Journal of Shellfish Research, Vol. 26, No. 3, 763–767, 2007.

EFFECT OF THREE PHOTOPERIOD REGIMES ON THE GROWTH AND MORTALITY OF THE JAPANESE ABALONE *HALIOTIS DISCUS HANNAI* INO

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ABSTRACT Feeding activity of the Japanese abalone *Haliotis discus hannai* is clearly diurnal, with an increase in feeding between the onset of darkness and midnight. The feeding rate increases slowly in the afternoon, approaching sunset and becomes constant between sunset and midnight. The abalone remain within shaded refuges during the day, and only emerge from them at night. The present study evaluated some effects of varying the photoperiod on a group of these abalone to determine its potential effects on their feeding behavior. Three groups of juvenile abalone with an initial mean length near 14 mm were maintained in raceway cage culture systems with a continuous flow of fresh seawater and abundant food where each group was exposed to different photoperiods. Growth of the abalone in these systems was monitored for a period of 106 days. Treatment A, considered "natural," included 12 h of light and 12 h of darkness. In Treatment B, the abalone were maintained in complete darkness, and Treatment C included four h of light and 20 h of darkness. The results showed that juveniles cultured in total darkness demonstrated some retardation in growth early in the experiment compared with abalone in a natural light regimen, and those with a partial light regimen. After 43 days, the abalone in the partial light regimen reached and surpassed sizes of the abalone maintained in the natural light regimen and in total darkness, although their feeding activity and growth patterns were erratic compared with abalone in the normal light and total darkness regimes. The results of the experiment were inconclusive as to which light regimen was most favorable when considering the growth patterns and comparative mortality rates within the systems.

KEY WORDS: abalone, tank culture, *Haliotis discus hannai*, photoperiod manipulation, growth rate, survival, Chile

INTRODUCTION

The Japanese abalone ("ezo awabi") *Haliotis discus hannai* was introduced to northern Chile in 1981, which at present is the only place it has been introduced on the South American continent. It became important as a potentially cultured marine resource with the initiation of the FONDEF 1102 project in 1997. A significant amount of research was required on developing culture technology and optimal foods for this species, particularly because its culture was restricted to land based tanks by law. Early emphasis was placed on obtaining the best growth rates for this species in these controlled systems (Uki 1989, Day & Fleming 1992, Greenier & Takekawa 1992).

This abalone species occupies rocky crevices near strong wave action in its natural environment, to a depth of about 5 m (Imai 1982, Hahn 1989). It was thus imperative to present similar water quality and physical conditions to the abalone in culture. An important environmental requirement was the presentation of a daily light and dark cycle, as the feeding of this abalone begins near sunset and becomes constant until midnight (Uki 1989). Studies on the light regimes required of this species in culture have been in relation to broodstock conditioning and postlarval feeding on benthic algae. Postlarvae of the blue abalone *H. fulgens* developed best at low light levels (Searcy-Bernal et al. 2003); those of the red abalone *H. rufescens* grew best with certain density levels of benthic microalgae, and a dark regimen, which was different from postlarvae of *H. diversicolor supertexta* in which low light regimes and darkness were not significant (Stott et al. 2004). Research related to the effects of light and darkness on juvenile abalone growth is scarce. Once *H. rufescens* juveniles stop grazing on benthic microalgae and begin feeding on macroalgae, they assume higher growth rates when cultivated under certain regimes of darkness (Ebert & Houk 1984), and with juveniles of *H. discus hannai* with certain types of refuge, which provide shading in the culture tanks (Rasse 2004).

The premise of this study is that once juveniles of *H. discus hannai* cultivated in tanks in Chile begin feeding on macroalgae, an increase in dark regimen may promote better growth based on known characteristics of their feeding behavior in Japan. Thus the objective of our study was to determine if optimal growth rates and survival could be obtained in the commercial culture of these abalone by manipulation of the light regimen in the culture tanks.

