

EFFECT OF DARKNESS AND WATER FLOW RATE ON SURVIVAL, GRAZING AND GROWTH RATES OF ABALONE *HALIOTIS RUFESCENS* POSTLARVAE

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ABSTRACT The effect of two light conditions (light at 10–13- $\mu\text{mol quanta/m}^2/\text{s}$ and darkness) in three water flow rates (0, 200, and 600 mL/min) on the survival, grazing, and growth rates of 6-day-old *Haliotis rufescens* postlarvae (pl) was evaluated. A factorial experiment with three replicates per treatment in blocks was conducted for 44 days in 2-L plastic containers with ca. 100 postlarvae each, inoculated every week with the cultured diatom *Navicula incerta*. Survival was highest (80%) in the treatment without water flow and with light, whereas the lowest (52%) corresponded to the 600-mL/min flow rate under darkness, but these differences were not significant. Initial grazing rate was significantly higher in darkness than in light (37 cell/pl/hr, SE = 1.6, and 27 cell/pl/hr, SE = 2.8, respectively). Growth rate was not significantly affected by the light treatments. However, in static conditions growth was higher in darkness (38- $\mu\text{m/day}$, SE = 2.0) than in light (34- $\mu\text{m/day}$, SE = 1.0). Growth rates of postlarvae were significantly affected by flow conditions, with means of 36 (SE = 1.3), 33 (SE = 0.7) and 31 (SE = 0.7) $\mu\text{m/day}$ in flows of 0, 200, and 600 mL/min, respectively). These results suggest that the benefits of dark conditions on the growth rate of abalone postlarvae shown in previous experiments might not occur under flow conditions. However, the flow rates tested here were apparently too high to allow an optimal postlarval growth and slower flows should be tested in future experiments. On the other hand, flow allowed the development of high diatom densities under the light condition, which were difficult to maintain in darkness.

KEY WORDS: postlarvae, *Haliotis rufescens*, growth, darkness, flow

INTRODUCTION

Abalone (*Haliotis* spp.) seed production includes the culture of postlarvae that feed mostly on biofilms dominated by benthic diatoms (Hahn 1989, Leighton 2000). Growth of postlarvae in culture systems is affected by several factors, including diatom strain (Kawamura et al. 1998, Roberts et al. 1999, Daume et al. 2000, Sawatpeera et al. 2004, Carbajal-Miranda et al. 2005, Gordon et al. 2006) and biofilm density (Searcy-Bernal et al. 2001, Day et al. 2004, Gorrostieta-Hurtado & Searcy-Bernal 2004). Light intensity is a key abiotic factor affecting postlarval growth of cultured abalone because it controls the growth of diatom films (Searcy-Bernal et al. 2003, Day et al. 2004, Watson et al. 2004, Watson et al. 2005).

Juvenile and adult abalone have nocturnal habits and display a photophobic behavior in the laboratory (Hahn 1989). The feeding and growth rates of *H. discus hannai* juveniles (ca. 30 mm shell length) increased 20% and 160%, respectively when grown in complete darkness rather than in light conditions and similar effects were detected on *H. discus* and *H. sieboldi* (Kim et al. 1997). For *H. rufescens* (ca. 40 mm shell length) feeding and growth rates improved 24% and 260%, respectively when grown in the dark (Ebert & Houk 1984). Similar findings were reported for *H. iris* (Clarke & Creese 1998).

It is not known when nocturnal habits of abalone first develop and it is generally considered that these arise after postlarval stages, because postlarvae do not exhibit a cryptic behavior (McShane 1992) and graze on diatom films under illumination (e.g., Martínez-Ponce & Searcy-Bernal 1998). Culture systems for postlarvae provide natural or artificial illumination to promote diatom growth, avoiding excessive light intensity by shading, because this condition may create

overdeveloped films and adverse ecological conditions (Ebert & Houk 1984, Hahn 1989, Searcy-Bernal 1996, Watson et al. 2004).

