Journal of Shellfish Research, Vol. 26, No. 3, 777–781, 2007.

EVALUATION OF THREE METHODS FOR TRANSPORTING LARVAE OF THE RED ABALONE *HALIOTIS RUFESCENS* SWAINSON FOR USE IN REMOTE SETTLEMENT

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ABSTRACT Three closed systems were evaluated for the transport of abalone larvae for use in remote settlement associated with mass culture activities. The first of these systems maintained the larvae in seawater, the second in seawater with oxygen added, and the third in wet condition without seawater but with oxygen added. Survival of the larvae was determined within each system over periods of 10, 16, and 36 h. In a separate experiment, larval settlement in aquaria was determined after holding the larvae in the three different transport systems for 10 h. The results showed the best survival was obtained for the larvae in water alone, with survival rates ranging from 97% at 10 h to 63% at 36 h. In the system without water these rates were 88–50% respectively. The use of oxygen in the transport systems only produced a positive effect at 36 h. Larval settlement after 10 h showed no significant differences in relation to transport system, from 60% settlement of larvae transported in water to 54% settlement of larvae in the wet condition, not suspended water.

KEY WORDS: abalone culture, *Haliotis rufescens*, competent larvae, transport systems, remote setting, Chile

INTRODUCTION

The California red abalone *Haliotis rufescens* was introduced to Chile in 1977 (Godoy & Jerez 1998) in an attempt to diversify the nation's aquaculture potential using a resource having a high market potential in overseas markets. Since its introduction, there have been various studies carried out to adapt this culture to local conditions. National production in 2003 was 120 tons, increasing in 2005 to 342 tons (SERNAPESCA 2005). Although this activity has an important potential for expansion, at present the availability of "seed" stock is limited, and the demand for these juvenile abalone is unsatisfied.

The technique of remote setting of abalone involves producing massive quantities of competent ("ready to settle") veliger larvae in hatcheries established in climatically favorable zones, and then transporting these larvae to different regions better adapted for growout of settled juveniles (Devakie & Ali 2000). This involves storage and transport of the competent larvae, in which their survival and capacity to settle and grow is maximized. The aim of this technology is to allow both hatcheries and growout systems to exist independently in the most favorable environment for each operation. (Bohn et al. 1995); it is less economical to have both systems established in the same environment. The advantage of this culture strategy is optimization of production of a constant supply of presetting larvae for growout systems in various regions of the country (Holiday et al. 1991, Jones et al. 1993), with larvae produced throughout the year in large quantities for transport to wherever they may be required (Donalson 1991). In Chile, remote setting technology is used in the commercial production of *Argopecten purpuratus*, and the Japanese Oyster *Crassostrea gigas;* the former species is used to supply culture systems within Chile, and the latter are produced for the export market. Mass hatchery culture of abalone larvae has allowed for scientific research (Hahn 1989, Roberts 2001), bioassays (Hunt & Anderson 1989), attempts at repopulation of natural habitats by diver release of larvae in target areas (Preece et al. 1997, Schiel 1991, Tong et al. 1987), and allowing for various larval culture centers to exist, which provide competent larvae for remote setting at distant growout centers (Kurita et al. 1978, Tong & Moss 1991, McBride 1998). Although various forms of transport have been described for abalone larvae (Kurita et al. 1978, Preece et al. 1997, Schiel 1991, Tong & Moss 1991) there are few data available on the survival of the larvae after transport over different time periods (resistance tests). Also, there have not been comparisons of different possible transport methods given within individual reports. An adequate larval transport method is required to decrease larval mortalities and thus improve the economics of remote setting technology. The present report presents data concerning the improvement of this technology through experimentation with several possible methods for successful transport of competent abalone larvae, maximizing their survival and success in settlement and post-larval growth.

MATERIALS AND METHODS

Abalone broodstock maintained at the Coastal Center of the Universidad Católica del Norte, Coquimbo, were induced to spawn, and the larvae were cultured following the methods of Mazón-Suastegui et al. (1991). The experimental design of the transport methods was tested using competent larvae (those showing crawling behavior), which had been cultured at 14°C. The larvae were collected on 100-μm mesh screen, and suspended in seawater at 50 larvae/mL preceding placement in transport units. The transport containers were one-liter polystyrene (0.01 mm gauge) bags. Two transport units were established with 400 mL of larval suspension in seawater, one of which contained only water, excluding all air and closed with an elastic band (SW); a parallel system received about 400 mL of pure oxygen in the bag over the water (SW + O_2). A third transport unit was established in which 400 mL of the larval suspension was screened off on a 10 × 10 cm piece of 100-μm screen, and deposited in a bag with seawater-wet paper towel to maintain humidity, plus one liter of pure, "dry" oxygen (Dry + O_2). Three replicates were run per temperature for the three

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transport systems. All the systems were placed in the refrigerator at 6 ± 2°C, with three systems per treatment removed for observation after periods of 10, 16, and 36 h.

