RELATIONSHIPS BETWEEN THE HARPACTICOID COPEPOD

TIGRIOPSIS CALIFORNICUS BAKER

AND POST LARVAL RED ABALONE, HALIOTIS RUFESCENS SWAINSON

by

Randall M. Hamilton

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TIGRIOPUS CALIFORNICUS BAKER
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by

Randall M. Hamilton

Approved by the Master's Thesis Committee

George M. Allen, Chairman

James Welsh

William Shaw

Director, Natural Resources Graduate Program

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Natural Resources Graduate Program Number

Approved by the Dean of Graduate Studies

Alba M. Gillespie
ABSTRACT

High post-larval abalone mortality is a major problem for abalone culturists today. The purpose of this study was to determine if the copepod (Tigriopus californicus) caused mortalities among larval abalone (Haliotis rufescens). A significant (P<0.01) relationship existed between the concentration of copepods and abalone mortality. However, no significant difference of mortality was found between abalone with copepods directly in cultures or abalone with only copepod effluent water passed into cultures. Therefore, copepods did not directly kill abalone.

Indirect exposure of copepods to abalone were investigated by analyzing water quality parameters. Bacterial studies showed a trend of increasing concentration of Vibrio anguillarum with increasing concentrations of copepods. Species in the genera Vibrio are deadly to fish and shellfish in the marine environment. Thus, V. anguillarum was suspected as the primary cause of young abalone mortality in this study.

Ultraviolet treated seawater and the antibiotic neomycin were tested in abalone cultures with and without copepods to determine if Vibrio free conditions increased abalone survival. Treated abalone cultures were Vibrio free and contained abalone which survived superior to non-treated, Vibrio present groups.
ACKNOWLEDGEMENTS

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To my wife, Cathy, I will always be indebted for in large measure it was through her patience and understanding that this thesis was completed.

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INTRODUCTION

Remarkable progress has been made during the past few years in the development of mass cultivation procedures for the red abalone (*Haliotis rufescens* Swainson; Ebert 1973, Rutherford 1976, Klopfenstein 1976, Leighton 1977). Induced synchronous spawning techniques have largely been perfected using heavily irradiated seawater (Kikuchi and Uki 1974) or hydrogen peroxide (Morse et al. 1977). Furthermore, larval stages are routinely cultivated in large scale production systems with 90 percent or more survivorship rates (Ebert 1982). However, the primary limitation on the abalone industry is the high unit-cost of production, resulting from extensive and largely uncontrolled early post-larval mortality (Morse et al. 1979a).

Several hypotheses have been suggested for young abalone mortality ranging from water quality parameters (Shibui 1972) to inherent genetic defects in the eggs (Leighton 1972). A commonly accepted theory was suggested by Imai (1967) and Kan-no (1975) who attributed the mortality to inadequate food supply and suggested that a system which continuously provided young abalone with preferred diatoms such as *Cocconeis* sp. and *Navicula* sp. would reduce this problem. However, research by Leighton (1977) and Morse (1979a) have shown that adequate nutrition alone does not prevent high mortality. Morse (1979a, 1979b) has proposed that post-larval mortality was primarily because of the inability of abalone to rapidly and completely metamorphose under artificial conditions, followed by eventual bacterial and protozoan overgrowth. Furthermore,
Morse (1979b) states that:

abalone larvae were not found to settle on clean inorganic surfaces or those coated with diatoms, bacteria, other algae, or various juvenile invertebrates, unless these larvae were artifically induced to settle with chemicals such as \( \lambda \)-aminobutyric acid (GABA) or extracts of the crustose coralline red algae.

Ebert (1982) successfully and routinely settled ("recruited") abalone under experimental and production systems without the use of chemical inducers. However, in production, the survival rates after three months ranged from 0 to 12 percent with a mean of 3 percent. Ebert (personal communication) postulated that a major source of early benthonic abalone mortality may be caused by the harpacticoid copepod (Tigriopus californicus Baker). This copepod enters the abalone culture system adventitiously, rapidly reproduces and presumably competes with the young abalones. Additionally, this copepod may be a carrier or transmitter of disease, and may release toxic metabolites. However, the factor(s) responsible for abalone mortality by copepods have not been determined, nor have techniques been developed to inhibit copepod entry into the cultures.

