Enhancement of populations of abalone in NSW using hatchery-produced seed

Michael Heasman, Rowan Chick, Nicholas Savva, Duncan Worthington, Craig Brand, Peter Gibson & John Diemar

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We thank Drs. Neil Andrew (NIWA), Patrick Hone (FRDC) and Geoff Allan and Steve Kennelly (NSWF) for their professional guidance and encouragement particularly during the first 12 months of this project. This precarious and exasperating period was dogged by extremely low and irregular supply of spawnable blacklip abalone broodstock and hence the where-with-all to begin the task of developing cost effective seed production and fisheries enhancement technology.

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NON-TECHNICAL SUMMARY

1998/219	**Enhancement of populations of abalone in NSW using hatchery-produced seed.**

PRINCIPAL INVESTIGATOR: Dr Mike Heasman

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OBJECTIVES:

Original objectives as specified in FRDC funding application:

1. Produce seed from wild collected blacklip abalone at a range of sizes and ages throughout the year.
2. Develop techniques to enable the successful deployment of seed to coastal reefs in NSW.
3. Develop techniques to maximise the settlement, survival and growth of seed on coastal reefs in NSW.
4. Commence deployment of fluorochrome-marked seed to depleted coastal reefs in NSW.

Modified objectives as agreed to in collaboration with external referee and endorsed by FRDC:

1. Produce seed from wild collected blacklip abalone at a range of sizes and ages throughout the year.
2. Develop techniques to enable the successful deployment of seed to coastal reefs in NSW.
3. Develop techniques to maximise the settlement, survival and growth of seed on coastal reefs in NSW.
4. Reseed reefs at Port Stephens, Sydney and at least one location further south, to be determined in conjunction with ABMAC.
5. Quantify the survival and growth of reseeded abalone for a minimum of 18 months.

OUTCOMES ACHIEVED TO DATE

Several critically important steps to achieving the central long-term aim of this project namely, cost-effective enhancement of abalone fisheries using hatchery produced seed stock, were achieved. The first was year-round controlled temperature conditioning and spawning of captive broodstock. This provided impetus for funding and guidance for practical implementation of a complementary R & D project (FRDC 2000/204) to refine and improve this technology in relation to blacklip and greenlip abalone. This controlled breeding technology is in turn providing a number of important flow-on benefits to the abalone aquaculture industry in southern Australia. Most importantly it is facilitating an industry-wide selective breeding program through facilitated synchronisation of spawning that will enable pair crossing and identification of specific genes for faster growth.

Another important benefit for abalone farmers has been the development of technology that will enable greatly improved nursery production systems at much lower initial capital costs. Uptake of

this technology is expected in the next generation of land-based farms that will also need to incorporate controlled reproductive conditioning systems and an additional intermediate nursery system for weaning and on-growing 1-3 mm post-larvae.

Controlled year-round conditioning and spawning has also enabled initiation of research to produce and evaluate triploid abalone that offer prospects of faster growth and perhaps reproductive sterility, both of which will be of major benefit to future fisheries enhancement and farming of this species.

Results of laboratory-based research on the use of one week old post-larvae as an alternative to competent larvae are sufficiently encouraging to extend this research into a field evaluation phase. This technology, if successful, would largely circumvent practical problems of weather, currents and wave surge in targeted seeding of larvae onto natural CCA-encrusted habitats. In any case, results of this particular area of research also demonstrated that even if natural survival rates of one legal size abalone per 20,000 competent larvae could be achieved, costs will still be prohibitive. Hopefully this demonstrated futility of larval seeding will circumvent needless further investment in this field of research.

Production of Seed Abalone

- Wild *Haliotis rubra* in NSW were shown to be a poor and unreliable source of ripe, ready to spawn broodstock even within the recognised late spring/summer spawning season. However, limited spawning success was achieved with captive broodstock maintained under ambient conditions in outdoor flow-through seawater tanks.
- For the first time in Australia, year-round spawning and hatchery production was achieved using captive broodstock maintained in a recirculating seawater system at a constant temperature of 15±2°C conducive to gametogenesis. Significant improvements were achieved in the reliability and efficiency of large scale seed production by combined use of multiple annual batches, high density stocking and rearing of post-larvae on nursery plates, early weaning and a two step intensive shallow raceway nursery rearing protocol.
- Scope for further improving seed production was provided with the identification of several key rearing factors including optimum larval stocking density on nursery plates and use of optimum temperatures that shift markedly with successive life stages (incubation, settlement, metamorphosis, post-larval growth and several phases of juvenile growth). These improved techniques collectively provide scope for greatly raising annual yields of nursery systems and hence for drastically reduced seed costs associated with fisheries enhancement.

Release of Seed Abalone onto Reefs

- Laboratory experiments identified several factors that had the potential to influence the settlement and survival of larvae released in the field. Low rates of water flow significantly affected larval settlement and the addition of a chemical GABA prior to release reduced the time larvae spend in the water column. Factors imposed during the handling and transportation of larvae, such as periods of refrigeration and exposure to temperature gradients of 5-10°C over short periods of time generally had minor effects on the settlement of larvae. In fact, combinations of these factors that were expected to compromise the settlement of larvae resulted in increased settlement. Other factors found to promote high rates of settlement of 80-90% were low current flow and minimum periods of 24 and preferably 48 to 96 hours.
- Methods of releasing hatchery produced larvae and juveniles to natural reefs were developed, and the effectiveness of these methods to deliver competent larvae and fit juveniles to these reefs was tested. In addition, methods were developed to monitor the settlement and early survival of larvae and juveniles within their release area. Field experiments demonstrated the ability of juveniles to disperse from their immediate area throughout the release site and very likely into surrounding habitat, and have provided information that should be considered when attempting to determine long term estimates of survival of juveniles from releases.

- In excess of 24 million larvae and about one million juvenile abalone were released to 57 sites within six locations along the NSW coast. The survival and growth of released larvae and juveniles were monitored for periods up to 30 months. Short-term (<2 months) sampling was used to investigate movement of juveniles and to compare alternative sampling techniques.
- As in previous studies, seeding of larval abalone was shown to be fraught with practical difficulties and likely to be cost-prohibitive even if these difficulties could be overcome.
- Juvenile abalone deployed in large clusters of 700 to 2,500 within predator protective release devices were mainly in the "button" size range of 5 to 15 mm. Average growth rates were similar at all sites and similar to that of wild stock with legal size of 115 mm projected to be reached after 4 years. By contrast, survival of juveniles after a year or so was only 0-4% and compared poorly with rates of 12-40% expected of wild counterparts.
- These low survival rates prompted a re-examination of published information on the natural biology of juvenile blacklip abalone. This review revealed that natural densities of 12 month old juveniles on natural rocky reef substrates were consistently low, commonly 2 or less per square metre of reef. This knowledge prompted a final seeding experiment to test the hypothesis that button size juveniles should be seeded sparsely to overcome much higher than normal losses experienced so far in this project. Results of this experiment one year after release supported this hypothesis. Average survival of button size juveniles seeded as 10 dispersed groups of 100 was 12.5%. This was within the expected normal range of wild abalone and more than 3 times the average rate of 3.8% for abalone seeded in single clusters of 1,000.
- Continuing seeding research will be aimed at confirming the need for and then improving the technology of dispersed low density seeding of juveniles.
- Major practical advantages of using post-larvae rather than settlement stage larvae for seeding operations were identified and investigated in the hatchery. Results showed that post-larvae can be seeded at very high densities onto small CCA (crustose coralline algae) pebbles (vector rocks) and retained for up to eight days without significant restriction of growth or ability to rapidly disperse into simulated small boulder habitats after release. An important recommendation arising from these results, in conjunction with published age and density dependent mortality data for wild abalone, are that post-larvae, pre-settled onto small rocks, should be seeded over large areas of juvenile habitat at low densities of 10-100/m^2 for best effect.
- A simple cost benefit model to compare the respective attributes of different age/size classes of seed abalone from ready-to-set larvae through to advanced (40-60 mm) 2 year old juveniles was based on mortality data of wild abalone. Results strongly suggested that button size juveniles and week old post-larvae offered best prospects of cost-effective seeding.

KEYWORDS:
Abalone, Fishery, Aquaculture, Hatchery, Broodstock, Production, Deployment, Rehabilitation, Reseeding, Enhancement

TECHNICAL SUMMARY

Seed production

Wild H. rubra as a source of ripe broodstock (Section 4.2)

A total of 41 attempts were made to induce wild stock spawning shortly after their collection from reefs between Port Stephens and the far southern coast of NSW. Of these, 34 were conducted immediately following collection while the remaining seven attempts were conducted 2-14 days later. A significant number of viable eggs (1.5 million) was produced on only one occasion. Given the high demand on the resources of staff and facilities and disappointing results of both this and earlier attempts by the authors and others in NSW, investigations of wild *H. rubra* as a source of ripe, ready to spawn hatchery broodstock were abandoned in December 1999. This was after a 21 month period (Jan. 1998 to Oct. 1999) of sporadic attempts. Seven successful spawnings with broodstock held in outdoor flow through seawater tanks yielded 26.8 million eggs (mean fecundity 1.12 million eggs/spawner) that in turn yielded 13.3 million competent larvae. However, these occurred at irregular intervals and were largely limited to the spring to early summer natural breeding season of *H. rubra* in NSW.

Conditioning broodstock (Sections 4.3 and 4.4)

Greatly improved results were achieved using captive broodstock maintained in a recirculating seawater system at 15 ± 2ºC. These were collected from 7 localities extending southward from Port Stephens, 150 km north of Sydney, to Disaster Bay near the Victorian border. From October 1999 to June 2001, 34 spawning inductions using 13 to 38 adult females were attempted. Half (17) of these, spread throughout the year, were successful and yielded 59.3 million eggs. Three epidemic spawnings triggered by accidental temperature shocks yielded a further 63 million eggs, which were unusable due to polyspermy and bacterial contamination.

Overall spawning response rate using conditioned broodstock was 11% (85 out of 785 females). The maximum number of spawnings by any individual female was five. Intervals between successive spawnings varied widely from 74 to 290 days. Mean ± s.e. fecundity was 1.25 ± 0.49 million eggs/spawner (range of 42,000 to 4.0 million). A total of 24.0 million competent to settle 7- or 8-day-old larvae were produced and used for larval seeding experiments or for nursery production of juvenile seed and related experiments. Yields of competent larvae from eggs averaged 40% (range 8 to 71%) falling within a range routinely reported by commercial hatcheries in Australia. Yields of juveniles produced from competent larvae averaged only 5.7%. (range 1.8 to 75%) These inconsistent yields raised the need for better knowledge of and control over nursery production.

Hatchery and nursery technology development (Section 4.5)

Regardless of season or seeding density, growth patterns of post-larvae on adventitious diatoms on standard nursery plates were best described by exponential relationships with high correlation coefficients (R^2) above 0.97. Within batches, daily growth rates progressively increased from 8 to 24 μm/day for 2-day-old post-larvae, to 24 to 50 μm/day for post-larvae approaching 1500 μm (the minimum mean size suitable for weaning onto a finely ground particulate diets). Plate residence time from settlement to the designated mean minimum weaning size ranged widely. Batches reared in summer/early autumn required only 32 to 35 days. At the opposite extreme, winter and early spring reared batches required 50 to 72 days. Speed of development thus appeared dependent on seasonal temperature and were in accordance with influence of temperature on post-settlement growth determined experimentally.

An important feature of growth on diatom plates was that it continued exponentially until either food ran out, in which case it abruptly stopped, or when mean shell length reached 1-2 mm, when they

were weaned onto artificial diets in raceways. A number of batches, having reached the minimum weaning size, were allowed to exhaust available food, stop growing and starve before being harvested from diatom plates. Substantial losses of post-larvae occurred following such episodes. The physiological condition of some batches was compromised due to the degree they were unable to endure the combined stress of anaesthesia, harvesting, handling, transfer to shallow exposed raceway habitats and weaning. Increasing seeding density progressively reduced the size and age at which growth stopped and starvation began.

Improving nursery production: Optimum nursery plate seeding rates (Section 4.6)

A number of experiments aimed to improve the reliability and cost efficiency of large-scale seed production. Effects of larval seeding density on settlement, metamorphosis and growth of post-larvae seeded onto conventional (300 x 600 x 1.5 mm) nursery plates at densities of 500 to 4000 larvae per plate were assessed. Absolute yields of post-larvae peaked after four to seven days and yields increased with increasing seeding density, from 26% to 71%. From day 7 to 28 after set, survival across all seeding densities progressively converged to a common rate of about 20% that persisted until the termination of the experiment at day 56. Growth remained independent of initial seeding density up to 14 days, averaging about 14 μm per day. Subsequent growth became progressively more density dependent. By day 56, growth rates at residual densities of 10, 100 and 1000 post-larvae per plate averaged about 40, 30 and 22 μm/day respectively. Minimum mean shell lengths of about 1400 μm at day 56 attained at the highest density of 4000 post-larvae per plate were nevertheless found to be acceptable for the purpose of early weaning of post-larvae. These improved techniques provide scope to increase output per plate from 1 to 4 or perhaps even 6 batches per year and to increase yield densities from 30- 80/plate up to 1000-1500/plate thereby greatly raising annual yields/plate.

Improving nursery production: Effect of temperature on settlement and on post-settlement growth and survival (Section 4.7)

Experiments investigated the effect of temperature on settlement, metamorphosis and early post-settlement growth and survival on artificial (conventional diatom coated settlement plates) and natural crustose coralline algae (CCA) coated rock substrates. Yields of juveniles on diatom plates devoid of CCA or alternative settlement inducing algae, such as *Ulvella lens*, was poor, ranging from 0 to 5.5%. By contrast, settlement rates on CCA rock ranged from 20 to 40%. Effect of temperature on yield and early growth of post-larvae were consistent for those reared on diatom plates and CCA rock. Peak settlement occurred at 18.4°C. Growth rates of zero to six day post-larvae increased progressively from zero at 10°C to a peak of ~30 μm/day at 23°C, but then fell dramatically back to zero with a further rise in temperature to 27°C.

Improving nursery production: Effect of temperature on 4 age classes of juveniles (Section 4.8)

Effect of temperature on growth of four age/size classes of *H. rubra* ranging from 4-5months (3-10 mm) to 31-32 months (56-73 mm) was investigated. A major downward shift in optimum temperature occurred with progressive age/size. Results also demonstrated a major decrease in the tolerances of juveniles to temperatures above 23°C and to other forms of physiochemical stress with progressive age and size.

Deployment of larvae and juveniles

Holding and transporting of juvenile H. rubra (Section 5)

The need to transport and deploy batches of up to several hundred thousand 6-9 month old (5 to 15 mm) juvenile *H. rubra* up to 500 km from the hatchery prompted investigations of suitable methods of storage and transportation. Key issues addressed were whether stock should be stored and transported wet or damp, optimum temperatures for storage and transportation and the maximum duration over which they can be safely stored and/or transported without compromising

the post-release vigour and survival. Juvenile *H. rubra* (mean SL, 14 mm) previously held at ambient temperatures of 18 to 22ºC survived at acceptable rates above 80% for up to 48 h when stored damp in pure oxygen between 13 and 16ºC. The best 48 hour survival rate of 95% was achieved by juveniles stored damp at 14ºC. Generally poorer rates of survival by counterparts stored wet were due to declining physiochemical conditions, especially dissolved oxygen that rapidly fell below an acceptable threshold of 95% saturation.

Factors that influence settlement in the field (Section 6.0)

Laboratory experiments were used to identify several important factors that could influence the settlement and survival of *H. rubra* larvae released in the field. Low rates of water flow across the settlement substrate significantly reduced larval settlement. In addition, exposure to GABA prior to release reduced the time that larvae spent in the water column. Various forms of stress imposed during the handling and transportation of larvae, such as refrigerated storage and temperature shocks of 5-10^{o}C had minor effects on the speed and extent of settlement. Moreover, combinations of these factors that were expected to compromise the settlement of larvae in fact promoted settlement.

Results of these small-scale experiments to optimise field deployment of competent larvae also served to complement experiments to optimise high density diatom plate nursery production of 1-3 mm weaning size post-larvae reported in sections 4.6 & 4.7. Collectively, results showed that diatom film alone, i.e. in the absence of other settlement promoters such as CCA *Ulvella* and neuroactive chemicals such as GABA (gabba amino butyric acid) can consistently attract high rates of larval settlement and metamorphosis of $\geq$80%. Nevertheless such high yields are subject to a combination of critical conditions being met. These include a minimum larval seeding density of 1/cm^2 to promote gregarious settlement but no higher than 2/cm^2 to ensure stock reach a minimum weanable size of 1 mm before grazing out diatoms; minimum substrate exposure times of 24 hours and preferably 48 to 96 hours; low current flow and turbulence at the surface of settlement substrates; and optimum temperatures of 17 to 19°C. An additional prerequisite of high rates of settlement and metamorphosis both in the field and hatchery is of course “batch quality”. Although most larval batches yielded post-larval yields of 80-95%, occasional yields as low as 38% were experienced and may relate to over or under ripeness of eggs in conditioned stock.

Field deployment and early survival of larvae and juveniles (Section 7)

Improved methods of releasing hatchery produced *H. rubra* larvae and juveniles to natural reefs were developed, and the effectiveness of these methods to deliver competent larvae and juveniles to reefs tested. Several alternative methods of monitoring the settlement and early survival of larvae, post-larvae and juveniles within their release area were developed. Field experiments were used to demonstrate the ability of juveniles to rapidly disperse from points of release and provided greater insight into factors that must be considered when attempting to estimate long term survival of seeded stock.

Survival and growth of released stocks (Section 8)

In excess of 24 million larvae and about one million juvenile abalone were released to 57 sites within six locations along the NSW coast. The survival and growth of released larvae and juveniles were monitored for periods up to 30 months. Short-term (<2 months) sampling was used to investigate movement of juveniles and to compare alternative sampling techniques.

The best survival for seeded larvae a year or more after release was 125 out of 1.1 million (1 in 8,800) recorded 553 days after release. As in earlier abalone seeding studies, seeding of ready-to-settle larvae was shown here to be fraught with practical difficulties and likely to be cost prohibitive even if the difficulties could be overcome and natural rates of survival (approximately 1 in 20,000 to legal size of 115 mm) achieved.

Juveniles were mainly deployed at button size (5-15 mm) in clusters of 700 to 2,500 in predator protective release devices (coralline algae coated rocks within wire mesh cylinders or simple rectangular boxes comprising sections of PVC down-pipe). Mean survival rates 1-2 years after release ranged from 0-4%. These compare poorly with rates of 12-40% expected of their wild counterparts based on published natural mortality data. Average growth rates were similar among and between sites with juveniles expected to reach maturity (>90 mm) after 2.5 to 3.5 years, and the legal catch size of 115 mm after 4 years.

These lower than expected survival rates prompted a review of published literature on the natural biology of blacklip abalone. This review revealed that regardless of original densities up to several 1000/m^2 at 1 month and at up to 30/ m^2 at 6 months of age, surviving juveniles converged to a narrow density range of 1-3/m^2 as 15-30 mm 1+ year olds. From this it was postulated that if natural survival rates from button size to legal size (calculated as 1 in 20-30) are to be achieved, then seed should be sparsely deployed rather than in large dense clusters. A final reef seeding experiment at Disaster Bay near the Victorian border was conducted to test this hypothesis. Two release treatments were compared namely; 10 evenly spread clusters of 100 juveniles as opposed to one central cluster of 1000 juveniles. A year later, mean survival across the 3 replicate sites seeded with 10 x 100 juveniles was within the above cited expected range for wild stock, namely 12.5% (range 5-23%), while that of the alternative treatment was again low at 3.8% (range 0-9%).

Major practical advantages of using post-larvae rather than settlement stage larvae for seeding operations were identified and investigation in the hatchery. Results showed that post-larvae can be seeded at very high densities on to small CCA (crustose coralline algae) pebbles (vector rocks) and retained for up to 8 days without significant restriction of growth or ability to rapidly disperse into simulated small boulder habitats after release.

Results of these investigations also suggested that the above cited convergence of 12 month old wild juveniles to densities of 1-3/m^2 can largely be explained by food related carrying capacity limitations of these habitats more than by other frequently ascribed factors, especially predation.

Evaluation of post-larvae for seeding operations (Section 9)

The potential use of post-larvae rather than settlement stage larvae for seeding operations was investigated in a succession of three laboratory experiments. In the first experiment, seeding of *H. rubra* larvae at low densities of ~1,000 larvae/L (1/cm^2) of CCA rock resulted in relatively poor and protracted rates of settlement, metamorphosis and initiation of post-larval growth and development. At the opposite extreme, seeding at densities of 4,000 to 16,000 larvae/L CCA rock promoted rapid gregarious settlement, metamorphosis and initiation of post-larval shell growth. However, once settlement and metamorphosis had been completed and post-larval shell-growth initiated, subsequent growth of *H. rubra* post-larvae on CCA rock became strongly density dependent. By day 56, yields of surviving post-larvae at all stocking densities had progressively converged to a narrow range of 100 to 250/L (0.1-0.25/cm^2) CCA rock.

The second experiment again showed that initial settlement success of day two post-larvae increases with seeding density to a peak yield of 80% at the highest tested seeding density of 32,000 larvae/L (32/ cm^2) CCA rock. Density had a moderate effect on growth rate during the first week but a drastic effect thereafter. Post-larvae could be sustained at high densities up to 16,000/L (16/ cm^2) CCA rock for periods of up to 8 days without significantly restricting their growth or ability to rapidly disperse from the rocks onto adjacent surfaces.

Results of the third experiment showed that post-larvae seeded at high density onto small CCA pebbles (vector rocks) can rapidly and efficiently disperse into simulated boulder fields. The results also confirmed that the carrying capacity of CCA rock declines with increases in the biomass of juvenile *H. rubra*, falling to levels of 100 to 200/L (1,000 to 2,000/m^2) for 56 day (1.5 mm) post-larvae. Moreover, typical low densities of $\leq$30/m^2 reported for wild 6 month old button size (6-10

mm) *H. rubra,* were consistent with this relationship. An important probable implication of these findings, when considered in the context of published age and density dependent mortality data for wild populations of *H. rubra,* is that to achieve efficient enhancement of wild stocks, post-larvae should be seeded in areas of juvenile CCA rock habitat at low densities in the range 10-100/m^2. Likewise, 6 month old button size juveniles are probably best deployed at even lower densities of 1-10/m^2 typical of productive reef but much lower than early experiments conducted during the development of this project and as practised to date elsewhere.

PRACTICAL RECOMMENDATIONS FOR STAKEHOLDERS ARISING FROM THIS PROJECT

Wild *H. rubra* as a source of ripe broodstock (Section 4.2)

Wild *H. rubra* in NSW should not be regarded as a ready nor reliable source of ripe, ready-to-spawn broodstock even during the very brief late spring/early summer spawning season. Accordingly, future abalone hatchery and farming operations in NSW must include provision of broodstock holding and conditioning facilities.

Conditioning of broodstock (Sections 4.3 & 4.4)

The incorporation of a seawater reuse system within a controlled temperature broodstock conditioning system offers considerable energy savings over simple flow-through alternatives. However, in order to attain consistent spawning success rates of 60-80% together with high fecundity levels of ≥1.5 million ova, high quality larvae and high and reliable re-spawning success, the broodstock unit should include the following features:

- Dual independent temperature controlled systems to ensure against system failure.
- Stocking rates of ≤1 kg of broodstock biomass per 50 L of standing seawater volume.
- A minimum net seawater exchange rate of 4 volumes per day.
- Provision of a dual cycle air conditioner that can maintain seawater and conditioning broodstock at 16±1°C in the event of a seawater supply or chiller failure.
- Provision of stand-by generator capable of powering the main seawater supply pumps, the dual cycle air conditioning system and (preferably) the seawater chiller.
- Maintenance of broodstock in a common tank that is easy to access and clean.
- Twice weekly drainage of the broodstock units to allow cleaning and flushing of uneaten food and faeces and to enable quick and easy assessment of the health status of stock and if required, removal of dead or moribund stock.
- Regular (at least twice yearly) inspection of broodstock is recommended as is treatment or replacement of mudworm infected stock.
- Strict maintenance of spawning records and current conditioning status of all brooders thereby enabling optimised re-spawning after 1,200-1,600 degree days (150-200 calendar days at 16°C) in accordance with the findings of Grubert & Rita (2003a) and of Plant (2002).
- Adoption of a conditioning temperature of 16ºC rather than 18ºC as the former provides a safer buffer of time to detect and rectify mechanical failures. The latter can lead to broodstock being exposed to elevated temperatures of ≥20ºC that can trigger epidemic spawning of ripe stock or gonadal egression (atresia) in others.

Improving the diatom plate phase of nursery production (Sections 4.5-4.7)

- Nursery plates should be conditioned for a minimum of 2 weeks in summer and 4 weeks in winter.
- All incoming seawater should be filtered to 10 µm.
- Operation of diatom plate nursery systems should be limited to temperatures above 15ºC and preferably maintained in the range 17-23ºC to ensure high levels of settlement and subsequent growth of post-larvae. For hatcheries located in southern NSW and the other southern states, this will entail greenhousing of nurseries to raise ambient temperatures as low as 10ºC by up to 5ºC.
- 8 day rather than 7-day-old larvae reared at 18°C should be used.

- Larval seeding rates need to be at least 1/cm^2 but less than 2/cm^2 to ensure that gregarious settlement will occur in the absence of other settlement inducers such as *Ulvella*. Seeding rates below 2/cm^2 will ensure that surviving post-larvae can attain a minimum mean weaning size of 1 mm before running out of food. Optimal larval seeding rate varies with season, being highest during the highest seawater temperatures and sunlight intensity periods of summer and early autumn (December to March) and lowest during the winter and early spring months of June to September. Accordingly, it is recommended that rates of 6,000 larve/plate (1.5/cm^2) be applied in December to March, 4,000/plate (1/cm^2) from June to September and an intermediate rate of 5,000/plate in the other months. Mean yields of weanable post-larvae will be about 20% of seeded larvae or 800-1,500/plate.
- All the usual plate visual inspections and light adjustment protocols for maintaining a balance between grazing rates of developing post-larvae and benthic diatom production (Hone *et al.*, 1997) need to be applied for the first 4 weeks. Thereafter a representative array of plates should be examined every few days in summer and every week in winter to ensure growth is not food limited and to see whether the bulk of stock have reached the minimum weaning size of 1 mm.
- Harvesting of post-larvae using 0.5-1.0 g/L benzocaine anaesthesia in accordance with protocols described in Section 4.5 should be applied as soon as mean size of 1 mm is reached or coincident with general over-grazing of plates (whichever is the sooner).
- Harvesting of post-larvae should be confined to seawater temperatures of $\leq$23ºC. A benzocaine concentration of 1.0 mg/L should be used at temperatures of $\leq$20ºC and of 0.5 mg/L at higher temperatures up to 23ºC.

Improving weaning and post-weaning phase of nursery production (Section 4.8)

- As an aid to weaning, first stage weaning raceways should be filled with seawater and aerated to promote development of benthic diatoms for at least 2 weeks prior to stocking.
- Stocking rates in first stage raceways and in which post-larvae are grown to a mean size of 5 mm, should not exceed 40,000/m^2.
- Stocking rates of the second stage raceways for button size juveniles up to a mean size of 10 mm should not exceed 10,000/m^2.
- Harvesting of juveniles for seeding or on-rearing should be subject to the same temperature and anaesthesia restrictions cited above for harvesting post-larvae from diatom plates.

Holding and transportation of juvenile *H. rubra* (Section 5)

Juvenile seed can be safely transported and stored damp at 14ºC in an atmosphere of pure oxygen for up to 48 h.

Factors that influence larval settlement in the field (Section 6)

Although optimised methods of transporting and seeding *H. rubra* larvae have been identified, it was also recognised that larval seeding is fundamentally flawed both in terms of practicality and economics. Accordingly, further pursuit of larval seeding R&D is discouraged.

Field deployment and survival of larvae and juveniles (Sections 7 & 8)

Preliminary economic modelling (see Section 15.7) suggests that button size (5-15 mm) juveniles offer the best prospects of cost-effective enhancement of depleted *H. rubra* fisheries in NSW and elsewhere. However, deployment of such seed in large clusters of 700-2,500 consistently resulted in poor yields of 0-4% within a year or so of release. By contrast preliminary experimental evaluation of dispersed low density seeding of button size juveniles resulted in an encouraging survival rate of 12.5% 12 months after release. Accordingly, it is recommended that dispersed low density seeding be the cornerstone of ongoing abalone fisheries enhancement R&D.

Evaluation of post-larvae for seeding operations (Section 9)

Encouraging results of laboratory based seeding experiments and of a preliminary economic model (Section 15.7) suggest that pre-settlement of *H. rubra* onto small rocks together with low density broadcast seeding of 7 – 10-day-old post-larvae on these vector rocks warrants further R&D.

1. BACKGROUND

1.1. Development of the project

On 12 April 1993, NSW Aboriginal Land Council (NALC) representatives met in Sydney with NSW Fisheries (NSWF) staff to discuss the scope and potential for future south coast Aboriginal community participation in commercial aquaculture and fisheries in NSW.

The NALC subsequently organised a study tour of relevant aquaculture farms and research facilities in New Zealand on 9-14 May 1993 and subsequently in Tasmania over the period 16-23 July.

A status report (Heasman 1993) of abalone farming and related R&D in New Zealand and Tasmania and implications for NSW was subsequently prepared. Principal conclusions and recommendations were that:

- Although development of reliable and cost effective technology required for successful fisheries enhancement and farming of abalone was well advanced in other southern states of Australia, such technology would in all probability need to be evaluated under local conditions and adapted to suit local stocks.
- Suitable hatchery, nursery and farming sites that were likely to be scarce or difficult to procure in NSW should be identified and set aside for future development.

These conclusions were consistent with those of another report independently commissioned by the NSW Abalone Divers Association. At that time, the NALC and several members of the NSW Abalone Management and Advisory Committee (ABMAC) had expressed an in principal agreement to a joint venture project to develop abalone enhancement with NSWF. An R&D application to NOFARIC (NSW OLMA Fisheries and Aquaculture Research Committee – a division of DEET) was accordingly prepared and submitted by NSWF on behalf of the joint venture partners. Though unsuccessful, it was reconsidered in 1994, 1995 and again in 1996.

In May 1996, ADFIN (formerly NOFARIC) advised NSWF that this proposal had, in recognition of its indigenous and regional significance, been approved ahead of other grant applications submitted by NSWF. By this time however the level of interest from both joint venture parties had waned. NSWF, NSW aboriginal community groups, and commercial abalone fishers met at Port Stephens Fisheries Centre on 6 June. This and several other meetings hosted by NSWF during July and August 1996 failed to elicit a commitment from either party for additional capital funds of $264,750 required.

The decision to proceed with a modified version of the project involving a major shift in emphasis from at sea farming to enhancement of the wild fishery was authorised by the Director NSWF, Dr John Glaister, in September 1996. This modified project was made possible by a reduced capital funding requirement (from $264,500 to $42,500) and by NSWF bearing this cost in addition to salary and operating costs that already totalled $261,300.

Factors that increased the probability of success of this revised project were its coupling with the PhD research of technical officer Mr Rowan Chick and linkages with UNSW and Latrobe University teams that were investigating key factors to successful settlement and post-larval survival of *H. rubra.*

This revised project plan was submitted and approved by NOFARIC on 4 November 1996 and commenced on 12th December 1996.

1.2. Status of the NSW Abalone Fishery in NSW and its scope for enhancement

The valuable abalone (*Haliotis rubra*) fishery in NSW is based largely on populations in the south of the state. Annual catch peaked at over 1200 t during 1971-1972, and remained above 600 t during the early 1980s. A long succession fishery controls have been imposed since 1973 including a reduction in effort through license regulation, limiting catch by quota allocation and introduction of size limitations. Area fishing closures were also imposed between Port Stephens and Jervis Bay following a major depletion of stocks by the disease *Perkinsus* in 2001/2002. A total allowable commercial catch (TACC) of 370 tonnes was introduced in 1989. By 1996, the TACC had been dropped to 333 t, by 2000 to 305 t, and in 2003 to 280 t.

Over this period it became apparent to commercial divers that previously productive areas of reef were now supporting high densities of the black (or purple) sea urchin, *Centrostephanus rodgersii*, and low densities of abalone (J. Smythe, pers. comm.). These observations are compatible with the findings of Andrew and Underwood (1992) that densities of *H. rubra* and *C. rodgersii* are negatively associated. Continuous grazing pressure exerted by dense aggregations of *C. rodgersii* can degrade complex community reefs (Shepherd 1973). Such degraded areas are commonly referred to as "barrens". They are characterised by an almost total absence of both foliose algae (seaweed) and associated complex communities of fish and invertebrates including abalone.

Andrew & O'Neill (2000) conducted a ground truth supported aerial photographic survey of sixty 4 to 5 ha reef sites. These spanned commercially fished populations of *H. rubra* from Port Stephens to the Victorian border. A critical finding was that barrens habitat constituted on average 50% of near-shore reef area. The massive extent of barrens in NSW appears to offer great scope for reef rehabilitation of *H. rubra* stocks. Natural re-colonisation of depleted areas by abalone is probably limited by the combination of competitive exclusion by *C. rodgersii* and the restricted dispersal of *H. rubra* larvae from their parents as demonstrated by Prince *et al.* (1988).

Hamer (1982) and Andrew *et al*. (1998) demonstrated that culling urchins from urchin dominated reef in Southern NSW resulted in rapid rehabilitation of abalone. The recovery included a dramatic increase in the coverage and biomass of foliose algae and an associated increase in biodiversity of floral and faunal assemblages. This included *H. rubra*, which attained densities typical of high producing reef within 3 to 4 years. However, urchin culling techniques are very labour-intensive and result in re-colonisation over small, localised areas. The task of culling *C. rodgersii* from up to 50% of coastal reefs in NSW, that according to Worthington *et al.* 2003, collectively comprise about 5000 ha, is daunting. On the other hand, the mass production and release of seed *H. rubra* potentially provides a powerful technique to rapidly enhance depleted populations of abalone over large areas. It (seeding) also addresses other factors limiting recruitment and consequentially sustainable yields. Such factors include the combined effects of commercial, recreational and illegal fishing pressure, diseases such as *Perkinsus* that has devastated stocks north of Jervis Bay (Worthington 2002) and pollution.

The efficacy of enhancing wild stocks of abalone with hatchery-produced seed has been investigated both internationally and within Australia. As stated by Shepherd *et al*. (2000), "Release of larvae and juveniles has often been proposed as a panacea for rehabilitating depleted or over exploited reef. Seeding of juveniles has been practised in Japan for several decades with some success (Kojima 1995; Masuda & Tsukamoto 1998). Larval release has been practised sporadically in Mexico since the 1960's (Ortiz-Quintanilla 1980).

