

## Abalone stock enhancement by larval seeding : effect of larval density on settlement and survival

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### Abstract

Abalone larvae of *Haliotis rubra* and *Haliotis laevis* were released experimentally at sites in South Australia to determine the effect of density of larval release on subsequent survival. In one experiment, densities of post-larvae of *Haliotis rubra*, 19 days after release, were highest at intermediate release densities (16,000 larvae m<sup>-2</sup>) compared with low (1,600 m<sup>-2</sup>) and high (80,000 m<sup>-2</sup>) release densities. Highest post-larval densities were about 4.5 times background densities at control sites. In a second experiment, densities of post-larvae of *Haliotis laevis*, 6 days after release, were three times higher (317 m<sup>-2</sup>) at high release densities of 120,000 larvae m<sup>-2</sup> than at low release densities of 2,000 m<sup>-2</sup>. After 49 days average survival of post-larvae across treatments was about 0.5% and mean density was 3.8 m<sup>-2</sup>. After 11 months the density of seed was about 0.6 m<sup>-2</sup>. In both experiments the most cost-efficient densities of larval release were the lowest in terms of the proportions of released larvae settling and surviving. Given the likely density-dependent mortality of post-larvae after settlement, larval release at even lower densities than those tried over larger areas is likely to be the optimal seeding strategy.

Keywords: abalone, *Haliotis rubra*, *Haliotis laevis*, reseeded, seeding, larvae, early juvenile, density-dependent mortality, survival, stock enhancement.

### Introduction

The decline of abalone fisheries in many parts of the world has stimulated interest in the enhancement of abalone stocks. One method of enhancement, seeding with juveniles, has been practised for decades in Japan to prop up its ailing abalone fisheries (reviewed by Saito 1984, Uki 1989) and has been tested elsewhere (Schiel 1993, Tegner and Butler 1985, reviewed by McCormick *et al.* 1994). The high mortality or excessive cost of juvenile seeding has attracted sporadic interest in larval seeding. In the 1960s Ortiz-Quintanilla (1980) developed a program called "mareas de cria" for seeding abalone reefs in Baja California with larvae, and larval seeding is still practised by some co-operatives there (reviewed by Mazón-Sastegui *et al.* 1996). Larval seeding trials in Japan are referred to by Salas-Garza and Searcy-Bernal (1990). Tegner *et al.* (1986) attempted larval seeding in California by transplanting larvae into the field on mesh screens, a technique developed by the oyster industry (Jones and Jones 1983). Later Tong *et al.* (1987) and Schiel (1992) did similar small scale experiments in New Zealand with variable results.

In this paper we describe the experimental larval seeding of two commercial abalone species, *Haliotis laevis* Donovan (greenlip) and *Haliotis rubra* Leach (blacklip) at two sites in South Australia. We first describe our technique for transporting larvae to the field site and then delivering known larval densities onto the bottom. We then describe preliminary experiments testing (a) the effectiveness of tents to retain larvae at the site (see below), and (b) different larval densities. Our purpose was to test the delivery of different densities of larvae in suitable habitat in order to determine the range of densities which produced the optimum survival 20-50 days later. An

understanding of the process of settlement and survival at a fine scale during this initial phase of the life history is a necessary prelude to seeding trials at commercially realistic scales.

## Materials and methods

### Site description

Two seeding sites, one for *H. rubra* and one for *H. laevigata*, were selected within 30 km of abalone hatcheries near Port Lincoln, South Australia to minimise larval transport time. The sites were chosen for the abundance of boulders of manageable size (about 10x15 cm diameter) for sampling post-larval abalone, with adequate cover of crustose coralline algae (CCA), and were at the transition from the sloping rocky shoreline to a sandy bottom, some 5–15 m offshore. Control sites in a similar habitat, two for the blacklip experiment and one for the greenlip experiment, were established within 150 m of the experimental sites. The blacklip site near Cathedral Rock (Lat. 34°59'42"S; Long. 135°59'42"E) was 7–9 m deep. *Cystophora* spp. and *Ecklonia radiata* were the dominants on granite blocks and an algal turf of filamentous species on boulders. The greenlip site on the western side of Taylor Island (Lat. 34°52'48"S; Long. 135°59'54"E) was 9–11 m deep, and the dominant algae were *Ecklonia radiata*, *Seirococcus axillaris* and *Sargassum spinuligerum*. Under the boulders at both sites was a diverse fauna of chitons which graze the CCA assemblage and maintain a suitable substratum for settling abalone larvae (Kangas and Shepherd 1984, Clarkson and Shepherd 1985, Shepherd and Daume 1996).

