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METAMORPHOSIS AND POSTLARVAL GROWTH OF ABALONE *HALIOTIS RUFESCENS* IN A MEXICAN COMMERCIAL HATCHERY

RICARDO SEARCY-BERNAL,[1]* ESTEBAN PÉREZ-SÁNCHEZ,[1] CASANDRA ANGUIANO-BELTRÁN[1] AND ROBERTO FLORES-AGUILAR[2]

[1]*Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Ensenada, Baja California, México;* [2]*Centro de Investigación y Desarrollo de Recursos y Ambientes Costeros i-mar, Universidad de los Lagos, Puerto Montt, Chile*

ABSTRACT Metamorphosis induction and postlarval growth of the red abalone (*Haliotis rufescens*) were evaluated in a commercial farm of Baja California, México. This hatchery settles larvae with gamma-aminobutyric acid (GABA, 1-μM final concentration) and culture postlarvae in 250-L tanks placed in a four-story structure inside a building with artificial illumination. Eight tanks (four at the top and four at the bottom of a culture structure) were sampled during four months after settlement. Upper tanks received more light than the lower tanks (means of 727 and 217 lux, respectively) and had a higher mean water temperature (14.9°C and 14.4°C, respectively). Estimates of metamorphosis induction were highly variable (37% to 99%) and mean values were higher in lower (76%) than in higher (54%) tanks. Potential causes of this unexpected variability are discussed. There was a significant positive linear relationship between metamorphosis induction and early (7-day) postlarval survival. Average postlarval growth rates were also highly variable among tanks (37–63 μm/day) and slightly higher in upper than in lower tanks (60 and 52 μm/day, respectively). During the sampling period, growth variability was positively associated with water temperature changes, especially after the formation of the first respiratory pore when growth increased abruptly.

KEY WORDS: postlarvae, metamorphosis, growth, abalone, *Haliotis rufescens*

INTRODUCTION

In abalone (*Haliotis* spp.) hatcheries, metamorphosis induction and postlarval culture are key issues, because most mortality occurs during theses stages (Hahn 1989, Searcy-Bernal et al. 1992a). Abalone larvae require exogenous cues to undergo metamorphosis and in nature larvae of most species settle on crustose coralline algae (Morse 1992). In most hatcheries, conventional techniques provide these cues by biofilms (dominated by benthic diatoms) and/or conspecific mucus covering the settlement surfaces, but these cues are less effective than coralline algae. Two systems are commonly used: rectangular tanks with racks of plastic plates and round tanks without plates. The first system provides more available area for larval settlement, whereas the second provides management advantages (Hahn 1989, Leighton 2000).

Morse et al. (1979) discovered that gamma-aminobutyric acid (GABA), an inexpensive amino acid, induces metamorphosis of abalone larvae as efficiently as the natural algal inducers. This result offered new perspectives for improving settlement efficiency in abalone seed production by using GABA, but early attempts were discouraging (Akashige et al. 1981), probably because of interference by bacterial metabolism (Morse 1992). Therefore, the use of antibiotics was apparently a requirement for using GABA, which would limit its commercial application (Slattery 1992).

The first successful use of GABA without antibiotics was achieved by Searcy-Bernal et al. (1992a, Searcy-Bernal et al. 1992b) settling larvae of *H. rufescens* and *H. corrugata* in 18-L buckets (used by some commercial hatcheries). They suggested that bacterial interference might be minimized by settling larvae in clean containers and adding diatoms as postlarval feed 1–2 days after GABA. Another alternative would be to increase the GABA concentration, within safe limits, to cope with bacterial degradation (Searcy-Bernal & Anguiano-Beltrán 1998).

At present, GABA has proved to be an efficient inducer of larval metamorphosis in many abalone species of different parts of the world (Roberts 2001), it is routinely used by commercial hatcheries of the United States and México to induce larval metamorphosis in *H. rufescens, H. fulgens and H. corrugata* and it has been successfully applied in some hatcheries of Chile (R. Flores-Aguilar, personal obs.). However, studies to evaluate its effectiveness in farms are lacking. Castro-Gálvez & Searcy-Bernal (1997) provide the only evidence supporting that GABA is a better inducer than diatom films, in a hatchery of Baja California, México, using the Japanese plate system to culture *H. fulgens*.