The objective of this study was to determine the physical, chemical and possible predator-competitor relationship of the harpacticoid copepod to the early post-larval stages of the red abalone. Abalone survival and growth rates were determined at three, four, five and six weeks of age at various copepod densities and exposures. Ultraviolet treated water and antibiotic variables were also tested on some post-larval abalone to determine if bacteria-free conditions increased abalone survival in the presence or absence of copepods.
MATERIALS AND METHODS

Laboratory Sea Water System

This study was conducted at the California Department of Fish and Game's Marine Culture Laboratory, Monterey. The water delivery system was designed and constructed to provide a flow-through, filtered, heated, or ultraviolet (UV) treated sea water to the experimental tanks (Ebert et al. 1974, and Ebert 1982).

Abalone Cultivation Techniques

Adult red abalone came directly from laboratory cultivated stocks. Parent stock was conditioned at ambient temperature (ca 11-15°C) in 15 μm filtered, continuous-flowing, seawater, and exposure to a natural photoperiod. Brood tanks were cleaned and supplied weekly with an excess of fresh giant kelp (Macrocystis spp.).

Red abalone may be spawned in the laboratory every month of the year (E. Ebert, personal communication). Mature parent stock was selected based on gonadal bulk and color. Male abalone gonad is cream white, while female gonad is green. One male-female pair was used for each test run. Each pair were held separately in 15 liter polyethylene containers. Spawning was induced by using UV irradiated seawater (Kikuchi and Uki 1974). A REFCO® water purifier (Model RL-10) was used to generate UV treated seawater and 3 μm filtered seawater for preventative predator control. Water flow from the purifier to each of the abalone pair was maintained at about 150 ml/min. Three to four hours after induction, abalone spawned synchronously.
At spawning, 50,000 to 75,000 ova were siphoned into 15 liter plastic containers (3-5 ova/ml) and 50 ml of sperm suspension was added (approx. 400,000 sperm/ml was estimated with a Hemacytometer; Stein 1973). After the fertilized ova had settled to the bottom, each container was decanted to remove excess sperm and unsettled ova and then refilled with fresh seawater. This process was repeated a minimum of three times. The containers were then placed into a water bath at 15°C for 24 hours without aeration.

After 24 hours, the fertilized eggs hatched and the larvae (veliger stage) began to swim. These swimming veligers were transferred to 8 liter flow-through culture tubes to a concentration of 5 larvae per ml (40,000 larvae/tube). The larvae were cultivated at 15°C, with 200-300 ml/min of 3 µm filtered and UV treated seawater. Supplemental food was not given to larvae. After six days the veligers were ready to metamorphose (attach to available substrate and begin active benthic feeding) and were transferred to experimental containers.

**Copepod Cultivation Techniques**

The copepods used for experimentation were collected from a high-splash pool at the south end of the Marine Culture Laboratory on July 15, 1981. The copepods were collected in a 15 liter plastic container from the pool. The captured copepods were then immediately transported to the laboratory and served as the main stock for all experiments.

Widely varied techniques have been used to successfully culture harpacticoid copepods (Hawkins 1962, Huizinga 1971, Battaglia 1970,
Kahan et al. 1982). Copepods are extremely tolerant to environmental fluctuations and are easily kept alive in the laboratory for long periods of time (Dethier 1980, Fraser 1936, Vittor 1971, Johnson and Olson 1943).

The stock copepods in this study were maintained in the laboratory in four, 15 liter plastic containers. These containers were 80 percent filled with 3 μm filtered and UV treated seawater and protected from direct sunlight to reduce temperature fluctuation. The cultures were maintained at room temperature (ca 16-20°C), without aeration.

Each week, the copepod cultures were 75 percent decanted through a 280 μm screen (NITEX) to retain adult copepods, refilled with 3 μm filtered, UV treated seawater. Copepods were fed an excess of laboratory cultured benthic diatoms, primarily Navicula spp.. The captured copepods were returned to cultures if densities were low. Copepod densities were not closely monitored; however, the densities were maintained at about 1000 to 3000 per plastic container.

Forage Cultivation Techniques

Laboratory-cultivated benthic diatoms were fed to copepods and juvenile abalone. The predominate diatoms (>90 percent) cultivated throughout this study were of the Naviculoid type. These diatoms were cultured in polyethylene containers (2,924 cm² surface area) with about 0.5 L/min of 1 μm filtered seawater at 11-15°C. Fluorescent lamps (General Electric, Chroma 50) were placed 30 cm above the containers.
Initially, non-filtered diatoms were collected by allowing diatoms that naturally pass through the laboratory to plate-out on plastic containers. Thereafter, containers were reinoculated with 68 million cells of harvested, filtered diatoms. Diatoms were harvested by decanting most of the water, wiping down the container sides with a latex glove and pouring the diatom suspension through a 1 μm filter bag (American Filter and Fiber Company). One container generally yielded 1 liter of diatom filtrate (5.0 x 10^5 cells/ml) after seven days.