Prior to this study, research in NSW into the efficacy of previously developed techniques for the release of seed abalone was limited to larval releases in Twofold Bay. A total of 105 attempts were made year round to induce apparently ripe wild abalone to spawn. These were selected from

commercial landings over a period of two years (August 1994 to June 1996). On each occasion, an average of 5 males and 7 females were subjected to induction, however significant numbers (>100,000) of viable eggs were produced on only 7 occasions. Of these, only 2 yielded usefully large batches, one of 4.0 million eggs on December 8, 1994 the other 6.2 million one year later (21/11/1995). These results highlighted the vagaries of relying on wild stock as a source of ripe, ready to spawn broodstock. The spawning season of *H. rubra* in NSW may be limited to just a few weeks in spring or early summer coincident with a rapid rise in sea temperature over the range 15 to 18 ºC (unpublished data supplied by Ross Werner, Abalone Shellfish Enterprises [ASE], pers. comm. 1998). The only documented release of larvae by ASE was of 70,000 four-day-old larvae at Oman Point, Twofold Bay (Keesing *et al.* 1994). Although survival of the larvae were not monitored, a localised high density aggregation of sub-legal size abalone was observed at the same site several years later. This information, though anecdotal and highly dubious, encouraged collaborative involvement of TACC quota holders in this project as mediated through the NSW ABMAC (Abalone Management and Advisory Committee).

Larvae of both *H. rubra* and greenlip abalone (*H. laevigata*) were released at different densities in South Australia by Preece *et al*. (1998). Generally low but variable survival in the range 0.02 to 7.8%, occurred 6 to 7 days after settlement. Such strongly density dependent rates of mortality of larvae and early post-larvae was also reported by McShane (1991) in relation to naturally recruited stocks of *H. rubra* in north eastern Victoria. On the basis of these reports, Shepherd *et al*. (2000) recommended against larval seeding as did Schiel (1993) based on similar results with seeding of *H. iris* (paua) larvae in New Zealand.

In a review of experimental releases of 7 month old (12 mm) hatchery produced *H. rubra* in Tasmania, Shepherd *et al.* (2000) concluded that very high rates of mortality over the first few weeks were due mainly to handling stress. They also concluded that high mortality up to one year after release was due to persistent density dependent mortality factors and predator naivety. The latter conclusion was supported by low rates of mortality when hatchery produced juveniles were seeded onto artificial reef modules deployed in seagrass habitats with few predators.

As discussed above, it was clear from the outset of this project that successful enhancement of abalone populations in NSW using either hatchery produced larvae or juveniles is likely to require considerable knowledge of the ecology of post-larvae and juveniles and adaptation and improvement of techniques developed elsewhere. Whilst many of the logistical difficulties are the same as elsewhere, differences in the ecology of the sub-tidal reefs in NSW needed to be considered in experimental releases.

2. NEED

Key prerequisites to successful enhancement of natural fisheries, in the case of the NSW blacklip abalone fishery, are that:

1. Long-term sustainable catches from those fisheries are restricted by natural recruitment.
2. Seed animals, in this case ready-to-settle (competent) larvae or juvenile blacklip abalone can be produced reliably, cheaply and preferably throughout the year.
3. Released seed consistently generate additional volume and value to sustainable catch to cover all costs of production, deployment and compulsory impact monitoring.
4. Cost effective enhancement of sustainable catch of the target species does not threaten the genetic integrity of wild populations nor the long-term sustainability and diversity of associated coastal reef communities.

As discussed above, there is strong evidence that the NSW blacklip abalone fishery is indeed recruitment limited thereby satisfying the first prerequisite. The same cannot however be said of the second prerequisite. Although large-scale hatchery production of seed abalone was being achieved by commercial abalone farms in southern Australia at the outset of this study in 1998, all were fully reliant on access to wild, ripe, breeding stock generally restricted to the period mid to late spring to early summer (October to December).

Such operations also involved very large diatom plate nursery systems that yield on average about 40-50 advanced (10-20 mm shell length) juveniles per plate per year. Quoted prices for excess stock of 2¢ per mm (20-40¢ each) were considered cost prohibitive to seeding operations. Natural mortality data (Appendix 7) suggests that even amongst wild juveniles of this size class, only one in 20-30 survives through to legal size adults (115 mm shell length). At best this translates to seed costs of $4-6 per potentially harvestable abalone valued at $8-10.

These facts and figures highlight the need of this project to reduce seed costs and to develop controlled year-round reproductive conditioning that would also enable multiple rather than single annual batch nursery production.

The need for this project to develop improved cost, time and space efficient nursery seed production, equipment and protocols was exacerbated by our research facility site that totals only 300 m^2. Of this, only about one third was available for initial diatom plate and shallow raceway propagation of large numbers of seed required for field seeding experiments of meaningful scale.

Equally important as reliable, low cost seed production (prerequisite 2), was the need to improve generally low post-release survival rates of hatchery produced juveniles so far achieved in Australia and elsewhere. In most cases survival rates were well below those reported of wild counterparts. This situation highlighted the need of this project to systematically evaluate a range of factors to enhance post-release survival of both competent larvae and juvenile seed, i.e. to address prerequisite 3.

The need to address prerequisite 4, namely to assess and minimise environmental risks associated with large-scale abalone seeding programs, was recognised. However, we also considered that to attempt to address such issues would be beyond the scope, resources and time frame of this study and should in any case be appropriately subcontracted to independent researchers without any vestige of self-interest in outcomes. Moreover, such research should be considered premature prior to the demonstration of potential cost effectiveness of enhancement.

3. OBJECTIVES

3.1. Original objectives as specified in FRDC funding application

1. Produce seed from wild collected blacklip abalone at a range of sizes and ages throughout the year.
2. Develop techniques to enable the successful deployment of seed to coastal reefs in NSW.
3. Develop techniques to maximise the settlement, survival and growth of seed on coastal reefs in NSW.
4. Commence deployment of fluorochrome-marked seed to depleted coastal reefs in NSW.

3.2. Modified objectives as agreed to in collaboration with external referee and endorsed by FRDC

1. Produce seed from wild collected blacklip abalone at a range of sizes and ages throughout the year.
2. Develop techniques to enable the successful deployment of seed to coastal reefs in NSW.
3. Develop techniques to maximise the settlement, survival and growth of seed on coastal reefs in NSW.
4. Reseed reefs at Port Stephens, Sydney and at least one location further south, to be determined in conjunction with ABMAC.
5. Quantify the survival and growth of reseeded abalone for a minimum of 18 months.

4. SEED PRODUCTION

M.P. Heasman, N. Savva, C. Brand & J. Diemar

4.1. Introduction

From the outset of the project it was recognised that a key to development of successful seeding techniques was a regular and large supply of larvae and juveniles. Two complementary strategies were pursued. The first strategy was to improve access to wild, spawnable *H. rubra* by determining the availability of such stock. The second strategy was to determine how to reliably ripen and spawn captive broodstock.

4.1.1. Background

During 1997, a joint venture between NOFARIC (NSW OLMA Fisheries and Related Industries Committee) and NSW Fisheries established an abalone hatchery on Tomaree Headland, Port Stephens. This facility included a controlled temperature broodstock conditioning unit and larval and juvenile rearing facilities. Major difficulties were encountered in acquiring and spawning abalone at the Port Stephens hatchery. These difficulties were probably the product of three interrelated factors. The first was limited seasonal availability (spring to early autumn) of ripe *H. rubra* at Port Stephens, which lies at the northern limit of abalone populations in NSW. The second factor was a poor understanding of the inhibitory effects of stresses (such as trauma of capture, transportation, handling and confinement) on the ability of broodstock to respond to spawning induction. The third factor was our reliance on non-intrusive but flawed methods gauge gonad developmental stages. These methods were based on those used by McShane *et al*. (1986) to assess the seasonal breeding activity of *H. rubra* in Victoria.

The gonadal development stages used (Table 1) were based on the assumption that a direct relationship existed between readiness to spawn and the size and fullness of the distal apical process of the ovary, viewed by deflecting the foot (McShane *et al.* 1986). Histological examination of gonads from abalone collected between late spring (October 1997) and early autumn (March 1998) revealed that many ovaries visually assessed as stage 4 and 5 (Figure 1), were in fact over-ripe (atretic). Moreover, very few of these atretic ovaries exhibited a successive brood of newly developing oocytes undergoing vitellogenesis (Heasman & Savva, unpublished data). These findings were supported by results of spawning induction trials conducted by Lleonart (1993) who found that ovaries and oocytes of over-ripe (atretic) greenlip abalone held at a constant temperature of 17ºC continued to increase in volume apparently well after mobilization of yolk material from the atretic eggs.

In a preceding study to the current project (Heasman *et al*. 1998a), a total of eighteen attempts to induce spawning (each involving from 6 to 16 adult females collected from the Tomaree Peninsula) were made over a four month period from the 9th October 1997 to the 19th March 1998. In all cases these broodstock were visually assessed as ripe (gonad scores 2 or 3 in Table 1). Results summarised in Figure 2 suggest that spawning in wild caught female *H. rubra* can be successfully induced from late spring to early Autumn but is largely confined to a period of about half of the lunar cycle beginning about 3 days prior to the final quarter and ending shortly after new moon, i.e. during the darks.

These observations revealed the complexities and vagaries of depending on wild *H. rubra* as a source of hatchery broodstock. They also illustrated the need to develop more reliable non-

destructive methods to evaluate the reproductive condition of *H. rubra*, particularly their readiness to spawn, and a need to develop reliable conditioning protocols. The latter need was viewed as highest priority as it alone could enable year-round supply of larvae and juveniles for trials to improve seed production and develop technology to enhance *H. rubra* populations.

Table 1. Visual criteria used to rank gonad condition in live abalone (G.I. = Gonadal Index).

Score (G.I.)	Gonad Appearance
1	Sex indeterminate
2	Sex determinate
3	Sex easy to determine, tip pointed
4	Tip of gonad rounded but not swollen
5	Tip of gonad rounded and swollen

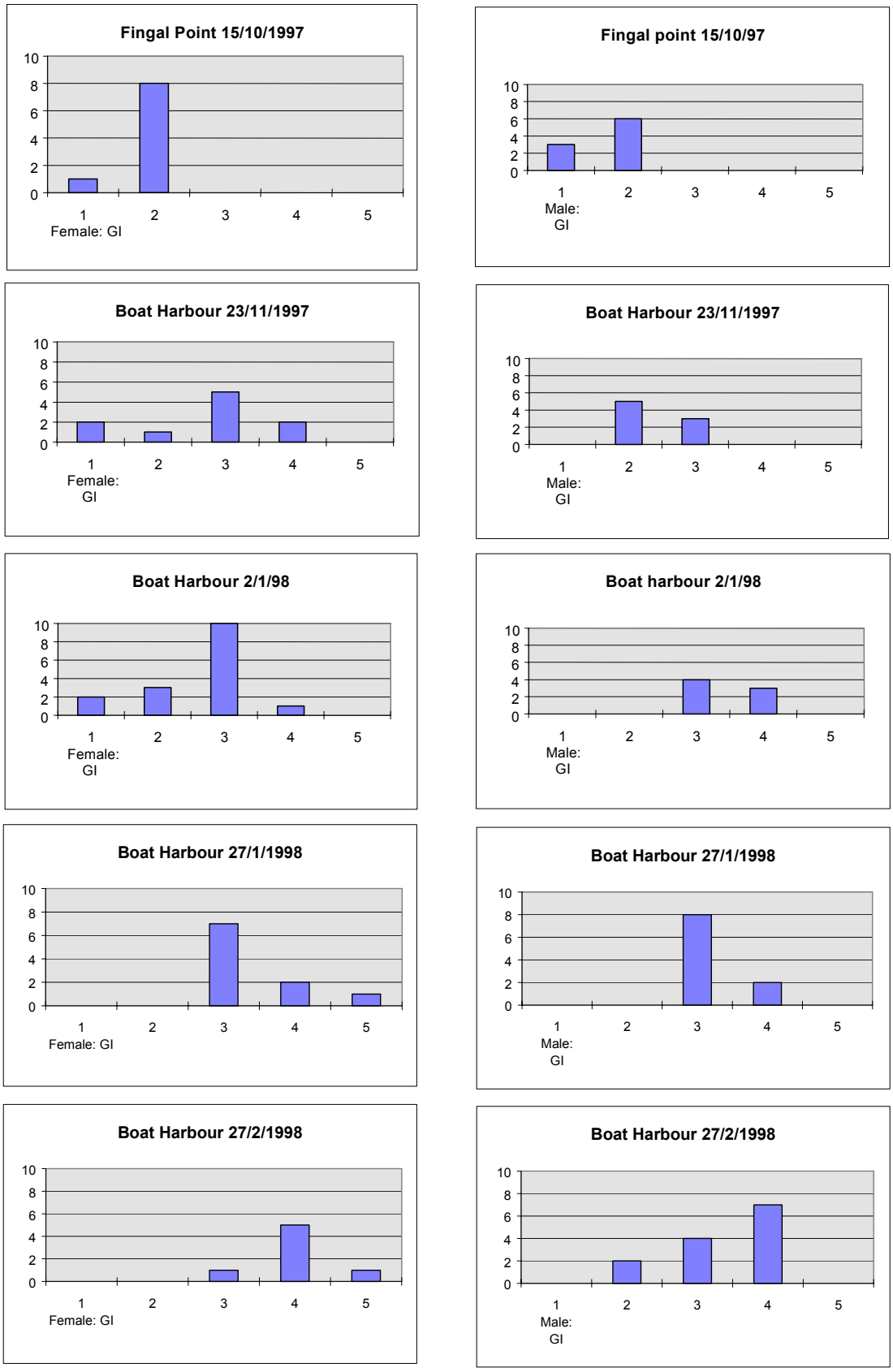


Figure 1. Size frequency distribution of wild broodstock of from Tomaree Peninsula classed according to visually assessed gonad stage (GI = Gonadal Index).

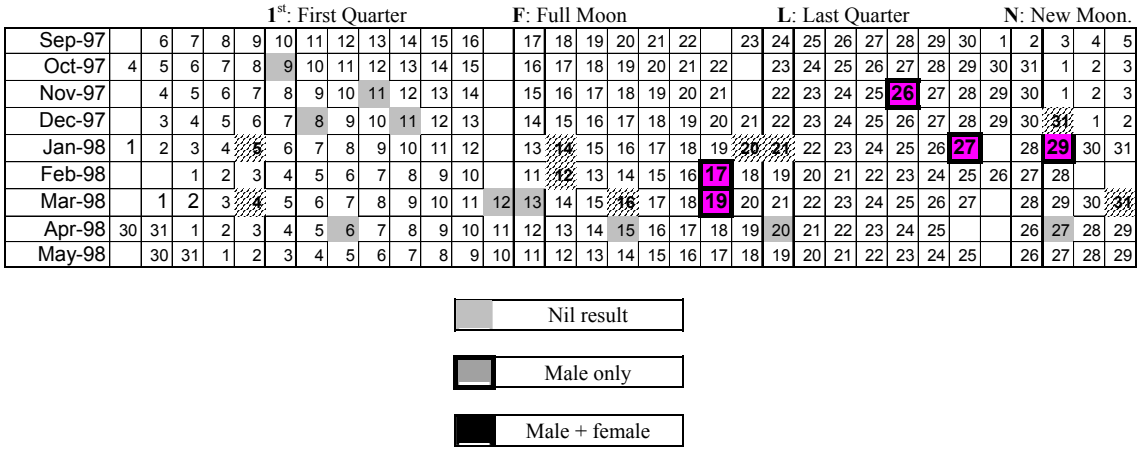


Figure 2. Induced spawning of wild female abalone from the Tomaree Peninsula in relation to moon phase. (Heasman *et al*. 1998a).

4.2. Further evaluation of wild populations as a source of ripe, ready to spawn broodstock

4.2.1. Methods

4.2.1.1. Field collection and spawning protocols for wild stock

An interim review of this project (Andrew 1999) recommended that investigations of wild populations of *H. rubra* in NSW as a source of ripe, ready to spawn stock be upgraded. In response an intensive and systematic program of collection and attempted spawnings of *H. rubra* was initiated using techniques described in Appendix 14.5.

Each month, adult *H. rubra* were collected by scuba divers from reefs on the Tomaree Peninsula, which lies near the northern limit for commercially fished stocks of *H. rubra,* and at Eden, in the far south of the state (Table 2). Additional spawning attempts were made using adult abalone collected opportunistically at Narooma, Tathra, Batemans Bay and Sydney (Table 2). Timing of collections was confined to the week prior to and following the new moon. This restriction was applied because successful spawning inductions of female abalone over the previous two years had largely been confined to this period (Figure 2).

A total of 29 attempts to induce spawning of wild abalone collected from southern NSW, primarily Eden, were completed between 28/8/99 and 2/12/99. Of these, 22 were conducted immediately following collection while the remaining 7 attempts were conducted 2-14 days later.

4.2.1.2. Lunar periodicity

Additional evidence was sought to confirm the influence of the lunar periodicity on spawning of both wild and captured adult females (Figure 2). Abalone were collected from a single site twice weekly (Table 1) and subjected to the spawning protocol. Logistical constraints dictated that the twice weekly sampling be confined to one area, namely reefs fringing the Tomaree Peninsula at Port Stephens. Twelve spawning attempts were conducted from 6/8/99 to 19/11/99. Continuity of this program was however disrupted by rough sea conditions that persisted from early to mid November.

4.2.2. Results

4.2.2.1. Field collection and spawning protocols for wild stock

Tomaree Peninsula (Table 1)

None of the twelve attempts to induce wild stock spawn immediately following their collection were successful. Accordingly, no further conclusions could be reached on the influence of the lunar cycle on spawning activity.

South Coast sites (Table 2)

No eggs were produced from attempts immediately following collection. Eggs were produced on two occasions with stock subjected to delayed induction following transportation back to the hatchery. On one occasion a negligible number of eggs were released but on the second, conducted with stock collected at Eden on 13/9/99, 1.5 million eggs were released. This batch was used in a

seeding trial at Eden consisting of about 120,000 juveniles (average shell length of 1.5 mm) in November 1999, and a further 30,000 (average 7.3 mm shell length) in March 2000.

4.2.3. Discussion

Prior to this study, seeding of reefs in NSW was limited to larval releases in Twofold Bay by Abalone Shellfish Enterprises (ASE). Over a two year period from August 1994 to June 1996, a total of 105 attempts were made by ASE to induce spawning of apparently ripe, wild abalone selected from commercial landings. On each occasion an average of 5 males and 7 females were subjected to spawning induction protocols with significant numbers (>100,000) of viable eggs produced on only 7 occasions. Of these, only two yielded usefully large batches, one of 4 million eggs on Dec 8, 1994 and the other of 6.2 million eggs almost one year later on 21/11/1995 (unpublished data, ASE; Ross Werner, pers. comm.1997).

Induction of spawning in wild abalone is often unreliable, which was of continuing concern for this project and indeed for many other research and commercial hatcheries around Australia (Fleming 2001). Given the high demand on resources of staff and facilities and disappointing results of both this and an earlier study by the authors and by ASE, further investigations of wild *H. rubra* as a source of ripe ready to spawn hatchery broodstock were abandoned and the project refocused on developing protocols for broodstock conditioning in a controlled environment.

Table 1. Attempted spawning induction of wild stock collected from reef sites on the Tomaree Peninsula.

Spawned	Collected	Location	Temp °C	Females	Males	x10^6 Eggs	Comments
6/08/99	Same day	Sunny Corner	17	7	6	Nil	
8/09/99	Same day	Cabbage Tree Is.	17	30	15	Nil	
7/10/99	6/10/99	Fingal Is.	16	12	8	Nil	
12/10/99	11/10/99	Fingal Is.	17	24	12	Nil	
15/10/99	14/10/99	Fingal Is.	18	16	12	Nil	
19/10/99	18/10/99	Fingal Is.	18	22	14	Nil	
22/10/99	21/10/99	Fingal Is.	18	20	12	Minimal	Very small egg release only, not continued
26/10/99	25/10/99	Fingal Is.	18	20	12	Nil	Thermal shock used as well, up to 21°C
29/10/99	28/10/99	Fingal Is.	18	22	14	Nil	Histology 1/11/99
2/11/99	1/11/99	Fingal Is.	18	21	13	Nil	Moderate male spawning. Thermal shock up to 21°C used
18/11/99	16/11/99	Fingal Is., N	19	21	13	Nil	
19/11/99	18/11/99	Fingal Is., S	19	24	12	Nil	

MOON	
New	Full
11-Aug	27-Aug
10-Sep	25-Sep
9-Oct	25-Oct
8-Nov	23-Nov

Table 2. Results of attempted spawning induction with wild stock collected from reef sites on the south coast of NSW.

Date of attempted Spawning	Collected	Site	Ambient Temp°C	Females	Males	Eggs x10^6	Comments
24/08/99	Same day	Narooma	16	12	6	Nil	
25/08/99	Same day	Tarthra		12	6	Nil	
26/08/99	Same day	Eden, Middle Head	14	12	6	Nil	
6/09/99	27/08/99	Eden, Middle Head	14	21	14	1.54	At Tomaree. Temp, 17°C. Batch 2, 200 000 to set
12/10/99	8/10/99	Sydney	15	2	5	Nil	At Tomaree. Temp, 17°C
13/09/99	27/08/99	Eden, Middle Head	14	21	14	Nil	At Tomaree. Temp, 17°C.
14/09/99	Same day	Bateman's Bay	17	20	10	Nil	
15/09/99	Same day	Eden, Mutrees Reef	15.5	20	10	Nil	
15/09/99	Same day	Eden, Lambourn Pt.	15.5	20	10	Nil	
16/09/99	Same day	Eden, Yellow Rock	15.5	20	10	Nil	
16/09/99	Same day	Eden, North Head	15.5	20	10	Nil	
20/09/99	17/09/99	Eden, Middle Head	15	24	12	Nil	At Tomaree. Temp 17°C.
29/09/99	17/09/99	Eden, North Head	15	24	12	Nil	At Tomaree. Temp 17.5°C. Small sperm release, 2 males
6/10/99	Same day	Eden, North Head	15.5	20	10	Nil	
6/10/99	Same day	Eden, S. Ben Boyd Tower	15.5	20	10	Nil	Small sperm release
6/10/99	Same day	Eden, Mutrees Reef	15.5	20	10	Nil	
13/10/99	27/08/99	Eden, Middle Head	14	15	21	Nil	At Tomaree. Temp, 18°C
13/10/99	17/09/99	Eden, North Head	15	21	21	Nil	At Tomaree. Temp, 18°C. Small sperm release
3/11/99	Same day	Eden, North Head	17	20	10	Nil	
3/11/99	Same day	Eden, Middle Head	17	20	10	Nil	
3/11/99	Same day	Eden, S Fisho Cull	17	20	10	Nil	
3/11/99	Same day	Eden, Mutrees Reef	17	20	10	Nil	
30/11/99	Same day	Eden, Leonards Is., N.	17.5	20	10	Nil	
30/11/99	Same day	Eden, N. North Head	17.5	20	10	Nil	
30/11/99	Same day	Eden, Yellow Rock, N Hd.	17.5	20	10	Nil	
1/12/99	Same day	Eden, S. Mowarry Pt	17.5	20	10	Nil	
1/12/99	Same day	Eden, N Mowarry Pt.	17.5	20	10	Nil	Minor sperm release from 2 males

Table 2. Continued

Date of attempted Spawning	Collected	Site	Ambient Temp°C	Females	Males	Eggs x10^6	Comments
2/12/99	Same day	Eden, Middle Head	17.5	20	10	Nil	
2/12/99	Same day	Eden, Mutrees Reef	17.5	20	10	Nil	
MOON							
New	Full						
11-Aug	27-Aug						
10-Sep	25-Sep						
9-Oct	25-Oct						
8-Nov	23-Nov						

4.3. Controlled reproductive conditioning of captive broodstock as an alternative source of ripe, ready to spawn broodstock

4.3.1. Introduction

Accessing ripe, ready to spawn wild *H. rubra* broodstock throughout its southern Australian distribution has proven highly problematic. Different regions pose different problems ranging from near or total absence of ripe animals in some years to unreliable supply within the spawning season during other years (Fleming 2000). As demonstrated in the previous sections, such problems are acute in relation to NSW populations, especially those nearing the northern limits of *H. rubra* from Sydney to Port Stephens and beyond to Coffs Harbour.

Critical prerequisites for successful reproductive conditioning of *H. rubra* and *H. laevigata,* reviewed by Fleming (2001), fall into four categories:

- non-traumatic or otherwise stressful handling of stock.
- adequate nutrition such as the use of high quality natural seaweed or formulated diets.
- maintenance of stock under favourable physico-chemical conditions especially, salinity, pH and concentrations of dissolved oxygen, un-ionised ammonia and nitrite.
- maintenance of stock under favourable temperature regimes.

Uki & Kikuchi (1984) concluded that temperature is the principal environmental factor affecting reproductive conditioning in *H. discus hannai*, and that gonad maturation may be regulated by manipulating the effective accumulated temperature (EAT). EAT is an expression in degree days of the time and temperature for which an animal is held above the biological minimum for gonad growth and may thus be a useful tool to predict spawning readiness. Uki & Kikuchi (1984) did not define an ideal conditioning temperature for H. discus hannai but predicted that maturation will proceed at any temperature over 7.6°C (the biological zero point [BZP] for gonad growth) and up to a maximum of 22.4°C.

At the outset of these investigations in July 1998, EAT requirements of *H. rubra* were not known but were subsequently the subject of at least two dedicated research projects described by Ritar (2000) and Freeman *et al*. (2000). Although the ideal temperature for reproductive conditioning and EAT for *H. rubra* thus remained to be defined, information then available (see Section 4.1) suggested that temperatures below 20°C are required for the gonads to fully ripen. This information also suggested that natural spawning events that occur from early spring to mid summer (September to January) in NSW, are triggered as sea temperatures rapidly rise from about 16 to 20°C.

4.3.2. Aim

The aim of this work was to create a year-round reliable supply of ripe ready-to-spawn *H. rubra* by controlled temperature conditioning of wild collected broodstock. This was critical to overcoming constraints to all key objectives of this research project as detailed in Section 3 of this report.

4.3.3. Methods

4.3.3.1. Broodstock conditioning

Except for very occasional equipment failures, conditioned broodstock were maintained at 15.5±1.0ºC (range of 15.5 to 17.5ºC) throughout this study. This temperature range was a best guess based on previous conditioning, and spawning attempts and on a similar mean temperature of 16.0°C successfully employed by Lleonart (1993) to condition *H. laevigata* in Tasmania. It was anticipated that this temperature would enable relatively rapid conditioning of gonads while providing a 3 to 5°C buffer against spontaneous spawnings triggered by sudden accidental temperature rises. Even with this buffer, high temperature induced spawnings of conditioned broodstock in the recirculation system did occur within a few hours following power cuts or failure of chiller equipment, especially during the warmer half of the year when daytime air temperatures commonly ranged from 25 to 35°C.

Two temperature controlled recirculating systems, hereafter referred to as broodstock units 1 & 2 (BU1 & BU2) were used. Each unit was housed in an insulated shipping container. The total seawater volume of each system was about 1500 L. A relatively high recycling rate of about 80 volumes per day was used and a net waste water to new water exchange rate of about 2 volumes per day. The systems included continuous foam fractionation and physical and biological filtration.

The abalone were held in eighteen 25 L buckets at a density of 5-10 per bucket. Batches of abalone, grouped according to original site of collection, were housed within 6 buckets, thereby enabling 3 separate batches of 30 to 60 abalone to be housed within each of the two conditioning units. Each bucket was aerated at 1-2 L/min and supplied with recycled filtered seawater at 6-8 L/min. A detailed description of system design and operation, including general broodstock husbandry and reproductive conditioning protocols, is provided as appendices (Sections 15.3, 15.4 & 15.5).

Adult *H. rubra* were collected from 5 sites off Tomaree Peninsula (Broughton Island, Cabbage Tree Island, Yacaaba Head, Fingal Island and Boat Harbour) and from sites off Sydney, Kiama, Ulladulla and Eden. The abalone were selected within a relatively narrow size range of 100-125 mm shell length, cleaned of excessive bio-fouling and individually tagged. Drained live-weight was recorded to the nearest gram and shell length and width to the nearest millimetre. Each abalone was sexed and assigned a visual gonad condition index (vGI) score from 1 to 3 according to modified visual criteria provided in Table 1 before being transferred to the conditioning units. The modified criteria better discriminates genuinely ripe from over-ripe (atretic) gonads with the latter tending to be more swollen, distended and spongy. Water temperatures were controlled at 15.5 ± 1.0°C. All broodstock were fed to slight excess on a commercial formulated diet, (Adam and Amos P/L, South Australia) at a rate of about 0.5% body weight per day; with the exception of abalone held in BU2 (July 1999-July 2000) during the dietary conditioning trial (Section 4.4).

Table 1. Modified visual criteria used to rank gonad condition in live abalone (Numbers in parenthesis are equivalent to scores used in earlier trials see Table 1 in Section 4.1).

Score	Gonad Appearance
0 (1)	Sex indeterminate
1 (2)	Sex determinate
1.5 (-)	Sex determinate. Tip of gonad tip pointed
2 (3)	Tip of gonad blunt and firm
2.5 (4)	Tip of gonad rounded, swollen and firm
3 (5)	Tip of gonad rounded, very swollen and/or spongy

Salinity, dissolved oxygen and ammonium concentrations and pH were routinely measured just prior to water changes for several months and thence from time to time, especially following periods of rough seas and protracted heavy rainfall. Dissolved oxygen and pH were assessed using a water quality meter (Pinpoint). Ammonium concentrations were assessed using colorimetric test kit (Merck 1.08024.0001). Temperature was recorded continuously using minimum/maximum thermometers or digital temperature loggers (Hastings Data Loggers). Full water changes including scrubbing of buckets and tanks were conducted weekly and additional half water changes were performed twice weekly as detailed in Section 15.3.

4.3.3.2. Induction of spawning

As elaborated in Section 15.6, hydrogen peroxide was used to initiate spawning following Morse *et al.* (1976) and Tong *et al*. (1992). Abalone were transferred to spawning tubs in a separate temperature controlled spawning and larval rearing unit on the afternoon prior to, or the morning of spawning initiation. There were usually 2 to 4 females per 14 L polyethylene spawning tub. They were held overnight in 1 μm filtered, UV irradiated, flowing seawater at the same temperature at which they were conditioned. The following morning the temperature was increased by 2°C. After one hour at the elevated temperature, the seawater flow was discontinued and 2M TRIS (Tris-hydroxy-methylamino methane, M.W. = 121.1.) was added at 6.6 mL/L to raise pH to 9.1. After a further 15 minutes, freshly prepared 6% hydrogen peroxide was added at 3 mL/L. Abalone were exposed to the hydrogen peroxide for 3 h before rinsing and refilling the tubs with isothermal seawater. Spawning generally occurred after a further 1-2 h. Despite having more than one female per spawning tub an effort was made to record which individual females spawned and how many eggs they each produced. Where this became difficult because of large multiple spawning events, the number of eggs produced were attributed evenly to those females that spawned within each tub.

4.3.4. Results

4.3.4.1. Water quality

Temperature generally remained at 15.5±1.0ºC, however occasional failures of the seawater chiller unit caused brief (1 to 12 h) exposure to elevated temperatures as high as 23°C. Salinity ranged from 33 to 36 g/L. Ammonium concentrations were always found to be below 0.2 mg/L, which was the minimum detection level of the kit. (equivalent to unionised ammonia NH_3 levels of less than 0.02 mg/L at the measured pH range of 8.0-8.3). Dissolved oxygen levels were at or slightly above 100% saturation at the corresponding temperature and salinity. All the above water quality criteria remained within ideal ranges as defined by Harris & Burke (1999) for *H. laevigata* and *H. rubra.*

4.3.4.2. Survival

Annual survival of conditioned stock, segregated according to site of collection over the period July 1999 to June 2000, was typically greater than 90% (Table 2). The lowest result was 87%. Overall survival of stock from July 2000 to July 2001 averaged 94%. While some mortalities were unexplained, most appeared to be associated either with injuries inflicted during handling, mudworm infections or due to culling prompted by shell fractures in turn induced by a boring sponge, *Cliona* sp.

Table 2. Broodstock survival.

Collection	Initial no.	Final no.	% survival
Broughton Island	31	27	87
Boat Harbour/Yacaaba Peninsula	32	29	91
Eden	66	62	94

4.3.4.3. Growth

Increases in mean shell length and width were consistent with those described by Worthington *et al.* (1998). Females that spawned did not appear to have grown any more or less than those that failed to spawn. However one female sourced from Broughton Island that spawned on 3 occasions over a 12 month period also grew considerably, increasing in length by 10 mm, in width by 7 mm and in live-weight by 71 g.

4.3.4.4. Spawning and egg production (Table 3)

During the three years, July 1999 to June 2001, a total of 34 controlled spawning induction attempts were made with groups of 13 to 38 adult females held in the conditioning systems for periods ranging from 47 to 721 days. Of the 34 attempted spawning inductions, half (17) were successful yielding significant batches of ova ranging from 0.8 to 19.3 million and totalling 59.3 million eggs. An additional three unplanned epidemic spawning triggered by high temperatures resulting from power or chiller failures, yielded a further 63 million eggs rendered unusable by polyspermy and bacterial contamination.

A total of 24.0 million competent to settle 7- or 8-day-old larvae were produced and used for larval seeding experiments or for nursery production of juvenile seed. This represented an overall mean yield of 40.5% from the fertilized egg stage. Overall mean ±s .e. fecundity was 1.25±0.49 million per spawner. This estimate was based on data collected on 14 occasions when exact numbers of spawning females (1 to 13) and total eggs generated by them in response to controlled induction of spawning, could be confirmed. Fecundity specific for 37 individual spawnings varied greatly over the range 42×10^3 to 4.0×10^6 but commonly ranged between 0.5 and 2.5 million per spawner.

Successful inductions of spawning were usually of limited success in that only one or a few *H. rubra* females from each group of 13 to 38 adult females subjected to controlled spawning induction, actually spawned. Overall verified rate of induced spawning success using conditioned broodstock was 10.8% (85 out of 785 females). Three individual abalone from a collection of 22 Broughton Island females held in the conditioning system from 15/4/1999 to 30/6/2001, spawned on either 4 or 5 successive occasions (Table 3). Intervals between consecutive spawnings of individual female broodstock varied widely from 74 to 290 days (Table 4).

Epidemic spawning of abalone occurred only on three occasions (Table 5). Subsequent synchronous spawning within the same groups of abalone occurred on two occasions. These episodes were fortuitous in that they provided useful indicators of reproductive conditioning time for *H. rubra*. A number of Broughton Island females were successfully induced to spawn 207 and 290 days after an initial mass spawning. Similarly, a group of Sydney sourced broodstock exhibited extensive spawning 206 and 233 days after a mass spawning event.

Appraisal of modified gonad staging criteria (Table 2) as a predictor of spawning success and fecundity.

Fecundity data grouped according to visual gonad score for individual spawnings of 34 female *H. rubra* (Figure 1) show that while fecundity increased with increasing size and prominence of the ovary, as assessed by visual index score, these scores were a poor indicator of fecundity as well as an unreliable indicator of spawning per se. Evaluation of the effects of time in captivity on spawning response (Figure 2), fecundity and larval yields (Figures 3 & 4 respectively) showed that while fecundity was not apparently affected, spawning response and egg and larval quality, (as indicated by larval yields from eggs), increased with increasing time in captivity.