In this study we evaluate the effectiveness of GABA in a commercial farm of México and describe the variability within tanks of metamorphosis induction and postlarval growth.

MATERIALS AND METHODS

This study was performed in the hatchery of Abulones Cultivados, a commercial abalone farm located in Eréndira, Baja California, México, currently producing ca. 25 metric tons a year. Since its establishment in 1993, this hatchery has been using GABA (1 μM) to settle larvae in fiberglass 250-L round tanks without plates. Settlement is carried out in clean tanks and the benthic diatom *Navicula incerta* is cultured and added to feed postlarvae after settlement. Seawater flow (filtered, UV treated, ca. 5 L/min) is open the next day.

The indoor postlarval facility includes 200 tanks placed in four-story structures. Each structure has 40 tanks arranged in columns of four with water flowing from top to bottom. These tanks receive natural light (through windows in the upper part of the facility's walls) and 24-h artificial illumination (fluorescent lamps above the tanks). For this study eight tanks, in the top and bottom levels of four adjacent columns, were selected.

*Corresponding author. E-mail: rsearcy@gmail.com.

The batch of postlarvae of *H. rufescens* sampled was from a spawning performed on February 3, 2004. Competent larvae were settled in 160 tanks at ca. 38,000 larvae per tank. *Navicula incerta* was added one day after settlement and powdered artificial food was added after 45 days.

Seven days after settlement dead shells from the eight selected tanks were washed off and collected by the hatchery staff. At the Instituto Investigaciones Oceanologicas (IIO) facilities these were counted and classified in stages with and without peristomial shell. Total early survival was estimated based on the number of initial larvae minus the total dead shells after seven days.

The rate of total metamorphosis induction was estimated as the number of early survivors plus the number of dead shells with peristomial shell, expressed as a percentage of the initial larvae. The early survival of postlarvae was estimated as the percent of early survival relative to total metamorphosis. That is, the number of live metamorphosed abalones as a fraction of total metamorphosis (live + dead).

Seven samplings were performed from February to June 2004 in the eight selected tanks. Temperature and light intensity (lux) were measured with conventional digital instruments. Postlarvae (ca. 20 per tank) were collected by brushing for length measurements (maximum shell length) to estimate growth, by the digital analysis of video-recorded images.

Data were analyzed by paired *t*-tests to compare tanks in the upper and lower levels (paired by tank column) and by linear regression to analyze relationships among variables. Percent survival data were subjected to the arcsine square root transformation before the analyses.

RESULTS

The estimates of total early survival and metamorphosis induction are presented in Table 1. Although early survival and total metamorphosis were higher in the lower than in the upper tanks, these differences were not significant ($t = 1.58$, $P = 0.211$ and $t = 1.48$, $P = 0.234$, respectively). At this first sampling (7 d) the average temperature and light intensity for the lower tanks were 13.5°C and 243 lux, whereas the values for the upper tanks were 14.5°C and 681 lux.

Figure 1 shows the relationship between total metamorphosis and total early survival (relative to initial larvae) and survival of postlarvae (relative to the number of larvae that completed metamorphosis). In both cases, linear regressions were significant ($P < 0.01$), although postlarval survival shows a lower slope at high metamorphosis values.

The growth rate of postlarvae, seven days after settlement, was inversely related to survival (Fig. 2) and this linear regression was significant ($P < 0.05$).

Variability in postlarval length increased over time as shown in Figure 3. It is interesting to note that in tank 68 postlarvae reached the lowest length at the end of the sampling period.

