicated that the use of parametric statistics on the growth data were appropriate.

A qualitative comparison of three samples from the total larval population used at the start of the experiment indicated there were no significant differences between the initial shell lengths (Table 2).

Manipulation of diet and light treatments over the 10 week period resulted in significant differences in abalone shell length for all factors and interactions tested (Table 3). The significance of the main effects and the interaction terms allows the analysis in the presence of the significant container effect (Underwood, 1981). However, the significant second order interaction warns that effects of one factor are not constant over levels of other factors (Scheffe, 1959; Underwood, 1981). For this reason each factor was tested for significant differences over each level of the other two factors.

There were no significant interactions between light and time at times 1, 2, and 3 (Figure 2a and b). The light and time interaction showed significant differences between means at times 4 and 5 for high irradiance. Abalone on

the *N. bilobata* diet had significantly greater shell length than those fed *N. zostereti* and *N. closterium.* In the low light treatment, *N. bilobata* exhibited greater growth than *N. zostereti* and *N. closterium* at times 3 and 4. At time 5, *N. bilobata* and *N. zostereti* had greater growth than *N. closterium* (Figure 2c, d and e). The significant differences are represented by the distance between vertically arranged points on all graphs. The interactions are represented by non-parallel lines (Winer, 1971). This interaction contributed 0.05 to the total explainable variation.

Diet and time interacted significantly at time 5. The abalone on the *N. bilobata* diet, high irradiance environment had significantly greater growth over all other treatments except *N. zostereti* high irradiance treatment. This interaction contributed 0.02 of the total variation (Figure 3a and b).

Neuman-Kuels test for differences between treatment means indicated significant differences between the diet and light interaction for times 3, 4 and 5 for *N. zostereti* and *N. bilobata* and for time 3 and 4 for *N. closterium.* This interaction is shown geometrically in the non-parallel lines of each diet at each irradiance. In all cases, growth was greater in the high irradiance treatment (Figure 4a, b and c). The proportion of total variance explained by the diet and light interaction is 0.01.

The main factors of diet, light, and time contributed 0.03, 0.06 and 0.65, respectively, of the total explainable variation in this analysis. When the main effects are averaged over the levels of the other two factors to test for effects of the third factor, the conclusion is a significant difference but no constant relationship can be inferred due to the significance of the first and second order interactions (Scheffe, 1959; Underwood, 1981).

Abalone Growth Rate

Sample means were used to calculate growth rates (μm/day) of the experimental post-larvae from all diet and irradiance treatments (Figure 5, Table 4). Abalone in all treatments decreased in growth rate between 3 and 5 weeks of age and increased in growth rate until 7 weeks of age. The decrease in growth rate for all treatments between 3 and 5 weeks may reflect developmental changes such as the protrusion of epipodial tentacles on both sides of the foot, enlargement of the digestive gland, and elongation of the intestine (Crofts, 1937; Oba, 1964; Koike, 1978). From 7 weeks until 11 weeks of age, the growth rate was variable between treatments. The post-larvae in the N. bilobata, high irradiance treatment had the greatest growth rate throughout the experiment.

The growth rate of the post-larvae from the N. bilobata, high irradiance treatment were similiar to those of the abalone from the two miscellaneous spawnings of 1988. It is interesting to note that abalone fed a unialgal diet in the test containers grew at a rate equivalent to abalone reared in a 260 liter tank with a mixed diatom diet (Figure 6). The shell lengths of these three groups of abalone were also similar (Figure 7).

Proportional Survival

Proportional survival data were collected for eight weeks as the number of live abalone divided by the total number of live and dead abalone per test container. The proportional survival data were transformed to degrees by arcsin $\sqrt{x}$ to remove heterogeneity of variance (Underwood, 1981). All following statistical analyses were performed on the transformed data. Chi Square Goodness of Fit for all observed survival data showed no significant differences

(X^2=13.088, df=7, p=0.07). There were no significant differences between sample variances at C=0.8643, df=2, k=4, p=0.05 (Cochran's Test, Winer, 1971).

Results of the 3 Way ANOVA showed significant second order interactions, requiring a separate analysis of each contributing first order interaction (Table 5). The light and time interaction showed significant differences between means at time 1 for *N. bilobata* and *N. closterium* in the high irradiance. At time 2, survival was greatest on *N. bilobata* for both high and low irradiance. There were no significant differences between sample means at time 3. Abalone survival was greater on *N. bilobata* than *N. closterium* and *N. zostereti* at the low irradiance for time 4. Survival of abalone fed *N. bilobata* was greater than survival on *N. closterium* in the high irradiance at time 4 (Figure 8a, b, c and d). The proportion of the variance explained by this interaction is 0.08.

The diet and light interaction for *N. zostereti* showed greater abalone survival in high irradiance at times 2 and 4. Abalone survival was greater on *N. bilobata* at time 2 and showed no significant differences at all other times (Figure 9a). Abalone fed *N. closterium* had greater survival at low irradiance, time 1 and no significant differences at all other times (Figure 9b). At time 3, there was a greater proportional survival of post-larval *H. rufescens* at the low irradiance (Figure 9c). This interaction contributed 0.02 to the total explainable variance and was not significant in the 3 Way ANOVA.

The diet and time interaction resulted in significant differences in mean proportional survival between *N. bilobata* and *N. closterium* at both high and low irradiances for times 1, 2, and 4. At times 2 and 4, *N. zostereti* and *N.*

closterium also had significant differences between their sample means at both high and low irradiances. At time 3, there were no significant differences between any sample means (Figure 10a and b). This interaction contributed 0.14 of the total variation and was not significant in the 3 Way Anova.

The main factors of diet, light, and time contributed 0.33, 0.02, and 0.08, respectively, to the total variation. Diet and time were both significant at the level tested. Light was not found significant in this analysis. Conclusions about light interactions are significant because the diet and time main effects are non-zero. Their contributions are sufficient in the interactions Sums of Squares containing light as a factor to show significant effects (Scheffe, 1959; Winer, 1971). The contributions to the total variance by the non-significant factors and interactions of the ANOVA are the lowest values of all sources.

Diatoms

Qualitative examination of cell densities in the control and abalone grazed containers (Table 6) relative to the inoculum (Table 1) indicates diatoms maintained their populations or reproduced in all but two cases. *N. closterium* decreased in cell density in the control, low irradiance treatment at time 2. *N. bilobata* decreased in cell density with abalone at low light, time 1. Neither of these exceptions corresponded with a significant multiple comparison in the abalone shell length or proportional survival analyses.

Chl *a* data were collected at times 1, 4, and 5. Chl *a* content increased with cell density over time (Table 7). The *N. closterium* sample at time 5, low irradiance with abalone may be a measurement error.

somewhat higher than for the other two species, but nitrogen values are similar. The C/N ratios of 5 to 6 indicate the diatoms were nutrient saturated (Jahnke, 1989; Table 8).