MATERIALS AND METHODS

Experimental Design

Preparation of tanks

Three smooth, white fiberglass tanks of 1,000-L capacity were used to hold culture baskets suspended at the surface. Water flow into the tanks came from 20-mm PVC tubing on the bottoms of the tanks, having 5-mm holes every 5 cm. Compressed air was introduced to the tanks with the same type of tubing on the bottom of the tanks, having 1-mm holes every 5 cm. The water tubing was installed such that injection of the water resulted in an overall rotary motion of the water along the horizontal axis of the tank from the entry zone to the exit zone. Water outflow from the tank occurred from a 60 mm standpipe fitted with a screen to prevent loss any abalone from the system. Nine identical culture baskets were used in the experiment, with three baskets per holding tank. The baskets measured $0.25 \times 0.3 \times 0.2$ m and were constructed of pearl net screen (6-mm mesh) and plastic-coated wire.

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Placement of Juvenile Abalone

Each culture basket received 20 juvenile abalone randomly selected from a single cohort produced at the Católica del Norte University (UCN) abalone culture facility. The total of 180 specimens initially measured 13.9 ± 0.3 mm and had an average drained wet weight of 0.409 ± 0.01 g each. The abalone were given a two-week acclimation period in the system prior to initiating the experiment to observe their behavior and condition. Once the abalone became acclimated and adapted to different photoperiod regimes, measurements were made of macroalgae consumption following Uki (1989).

Treatments

The abalone juveniles were cultured in three different photoperiod regimes, including total darkness, partial light, and normal light periods at 30°S Lat. Each tank was established to administer one of three different treatments designated by the letters A, B, and C (Fig. 1).

Treatment A

Culture of juveniles under normal photoperiod conditions at 30° S Lat. during November and March = 14L: 10D (hours).

Treatment B

Culture of juveniles in total darkness =0L: 24D (hours). (Tanks covered with 2-mm thick black polyethylene plastic).

Treatment C

Culture of juveniles with a photoperiod consisting of 20 h of darkness and four hours of light daily = 4L: 20D (hours). Black plastic cover removed daily between 14:30 and 18:30 h

The tanks were cleaned once a week, at which time the flow rate of water through each tank was measured by timing the fill rate of a 20-L bucket. Water temperatures were also measured, using a hand-held thermometer. Every two weeks general observations were carried out at night in one of the systems to observe any aberration in the system which might affect the progress of the experiment.

Food given to the abalone included *Ulva lactuca* and *Lessonia trabeculata* in varying proportions based on local availability of these algae at any given time. Algae given as food were renewed every three days to avoid decomposition, because the algae failed to survive for more time in the dark or short photoperiod replicates.

Sampling

Every three weeks all the abalone from all the treatments were measured for total shell length using a calliper accurate to 0.05 mm, and weight using a semianalytical balance accurate to 0.01 g. All the abalone were removed from each system in turn, and taken to the laboratory in seawater for measurement.

Every three days algae from the water storage tank at the Center for Abalone, was drained dry and weighed, to be subsequently deposited into the culture baskets and the control.

Data Analysis

Mortality was calculated as follows:

$$X\% = \frac{N^\circ \text{ non living indiv.} \times 100\%}{\text{Initial } N^\circ \text{ of indiv.}}$$

To determine if there were significant differences between the treatments, a one way analysis of variance was used to compare with the data on lengths and weights of the juveniles from each different culture basket at the end of the experiment. A multifactorial analysis of variance was applied among treatments and baskets to determine the existence of any significant differences at a 95% confidence limit, using SYSTAT Version 6.0.1 and Stadistica software.

The average specific growth rates (SGR) for the three week length and weight intervals were determined with the formulas used by Corazani (1997):

$$SGR(\%/d) = \frac{100 \times [ln(Lf) - ln(Li)]}{day}$$

where:

L_f = Final length (cm.)
L_i = Initial length (cm.)
ln = Natural log.