### Transport and release of larvae

Larvae were collected from the hatchery when they were competent to settle i.e. at stage 39 when the 3rd tubules on the cephalic tentacles appear (Hahn 1989, p.82). The larvae were placed onto damp 100 µm mesh screens and transported in insulated foam containers at about 14°C (Jones and Jones 1983). At the release site the larvae were resuspended in seawater in four 60 l barrels at the desired densities. The barrels were then sealed and the seawater was aerated by forcing air through weighted airstones connected to a SCUBA bottle. Just before release, the barrels were pressurised to about 28 kPa with a SCUBA tank. A 50 m plastic hose of 13 mm diameter was connected to the barrel at one end and fitted with a tap at the other end, so that the diver could control the release density of larvae by timing the flow of seawater and suspended larvae from the tap *in situ*. The diver released the larvae close to the bottom according to the experimental protocol at the pre-marked sites.

### Seeding experiment

To ensure that our experiments tested a range of release densities, one treatment was to use tent-like enclosures designed to retain larvae injected under their canopy. For this treatment pyramid-shaped tents made of 125 µm nylon mesh, with a basal area of 1 m<sup>2</sup> were placed on boulder substrata. The basal perimeter was weighted with chain and the apex was kept elevated with a buoy. The tents were removed after one day at the greenlip site but after 7 days at the blacklip site due to rough seas.

We planned to test a range of larval densities over two orders of magnitude. As our pre-release estimates of density were necessarily crude, we took samples (N=5–8) either at the point of release *in situ* or from the barrels, counted the larvae in them, and so derived mean release density values. Mean densities of larvae of *H. rubra* released were: 1600, 16000 and 80000 m<sup>-2</sup>, and those of *H. laevigata* were: 2000 and 120000 m<sup>-2</sup>. The mean coefficient of variation was 7% for the density estimates in the *H. rubra* experiment and 11.8% in the *H. laevigata* experiment. For each density treatment there was a "tent" and "no-tent" treatment. Each treatment was carried out in four replicate 1 m<sup>2</sup> plots for *H. rubra* and 8 replicate 1 m<sup>2</sup> plots for *H. laevigata*. Each plot was sampled twice in different parts (the micro-site effect). There were twice as many replicate plots for the *H. laevigata* experiment because samples were taken from 4 plots at the first sampling and from the other 4 plots at the second sampling (see below). In all, the experimental area covered 24 m<sup>2</sup> for *H. rubra* and 32 m<sup>2</sup> for *H. laevigata*.

Each sample consisted of a set of 5–6 boulders with a mean surface area per boulder of 300 cm<sup>2</sup>. Each sample set of boulders was sealed in a numbered plastic bag *in situ*, transported to the laboratory, and frozen for later sorting. Subsequently, the boulders were defrosted, rinsed thoroughly with freshwater, and the washings sieved through a nest of sieves. The residues retained in the sieves were stained with Rose Bengal, fixed in alcohol, and sorted under a binocular microscope. Post-larval abalone shells were counted and their shell lengths measured to the nearest 25 µm.

The *H. rubra* larvae were released at the blacklip site on 20 August 1994, nine days after spawning, and the inoculated sites were sampled 19 days later. The two control sites for this experiment were each sampled twice, one week before and one week after the sampling of the blacklip site and in the same manner. The *H. laevisgata* larvae were released on 7 December 1994, five days after spawning, and the inoculated sites were sampled twice, 6 days and 49 days after seeding. The two control sites were sampled 5 days after the day of seeding.

#### Later surveys

The greenlip site was later surveyed twice to estimate the density of survivors in the seeded area. At 153 days after seeding, we searched 16m<sup>2</sup> with a 1 m<sup>2</sup> quadrat by using an underwater magnifier *in situ*, and taking care to replace overturned rocks with minimal disturbance. At 335 days we searched an area of 10 m<sup>2</sup> which was estimated from the number of boulders searched; in this survey we selected boulders haphazardly throughout the entire seeded area.