Recent research suggests that nocturnal habits of abalone may develop early in postlarval stages. In small-scale experiments (10-mL containers) under static conditions with newly-settled postlarvae (2–14 d) of *H. rufescens*, growth rate increased up to 300% in darkness compared with constant illumination (Gorrostieta-Hurtado & Searcy-Bernal 2004) and similar results were obtained with *H. corrugata* (Gorrostieta-Hurtado 2005). The main objective of this study is to determine whether these results would also be obtained under flow conditions.

MATERIALS AND METHODS

Abalone, *Haliotis rufescens*, larvae were provided by a local commercial hatchery (Abulones Cultivados, Eréndira, B.C.) and settled with gamma-aminobutyric acid (GABA, 1.5- μM final concentration, Searcy-Bernal & Anguiano-Beltrán 1998) at the Instituto de Investigaciones Oceanológicas (I.I.O.) facilities. Postlarvae were fed the cultured benthic diatom *Navicula incerta* before the experiment.

An experiment following a two-factor randomized block design was conducted using 2-L plastic containers (bottom area = 196 cm²) with ca. 100 postlarvae (6 d, mean shell length = 332 μm , SE = 2.1) as experimental units. Factors considered were water flow rate (0, 200 and 600 mL/min) and light condition (light and darkness). Three blocks were considered along the water table where the units were placed, because irradiance at the center was higher than at the edges (13- and 10- $\mu\text{mol quanta/m}^2/\text{s}$, respectively). One replicate per treatment was randomly assigned to each block (i.e., three replicates per treatment). In the darkness treatments, units were enclosed in black plastic bags. Seawater was filtered to 1 μm and

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UV-sterilized. Water from the static treatments was changed daily. Two days before introducing the postlarvae into the experimental vessels, these were inoculated with *N. incerta* (ca. 280 cells/mm²).

The experiment was conducted for 44 days. During this period seawater temperature was 17°C ± 1°C. Survival was determined by counting live postlarvae in the containers weekly. Grazing rates were estimated 2–3 h after the introduction of postlarvae into vessels, by measuring the grazed area (digital analysis of video-recorded images) and the diatom density (Martínez-Ponce & Searcy-Bernal 1998, Searcy-Bernal et al. 2001). This method provides only an estimation of initial grazing rates. Feeding activity is hard to quantify after this period because of the movement of diatoms into grazed areas.

Growth rates were determined based on the weekly measurements of the shell length of 15–20 postlarvae per unit, also by the digital analysis of images (Scion Image 4.0.2) recorded directly from the bottom of containers (nondestructive method) on an inverted microscope using a high-resolution camera (Sony SSC-C374).

The density of the diatom *N. incerta* was determined periodically by counting three ungrazed microscopic fields (×400 final magnification). These measurements reflect the highest diatom density achieved in each treatment and may be used to compare, at least qualitatively, the food supply for postlarvae among treatments. The actual food availability is difficult to estimate because of the patchiness of the distribution of diatoms and abalone postlarvae. However, these usually concentrate at the edges of ungrazed films for feeding.

Re-inoculations of diatoms were performed in those containers with low densities, trying to reach adequate levels suggested by previous experience. During the first four weeks, diatom densities were attempted to be kept around 3,000 cells/mm² and during the last two weeks around 8,000 cells/mm². In previous trials these densities promoted optimal postlarval growth of this abalone species (see Gorrostieta-Hurtado & Searcy-Bernal 2004). The new density after reinoculation was also determined. This procedure was required especially in the static and dark treatments (Table 1).

Data were analyzed by two-factor ANOVAs for block designs (Steel et al. 1997). Percent survival data were subjected to the arcsine square root transformation before the analyses.

Block effects were never significant and are not reported here. In all tests the error degrees of freedom were 10.

RESULTS

Survival at the end of the experiment was 70% to 80% in all treatments except for the dark-600 mL/min treatment where the lowest survival (52%) was observed. These differences were not significant for light condition (F = 3.07, P = 0.11), flow rate (F = 2.40, P = 0.14) or interaction (F = 0.32, P = 0.72).

Grazing rates were significantly higher in darkness than in light (37.0 cell/pl/hr, SE = 1.6 and 27.1 cell/pl/hr, SE = 2.8, respectively; F = 13.74, P = 0.004). There was no significant effect of flow rate (F = 2.66, P = 0.11) but a strong interaction was detected (F = 3.62, P = 0.06) (Fig. 1).