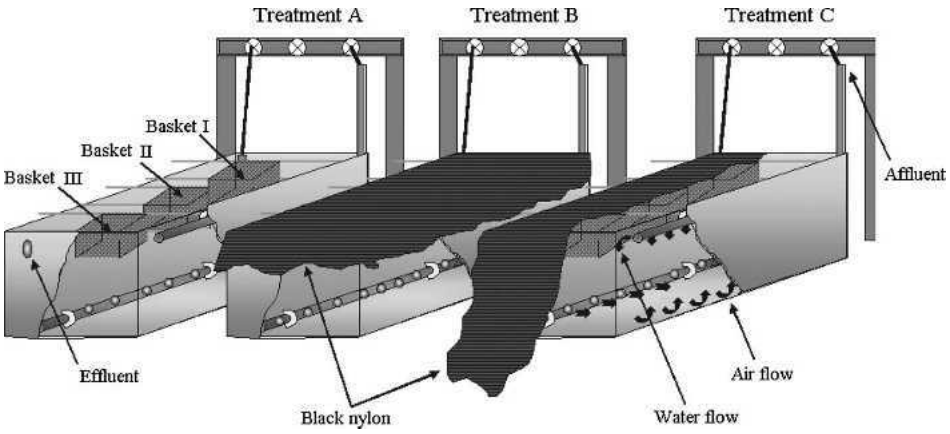

Figure 1. Scheme of treatments and the distribution of culture baskets within the culture tanks. Treatment A (14L;10D), Treatment B (OL;24D), and Treatment C (04L;20D).

$$SGR(\%/d) = 100 \times \frac{[ln(Pf) - ln(Pi)]}{days}$$

where

P_f = Final weight (g.)
P_i = Initial weight (g)
ln = natural log.

Daily food consumption per abalone was estimated using the following formula:

$$FC = F_1 - F_2(g)$$

where:

F_1 = Initial weight of the food (g)
F_2 = Final weight of the food (g).

This calculation was made for each culture basket in the experiment. Calculation of food consumption per abalone was done using the following formula:

$$\frac{F.C}{N^{\circ}\ ofind/day}(g/abalon/day)$$

After these values were obtained we proceeded to determine the average food consumption in each tank.

RESULTS

Culture Conditions

The water temperature of the culture ranged from 15.5°C to 17.0°C, with the highest temperatures found from January to March. Water flow rates through the main tanks were about 1.5 L/min for Treatment A, 0.75 L/min for Treatment B and 1.05 L/min for Treatment C.

Mortality

The percentage mortality of the abalone juveniles (Fig. 2) for each treatment was:

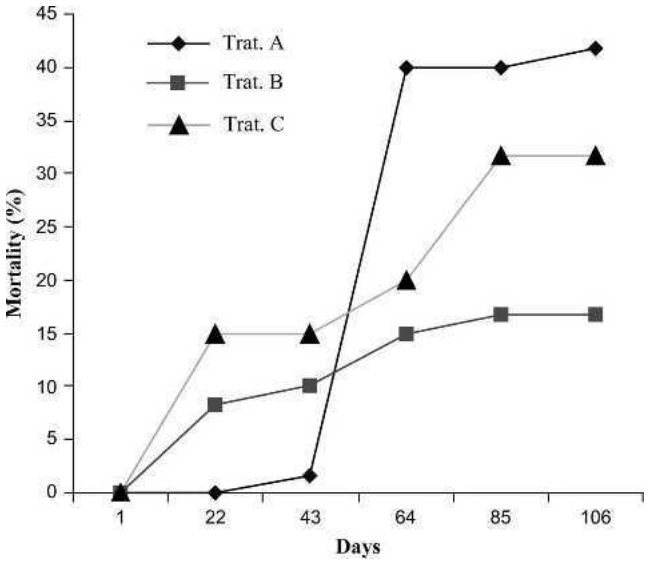

Figure 2. Percentage of accumulated mortality of juvenile Japanese abalone in each photoperiod treatment (see text) thin the culture tanks.