The diatom filtrate was microscopically examined (100x) before feeding to abalones. Densities were estimated using a hemacytometer (Stein 1973). Only diatom filtrates which were free from organisms such as copepods, amphipods, polychaetes and nematodes were used.

Test Culture Apparatus

Testing was conducted in a water table which accommodated the culture apparatuses. For the first three experiments, a lucite-plastic water table was used which measured 244 cm by 43 cm by 30 cm deep. For the fourth experiment, a black polyethylene plastic-hooded water table was used which measured 214 cm by 91 cm by 30 cm deep. Each test culture apparatus consisted of a PVC pipe section measuring 20 cm in diameter by 15 cm high, which was positioned in the water table to support a cut plastic container measuring 25 cm in diameter by 9.5 cm high. Inside each cut plastic container were placed two culture containers. Each culture container consisted of a 10 cm inside diameter, clear PVC pipe section, 11.3 cm high and screened at the base with 90 μm NITEX to retain test animals. The culture
containers were placed on plastic grate inside the cut plastic container to give each culture a water volume of 0.5 liter (Figure 1).

One μm filtered seawater, the pH (8.0) and salinity (34 parts per thousand), was supplied continuously to each culture container at a rate of about 200 ml/min. The temperature was maintained at 15 ± 2°C and an airstone was placed into each cut plastic container. Throughout this study, the seawater was UV treated with a 10 lamp system (steri-tromics International, Model SWL 80/120); however, in an experiment (fourth), a secondary UV lamp (Refco, Model RL-10) was used for increased bacterial control.

Illumination using fluorescent lamps (General Electric, Chroma 50) was provided 75 cm above the containers in Experiment 1 and 30 cm above the containers in Experiments 2, 3 and 4. The photoperiod was adjusted to the diatom density of cultures by covers which were placed over appropriate containers. A thin, uniform diatom film was considered optimum.

Abalone Survival and Growth Under Copepod Variables

From July 1981 through February 1982, four experiments were conducted. The first three experiments were conducted to determine if the copepods had an effect on abalone survival or growth. The fourth experiment was conducted to investigate the bacteriology of abalone cultures grown in the presence or absence of copepods.

Five test culture apparatuses were used to study the effects of copepod density in the first three experiments. The first container held only post-larval abalone and served as the control. The second container held post-larval abalone and a low density of 50 copepods (one
Figure 1. Test apparatus used to hold abalones and copepods for experimentation.
copepod per 10 ml seawater). The third container held post-larval abalone and a high density of 300 copepods (six copepods per 10 ml seawater). The fourth container held only post-larval abalone and the fifth container held only 300 copepods; however, the copepod effluent water of the fifth container was passed into the abalone cultures of the fourth container for indirect exposures.

In experiment four, four test culture apparatuses were used to determine abalone survival and growth under effects of secondary UV treated (100 percent bacteria free) seawater, an antibiotic (neomycin), and copepods. Containers 1 and 2 received secondary UV treated, one μm filtered influent seawater and a dose of neomycin (50 mg/L for 24 hours) at the onset of experimentation. Containers 3 and 4 received primary UV treated (non-bacteria free), one μm filtered influent seawater. Containers 1 and 3 contained 50 abalone and served as the controls. Containers 2 and 4 contained 50 abalone and 300 copepods.

Experiment 1 was conducted for 49 days (July 22, 1981 to September 9, 1981). Spawning induction denoted the onset of the experiment. On the sixth day, 40 million diatom cells were added to each culture container. Two hours later, one hundred abalone veligers were pipetted into appropriate cultures. On the 13th day, an abalone chemical settling inducer (Gaba Amino Buteric Acid) was used to assure maximum settling by submersing cultures into 10^{-3}M solution for three minutes. Adult copepods were added to appropriate cultures on the 14th day. These copepods were obtained by rinsing stock cultures through a 280 μm NITEX screen to retain adults and allow copepodite and naupliii stages to pass through. The copepods were then thoroughly
rinsed with UV treated seawater and placed into a 500 ml beaker. Copepods were then withdrawn into a 5 ml wide-bore pipet, counted and placed into appropriate containers. Diatoms were added to various cultures throughout the experiment to assure that food was not limiting to animals. The densities were not closely monitored; however, an attempt was made to maintain a uniform diatom film throughout the culture by adding diatoms to needed cultures or covering overgrown cultures from light to reduce diatom growth.