Discussion

   The main objective of this study was to determine the effects of diet and irradiance on post-larval *H. rufescens* growth and survival. The results indicate *H. rufescens* growth and survival are higher on *N. bilobata* than *N. zostereti* or *N. closterium*. Even though growth was not significantly different until time 3, (age 5 weeks), qualitatively, growth was greater on *N. bilobata* regardless of age. Survival was always greater on *N. bilobata* at high irradiance.

   Slow growing abalone have been shown to remain so compared to their siblings (Leighton, 1974). This is usually attributed to genetics but in this case it may be partially from the nutritional history of the abalone. If the post-larvae did not receive sufficient energy from the diet offered, the developmental process would slow or halt and the abalone die or become stunted. From the data, it appears that stunting and a higher mortality occurred on the *N. closterium* diet.

   Time was the most pronounced factor affecting post-larval abalone growth in this study. Differential growth on the three diets and the increase in growth rate of the abalone fed *N. bilobata* in the last two weeks of the study were responsible for the significance of the time factor. *N. closterium* fed abalone remained slow growing. The abalone fed *N. zostereti* were intermediate and somewhat more erratic in their growth, appearing to approach growth rates of the abalone fed *N. bilobata* at the end of the experiment. The abalone fed *N. bilobata* were consistently faster growing. Due to the faster growth rate, the abalone fed *N. bilobata* reached the end of the post-larval life stage and began the accelerated growth of juvenile abalone during the last two

weeks of the experiment (Clavier and Richard, 1986; Koike, 1978; Hayashi, 1982) . This increased growth rate added to the significance of time as a factor affecting growth of the abalone in this study.

Post-larval *H. rufescens* are often observed feeding within 20 hours of settlement (E. Ebert, pers. comm.). In two other studies, feeding began from 2 to 6 days post-settlement (Morse, 1981; Norman-Boudreau et al, 1986). At settlement, the mouth diameter is 10 μm and by 48 hours it is 30 μm (Hahn, 1989). The diatoms offered in this study were 5 to 8 μm in width and 20 to 35 μm in length. This is within the size range of diatoms found in the digestive tract of *H. rufescens* two days post-settlement (Norman-Boudreau et al, 1986). When the abalone feed during the first two days post-settlement, their nutritional needs may be met by bacteria associated with the diatoms. Post-larval *H. ruber* are known to feed on the bacteria associated with coralline algal crusts. The importance of the bacteria to the post-larvae is unknown (Garland et al, 1985).

The first 2 to 4 days post-settlement may also be influenced by parental yolk allotment (Leighton, 1972). The high fecundity of these animals lends some support to this idea. At the time of first reproductive maturity, female *H. rufescens* are 40 mm in length and release approximately one million eggs (Ebert and Houk, 1984). A twelve to thirteen cm female *H. rufescens* may release up to 11 million eggs (Hahn, 1989). The amount of yolk material in each egg may not be equal. Post-larvae with energy reserves remaining from the yolk may have increased survival and/or growth potential for the first days following settlement.

The first 100 hours of post-larval growth was enhanced by providing specific physiological requirements (Morse,1981). Efficient induction of

settlement and metamorphosis and the synchronous start of post-larval growth was achieved by providing competent *H. rufescens* larvae with a specific amino acid, g-aminobutyric acid. The subsequent growth was accelerated during this 4 day experiment with the addition of mammalian growth hormones and insulin resulting in an average growth rate of 65 μm/day. In the present study, diatom diet had a significant effect on growth and survival of post-larval *H. rufescens* without the addition of exogenous compounds although such high growth rates were not achieved (Table 4).

The feeding organ of the post-larvae, the radula, consists of 8 to 10 rows of broom like marginal teeth which sweep the substrate to remove food items (Dinamani and McCrae, 1986). Cell size, morphology and cell wall thickness may mechanically affect the ability of the radula to remove particles from the substrate. The apical extremities of *N. closterium* may also have presented a mechanical problem and affected their overall digestibility. Relative cell wall thickness of the three diatom species used in this study are unknown. Thicker cell walls may reduce the digestibility of cell contents by digestive enzymes present in the post-larval stomach (Crofts, 1937).

If the abalone were feeding at equal rates on all diets, the differential growth and survival may have reflected a lag time in development of digestive enzymes from nutritional deficiencies of *N. closterium* and *N. zostereti*. Specific unialgal diets of diatoms and green algae have been used to increase production of mass cultured larval oysters, clams, and mussels (Manzi et al,1985, Bayne et al, 1987,). These planktonic larve feed after depleting yolk reserves. During this period, the quality of the food, determined by growth of the larval bivalves and the survival of the resulting spat, is greatly influenced by

storage of energy reserves from the food consumed (Helm et al, 1973). There is however a lag phase when yolk material is depleted concurrent with increased morphogenic activity and a lowered growth rate (Whyte et al, 1987; Holland and Spencer, 1971). This response is similiar to the post-larval *H. rufescens* settlement and early growth. When the parental yolk reserves are used during settlement and metamorphosis the following growth is slow probably due to similar delay in metabolic activity and development of digestive processes. This could partially explain the significant differences occurring in shell length at 5 weeks of age for post-larval abalone.

Overall the abalone in this study grew more rapidly in the high irradiance treatment. Diatom cell biochemistry may have contributed to this accelerated abalone growth. In general, when diatoms are placed in a different irradiance environment, the cells respond by increasing the amount of chl *a* per cell or increase the rate of photosynthesis (Beardall and Morris, 1976). Chl *a* analysis suggests the amount of chl *a* per cell did not increase in the low irradiance treatment. However, the photosynthesis rate may have increased in the high irradiance treatment. The abalone in the high irradiance treatment would then benefit from increased oxygen concentration. Inefficient respiration is one factor that may limit post-larval abalone growth (Crofts, 1937).

In the invertebrate phyla, irradiance effects have been most intensively studied in insects. Insect response to light ceases at the lower limit of bud initiation in plants, 1 μE (Lees, 1968). For example the Oriental fruit moth, *Grapholitha molesta*, feeding activity inside apple fruit continues at 3 μE but not at 1 μE. Also as irradiance is progressively reduced, the growth response of larval rice stem borers, *Chilo suppressalis*, ceases to occur. A parallel situation

may have occurred with the abalone in the low irradiance treatment fed *N. closterium*. The growth rate found on the *N. closterium* diet was nearly constant for the duration of the experiment suggesting a stunted growth, possibly in response to low irradiance.

It is generally accepted that the nutritional value of different algal diets is not the same. However, studies on marine phytoplankton cultured in the same experimental conditons have very similar organic chemical composition (Parsons et al, 1961; Chu et al, 1982). Nevertheless, two of the four diatoms studied by Parsons et al (1961) did contain 10% more crude carbohydrate than the other 9 species representing four families. In marine, pennate diatoms this material may be present on the outside of diatom cells as they move over the substrate. Diatoms are known to contain carbohydrate associated with the silica of frustules (Fogg, 1965). This material would be more accessible to the abalone than carbohydrate inside the frustule. It seems reasonable to suggest *N. bilobata* cells produced more of this 'crude' carbohydrate than the other two species. The food value of phytoplankton for the scallop, *Patinopectin yessoensis*, showed a high correlation between carbohydrate content of the cells and larval growth (Whyte et al, 1989).