4.3.5. Discussion

At the time of this study (July 1999 to June 2001) the optimal reproduction conditioning temperature for *H. rubra* was yet to be determined as was biological zero point (BZP) and EAT for this species. Subsequently all three parameters have been accurately identified by Grubert & Rita (2003a & b) in a project dedicated to this task. The main findings of Grubert & Rita (2003a & b) were as follows:

- Periods of conditioning *H. rubra* for successful spawning is linearly related to temperature from the BZP (determined as 7.6-7.8ºC) up to and including the highest tested temperature of 18ºC.
- Mean spawning rate of both sexes was higher in groups held at 18ºC than at 16ºC as was the repeat spawning rate.
- Conversely, animals held at 16ºC produced significantly more gametes than those at 18ºC.
- Mean fecundity of 0.2 to 2.0 million eggs per female was achieved with total egg production peaking in groups held at 16ºC for >1350 EATºC-days equivalent to $\geq$165 days.

These results prompted the authors to conclude that…"Conditioning of blacklip on artificial diets for as little as 114 days at 18ºC and as long as 235 days at 16ºC before induction generally yielded production rates that can be considered commercially acceptable."

These results are comparable to those of the present study (Table 4) and confirm that our best-guess choice of conditioning temperature of 15.5±1.0ºC was appropriate. However, the results did expose a relatively poor average spawning response of only 11% compared with a range of 20-40% at 16ºC and of 30-80% at 18ºC achieved by Grubert & Rita (2003a).

Plant *et al*. (2002) found that both spawning response (92%) and fecundity (2.2 million/eggs/female/spawn) peak in *H. rubra* conditioned at 18ºC after 120 days (1220 EATºC days). Corresponding performance decreased significantly after 1,500 degree days to 65% spawning response and mean fecundity fell to 1.1 x 10^6 eggs/female.

Since completion of this study, a number of major changes have been made to the conditioning system and operating protocols. These have resulted in an elevation of the average spawning response to 60% during 2003. These changes include:

1. Removal of broodstock from 25 L buckets to a common 1,200 L reservoir that enables continuous removal of uneaten food and faeces.
2. Increased daily net seawater input from 2 to 4 exchanges per day.
3. Upgrading of seawater chillers from cheap off-the-shelf systems to purpose-built units.
4. Individual tracking of tagged broodstock that are subjected to re-spawning induction every 150 days at a holding temperature of 16±1ºC (equivalent to 1,200 degree days).

Although the two conditioning units used in the present study were generally reliable, significant problems developed when the electrical power supply, seawater chillers or their temperature controllers failed. These failures exposed conditioned broodstock to high temperatures above 20ºC. This in turn triggered epidemic spawning on three occasions during the reporting period of July 1999 to June 2001.

Abalone are particularly sensitive to ammonia. Total ammonium nitrogen concentrations (TAN) in the brood units were routinely checked and always found below 0.2 mg/L. This translates to a toxic free ammonium nitrogen (FAN) level of below 0.001 mg/L. This level is well below a recognised and acceptable level of 0.04 mg/L defined by Harris *et al*. (1999) and cited by Fleming (2000) and also below an EC_5 level of 0.004 mg/L cited by Huchette *et al*. (2003).

Foam fractionation in conjunction with the high turnover rate bio-filtration, high daily net seawater exchange and conservative stocking and feeding rates were critical to maintaining good water quality including low TAN and FAN concentrations. Without the fractionators, foam rapidly developed on the surface of the holding tanks. Regular cleaning of the units to remove faeces and uneaten food from the systems and the continual throughput of seawater also helped to prevent overloading of the bio-filters. The nature of seawater flow and aeration provided in buckets used to accommodate small groups of abalone in BU1 was found to facilitate continuous flushing of faeces and food remnants away from the abalone and ensured that dissolved oxygen was continuously maintained at ≥100% saturation.

The most unsatisfactory aspect of controlled conditioning and induction of spawning was the relatively low overall spawning response of 10.8% by female *H. rubra*. On the other hand, data presented in Figure 4 suggests that much improved spawning response in the order of 50% can be achieved once stock are fully domesticated. In this case it would appear that adult *H. rubra* required at least one and perhaps up to two years (720 days) to recover from trauma and stress of capture and to fully acclimatise to conditions of communal confinement in dark, noisy, turbulent containers and to adapt to an artificial diet and to regular handling.

Some additional evidence that the captive broodstock were well nourished and maintained was that mean fecundity (Figure 3) did not change over time. Even more compelling evidence was that egg and larval quality, as indicated by the percentage of fertilized eggs that yielded competent to set larvae (y in Figure 4), also increased with holding period. The relationship between holding period (x in Figure 4) was better described by a power equation ($y = 3.7028x^{0.4581}$; $R^2 = 0.5295$) than by equivalent best fit linear, logarithmic, exponential or second or third order polynomial equations.

Table 3. Spawning summary of conditioned broodstock, May 1999 to June 2001 (excluding dietary trial) (BU = Broodstock Unit).

Collection location	Date Collected	BU	Date allocated to BU	Date of attempted spawning	Days conditioned	No. Females	No. Females spawned	Number of eggs x10^6	Average fecundity 10^6 eggs	Competent to set larvae x 10^6
Broughton	15-04-99	1	21-05-99	07-07-99	47	23	0	0.00		
Broughton	15-04-99	1	21-05-99	11-08-99	82	23	0	0.00		
Broughton	15-04-99	1	21-05-99	10-09-99	112	23	0	0.00		
Broughton	15-04-99	1	22-05-99	05-10-99	136	22	0	0.00		
Broughton	14-04-99	1	23-05-99	05-11-99	176	23	3	3.29	1.10	Nil
Broughton	15-04-99	1	24-05-99	19-01-00	240	22	0	0.00		
Eden	27-08-99			14-01-00		20	0	0.00		
Broughton	15-04-99	1	24-05-99	14-03-00	295	21	1	2.10	2.10	Nil sperm
Broughton	15-04-99	1	24-05-99	06-04-00	318	16	2	3.46	1.73	2.16
Sydney	03-04-00			26-04-00		13	0	0.00		
BI, BH, YA	15-04-99	1	24-05-99	08-05-00	350	16	12	19.30	1.61	9.12
Broughton	15-04-99	1	24-05-99	19-06-00	392	(22)	?		?	
Eden	27-08-99	1	14-12-99	20-06-00	189	38	6	10.80	1.80	2.94
Eden	27-08-99		14-12-99	01-08-00	231	27	0	0.00		
Sydney	03-04-00			08-08-00		14	0	0.00		
Ulludulla	11-07-00	1	12-07-00	26-09-00	76	25	0	0.00		
Eden	27-08-99	1	01-08-00	28-09-00	58	26	3	4.00	1.33	0.57
Sydney	03-04-00	2	13-09-00	23-10-00	40	14	13	40.0*(3.6)	3.08	0.59
Ulladulla	11-07-00	1	12-07-00	31-10-00	111	25	0	0.00		
Sydney	03-04-00	2	13-09-00	01-11-00	212	14	0	0.00		
Kiama	15-09-00	2	19-09-00	08-11-00	54	30	0	0.00		
Eden	28-08-99	1	01-08-00	15-11-00	106	26	4	2.5	0.63	0.89
Kiama	15-09-00	2	19-09-00	05-12-00	81	30	1	1.57	1.57	1.10
Ulladulla	11-07-00	1	12-07-00	10-01-01	183	24	12	11.10*(0.0)	0.93	
Kiama	15-09-00	2	19-09-00	11-01-01	118	30	0	0.00		

Table 3. Continued.

Collection location	Date collected	BU	Date allocated to BU	Date of attempted spawning	Days Conditioned	No. Females	No. females spawned	Number of eggs x10^6	Average fecundity1 0^6eggs	Competent to set larvae x 10^6
Broughton	15-04-99	1	24-05-99	12-01-01	638	22	4	3.89	0.97	2.52
Ulladulla	11-07-00	1	12-07-00	27-03-01	259	24	0	0.00		
Sydney	03-04-00	2	13-09-00	28-03-01	359	14	0	0.00		
Kiama	15-09-00	2	19-09-00	29-03-01	195	30	0	0.00		
DBX	30-11-00	1	14-12-00	03-04-01	124	25	0	0.00		
BH	22-09-00	2	22-09-00	04-04-01	194	21	0	0.00		
Broughton*	15-04-99	1	24-05-99	05-04-01	721	22	9	10.20*(0.0)	1.13	
Ulladulla	11-07-00	1	12-07-00	15-05-01	308	24	0	0.00		
Kiama	15-09-00	2	19-09-00	16-05-01	243	30	3	0.80	0.27	0.40
Sydney	03-04-00	2	13-09-00	17-05-01	409	14	8	5.60	0.70	3.36
Sydney	03-04-00	2	13-09-00	13-06-01	436	14	4	3.80	0.95	0.41*
Totals						785	85	122.4(59.3)		24.0
Means							10.8%		1.25	

* batches failed due to extraneous factors especially failure of seawater flow and/or UV sterilizer.
** included unplanned spawning of 36.4million eggs triggered by failure of chiller.

Table 4. Days between successive spawnings of females that spawned on multiple occasions in response to the standard induction protocol.

Tag No.	Days between successive spawnings by individual females			
	1st	2nd	3rd	4th
1009	143	74	207	83
1010	120	97	290	
1012	143	281	83	

Table 5. Days elapsed between successive synchronous (epidemic) spawning events.

Spawning Date	Source of stock	Days Conditioned	No. females	No. females spawned	No. eggs x106
*19-06-00	Broughton Is.		22	Most if not all	Not quantified but tens of millions
12-01-01		207		4	3.9
05-04-01		290		9	10.2
23-10-00	Sydney		14	13	40.0
01-11-00		9		0	0.0
28-03-01		156		0	0.0
17-05-01		206		8	5.6
13-06-01		233		4	3.8
10-01-01	Ulladulla		24	12 or more	11.0
27-03-01		76		0	
15-05-01		125		0	
16-07-01		187		0	

* Unplanned spawning triggered by system failure and elevated temperature shock.

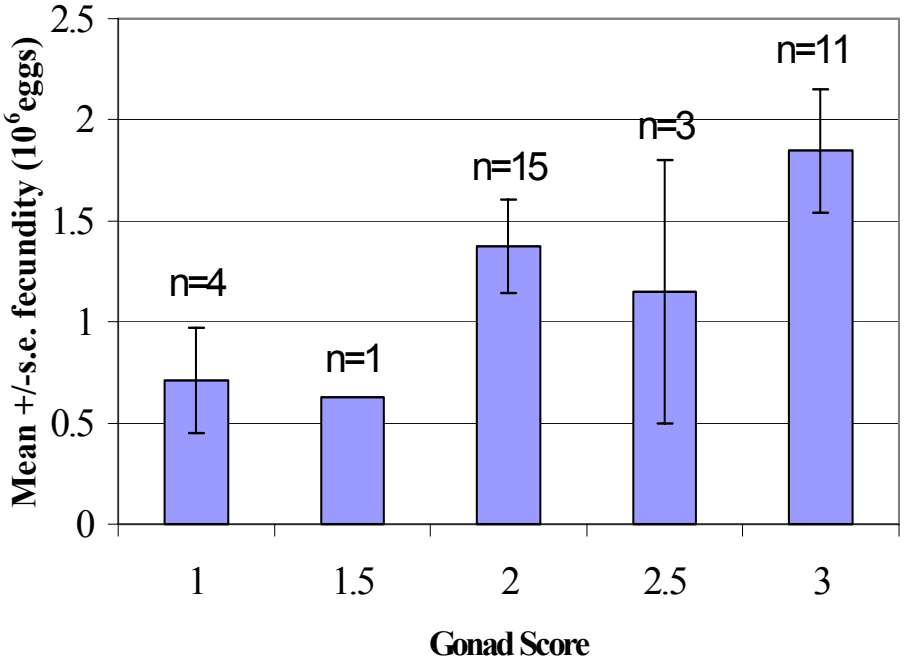


Figure 1. Relationship between visual gonad score and mean fecundity.

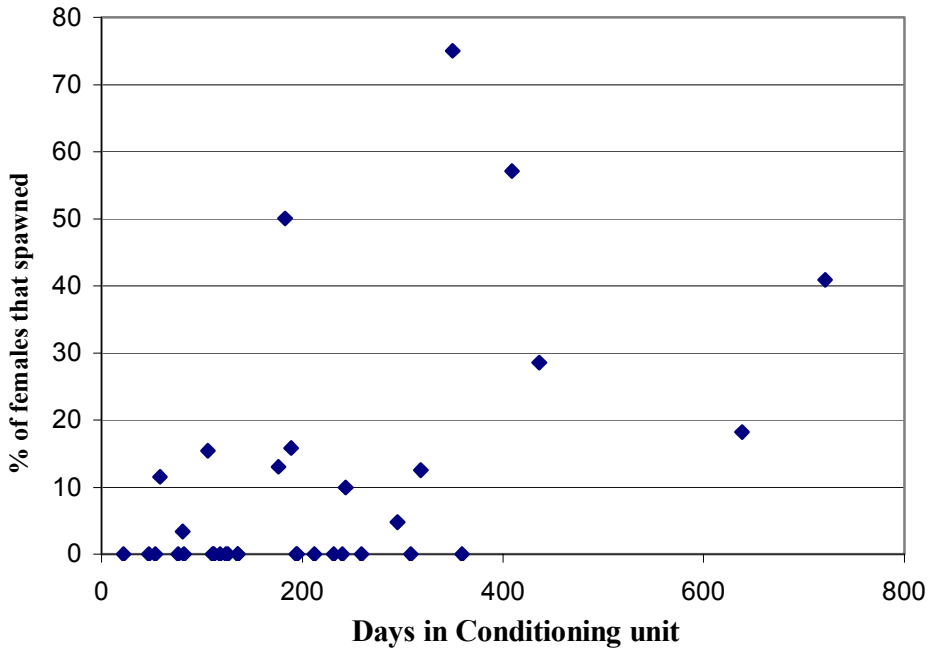


Figure 2. Effect of cumulative time in conditioning units on spawning response.

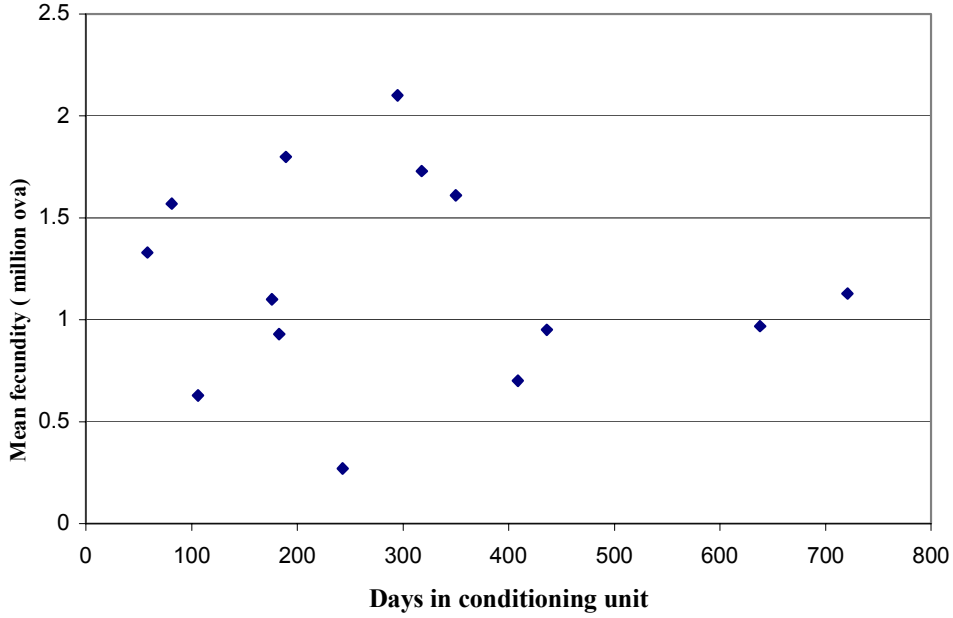


Figure 3. Effect of time in conditioning unit on fecundity.

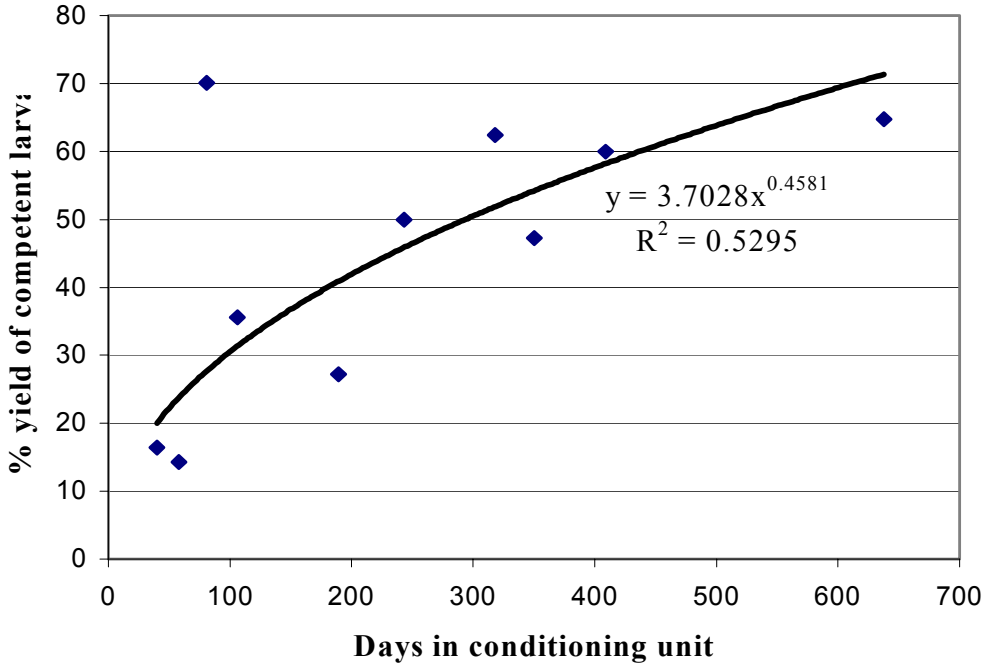


Figure 4. Effect of conditioning period on egg/larval viability.

4.4. Evaluation of three diets on reproductive conditioning

4.4.1. Introduction and aims

Although some investigations have been made on the effects of diet on abalone reproduction, breeding of captive stock is poorly understood and difficult to control (Fleming 2001). Temperature is recognised as the main exogenous factor that regulates breeding in many marine gastropods (Hahn 1989), but adequate nutrition is also critically important in gametogenic development of abalone (Uki & Kikuchi 1982). As articulated by Fleming 2001, artificial diets may be deficient in some nutrients required for gonadal development and it is therefore advisable to supplement them with algae when preferred species are available. Total reliance on a commercial formulated diet to condition captive *H. rubra* over protracted periods was accordingly viewed with some concern.

To address this concern, a range of alternative seaweed species were considered for evaluation as supplements to commercial feed manufactured in accordance with recommended formulation developed under the FRDC Abalone Aquaculture Subprogram. Based on results of Fleming's (1995) appraisal of the algal feeding preferences of *H. rubra,* and relative growth performance thereon, it was anticipated that a mixture of fresh, fleshy green and red seaweeds would constitute an ideal supplement for *H. rubra* broodstock. However this preferred option was precluded by very high labour demands and practical constraints to frequent year round collection. Practical constraints included OH&S and departmental operational codes of practice that dictate the involvement of at least 3 commercially accredited SCUBA divers for all collections, favourable sea and weather conditions and seasonal variability in availability of suitable seaweeds.

Instead dried *Phyllospora comosa* was selected as a supplementary diet. This decision was supported by anecdotal information that commercial fishers routinely stockpiled and maintained captive *H. rubra* in good market condition on a diet of fresh or air-dried *P. comosa*. Other contributing factors to the selection of *P. comosa* was its ubiquitous distribution and year round availability on reefs throughout the southern and central coasts of NSW.

Uki & Kikuchi (1984) noted that diet must be adequate for EAT to be a practical guide for predicting gametogenic conditioning. They supplied seaweed (*Undaria pinnifitada* and *Laminaria religiosa*) at 20% of body weight per day during conditioning and recorded an average daily ingestion rate for *H. discus hannai* of 5% of body weight per day. The quantity of seaweed ingested increased with gonadal development (Uki & Kikuchi 1984). Despite feeding only a single species of seaweed at a time, Uki & Kikuchi (1984) and Ault (1985) found a relationship between food availability and gonad development only when food quantity was limited. Buchal *et al*. (1998) did not detect significant differences in the growth rates (shell length and live-weight) in the number and diameter of eggs produced by the red abalone *H. rufescens* fed alternative seaweeds, dulse (*Palmaria mollis*) and kelp (*Nereocystis luetkeana*). However, the overall dry weight of eggs, protein and lipid content and subsequent viability and metamorphic success was significantly greater for abalone fed *P. mollis* (Buchal *et al*. 1998).

The aim of this experiment was thus to evaluate the long-term efficacy of commercial formulated diet and to evaluate dried *P. comosa* as either an alternative diet or a supplement to the formulated diet for reproductive conditioning of captive broodstock.

4.4.2. Methods

4.4.2.1. General

Adult female *H. rubra* from 100-125 mm shell length were collected from reefs on the southern side of Fingal Island on 30/6/1999 and after cleaning, tagging and sizing were transferred to the reproductive conditioning unit at a controlled temperature of 15.5 ± 1.0°C. Apart from variations in diet, all other aspects of reproductive conditioning, spawning induction and hatchery husbandry were in accordance with those already described in Section 4.3.

4.4.2.2. Dietary treatments

Abalone were fed to slight excess on one of three alternative diets:
1. Formulated diet (Adam and Amos PL, South Australia) at 0.5% live-weight per day.
2. Mixed diet 50% seaweed and 50% formulated diet at 1% & 0.25% live weight/day, respectively.
3. Seaweed diet (sun and air dried *P. comosa*) at 2.0% live weight/day.

Feeding rate of 0.5% live weight per day with the commercial formulated diet was set on the basis of earlier observations that this was in slight excess of consumption for adult *H. rubra* maintained at 15.5°C. The feeding rate for dried *P. comosa* of 2% live weight per day was equivalent of a fresh seaweed rate of about 10%. This rate was comparable to the mean ingestion rates of 5% live weight/day for fresh seaweeds reported by Uki & Kickuchi (1984) and consistent with generalised fresh seaweed feeding rates of at least 7% live weight recommended by Fleming (2001) on the basis of a review of commercial abalone hatchery practices and published research findings from Australia and elsewhere.

For each of the three dietary treatments, 25 abalone (75 abalone in all) were housed in five 30 L black plastic barrels with 5 abalone per barrel. In each dietary treatment, 3 replicate groups of 8 abalone were randomly assigned to a spawning batch. Every 4 weeks a different group (8 abalone from each dietary treatment) were subjected to a spawning stimulus. Each replicate group of 8 abalone was thereby subjected to a spawning induction stimulus at intervals of 12 weeks. The remaining (25^{th}) abalone in each dietary treatment remained unassigned to replace mortalities.

Spawning induction attempts were initiated after 10 weeks and then every 4 weeks for a period of one year. By this time, two of the three replicate groups of 8 abalone from each diet treatment had been subjected to four attempted inductions of spawning and the third replicate group to three attempts. All attempted spawnings were conducted around new moon (range 7 days prior to 5 days after) coincident with the highest rate of spawning success exhibited by wild brood stock (Section 4.1) and to thereby avoid possible confounding effects of lunar cycle. On each attempted spawning induction, visual gonad index scores of all abalone were assessed as were the number of females spawning, the number of eggs produced per female. Overall fertilisation rate, quality of larvae produced (% normal) and yield of competent veligers were also estimated.

After a mean holding time of approximately 12 months (June 1999-June 2000), a census was made of all broodstock in the conditioning unit together with an assessment of individual growth.

4.4.3. Results

4.4.3.1. Survival

A failure of the seawater chiller unit of BU2 coincident with failure of the over temperature alarm on 23/7/00 5 days prior to the final planned spawning, resulted in mortality of all the abalone in the experiment. As a consequence, final live-weights could not be determined. Prior to this overall survival was 94%, a total of only 3 deaths having occurred amongst the 75 female abalone. Two of the dead had been maintained on the mixed diet and the third on the seaweed diet.

4.4.3.2. Growth

Mean length and width data for the abalone at the commencement and conclusion of the experiment are presented in Table 1. A mean ± s.e. annual shell length increment of 4.4 ± 0.7 mm/yr achieved on the formulated diet were significantly greater than broodstock fed the mixed diet which only increased by 1.5 mm and those of seaweed fed stock that marginally lost shell length and width and appeared visibly emaciated after one year. Growth increments of the formulated diet fed stock were consistent with natural growth rates of wild *H. rubra* in this size range (112 to 116 mm) reported from Victoria (McShane *et al*. 1988), Tasmania (Prince *et al*. 1988) and NSW (Worthington *et al*. 1995).

Table 1. Mean ± se length, width (mm) and weight (g) data for stock reared in dietary experiment.

		Initial size 24/5/00	Final size 5/7/01	Shell length increment
		Mean ± SE	Mean ± SE	Mean ± SE
Formulated Feed	Length	112.5 ± 0.7	116.9 ± 0.9	4.4 ± 0.7
	Width	87.4 ± 0.6	91.9 ± 0.8	4.5 ± 0.6
Mixed Diet	Length	111.1 ± 0.6	112.8 ± 0.7	1.7 ± 0.5
	Width	87.7 ± 0.5	88.6 ± 0.6	0.9 ± 0.4
Seaweed	Length	111.9 ± 0.8	109.9 ± 0.8	-1.5 ± 0.3
	Width	87.5 ± 0.6	85.5 ± 0.6	-1.7 ± 0.2

4.4.3.3. Visual Gonad Index

The mean visual gonad index (vGI) data are presented in Figure 1. Over the course of the experiment the mean vGI increased from an initial value of 1.2 rising over about 225 days to peak values of 3 in formulated diet fed females and to 2.5 for the females fed the mixed diet. Thereafter vGI stabilized within a narrow range of 2.5 to 2.8. By contrast vGI in seaweed fed stock increased over the first 125 days then decreased to stabilize at a value of around 1.5 from about 200 days onward.

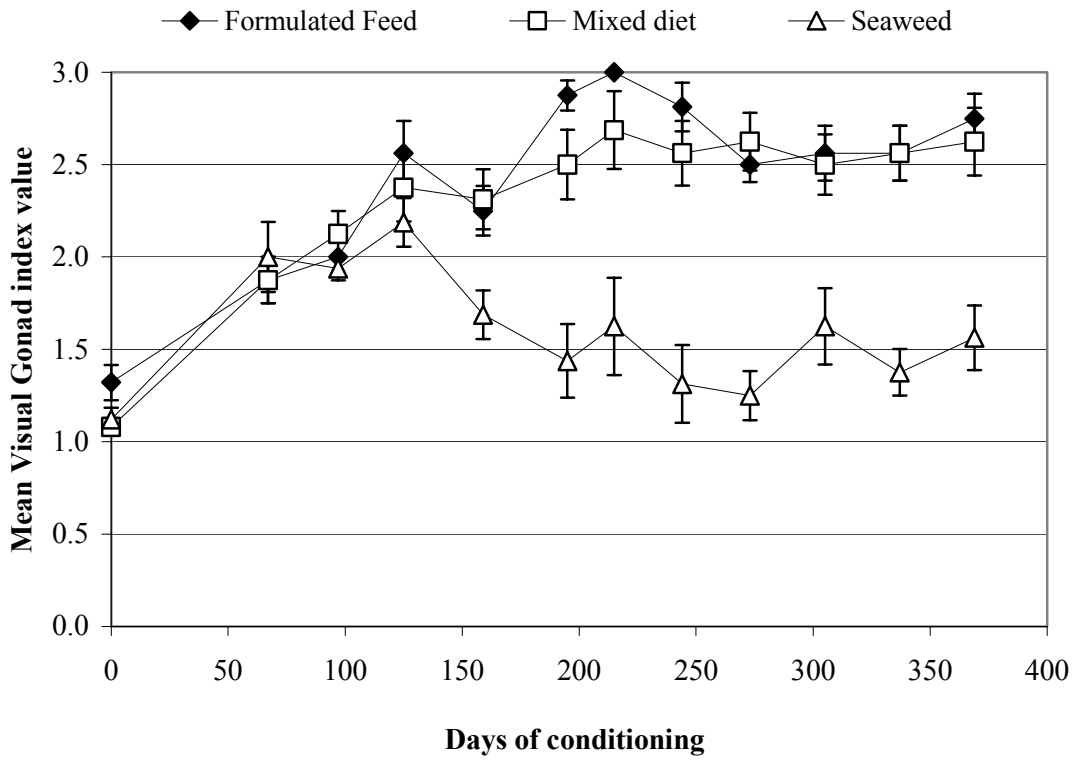


Figure 1. Effect of diet on mean apparent gonad condition.

4.4.3.4. Spawning results

Of the 11 spawning attempts during this 369 day experiment (Table 2), 8 resulted in the successful spawning of good quality eggs. A total of 264 female abalone were exposed to the spawning stimuli, resulting in the spawning of 27. This overall success rate of 10.3%, i.e. similar to that achieved in the general conditioning and spawning trials discussed in Section 4.2.

Of 35.6 million eggs produced, 15.24 million were spawned by abalone fed the formulated diet. A similar 13.68 million eggs were produced by abalone fed the mixed diet, while only 6.63 million from seaweed fed stock. Patterns of spawning behaviour, as illustrated by frequency of spawners and eggs produced (Figures 2 a & b), varied markedly between the three diets. The majority of eggs produced by seaweed fed abalone were generated in a single mass spawning after only 125 days of conditioning followed by a small number of very reduced fecundity spawnings. These same stock failed to spawn on the final 4 induction attempts beyond 244 days of conditioning These results were consistent with corresponding growth data discussed above and reflect long term effects of this deficient diet.

The great bulk of eggs produced by stock fed the mixed di*et al*so occurred in a single mass spawning but this happened after a much longer 244 day period of conditioning. The formulated diet fed abalone exhibited a more consistent pattern. Of the 7 successful spawning attempts, 1 to 2 females spawned with no apparent decrease in fecundity.

Two female abalone, one each from the mixed and single algae diets spawned twice while a third formulated diet fed female spawned 3 times. As with the conditioning results presented in Figure 5, periods between successive spawnings were highly variable ranging from 90 to 244 days. Similarly vGI scores for the 27 spawners were similar to those previously recorded for routine conditioning

and spawning operations using the formulated diet. While commonly scored as 2 (15 cases) or 3 (9 cases), spawners also included stock with gonads scored as low as 1 (3 cases).

Five of the 8 spawnings yielded batches of good quality (as indicated by low rates of deformity, detachment of the twin retractor muscles and vigorous phototactic rafting) 7-day-old competent to set veliger larvae. No sperm was available on two occasions and a system failure resulted in the loss of another batch. Of the 27 abalone that spawned, 9 were from each of the three dietary treatments.

Table 2. Summary of results of the *H. rubra* dietary experiment.

Date	6-09-99	6-10-99	3-11-00	7-12-99	12-01-00	1-02-00	1-03-00	30-03-00	1-05-00	2-06-00	3-07-00	**Overall Means se or Totals**
Days of conditioning	67	97	125	159	195	215	244	273	305	337	369	
Group	1	2	3	1	2	3	1	2	3	1	2	
						FORMULATED DIET						
Females spawned	0	1	2	2	0	1	1	1	0	0	1	**9**
No's females induced	8	8	8	8	8	8	8	8	8	8	8	**88**
% spawned	0	12.5	25	25	0	12.5	12.5	12.5	0	0	12.5	**10.3**
n eggs x10^6	0	2.95	2.5	2.4	0	1.1	2.42	2.52	0	0	1.35	**15.24**
mean fecundity 10^6		2.95	2.50	1.2		1.1	2.42	2.52			1.35	**1.69**
						MIXED DIET						
Females spawned	0	0	2	0	0	0	5	1	0	1	0	**9**
No's females induced	8	8	8	8	8	8	8	8	8	8	8	**88**
% spawned	0	0	25	0	0	0	62.5	12.5	0	12.5	0	**10.3**
eggs x10^6	0	0	2.2	0	0	0	10.91	0.07	0	0.5	0	**13.68**
mean fecundity 10^6			1.1				2.18	0.07		0.5		**1.52**
						SEAWEED						
Females spawned	0	0	6	0	0	1	2	0	0	0	0	**9**
No's females induced	8	8	8	8	8	8	8	8	8	8	8	**88**
% spawned	0	0	37.5	0	0	12.5	25	0	0	0	0	**10.3**
n eggs x10^6	0	0	4.7	0	0	0.84	1.092	0	0	0	0	**6.63**
mean fecundity 10^6			1.2			0.84	0.55					**0.74**
						TOTALS						
Females spawned	0	1	10	2	0	2	8	2	0	1	1	**27**
No's females induced	24	24	24	24	24	24	24	24	24	24	24	**264**
% spawned	0	4	42	8	0	8	33	8	0	4	4	**10.3**
n eggs x10^6	0	2.95	11.9	2.4	0	1.94	14.4	2.59	0	0.5	1.35	**35.55**
mean fecundity x 10^6		2.95	1.57	1.20		0.97	2.06	1.30		0.50	1.35	**1.32**

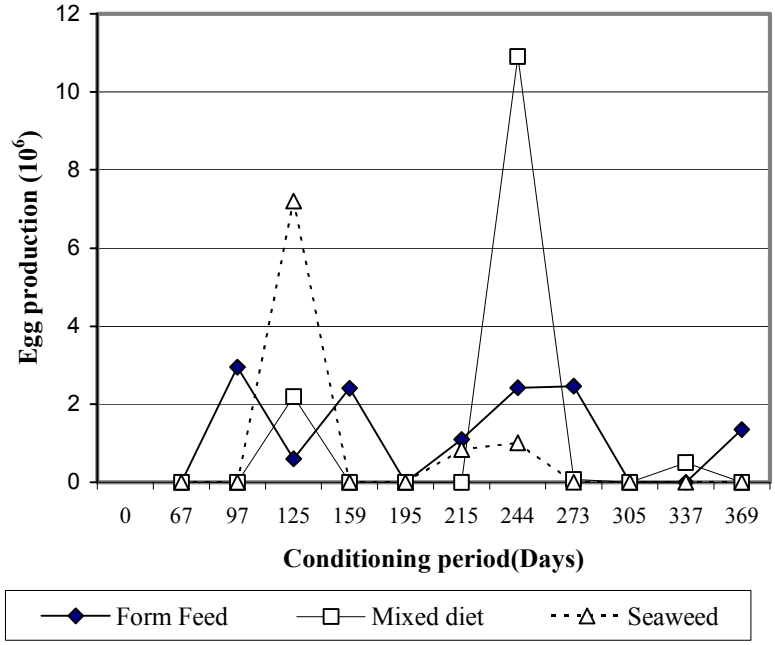


Figure 2a. Effect of conditioning time and diet on reproductive performance of female *H. rubra.*

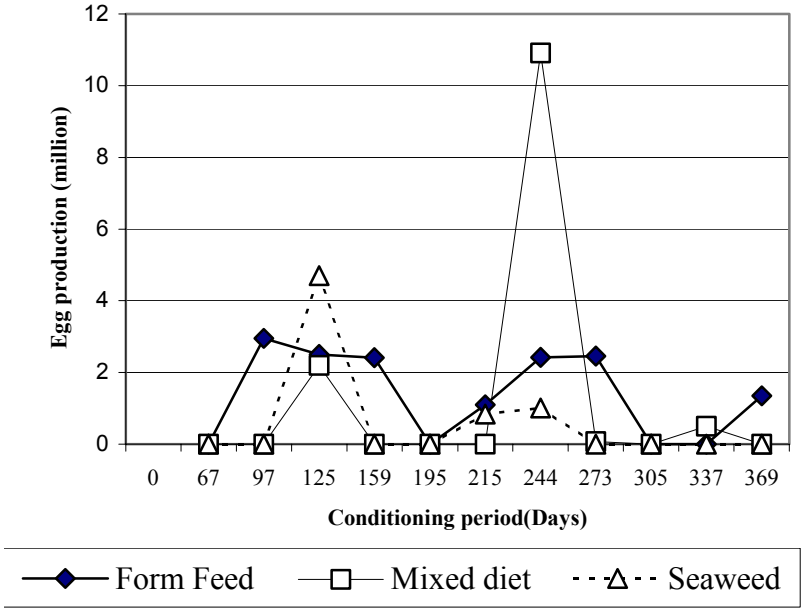


Figure 2b. Effect of conditioning time and diet on spawning frequency of female *H. rubra.*

4.4.4. Discussion

H. rubra broodstock fed *P. comosa* lost body weight in complete contrast to their formulated diet and mixed diet fed counterparts. They also produced less than half as many eggs as broodstock fed mixed and formulated diets (Table 1). Initial spawning results from the *P. comosa* fed broodstock early in the experiment (after 121 days of conditioning) were nevertheless encouraging. However, the fact that the majority of the eggs produced by these seaweed fed abalone were spawned early in the experiment, that these same stock failed to spawn over the second half of the experiment and that they had not grown and were visibly emaciated at the conclusion suggests that *P. comosa* is a poor diet for *H. rubra*. Additional evidence of the poor nutritional status of *P. comosa* as a conditioning supplement, was the fact that the formulated and mixed diets significantly outperformed *P. comosa* in respect to visual gonad index scores.