General qualitative bacterial determinations (spread plate technique) were conducted on the 40th, 41st and 44th days to determine if Vibrio-type bacteria were present.

Experiment 2 was conducted for 22 days (September 16, 1981 to October 8, 1981). Three days post spawning, 270 million diatom cells were added to each culture container and served as the only inoculum for the test. On the fourth day, 50 abalone veligers were pipetted into appropriate cultures. On the eighth day, adult copepods were added to appropriate cultures as described in Experiment 1. Quantitative bacterial analysis (membrane filtration) was conducted throughout this experiment. Bacteria samples were obtained from influent and effluent waters and within the cultures at the surface and bottom.

Experiment 3 was conducted for 39 days (October 15, 1981 to November 23, 1981). Four days post spawning, 100 million diatom cells were added to each culture container and served as the only inoculum needed. On the fifth day, 50 abalone veligers were pipetted into appropriate cultures. On the sixth day, copepods were pipetted into appropriate cultures (as described in Experiment 1). Quantitative bacterial analysis was conducted throughout this experiment.
Experiment 4 was conducted for 30 days (December 23, 1981 to January 22, 1982). Five days post spawning, 50 million diatom cells were added to each culture container and served as the only inoculum. On the sixth day, 50 abalone veligers were pipetted into appropriate cultures. On the eighth day, 300 copepods were pipetted into appropriate cultures (as described in Experiment 1) and neomycin (50 mg/L) was used in appropriate containers. Quantitative bacterial analysis was conducted thoroughly during this experiment. Only influent and effluent water samples were tested.

Abalone and copepods were killed at the end of experimentation for counting and measuring. In the first experiment, death was accomplished by submersing cultures in 40°C fresh water for one minute. In the 2nd, 3rd and 4th experiments, death was accomplished by submersing cultures in 5 percent formalin for one minute. After animals were dead, they were rinsed into a finger bowl and placed under a dissecting scope (12x) where abalone were easily observed. They were then pipetted onto a slide and observed with a compound microscope (100x). Abalone were measured with an optical micrometer which was calibrated with a stage micrometer. Abalone with all soft parts fully developed within the shell were considered alive when the experiment was terminated.

A three-way Goodness of Fit test (Sokal and Rohlf 1969) was used to determine if abalone mortality was correlated to copepod densities for Experiments 1, 2 and 3. Survival data for each container were used for analysis with missing values and live abalone larvae which failed to metamorphose counted as dead. Different orthogonal contrast sets were calculated to further analyze which
experiments and copepod concentrations were significant (P<0.05) in showing abalone mortality.

Growth was assessed using a two-way nested factorial analysis of variance on live post larval lengths for Experiments 1, 2 and 3. This analysis was conducted using the computer software statistical program SPSS (Nie et al. 1975). Difference between experiment number and treatment were considered significant when P<0.05. Specific treatment effect comparisons were conducted to further analyze which experiments and copepod concentrations were significant in showing abalone growth differences.

**Bacterial Determination for Vibrio**

Water samples from various culture containers were taken from the bottom of containers with a one ml pipet, taken from influent water with a one ml pipet, or taken from the effluent water collected into sterile 25 ml glass test tubes. Logarithmic dilutions were made using sterile seawater. All samples were immediately processed for bacterial counting in two ways: the spread plate method which used 0.1 ml to one ml volumes of sample spread onto the surface of prepared agar; and the membrane filtration method where various volumes of samples were filtered through a Millipore® sterile, 0.45 mm gridded, 47 mm membrane (Patrick 1978). The membranes were placed onto Thiosulfate citrate bile salt (TCBS) agar (Difco Laboratories, Detroit, Michigan) in 60 mm sterile, disposable plastic petri dishes. All filtrates were incubated at 22°C for 24-48 hours. Colonies were counted using a compound microscope under 25x magnification. Colonies
grown on TCBS were identified by Renee Rosemark at University of California Bodega Marine Laboratory, California.

**Larval Counting**

Total observed abalone mortalities and projected abalone mortalities were calculated after each of the four experiments. Projected mortalities may be a more realistic representation to the effects of copepod densities in abalone cultures than recorded mortalities. Projected mortalities includes unrecorded values and live abalone larvae which have failed to metamorphose, along with abalone which were recorded as dead. Unrecorded values may have occurred because (1) dead abalone shells are fragile and easily broken, resulting in fragmented pieces which either pass through the screen bottom or were overlooked when counting, or (2) unbroken, dead abalone shells which tend to entangle with container debris, were difficult to locate when counting. Unmetamorphosed live abalones were projected as dead because larvae which fail to metamorphose after 10 days post fertilization at 15°C, probably never will metamorphose (E. Ebert, personal communication). This presumption was strengthened by the fact that the shortest experiment (#2, 22 days) had the greatest number of live larvae while the longest experiment (#1, 49 days) had no live larvae at time of test termination. The normal larvae stage was about seven days.
RESULTS