Shell length data are presented in Figure 2. By the end of the experiment the largest postlarvae corresponded to the static treatments. Figure 3 shows postlarval growth rates in different periods during the experiment. During the first 12 days there was an increased growth at moderate flow rates (200 mL/min) (Fig. 3a) but after this period growth decreased in flow conditions. The highest final growth rates were obtained in static conditions (34.4 µm/d, SE = 1.0, and 37.9 µm/d, SE = 2.0, in light and dark conditions, respectively) and the lowest in the 600 mL/min flow rate (30.9 µm/d, SE = 0.9 and 30 µm/d, SE = 1.0, in light and dark conditions, respectively) (Fig. 3d). Flow effects on final growth rates were significant, but light effects and interactions terms were not significant at any time period tested (Table 2).

Diatom density increased rapidly in the light treatments under flow, reaching values above 6,000 cells/mm² after the first week (Fig. 4) but in static conditions reinoculations were required to keep densities above 3,000 cells/mm² (Table 1). In dark conditions diatom densities were low during most of

TABLE 1. Inoculation scheme of *Navicula incerta* in experimental treatments. Those with an asterisk were reinoculated in the specified day after the start of the experiment, because of low diatom densities.

Day	Light			Darkness		
	0 mL/min	200 mL/min	600 mL/min	0 mL/min	200 mL/min	600 mL/min
2				*	*	*
6				*	*	*
12				*	*	*
19	*			*	*	*
26	*			*	*	*
29	*			*	*	*
37	*	*	*	*	*	*

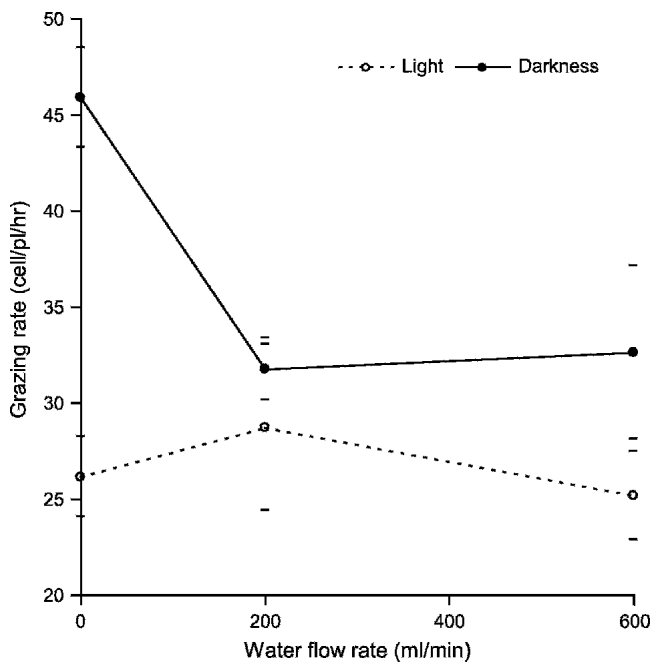


Figure 1. Grazing rates (mean ± std. error, n = 3) of *H. rufescens* postlarvae fed with *N. incerta* in different water flow rates and light conditions.

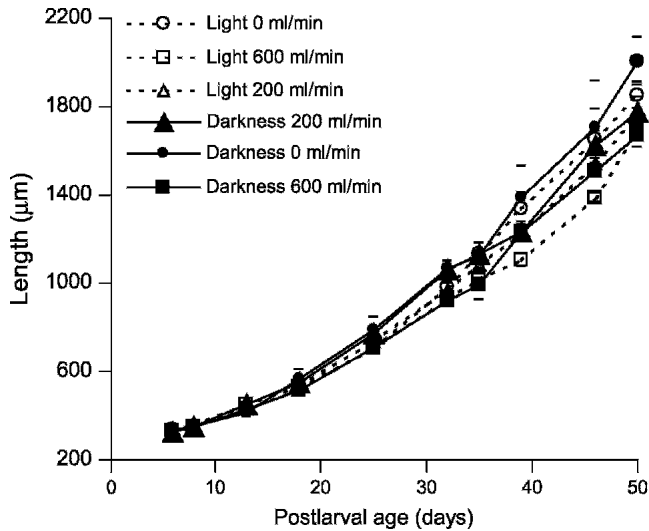


Figure 2. Shell length (mean \pm std. error, $n = 3$) of *H. rufescens* postlarvae at different ages during the experimental period, in the different treatments.

the experiment, despite reinoculation efforts (Fig. 4, Table 1). During the experimental period no other diatom species were detected in containers.