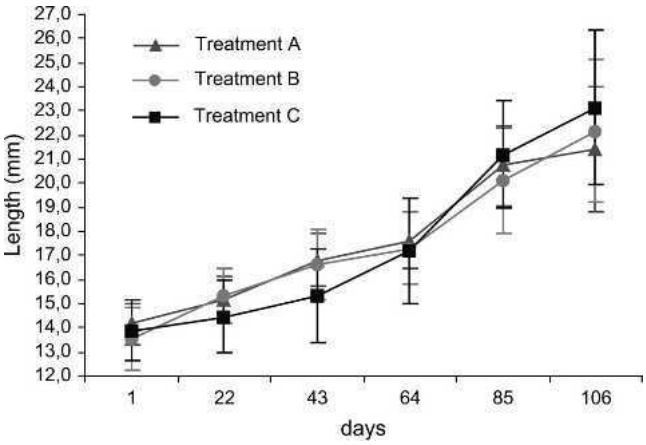

Figure 3. Average increase in shell length of cultured *H. discus hannai* in the different treatments. Treatment A (14L;10D), Treatment B (OL;24D), and Treatment C (04L;20HD).

(a) Treatment A: Figure 2 shows an anomalous (unexplained) jump in mortality from day 43 to day 64, with otherwise low and constant mortality.
(b) Treatment B: The mortality remained at 8% to 22 days in culture, remaining stable until the end of the experiment, reaching a maximum of 16%.
(c) Treatment C: The highest mortality occurred over the first 43 days at 15%, rising at 60 days to 20%, and to 31% at the end of the experiment.

Growth

Growth in Length

The mean length of the juveniles at the beginning of the experiment was 13.84 ± 0.31 mm, and the weight was 0.409 ± 0.018 g (Fig. 3). A Bartlett test was carried out on the homogeneity of the variances of the final sizes in each culture basket as a precondition for the various other statistical tests, which were applied (Sokal & Rohlf 1995).

The smallest average length reached at the end of the experiment was 21 mm, observed in Treatment A, slightly higher average length was reached in Treatment B at 22.1 mm and for Treatment C it was 23.1 mm. During the first weeks of culture the least growth in length was recorded for Treatment C,

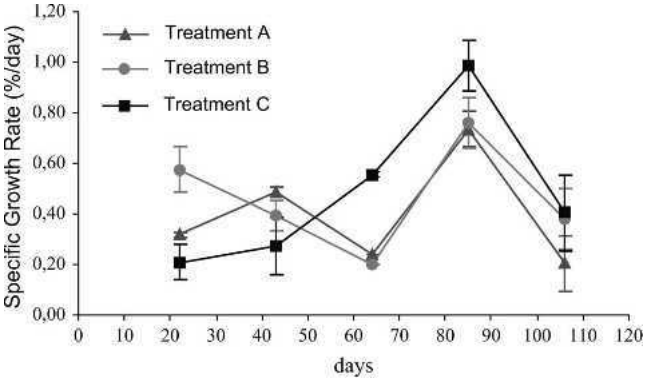

Figure. 4. Specific growth rate for juvenile Japanese abalone exposed to three different photoperiods. Treatment A (14L;10D), Treatment B (OL;24D), and Treatment C (4L;20HD).

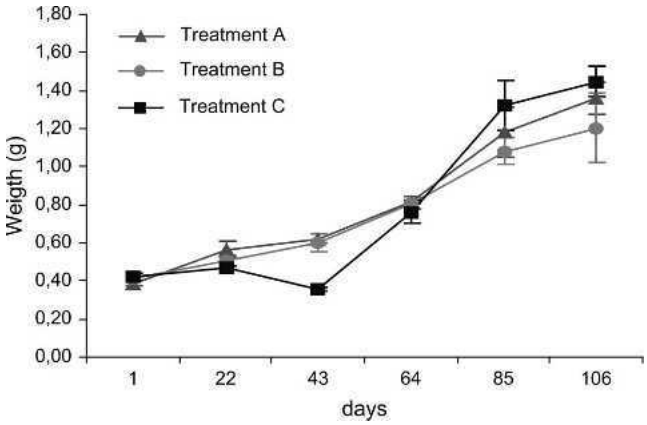

Figure 5. Average increase in total weight and total weight of cultured *H. discus hannai* in the different treatments. Treatment A (14L;10D), Treatment B (OL;24D), and Treatment C (04L;20HD).

which increased markedly between days 43 and 85. The differences in growth in length at the end of the experiment between the three treatments were significant ($P < 0.05$) (Fig. 3).