These results were consistent with those of Fleming (1995 a & b) who also found that despite high rates of ingestion of *P. comosa* by *H. rubra*, growth rates were poor. Buchal *et al*. (1998) showed that growth and fecundity data alone may not be sufficient to elucidate differences between the quality of feeds for broodstock abalone. In this context it is acknowledged that supplementation of the formulated diet with a variety of red and green seaweeds more nutritious than *P. comosa* might have enhanced reproductive conditioning of *H. rubra*. Harrington (2001) showed that reproductive performance of yellow footed paua *H. australis* was not improved when fresh *Macrocystis pyrifera* and *Gracilaria chilensis*, two seaweed species preferred by (*H. australis*) were used as an alternative diet to "Makara", a casein based formulated diet. As in the present study with *H. rubra*, Harrington (2001) showed that reproductive performance of seaweed fed *H. australis* was lower than animals fed both formulated and mixed diets.

Compelling evidence that artificial diets alone can be used indefinitely to condition captive broodstock was that fecundity and % yield of competent larvae from *H. rubra* fed this diet tended to increase rather than diminish as the cumulative conditioning period stretched into its second year. Finally, annual growth rates of 4 to 5 mm by the 105 to 120 mm *H. rubra* fed the formulated diet in this experiment and elsewhere during this project (see Section 4.2.) fell within ranges reported for wild *H. rubra* in NSW and elsewhere in southern Australia (Worthington *et al*. 1995).

4.5. Hatchery and nursery production and technology development

4.5.1. Introduction

Hatchery and nursery production of *H. rubra* by commercial farms in southern Australia have been confined to natural spawning seasons of local stocks. Annual production has thus been reliant on a small number of very large batches. Raising juveniles on standard 300 x 600 mm nursery plates until they reach 5-10 mm after 5 to 7 months limits yields to 30 to 100 per plate (Fleming 2000). This translates to 100-300 juveniles per square metre of shallow tank nurseries (Roberts 2000). As minimum and optimum economic scales of production of farms in Australia are in the order of 40 and $\geq$80 tonnes per annum respectively (J. Morrison, pers. comm.; S. Rodis, pers. comm.; ABARE 2003; Weston *et al.* 2001), nursery output capacity of farms must be in the order of one to several million juveniles per annum. To meet such requirements farms employ 10,000 to 25,000 standard 300 x 600 mm plates and site areas of about 1,000 m^2 (S. Rodis, pers. comm.).

A critical requirement of this project was to produce as many competent larvae and juveniles as possible over as long a production season as possible. Moreover, juveniles needed to be small enough to be easily and cheaply produced, handled, transported and seeded in usefully large batches of tens to hundreds of thousand. At the same time they needed to exceed a minimum size of about 5 mm.

A preliminary economic model was developed by the authors (Appendix 15.7) to compare relative benefits of costs of enhancing *H. rubra* fisheries using hatchery produced larvae and several age/size classes of juveniles. This model showed that inter-relationships of costs and yields based on published age/size and natural mortality data for *H. rubra* strongly favoured newly cryptic $\geq$5 mm juveniles as providing the most cost effective seeding. This model also exposed scope for further improving cost effective seeding by reducing current quoted price of 2 cents per mm.

An equally compelling reason for improving production efficiency of button size juveniles was that production of hundreds of thousands required by an ambitious experimental field seeding program had to be achieved by technical staff of only two and an available nursery production area of about 100 m^2.

The advent of year round spawning and hatchery production of *H. rubra,* achieved in this study in October 1999, was the first critical step to achieving the high levels of seed production within these strict constraints of space and labour. The second was to achieve greatly elevated nursery stocking densities, coupled with multiple annual batch production using otherwise conventional diatom plate systems. Achievement of the latter in turn required successful early weaning of juveniles onto formulated feeds coupled with successful development of a space and labour efficient post-weaning nursery rearing system.

4.5.2. Aims

The aims were to:

1. Develop space and labour efficient nursery rearing equipment and procedures that would enable year round production of large batches of competent larvae and juvenile *H. rubra* with which to conduct systematic fisheries enhancement experiments.
2. Investigate the influence of broodstock conditioning history, season, etc. on survival and growth of post-larvae on diatom nursery plates. Also the influence of factors (such as age and size and condition of post-larvae) on subsequent weaning success.

4.5.3. Methods

Broodstock collection, conditioning, induced spawning and fertilisation protocols were as described in Section 4.3. A flow-through incubation and larval rearing system and operational protocols, similar to that described by Hone *et al.* (1997), was used for incubation of eggs and larval rearing. Larvae tanks were cylindro-conical, either 180 or 360 L in volume and served by 200 mm or 300 mm diameter banjo water discharge screens fitted with 85 μm polyester mesh.

Seawater, preheated or pre-cooled to 17 ± 1°C, was filtered serially through an anthracite filled swimming pool filter; wound cartridge depth filters, rated at 10 and to 1 μm nominal and finally irradiated using a 40 watt UV sterilizer. This was supplied at the rate of 20 to 40 exchanges per day. Larvae were stocked at densities in the range 10-30/mL or a maximum of $5.4x10^6$ per 180 L tank and $10x10^6$ per 360 L tank. They were wet sieved, rinsed, counted and transferred on alternate days to clean disinfected rearing tanks. Banjo screens were changed daily.

Flow rate, pH, and DO were also checked, recorded and if necessary, adjusted daily. At 17±1 °C, the larvae were ready to settle after 6-7 days, however many larvae usually began to crawl on the base of the vessel long before this. Larvae were examined at least once a day. Developmental stage was assessed, as was level of activity, malformations and any obvious dropout or unusual aggregations of larvae. The most common malformation seen in larvae is a breakdown of the twin retractor muscle integumental attachments. Loss of one or both attachments, hereafter referred to as IDS (integument/muscle detachment syndrome), is symptomatic of bacterial infection arising from poor quality seawater or inadequate hygiene. Total reliance was placed on high flushing rate, physical filtration and UV irradiation to maintain bacteria (especially potentially pathogenic *Vibrio* spp.) at low densities to prevent and control IDS. Antibiotics were never used.

Competent larvae destined for field seeding experiments were calcein labelled. The calcein treatment was adapted from that developed by Day *et al*. (1995) for adult *H. rubra*. Six to 8-day-old abalone larvae were bathed in seawater with calcein ($C_{30}H_{26}N_2O_{13}$, FW 622.5, Sigma Chemicals) at a concentration of 50 mg L^{-1} for 24 h at 18°C in accordance with procedures described in Section 15.6.

4.5.3.1. Nursery rearing

General

Nursery equipment and procedures were similar to those prescribed by Hone *et al*. (1997) for *H. rubra* and *H. laevigata,* except for the size and age at which the juveniles were detached from the plates and stocked into shallow raceways for weaning and on-growing. Competent, 6- to 8-day-old (usually 7) larvae were settled and reared on conventional 600 x 300 x 1.5 mm clear PVC plates mounted vertically on their long edge and supported within plastic coated wire racks, each accommodating 17 plates (Plate 1).

Eight such racks in two rows of four were accommodated in one of 10 fibreglass tanks of overall dimensions 2.7 x 1.2 x 0.6 m fitted with standpipes to maintain a water depth of about 500 mm (Plate 2). Plates within the racks were oriented at right angles to the long axis of the tanks. The tanks were equipped with seawater spray bars mounted centrally over each row of racks and floor mounted 15.5 mm air diffuser tube (Barfell Industries P/L) in a ladder array with two “rungs” of diffuser tube running beneath and transecting each rack of 17 plates.

Plate 1. Nursery racks holding 17 diatom plates.

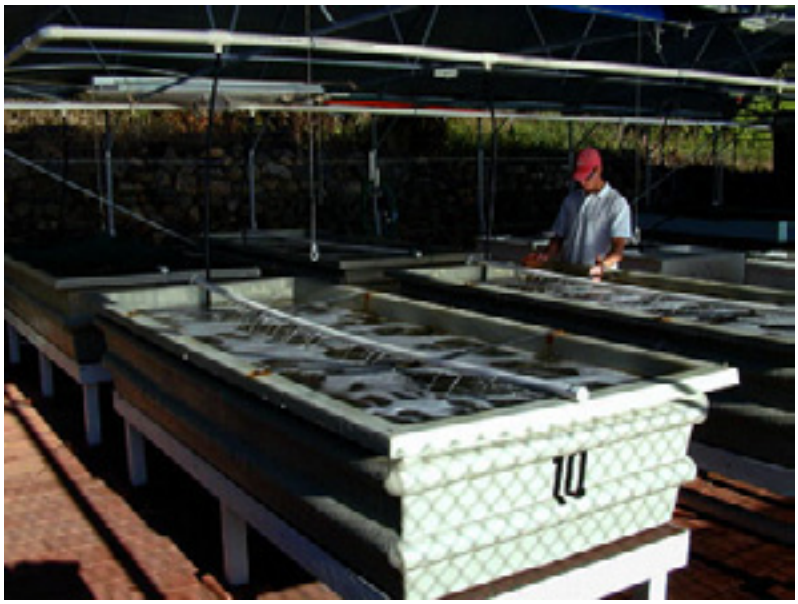


Plate 2. Diatom plate nursery tanks in operation - 136 plates/tank.

The settlement plates, loaded in racks, were placed in aerated nursery tanks supplied with 10 μm (nominal) filtered seawater, for 2 to 4 weeks prior to use. This standardised procedure was to ensure development of light to moderate biofilms comprising adventitious mixed benthic diatom communities dominated by *Cocconeis* and *Navicula* spp. The diatoms were interspersed with low to moderate colonies of crustose (= non-geniculate) coralline algae (*Sporolithum* spp.) (hereafter referred to as CCA) or of the colonial green alga, *Ulvella lens*. Both of the latter are known to significantly enhance settlement and metamorphosis of abalone larvae (Takahashi & Koganezawa 1988; Roberts 2000) including *H. rubra* larvae (Daume *et al*. 1999). Methods to colonise by *U. lens* were similar to those described by Krsinich *et al*. (2000). Colonisation of the plates by CCA algae involved placing shallow trays containing a single layer of washed CCA coated pebbles and small rocks beneath the racks of plates at the beginning of the conditioning period and promoting spore release using techniques described by Daume *et al.* (1999).

Competent larvae were evenly dispersed at densities of 735 to 7625 per plate (Table 1a) depending on the relative numbers of available larvae and plates, the quality of larvae and/or diatom films, or the coverage of *Ulvella* sp. or CCA colonies on the plates. At seeding, seawater flow was stopped for 24 to 48 h and trickle aeration applied until complete settlement, as indicated by the absence of residual free-swimming larvae (Hone *et al*. 1997). The nursery tanks were at ambient temperature, determined by incoming seawater, heat losses or gains via the air, and to a lesser degree, solar heating. The latter was moderated by the use of removable 80% shade-cloth covers and a permanent green house canopy comprising 80 % shade-cloth underlaid with opaque reinforced polyethylene film.

Preliminary weaning trials in late August and early September 1998 revealed that the minimum mean size at which juveniles could be safely removed from diatom plates, transferred to shallow raceways and successfully weaned onto finely ground formulated feed (Adam and Amos P/L) with high rates of survival, was about 1 mm shell length (Plate 3). This size coincided with a mean shell length of about 1.5 mm at which about 90% of all individuals fell within the range 1.0 to 2.0 mm. Consequentially, a mean shell length of 1.5 mm (1500 μm) was adopted as the minimum acceptable weaning size.

Juveniles were removed from the diatom plates by firstly immersing the plates in a bath of seawater containing the anaesthetic benzocaine at either 0.5 or 1 mg/L for periods of 5 to 15 minutes then detaching any remaining stock with a jet of seawater. Harvesting operations during warmer months were confined to cooler days when temperatures of baths could be maintained at or below 24°C. At temperatures ≥22°C concentration of benzocaine was always reduced to 0.25-0.5 mg/L.

Maximum and minimum water temperatures in the diatom plate tanks and post weaning shallow nursery raceways were recorded daily.

Plate 3. *H. rubra* post-larvae approaching optimum mean weaning size of 1.5 mm at a density of ≥1000/ plate (0.25/cm^2). Note typical pink mauve cryptic colouration imparted by diet of benthic diatoms (patch at top left of photo) that mimics that of natural coralline algae encrusted rock habitats.

4.5.3.2. Weaning and raceway nursery rearing

On-rearing of post-larvae removed from diatom plates was completed in two stages. The first comprised 4 small rectangular (2.2 x 0.5 x 0.2 m) raceways (Plate 4) that accommodated batches of up to 100,000 ex-plate post-larvae (Plate 5). These were immediately weaned onto a formulated diet (Adam and Amos P/L) and grown to a mean shell length of 4-5 mm. These juveniles were next transferred to one of eight larger rectangular (2.7 x 1.2 m) raceways (Plates 6 & 7) until reaching final deployment size that commonly ranged from a mean of 7 to 14 mm shell length (Plate 8). Maximum stocking rate was limited to 60,000 per raceway.

Small and larger raceways were maintained at shallow depths of 10 to 15 cm (depending on types of shelters provided) using removable stand-pipes fitted with plastic mesh to prevent juveniles from escaping. A high continuous level of diffuse aeration was provided by two 20 mm diameter air diffuser hoses (Barfell Industries P/L) set parallel to the long axis of raceways. Day time shelters (hides) were either 300 x 200 x 100 mm deep concrete "abalone tiles" (Besser P/L, Tasmania – Plate 7) or smaller 220 x 120 x 30 mm concrete pavers. The latter were fitted with "feet" comprising 20 x 20 mm PVC plastic angle that were glue attached to the opposite ends of each paver.

The shelters were arranged in a regular grid pattern (Plate 6) and oriented to facilitate flushing of faeces and uneaten food. A fall of about 1 in 60 along the long axis of the raceway tanks towards the drain also facilitated cleaning and draining.

The raceways were drained and flushed of residual food and faeces daily. Flow-through anthracite filtered seawater was supplied at the rage of 2-10 L/minute to the smaller raceways and at rates of

10-50 L/minute in the larger raceways. These rates ensured that total biomass of juveniles at all times was matched by a two-fold or greater rate of net seawater exchange per minute which is about twice commercial farm flow rates (S. Rodis and A. Krsnich, pers. comm., 2004).

Draining, cleaning and refilling of raceways, operations were completed in a few minutes to minimise exposure of juveniles to ambient air temperatures especially during summer. These provisions, together with shading of the entire nursery system under a translucent greenhouse canopy ensured that stock were never exposed to excessive heat stress nor direct sunlight.

Wherever possible, adventitious benthic algae biofilms were allowed to develop on the floor of the raceways 1-2 weeks prior to stocking with ex-plate post-larvae. Although not quantitatively assessed, these biofilms mainly comprising benthic diatoms, appeared to promote better and faster weaning and subsequent growth and survival. All formulated feed used was supplied by Adam and Amos P/L. "Plate powder" used as the weaning diet was added to the tank the same day as stocking. This was followed by 1.5 and 2.5 mm crumb for juveniles in the range ~3-14 mm shell length. Either 3 mm chip or noodle was fed to larger juveniles.

4.5.3.3. Monitoring of growth

Growth and survival data of animals during the diatom plate and post-weaning nursery phases were collected to evaluate effect of season and stocking density on the times required to attain a mean weanable shell length of 1.5 mm. Shell lengths of post-larvae (up to 3 mm) were measured with the aid of a low power stereo microscope fitted with a calibrated eye-piece graticule. Juveniles (>3 mm) were measured using vernier callipers. Progressive estimates of total surviving numbers of post-larvae and juveniles within batches were made from *in situ* randomised replicate counts on plates or from total biomass data - in the case of juveniles, immediately prior to deployment to field seeding trials. The latter were accompanied by weighing and counting 4 or 5 sub-samples comprising 100 to 200 juveniles.

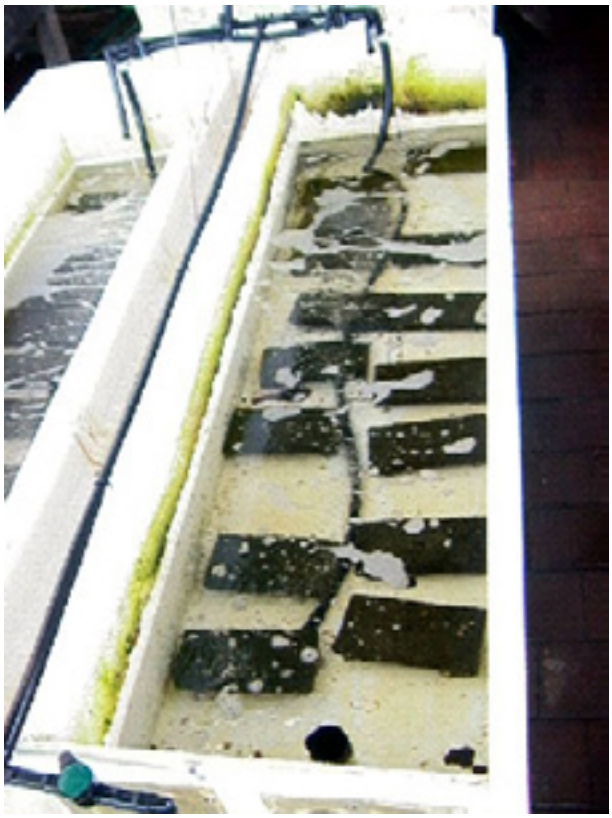


Plate 4. First stage weaning nursery showing central air diffuser and day time hides.

Plate 5. Close up of 1-2 mm post-larvae recently moved from diatom plates and undergoing weaning in first stage nursery.

Plate 6. Second stage flow-through nursery raceway for on-growing of juveniles to button size.

Plate 7. Button size juveniles sheltering on under side of day time hides within the second stage raceway.

Plate 8. Close up of ready to release button sized (commonly 7-14 mm) juveniles.

4.5.4. Results

4.5.4.1. Temperature and water quality

Seawater temperatures within the nursery tanks commonly ranged from 14 to 23ºC and generally remained within ±2°C of incoming seawater. Nevertheless, ambient seawater temperatures as high as 24°C were experienced in late summer and early autumn resulting in nursery systems temperatures as high as exceeded 26°C.

4.5.4.2. Seed production overview

Hatchery operations during the three year project collectively yielded more than 100 million fertilised eggs and more than 40 million competent 7-day-old larvae. An additional 6 broods of eggs totalling 57.6 million were produced but failed to yield competent larvae. Causes of these failures included unplanned spawning events triggered by accidental high temperature shocks (2 cases), absence of viable sperm (1 case) and power or equipment failures (3 cases). Yields of competent larvae from eggs ranged from 8 to 71% and averaged 40%, which fell within a range routinely reported by commercial hatcheries (Fleming 2000).

Yields of juveniles produced from competent larvae ranged from 1.8 to 75% and averaged 5.7%. These highly variable results raised the need for better knowledge of and control over nursery production. Key elements included optimum seeding rates on nursery plates and improved production of diatoms on the plates. In spite of these shortcomings, 14 batches of competent larvae totalling 23 million were produced. These in turn yielded 20 batches or sub-batches of juveniles used for field seeding trials and complimentary laboratory experiments. The parents of these

juveniles were collected from a wide array of sites stretching southward from Broughton Island and 4 sites around the Tomaree Peninsula, to Sydney, Kiama, Ulladulla, Eden and Disaster Bay.

4.5.4.3. Seed production using broodstock maintained at ambient temperature

As reported in Section 4.3, temperature controlled conditioning and induced spawning of captive *H. rubra* brood stock was not achieved until mid October 1999. The preceding period, (see Section 4.1), was characterised by largely failed attempts to acquire ripe ready to spawn broodstock from the wild. During this same period, some limited spawning success was achieved with captive broodstock maintained under ambient temperatures in outdoor flow through seawater tanks. As summarised in Table 1a, a total of 9 spawnings were achieved in the 22 month period from January 1998 to early October 1999. The first two spawnings of captive broodstock were achieved in January and February 1998 in the lead-up to the current project. Two small broods of 0.915 and 0.5 million eggs were fertilised, incubated and hatched but the larvae subsequently died of bacterial induced IDS, the cause of which was traced to a malfunctioning UV steriliser.

Seven batches of competent larvae and five batches of small juveniles used for seeding trials were subsequently produced. However these occurred at irregular intervals and were largely limited to the natural spring to early summer natural breeding season of *H. rubra* in NSW.

Total egg production from ambient temperature conditioned broodstock was 26.84 million at mean fecundity of 1.12 million. The seven successful hatchery batches collectively yielded 13.32 million competent larvae. More than 5 million of these larvae, including one complete batch were deployed in reef seeding experiments. Most (6.3 million) of the remaining larvae from six of the seven batches were seeded onto nursery plates. Five of the six batches were successfully on-grown to a size suitable for weaning. These were subsequently used for fisheries enhancement experiments (see Sections 6 to 8) or on-gown in raceways. As discussed below, the sixth batch although potentially successful, stalled in growth just below the minimum wean-able size and starved.

Two of the remaining weaned batches died of gas bubble disease caused by an air leak on the suction side of the main seawater delivery pump. A total of 338,000 juveniles were deployed in reef seeding trials. These ranged from 132,000 ex-nursery plate 1 to 2 mm stock to a small batch of 3000 advanced juveniles averaging 43.3 mm. The bulk (201,000) of these seed however comprised intermediate 5 to 20 mm SL, “button sized” juveniles.

4.5.4.4. Seed production using broodstock conditioned at constant temperature

Controlled temperature reproductive conditioning, and hence year-round large-scale spawning and hatchery and nursery production (Table 1b), resulted in greatly improved hatchery and nursery output. Three times as many batches (18), four times as many eggs (83.6 million) and three times the number competent larvae (31 million) were produced in 21 month period from October 1999 to June 2001 than during the preceding 21 months. Likewise, twice the number of competent larvae (12.7 million) were seeded onto nursery plates.

4.5.4.5. Post settlement growth and survival on diatom plates

A summary of diatom plate nursery growth data documented for 10 separate batches is provided in Table 2. Regardless of season or seeding density, patterns of growth were best described by exponential relationships accompanied by consistently high correlation coefficients of 0.9731 or greater (Figures 1A to J). Within batches, daily growth rates progressively increased from 8 to 24 μm/day for newly metamorphosed post-larvae on day 2 after settlement, to rates of 24 to 50 μm/day for juveniles approaching the minimum mean size of 1500 μm suitable for weaning onto a finely ground particulate diet.

Plate residence time from settlement and metamorphosis to attainment of the minimum weaning size ranged widely. Batches B6-0299, B1-0200 and B1-0200 reared in summer/early autumn required only 32 to 35 days. At the other extreme winter/early spring reared batches B4-0898, B3-0798 and B2-09 required 50 to 72 days. Overall speed of development thus appeared mainly dependent on seasonal temperature. These results were similar to experimentally determined influence of temperature on post-settlement growth and survival presented in Section 4.7. The high degree of variability in seasonal growth rates observed during the diatom plate nursery phase is further illustrated in a collective plot of growth data for all 10 batches provided in Figure 2.

An important feature of diatom plate phase growth data for individual batches presented in Table 2 and Figures 1A to J, was that it continued exponentially until either food ran out, in which case it abruptly stopped, or when (and if) mean shell length reached approximately 1.5 mm when they were harvested and weaned onto artificial diets in raceways. In the case of batch B4–0898, the second to be produced (Figure 1B), growth stalled at a mean shell length of about 1400 μm just short of the designated 1500 μm. This batch, having exhausted available food was left on the plates and inadvertently starved to death over a period of about 8 weeks.

A number of other batches, having reached weaning size, were also inadvertently allowed to exhaust available food, stop growing and starve before being harvested from diatom plates and stocked into shallow raceways. Substantial losses of stock were sustained as a consequence of such episodes. Presumably the physiological condition of many of these juveniles was so compromised that they were unable to endure the combined stress of anaesthesia, harvesting, handling, transfer to shallow exposed raceway habitats and of weaning. Plate seeding density was expected to influence growth rate but did not apparently do so. However, increasing seeding density did progressively reduce the size and age at which growth stopped and starvation began. This effect is well demonstrated by data of batch B6 0199 presented in Figure 1D. Although stocked at a very high density of 7625 larvae/plate, this batch exhibited the fastest growth recorded. However growth abruptly stopped at mean shell length of 1200 μm only 28 days after seeding. At the opposite extreme, batch B3-0999 (Figure 1F) that was seeded at the very low density of 735 larvae/plate, continued to grow exponentially until removed from diatom plates at a mean shell length of 2520 μm.

4.5.4.6. Growth and survival of weaned juveniles reared in shallow raceways

Because of a high and constant demand for seed abalone with which to conduct fisheries enhancement experiments, intensive long term monitoring of the growth of juveniles in shallow flow through raceways was limited to only two batches, B3-0798 (Figure 3A) and B2-0999 (Figure 5B). Small (1 to 2 mm) juveniles exhibited a period of reduced growth after being harvested from plates, stocked into shallow relatively exposed raceways, and weaned onto a formulated diet.

In the case of batch B3 0798, subjected to this transition in spring 1998, growth persisted at a low rate of 20 μm/day for the first 52 days. Over this period mean shell length increased form from 1245 to 2297 μm. This in turn was followed by a period of accelerated growth that peaked at about 60 μm/day. This rate of growth persisted for the next 10 months by which time remaining stock had reached a mean shell length of 16.8 mm and were released.

Batch B2-0999 exhibited a similar pattern of growth. The initial 9 week early post weaning period through spring and early summer was accompanied by a modest growth rate of 31 μm/day. This was followed by a period of elevated growth of 71 μm/day that continued for a full year. The mean shell length increasing from 4.0 mm to 30.5 mm. In both these cases, growth over a full year was best described by linear relationships as indicated by correlation coefficient (R^2) values above 0.99. Growth rates did vary over shorter sampling intervals.

Within batch B3-0798, growth rates over short-term sampling intervals of 12 to 63 days ranged from 40 to 88 μm/day. For B2-0999 mean growth rates calculated for sampling intervals 63 to 106 days ranged from 47 to 99 μm/day. These growth data together with more limited data from an additional five batches of juveniles produced during this study are presented in Figure 4 and Table 3.

Shorter-term growth rate fluctuations appeared to be influenced by season. Periods of slow growth (40 to 57 μm/day) mainly coincided with lowest temperature mid to late winter months (July and August). Periods of rapid growth (78 to108 μm/day) occurred from mid autumn to early winter months (April to June) and again in spring and early summer months (September to December).

4.5.4.7. Length weight relationships

Shell length and live weight data for hatchery produced juvenile *H. rubra* are presented in Figure 5a. These data illustrate a relatively low degree of variability for stock below 30 mm but a high degree of variability in larger stock up to 60 mm. Mean shell length (L) and live-weight (W) data for 15 batches of button size abalone in the range 5.2 to 17.8 mm are presented in Figure 5b. These data appear to conform to a common cubic relationship ($W = 0.0002\ L^{2.954}$) as indicated by a high correlation coefficient r^2 value of 0.9824. These data also suggest that marked differences in length weight relationships between wild fished *H. rubra* stocks along the NSW coast (Worthington *et al*. 1998) either do not manifest during early juvenile stages or are phenotypic rather than genotypic in origin.

4.5.5. Discussion

Two weaknesses in current hatchery and nursery production technology employed by commercial abalone farms in Australia were apparent at the outset of this project. Spawning was limited to use of local wild broodstock during the natural breeding season thereby limiting production to a few large batches per year. Juveniles are retained on diatom nursery plates for 5-7 months (5-10 mm SL) limiting the density of juveniles per plate to about 30-100 per plate. For a restocking programme to be cost effective, the production of juveniles must be extended over the longest possible production season and be space efficient. The latter was of special importance for this project where available space for nursery production was limited to a total area of less than 100 m^2.

Induced spawning from captive, temperature control conditioned stock was first achieved in October 1999. This greatly increased the yield and seasonality of hatchery production. Numbers of spawnings, eggs and numbers of competent larvae produced were three times higher than with broodstock maintained in flow through seawater systems under ambient temperatures and photoperiods. There appeared to be no difference in the percentage yield or growth of larvae from naturally or artificially conditioned broodstock.

One of the disappointing aspects of the production of post-larvae in the current study was the low and variable rate of successful metamorphosis of larvae onto conditioned nursery plates. This is a chronic problem in abalone production and is the focus of much research (reviewed by Roberts 2001). All abalone production facilities around the world are to some degree reliant on diatom dominated biofilms to induce settlement and metamorphosis. Growth of CCA, *U. lens* and other algae, pre-grazing by con-specifics and artificial chemical cues have all been used to enhance metamorphosis of larvae onto diatom biofilms. For *H. rubra* laboratory experiments have shown that CCA, *U. lens* and a variety of macroalgae can increase the rate of settlement and metamorphosis of larvae (Daume *et al*. 2000; Roberts 2001). However, hatchery scale studies on *H. rubra* (and most for other abalone species) that incorporate some of the practical constraints of using these cues are absent from the literature. In the present study diatom conditioned plates were conditioned with CCA and other macroalgae (Krsinich *et al*. 2000). Despite this, settlement and

metamorphosis was often low and variable possibly because of the difficulty in producing consistent CCA and other algal settlement cues such as *Ulvella*.

The growth of post-larvae on diatom plates from both naturally and artificially conditioned broodstock, showed consistent and predictable growth rates. Post-larvae from all batches grew exponentially. The time to weaning (commonly 1-3 mm SL) was strongly dependent on water temperature. In the warmer water conditions of summer/autumn it took between 32-35 days to obtain the weaning size while during winter/spring plate residence times increased to 50-75 days. Exponential growth of juveniles on diatom plates only stopped when food ran out or when they reached a weanable size and were harvested. Interestingly, seed density did not generally affect growth providing that the biofilm was not totally consumed before harvesting.

This consistent exponential growth is not surprising given the feeding physiology of abalone post-larvae. Abalone post-larvae have three ontogenetic phases (Kawamura *et al.* 1998). Up to approximately day ten, post-larvae are still heavily reliant on yolk and to a small degree on dissolved organic matter (DOM) from diatom biofilms for nutrition. From day 10 to 20, corresponding to 0.8-2 mm SL, post-larvae feed mostly on DOM from diatom biofilms. At this stage the digestibility of diatom film has only a small effect on growth. Given an adequate supply of diatoms growth rates are similar on different species (Kawamura & Takami 1995; Kawamura *et al.* 1998; Daume *et al.* 1998). Only after reaching a SL of approximately 3 mm do juvenile abalone start to digest whole diatom cells. At this stage that defines juvenile status, growth is heavily influenced by diet quality. Thus the growth of post-larvae before weaning will be only subtly affected by food quality as they develop from lecithotrophy and dissolved nutrient uptake to ingestion feeding. The growth rates of 24-50 μm/day for larvae approaching weaning, as measured in this study, are at the upper end of recorded growth rates indicating that the biofilm presented was of good nutritional quality (Reviewed by Kawamura *et al*. 1998; Daume *et al.* 2000).

After transfer into shallow raceways, post-larvae often experienced a period of slow growth apparently associated with weaning. This slow growth may have also been caused by shock involved in transferring the animals from plates to the raceway. Similar handling effects have been documented in other studies (e.g. Greenier & Takekawa 1992). Also, at that size animals may still be dependant to some extent on diatom films that comprise about 60% protein and take some time to adjust to what is essentially the adult diet comprising only 20% protein. This explanation is strongly supported by the work of Dunstan *et al.* (1996) who showed that more natural diets of benthic diatoms on nursery plates were markedly different in unsaturated fatty acids and sugars than commercial formulated diets. Dunstan *et al.* (1996) also showed that growth of small juvenile *H. rubra* following removal from diatom plates and weaning onto specially formulated diets increased from 60 to more than 80 μm/day with increasing dietary protein over the range 25 to 45%. As cited in the methods section, pre-conditioning of nursery raceways with algae biofilms appears to mitigate such growth checks. Beyond the first few weeks after weaning, length increased in a linear manner over the following year (Figures 3A & B) averaging between 50 and 100 μm/day. Growth rates of 60 and 75 μm/day depicted in Figures 3A & B are in the median range expected for juvenile *H. rubra* in this size range. In contrast with smaller post-larvae, growth rates were relatively consistent with seasonal variation in seawater temperature.

Table 1a. Competent larvae and juveniles produced from broodstock held in outdoor flow through tanks at ambient temperatures.