DISCUSSION

Effect of Darkness (Evidence of Nocturnal Habits in Abalone postlarvae?)

Overall, darkness stimulated postlarval growth of *H. rufescens* under static conditions (Figs. 2, 3), which is consistent with other small-scale trials on this species (Gorrostieta-Hurtado & Searcy-Bernal 2004) and *H. corrugata* (Gorrostieta-Hurtado 2005). This effect was detected despite the fact that food supply

TABLE 2.

Result of ANOVAs for growth rates in different periods after the start of the experiment. Initial age of postlarvae was 6 days. Block effects were not significant (error df = 10).

Period (days)	Light Condition		Water Flow Rate		Interaction	
	F	P	F	P	F	P
0–12	1.43	0.26	0.81	0.47	1.24	0.33
12–29	0.17	0.68	2.17	0.17	0.56	0.59
29–44	0.58	0.46	2.05	0.18	0.89	0.44
0–44	0.65	0.44	8.3	0.008	0.92	0.43

was generally lower in the dark treatment even after periodic reinoculation effort (Fig. 4, Table 1).

The most obvious explanation of this pattern is a difference in feeding activity. Grazing rates were also higher in dark conditions (Fig. 1) and this result is consistent with small-scale trials with *H. corrugata* (Gorrostieta-Hurtado 2005). Although these studies measure grazing rates only during the first 2–3 h of the experimental period, they support the hypothesis of an early development of nocturnal feeding habits even though, during the first weeks after settlement, abalone postlarvae do not display photophobic behavior and show feeding activity even under constant illumination (e.g., Martínez-Ponce & Searcy-Bernal 1998, Searcy-Bernal et al. 2001). This is also partially supported by a preliminary trial by Vélez-Espino (1999) on grazing rates of *H. fulgens* during a 24-h cycle. He reported a significant increase in feeding activity of early postlarvae (15- and 30-day-old) about an hour before sunset but in older postlarvae no significant differences between day and night hours were detected.

The only other paper available to the authors, comparing abalone postlarval growth in dark and light conditions is that

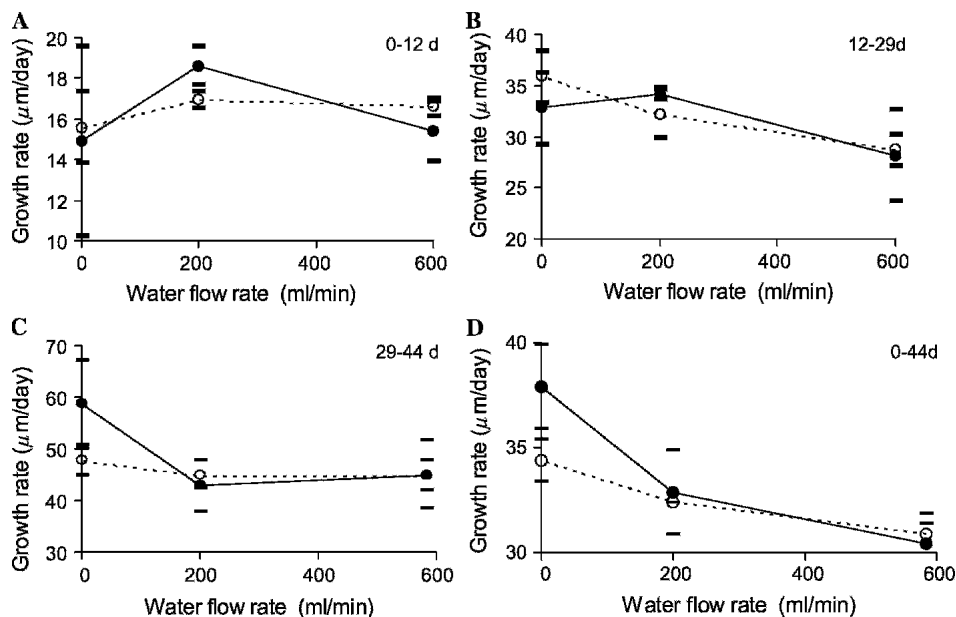


Figure 3. Growth rates (mean \pm std. error, $n = 3$) of *H. rufescens* postlarvae in different periods during the experiment. The initial postlarval age was 6 d. ---○---, Light; —●—, Darkness.