Specific Growth Rate (SGR) for Total Shell Length

The highest rates of growth were obtained with Treatment C from the beginning of the experiment until day 85, after which there was a decrease, although the rate remained higher than in Treatments A and B (see Fig. 4 later).

Growth in Weight

Figure 5 shows that the average final weights of the abalone in the three treatments were 1.2 g (A), 1.3 g (B), and 1.45 g (C), with the (C) value significantly higher than the other two values ($P < 0.05$). The specific growth rate (see Fig. 6 later) showed growth in weight of abalone in Treatment C was not uniform throughout the experiment, however, and began with negative values to 40 days, which later became positive to 64 days in culture, followed by a drop in the growth rate to the end of the experiment. Growth rates in the other two treatments began at about 85 days, lasting to the end of the experiment.

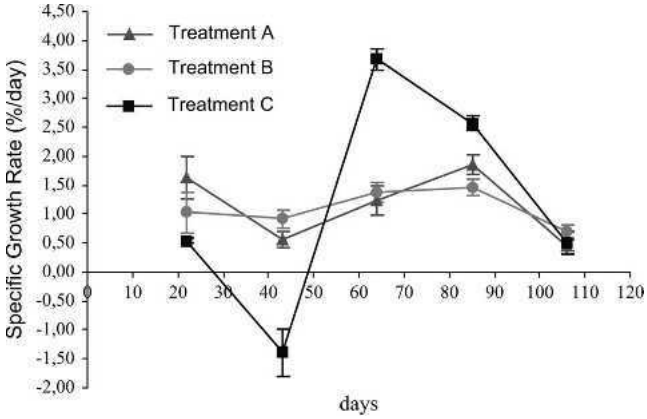

Figure 6. Specific growth rate in weight for juvenile Japanese abalone under three different photoperiods.

Feeding

The average daily food consumption by abalone in Treatment A was 0.091 ± 0.067 g, whereas for Treatment B it was 0.092 ± 0.062 g and Treatment C 0.115 ± 0.097 g. The consumption between Treatments A and B were not statistically different ($P = 0.438$).

The variation in consumption of microalgae by the juveniles during the experiment was similar between Treatments A and B compared with Treatment C which was characterized by periods of pronounced high rates of consumption during the experiment (Fig. 7).

DISCUSSION

Juvenile Japanese abalone maintained under extended periods of darkness demonstrated lower mortality than those