Batch	Spawn date	Days in captivity	Source	Eggs x10^6	Mean fecundity	Competent larvae x10^6	Yield %	Larvae to field x10^6	Larvae to set x10^3	Plate set rate	Days on plates (age at weaning)	Date juveniles deployed	Juveniles to field x10^3	% yield from competent larvae	Mean shell length mm
1	29/01/98	20	Mallacoota	0.915	0.0.915	0.022[1]	2.4[1]		22*			NA	NA	NA	
2	17/02/98	78	CT	0.5	0.5	0.0[1]	0.0								
3	**23/07/98**[2]	153-,193,234	BH, CT	5.32	1.77	2.7	51	0.335	2400	5882	67-105	27-Oct-98	12		1.5
													75		6.0
													3	3.75	43.3
4	**27/8/98**[2]	140-239	BH TP	6.0	1.20	3.6		0	2100	5147	131		?	?	
5	**25/12/98**[22]	209	Broughton Is	1.654	0.827	0.470	28.4		470	1618	61	?	?	?	
6	**20/01/99**[2]	235	Broughton Is	2.91	0.582	1.48	51	0.93	610	7625	50	?	?	?	
7*	6/09/99	10	Eden	1.54	0.513	0.2	13	0.00	200	1472	56	3-Nov-99	120	75	1.5
												20-Mar-00	30		7.3
8	14/09/99	?	CT, FI	6.7	2.23	4.17	62	3.40	500	735	90	7-Feb-00	82		5.2
												30-May-00	12		13.6
												7-Jun-00	4	19.2	13.6
9	4/10/99		CT, FI	1.3	1.3	0.67	52	0.61							
Totals & means				**26.84**	**1.12**	**13.32**	**49.6**	**5.28**	**6302**				**338**		

Footnotes

1. *UV lamp failure resulting severe integumental muscle detachment syndrome.*
2. *Batches regularly monitored for growth.*

Table 1b. Competent larvae and seed juveniles produced from broodstock conditioned at 15.5°C at the Tomaree facility.

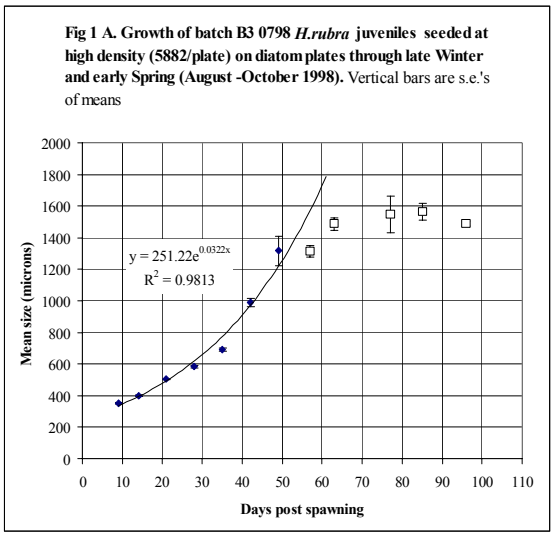
Batch	Spawn date	Source	Eggs x 10^6	Mean fecundity	Competent Larvae x10^6	Yield %	Larvae to field x10^6	Larvae to set x10^3	Plate seeding density/plate	Date juveniles deployed	Juveniles to field x10^3	% yield from competent larvae and from (eggs)	Mean shell length mm
10	6/10/99	FI	2.95	2.95	2.1	71	2.00						
11	3/11/99	FI	10	1.11	1.25	13	1.00	250	1838	5-Sep-00	11	4.4(0.57)	17.8
12	5/11/99	BI	3.29	1.11	0.27	8	0.2						
13	24/11/99	BH, Ya	2.39	2.39	1.44	60	0.29	360	1323	30-May-00	34	11.7(7.0)	9.3
										26-Jul-00	8		11.8
14	7/12/99	FI	2.41	1.21	0.57	24	0.00	570	4191	7-Sep-00	16	2.8(0.67)	11.2
15	1/02/00	FI	1.94	0.97	0.83	43	0.00	830	2507	30-May-00	39	5.4(2.32)	4.0
										26-Jul-00	6		7.6
16	6/04/00	BI	3.4	1.7	2.16	64	0.25	1910	1800	6-Dec-00	85	4.9(3.14)	9.8
										5-Feb-01	8		13.0
17	8/05/00	BI,BH,Ya	19.3	1.6	9.12	47	8.00	610	4485	31-Jan-01	20.0	3.28(1.54)	10.3
18	20/06/00	Eden	10.8	1.8	2.94	27	1.96	860	1581	29-Nov-00	152	17.9(4.83)	6.1
19	3/07/00	FI	1.35	1.35	0.69	51	0.00	690	1691	31-Jan-01	48	7.0(3.57)	6.8
20	28/09/00	Eden	4.02	1.34	0.45	11	0.00	450	1654	26-Jun-01	25	5.55(0.61)	14.1
Sub totals or means											**452**	**7.0(2.52)%**	
21	24/10/00	Sydney	3.6	3.625	0.59	16	0.00	590	4338				
22	14/11/00	Eden	2.5	0.63	0.89	36	0.00	890	1636				
23	5/12/00	Kiama	1.6	1.565	1.1	69	0.00	1100	3083				
24	12/01/01	BI	3.89	0.973	2.52	65	1.50	820	2007				
25	16/05/01	Kiama	0.8	0.8	0.43	54	0.00	466	1670				
26	17/05/01	Sydney	5.6	0.7	3.2	57	2.22	940	1382				
27	14/06/01	Sydney	3.8	0.95	0.41	11	0.00	440	3015				
Sub-totals & means			**83.6**	**1.49**	**31.0**	**37%**	**17.8**	**12726**					
Totals & means			**101.4**		**40.2**	**40%**	**23.1**	**16.1**					

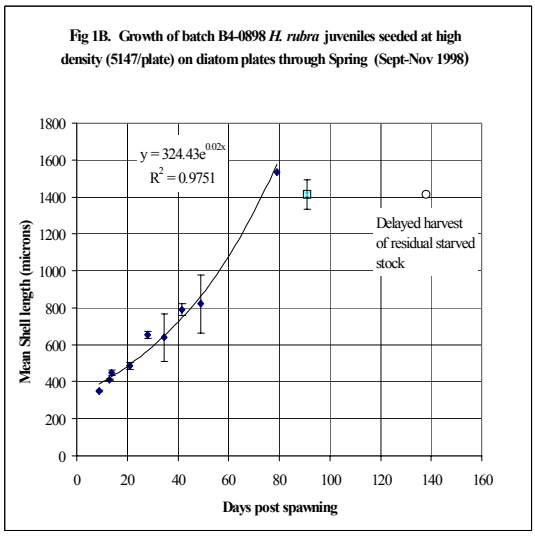
Table 2. Post settlement growth data of *H. rubra* on diatom nursery plates.

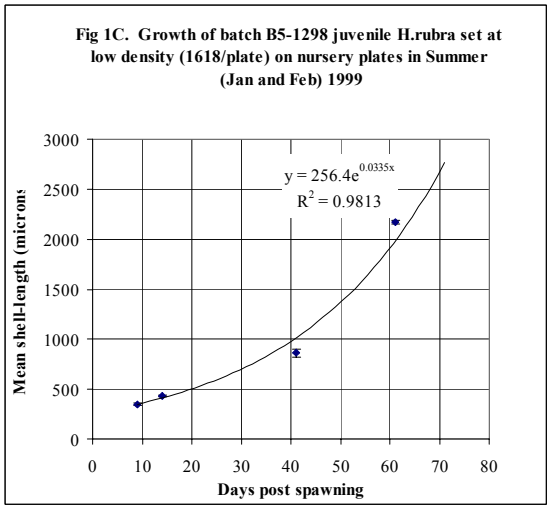
Batch. No. See Table 1a	Batch reference code	Spawning Date	Growth season and Temp range	Seeding density	Initial post-set growth rate	Growth rate at mean minimum weanable size of 1500 micron	Estimated period on plates to reach minimum mean weaning size of 1500 microns	Estimated mean growth rate on plates to reach minimum mean weaning size of 1500 microns	Comments
3	B3 – 0798 Tomaree Peninsular	23/07/98	Late Winter early Spring	High 5882/plate	10 um/day	43 um/day	50 days	23um/day	Barely reached minimum wean-able size when growth abruptly stalled, reflecting the high seeding density – See Figure 1A.
4	B4 – 0898	27/08/98	Early to mid Spring	High 5147/plate	8 um/day	24 um/day	72 days	17um/day	Barely reached minimum weaning size when growth abruptly stalled at about day 62 Batch had largely starved to death at time of attempted harvest at day 138 – i.e. after 8 weeks of stalled growth. See Figure 1B.
5	B5-1298	25/12/98	Summer	Low 1838/plate	16 um/day	45 um/day	45 days	26um/day	Grew well beyond minimum weaning size with no check to growth at time of harvest and weaning on day 61 (= 54days on the plates). By this time daily growth rate was about 65um/day and mean size 2171um. See Figure 1C.
6	B6 – 0199	20/01/99	Late Summer	Very high 7625/plate	14 um/day	55 um/day	32 days	36um/day	A consequence of the very high stocking rate batch failed to reach wean-able size growth abruptly stalling at mean size of 1200um at day 35 i.e. after only 28days on the plates - See Figure 1D.
7	B2 – 0999 EDEN	6/09/99	Early to mid Spring	Low 1472/plate	17.6/day	24 um/day	52 days	22um/day	Bulk of on grown juveniles weaned day 56 stock left on plates did not exhibit stalled growth until about day 84 at a mean size of about 4mm a reflecting the low initial stocking density - See Figure 1E.
10	B3 – 0999	14/09/99	Mid to late Spring	Low 735/plate	17um/day	40um/day	47days	24um/day	In keeping with the extremely low stocking rate, growth continued exponentially up to the time of harvesting and weaning on day 79 (72 days after set) by which time mean shell length was 2562um and daily growth was about 66um/day. See Figure 1F.
11	B6 – 1199	3/11/99	Late Spring early Summer	Low 1838/plate	18 um/day	46 um/day	35 days	33um/day	Growth stalled about day 55 at about 2400um but not harvested and weaned until day 84.ie a stalled growth /starvation period of 1 month. See Figure 1G.
13	B8 – 1199	24/11/99	Early to mid Summer	Low 1323/plate	22 um/day	46 um/day	41 days	28um/day	Reached minimum weaning size at day 46 and stalled in growth on day 60 at about 2300um but not harvested until day 86 leaving 4 weeks of stalled growth. See Figure 1H.
14	B9 – 1299	7/12/99	Early to mid Summer	4192/pate	18um/day	45um/day	41days	28um/day	Growth remained exponential up to about day 65 and at a mean size of about 2300um had been stalled for about 2 weeks when the stock were transferred to a raceway for weaning on day 79. See Figure 1I.
15	B1 – 0200	1/02/00	Late Summer	Moderate 2507/plate	24 um/day	50 um/day	35 days	33um/day	Fastest of all batches to weaning size (33days on plates) and continued to grow exponentially for about 65 days to a mean size of 4100 um. However this batch then at about 286/plate was allowed to stall at this size and starve for an additional 7 weeks and in a weakened state suffered high handling mortality. See Figure 1J.

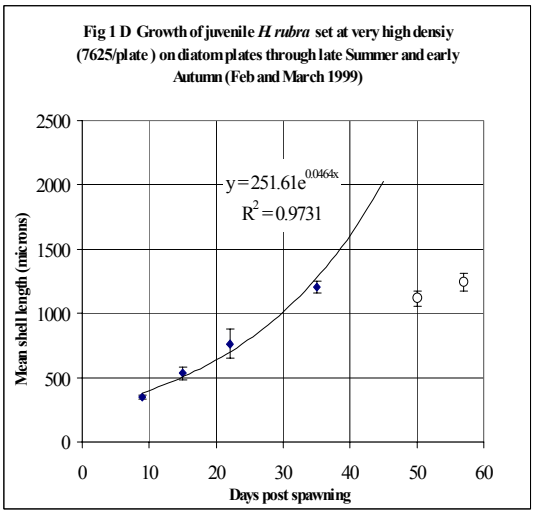
Table 3. Post weaning growth data of 7 batches of juvenile *H. rubra* reared in shallow outdoor raceways at ambient temperatures.

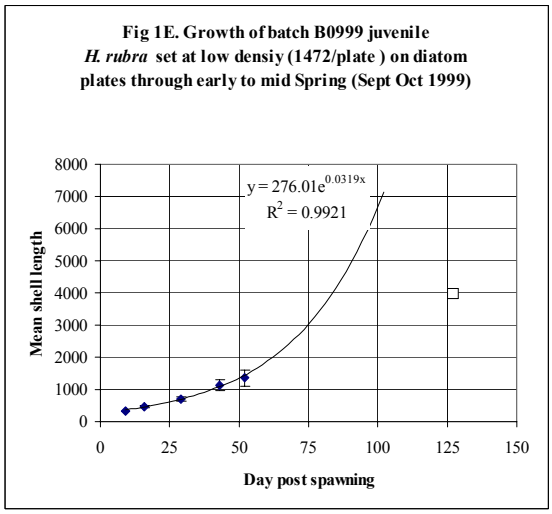
Batch	Date	Days post spawning	Mean shell length (μm)	Growth rate (μm/day)	Season
B3- 0798	2/12/98	132	3354		
	8/01/99	169	5959	70	early summer
	5/02/99	197	7805	66	mid summer
	9/04/99	260	10320	40	late summer to mid autumn
	18/05/99	299	12745	62	mid to late autumn
	21/06/99	333	15507	81	late autumn early winter
	14/07/99	356	16822	57	early to mid winter
B2-0999	11/01/00	127	3980		
	20/03/00	197	7300	47	mid summer to early autumn
	3/07/00	303	17800	99	early autumn to early winter
	8/09/00	368	20900	48	early to late winter
	10/11/00	431	25800	78	early to late spring
	12/01/01	494	30800	79	late spring to mid summer
B4-1099	28/02/00	147	5160		
	9/04/00	188	8490	81	early to mid autumn
	13/10/00	284	13580	53	mid autumn to mid spring
B6-1199	16/04/00	148	4200		
	24/06/00	217	11664	108	mid autumn to early winter
	23/09/00	308	17340	62	early winter to mid spring
B8-1199	30/05/00	188	9321		
	26/07/00	245	11776	43	early to mid winter
B9-1299	27/06/00	184	6393		
	27/09/00	277	11288	53	early winter to early spring
B1-0200	28/05/00	119	4017		
	26/07/00	176	7661	64	early to mid winter











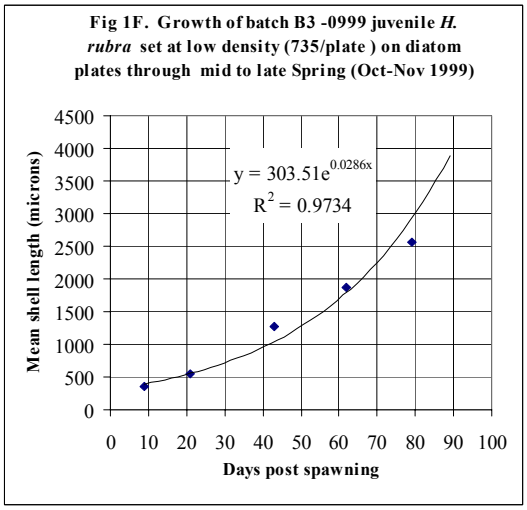
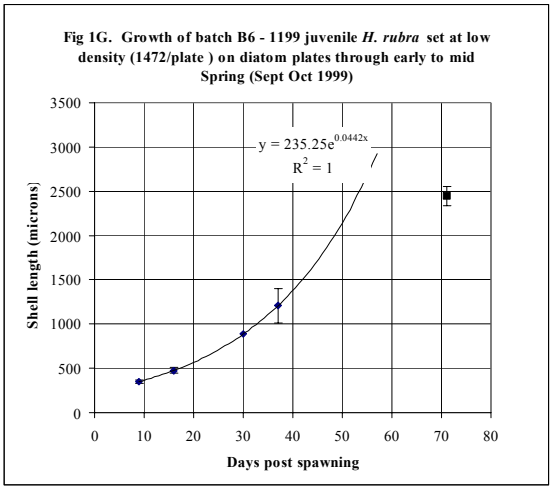
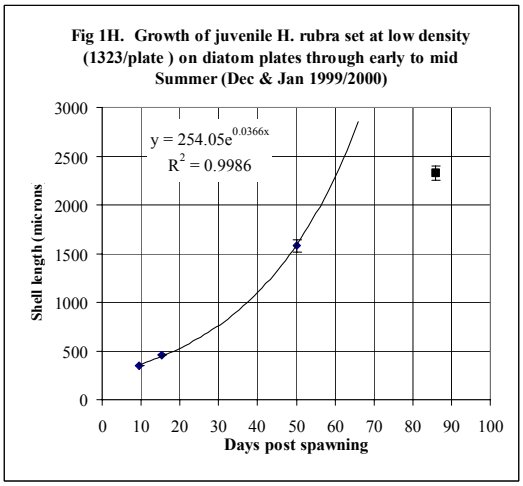
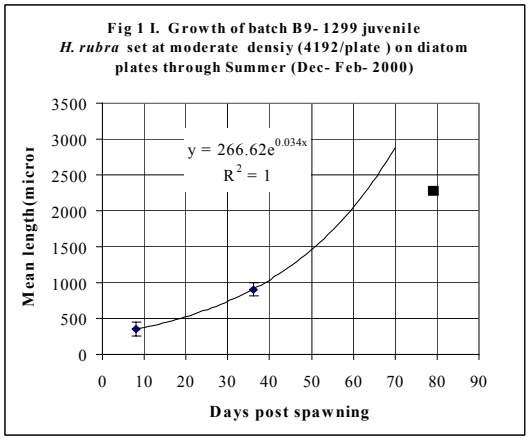


Figure 1. Growth of batches of juvenile *H. rubra* at different densities.







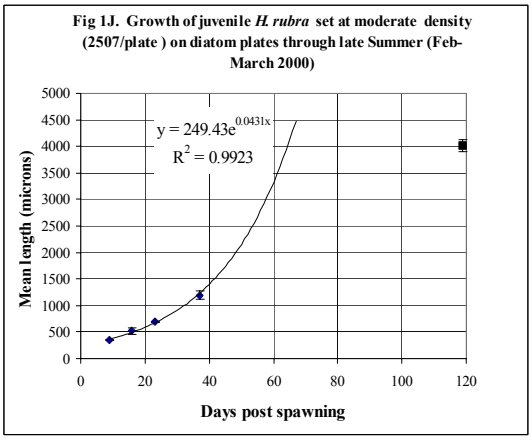


Figure 1. Continued.

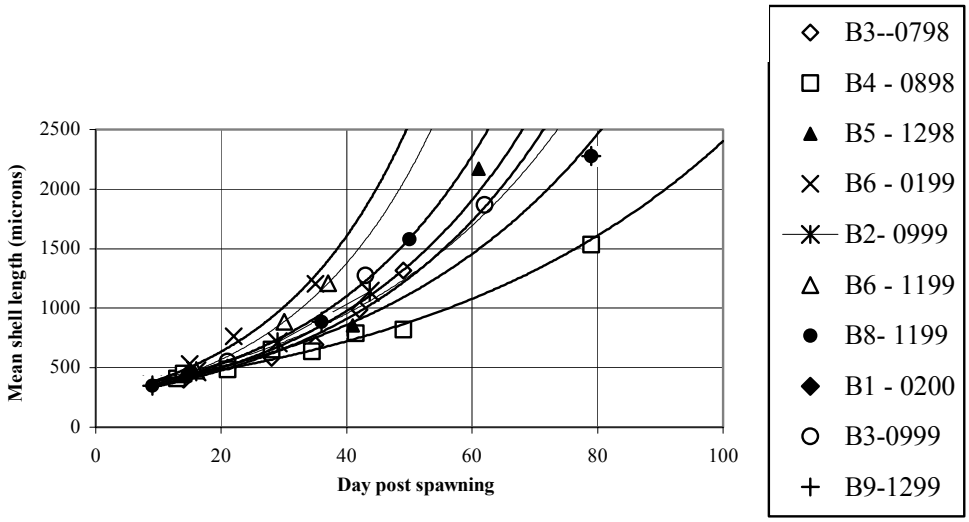


Figure 2. Batch variation in growth of post settlement juvenile *H. rubra* on diatom nursery plates.

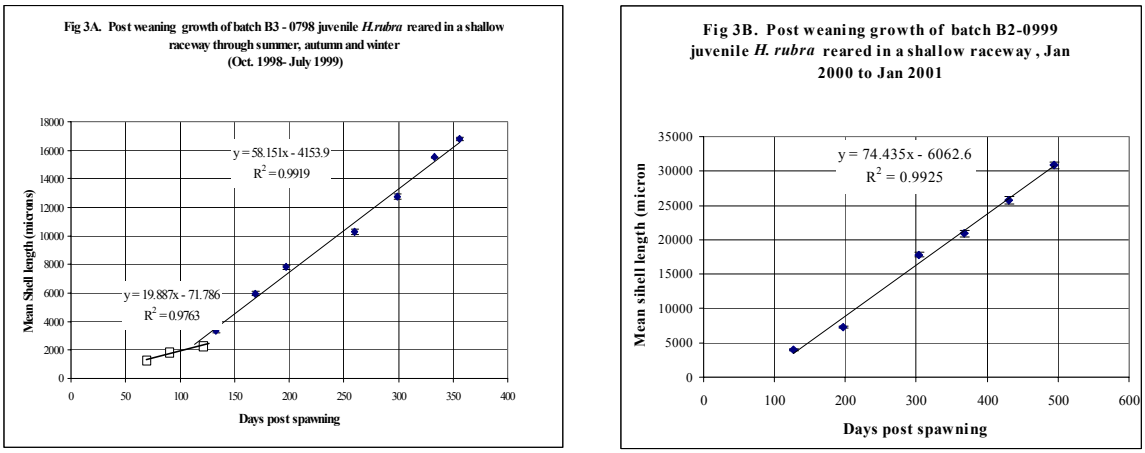


Figure 3. Post weaning growth of batches of juvenile *H. rubra*.

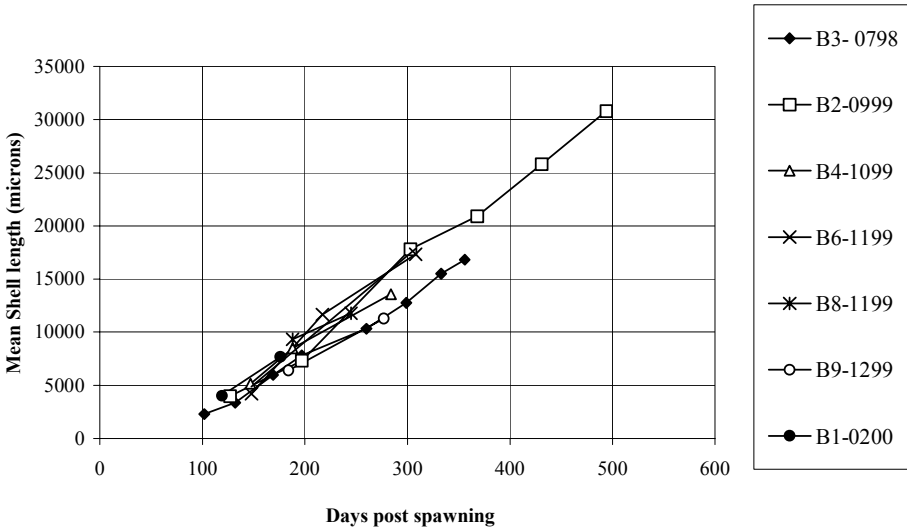


Figure 4. Post weaning growth performance of 7 batches of juvenile *H. rubra* reared in shallow raceways.

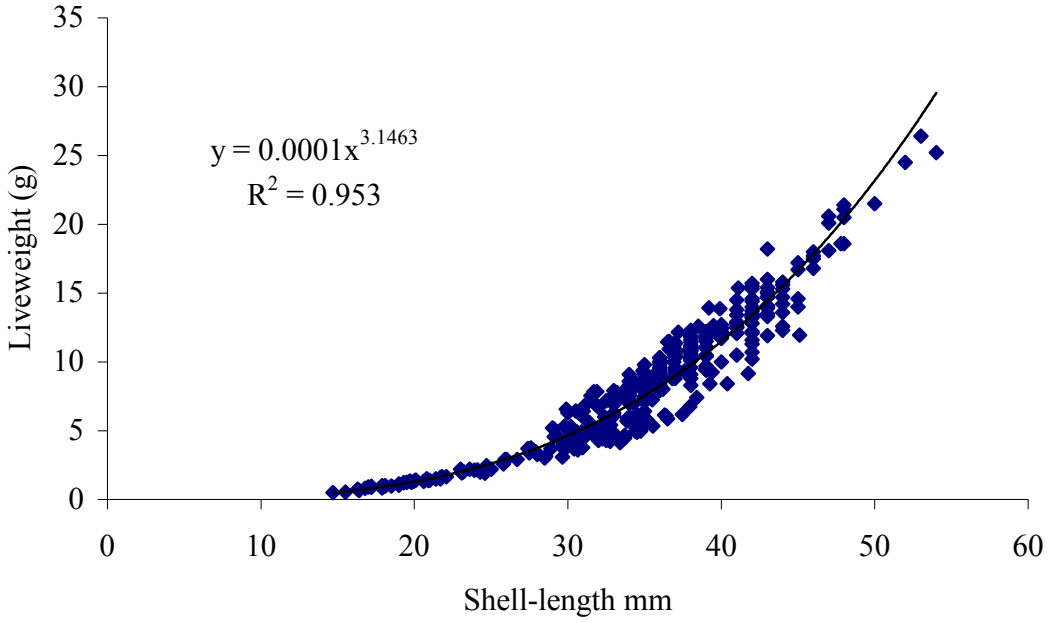


Figure 5a. Length weight relationship for hatchery produced juveniles.

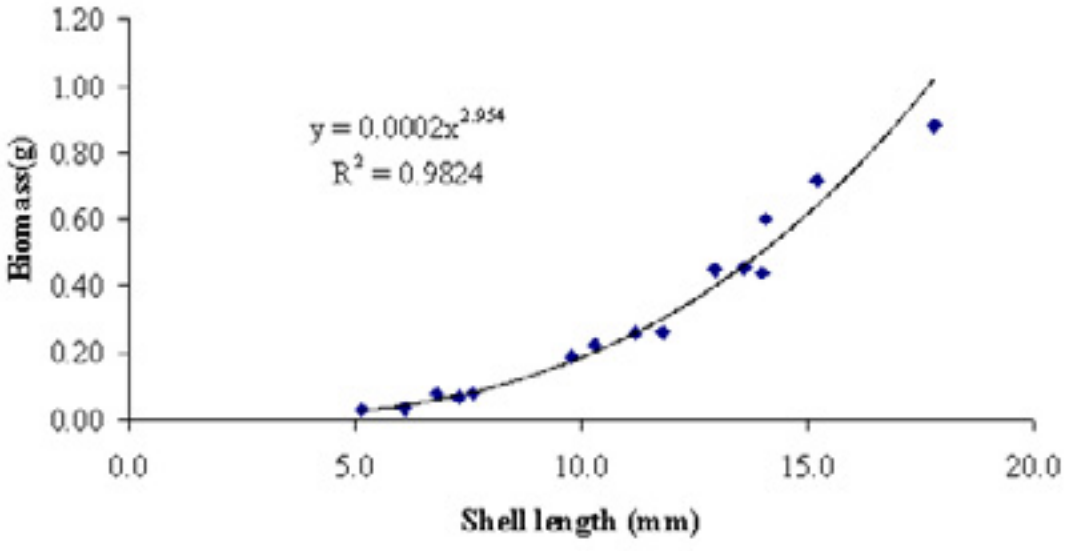


Figure 5b. Shell length and live weight relationship of small hatchery produced juveniles.

4.6. Improving nursery production technology: Determination of optimum nursery plate seeding rates

4.6.1. Abstract

This research aimed to optimise larval seeding density by to evaluating effects of seeding density on settlement, metamorphosis and growth of blacklip abalone (*H. rubra*) larvae when set onto conventional 300 x 600 mm clear PVC diatom coated plates. Four seeding density treatments used were 500, 1000, 2000 and 4000 per plate. Subsequent survival and growth was assessed 2, 4, 7, 14, 28, 42 and 56 days after seeding. Yield of juveniles peaked after 4 to 7 days at all seeding rates. Yields of juveniles increased with seeding density, from 26% for larvae seeded at 1000 per plate to 71% for those seeded at the maximum rate of 4000 per plate. From day 7 to day 28, survival across all seeding densities progressively converged to a common rate of about 20% (18 to 22%) that persisted until the termination of the experiment on day 56. Growth remained independent of actual density of juveniles surviving on the plates up to and including day 14, averaging about 14 μm per day. Beyond day 14 however, growth became progressively more density dependent, presumably due to food limitation. By day 56, growth rates at residual densities of 10, 100 and 1000 juveniles per plate, averaged 40, 30 and 22 μm per day respectively. Minimum mean sizes of around 1400 μm at day 56 attained by juveniles at the highest density of 4000 per plate are nevertheless acceptable for the purpose of early weaning of juveniles off diatom plates as a means of significantly enhancing nursery production of *H. rubra*.

4.6.2. Introduction and aims

As reported previously (Section 4.5), yields of *H. rubra* juveniles produced from competent larvae averaged only 5.7% and varied over a wide range (1.8 to 75%). These results highlighted the need to gain a knowledge of, and control over, nursery plate production. One key element for improving performance was to gain a better knowledge of the effects of the density at which larvae are seeded onto nursery plates. Prior to this study, "best guess" seeding rates in the range 500 to 2000 competent larvae per standard 600 x 300 x 1.5 mm nursery plate were practised. This study sought to quantify effects of seeding density on settlement success and subsequent juvenile growth and survival of *H. rubra* as a means of increasing mean yields and reducing interbatch variability.

4.6.3. Methods

4.6.3.1. Broodstock acquisition, conditioning, induced spawning and larval rearing

Larvae used in this experiment were sourced from a batch of 3.5 million eggs spawned on the April 6, 2000 by conditioned broodstock originally collected from Broughton Island on 15th April 1999. *H. rubra* broodstock were collected from Broughton Island, NSW (32°37'S, 152°19'E). Broodstock collection, conditioning, induced spawning, fertilisation and larval rearing protocols were as described in Section 4.2.

4.6.3.2. Experimental design, equipment and protocols

The experiment was conducted between April and June 2000 and comprised a simple 4 treatment (stocking density) x 4 replicate design. Each replicate comprised a set of 7 conventional clear PVC plates (600 x 300 x 1.5 mm) supported by plastic coated wire racks. Six weeks prior to settlement, the plates were placed in a nursery tank supplied with 10 μm (nominal) filtered seawater and aeration. This standard nursery procedure (as described more fully in Section 4.2) was to ensure development of light to moderate biofilms comprising adventitious mixed benthic diatom communities dominated by *Cocconeis* and *Navicula* spp. These were interspersed with low to moderate numbers of colonies of CCA and of the green alga *Ulvella lens* both of which are known to enhance settlement and metamorphosis of *H. rubra* larvae (Daume *et al*. 1999; Krsinich *et al*. 2000).

Nursery plates prepared by this standard procedure had consistently supported acceptable growth in *H. rubra* during the preceding year (see Section 4.4). To minimise variability in the type and density of diatom films on the plates, each replicate set of test plates comprised one plate taken from each the original seven conditioning racks, the complete set being a representative of inner, outer and intermediate slot positions.

Prior to settlement, each of the sixteen 7 plate racks was inserted into an open top box-shaped envelope constructed of 150 μm clear polyethylene film (Plate 1). All 16 envelopes had been previously maintained in a single (2.7 x 1.2 x 0.6 m) nursery tank. Seawater flow was discontinued immediately prior to seeding with larvae. The four seeding densities applied were 500, 1,000, 2,000, 4,000 competent larvae of 295 ± 0.8 μm (mean ± s.e. SL) per plate. The larvae were seeded using an automatic pipette. From 1 to 8 aliquots, (depending on target density) containing 3500 ± 160 (mean ± se) larvae were dispensed to each replicate. Care was taken to disperse the larvae evenly amongst the 7 plates within each replicate. Positions of the 16 replicate sets of 7 plates within the common nursery tank were randomised. To ensure successful settlement, the sets of plates, together with added larvae were retained for two days in static gently aerated seawater within the plastic envelopes. On the second morning after seeding a scalpel was used to insert 1-2 cm slashes in the sides of the envelopes. Moderate aeration and continuous flow-through of 10 μm filtered seawater was then initiated.

Settlement success, growth and survival were monitored progressively by sampling all replicates at days 2, 4, 7, 14, 28, 42 and 56 after seeding. On each of the first 4 sampling occasions, 2, 4, 7 and 14 days after seeding, post-larval abalone were sampled by removing a single plate from each replicate. A stratified sampling method was used to select plates from each replicate. This strategy was adopted to negate possible positional effects within the racks. For each seeding density, plates sampled from each of two replicates were taken from outer rack positions 1, 2 or 6, 7. Plates sampled from the two other replicates were sourced from inner rack positions 3, 4 or 5. All adhering post-larvae were harvested using a soft bristle brush and rinsing the plates with 70% ethanol dispensed from a 500 mL squeeze-bottle. Twenty post-larvae selected at random from each replicate plate were measured at 25x or 100x, using a compound stereo microscope fitted with a calibrated eyepiece micrometer.

On each of the three final sampling occasions, 28, 42 and 56 days after seeding, post-larvae had grown large enough to be counted *in situ*. Full bilateral counts of the remaining 3 plates were made to determine numbers of surviving post-larvae. On the same three final sampling occasions, 20 juveniles were randomly collected for sizing. These were taken from only one of the three remaining plates. This ensured that size data for particular plates could be linked to actual residual density on that same plate. The temperature of seawater flowing though the nursery tank was continuously recorded with a submersible data logger (Tidbit/TID 32-2050).

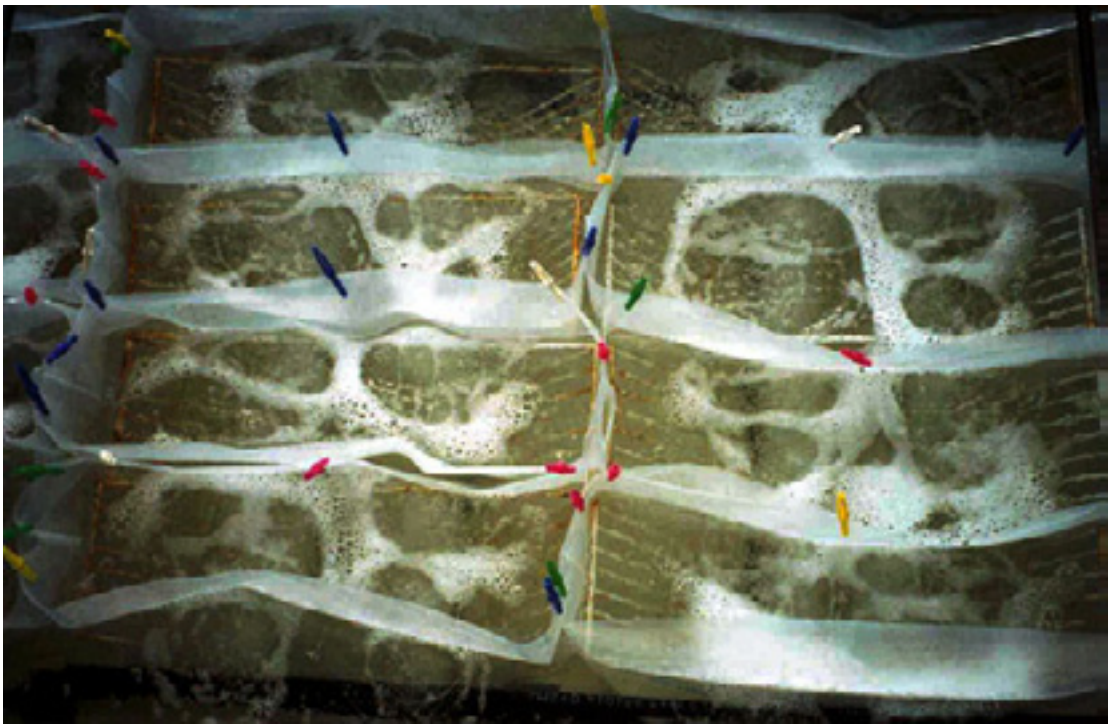


Plate 1. Replicates of 7 nursery plates separately housed in plastic film envelopes.