by Stott et al. (2004), using plates coated with artificial feeds. They reported no differences in growth rate of *Haliotis diversicolor supertexta* postlarvae between light and darkness (four weeks after settlement) and explained this result by considering that microalgae that may have grown on illuminated plates provided postlarvae no advantages in terms of growth. However, an alternative hypothesis would be that postlarvae in the dark grew as well as those in light despite the absence of microalgae, probably because of an increased feeding activity.

Evidence on photophobic habits in abalone postlarvae can also be extracted from studies comparing different light intensities by shading procedures. For instance, postlarvae of *H. fulgens* grew better in low irradiances of 6 $\mu\text{mol quanta/m}^2/\text{s}$ than in higher light intensities (24–75 $\mu\text{mol quanta/m}^2/\text{s}$) (Searcy-Bernal et al. 2003).

In a commercial-scale trial with *H. rubra*, Huchette (2003) reported a higher growth rate of postlarvae (ca. 1 mm shell length) in shaded tanks than in unshaded tanks (13.3 and 11.5 $\mu\text{m/d}$, respectively) during the first weeks of the trial (although growth in unshaded tanks was higher thereafter).

Watson et al. (2004) conducted an experiment with early juveniles (ca. 3.5 mm) of *H. laevigata*, fed two diatoms in shaded and unshaded aquaria (77 and 270 lux in the bottom, respectively). When fed *Cocconeis* sp. postlarvae grew better in shaded aquaria, despite a lower food supply. Moreover, these authors documented a negative phototactic behavior of juveniles, positioning themselves under airlines, under plate racks or in corners of the aquaria during the day, especially in the unshaded aquaria, although grazing during the night was evident (cleared areas over plates).

On the contrary, Day et al. (2004) reported that postlarval growth of *H. rubra* was higher in unshaded than in shaded containers, during the first five weeks after settlement. This experiment was conducted with water flow, but apparently without reinoculation of food. Therefore, food limitation in shaded containers might help to explain differences in growth

rates. Watson et al. (2005) reported that grazing rates of *H. laevigata* juveniles feeding on *Navicula jeffreyi* were higher in unshaded (22 $\mu\text{mol quanta/m}^2/\text{s}$) than in shaded (4.7 $\mu\text{mol quanta/m}^2/\text{s}$) containers. However, biofilms were denser in unshaded conditions and that result might reflect a positive relationship between grazing rates and diatom density as reported for *H. fulgens* postlarvae (Searcy-Bernal et al. 2001).

Other hypotheses may also help to explain the higher postlarval growth in the dark (or shade) than in the light, or the similar growth despite reduced food supply. High light intensities may result in high oxygen supersaturation levels in the boundary layer, which might affect postlarval fitness (Searcy-Bernal 1996). These levels can be more extreme than the subsaturation concentrations found under darkness (Roberts et al. 2006). Although there is some evidence that abalone postlarvae can survive in up to 150% oxygen saturation (Loipersberger 1996), information on potential sublethal effects is lacking.

On the other hand, the nutritional value of diatoms for abalone postlarvae might change in different light regimes. For instance, it has been reported that the benthic diatom *Navicula perminuta* produces mucus at a higher rate in darkness than under light conditions (Smith & Underwood 2000) and diatom mucus plays an important role in early postlarval nutrition of abalone (Kawamura et al. 1998). In a recent study, Watson et al. (2005) reported higher protein and lower fat contents of the benthic diatom *Navicula jeffreyi* in shaded (4.7 $\mu\text{mol quanta/m}^2/\text{s}$) than in unshaded (22 $\mu\text{mol quanta/m}^2/\text{s}$) culture conditions.