Evaluations of settlement were carried out in 20-L transparent plastic aquaria having a total internal surface area of about 3,200 cm^2. A density of 2 veligers/cm^2 was used in each test following the method of Ebert & Houk (1984). Based on the available surface and density chosen for settlement, we calculated a requirement for 6,400 veliger larvae per aquarium, and 2×10^4 larvae per bag to prepare three replicates for each system.

The test aquaria were numbered and prepared to receive larvae three days prior to settlement tests. Aquaria were washed with dilute HCl, rinsed with freshwater and left to dry for one day to eliminate microbial contamination. They were then filled with 100- μm filtered UV irradiated seawater, and they inoculated with axenic laboratory cultures of *Nitszchia* sp. and *Tetraselmis* sp. to produce microalgal films on the aquarium surfaces.

Three transport units representing each storage time cold were removed from the refrigerator and allowed to come to room temperature over a one-hour period. The systems were then opened, and the larvae without water were resuspended in 400 mL of microfiltered seawater. The larvae within the bags were then resuspended by agitation with a pipette, and 3 mL samples were deposited in watch glasses and observed under a stereoscopic microscope to determine the percentage of survival in each sample.

Prior to carrying out the sampling to determine settlement, all aquaria were completely rinsed to assure elimination of all dead and nonsettled larvae Five 25-cm^2 quadrates in each aquarium were selected randomly, and all larvae within the quadrates were counted by naked eye. The average number of settled larvae per quadrate was used to calculate the approximate number of larvae settled per aquarium. Evaluations of settlement after holding the larvae for 10 h. were done in three replicates, as well as the survival of the larvae in the maintenance systems over the three time periods tested. In summary, a total of 36 larval transport systems were used, plus 9 aquariums for settlement, and a total of 7.2×10^5 larvae.

A two-way analysis of variance (ANOVA) was used to determine significance among survival in the different types of transport systems and for different storage times. All percentage values were transformed by arcsine root transformation prior to the analysis to normalize the variances and reduce the homocedasticity (Snedecor & Cochran 1989). This was followed by use of a Tukey range test (Zar 1984) for comparisons of differences. The evaluation of larval settlement in the aquaria was carried out using a simple ANOVA, which permitted determination of differences because of their stays in the different transport systems. The analyses were carried out using SYSTAT 8.0 statistical software.

RESULTS

Survival of the larvae (Table 1, Fig. 1) in the "SW + O_2" system was 99 ± 1%, 89 ± 3% and 76 ± 2% at 10, 16, and 36 h. respectively. With the "Dry + O_2" system we obtained 88 ± 2%, 72 ± 3% and 50 ± 1%; the "SW" system gave survival values of 97 ± 2%, 89 ± 2% and 63 ± 4%, which fell between the two preceding data sets over the same test periods. Survival of larvae in the oxygen-enriched transport system without water was not much lower than in the preceding systems, with a minimum at 36 h with 50% survival. The two-way ANOVA, which related to the system used and times used showed significant differences among systems and times ($P < 0.001$). No interaction was observed between the systems and the times ($P < 0.05$). The Tukey test showed that the results between systems with water and oxygen-enriched water were not significantly different at the 10 and 16 h times ($P > 0.05$), with the means of these results superior to those obtained without water. Whereas in the third time period (36 h.) each system was different, with the highest mean value obtained from "SW + O_2". Survival was significantly different among all the times incubated ($P < 0.05$), for each maintenance system.

Results of the settlement trial carried out over 10 h are presented in Table 2. No significant differences were encountered among any of these results ($P > 0.05$).

TABLE 1.

Percentage survival of *Haliotis rufescens* pediveliger larvae during maintenance in three different transport units over three different time periods.

	Transport Units		
Time (h)	SW + O_2	SW	Dry + O_2
10	99 ± 1[a]	97.33 ± 2.08[a]	88 ± 2[b]
16	89 ± 3[c]	89 ± 1.73[c]	72 ± 3[d]
36	76.33 ± 2.31[e]	63 ± 3.6[f]	50 ± 1[g]

SW = seawater ± = 1 standard deviation.
In the columns, the means with the same superscripts showed no significant difference (Tukey Test $P > 0.05$).