4.6.4. Results

4.6.4.1. General

This batch of eggs yielded 2.2 million competent veliger larvae 7 days later. Very few swimming larvae were observed 2 days after seeding, indicating that the very large majority had settled. Daily temperature progressively fell from 22° ± 1°C at set (13 April, 2000) to 16° ± 1°C during the latter stages of the study 50 to 56 days later.

Initial settlement and metamorphosis

Peak yields of post-larvae occurred between day 4 and 7 after seeding at all seeded densities (Figures 1 & 2). Relative yields of post-larvae increased with seeding density, from 26% for larvae stocked at 1,000 per plate, to 71% for those seeded at the maximum rate of 4000 per plate (Figure 2).

Size frequency distributions for 2-day-old post-larvae over this period (Figures 3a-d) did not reveal differences between density treatments nor did an ANOVA of size data. From day 7 to day 28, survival progressively converged to a common yield of about 20% (17-22%) that persisted until the conclusion of the experiment on day 56 (Figure 2).

Growth data (Figure 4a) show that up to day 42 there is no significant difference in growth of post-larvae between the four stocking densities. At the last sampling date (day 56), the post-larvae from the lowest stocking density of 500 larvae/plate were significantly larger (ANOVA; $F = 6.03$, df 3 $P = 0086$, followed by Tukey's test). An alternative presentation of these same data in Figure 4b enables a different interpretation, namely that regardless of initial stocking rate over the range 500-4,000 larvae/plate, growth of post-larvae up to day 42 was strictly exponential, as indicated by regression correlation coefficients (R^2) of 0.99. These same data also show that up to day 42,

density dependent differences in growth rate were marginal except for post-larvae stocked at the highest initial density of 4000 larvae/plate.

Growth data specific to actual numbers of stock counted on individual plates (i.e. regardless of initial seeding density), collected on each sampling occasion are presented in Figure 5. These data show that a progressively pronounced inverse relationship between growth and actual density developed over the second half of the experiment (day 28 to 56). Between days 42 and 56, mean growth rates at reference densities of 10, 100 and 1000 juveniles per plate were 40, 30 and 22 μm per day respectively. However in spite of this pronounced density dependent restriction to growth, by day 56 the majority of post-larvae (Figure 6) had attained shell lengths above 1000μm, even at the highest densities up to about 1000/plate. Practical experience gained during the course of routine nursery production operations (see Section 4.4) showed this size to be about the minimum size needed to ensure high rates of survival of post-larvae following harvest from diatom plates, transfer to shallow raceways and for weaning onto commercially available formulated diets.

Another useful observation from the data (Figure 6) is that even as late as day 56, density related effects on growth appeared relatively minor. Indeed, the mean shell lengths of post-larvae at densities of 100 and 1000/plate were of about 2000 and 1500 μm respectively.

4.6.5. Discussion

Results of this study showed that initial settlement rates and subsequent rates of metamorphosis and post-larval survival of *H. rubra* up to day 14 after seeding is enhanced by increasing initial stocking density of larvae. This apparent gregarious behaviour was not entirely unexpected in that it was consistent with behaviour exhibited by *H. rubra* larvae when seeded at variable density onto crustose coralline algae coated rocks freshly collected from natural boulder zone habitats of exposed rocky coasts (see Section 9).

A mean yield of 71% recorded on day 7 for larvae seeded at 4000/plate is the highest rate reported to date for this species on diatom coated plates. This rate also exceeds a maximum rate of 52% reported by Daume *et al*. (1999) for *H. rubra* set on plates with pure films of *U. lens*. Such high rates of settlement and metamorphosis may however be regarded as an inadvertent consequence of methodology rather than the product of improved husbandry or superior stock.

Two aspects of methodology may have contributed to the high rates of settlement and metamorphosis. One was the use of seeding rates higher than those commonly practiced by most commercial *H. rubra* hatcheries. The other was the longer period for assessing settlement success, which is usually within 1 to 3 days of larvae being set. Krsinich *et al*. (2000) reported a mean yield of 34±3% for *H. rubra* larvae 3 days after seeding onto wild algae + *U. lens* coated nursery plates at a density of 1917/plate. This result compares favourably with that achieved under the nearest equivalent conditions in the present study, namely a yield on day 4 of 29±6% for larvae seeded at 2000/plate.

The convergence in survival to a common level of about 20% between day 14 and 28 across all seeding density is of particular interest as is stabilisation of survival rate that persisted from day 28 to the conclusion of the experiment on day 56. As individual biomass of *H. rubra* increased by more than 2 orders of magnitude from settlement to day 56, the influence of non-genetic factors such as nutrient reserve status of eggs and larvae seems unlikely. Mean survival of 23.6% was reported by Krsinich *et al.* (2000) for 42 day *H. rubra* set on standard wild diatom + *U. lens* coated nursery plates. This is similar to survival rates of 17 to 22% achieved in the current study over a wide a range of seeding densities, including that used by Krsinich *et al.* (2000).

In contrast to survival, growth rate of post-larvae seeded at the highest initial rate of 4000 larvae/plate became increasingly density dependent during the second four week period (days 28 to

56). Growth rates of post-larval *H. rubra* seeded at the four densities (500 to 4000 per plate) were exponential for the first month. This pattern of exponential growth was consistent with that exhibited by many large-scale batches produced at the hatchery over the previous two years (see Section 4.1). Accordingly, daily growth of post-larvae stocked at 500 to 2000/plate progressively increased from an initial rate of about 14 μm/day to a rate of about 40 μm/day by day 28.

These growth rates were also generally consistent with those recorded for batches of post-larvae by Daume *et al.* (2000). By contrast, growth rates reported by Krsinich *et al.* (2000) for *H. rubra* reared at equivalent densities and on wild diatom film + *U. lens* coated plates, are almost twice that of the present study (averaging 30 μm/day between days 0 to 14 and 50-60 μm/day from day 14 to 32). As growth rate of day 0 to 7 *H. rubra* post-larvae increase from zero at 10°C to the maximum rate at 22°C (see Section 4.7), temperature alone could account for these differences. A second likely contributing factor is diet, especially the composition and density of wild diatoms available. Continuous addition of algal fertilisers to incoming seawater by Krsinich *et al.* (2000) in combination with application of controlled temperatures around 20°C may be the key to promoting fast growth of *H. rubra* post-larvae and warrants further investigation.

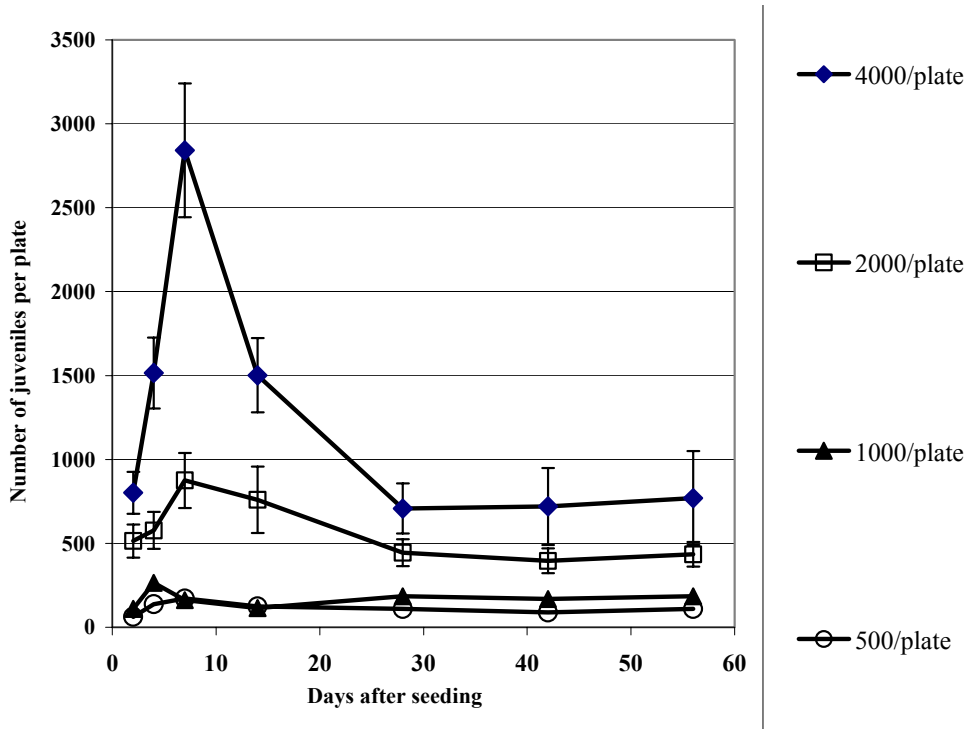


Figure 1. Effect of initial seeding density on number of juvenile *H. rubra* set on conventional diatom plates (bars are standard errors of means, $n = 4$).

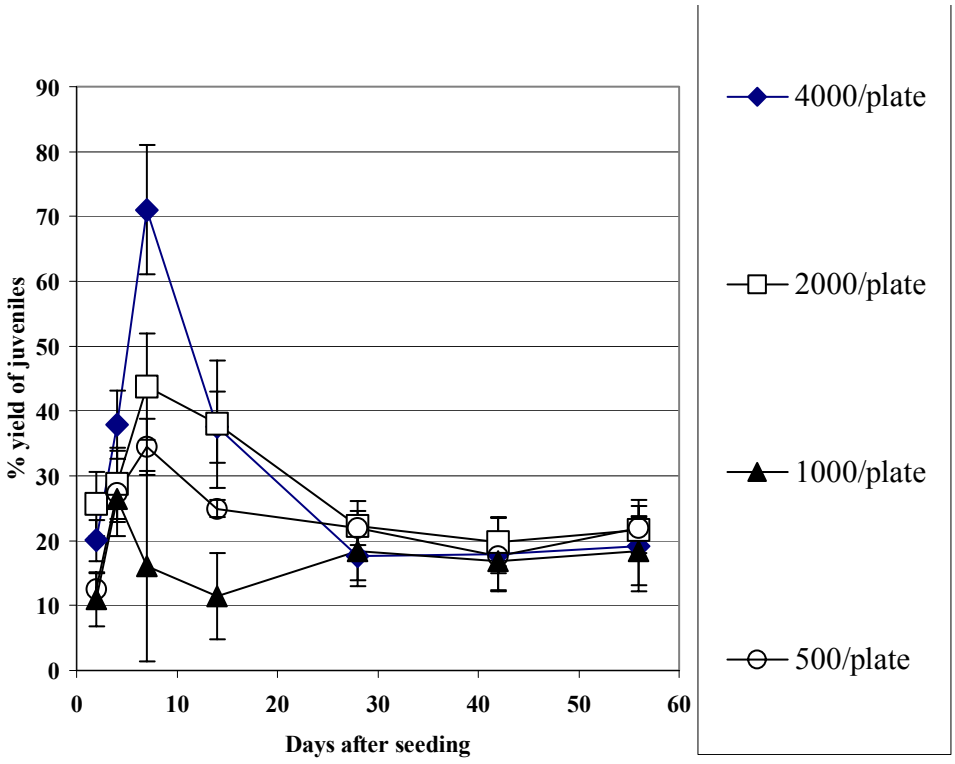


Figure 2. Effect of initial seeding density on relative yield of *H. rubra* set on conventional diatom plates (bars are standard errors of means, $n = 4$).

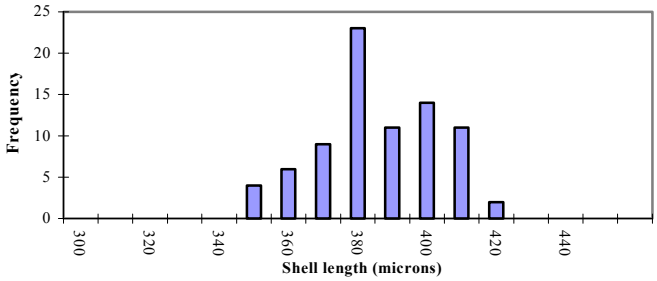


Figure 3a. Day 2 length frequency distribution of *H. rubra* post-larvae set at 500 per plate.

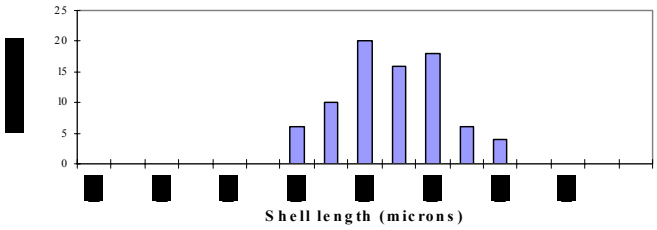


Figure 3b. Day 2 length frequency distribution of *H. rubra* post-larvae set at 1,000 per plate.

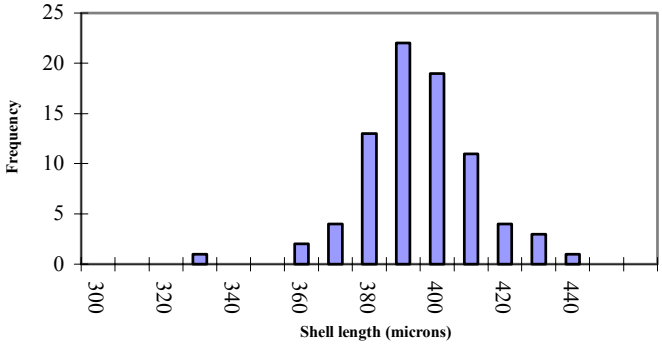


Figure 3c. Day 2 length frequency distribution of *H. rubra* post-larvae set at 2,000 per plate.

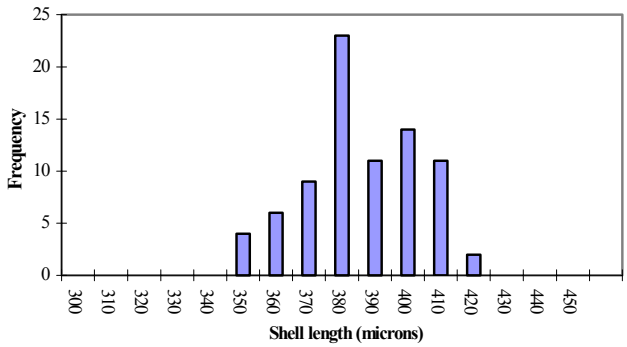


Figure 3d. Day 2 length frequency distribution of *H. rubra* post-larvae set at 4 000 per plate.

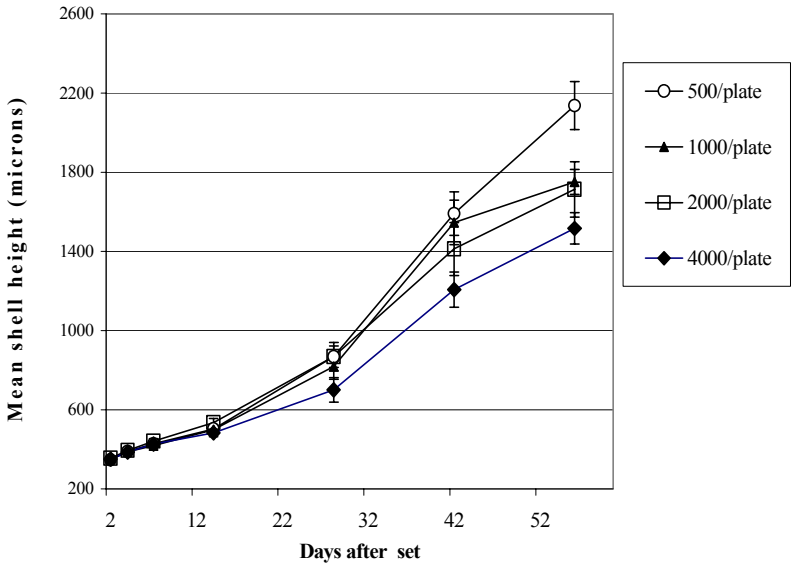


Figure 4a. Effect of initial seeding density on shell length of *H. rubra* set on conventional diatom plates (mean ± s.e., $n = 4$).

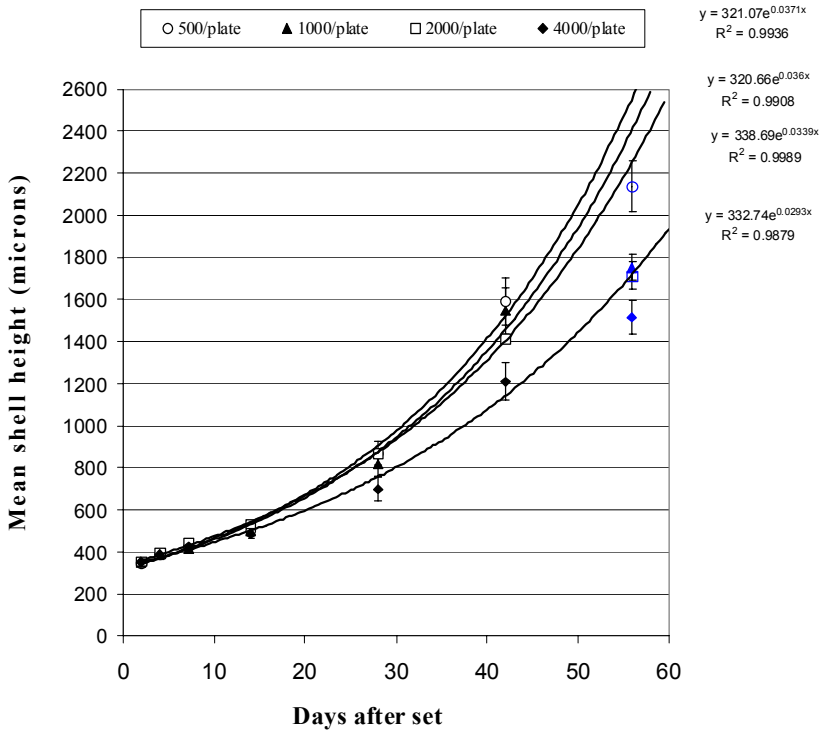


Figure 4b. Effect of initial seeding density on shell height of *H. rubra* set on conventional diatom plates (bars are standard errors of means, $n = 4$).

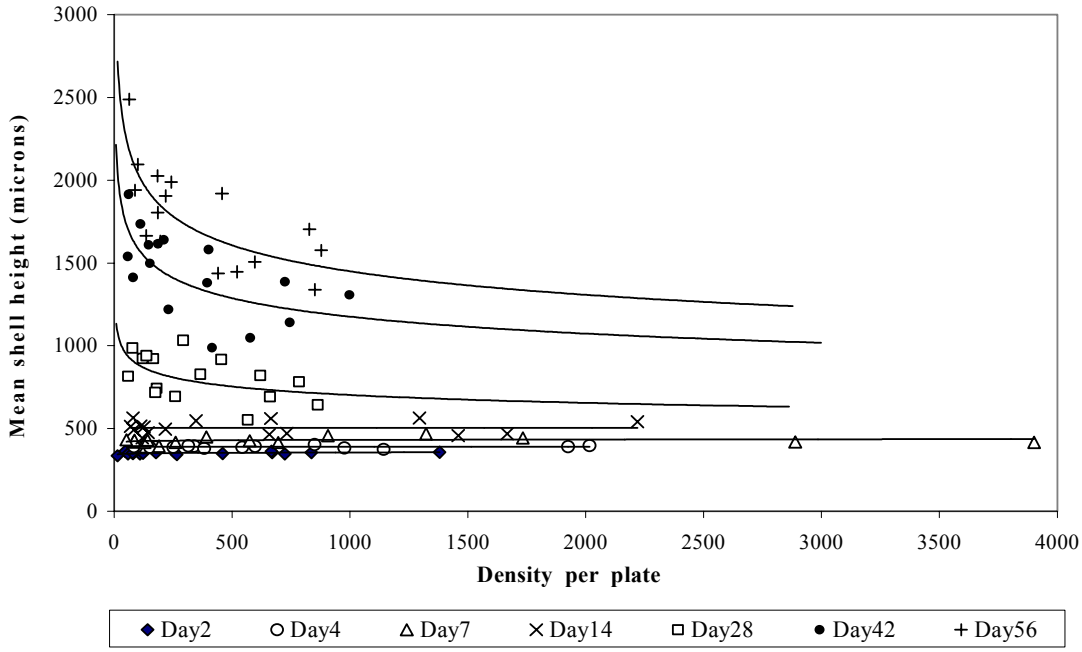


Figure 5. Effect of density on growth of *H. rubra* on diatom plates 2, 4, 7, 14, 28, 42 and 56 days after set.

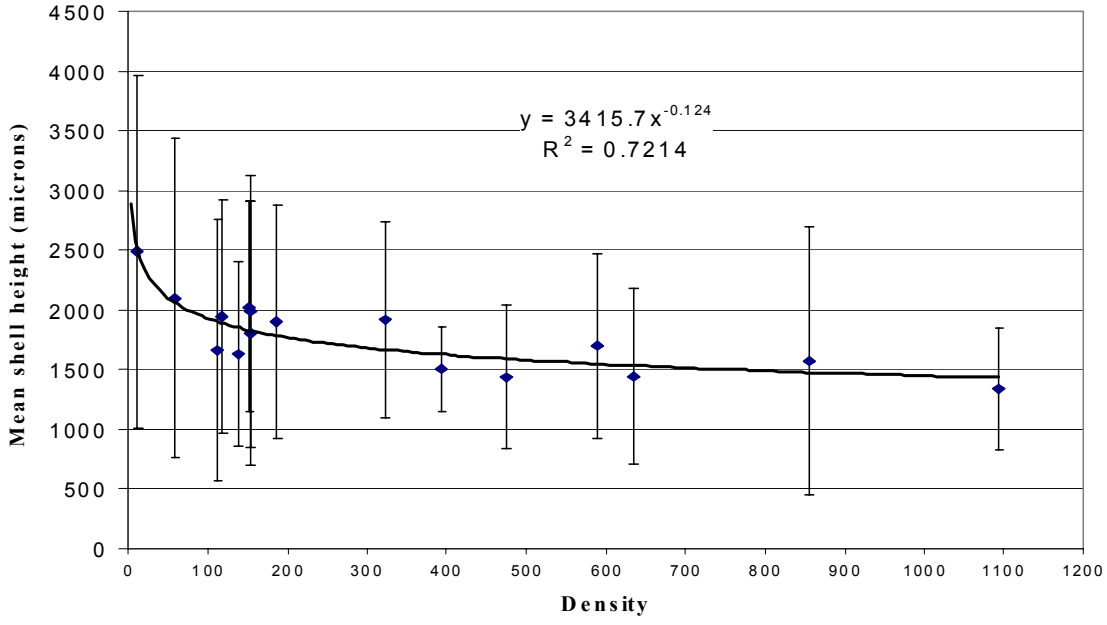


Figure 6. Effect of stocking density on growth at day 56. Mean size data points relate to individual plate counts. Bars are 95% confidence limits about means based on a sub-sample of 20 post-larvae/plate.

4.7. Improving nursery production: Effect of temperature on settlement and post-settlement growth and survival

4.7.1. Abstract

Experiments were conducted to evaluate the effect of temperature on settlement success and early post-settlement growth of blacklip abalone, *H. rubra* sourced from Tomaree Peninsula, NSW (32°45'S, 152°10'E). The first experiment was conducted with conventional diatom coated settlement plates and the second with natural settlement substrates comprising crustose coralline algae (CCA) coated rocks. Yields of juveniles on diatom plates devoid of CCA or alternative settlement inducing algae such as *Ulvella lens*, was universally poor, ranging from 0 to 5.5%. By contrast, settlement rates on CCA coated rock mainly ranged from 20 to 40%. General effects of temperature, as indicated by relative yields and early juvenile growth of *H. rubra* whether set on diatom plates or CCA rocks, were nevertheless consistent. The estimated full temperature range over which *H. rubra* larvae can successfully set on CCA is 7 to 26°C and on diatom plates, 12 to 26°C. Peak settlement occurred on diatom plates at 19°C and at 17°C on natural CCA rock substrates. The estimated temperature range over which early *H. rubra* juveniles can grow is 11 to 26°C and is independent of substrate type. In spite of marked differences in settlement success, growth in response to temperature and absolute growth rate of juveniles grown on CCA rocks and diatom plates were essentially identical. Growth rate of post-larvae measured 6 days after settlement increased progressively with increasing temperature from zero at 10°C to 25 to 30 μm/day at 22 to 24°C but then declined rapidly back to zero as temperature rose further over the range 25 to 27°C. Important practical implications of these results for commercial nursery production of *H. rubra* are discussed.

4.7.2. Introduction and aims

At the time of this study, published information on the effects of seasonal temperature variation on *H. rubra* in general and on larval settlement, metamorphosis and early juvenile growth and survival in particular, was confined to that by Shepherd (1986) and Gilroy & Edwards (1998). The latter estimated a temperature of about 17°C as that likely to optimise growth in *H. rubra*. This estimate was based indirectly on preferred temperatures exhibited by *H. rubra* when presented with a free choice to move between five alternative temperatures in the range 12 to 21°C.

This study aimed to quantify effects of temperature on settlement success and early post-settlement growth and survival of blacklip abalone *H. rubra.* Such knowledge is critical for optimisation of seeding operations associated with ranching or enhancement of natural fisheries populations. This knowledge was also sought as a means of improving efficiency and reliability of commercial hatchery and nursery production of larvae and juveniles for the burgeoning abalone farming industry in southern Australia.

4.7.3. Methods

4.7.3.1. General

Two experiments were conducted. The first employed conventional diatom coated settlement plates as used by commercial abalone nurseries. The second experiment employed CCA coated rocks, a preferred natural settlement substrate of *H. rubra* larvae (Daume *et al*. 1999).

Both experiments were conducted in a controlled environment room within the bivalve hatchery at the Port Stephens Fisheries Centre (Plate 1). Air temperature was maintained within the range 11 ± 1°C under a 12 h:12 h light/dark regime. Cylindrical 25 L plastic buckets were used as temperature baths. Those maintained above room temperature were each heated with a thermocouple controlled 300-watt submersible aquarium heater.

Plate 1. Experimental array used for temperature trials.

4.7.3.2. Experiment 1

Broodstock acquisition, conditioning, spawning induction and hatchery rearing of larvae

Larvae were sourced from the same batch as used to conduct the diatom nursery plate stocking density experiment (Section 4.6). Methods used to condition and spawn broodstock and to rear larvae are described in Section 4.3.

Experimental design and protocols

Four replicates were initially established for each of five temperatures (12.0, 15.0, 18.0, 21.0 and 24.0°C.) Each replicate was held in a separate, randomly positioned temperature bath. In practice, the temperature baths could not be precisely set nor maintained at the prescribed temperatures. Actual mean temperatures varied by up to 1.9°C below, and by up to 1.8°C above prescribed levels. Consequently each temperature bath was regarded as a separate treatment.

Settlement substrate for each treatment comprised four 150 x 200 mm clear PVC plates. These were one sixth segments cut from standard 600 x 300 x 1.5 mm clear PVC abalone nursery plates used by most commercial abalone farms throughout southern Australia (Hone *et al*. 1997). The plates had been aged in aerated flowing seawater for three weeks prior to the experiment to ensure thorough leaching of any potentially toxic solvents. It also enabled development of biofilms of mixed benthic diatom communities dominated by *Cocconeis* spp. and *Navicula* spp. that had consistently supported good growth in *H. rubra* (Section 4.5). For larval settlement, four of the plates were mounted vertically on their long edge (30 mm apart) using a simple slotted rack positioned centrally on the floor of a 9 L plastic bucket in turn suspended in an individual 25 L temperature bath. Each rack of four plates straddled a pair of 100 mm air-stones that maintained a constant flow of water and air across the surface of the plates. Aeration was reduced during the initial 24 h to facilitate larval settlement. A second air-stone was mounted directly beneath the aquarium heater in the outer 25 L temperature bath to ensure even temperature distribution. Temperature in each replicate bucket was recorded to the nearest 0.1°C twice daily.

At the outset of this experiment, an estimated 1600 day 7 competent veliger larvae shell length 295 ± 0.8 μm, were stocked into each replicate 9 L bucket. This represented a stocking rate equivalent to 2400 per standard 600 x 300 mm PVC nursery plate (~0.67/cm^2). This falls within the range of 735 to 7625/plate (0.2-2.0/cm^2) routinely used in large scale diatom plate nursery operations over the previous 2 years (Table 1a, Section 4.2). Seawater in the 9 L buckets was replaced with freshly 1 μm filtered isothermal seawater on the 2nd and 4th days after stocking.

One of the four 150 x 200 mm test plates from each replicate was sampled after 2 days. The remaining three plates were sampled 6 days after stocking as were larvae that had failed to metamorphose and any post-larvae that had settled and metamorphosed on inner surfaces of the buckets. In all cases, post-larvae adhering to surfaces of the PVC plates or the buckets were harvested using a medium bristle 40 mm paint brush and by jetting with 70% ethanol applied with a squeeze bottle. Post-larvae were collected on a 45 μm mesh screen, preserved and stored in 70% ethanol in labelled 70 mL screw-cap sample bottles. For each sample, post-larvae (defined by the presence of peristomal shell growth) were counted and shell length of up to 30 (depending on availability) randomly selected individuals measured to the nearest 5 μm.

4.7.3.3. Experiment 2

This follow up experiment was prompted by poor overall post-larval yields and some ambiguous results achieved with diatom plates in the first experiment. Daume *et al*. (1999) demonstrated that competent *H. rubra* are strongly attracted to settle and undergo metamorphosis on CCA substrates. Rates of initial settlement and successful metamorphosis were shown (Section 9) to be highest and most stable for CCA rocks in the range 5 to 60 mL displacement volume. It was also shown (Section 9) that *H. rubra* larvae are gregarious when at high density during this process and that relative yields of post-larvae actually rise with increasing stocking density up to 4000 larvae/L (~1/cm^2) of CCA rock, but stabilise at higher densities up to 16,000/L (~4/cm^2).

Settlement substrate used in each treatment of this follow-up experiment comprised 200 ± 10 mL displacement volume of CCA rock with volumes of individual rocks ranging from 5 to 60 mL. The latter were collected on the previous day from a shallow sub-tidal "boulder zone" of a nearby oceanic embayment (Boulder Bay). CCA rocks used in the experiment were selected for uniformity of growth forms of CCA and vigorously washed rinsed with high-pressure seawater to remove invertebrate predators and competitors, especially polychaete worms and small crabs. Finally, 200 ± 10 mL lots of rinsed CCA rock were stocked into 0.5 L (miniature) down-wellers (Heasman *et al*. 1998b). These down-wellers were individually mounted directly within previously described 25 L temperature baths that also served as seawater reservoirs.

Four replicates were initially established for each of the six rearing temperatures of 12.0, 15.0, 18.0, 21.0, 24 .0 and 27.0°C. However as in Experiment 1, actual temperatures could not be precisely maintained over the 6 day experiment. Mean temperatures for individual buckets varied by up to 2°C around the set points.

Each down-weller was fed with recycled seawater airlifted from the 25 L reservoir at a uniform rate of about 0.5 L/min. The airlift was left off during the first day of the experiment to facilitate larval settlement. During this period a low rate of aeration was provided to ensure that settling and metamorphosing larvae were maintained under dissolved oxygen levels at or near 100% saturation.

At the outset of the experiment, 1,000 seven-day-old competent larvae were stocked into each of the 24 down-wellers. This stocking rate was equivalent to 5,000/L of CCA rock (~1.25/cm^2) and fell within a density range found optimal for successful settlement, metamorphosis and early growth in *H. rubra* (Section 9). Seawater in the 25 L reservoir was drained and replaced with fresh 1 μm filtered isothermal seawater on the 2nd and 4th days after stocking.

All 24 treatments were sampled on day 6 after stocking. As in Experiment 1, larvae and post-larvae that had settled and metamorphosed on inner surfaces of the down-wellers were separately harvested and stored from those that had settled on the CCA rocks. Temperature was recorded to the nearest 0.1°C for each bucket twice daily. As in Experiment 1, juveniles from each sample were counted and shell length of up to 30 (if available) randomly selected individuals measured to the nearest 5 μm. Settlement success was defined as yield of live post-larvae as a percentage of larvae applied.

4.7.3.4. Analyses of data

The relationship between temperature, as a fixed factor, and yields of post-larvae and growth (as measures by mean shell length), were compared by ANOVA, (Statgraphics Version 4.1) once homogeneity of variance had been established. In some cases the latter entailed arcsine transformation of data. To aid discussion and interpretation of results, mean temperature response data for post larval yield were plotted as scattergrams to which best fit regressions curves were fitted using a standard "Analysis Toolpak" package provided in Excel version 6 (Microsoft®). This

process was accomplished by calculating and comparing respective correlation coefficient values and by simple inspection and fitted regressions. Post-larval yield vs temperature relationships were found to be better described by second order polynomial regression equations than by linear, power, exponential, logarithmic or alternative (higher or lower) order polynomials. For growth vs temperature data, fourth order polynomials were generally found to best describe relationships between the two variables.

4.7.4. Results

4.7.4.1. Experiment 1

The usefulness of day 2 results was undermined by the fact that larvae held at temperatures of 12 and 15ºC had not had sufficient time to undergo metamorphosis and subsequent growth. Nevertheless the remainder did exhibit an upward trend with rising temperatures both in terms of post-larval yields that ranged up to about 4% (Figure 1) and in terms of growth (Figure 2). Growth rates of up to 30 μm/day were exhibited by post-larvae subjected to an optimum temperature of 22ºC.

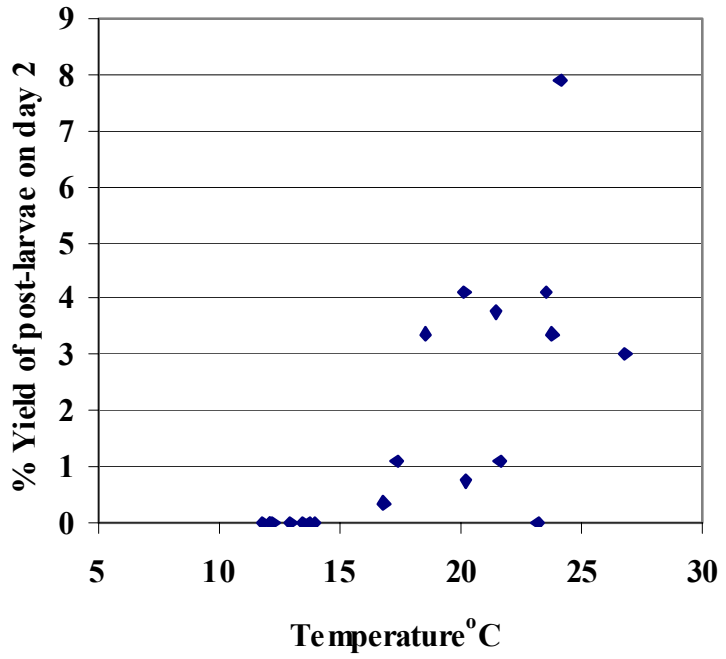


Figure 1. Effect of temperature on yield of day 2 *H. rubra* post-larvae as a % of larvae seeded onto diatom plates.