In Table 2, the average growth rates over the sampling period are shown, along with average temperature and light intensity data. Growth rates were higher in the upper tanks (59.6 μm/d) than in the lower tanks (52.0 μm/day), but this difference was not significant ($t = 1.73$, $P = 0.182$). The average temperature was 14.9°C and 14.4°C in the upper and lower tanks, respectively ($t = 10.67$, $P = 0.002$) and the average light intensity was also higher in the upper tanks (727 lux) than in the lower level (217 lux) ($t = 3.06$, $P = 0.055$). A multiple regression of average growth rate of the eight tanks on temperature and light intensity was not significant ($R^2 = 0.42$, $F = 1.78$, $P = 0.260$).

Average postlarval growth rate, across all tanks, increased over time following a similar pattern than temperature, and the main growth rate increase occurred after the formation of the first respiratory pore (Fig. 4). In this figure, growth rates and temperature data are estimated for periods between two sampling dates.

TABLE 1.

Number and stages of dead shells collected from each tank seven days after settlement and estimates of total early survival (% surv 7 d) and metamorphosis induction (% met).

	Shell Stages				
Upper Tanks	Larval	Postlarval	Total	% Surv 7 d	% Met
65	7,201	1,231	8,256	78.3	81.1
69	18,089	2,204	20,293	46.6	52.4
73	23,984	4,085	28,069	26.1	36.9
77	21,078	3,555	24,632	35.2	44.5
mean				46.5	53.7
s.dev.				22.7	19.3
Lower tanks					
68	14,428	1,662	16,566	56.4	62.0
72	323	101	424	98.9	99.2
76	14,647	1,208	15,854	58.3	61.5
80	7,067	686	7,753	79.6	81.4
mean				73.3	76.0
s.dev.				20.0	18.0

DISCUSSION

Induction of Metamorphosis

Although the average estimated rate of metamorphosis was acceptable, from the hatchery perspective, the high variability

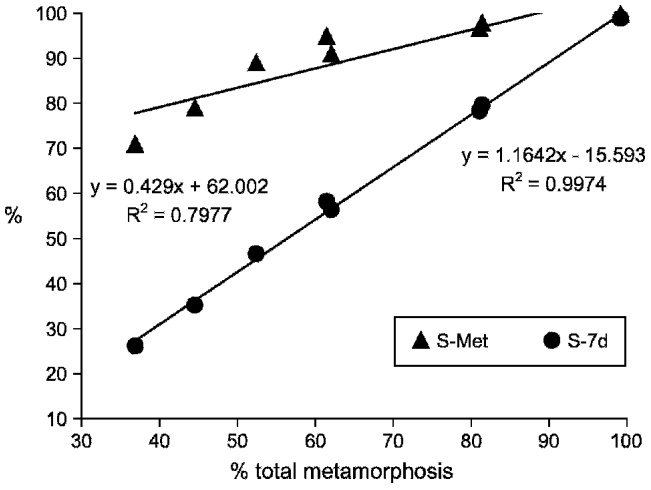

Figure 1. Relationships between the estimates of total metamorphosis and early total survival (S-7 d) and postlarval survival (S-met) seven days after settlement. Linear regression equations and lines are shown. Both regressions are significant ($P < 0.01$). Each dot corresponds to a tank sampled.

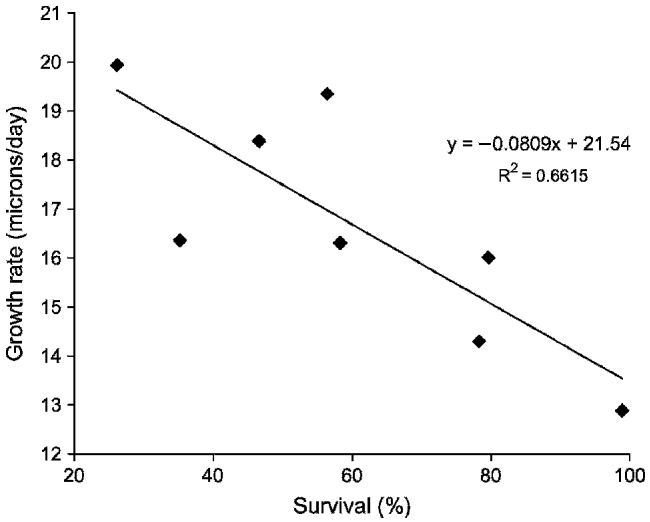

Figure 2. Relationship between postlarval growth rates and early survival. Linear regression equation and line is shown. This regression is significant ($P < 0.05$). Each dot corresponds to a tank sampled.