Effect of Water Flow Rate

The pattern of postlarval growth observed in static conditions (higher growth in darkness than in light) was not maintained under flow conditions. Overall, growth rates were similar in dark and light under flow conditions (Fig. 3). This is consistent with results from a preliminary trial testing the effects of darkness and diatom density on the growth of *H. rufescens* postlarvae, at a similar flow rate than the highest in this experiment (Gorrostieta-Hurtado 2005).

Diatom densities increased rapidly with flow only under light conditions (Fig. 4) and food supply remained low in the dark treatments during most of the experimental period, despite reinoculation efforts (Table 1). Growth rates of postlarvae in the dark (under flow) were similar to those in the light, despite the reduced food availability.

Although the flow rates tested here had been used in previous trials in our laboratory (Gorrostieta-Hurtado 2005), these were probably too high for optimal postlarval development, probably because of reduced feeding and the energetic costs of staying in place or move against the water flow (Donovan & Carefoot 1997). This is supported by the observation that grazing rate decreased in dark conditions under flow, falling to levels similar to those observed in light. (Fig. 1).

Water flow stimulated diatom growth under light but not in the dark (Fig. 4) and denser diatom films probably provided some protection for postlarvae against water flow. This would explain the lowest survival (52%) and lowest growth rate (30 $\mu\text{m/day}$, Fig. 3) in the highest flow rate (600 mL/min) under the dark condition.

Besides stimulating diatom growth, water flow replaces nutrients and gases, washes wastes away and reduces boundary

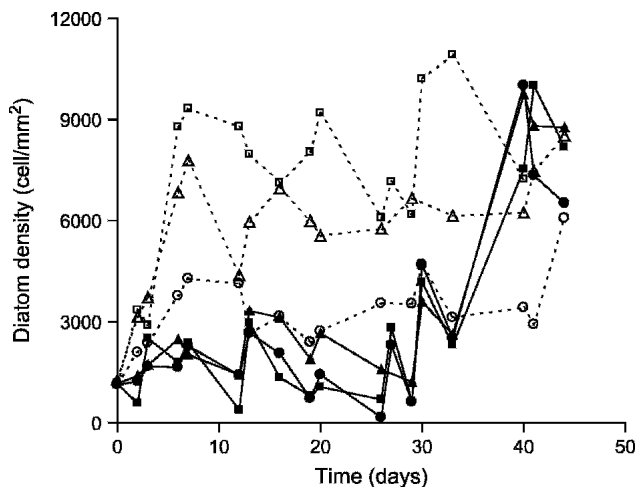


Figure 4. *Navicula incerta* density in the plastic containers during the experimental period. Day 0 corresponds to the introduction of postlarvae into vessels, but these were first inoculated with the diatom two days before with ca. 280 cells/mm². - - ○ - - , Light 0 ml/min; - - △ - - , Light 200 ml/min; - - □ - - , Light 600 ml/min; —●— , Darkness 0 ml/min; —▲— , Darkness 200 ml/min; —■— , Darkness 600 ml/min.

layer problems (Roberts et al. 2006), so there should be slower flow rates than those tested here, that would provide these benefits without interfering with postlarval locomotion and grazing, and thus resulting in increased growth. Water flow can also increase feeding rates and growth in larger juveniles of *H. laevigata* (Fleming et al. 1997, Higham et al. 1998).

It is difficult to compare the flow rates tested here with those used in hatcheries or other experiments. Water exchange rates were probably higher in this study but the water velocity over culture surfaces may be more important to compare and this is usually not available.

In conclusion, this study confirms previous trials showing increased grazing and growth rates of abalone postlarvae when grown in the dark without water flow, and these results support the hypothesis of the development of nocturnal habits earlier in the life history of abalone than previously considered. This pattern was not observed in flow conditions probably because flow rates were too high, and more research with slower flows is required. The potential benefits of culturing postlarval abalone

in the dark (or very low light intensities) would depend on the practicability of maintaining an adequate food supply, which may be a difficult task. Possible approaches to maintain biofilms, whereas benefiting from the photophobic behavior of postlarvae, besides intensive diatom cultivation, would include to either alternate periods of light and darkness or to increase the duration of nocturnal periods.

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