#### Statistical analysis

To estimate the surface area of sampled boulders we weighed a subsample of 25 boulders and measured their individual surface areas (SA) by carefully covering each with a single layer of paper. After logarithmic transformation of the data on weight (W) and surface area of boulders, a least squares regression of SA in cm<sup>2</sup> vs W in g gave the equation:

$$SA = 4.106 W^{0.643} \quad (R^2 = 0.98)$$

We then standardised the sample data to numbers of post-larvae m<sup>2</sup> surface area by using the above equation.

The data were analysed as a three factor (for *H. rubra*) and four factor (for *H. laevisgata*) analysis of variance (ANOVA) in which release density and tent/no tent (and time for *H. laevisgata*) treatments were fixed factors and micro-site was a random factor. Cochran's test showed that the untransformed data were significantly heteroscedastic ( $p < 0.05$ ) so we applied a  $v(x+1)$  transformation which satisfactorily corrected the variance structure. We applied Dunnett's test (see Zar 1974) for the *H. rubra* seeding experiment to compare the treatment means with control means, and Tukey's test for comparison of treatment means.

#### Survival

The 6- and 49-day density estimates for *H. laevisgata* were converted to numbers m<sup>2</sup> planar surface of the seabed for comparison with the later surveys expressed in the same units. The mean density of boulders at the greenlip site was 21.9 (s.e. 1.4) m<sup>2</sup> (N=66), and the mean total surface area of boulders was 7028 (s.e. 576) cm<sup>2</sup> m<sup>-2</sup>. Assuming the underlying substratum was flat its area is 10,000 cm<sup>2</sup>, giving a total mean surface area of about 1.7 m<sup>2</sup> m<sup>-2</sup>. So we multiplied the 6- and 49-day densities by 1.7 and then calculated survival. Estimates of larval survival in both experiments were obtained in the same way.

## Results

#### Growth rates

The abalone larvae were about 250 µm long on release. After 19 days the modal mean for post larvae of *H. rubra* was 593 µm (range 400–950 µm) giving a mean growth rate of 18.1 µm day<sup>-1</sup>,

after pooling of the three density treatments which showed no significant differences in growth rate ( $t_{178}=0.6$ ; ns). For *H. laevigata* the modal mean at 6 days was 433  $\mu\text{m}$  (range 350–500  $\mu\text{m}$ ) and at 49 days was 1.06 mm (range 0.6–1.5 mm) giving a mean growth rate of 30.5  $\mu\text{m day}^{-1}$  for the first 6 days and 14.7  $\mu\text{m day}^{-1}$  for the next 43 days. The growth rates of the high and low density treatments did not differ significantly ( $t_{32}=0.02$ ; ns). These mean growth rates are similar to those recorded by Rodda *et al.* (1997) for *H. laevigata* i.e. 19  $\mu\text{m day}^{-1}$ , and only slightly less than those recorded by Tong *et al.* (1987) for *H. iris* i.e. 22  $\mu\text{m day}^{-1}$  during the first 96 days after settlement.

#### *H. rubra* seeding experiment

Enhancement of densities of post-larvae was highest at intermediate (16,000  $\text{m}^{-2}$ ) release densities, compared with low and high release densities (Fig. 1), and significantly higher than controls ( $\times 4.5$ ) for both tent and no-tent treatments (Dunnett's test: tents  $q'=2.23$ ,  $p<0.05$ ; no tents  $q'=2.31$ ,  $p<0.05$ ). At low and high release densities only the tent treatments enhanced ( $\times 2.5$ ) post-larval densities significantly above the controls (Dunnett's test: low density, tents  $q'=1.97$   $p<0.05$ ; high density, tents  $q'=2.13$   $p<0.05$ ). The ANOVA (Table 1) for *H. rubra* shows that both fixed factor treatments, density and tents, were significant, as well as the interaction between them. The interaction term was significant because the effect of tents which increased settlement densities at low and high larval release densities was reversed at intermediate release densities.