TABLE 2.

Average *H. rufescens* postlarval growth rate, temperature, and light intensity in each tank sampled.

Tanks: Upper	Growth Rate (μm/d)	Temperature (°C)	Light Intensity (lux)
65	57.67	14.9	493
69	63.11	14.8	743
73	61.62	14.8	493
77	56.04	15.0	1179
mean	59.6	14.9	727
s.dev.	3.3	0.1	323
lower			
68	37.00	14.3	183
72	61.62	14.4	337
76	56.04	14.4	176
80	53.16	14.4	173
mean	52.0	14.4	217
s.dev.	10.6	0.1	80

observed among tanks (Table 1) was not expected, because GABA induces metamorphosis of *H. rufescens* larvae with high efficiency and low variability in small-scale trials (Morse 1992, Searcy-Bernal & Anguiano-Beltrán 1998) and up to 18-L volumes (Searcy-Bernal et al. 1992a, Searcy-Bernal et al. 1992b).

Several factors might influence this variability. Because the estimation of metamorphic rate is partially based on the initial number of larvae, variability of these would strongly affect estimates. This variability could arise from larval counting, homogenizing and stocking procedures. Also, the collection of dead shells (used to quantify dead metamorphosed organisms) might not have been as uniform as desired. All these procedures were conducted by hatchery staff according to efficient standard methods, but variability can be often introduced because the demands of commercial operations (e.g., minimizing the time and labor required for procedures).

Variability in the number of larvae per tank would also affect results on metamorphosis induction if this process is influenced by larval density. Daume et al. (2004) report no significant effects of larval density on the induction of *H. rubra* with the macroalga *Ulvella lens*. However, they used tanks with settlement plates and their higher larval density (100/L, 0.5/cm^2) was below that used in this study (152/L, 4/cm^2). Although research is needed on the effect of larval density on the induction of metamorphosis with GABA, we have used this inducer successfully at larval densities up to 15/cm^2 (unpublished data), so probably this was not an important factor in this study.

Larval quality is another important issue to consider. In this hatchery, larvae are concentrated in buckets, and aliquots are taken to pour into tanks at the desired stocking densities. Despite efforts to homogenize larvae, those less active or dead tend to concentrate in the bottom of containers (R. Searcy-Bernal, personal obs.) and tanks receiving these larvae would probably have a lower metamorphic performance. This would

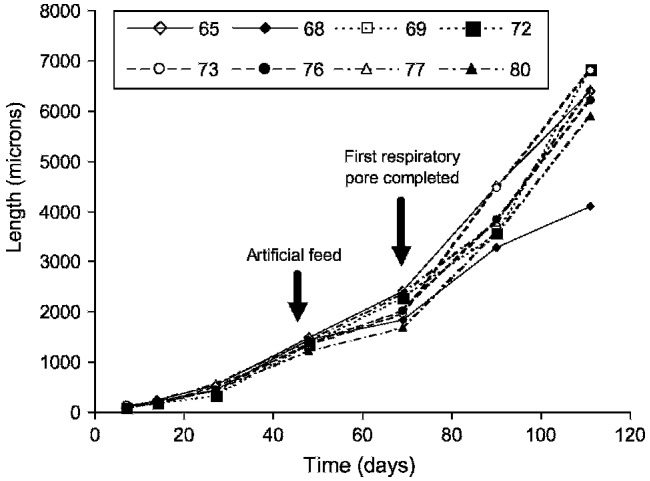

Figure 3. Shell lengths of *H. rufescens* postlarvae during the sampling period in individual tanks. The time when artificial feed was first added is shown. The first respiratory pore was completed in all postlarvae in the date shown, but the process started since the previous sampling.