Survival

Experiments 1, 2 and 3

Frequencies of abalone mortality for each treatment (Table 1) were analyzed by a three-way Goodness of Fit test (Table 2). There was a significant dependence (P<0.01) among experiments, treatments (various copepod concentrations) and mortality, indicating that abalone survival negatively correlated with copepod concentration. There was no significant dependence between treatments and experiments. Interactions among experiments, treatments and mortality were also not significant. There was dependence though between treatments and mortality (P<0.01). Furthermore, three treatment contrasts are particularly interesting: (1) abalone alone survived better than abalone plus 300 copepods, (2) abalone plus 50 copepods survived better than abalone plus 300 copepods, (3) abalone plus 300 copepods survived no differently than abalone plus copepod effluent.

Mean percentage mortality of the abalone in the various experiments were determined (Fig. 2). As expected, the shortest experiment (#2) has the lowest overall mortality (19 percent) with the longest experiment (#1) with the highest overall mortality (75 percent).

The observed and projected mortalities for Experiments 1, 2 and 3 clearly shows a trend of increasing mortality with increasing concentrations of copepods. Also, copepod effluent water has an adverse effect on abalone survival.
Table 1. Frequencies of Abalone Condition (dead versus alive) Classified to Treatment (abalone subjected to various copepod densities and exposures) and Experiment Number (each experiment subjected to similar treatments for various durations). Unmetamorphosed Live Abalone (8.0%) and Unrecorded Values (9.8%) Were Assumed Dead (ab abbreviates abalone).

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Table 2. Three-way Test of Independence (G-test) and Three
Orthogonal, A-priori Tests of Partitions for Experiments 1, 2 and 3. Abalone are Classified According to Treatment
(abalone subjected to various copepod densities and exposures), Experiment (each experiment subjected to similar
treatments for various durations; #1 for 49 days, #2 for 22
days, #3 for 39 days) and Mortality. Symbols are:
T = treatment, E = experiment, M = mortality, ab = abalone,
cop = copepods, eff = effluent.

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<td>ab vs ab + 300 cop</td>
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<td>3.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ab + 50 cop vs ab + cop eff</td>
<td>1</td>
<td>0.07</td>
</tr>
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<td>ab &amp; ab + 300 cop vs ab + 50 cop &amp; ab + cop eff</td>
<td>1</td>
<td>18.41&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
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<td>1</td>
<td>10.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ab vs ab + cop eff</td>
<td>1</td>
<td>9.49&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>1</td>
<td>2.69</td>
</tr>
<tr>
<td>ab vs ab + 40 cop</td>
<td>1</td>
<td>1.51</td>
</tr>
<tr>
<td>ab + 300 cop vs ab + cop eff</td>
<td>1</td>
<td>1.26</td>
</tr>
<tr>
<td>ab &amp; ab + 50 cop vs ab + 300 cop &amp; ab + cop eff</td>
<td>1</td>
<td>19.59&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>E x M independence</td>
<td>2</td>
<td>60.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>E x T x M interaction</td>
<td>6</td>
<td>12.00</td>
</tr>
<tr>
<td>E x T x M independence</td>
<td>17</td>
<td>94.74&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a</sup>P<0.05  
<sup>b</sup>P<0.01  
<sup>c</sup>P<0.001
Figure 2. Mean Percent Mortality of Abalone for the Four Experiments. Projected Mortality Includes Dead, Missing and Live Veliger Stage Abalone. Symbols are: AB=abalone, COP=copepod, EFF=effluent water, UV=ultraviolet treated water, EXP=experiment.
Figure 2. Mean Percent Mortality of Abalone for the Four Experiments. Projected Mortality Includes Dead, Missing and Live Veliger Stage Abalone. Symbols are: AB=abalone, COP=copepod, EFF=effluent water, UV=ultraviolet treated water, EXP=experiment.
For abalone which failed to metamorphose and died at the veliger stage, higher concentrations of copepods had greater numbers of dead veligers (Fig. 2).

**Experiment 4**

Mean percentage abalone mortality were determined for the four treatments in Experiment 4 (Fig. 2). The largest abalone mortality was in the copepod groups: with the group containing abalone treated with ultraviolet light and neomycin having a slightly smaller mortality. Abalone cultures treated with neomycin and supplied with bacteria free, UV treated seawater survived better than non-treated groups (in copepod-free abalone culture, mean survival was 93 percent in the treated and 66 percent in the non-treated groups).