Two other studies have shown little correlation between the carbohydrate, lipid, and protein content of algae used as food items to cultured bivalves and their nutritional value. Epifanio (1979) reported growth of hard and soft tissue on juvenile American oysters, *Crassostrea virginica*, and hard clams, *Mercenaria mercenaria*, was not correlated with the amount of carbohydrate, lipid, protein or amino acid in the diets. Instead it was related to the presence or absence of a particular algal species in the diet. It is possible

once the biochemical balance of protein, lipid, and carbohydrate has been achieved by the combination of several species of algae, other biochemical components such as trace metals and vitamins are present in adequate amounts to promote growth (Chu et al, 1982). If this is the case, *N. bilobata* most closely matched the virtually unknown nutritional requirements of post-larval abalone and use of this diatom species in conjunction with other diatoms may enhance post-larval abalone growth.

Comparison of mean, post-larval, abalone shell lengths between this study and miscellaneous spawnings at the Marine Resources Laboratory indicate that use of unialgal cultures did not increase growth. For example, mean size of 11 week post-larvae spawned in February and June 1988 were, 1.86 and 1.74 compared to 1.78 for the abalone in the *N. bilobata*, high irradiance environment. However, the use of unialgal cultures at this critical life stage shows a pronounced benefit towards their survival, indicating post-larval abalone need the diatoms to continue their existence. Proportional survival of abalone fed *N. bilobata* was 55-70% while the two spawnings mentioned above had 6% and 10% survival, respectively. In a commercial operation, consistent yield of post-larvae would allow adjustment of the hatchery system to known levels of production and greater efficiency.

When energy is sufficiently converted to allow for growth, time was the most pronounced factor influencing growth of post-larval abalone. The three diets supplied to the post-larvae allowed their continued growth but at different rates. At the completion of the experiment, the abalone fed *N. bilobata* were 20% greater in shell length than abalone fed *N. zostereti* and 25% greater than those fed *N. closterium*. Survival on *N. bilobata* was 52% greater than *N.*

closterium and 47% greater than *N. zostereti*. Growth and survival were overall greater in the high irradiance environment and diet also had a significant effect on these two parameters.

In the biological world, we usually have no knowledge that any one factor will exert its influence independently of all others that could be manipulated. In this experiment two factors which could be controlled (diet and light) and one which could be measured (time) were chosen for comparison of their effect on post-larval red abalone growth and survival. More knowledge of the effect of these factors was gained using the multifactorial design because every trial supplied information on the main factors as well as an estimation of the interactions between them. In a traditional one factor design or if a series of factors are tested singly, no interactive effects can be estimated. A factorial experiment is more informative and possibly more powerful and economical than smaller experiments.

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Table 1. Diatom inocula (cells/cm^2 x 10^3) for test containers. Time 0 is one day before the beginning of the experiment. Time 1 is the first sampling time or two weeks after the start of the experiment.

Time	N. closterium	N. zostereti	N. bilobata
0	72	79	67
1	28	28	33
2	41	135	107
3	68	73	66
4	106	65	73

Table 2. *Haliotis rufescens.* Mean shell length at the beginning of the experiment (x ± SE, n= 5).

	Sample 1	Sample 2	Sample 3
Mean Abalone Shell Length (mm ± SE)	.265 ± .002	.261 ± .002	.261 ± .002

Table 3. *Haliotis rufescens*. ANOVA for Shell Length (* denotes significance at p < .05).

Source	df	Mean Square	F ratio	Importance
Diet (D)	2	.986	30.81*	.033
Light (L)	1	3.656	114.25*	.061
D x L	2	.376	11.75*	.012
Time (T)	4	9.761	305.03*	.652
D x T	8	.166	5.18*	.022
L x T	4	.691	21.59*	.046
D x L x T	8	.201	6.28*	.026
C (DLT)	60	.032	1.68*	.032
error	360	.019		.113

Table 4. *Haliotis rufescens.* Growth rate in μm/day for each diet, irradiance and time. Values are differences between sample (n=3) means.

Age (weeks)	N. closterium		N. zostereti		N. bilobata	
	High	Low	High	Low	High	Low
1-3	11	13	13	13	14	14
3-5	8	5	7	7	11	7
5-7	22	6	22	4	20	13
7-9	11	18	21	19	28	17
9-11	7	17	23	4	35	12

Table 5. *Haliotis rufescens.* ANOVA for Proportional Survival (* denotes significance at (p < .05).

Source	df	Mean Square	F ratio	Importance
Diet (D)	2	2973.755	31.971*	.33
Light (L)	1	366.592	3.941	.02
D x L	2	199.344	2.143	.02
Time (T)	3	512.042	5.505*	.08
D x T	6	164.200	1.765	.05
L x T	3	849.880	9.137*	.14
D x L x T	6	264.800	2.847*	.08
error	48	93.015		.25

Table 6. Mean diatom cell density (cells/cm^2 x 10^3 ± SE). Controls represent diatom growth for each two week sampling period in test containers without abalone. 'Abs' represent diatom growth from inoculum added successivley each two weeks. Times 1, 2, 3, 4, and 5 are shown in the treatment column.

Treatment	N. bilobata	N. closterium	N. zostereti
control-high-1	448±125	459±28	164 ± 23
control-low-1	269±66	204±51	45 ± 20
abs-high-1	128±21	540±105	434 ± 162
abs-low-1	34±12	258±52	95 ± 19
control-high-2	999±145	154±18	105 ± 3
control-low-2	366±213	19±4	79 ± 21
abs-high-2	390±142	267±74	288 ± 88
abs-low-2	268±14	203±128	109 ± 2
control-high-3	433±201	1017±41	no data
control-low-3	258±46	2159±857	no data
abs-high-3	1045±389	666±477	1353 ± 190
abs-low-3	393±97	441±223	1006 ± 24
control-high-4	867±72	1230±50	620 ± 103
control-low-4	431±104	2413±343	95 ± 13
abs-high-4	2356±998	5853±675	3620 ± 450
abs-low-4	465±70	1963±206	2433 ± 733
control-high-5	347±7	901±6	478 ± 3
control-low-5	214±3	706±10	289 ± 5
abs-high-5	3159±289	12600±260	3370 ± 614
abs-low-5	1147±239	5570±230	1210 ± 251

Table 7. Mean Chl a content (µg/ml ± SE). Controls represent diatom growth for each two week sampling period. 'Abs' treatment are diatom growth with successive additions of inoculum. Times 1, 4, and 5 are shown in the treatment column.