Pairwise comparisons between all treatments showed that only three treatments differed significantly (Tukey's test,  $p<0.05$ ). With the treatments numbered 1–6 from the left in Figure 1, Nos 3 and 4 (intermediate density) were different from No.6, and Nos 2 and 4 differed.

A comparison of the mean proportion of surviving larvae in the three density treatments, after combining tent and no-tent treatments, shows that after 19 days the survival of released larvae was 1.9%, 0.5% and 0.03% for the low, intermediate and high density treatments respectively.

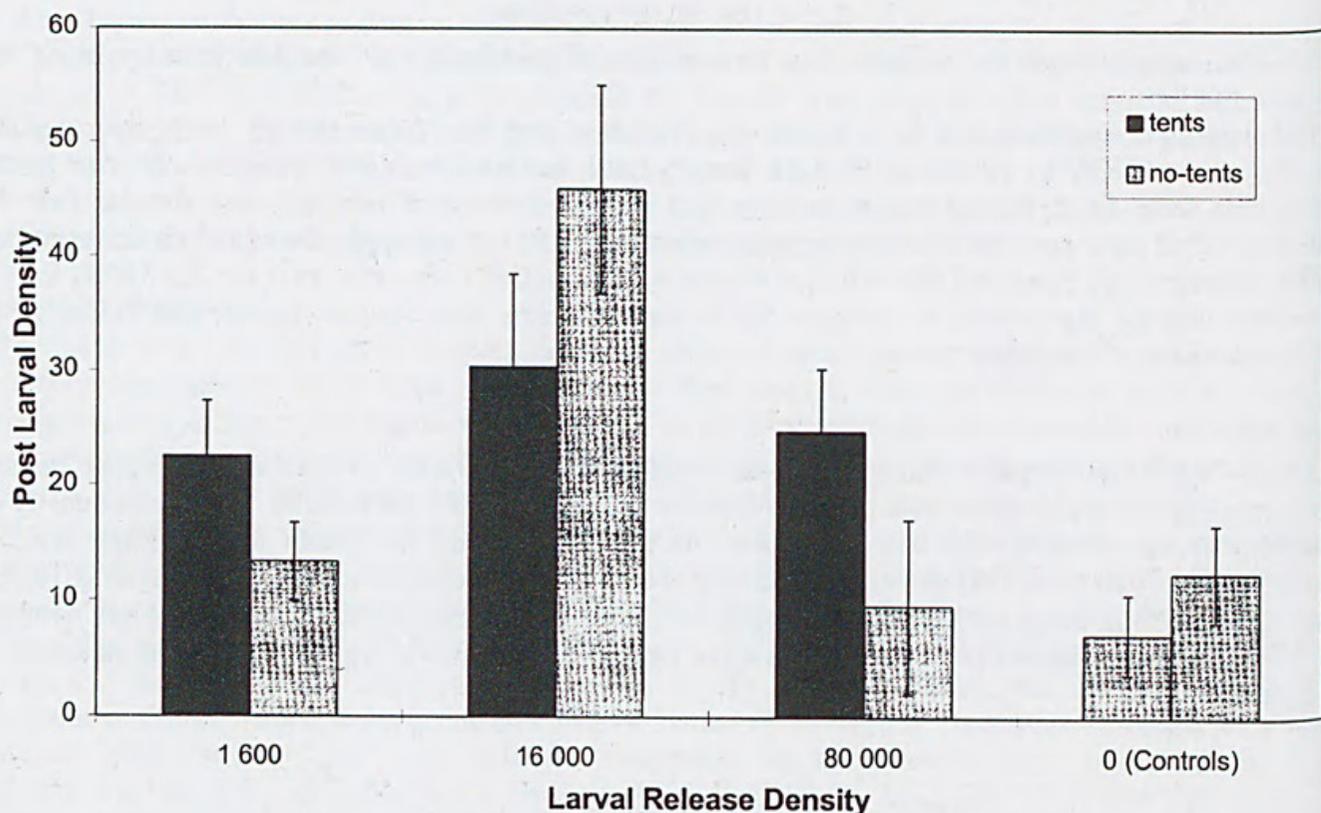


Figure 1. Mean densities of *H. rubra* 19 days after release at three release densities for tent and no-tent treatments. Vertical bars are standard errors. Post-larval densities are in numbers  $\text{m}^{-2}$  of surface area of substratum. Larval release densities are in numbers  $\text{m}^{-2}$  of the planar area of the substratum. The two control sites, Cathedral Rock and Shag Cove, were to the south and north respectively of the experimental site.