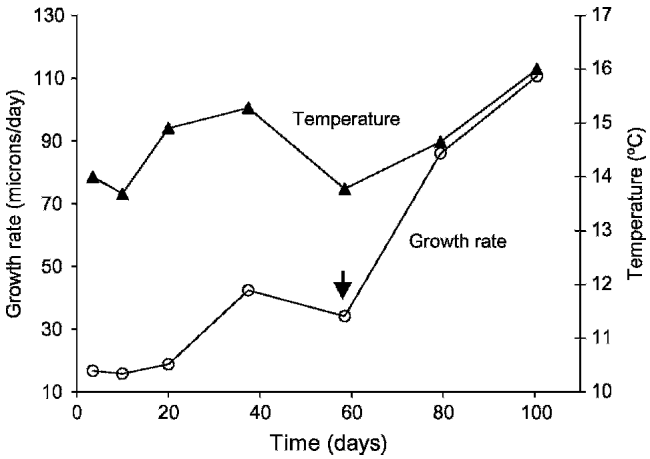

Figure 4. Average growth rates of all tanks (circles) and seawater temperature (triangles) during the sampling period. The arrow suggests the average date of formation of the first respiratory pore, which occurred between the samplings of days 48 and 69 after settlement.

explain the large quantity of dead larvae in some tanks (e.g., 73 and 77 in Table 1).

It is hard to explain why metamorphosis induction was higher in the lower tanks (Table 1). These tanks had a slightly lower temperature and received less light, but there are no previous reports on the effects of these variables on GABA induction. Further experimental studies would be required to determine their potential effect on the induction of metamorphosis in abalone larvae.

The usual method for determining the success of metamorphosis is based on sampling the settlement surfaces 1–3 days after induction, to witness the loss of velum or the secretion of peristomial shell (attachment is not a valid criteria). (Morse 1992, Searcy-Bernal et al. 1992a, Daume et al. 2000, Roberts 2001, Takami et al. 2002, Daume et al. 2004, Sawatpeera et al. 2004, Gapasin & Polohan 2005). This method may underestimate metamorphosis induction by not including larvae that might metamorphose after the evaluation or that metamorphosed and died before. The collection of dead shells after a few days to quantify dead postlarvae, as done in this study, would solve part of that problem but requires careful (and time-consuming) counting procedures of both live and dead organisms. Probably a combination of methods would be a better option as suggested by Searcy-Bernal et al. (1992a, Searcy-Bernal et al. 1992b); however, in this study it was not possible to sample the tanks surfaces to avoid interference with commercial production.

The concentration of GABA used in this hatchery (1.0 μM) is recommended for small-scale trials with *H. rufescens*, but may not be optimal for commercial production, because of potential bacterial interference (which may also differ among tanks) (Searcy-Bernal et al. 1992a, Searcy-Bernal et al. 1992b). In these cases, GABA can be increased within safe limits and a concentration of 1.5–2.0 μM has been recommended (Searcy-Bernal & Anguiano-Beltrán 1998).

GABA induces metamorphosis successfully in more than a dozen of abalone species (Morse 1992, Roberts 2001, Sawatpeera et al. 2004, Gordon et al. 2006). Although a concentration of 1 μM is usually successful for most species, the tropical abalone *H. asinina* responds better to 0.45 μM (Gapasin & Polohan 2004). *Haliotis virginea* is the only species reported that is not induced to metamorphose by a range of GABA concentrations (Roberts 2001).

Some authors have reported poor results with GABA probably because of experimental conditions. Akashige et al. (1981) tested GABA on *H. discus hannai* in a system with potential bacterial interference, as suggested by Morse (1992). Slattery (1992) concluded that GABA was not effective on *H. rufescens* in an experimental system that allowed the interaction of other cues (i.e., diatoms and abalone mucus) and bacteria with GABA. However, GABA has shown to be effective in many studies with *H. rufescens* (e.g., Morse 1992, Roberts 2001) even without antibiotics (Searcy-Bernal et al. 1992a, 1992b; Gorrostieta-Hurtado & Searcy-Bernal 2004) and recent studies have also confirmed the efficiency of GABA on *H. discus hannai* (Takami et al. 2002, Gordon et al. 2006).