Treatment	N. bilobata	N. closterium	N. zostereti
control-high-1	275±.275	389 ± 1.051	155 ± 2.99
control-low-1	195±7.57	23 ± 2.278	26 ± 1.09
abs -high-1	112±.375	37 ± 1.873	60 ± .668
abs -low -1	23±2.01	264 ± 2.59	74 ± 2.23
control-high-4	291±9.03	101 ± 1.725	238 ± 10.23
control-low-4	140±2.37	93 ± 1.845	231 ± 1.059
abs -high- 4	590±2.27	302 ± 2.35	496 ±3.04
abs -low- 4	370±2.66	178 ± 2.83	121 ± 2.82
control-high-5	306±4.33	407 ± .39	312 ± 3.16
control-low-5	230±2.97	301 ±2.28	195 ± .667
abs -high -5	257±1.53	577 ± 5 57	340 ± 17.11
abs -low -5	579±7.51	7 ± 1.864	380 ± 1.93

Table 8. CHN Analysis. Each value represents a mean of three subsamples taken from one sample of each diatom species ± one standard error.

Organism	% C	% H	% N	C/N
N. closterium	9.107 ±.454	3.260 ±.670	1.584 ±.029	5.749
N. zostereti	6.593±.287	1.433±.031	1.223±.041	5.391
N. bilobata	7.984±.120	1.618±.077	1.465 ±.200	5.449
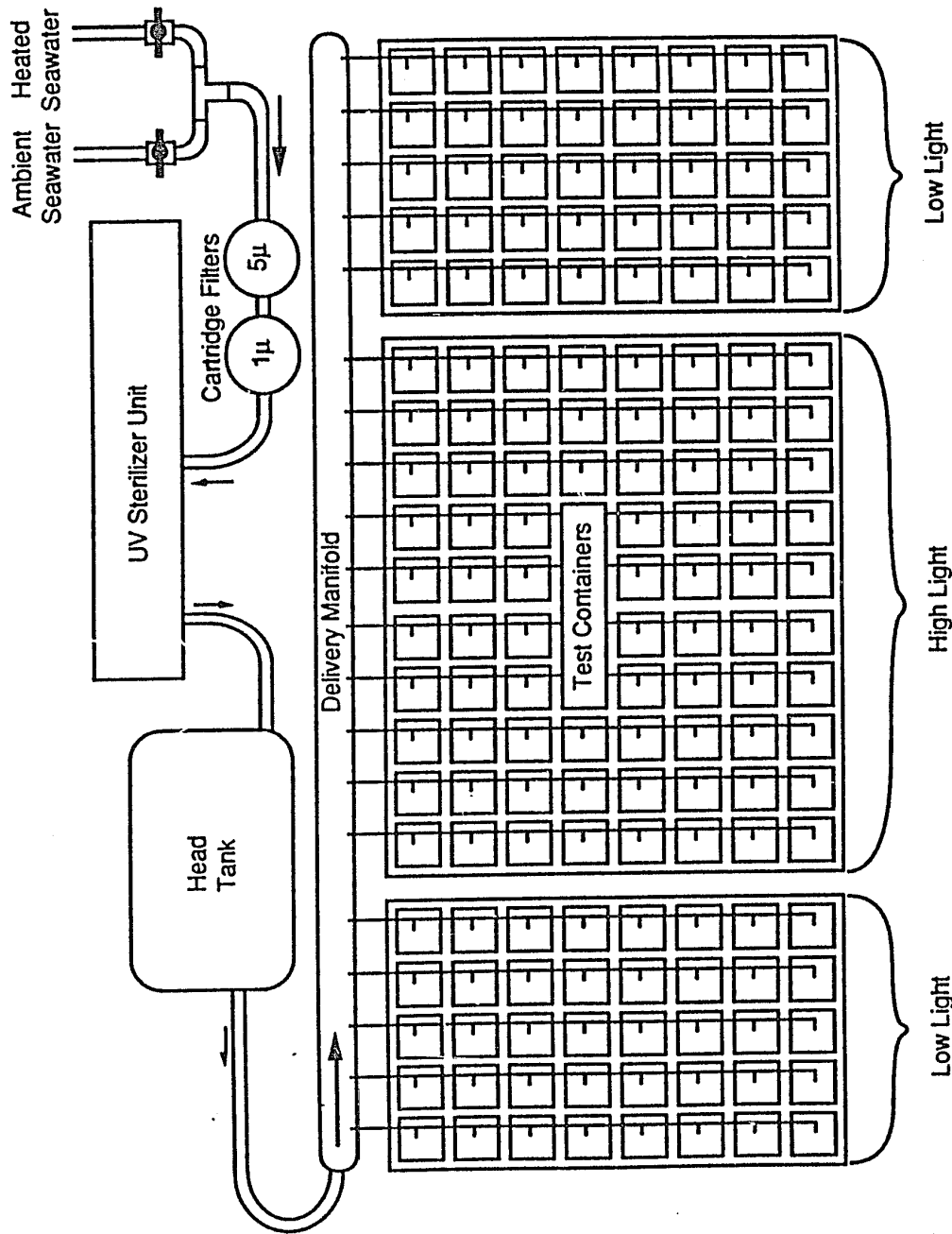
Figure 1. Design of diet and irradiance experiment showing 162 test containers. Three replicate containers from each of 6 treatment combinations (3 diets, 2 light levels) are removed every 2 weeks, sampled for abalone growth and survival and preserved. All diets are randomly interspersed in each light treatment area.
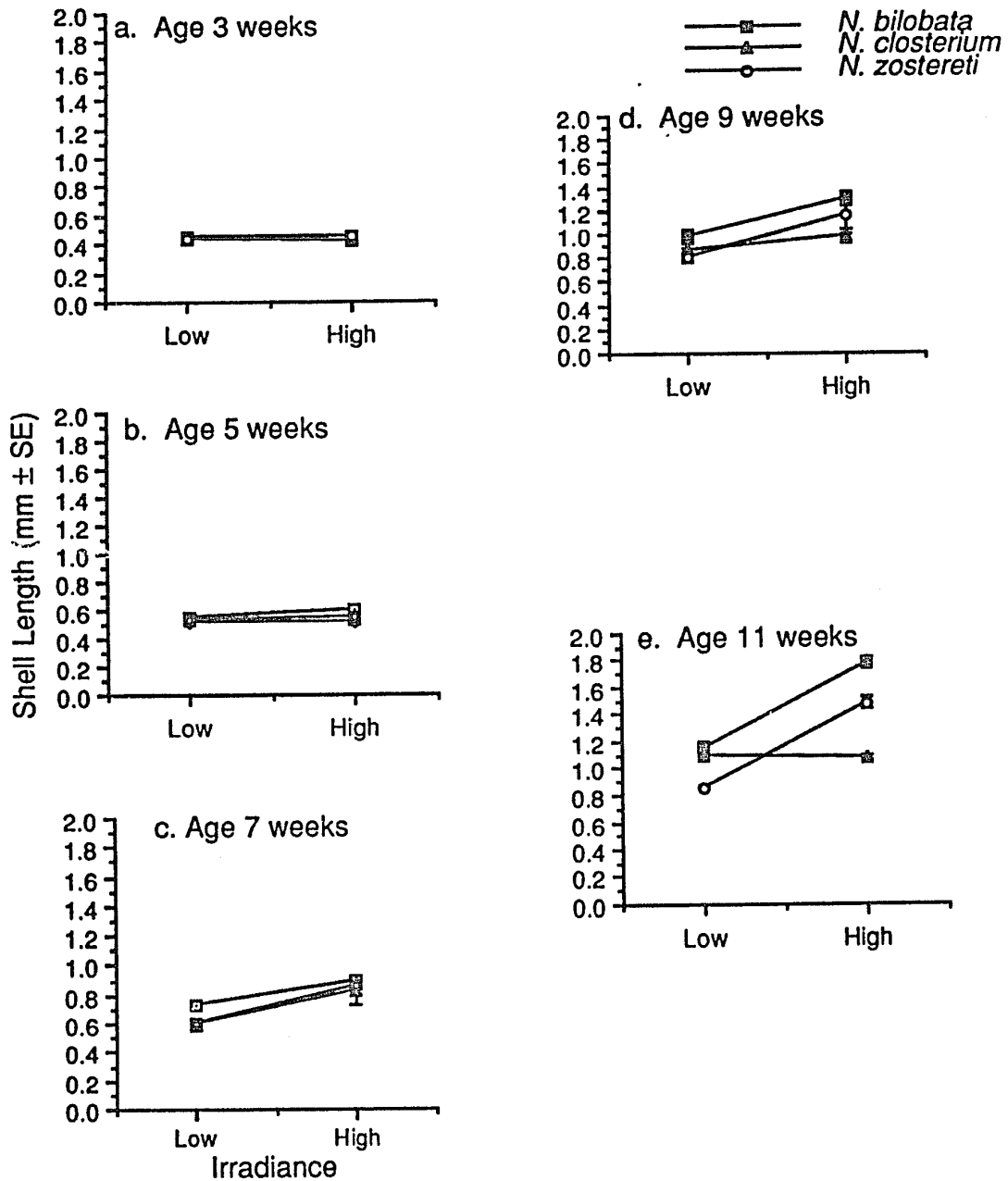
Figure 2. Effect of irradiance and time on mean abalone shell length for all diets at each two week sampling interval ($x \pm$ SE, n=3).
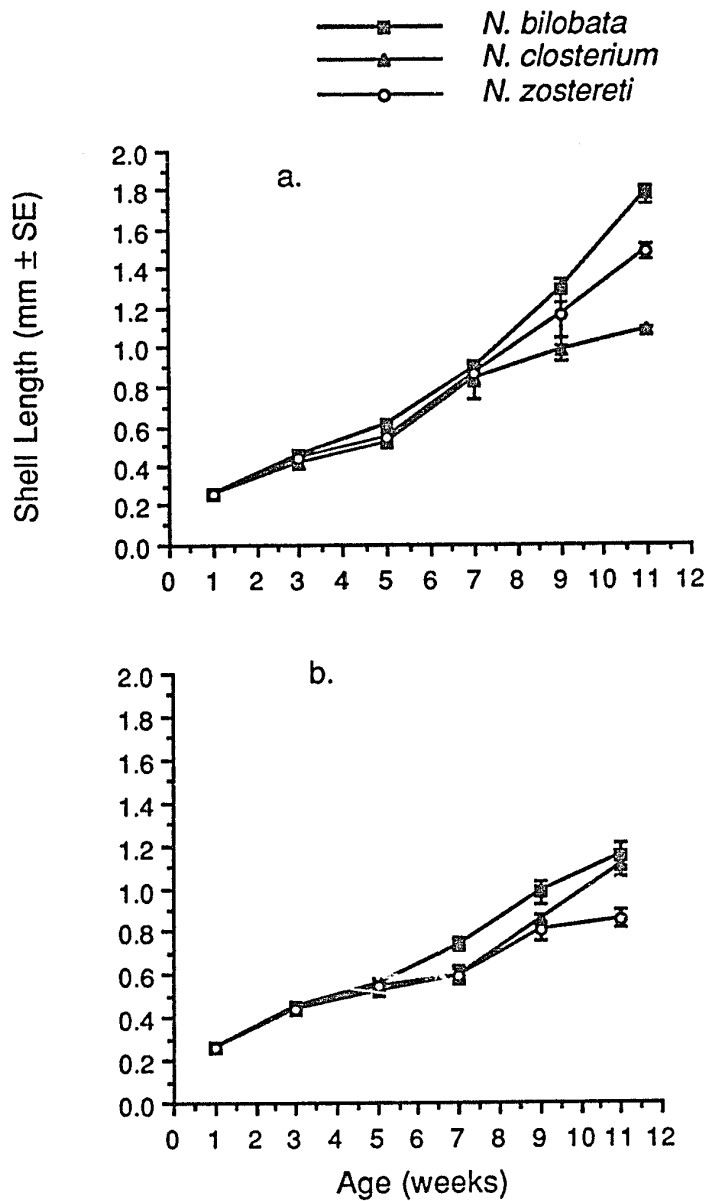
Figure 3. Effect of diet and time on abalone shell length for both light levels, a. high irradiance (50 - 60 µE); b. low irradiance (15 - 25 µE). Data points indicate mean shell length, $x \pm$ SE, n=3.