Table 1. ANOVA table for larval seeding experiments  
 \*\* :  $p < 0.01$ . \* :  $p < 0.05$

Source of variation	SS	<i>H. rubra</i>			F-ratio	SS	<i>H. laevigata</i>		F-ratio
		df	MS	df			MS		
tents	9.49	1	9.49	20.8**	3.16	1	3.16	2.10	
density	62.86	2	31.43	68.9**	16.49	1	16.49	10.99**	
site	2.73	6	0.46	0.09	15.65	3	5.22	3.48*	
time					197.29	1	197.29	131.49*	
tents x density	21.61	2	10.81	23.7**	3.83	1	3.83	2.55	
tents x time					1.38	1	1.38	0.92	
density x time					7.35	1	7.35	4.90*	
tents x density x time					0.39	1	0.39	0.26	
tents x density x time x site					31.51	21	1.50		
residual	183.31	36			33.52	32	1.05		
total	280.02	47			310.57	63			

### *H. laevigata* seeding experiment

No post-larvae  $< 500 \mu\text{m}$  were found at the two control sites at the 6 day sampling, so we attributed all the post-larvae found at the 6 and 49 days samplings to the seeding experiment. The ANOVA (Table 1) shows that density, sites and time were significant as well as the interaction term, time x density. The tent treatments resulted in higher average densities both at 6 and 49 days (Fig. 2) but the differences were not significant due to the large within-site variability.

The mean proportion of larvae that settled and survived 6 days, after combining tent and no-tent treatments, was 6.5% in the low density treatment and 0.4% in the high density treatment.

Mean densities of settlers declined from  $272 \text{ m}^{-2}$  at 6 days (after averaging all treatments) to  $6.5 \text{ m}^{-2}$  at 49 days (Table 2), giving a mean survival of about 2.4% over 43 days. However, if the high and low density treatments are partitioned and survival estimated for each, survival in the low density treatment was 3.0 (s.e. 1.9)% and in the high density treatment was 2.1 (s.e. 1.1)%; these do not differ significantly. At 335 days overall survival of settlers was about 0.2% (Table 2).

### Discussion

The *H. rubra* experiment took place against a background of natural abalone settlement. At a size of  $400\text{--}1,000 \mu\text{m}$  we cannot distinguish newly settled post-larvae of the four species of abalone *H. laevigata*, *H. roei*, *H. rubra* and *H. scalaris* whose adults were present at the blacklip site and control sites. Our data could therefore have included post-larvae of any of these species. However, *H.*

Table 2. Mean density of post-larval/juvenile seed of *H. laevigata* at the greenlip site for 4 sampling periods. Densities are standardised to numbers  $\text{m}^{-2}$  of the planar area of the substratum. Survival values and instantaneous mortality rates (M) of settlers are given after 49 and 335 days. Standard errors in brackets.

	6 days	49 days	153 days	335 days
Mean density	272 (56)	6.5 (1.2)	0.61 (0.18)	0.63 (0.13)
Survival (%)		2.4		1.3
M $\text{mth}^{-1}$		2.6		0.4