Experiment 4 abalone without secondary UV treated seawater fit the general trend of mortality shown by abalone without copepods in Experiments 1, 2 and 3, (i.e., the longer the experiment duration the greater the abalone mortality, Fig. 2). However, Experiment 4 abalone with secondary UV treated seawater did not fit this general trend of mortality. The projected mortality of abalone grown for 30 days in UV-treated seawater was less than abalone grown in non-UV treated seawater for 22 days (Fig. 2).

**Growth**

**Experiments 1, 2 and 3**

A two-way nested analysis of variance on length (μm) data for live post larval abalone shows significant differences between experiments and treatments (Table 3). Because no significant
Table 3. Two-way Nested Analysis of Variance (Sokal and Rohlf 1969) on Growth (length) Data in Experiments 1, 2 and 3 (each experiment subjected to similar treatments of copepod densities and exposures for various durations: #1 for 49 days, #2 for 22 days, #3 for 39 days).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F_r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>2</td>
<td>717143.55</td>
<td>35871.77</td>
<td>22.01\textsuperscript{b}</td>
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<tr>
<td>Treatment</td>
<td>3</td>
<td>293943.87</td>
<td>97981.29</td>
<td>14.79\textsuperscript{a}</td>
</tr>
<tr>
<td>Experiment by Treatment</td>
<td>6</td>
<td>390134.57</td>
<td>65022.43</td>
<td>3.99\textsuperscript{a}</td>
</tr>
<tr>
<td>Screen within Treatment</td>
<td>4</td>
<td>26505.55</td>
<td>6626.39</td>
<td>1.62</td>
</tr>
<tr>
<td>Experiment by Screen within Treatment</td>
<td>8</td>
<td>130322.56</td>
<td>16290.32</td>
<td>3.98\textsuperscript{b}</td>
</tr>
<tr>
<td>Within Cells (Error)</td>
<td>319</td>
<td>1305868.99</td>
<td>4093.63</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}P<0.05  
\textsuperscript{b}P<0.01
difference was detected between screen tubes within treatments, these values were lumped together for further testing. A priori contrasts (Table 4) shows significant differences (P<0.01) between abalone only compared to the other treatments, between abalone plus 50 copepods and abalone plus 300 copepods (P<0.01), but not between abalone plus 300 copepods and abalone plus copepod effluent.

Mean and range of length measurements for live post larval abalone in the various experiments were compared graphically in Figure 3. As expected, the shortest growing period (experiment #2) produced the smallest abalone (overall mean lengths 373 μm) while the longer rearing periods (e.g. Experiment 1) resulted in largest larvae (overall mean lengths of 495 μm). However, within each experiment, abalone grew larger in those containers which had copepod exposure either directly (copepods in abalone cultures) or indirectly (only copepod effluent water passed into abalone cultures).

Experiment 4

Mean and range of length measurements for abalone in the various treatments were determined to investigate the bacteriology of abalone cultures grown in the presence or absence of copepods (Fig. 3). Growth of abalones were best in cultures which contained copepods and treated with UV light (507 μm). The aforementioned container also had the fewest live abalones at end of experiment (50 percent dead).
Table 4. Treatment Contrasts for Experiments 1, 2 and 3 (each experiment subjected to similar treatments of copepod densities and exposures for various durations; #1 for 49 days, #2 for 22 days, #3 for 39 days); Two-way Nested Analysis of Variance (Sokal and Rohlf 1969) on Growth Data. Symbols are: ab = abalone, cop = copepod, eff = effluent water.

<table>
<thead>
<tr>
<th>Treatment Contrasts</th>
<th>df</th>
<th>Standard error</th>
<th>T-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ab vs ab, ab + 50 cop, ab + 300 cop, ab + cop eff</td>
<td>1</td>
<td>30.57</td>
<td>-5.82^a</td>
</tr>
<tr>
<td>ab + 50 cop vs ab + 300 cop</td>
<td>1</td>
<td>10.39</td>
<td>-4.55^a</td>
</tr>
<tr>
<td>ab + 300 cop vs ab + cop eff</td>
<td>1</td>
<td>20.69</td>
<td>2.51</td>
</tr>
</tbody>
</table>

^aP<0.01
Figure 3. Mean of Growth Rates Shown by Horizontal Bars and Range in Length by Vertical Lines. Only Live Post Larvae Abalone were used in Calculation. Symbols are: AB=abalone, COP=copepod, EFF=effluent, UV=ultraviolet treated water, EXP=experiment.
Bacteriologic Studies