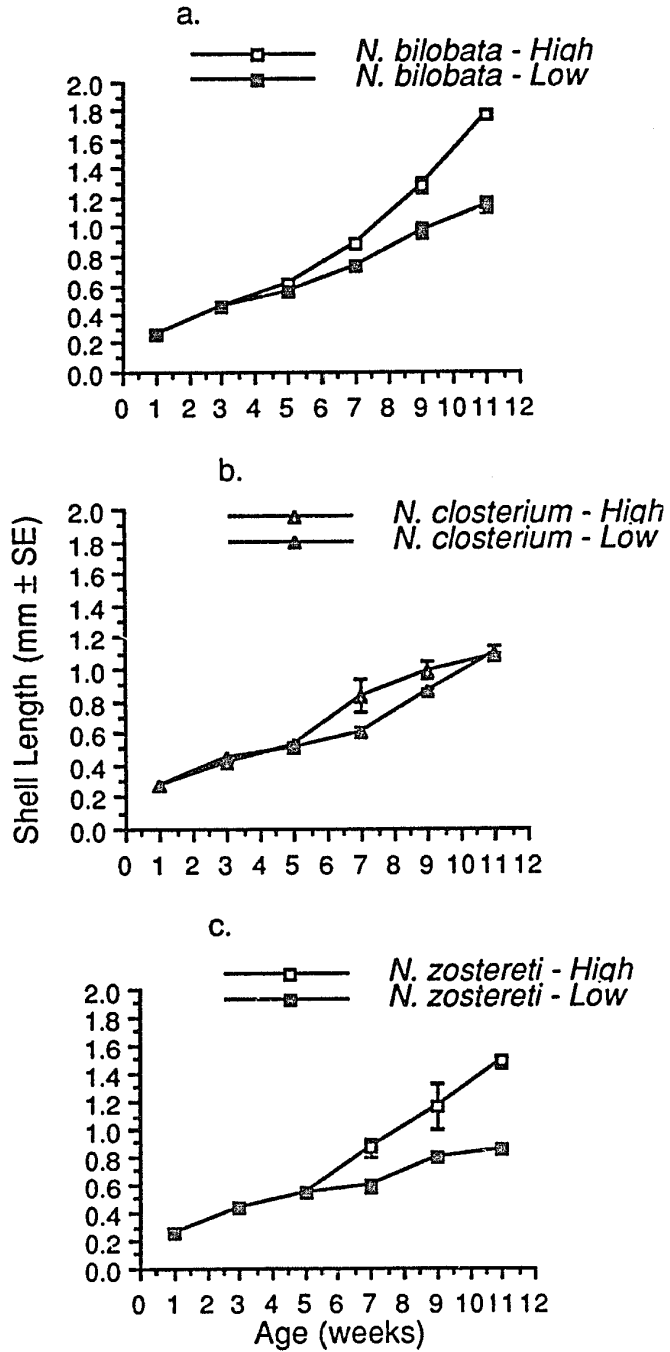
Figure 4.   Effect of individual diet and irradiance level on mean abalone shell length for the ten week experiment, $x \pm$ SE, n=3.
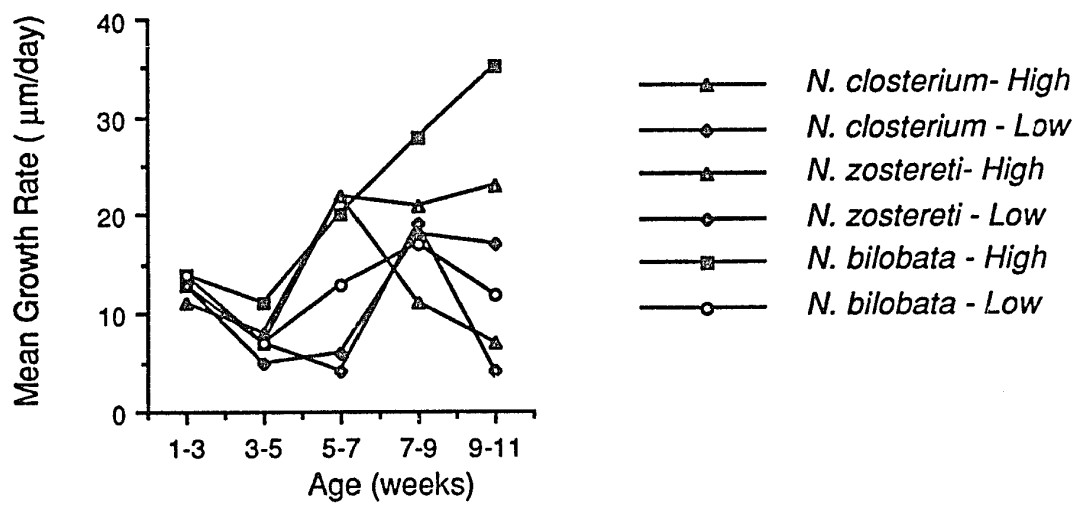
Figure 5. Mean abalone growth rates for all diet and light treatments calculated from sample means (n=3).
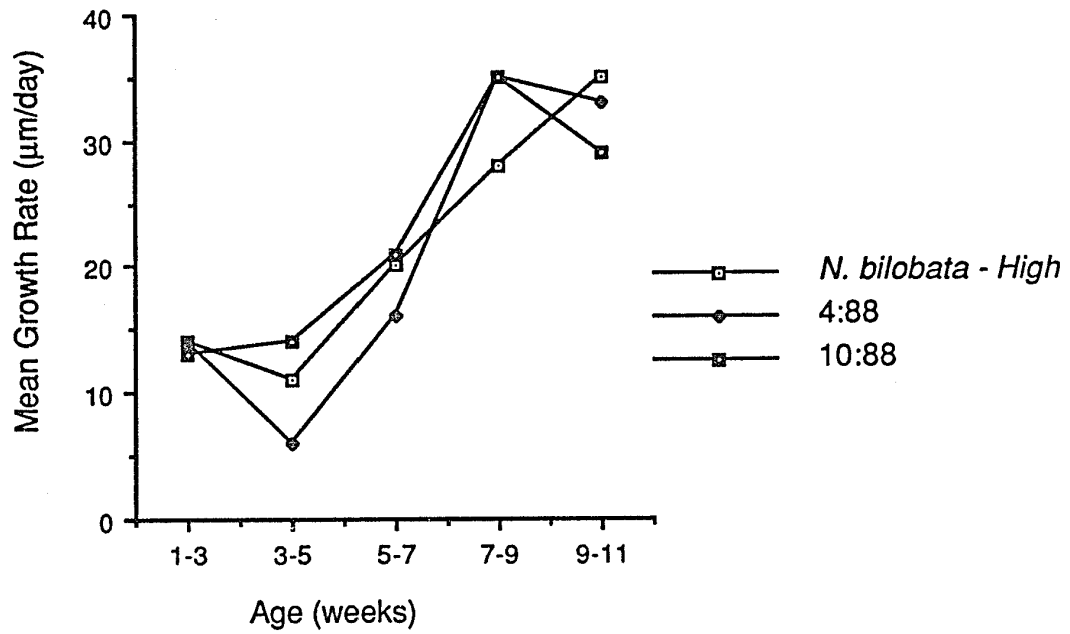
Figure 6. Mean growth rates of abalone from N. bilobata, high light treatment and from two miscellaneous spawnings of 1988 (4:88 and 10:88).
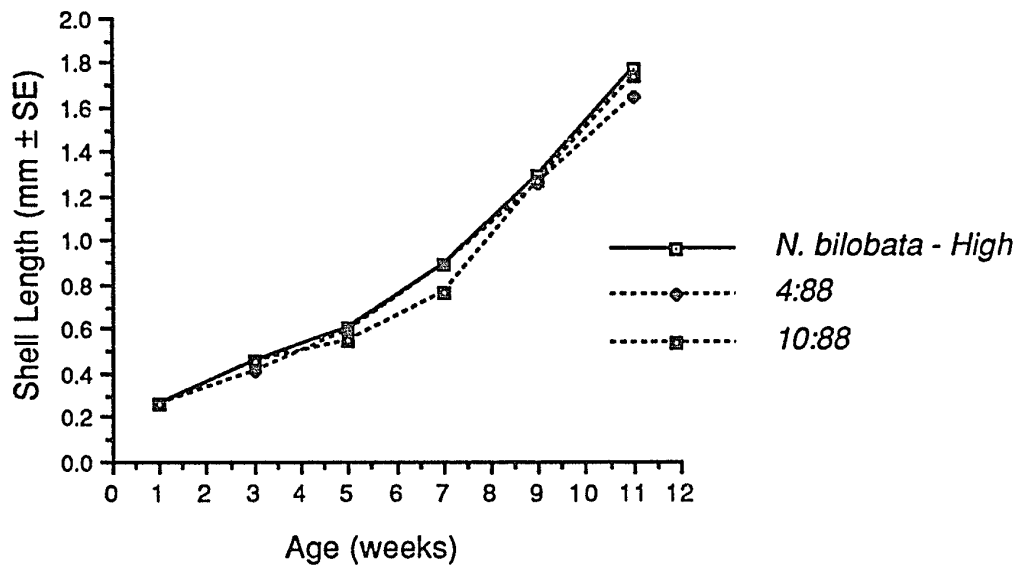
Figure 7. Mean shell length of abalone from N. bilobata, high irradiance treatment and two miscellaneous spawnings of 1988 (4:88 and 10:88)