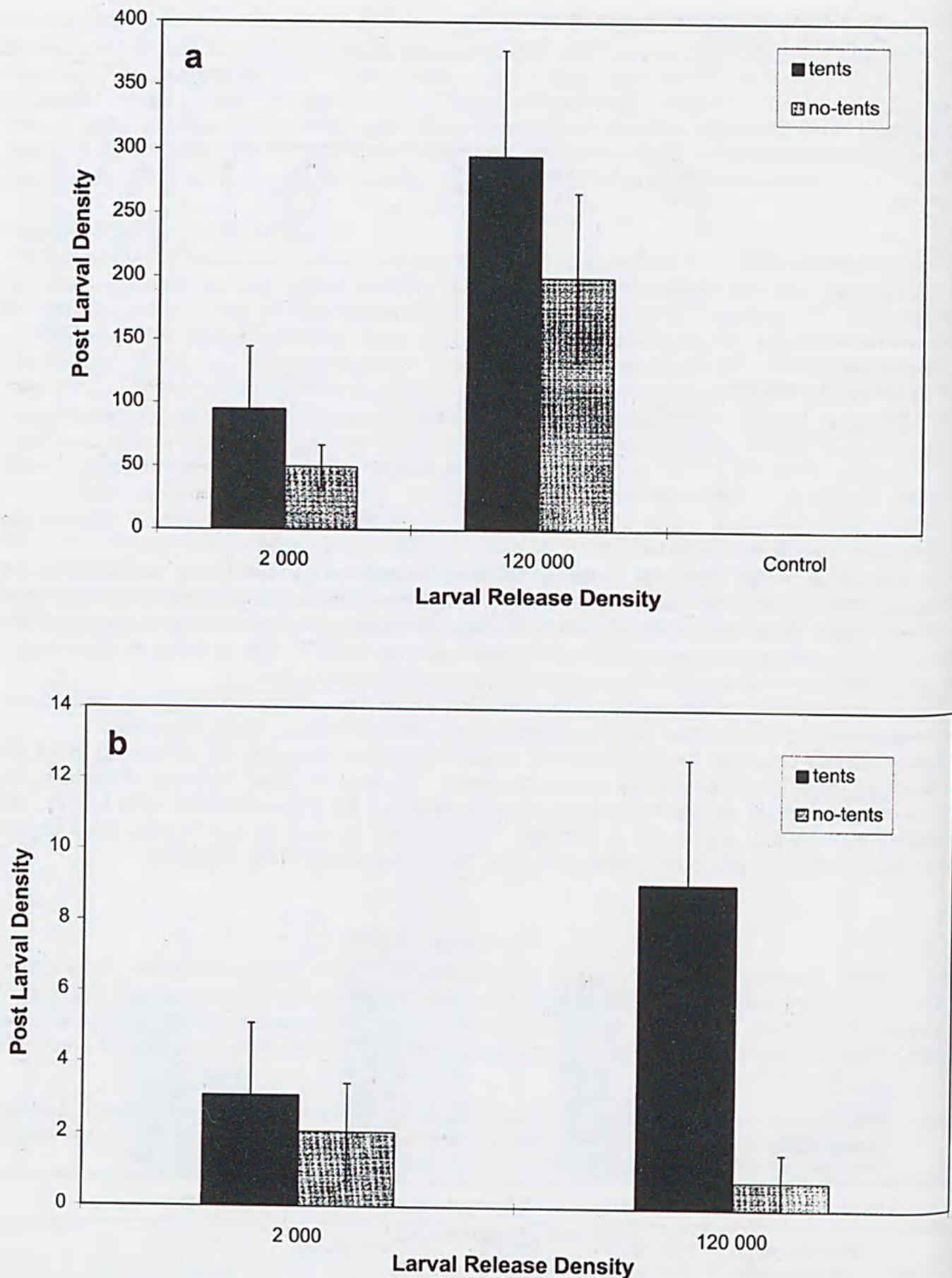


Figure 2. Mean densities of *H. laevigata* (a) 6 days, and (b) 49 days, after release at two release densities for tent and no-tent treatments. See Fig. 1 for meaning of density scales.

*roei* larvae settle only at 1–2 m depth (see Shepherd 1973, Shepherd unpublished data). *H. laevigata* and *H. scalaris* both spawn mainly from October–May (Shepherd and Laws 1974, Shepherd *et al.* 1986) so their post-larvae are unlikely to have been present during our seeding experiment in August. *H. rubra* spawns from July–September (Shepherd and Laws 1974, Rodda *et al.* 1997), and no spawning occurred at the date of our seeding experiment although minor spawning occurred at nearby sites in July 1994 (Rodda *et al.* 1997). We surmise that the background post-larval densities probably resulted from these minor spawning episodes of *H. rubra*.