*Vibrio anguillarum* was detected throughout this study. During experiments 1, 2 and 3 levels of *V. anguillarum* increased with increasing concentrations of copepods. Furthermore, abalone cultures which had copepod-effluent water passed into them, also had levels of *V. anguillarum* similar to or greater than cultures with 300 copepods. The greatest *V. anguillarum* concentration (240 organisms/ml) was obtained when sampling on container surfaces; however, surface sampling techniques were discontinued because of the possibility of removing or damaging young abalone. Although effluent water samples were generally very low in *V. anguillarum* concentration (<50 organisms/ml), effluent sampling enabled monitoring of bacteria levels without disturbing experimental cultures.

In Experiment 4, a consistent, effluent water sampling program was conducted throughout the test period (Fig. 4). Abalone cultures not treated with neomycin or supplied with UV treated water, contained *V. anguillarum* levels greater than treated groups. The greatest *V. anguillarum* levels were obtained in non-treated cultures which contained copepods. No *V. anguillarum* were detected in the treated groups. Except for the non-treated copepod groups, influent water samples were generally higher in *V. anguillarum* concentration than effluent.
Figure 4. Mean *Vibrio anguillarum* densities for test cultures in experiment 4. AB-COP-UV and AB-UV cultures were *Vibrio* free throughout test run. Symbols are: AB=Abalone, COP=Copepod, UV=secondary ultraviolet treated seawater plus neomycin.
DISCUSSION

Larval mortality is still a major problem for abalone mariculturists today. Researchers throughout the world are currently investigating this problem of high post-larval mortality (Leighton 1981, Morse 1979a, Seki and Kan-no 1981, C. Sumner, personal communication). Once juvenile abalone reach 3 mm, survival under hatchery conditions is greatly increased and mortality is very rare in abalone larger than 10 mm (Ebert 1982, Leighton 1981).

My study has contributed to the understanding of post-larval mortality. Copepods clearly caused abalone mortality. Increased concentrations of copepods were correlated with increased abalone mortality and increased numbers of dead veliger larvae.

Abalones grown with copepods resulted in larger but fewer live abalones. This suggests that abalone population densities may be an important factor in abalone growth. I found no studies on population density and growth of young red abalone in the literature; however, Shibui (1972) also noted faster growth rates of Japanese abalones in lesser densities. Although initial stocking densities of abalone in my study were low (2.5 cm$^2$ of culture container/abalone) increased mortalities in copepod contaminated cultures resulted in increased food availability to remaining abalones. Apparently, very low abalone densities resulted in the highest growth rates.

Growth rates in abalones were similar to results obtained by other researchers (Leighton 1974, Morse 1979b, J. McMullen, personal communication). Although growth rates may have been increased by
increasing water temperature to 18°C (Leighton 1974), 15°C has been determined to be the overall safest temperature for abalone culture at the Marine Culture Laboratory (Ebert 1982, Ebert and Hamilton 1983). Growth rates may also have been improved with a different diet. Further research on post-larval abalone diet is required to determine optimum species, sizes, densities, nutrient values, etc.

Copepods probably do not directly cause abalone mortality. This was supported by two facts: (1) abalone mortality was not significantly different (Table 2) between abalone cultures containing copepods directly (300 copepods within abalone cultures) or indirectly (300 copepods held in separate containers with their effluent water passed into abalone cultures) and (2) numerous hours of direct observations of abalone-copepod interaction failed to reveal copepod predation on live abalone. Factors other than predation or competition for food and space contributed to abalone mortality.

Water quality values could not be related to mortalities. Periodic tests of dissolved oxygen (6.9-8.2 mg/L), pH (7.9-8.1), salinity (33-34 parts per thousand) and temperature (13.5-16.1°C) were similar in all containers and within normal ranges. Ammonia levels were assumed insignificant because the water volume in test containers were exchanged about every five minutes. Disease was the most probable agent causing mortality.

The highest concentrations of *V. anguillarum* were found in abalone cultures which contained the greatest concentration of copepods, either directly in cultures or indirectly as effluent water passed into cultures. Rinsing copepods in sterile seawater for five minutes failed to remove high levels of *V. anguillarum*. Therefore,
copepods probably carry *V. anguillarum* internally and transmit it to the cultures which proliferate bacteria growth.