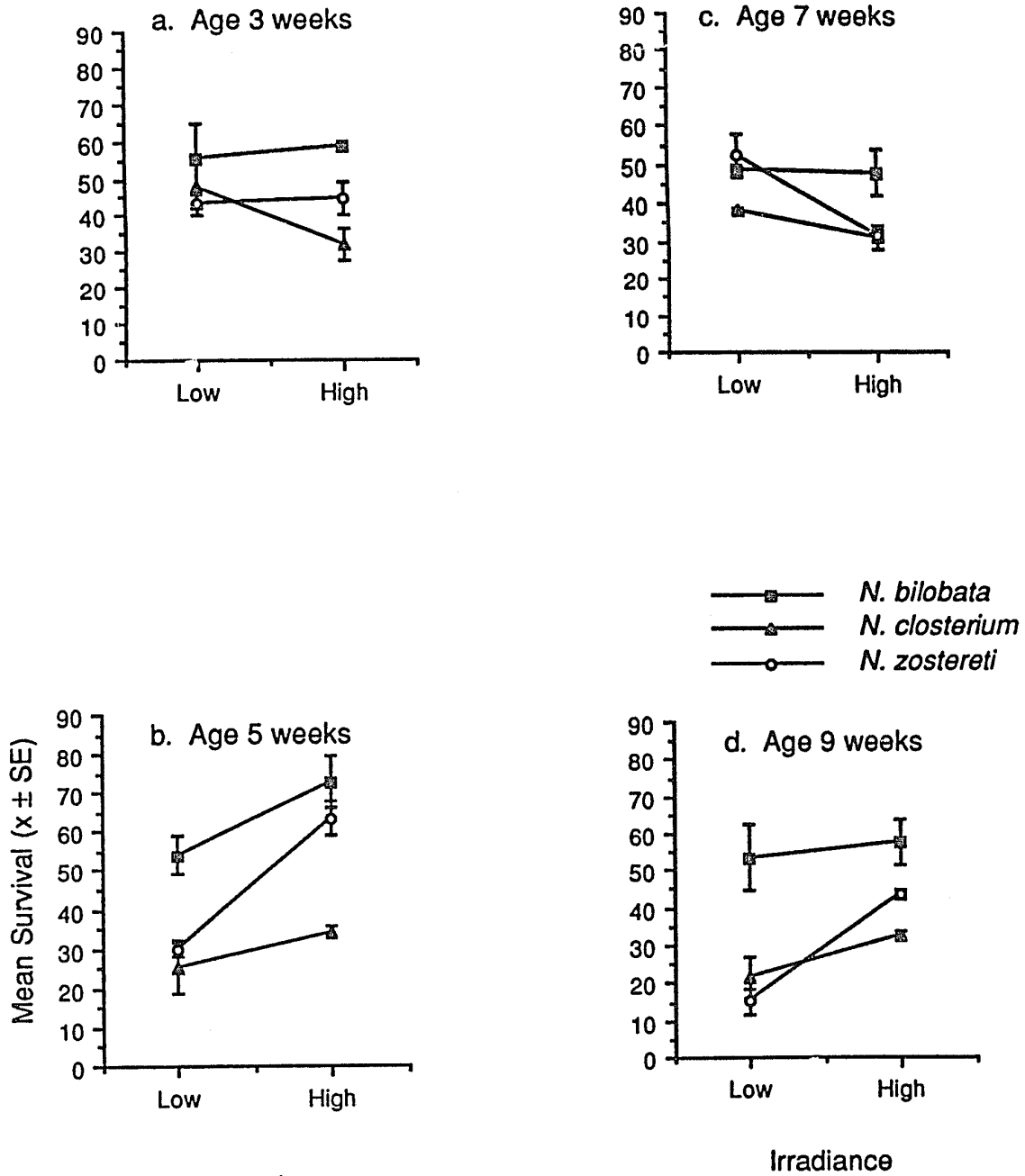
Figure 8. Effect of irradiance and time on mean proportional abalone survival for each diatom diet and at each two week sampling interval, $x \pm$ SE, n=3. Data presented are arcsin $\sqrt{x}$ where 90° = 100%.
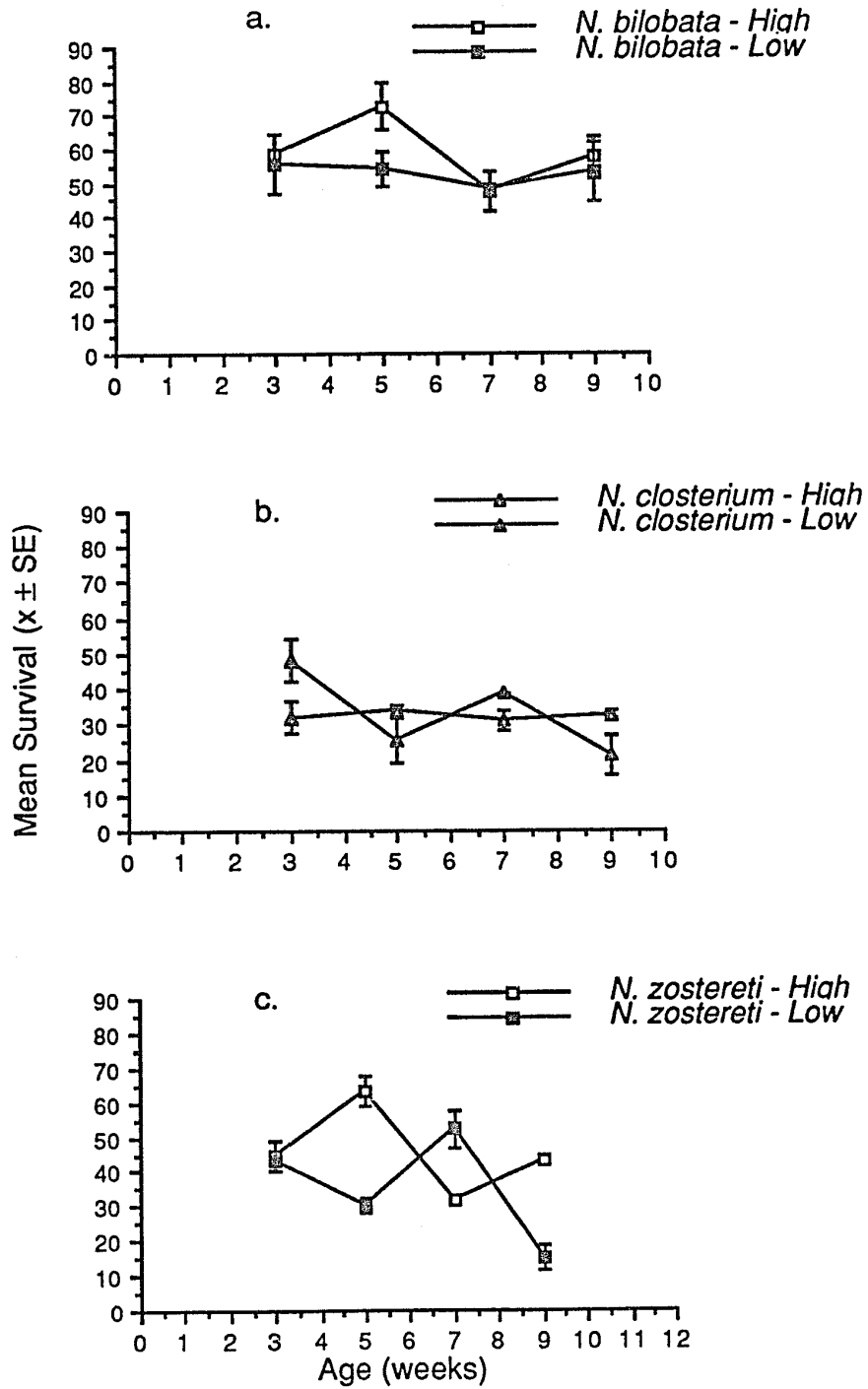
Figure 9. Effect of individual diet and irradiance levels on mean proportional abalone survival for the ten week experiment, $x \pm$ SE, n=3. Data presented are transformed by arcsin √x.
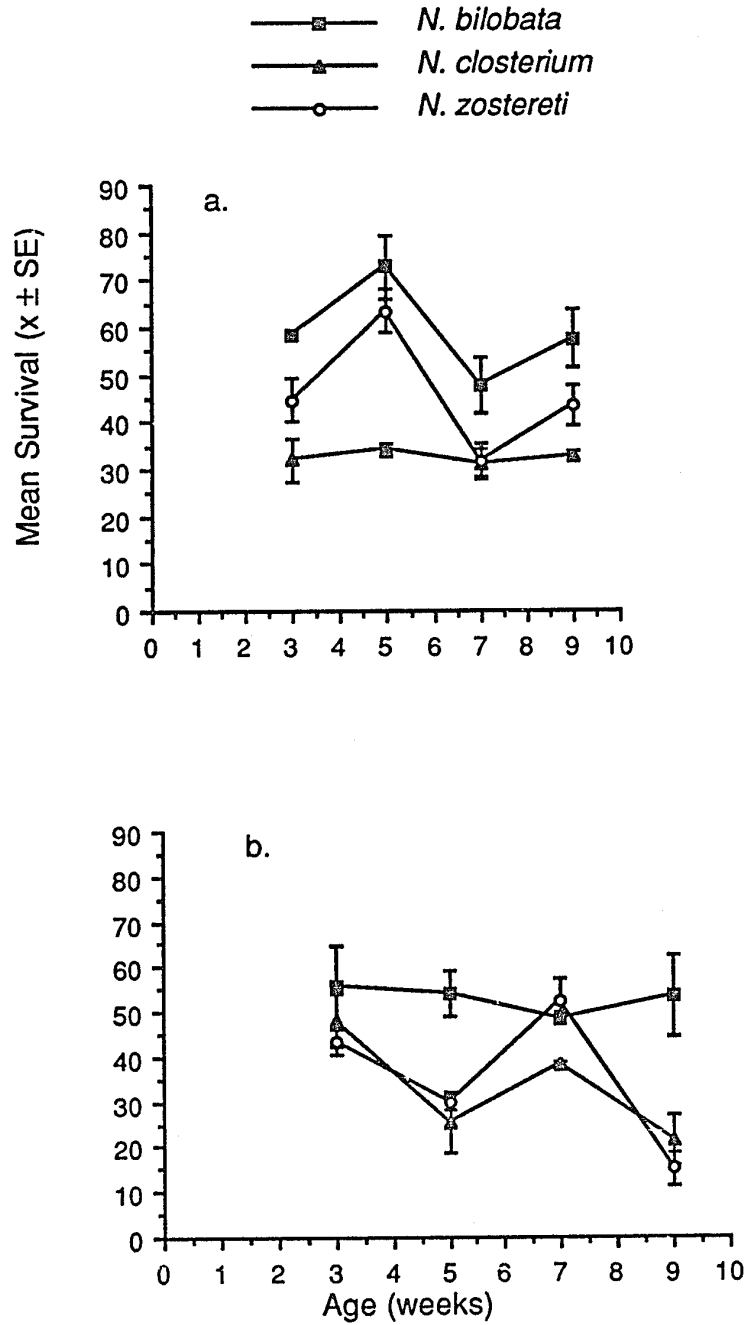
Figure 10. Effect of individual diet and time on mean proportional survival for both light levels, a. high irradiance, 50 - 60 µE; b. low irradiance, 15- 25 µE. Data presented are arcsin √x transformed, x ± SE, n=3.

Appendix

Mean Square Estimates for Analysis of Variance on Abalone Shell Length

Diet: $\vartheta_e + n\vartheta C(DLT) + ltcn\vartheta D$

Light: $\vartheta_e + n\vartheta C(DLT) + dtcn\vartheta L$

Diet x Light: $\vartheta_e + n\vartheta C(DLT) + tcn\vartheta LD$

Time: $\vartheta_e + n\vartheta C(DLT) + ldcn\vartheta T$

Light x Time: $\vartheta_e + \vartheta C(DLT) + dcn\vartheta LT$

Diet x Time: $\vartheta_e + \vartheta C(DLT) + lcn\vartheta DT$

Diet x Light x Time: $\vartheta_e + \vartheta C(DLT) + cn\vartheta DLT$

Containers: $\vartheta_e + \vartheta C(DLT)$

Error: ϑ_e

For these mean square estimates, d, l, t, and c are levels of the factors, diet (D), light (L), time (T), and container (C), respectively. The symbol ϑ represents variance, the letter e signifies error, and the letter n represents each individual shell length measurement.