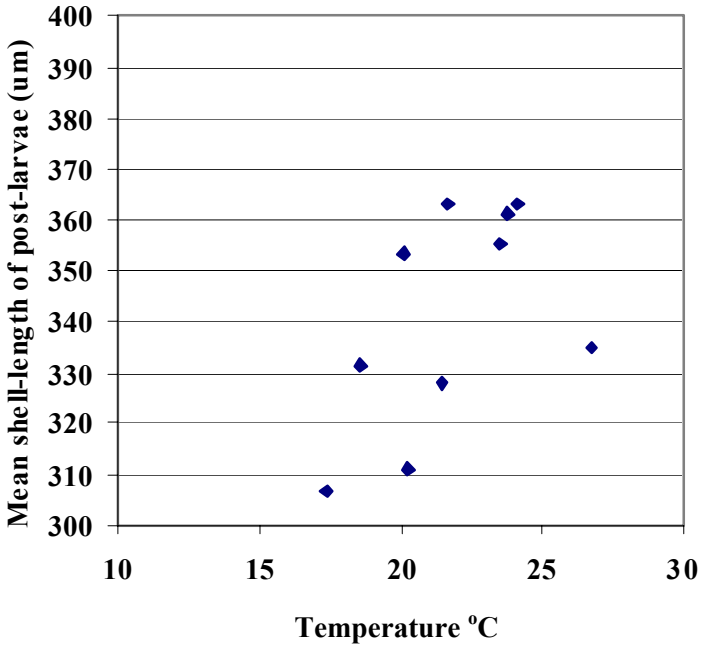


Figure 2. Effect of temperature on growth of day 2 *H. rubra* post-larvae seeded onto diatom plates.

By day 6, however, a somewhat different but equivocal pattern had emerged (Figure 3) with highest post-larval yields of about 8% coinciding with an intermediate temperature of 18°C. These data, though confused, suggest that at the larval seeding density of 0.67/cm^2 and in the absence of settlement attractors such as *Ulvella* sp. on crustose coralline algae, little if any settlement and metamorphosis occurs at temperatures below about 12°C. These data also suggest that a progressive increase in settlement and metamorphosis occurs at higher temperatures peaking at about 19°C and finally, that a progressive decline to zero again occurs with a further temperature rise over the range 20 to 26°C.

As illustrated in Figure 4, by day 6, growth data of post-larvae exhibited a much clearer relationship with temperature than at day 2. This relationship was best described by an asymmetrical fourth order polynomial equation accompanied by a high correlation coefficient (R^2) value of 0.9431. As with post larval yield data, this equation predicts zero growth at temperatures below about 12°C followed by a progressive increase of growth rate peaking at about 30 µm/day at 22°C. However, in contrast to the yield data, it predicts continuing high growth rates at temperatures up to 24°C. A single growth data point in Figure 4, coincident with the highest temperature tested, 25.4°C, though equivocal, was consistent with the prediction of this equation that growth plummets towards zero over a small additional temperature rise of from 24 to 26°C.

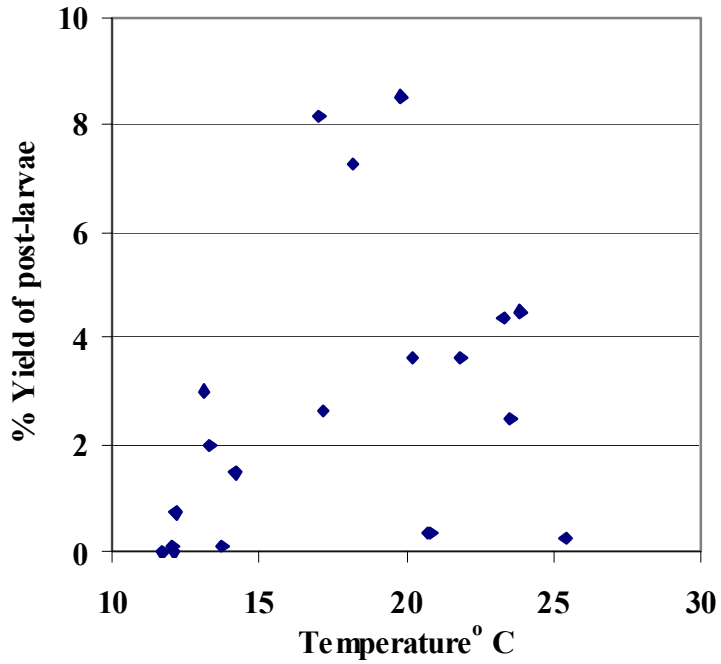


Figure 3. Effect of temperature on yield of day 6 *H. rubra* post-larvae as a % of larvae seeded onto diatom plates.

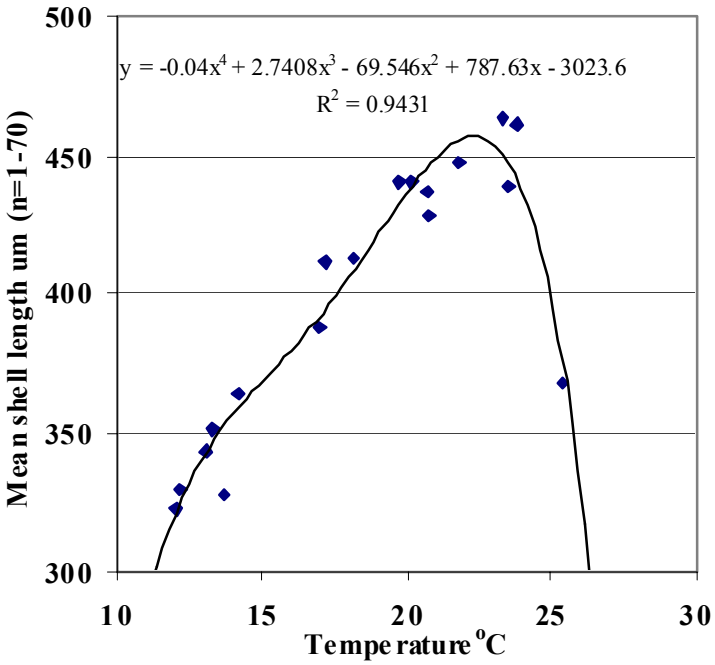


Figure 4. Effect of temperature on growth of day 6 *H. rubra* post-larvae seeded onto diatom plates.

4.7.4.2. Experiment 2

Settlement rates on the CCA rocks were mainly in the range of 20 to 40% (Figure 5), which was an order of magnitude higher than those achieved with larvae seeded onto diatom plates in Experiment 1. Yields of post-larvae seeded onto CCA rocks were more closely linked to temperature than diatom plate seeded larvae, and the relationship was found well described by a symmetrical second order polynomial equation with a correlation coefficient (R^2) value of 0.8468. This pattern mirrors that exhibited by the diatom plate seeded larvae in Experiment 1, yields of post-larvae rising to a peak then diminish back to zero with increasing temperature. However the indicated temperature range over which some degree of successful settlement occurs is considerably broader (≥7 to ≤ 27°C) than that exhibited on diatom plates (≥12 to ≤ 26°C) with peak yield occurring at 17°C rather than at 19°C.

Growth of *H. rubra* post-larvae on CCA rocks by day 6 (Figure 6) was almost identical in response to temperature as that of counterparts seeded onto diatom plates in Experiment 1 (Figure 4). Also as in Experiment 1, this relationship was best described by an asymmetrical fourth order polynomial equation that was again accompanied by a high correlation coefficient (R^2) value of 0.9484. The predicted lower and upper temperature limitations to growth for diatom plate and CCA rock seeded larvae were within 1°C of each other as was the predicted optimum temperature for growth (22°C in both cases). Most importantly, a dramatic fall to zero growth coincident with a small rise in temperature from 25°C to 27°C, equivocally indicated by the results of Experiment 1, was strongly affirmed by the results of this follow-up experiment.

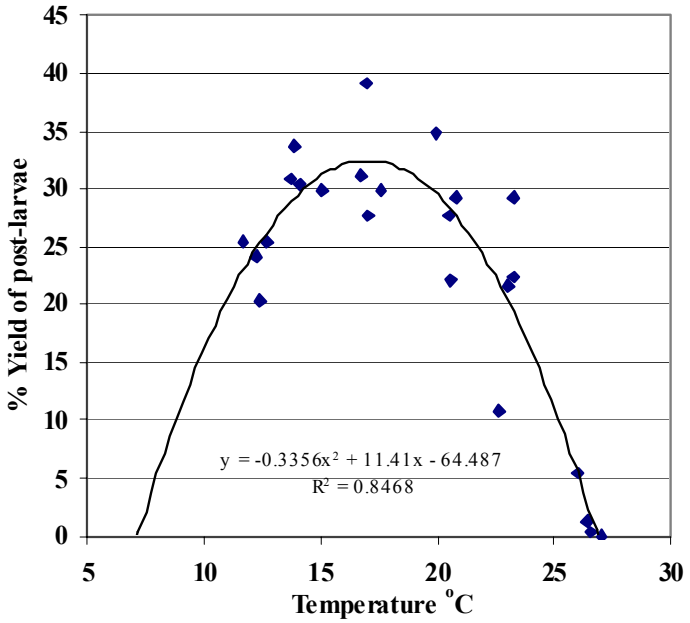


Figure 5. Effect of temperature on yield of day 6 *H. rubra* post-larvae as a % of larvae seeded onto CCA rocks.

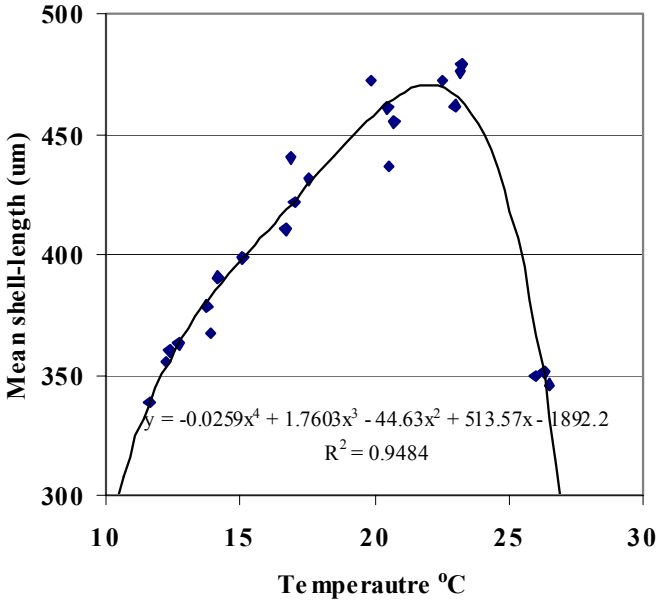


Figure 6. Effect of temperature on growth of day 6 *H. rubra* post-larvae seeded onto CCA rocks.

4.7.5. Discussion

Mean monthly inshore sea temperatures experienced along the central and southern coasts of NSW commonly vary in the range 15 to 22°C. Extreme inshore temperatures as low as 11°C are occasionally experienced on the far south coast in winter. In mid summer to early autumn, extremes as high as 25°C are encountered on the central coast of NSW, including Tomaree Peninsula and adjacent Islands. As these experiments were conducted with abalone stocks from these areas which lie toward the northern limit of commercially fished stocks of *H. rubra* in NSW, some caution needs to be applied before extrapolating results to more southerly stocks in NSW, Victoria, and Tasmania where sea temperatures are significantly lower.

An important implication of these results in conjunction with those of Section 4.6 for nursery production, is the need to use CCA, *U. lens* or other settlement promoters to facilitate high and consistent rates of settlement and metamorphosis on nursery plates when applying *H. rubra* larvae at densities below 1/cm^2. An equally important implication for farming and fisheries enhancement using hatchery produced *H. rubra* seed is the broad range of temperature over which larvae exhibit high rates of successful settlement and metamorphosis. For instance, under an annual seawater temperature range of 14 to 22° C, potential yields of 6-day-old post-larvae will remain at or above 75 % of the maximum rate that coincides with an optimum temperature for settlement and metamorphosis of about 18°C.

The optimum temperature for growth of 22°C determined in this study for recently settled *H. rubra* post-larvae is 5°C higher than an optimum temperature of 17.0°C calculated for sub-adult *H. rubra* from northern Tasmania by Gilroy & Edwards (1998), based on behavioural responses of *H. rubra* to temperature. These contrasting results are however in line with those obtained in a study by Uki *et al*. (1981) on the effects of temperature (10.8 to 25.4°C) on the growth of four different size/age classes of *H. discus hannai* ranging from early post-larvae to advanced juveniles. These findings have potentially important implications for hatchery and nursery rearing of *H. rubra* and prompted additional investigation of age/size related changes in effects of temperature on growth and survival of *H. rubra* (Section 4.8).

4.8. Improving nursery production: Effect of temperature on growth and survival of different juvenile size/age classes

4.8.1. Abstract

This study investigated the effect of temperature in the range 12 to 23 °C on growth of four age size classes of the blacklip abalone, *H. rubra*. Classes comprised juveniles in the age and size ranges 4-5 months (3-10 mm); 8-9 months (10-15 mm); 18-19 months (30-45 mm) and 31-32 months (56-73 mm). Results indicate a major downward shift in optimal temperature for growth with progressive age/size grade from 18.4°C for class 1, 17.1°C for class 2, and 14.0°C for classes 3 and 4 under the array of conditions provided. Results also demonstrated a major apparent downward shift in the physiological tolerances of juveniles with progressive age and size. For example, although classes 1 to 4 were reared within common static water baths, class 1 stock grew at a rate of 125% that of raceway reared counterparts, while class 2 stock grew about 80% that of raceway reared counterparts. This trend continued with class 3 and 4 stock, growing at rates of only 63% and 30%, respectively, of raceway reared counterparts. These results might have important implications for intensive land based farming of this species.

4.8.2. Introduction and aims

Published information on the effects of seasonal temperature variations on *H. rubra* in general and on larval settlement, metamorphosis and early juvenile growth and survival in particular, is limited to that of Shepherd (1986) and Gilroy & Edwards (1998). The authors forecast a temperature of about 17°C as that likely to optimise growth in for sub-adult *H. rubra* from northern Tasmania. This prediction was based indirectly on preferred temperatures exhibited by *H. rubra* when presented with a free choice to move between five alternative temperatures in the range 12 to 21°C. By contrast, the optimum growth temperature determined for 1 to 6-day-old *H. rubra* post-larvae in the present study (see Section 4.7) was 5°C higher namely 22°C.

This study aimed to quantify effects of temperature on growth and survival of a range of size/age classes of juvenile blacklip abalone. Such knowledge was also sought as a means of improving efficiency and reliability of commercial hatchery and nursery production of larvae and juveniles for the burgeoning abalone farming industry in southern Australia.

4.8.3. Methods

4.8.3.1. Experiment 1: Effect of temperature on growth and survival of four size classes of juvenile H. rubra

Experimental design and protocols

This experiment comprised 4 sub-experiments in which effects of temperature on growth and survival were simultaneously assessed across four disparate size/age classes of juvenile *H. rubra* maintained under a common set of physio-chemical conditions that in turn mimicked those commonly imposed under land based farming operations. It was conducted in a controlled environment room within the bivalve hatchery at the Port Stephens Fisheries Centre (PSFC), Taylors Beach, NSW. The room was maintained at an air temperature of 11.0 ± 1.0°C, under a 12 h:12 h light/dark regime. The 4 age size/age classes of juvenile *H. rubra* were subjected to alternative temperatures. Four replicates were established for each temperature x size class treatment. Initially targeted temperatures were 12.0, 15.0, 18.0, 21.0, 24.0 and 27.0 ± 0.5°C.

Juveniles previously held at an ambient temperature of 20°C, were progressively acclimated to allocated test temperatures at the rate of 1.0°C per day. However stock of all four size classes destined to be allocated to the 27.0°C treatment became progressively moribund during the period of acclimation and died before reaching this temperature. Moreover, many class 3 and class 4 stock exhibited overt symptoms of stress, culminating in some deaths at temperatures as low as 24.0°C. As a consequence, the highest test temperature of 27.0°C was abandoned and the second highest temperature of 24.0°C reduced to 23.0°C.

Juveniles used were all progeny from locally sourced wild broodstock reproductively conditioned and spawned at the Abalone Research Facility at Tomaree Head, Port Stephens, NSW (32°45'S, 152°10'E). They were also reared through larval and post-larval stages, weaned onto a formulated diet and on reared as juveniles in shallow flow-through raceways using methods previously described in Section 4.3. All four size/age classes were selected from batches exhibiting good growth, general health and vigour. Great care was taken to minimise stress during handling and to ensure that juveniles within each size/age were as uniform in size as possible at the outset of the experiment. The initial post-spawning age and shell length range of the four classes of juveniles were: 3 month old and 2 to 6 mm (class 1); 7 month old and 6 to 8 mm (class 2); 17 month old and 20-25 mm (class 3); 30 month old and 50 to 60 mm (class 4).

Immediately before being transported to an aquarium facility at PSFC, the juvenile stock were assembled into groups of 200 class-1, 100 class-2; 20 class-3 or 16 class-4. Four groups of each size class were held overnight in small experimental scale down-wellers in readiness for wet transportation to PSFC. On arrival at PSFC on 3rd of April 2001, the 16 mini down-wellers were accommodated in a single 1200 L rectangular seawater tank (2.6 x 1.2 x 0.45 m) at the same ambient temperature (20^{o}C). Each down-weller was connected to an air-supply to provide stock with a constant stream of turbulent aerated seawater.

Experimental sub units initially comprised 20 cylindrical 25 L plastic buckets that served both as temperature baths and seawater reservoirs. Submersible 300 watt aquarium heaters were used to heat individual temperature baths. However, as the adjustable range of these heaters was limited to 22.0 to 35.0 ± 0.5°C, those used to maintain temperatures of 15.0, 18.0 and 21.0 ± 0.5°C were powered by electrical outlets controlled by Honeywell temperature controllers and associated sensors. Provision of the lowest experimental temperature of 12.0°C was achieved by exposing unheated 25 L water baths to air cooling and to incidental radiant heating by warmer neighbouring temperature baths.

Maintenance and monitoring of stock, hygiene, food consumption and water quality

The general appearance and health of stock in all replicates was evaluated daily as were key physio-chemical parameters. Dead or moribund stock were checked for daily and if detected, were immediately replaced with a similar sized animal from reserve stock fed and maintained at low density in auxiliary temperature baths.

An *Horiba* (Model U10, Water Quality Checker) water analyser was used daily to measure temperature to the nearest 0.1°C, salinity to the nearest 0.1g/kg, dissolved oxygen to the nearest 0.01 mg/L, and pH to the nearest 0.1 of a unit, across all temperature baths. On alternate days, down-wellers and temperature baths were drained, thoroughly cleaned with a soft sponge and rinsed before refilling with new isothermic (±0.2°C) coastal seawater. To avoid any risk of temperature shock, temporary storage, cleaning and rinsing operations of down-wellers, and their enclosed stock, were performed in isothermic seawater.

Shell lengths of size class 1 juveniles were measured to nearest 0.1mm at 10x using a stereo microscope fitted with a calibrated eyepiece graticule. Shell lengths of juveniles within the three other size classes were measured to the nearest 0.1mm using Mitutoyo, "Digimatic" callipers.

Drained and blot dried live-weights of all class 3 and 4 stock were also measured to the nearest 0.1g using a *Mettler* (Model-PB303) top loading balance. Size classes 1 and 2 stock were always anaesthetised with benzocaine in accordance with procedures described in Section 5.2 prior to handling, measuring and weighing. Total ammonium nitrogen (TAN) concentration of all temperature baths was assessed at probable peak levels immediately prior to alternate day water changes, using colorimetric test kit, (Merck 1.08024.0001) suitable for determining total ammonium nitrogen concentrations within the range 0.2 to 5 mg/L. Determination of temperature, salinity and pH also enabled corresponding concentrations of the more toxic unionised free ammonium nitrogen (FAN) determined using tables of Liao & Mayo (1972).

Various pelleted forms of a formulation diet, manufactured by Adam and Amos P/L (South Australia), were used to feed the different size classes. Class 1 and 2 juveniles were fed a crumb form while class 3 and 4 juveniles were provided with small and large grade noodles respectively. To aid general hygiene and to reduce the risk of screen blockages, mesh size of removable screens on the floors of down-wellers were increased in concert with food particle size, being maintained at the largest mesh aperture capable of retaining all uneaten food particles. Feeding status of stock in all replicate down-wellers was visually assessed in conjunction with successive water changes. Feeding rates were increased by 5% if all four replicates of any treatment had been devoid of residual food. Likewise, feeding rates was reduced by 5% if all four replicates of any treatment contained substantial amounts of uneaten food. While amounts of feed administered to all replicates were recorded daily, formal assessment of effects of temperature on net food consumption by animals in size class 2 was addressed in a separate experiment described below.

Four down-wellers, comprising a replicate of each of the four size classes, were initially accommodated in each 25 L temperature bath (Plate 1). Numbers of juveniles per replicate varied with size class. Numbers per replicate initially allocated to classes 1, 2, 3 and 4 were 40, 20, 10 and 5 respectively. However during the first week of the experiment, water quality parameters within all replicates maintained at the two highest test temperatures of 21.0 and 23.0°C were found to deteriorate to levels outside EC_5 levels recognised as conducive to good health and normal growth of *H. rubra*, namely DO $\geq$95% saturation (Fleming 2001), pH 7.9 to 8.5 (Harris *et al*. 1998), and free ammonia nitrogen concentrations below 0.041 mg/L (Harris *et al*. 1999). This deterioration in water quality was principally ascribed to relatively high biomasses and associated high feeding requirements of class 4 stock. Indeed satiation feeding requirements of these large juveniles far exceeded those of the other three smaller size classes combined. This in turn prompted the reallocation of class 4 replicate down-wellers that were being maintained at 21 and 23.0°C to individual 25 L temperature baths. Provision of separate accommodation for these replicates on 1st May 2001 required another 8 temperature baths. Also in deference to these overstocking problems, numbers of juveniles per replicate for size classes 3 and 4 were drastically reduced from 10 and 5 respectively to 4 and 3 respectively during the early stages of the experiment.

A succession of other problems were encountered during the first weeks of the experiment. These included difficulties in quantifying and maintaining feeding rates just above satiation levels, prevention of escapement of class 1 and 2 stock and failure of some heaters and controllers. As it was strongly suspected that growth and survival of stock during this initial phase of the experiment had been compromised, the experiment was reinitiated after 27 days on the 9th May 2001 following re-measurement of stock within all replicates. The experiment was terminated after a further 34 days on 12th June 2001.

Plate 1. Experimental array showing some of the 20 temperature bath/seawater reservoirs housing 4 mini-downwellers that in turn each accommodated a replicate of the four discrete age/size classes of juvenile *H. rubra*.

Statistical methods

The relationship between temperature, as a fixed factor, and yields of post- larvae and growth (as measures by mean shell length), were compared by ANOVA, (Statgraphics Version 4.1) once homogeneity of variance having been established, in some cases entailing arcsine transformation of data. To aid discussion and interpretation of results, mean temperature response data for post larval yield growth were plotted as scattergrams to which best fit linear, second third and fourth order polynomials, power, exponential and logarithmic regressions were fitted using a standard "Analysis Toolpak" package provided in Excel version 6 (Microsoft.®). This process was accomplished by calculating and comparing respective correlation coefficient values and by simple inspection and fitted regressions.

4.8.3.2. Experiment 2: Effect of temperature on food consumption

This 4 day companion experiment employed the same generalised methods as described above for Experiment 1. Additional and/or altered procedures were as follows. Stock used comprised residual class 2 juveniles from all 6 temperature treatments (12.0, 15.0, 18.0, 21.0, 23.0° C) and replicates from Experiment 1. These stock, which ranged in shell length from 10 to 17 mm, were retained in their original replicate groupings of 15 within the same experimental down-wellers as used in Experiment 1. The down-wellers in turn were located singly in each of their original randomly positioned 25 L temperature baths as used in Experiment 1.

The day before commencing the experiment, the collective live weight of each replicate group of juveniles was determined after anaesthetising them in isothermic seawater. To avoid toxic side effects (Section 5.2), anaesthesia was performed using either 0.5 mg/L of benzocaine for replicates in treatment temperatures 21.0 and 23.0°C or 1.0 mg/L for replicates in treatment temperatures of 12.0, 15.0 or 18.0ºC.

The experiment was initiated on the morning of Sunday 24th June 2001 and terminated four days later. Each morning, daily rations of the "noodle" diet fed to each replicate were weighed to the nearest 0.1 mg. Pre-weighed removable mesh circles that had retained uneaten food from the previous day's ration were drained and blot dried on absorbent paper towelling. After determining their damp weight to the nearest 0.1 mg, they were placed in an oven and dried to constant weight at 100°C overnight and reweighed.

On the final day of the experiment, stocked down-wellers in each temperature bath were accompanied by a second but unstocked down-weller to which an equal ration of food was added. These empty companion down-wellers thereby served as "controls" to determine extraneous food weight losses due to leaching and microbial degradation. Food consumed each day was estimated from dry weights of daily rations after deducting the moisture content of the ration (predetermined as 10.0%), dry weight of uneaten food, extraneous losses due to leaching and microbial degradation and dried sea salts retained on the screens and in uneaten food. The latter was estimated by assuming that sea-salt comprised 3.5% of retained moisture on the screens and in uneaten food. Retained moisture was in turn determined as the difference between damp and oven dried weights of uneaten food on screens.

4.8.4. Results

4.8.4.1. Experiment 1: Physio-chemical conditions

Temperature

Mean daily temperatures for all replicates at prescribed temperatures of 12.0, 15.0 and 18.0 ºC were generally held within 0.2ºC of prescribed levels. A single failure of a heater serving one of four replicates set at 18.0ºC on day 20 that resulted in a surge to 21.3ºC was the only major aberration recorded over the 34 day duration of the trial. Occasional daily over-temperature spikes of 0.5 to 1.0ºC were recorded within individual replicates set at 21.0 and 23.0ºC. Overall mean ± s.e. treatment temperatures, all of which were within 0.2ºC of prescribed levels, are provided in Table 1 together with standard deviations within replicates and extreme ranges across all replicates for each treatment temperature.

Table 1. Summary of temperature data for Experiment 1.

Treatment and replicate	mean	st. dev.	Minimum	Maximum
12a	11.9	0.1	11.8	12.0
12b	11.9	0.1	11.8	11.9
12c	11.8	0.1	11.7	11.9
12d	11.8	0.1	11.6	11.9
All replicates pooled	11.8	0.1	11.6	12.0
15a	14.9	0.0	14.4	14.9
15b	14.9	0.2	14.6	15.5
15c	14.9	0.1	14.8	15.2
15d	14.9	0.1	14.6	14.9
All replicates pooled	14.9	0.1	14.4	15.5
18a	17.9	0.6	17.0	21.3
18b	18.2	0.2	17.8	18.5
18c	17.9	0.3	17.5	18.8
18d	17.8	0.1	17.6	17.9
All replicates pooled	17.9	0.4	17.0	21.3
21a	21.0	0.5	19.2	21.7
21b	21.6	0.6	20.0	23.5
21c	20.9	0.1	20.6	21.2
21d	21.0	0.2	20.5	21.5
All replicates pooled	21.1	0.5	19.2	23.5
24a/1-3	23.1	0.1	23.0	23.5
24b/1-3	23.1	0.2	22.5	23.5
24c/1-3	23.1	0.4	22.2	23.7
24d/1-3	22.8	0.5	21.1	23.2
All replicates pooled	23.0	0.4	21.1	23.7
24a/4	22.9	0.1	22.7	23.3
24b/4	23.2	0.1	22.9	23.4
24c/4	23.1	0.3	22.6	23.8
24d/4	22.7	0.6	21.2	23.1
All replicates pooled	23.0	0.3	21.2	23.8

Salinity

Mean ± s.e salinities measured just prior to alternate day seawater changes for the four treatments over the 34 day trial varied over the narrow range 34.5 ± 0.02 to 36.0 ± 0.18 g/kg. As indicated in Figure 2, mean salinity increased progressively and significantly ($P < 0.01$) with each successive 3ºC increment from 12 to 21ºC, but stabilised thereafter.

Comparative alternate day mean ± s.d. salinity time series data for treatments presented in Figure 3, reveal a synchronous pattern of rises and falls. This pattern probably reflects background fluctuations in source seawater collected from sites close to the mouth of Port Stephens. Absolute lower and upper extremes of salinity measured across all treatments and replicates were 32.2 to 37.7 g/kg. These extremes fell within a range reported as conducive to good health and growth performance for *H. rubra* and *H. laevigata* by Fleming (2000).

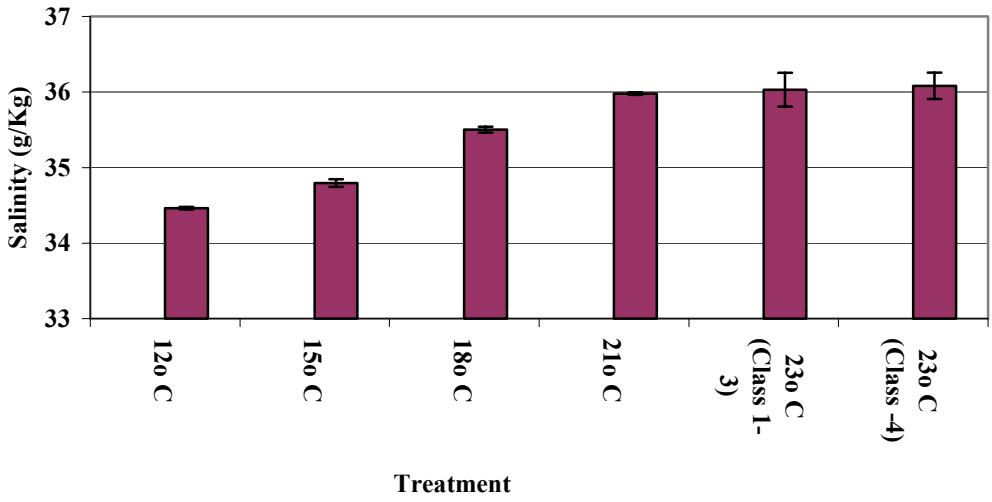


Figure 2. Mean salinity data measured immediately before alternate day water changes (error bars = s.e.).

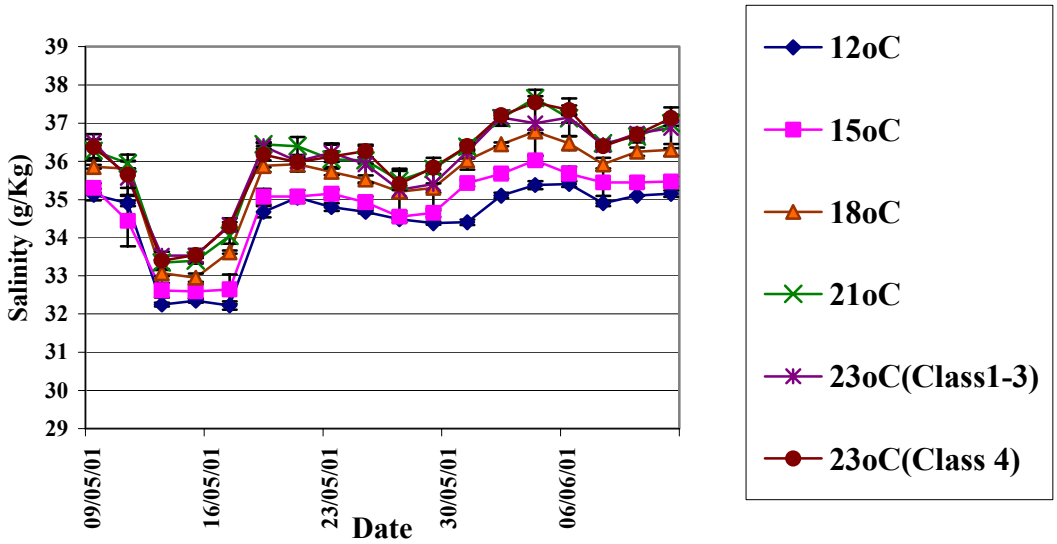


Figure 3. Mean salinity data measured immediately before alternate day water changes (error bars = s.d.).

pH

Mean ± s.e. pH values for various treatments over the 34 day duration of the trial (Figure 4) varied within the narrow range of 7.94 ± 0.06 to 8.07 ± 0.01. As with salinity, comparative alternate day mean ± s.d. time series pH data for treatments (Figure 5) exhibited apparently synchronous rises and falls that probably reflect background fluctuations of source seawater. Lower and upper extremes of pH measured across all treatments and replicates were 7.83 to 8.24. These extremes, as well as mean values, fell within a range reported as conducive to good health and growth performance for *H. rubra* and *H. laevigata* by Harris *et al*. (1998).

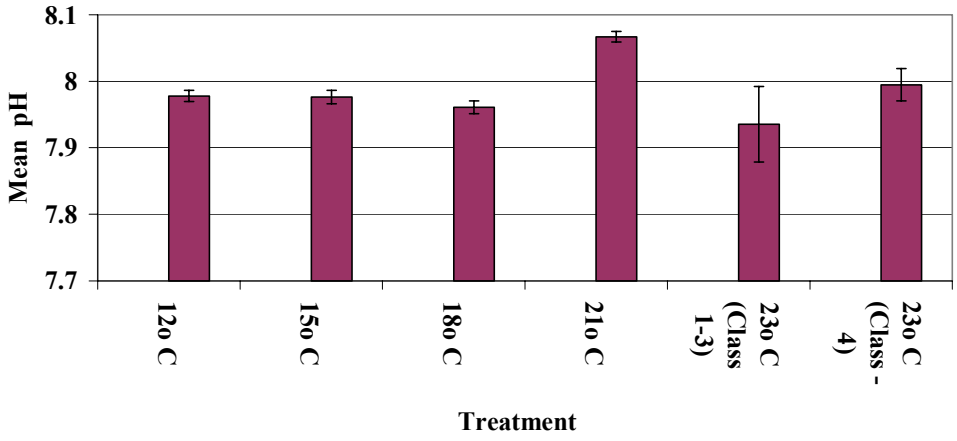


Figure 4. Mean pH data measured immediately before alternate day water changes (error bars = s.e.).