More recently, Stott et al. (2004) failed to induce successful metamorphosis in *H. discus discus* larvae with GABA (with and without antibiotics) at concentrations of 2 ppm and 20 ppm, apparently confounding these with micromolar concentrations, actually testing doses one and two orders of magnitude higher than those recommended, which are known to be toxic for abalone larvae (Morse 1992, Searcy-Bernal & Anguiano-Beltrán 1998, Roberts 2001).

Early Survival and Growth

Early survival was also variable among tanks (Table 1) and this is partially explained by the variability in metamorphosis induction. There was a strong positive relationship between metamorphosis and total survival (Fig. 1) and this is explainable, because larvae that do not metamorphose eventually die (Morse 1992).

However, the relationship of metamorphosis with postlarval survival (Fig. 1) is more difficult to explain. Larval mortality might deteriorate the ecological conditions in the bottom of tanks (e.g., proliferation of bacteria or ciliates) causing mortality of postlarvae already settled.

During the first week of postlarval life, abalone growth is mostly fueled by their energy reserves (Kawamura et al. 1998). Therefore it is unlikely that diatom density would explain differences in survival or growth among tanks. However, it is interesting to note the inverse relationship between survival and growth (Fig. 2), suggesting some kind of early density-dependence not previously reported.

Density-dependent growth has been reported in larger postlarvae (ca. 660 μm) of *H. rubra*, even in conditions of abundant food (Day et al. 2004). These authors explain this pattern by exploitative (indirect) competition, because postlarval grazing in high-density conditions may increase patchiness in the diatom film, reducing the availability of food for postlarvae entering theses patches. They also consider that contacts between individuals (more intense at higher densities) might reduce growth because of elevated levels of stress. In this study, the second mechanism (or other) might be most likely operating, because particulate food is probably not critical for growth of early postlarvae.

Postlarval Growth

Average postlarval growth was lower in the lower tanks mainly because of the effect of tank 68 (Table 2, Fig. 3). In this hatchery, lower tanks receive less light despite the fact that all tanks have fluorescent lamps above. This is explained by the natural illumination during the day (when samplings were done) from windows closer to the upper tanks and the shadow of these over lower tanks. During the night these differences might disappear. The temperature in the lower tanks was also lower probably because during the sampling period (winter and spring), the average air temperature was lower than that of seawater and there was a cooling effect as water flowed from the upper to lower tanks. Probably an opposite effect would be observed during summer months. This difference of temperature between upper and lower tanks could be decreased by improving the water flow system.

However, these environmental factors probably had only a slight effect on growth variability among tanks (nonsignificant multiple regression), which was more likely affected by biofilm density and quality, among other factors.

Overall, the average growth rate increased over time and was probably influenced by temperature and developmental factors (Fig. 4). During the first two months, the growth rate followed a

similar pattern than temperature but increased sharply after the development of the first respiratory pore, when an increase in temperature was also recorded (Fig. 3, Fig. 4). The effect of these two factors on abalone growth has been documented elsewhere (e.g., Hahn 1989).

In conclusion, GABA was an efficient inducer of metamorphosis in the hatchery studied, but variability among tanks might be reduced by improving methods of larval stocking and increasing the concentration of GABA to 1.5–2.0 μM. This inducer is being applied mostly in commercial hatcheries using settlement tanks without plates. However, it has also been used to improve settlement rates in hatcheries using tanks with plates in México (Castro-Gálvez & Searcy-Bernal 1997) and Chile (R. Flores-Aguilar, personal obs.), but more research is needed on this system, which is dominant in abalone culture in the world. More research is also required in hatcheries on the factors controlling postlarval growth including biofilm density and composition, abiotic factors, and density-dependence.

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