DISCUSSION

When maintaining the larvae in the water for more than 10 h we observed an increase in suspended organic matter, and groups of larvae became trapped in mucus strands from which

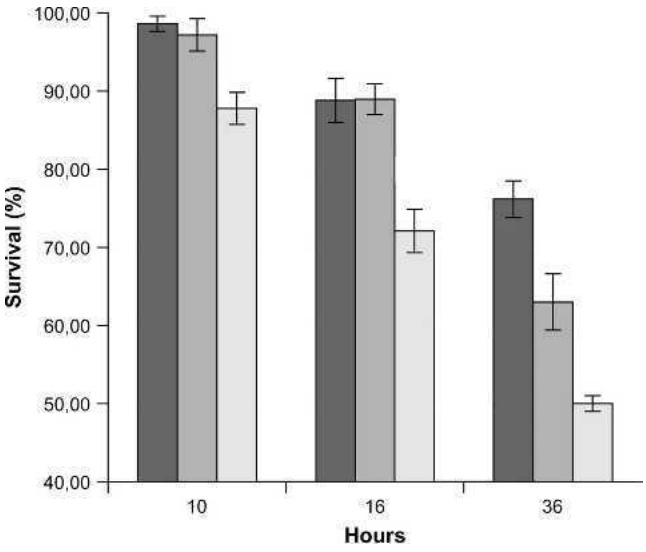

Figure 1. Percentage survival of *Haliotis rufescens* veliger larvae in three experimental transport units described in text over three test periods. ■, SW + O_2; ■, SW; □, Dry + O_2.

TABLE 2.

Mean percentage settlement of *Haliotis rufescens* veliger larvae treated in three transport units as listed in Table 1.

	Transport Units		
Time (h)	SW + O_2	SW	Dry + O_2
10	54.08 ± 9.61	60 ± 6.46	52.38 ± 11.61

SW = seawater ± = standard deviation.

they could only be liberated with difficulty; successful continuation of the settlement process became impeded. Ebert & Houk (1984) described this condition, and its association with the proliferation of pathogenic bacteria (*Vibrio* spp.). Some authors reported that the use of antibiotics prevented contamination of the medium, allowing maintenance of abalone larvae without changing the water for several days (Roberts 2001). The few studies on the transport of larval abalone available from the literature are listed in Table 3. Schiel (1991) described the transport of *H. iris* larvae in plastic bags in cold water, Kurita et al. (1978) transported *H. discus* for four hours in bags with seawater plus oxygen at 18°C, achieving high survival rates. Tong et al. (1987) and Tong & Moss (1991) achieved a high survival rate in the transport of *H. iris* larvae in a system originally designed for the transport of oyster larvae. McBride (1998) described transport of abalone on screens in a humid condition at 4°C under oxygen and achieved 90% survival although not specifying the time held. In conclusion, this study describes the various means of transport used by other authors, making comparisons with our methods.

The authors who tested transport in water used lower densities than those of our study (20/mL maximum) and Kurita et al. (1978) transported larvae in "SW + O_2" for four hours (density 10/mL at 18°C) and obtained 85% survival, which was similar to our results with water and oxygen-enriched water after 16 h. The positive effects on the survival of the larvae by supplying oxygen to water-containing transport units was noted for all the different methods and for all the different times, with significant difference from the nonoxygenated systems noted at 36 h ($P < 0.05$). The "Dry + O_2" method of storing the larvae of other species of abalone reported good survival results without the use of oxygen. This agrees with the opinion of McBride (1998) that the use of in-water transport of larvae greatly prolongs their survival. Results from the "Dry + O_2" transport units of the present study were similar to those given by McBride (1998) and obtained comparable survival values (90%) but only over a 10 h time period. This survival also resembles the results obtained by Tong & Moss (1991), but it is important to indicate that these authors obtained 86% survival, whereas transporting the larvae under more crowded conditions ("clump of larvae") and without oxygen for 24 h.

It is known that abalone are capable of using alternate biochemical mechanisms for carrying on metabolism independent of oxygen concentration, which permits them to survive periods of hypoxia and even anoxia (Hindrum et al. 2001). Abalone are also capable of obtaining environmental oxygen by diffusion through the mantle under humid conditions (McBride 1998). Both these conditions may have occurred in the "Dry + O_2" transport of larvae, as long as the larvae were able to absorb oxygen through their tissues. In the present study, the larvae were observed to be retracted into their shells under "Dry + O_2" transport conditions. Ebert & Houk (1984) reported that under normal conditions at culture centers, 90% of the larvae can reach the presettlement stage; settlement and metamorphosis of these larvae is variable however, often reaching only 50% success. Some observations have shown that only 30% of settled larvae have lost the velum and developed peristomial teloconch, with the remaining larvae failing to pass metamorphosis. Sampling carried out over 72 h of exposure of competent abalone larvae to a substrate showed that this time period was sufficient for the abalone to settle and pass metamorphosis (Hahn 1989), although only 30% of the settled larvae indeed passed metamorphosis. The preceding represents a "normal" situation in which the cultures included settlement surfaces coated with microalgae known to induce settlement, on which the larvae metamorphosed slowly, with most of the larvae occupying the water column for several days although competent for metamorphosis (Slattery 1992, Morse 1984). The fact that there were no significant differences between settlement percentages of larvae maintained for 10 h in each of the three transport units tested ranging from 52.38 to 60.00 (Fig. 2) indicated a degree of success in using all the systems, although there were high standard deviations associated with the use of

TABLE 3.