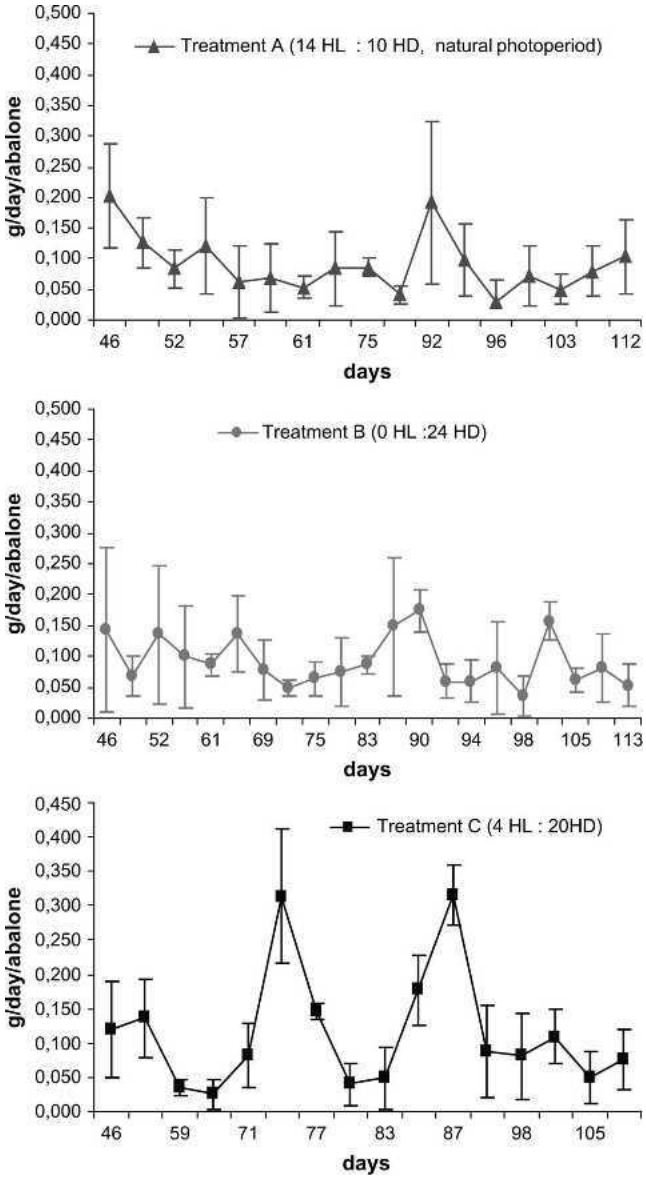

Figure 7. Daily consumption of *Gracilaria chilensis* and *Ulva sp* by juvenile Japanese abalone maintained under different photoperiods between November 1998 and March 1999.

maintained under a normal light regimen. Nie et al. (1996) working with the same species and size of abalone obtained 47.6% mortality after 165 days in culture, similar to our abalone in the natural light regimen (Treatment A, 41.7%), but higher than our rates for Treatments B and C (16.7% and 31.7% respectively). These authors reported a similar tendency in the variation of the mortality when exposed to artificially extended periods of darkness where differences in mortality between treatments may be attributable to covering and uncovering the tanks daily as in C, this study. In this study there could have been a source of stress for the partially lighted animals C compared with those maintained in total darkness B. This possibility was mentioned by Fallú (1991), where the absence of a cover to protect the abalone from light produced a state of stress, and even death in the early growth stages.

Rasse (2004), reported on the deleterious effects of light on the growth of juvenile *H. discus hannai*. In our study, abalone in Treatment A tended to agglomerate into high density groupings, potentially explaining their lower degree of growth in length and weight compared with their congeners in extended dark regimes.

Abalone juveniles in increased darkness (Treatment C) had low growth rates compared with those of the other two treatments, suggesting that acclimation to these culture conditions was slower than for animals in total darkness (Treatment B). Day and Fleming (1992) suggested that abalones required at least two months to acclimate to changes in culture conditions. This was in agreement with our observations of changes in mortality in the dark systems after 64 days.

There was an uncontrolled source of error, which may have affected the early results where, during the first three weeks, a few of the abalone in the treatments with extended darkness (Treatment C) climbed algal strands to the surface of the cages and became directly exposed to air, and were unable to return to the water. This situation was corrected by weighing the food algae to the bottom of the culture cages, and probably did not affect the overall results of this experiment. This observation, plus the observation of erratic feeding over the length of the experiment by abalone in the extended period of darkness (see Fig. 7 later) as well as the erratic pattern of specific growth in the weight of these individuals (Fig. 6) suggested this unusual light regimen caused stress in the animals, which did not resolve to a steady state during the period of observation and was the least desirable condition of the three treatments. This is further supported by their having the second highest mortality rate (Fig. 2).

The present study has emphasized the need for long periods of acclimation of the abalone prior to beginning similar studies, as well as a need for carrying out much longer term experiments, such that the cultured animals reach a steady state in their feeding and growth patterns to decide which are the more favorable culture management strategies. Remarkably, in the present study, the feeding and weight increase between abalone with the natural light regimen and those in total darkness were highly similar (Figs. 6, 7), only differing in the disparate mortality rates, which remain unexplained. Thus, until long-term future results can be obtained on the effects of presenting various light regimes to the abalone in culture this factor remains unresolved.

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