Explanation of the results of both experiments must take into account the dispersal of larvae before settlement and post-settlement mortality. The fact that the tent treatments generally elevated post-larval densities above those of no-tent treatments indicates that larval advection from the sites, especially in no-tent treatments, must be substantial. The non-significance of the tent treatments in the *H. laevigata* experiments appears to be due to the great variability in the number of post-larvae found at 6 days. Besides the variability in larval loss, a variable micro-scale post-settlement mortality (Keesing and Halford 1992) seems very likely. The latter explanation would also account for the significant interaction between density and tent treatments in the *H. rubra* experiment.

Our survival estimates over 335 days are almost certainly low for the following reasons. First, diver efficiency must have been <100% at the 153- and 335-day samplings. Shepherd (1997) recorded only about 40% efficiency of divers at finding juveniles <20 mm long but that was in habitat where boulders were two layers deep whereas they were only one layer deep at this site. We expect our efficiency at this site to have been 60–70%. Second, some juvenile seeded abalone would have dispersed from the seeded island plots into the surrounding unseeded areas (c. 200 m<sup>2</sup>) when the 153- and 335-day samplings were done. This dilution effect would cause an under-estimate of survival. Shepherd and Godoy (1989) recorded random movement of about 0.5 m mth<sup>-1</sup> for 1-yr old *H. laevigata*, but we would expect less movement than this for younger ones. Thirdly, seeded larvae could have settled outside the plots in the surrounding areas so enhancing densities. Taking these considerations into account our survival estimate at 335 days (0.4%) is a minimal value and the true value could be as high as 1% of those alive at 6 days.

This paper is the first that considers post-larval survival of abalone at different initial densities, and so provides further evidence of density-dependent mortality (M) from settlement as previously shown by McShane (1991). Shepherd and Daume (1996) reported density-dependent M from two months after settlement, but their technique could not estimate M from the time of settlement. There are four other published studies of natural mortality of post-larval abalone in the first few months from settlement. McShane (1991) recorded M rates of 0.6–0.8 mth<sup>-1</sup> for *H. rubra* in studies of natural settlement. Schiel (1992) seeded larvae of *H. iris*, and also used tents to retain them. About 10% of the larvae settled and subsequent M was 1.0–1.3 mth<sup>-1</sup>. Sasaki and Shepherd (1995) found M of 1.4–2.7 mth<sup>-1</sup> for *H. discus hannai* in a study similar to McShane's, and McShane and Naylor (1995) recorded M = 0.6 mth<sup>-1</sup> in a seeding experiment also for *H. iris*. Our estimate of M=2.6 mth<sup>-1</sup> for the same initial period of 2 months is higher than in Schiel's (1992) experiments and in the upper part of the range of the above published values, but is similar to that of many other gastropods (reviewed by Gosselin and Qian 1997).

Was mortality in the first 50 days from settlement due to intra-specific competition for bacterial and diatom food on CCA (Kawamura 1996) or was it due to predation or other factors (see Gosselin and Qian 1997)? Post-larval growth rates did not differ significantly between any of the density treatments for *H. rubra* or *H. laevigata*, implying that competition may be less important than predation during this early phase of the life history, at least over the range of densities tested.

Six months after settlement M declines sharply, although it still seems to be very variable. For this period Shepherd (1997) has recorded M in the range 0.1–3.2 yr<sup>-1</sup>. So our maximum estimate of M 2.9 yr<sup>-1</sup> for the period 49–335 days for *H. laevigata* is plausible. Our data for *H. laevigata* suggest that, if larvae are seeded at 2000 m<sup>-2</sup>, about 0.03% of them will survive to one year. This assumes, conservatively, that larvae which did not settle in our experimental plots died. Thereafter, if we assume that M is 0.4 yr<sup>-1</sup> from age 1 to 3, and 0.2 yr<sup>-1</sup> from age 3 to 6 (Shepherd and Breen 1992), about 74 individuals per million seed would survive to 6 years, when harvested.

Both seeding experiments showed that the lowest density treatments produced the highest proportion of survivors when first sampled after settlement. Thus they imply density-dependent *M*. However, the evidence is inconclusive because the extent of larval loss from the plots before settlement is not known. If *M* proves to be strongly density-dependent at settlement then the best strategy for future seeding would be to seed at lower densities than those we have so far tested. The overall practical and economic feasibility of seeding in the light of our experience in these and related experiments will be considered in a later paper.

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