Data from the literature on transport of abalone larvae under various conditions.

Author(s)	Species	System	Density/N°	T	S	A
Tong et al. (1987)	*Haliotis iris*	SW	12.5/mL			
Schiel (1991)	*Haliotis iris*	SW	20/mL			10
Tong & Moss (1991)	*Haliotis iris*	Dry	200,000	24	86	
Kurita et al. (1978)	*Haliotis discus*	SW + O_2	10/mL	4	85	
McBride (1998)	*Haliotis sp*	Dry + O_2			90	
Preece et al. (1997)	*Haliotis rubra*	Dry				
	Haliotis laevigata	Dry				
Kawamura et al. (1998)	*Haliotis discus*			5	100	
		SW	50/mL	10	97	60
Present study	*Haliotis rufescens*	SW + O_2	50/mL	10	99	52
		Dry + O_2	20,000	10	88	54

T: Time (hr) S: Survival (%) A: Settlement (%).

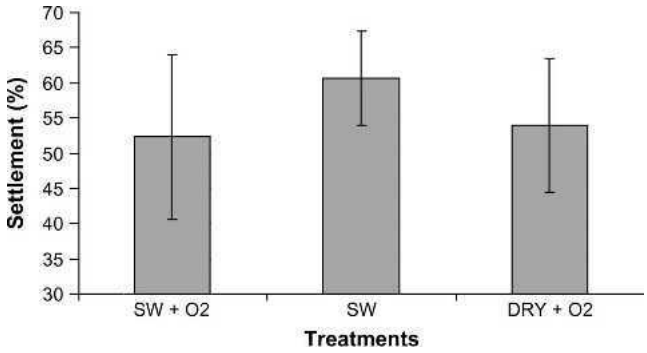

Figure 2. Settlement over a 10 h period of larvae, which had been maintained in three experimental transport units as described in text.

the "Dry + O_2" transport units. Among the authors who reported on larval transport, none made methods comparisons, nor reported on settlement success (Schiel 1991) mainly because the larvae were transported to the coast and released into the environment with the aim of repopulation. Only Preece et al. (1997) observed the decrease in settlement with extension of the times the larvae were maintained in the "Dry + O_2" condition. This occurrence could have possibly been avoided by maintaining the larvae under refrigeration overnight prior to making their trials, rather than directly transporting the larvae from cultures and carrying out an immediate "seeding" trial. Results of the present study were within the ranges of survival results obtained in previous studies (50% to 90%) by other authors (Ebert & Houk 1984).

Although good survival of larvae was obtained when they were stored in water rather than on a wet screen, at least up to a period of 10 h, there was no clear distinction between the two methods in terms of the subsequent settlement rates of these larvae. Thus, of the three methods tested, it is suggested that the "SW" or "Dry + O_2" method be used for transport, depending on its duration. If there is a need to transport a large quantity of larvae in a short time, the recommended mode of transport is the "Dry + O_2" method, because it economizes on volume and weight. If no rapid transport methods are available, it is safer to use water. The "SW + O_2" method can be discarded, because it is no different from the "SW" method. Distances of up to 1,000 km can be traversed within a 10 h period by ground transport in Chile's northern region, within which most of the abalone culture centers are located. Supply of the culture centers, especially those that have been completed recently, solve some normal problems during the startup period of a commercial culture system, such as developing sufficient seawater supply to the various working areas, broodstock conditioning, spawning, environmental requirements, and purchase of juveniles whose origin is in doubt (first or second settlement batch). With the accelerated production of abalone in recent years (practically 100% since 2002), and the great potential that this activity enjoys in northern Chile, the production of seed organisms is one of the most important activities in this endeavor.

ACKNOWLEDGMENTS

The authors of this study thank the Mollusc Unit of the U. Católica del Norte, Coquimbo, for providing the materials and space required for our work, and the AWABI Center for Abalone Production at this university for providing adult broodstock. The authors also thank Dr. L. H. DiSalvo for aid in translation and critical reading of the manuscript, as well as the anonymous reviewers who provided valuable suggestions for improvement of the manuscript.

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