_Vibrio_ species are widely recognized as one of the deadliest bacteria to fish in the marine environment (Sinderman 1970). *V. anguillarum* has been implicated in a disease of hatchery reared larval shellfish (DiSalvo et al. 1978). Several _Vibrio_ species have been found as pathogens of oyster larvae, with highest concentrations recorded in the spring or summer (Leibovitz 1978; Elston et al., 1981). Recent investigations have also determined that *V. alginolyticus* can cause death in young hatchery reared abalones (Elston and Lockwood 1983). Thus, *V. anguillarum* was presumed to cause post-larval mortality in this study.

Results of Experiment 4 strongly supported the disease-related mortality concept. The greatest abalone survival occurred in Experiment 4 cultures grown in bacteria-free conditions. Furthermore, Experiment 4 abalone without seawater treated with secondary UV light fit the general trend of mortality shown by abalone without copepods in other experiments where the long experiment duration was associated with high abalone mortality (Fig. 1). However, in Experiment 4, abalone with seawater treated with secondary UV light did not fit this general trend of mortality. The projected mortality of abalone when grown with UV treated seawater for 30 days was lower than abalone grown without UV treated seawater for 22 days.

Experiment 4 also supports the theory that copepods enhanced bacterial growth. The abalone-copepod group not treated with neomycin or UV light was the only container in which effluent bacteria levels were greater than influent levels. Greer (1981) reported, "there are
definite relationships between pathogenic vibrios and chiton, a major structural component of crabs and other aquatic invertebrates. This relationship involves a natural recycling of chiton in marine ecosystems. Because the copepod is known to molt this chitonous material 12 times from egg to adult in 25 days (Vittor 1971), copepods may have increased bacteria growth by supplying existing vibrios with additional media.

**Suggested Future Studies**

In order to confirm bacteria as the main factor in abalone mortality, further tests are required. The first test should challenge abalone directly with different concentrations of *Vibrio* sp. without copepods present and the second test would separate the bacteria effects from ammonia effects. The later test could be conducted by filtering out the bacteria in copepod effluent water with an inline 0.2 μm filter in one line while leaving non-filtered effluent water in a second line. Each copepod line would originate from a common copepod pool and end in separate abalone cultures.

In future experiments, new bacteriological test methods should be investigated. *Vibrio*-type bacteria tend to progressively attach to tank surfaces and persist there while numbers decline or disappear from the water column (Elston et al. 1981). This phenomenon was also observed in Experiment 4 abalone control group where bacteria numbers in influent samples often were greater than effluent samples. Effluent sampling was conducted because surface substrate type sampling might remove or damage young abalone. Although a trend was established, the exact concentration of bacteria on substrates was
unknown and therefore numbers of bacteria consumed by abalone larvae feeding on benthic diatoms could not be estimated. Abalone at 2.3 mm in length are known to consume 300,000 organisms per day (Shibui 1972). At this high rate, even low numbers of pathogenic bacteria in the water column may be hazardous to abalone. Thus, future bacteriological tests should include surface sampling techniques developed by Elston (personal communication). His technique included hanging glass plates with one square cm grids placed into test containers. Surface samples were made by wiping a known area with a sterile cotton swab. The swab was subsequently rinsed in 5.0 ml of seawater. Quantitative water column samples were made using 0.01 ml loops.

Possible Control Measures

Copepod concentrations significantly effect larval abalone survival rates. Therefore, elimination of copepods from water in which young abalones are cultured is desirable. Prophylactic measures most certainly should include filtration; however, biological control and chemical treatments could also be tested. Biological control methods might use types of animals known to prey on copepods such as sculpins and sea anemones (Dethier 1980). Chemical control methods could employ those chemicals which kill parasitic copepods such as potassium permanganate, malachite green, formalin and terramycin (Roberts 1978, Leitritz and Lewis 1976, Kabata 1970). Furthermore, a specific crustacean killing chemical such as Lindane could be tested (Hanks 1961).

Antibiotics and ultraviolet treated seawater should be used if pathogenic bacteria are present. An initial treatment of neomycin to
larval cultures is recommended because neomycin completely eliminates vibrios without harming abalone development and is cost effective when administered to animals confined in small volumes. UV treated seawater should be used to eliminate incoming bacteria until abalone reach 2.1 mm (3 months); this is the size at which abalone have high survival under hatchery conditions.
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PERSONAL COMMUNICATIONS

Ebert, E. Marine Culture Laboratory, Granite Canyon, CA 93940.

Elston, R. Battelle Marine Research Lab., Route 4, Box 1000, Sequim, WA 98382.

McMullen, J. Ab Lab, Port Hueneme, CA 93043.

Sumner, C. 23 Old Wharf, Hobart, Tasmania 7000, Australia.