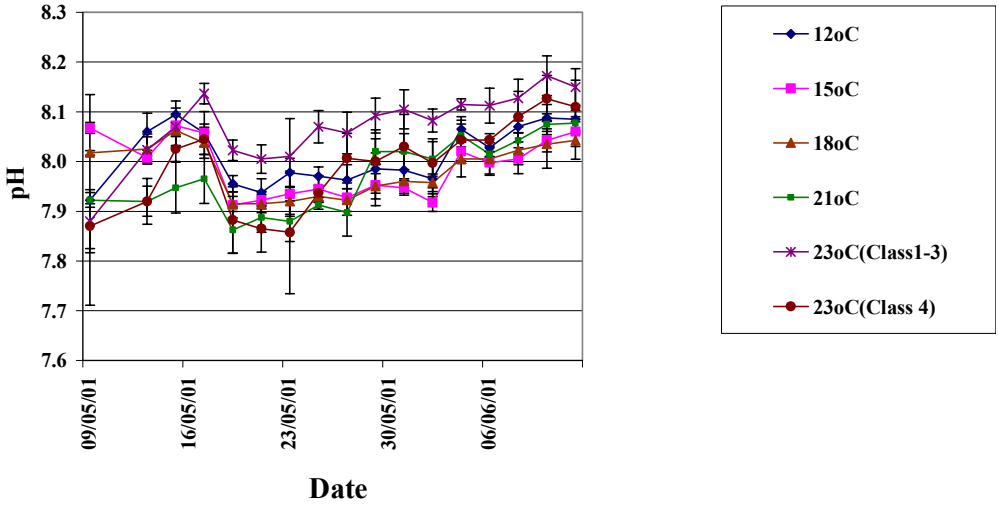


Figure 5. Mean pH data measured immediately before alternate day water changes (error bars = s.d.).

Dissolved oxygen (DO)

Mean ± s.e. DO values for various treatments over the full 34 day duration of the trial (Figure 6) diminished progressively with increasing temperature from 10.13 ± 0.03 mg/L in the 12ºC treatment 8.25 ± 0.04 and 7.95 ± 0.16 mg/L in sub treatments 23ºC (classes 1-3) the 23ºC (class 4) respectively. These results were nevertheless in accordance with temperature related reductions in the solubility of oxygen in of seawater of equivalent mean temperatures and salinities. Indeed a plot of these same data transformed to equivalent percent saturation values (Figure 7) show that mean ± s.e values across the different treatments varied over the narrow range 113.7 ± 2.2 to 118.0 ± 0.6% saturation, with no clear trend with temperature.

As with salinity and pH, comparative alternate daytime series DO data, expressed in absolute terms (Figure 8), or as % saturation (Figure 9), reveal synchronous rises and falls that probably reflect background fluctuations in pressure of compressed air being supplied to the experimental array. Discounting replicates of class 3 and 4 juveniles held at 23ºC, that contained multiple mortalities at the time of measurement, the lowest DO measured across all treatments and replicates was 106% saturation. Moreover, even in the case of the multiple mortalities, the lowest recorded DO level was 96% of saturation. These data suggest that DO was at all times maintained at levels reported as conducive to good health and growth performance for *H. rubra* by Fleming (2000).

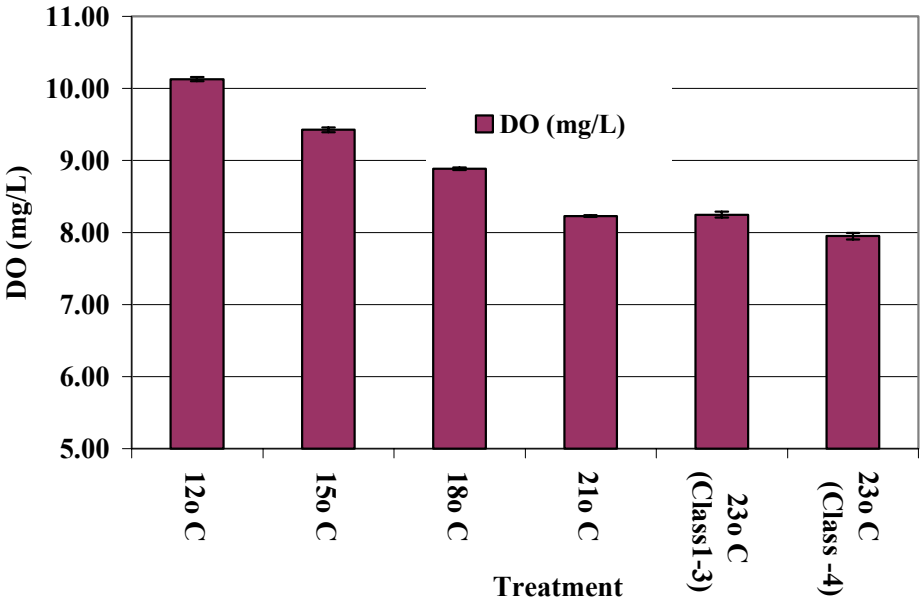


Figure 6. Mean dissolved oxygen data measured immediately before alternate day water changes (error bars = s.e.).

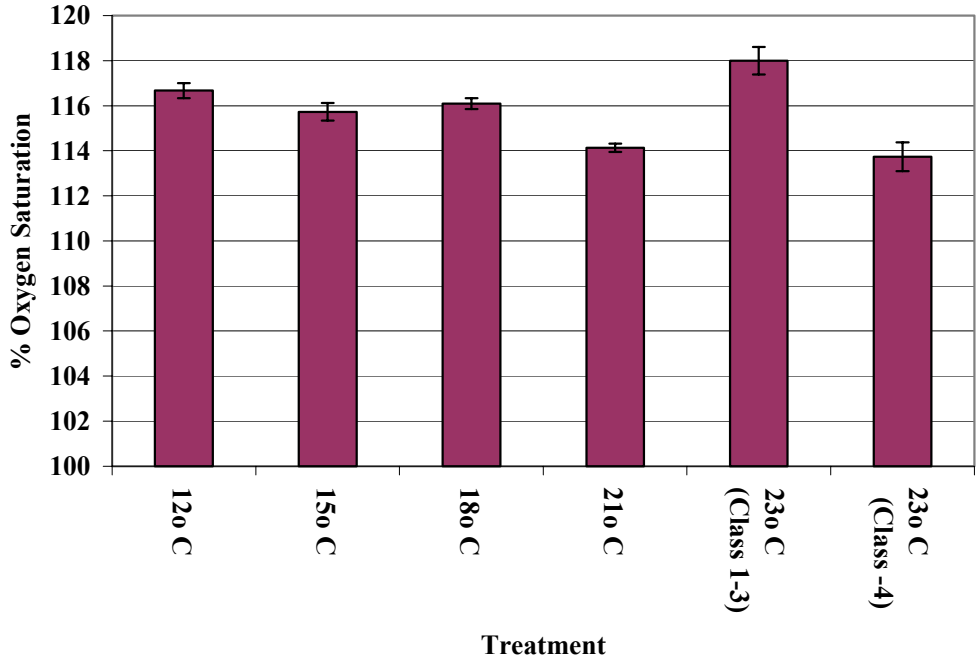


Figure 7. Mean % oxygen saturation data measured immediately before alternate day water changes (error bars = s.e.).

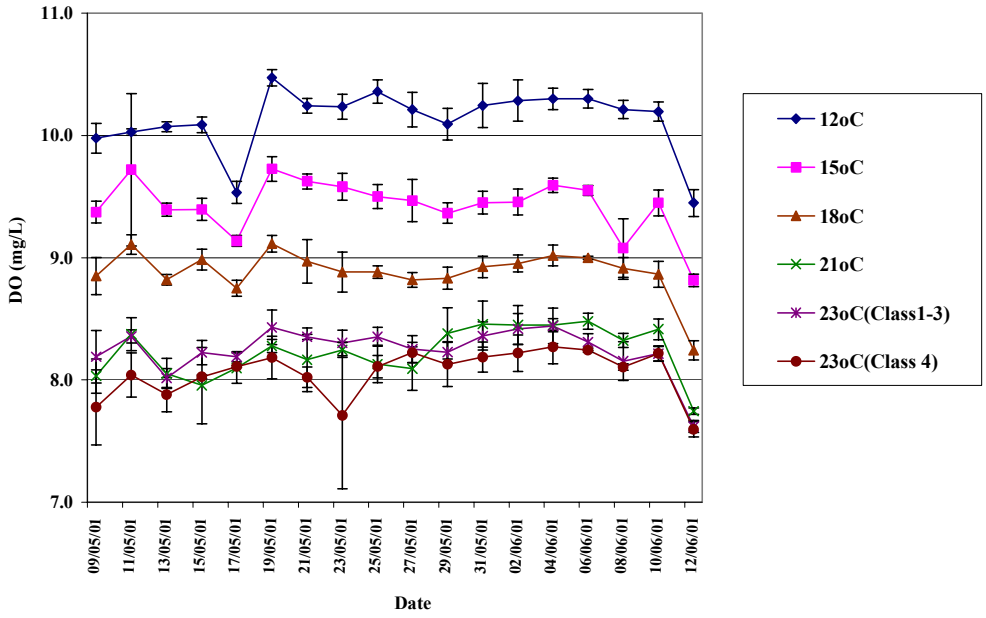


Figure 8. Mean dissolved oxygen data measured immediately before alternate day water changes (error bars = s.e.).

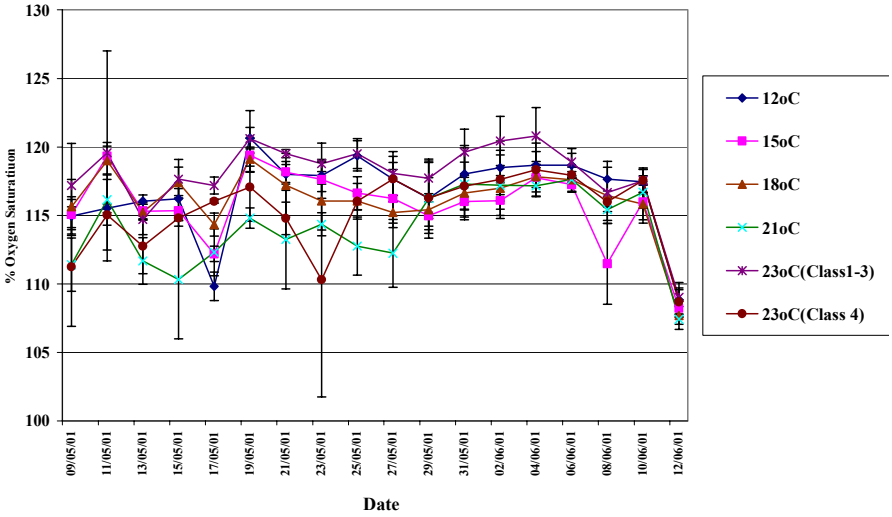


Figure 9. Mean % oxygen saturation data measured immediately before alternate day water changes (error bars = s.d.).

Ammonia

Mean ± s.e. total ammonium nitrogen (TAN) and free ammonium nitrogen (FAN) levels for various treatments averaged over the 34 day duration of the trial are presented in Figure 10 and Table 2. Levels of TAN increased progressively with temperature from 0.121 ± 0.009 mg/L in the 12ºC treatment to 0.389 ± 0.047 mg/L in the 21ºC treatment. In contrast, intermediate TAN values occurred at the highest treatment temperature of 23ºC where size class 4 juveniles were separately accommodated in a subset of buckets from their size class 1 to 3 counterparts. As previously discussed (see methods), this was to circumvent excessive bio-load problems encountered during the preliminary phase of the experiment. Mean ± s.e TAN values, recorded for sub treatments 23ºC (Class 1-3) and 23ºC (Class 4), were 0.238 ± 0.010 mg/L and 0.252 ± 0.055 mg/L respectively.

Using corresponding pH and temperature data, levels of the highly toxic free unionised (NH_3) form of ammonium nitrogen (FAN) were calculated (Liao & Mayo 1972) as comprising 2, 2.5, 3.0, 3.5 and 4.0% of TAN at treatment temperatures of 12ºC, 15ºC, 18ºC, 21ºC and 23ºC respectively.

Mean ± s.e. FAN levels for various treatments averaged over the 34 day duration of the trial, also presented in Figure 10, increased progressively with temperature in parallel with TAN from 0.0024 ± 0.0004 mg/L in the 12ºC treatment to 0.0067 ± 0.0013 mg/L in the 21ºC treatment. Intermediate FAN values occurred at the highest treatment temperature of 23ºC where large size class 4 juveniles were separately accommodated in a subset of buckets from their size class 1 to 3 counterparts. This was to circumvent excessive bio-load problems encountered during the lead in phase of the experiment (see methods). Mean ± s.e TAN values recorded for sub treatments 23ºC (Class 1-3) the 23ºC (Class 4) were 0.238 ± 0.010 mg/L and 0.252 ± 0.055 mg/L respectively.

In contrast with salinity, pH and DO, alternate daytime series TAN and FAN concentrations (Figures 11 & 12) for the four temperature treatments exhibited asynchronous rises and falls. Lower and upper alternate day extremes of FAN across all treatments and replicates were 0.001 mg/L and 0.016 mg/L respectively. The upper extreme, as well as mean levels of FAN for all treatments, appear well within those conducive to good growth performance of juvenile *H. rubra* as indicated by a EC_5 value of 0.041 mg FAN l^{-1} determined for 3 year old, 32 mm mean shell length, juvenile *H. laevigata* reared at ambient temperatures of 13.3 to 19.6ºC by Harris *et al*. (1999).

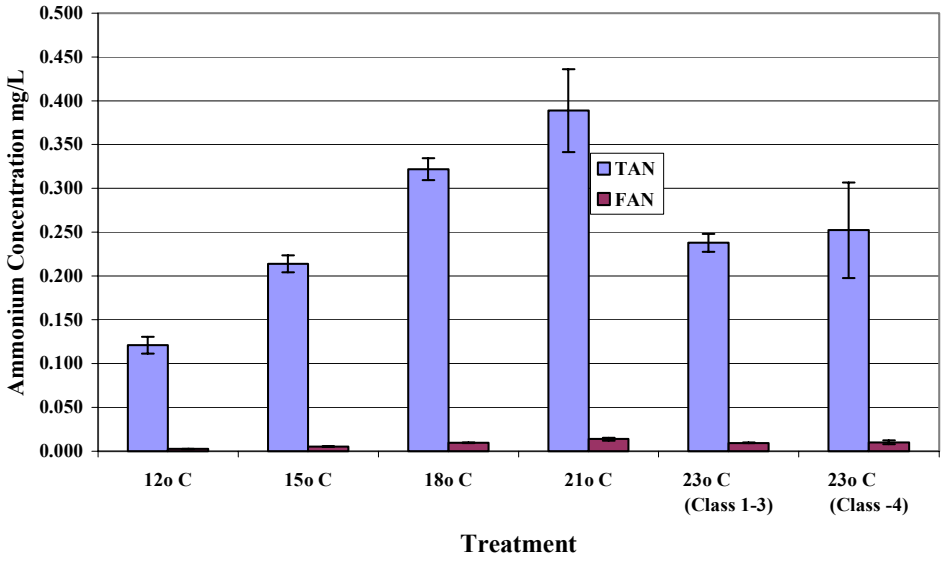


Figure 10. Mean TAN (total ammonium nitrogen) and FAN (free ammonium nitrogen) levels measured immediately before alternate day water changes (error bars = s.e.).

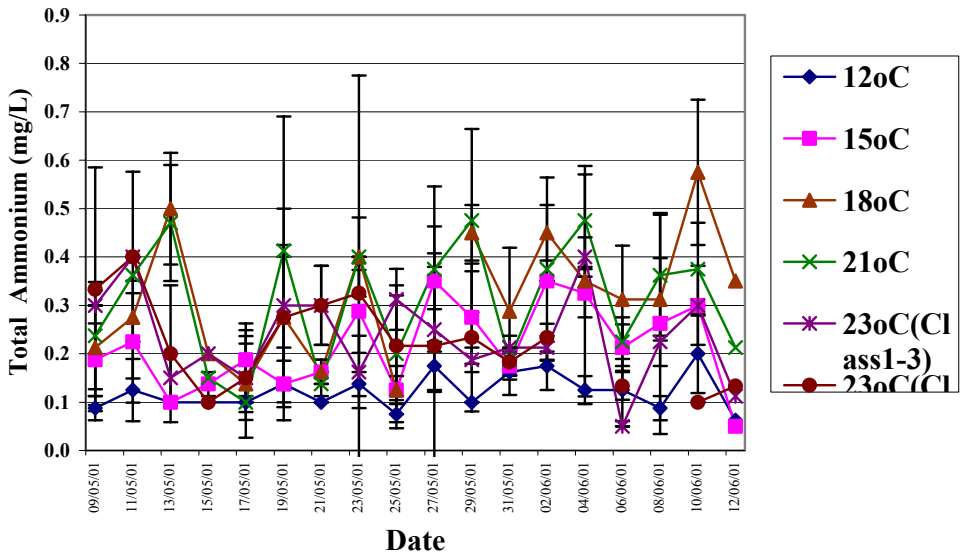


Figure 11. Total ammonium nitrogen (TAN) levels measured immediately before alternate day water changes (error bars = s.d.).

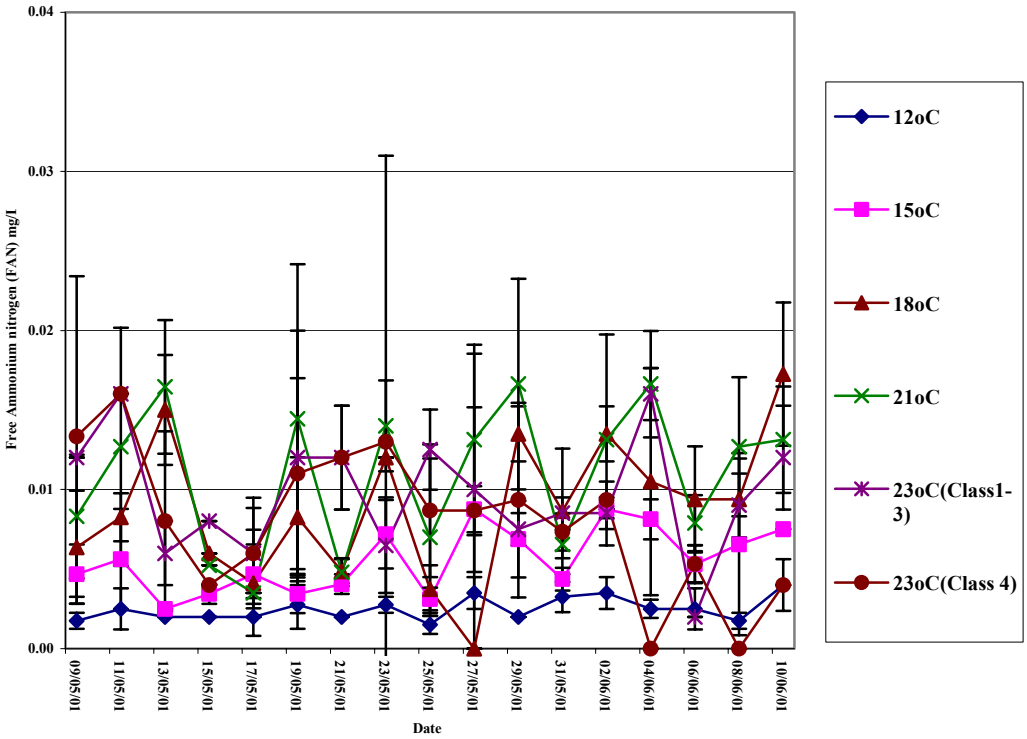


Figure 12. Free ammonium nitrogen (FAN) levels measured immediately before alternate day water changes (error bars = s.d.).

Table 2. Ammonium concentration data.

Treatment and replicate	TAN mg/L				FAN mg/L			
	Mean	St. dev.	Minimum	Maximum	Mean	St. dev.	Minimum	Maximum
12a	0.139	0.063	0.050	0.300	0.0028	0.0013	0.0010	0.0060
12b	0.128	0.055	0.050	0.200	0.0026	0.0011	0.0010	0.0040
12c	0.122	0.046	0.050	0.200	0.0024	0.0009	0.0010	0.0040
12d	0.094	0.034	0.050	0.150	0.0019	0.0007	0.0010	0.0030
Pooled replicates	**0.121**	**0.019**	**0.050**	**0.300**	**0.0024**	**0.0004**	**0.0010**	**0.0060**
15a	0.225	0.127	0.050	0.600	0.0045	0.0025	0.0010	0.0120
15b	0.186	0.097	0.050	0.400	0.0037	0.0019	0.0010	0.0080
15c	0.214	0.107	0.050	0.400	0.0043	0.0021	0.0010	0.0080
15d	0.231	0.111	0.050	0.400	0.0046	0.0022	0.0010	0.0080
Pooled replicates	**0.214**	**0.020**	**0.050**	**0.600**	**0.0043**	**0.0004**	**0.0010**	**0.0120**
18a	0.297	0.209	0.050	0.800	0.0059	0.0042	0.0010	0.0160
18b	0.322	0.146	0.100	0.500	0.0064	0.0029	0.0020	0.0100
18c	0.313	0.152	0.100	0.600	0.0063	0.0030	0.0020	0.0120
18d	0.356	0.134	0.150	0.600	0.0071	0.0027	0.0030	0.0120
Pooled replicates	**0.322**	**0.025**	**0.050**	**0.800**	**0.0064**	**0.0005**	**0.0010**	**0.0160**
21a	0.267	0.153	0.050	0.600	0.0053	0.0031	0.0010	0.0120
21b	0.315	0.181	0.050	0.600	0.0063	0.0036	0.0010	0.0120
21c	0.421	0.447	0.100	2.000	0.0084	0.0089	0.0020	0.0400
21d	0.347	0.158	0.100	0.600	0.0069	0.0032	0.0020	0.0120
Pooled replicates	**0.337**	**0.065**	**0.050**	**2.000**	**0.0067**	**0.0013**	**0.0010**	**0.0400**
24a/1-3	0.219	0.094	0.050	0.400	0.0044	0.0019	0.0010	0.0080
24b/1-3	0.264	0.150	0.050	0.600	0.0053	0.0030	0.0010	0.0120
24c/1-3	0.224	0.103	0.050	0.400	0.0045	0.0021	0.0010	0.0080
24d/1-3	0.244	0.120	0.050	0.450	0.0049	0.0024	0.0010	0.0090
Pooled replicates	**0.238**	**0.021**	**0.050**	**0.600**	**0.0048**	**0.0004**	**0.0010**	**0.0120**
24a/4	0.166	0.094	0.050	0.400	0.0033	0.0019	0.0010	0.0080
24b/4	0.155	0.069	0.100	0.300	0.0031	0.0014	0.0020	0.0060
24c/4	0.313	0.224	0.100	1.000	0.0063	0.0045	0.0020	0.0200
24d/4	0.375	0.222	0.100	0.600	0.0075	0.0044	0.0020	0.0120
Pooled replicates	**0.252**	**0.109**	**0.050**	**1.000**	**0.0050**	**0.0022**	**0.0010**	**0.0200**

4.8.4.2. Effect of temperature on growth

Size class 1 juveniles

A symmetrical second order polynomial regression equation (Figure 13) accompanied by a correlation coefficient value (R^2) of 0.7524 was found to better describe the relationship between temperature and mean daily shell length increment data than equivalent linear, higher order polynomial, power or exponential equations. The optimum temperature for shell growth, as determined by the point of inflexion of the regression equation, was 18.4ºC. This temperature supported a daily shell growth increment estimated as 69µm/day. The approximate temperature range over which positive growth was indicated was 9 to 27ºC.

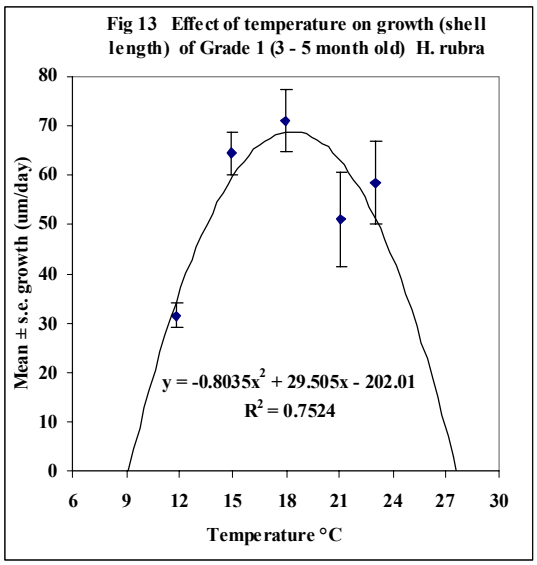
Size class 2 juveniles

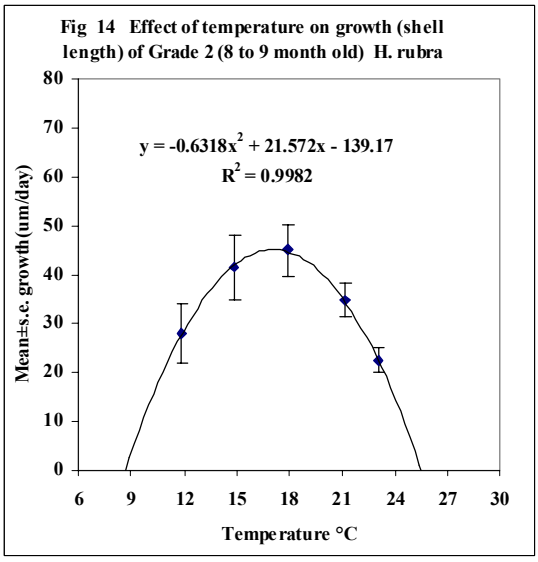
As for size class 1 juveniles, a symmetrical second order polynomial equation (Figure 14) with a correlation coefficient value of 0.9982 was found to best describe the relationship between temperature and mean daily growth rate. Optimum temperature, as determined by the point of inflexion of the fitted equation, was 17.1ºC, i.e. more than a degree lower than for size class 1 juveniles. However, this temperature supported a daily shell growth increment of 45µm/day that was markedly lower than the equivalent rate of 69 µm per day for class 1 juveniles. While the lower limit of temperature needed to support positive growth was the same as that of class 1, the upper limit at 25.5ºC was 1.5ºC below that for size class 1 juveniles.

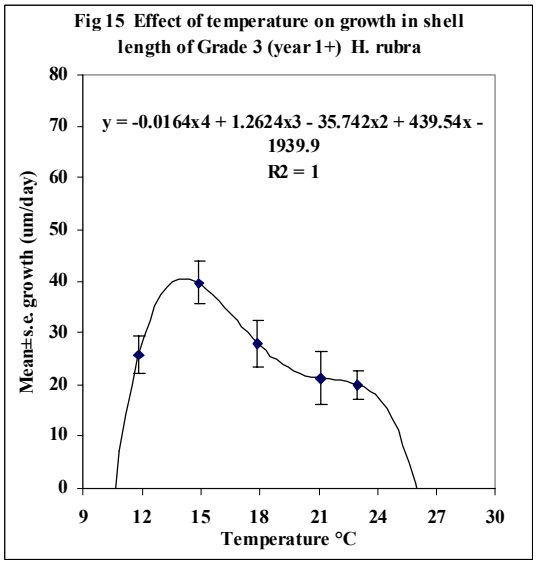
Size class 3 and 4 juveniles

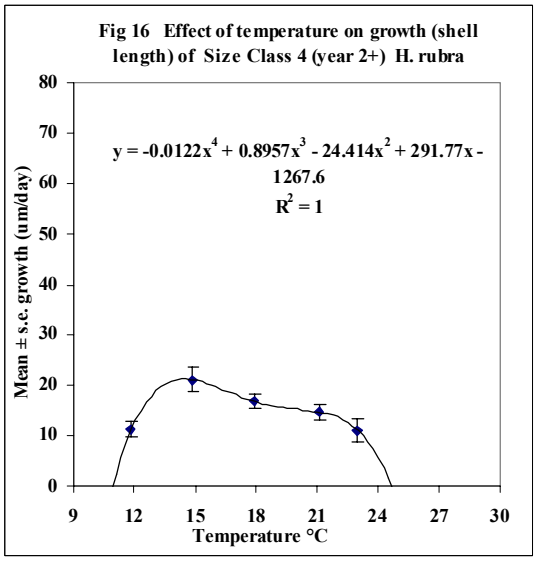
In contrast to size class 1 and 2 juveniles, shell length growth rates of size class 3 and 4 juveniles (Figure 15 & 16) exhibited a more complex relationship with temperature best described by an asymmetrical 4th order polynomial regression equation. Maximum shell growth rates predictions of only 40 and 22 µm per day occurred at 14.0ºC for both size classes. This was 3 and 4ºC lower than corresponding temperatures for size class 1 and 2 juveniles respectively.

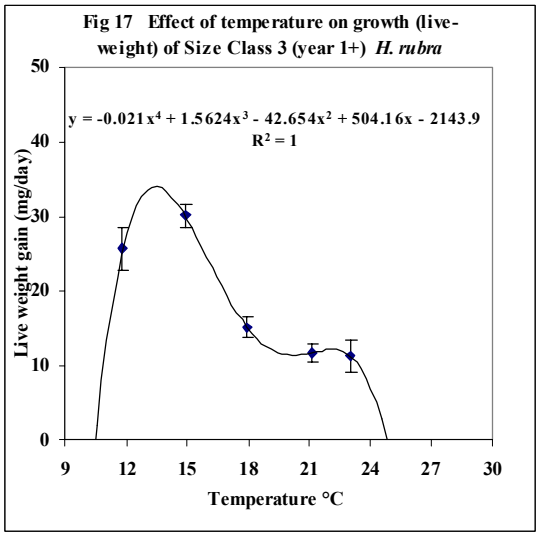
Maximum mean daily shell growth rates of 40 and 22 µm per day for class 3 and 4 juveniles respectively were consistent with a trend of diminishing growth with increasing age and size of juvenile *H. rubra* when reared under conditions provided in this experiment. The same trend is evident in live-weight growth rate data presented in Figures 17 & 18. As with shell growth, 4th order regression equations for class 3 and 4 juveniles were again both accompanied by a high correlation co-efficient value of 1.0.











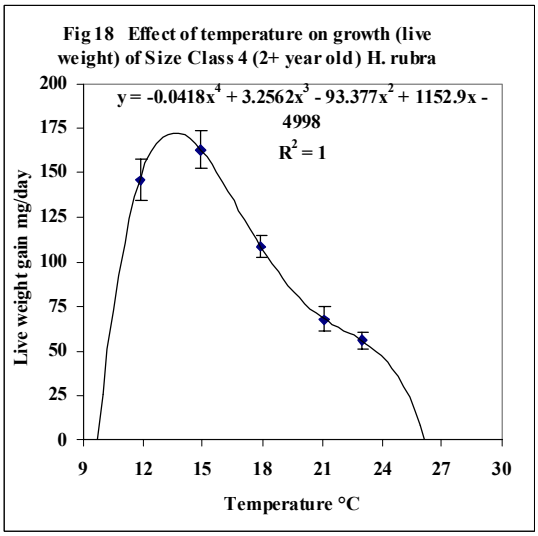


Figure 13-18. Effect of temperature on growth of *H. rubra*.

4.8.4.3. Effect of temperature on survival

Size class 1 and 2 juveniles did not sustain any mortality during the 34 days of the experiment (Figure 19). In stark contrast, size class 3 juveniles sustained high losses of 20 and 25% at rearing temperatures of 21 and 23ºC respectively. Size class 4 juveniles also suffered high losses of 25% at 21ºC and catastrophic losses of 83% at 23ºC. These mortality results for the four size classes of juvenile *H. rubra* thus followed the same general trend with increasing temperature as growth rate.

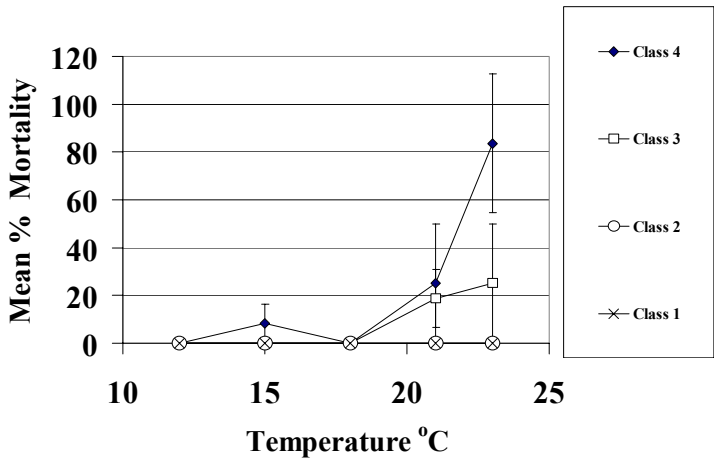


Figure 19. Mortalities over the 4 size / age classes (bars = s.e.).

4.8.4.4. Experiment 2: Effect of temperature on food consumption

Net relative feeding rate data for size class 2 juveniles held at the five temperature treatments are presented in Figure 20. Food consumption increased progressively with temperature from 12.0 up to 21.0ºC but diminished at the highest test temperature of 23.0ºC. A fourth order polynomial fitted to these data predicted zero food consumption at temperatures above 25ºC and below about 10ºC.

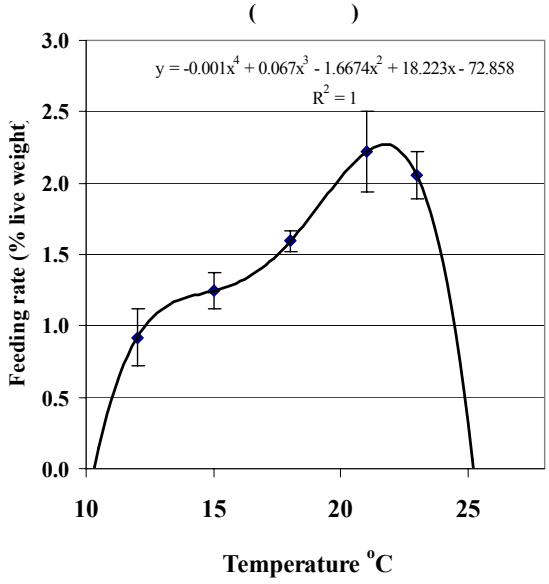


Figure 20. Effect of temperature on relative feeding rate (bars = s.d.).

4.8.5. Discussion

As indicated in Figure 21, a second order regression equation characterised by a correlation coefficient (r^2) value of 0.9752 adequately describes pooled growth data for 13 batches of juvenile *H. rubra* reared under ambient conditions in outdoor diatom plate and shallow flow through raceways at the Tomaree Headland research facility between July 1998 and June 2001. An estimation of mean daily growth rates for juvenile up to 70 mm in shell length is provided by the slope of the above regression, that in turn, is defined by the linear equation depicted in Figure 22. Peak growth rates achieved by each of the four size classes in Experiment 1 have been added to Figure 22 as a guide to their respective performances under static water conditions provided. These comparative data together with comparative survival data for the four size classes, provided in Figure 19, show that rearing conditions provided in Experiment 1 had no detrimental effect on growth and survival of small (3-7 mm) size class 1 juveniles. Indeed the peak mean growth rate of 69 µm/day, estimated to occur at a temperature of 18.4ºC, was well above the average achieved by raceway-reared counterparts. By contrast, progressively more pronounced retardation of growth and survival were exhibited by larger older juveniles culminating in the case of size class 4 juveniles in drastic growth reduction (22 cf 68 µm/day) and catastrophic (82%) mortality.

Key water quality parameters measured at most unfavourable times immediately preceding water changes remained inside limits generally recognised as conducive to good health, vigour and growth of temperate abalone. Dissolved oxygen was maintained above 100% saturation for all temperature treatments and replicates. Mean pH ranged from 7.94 to 8.07 across all treatments while extremes across all replicates ranged from 7.72 to 8.13. These compared with chronic pH thresholds of $\leq$7.93 or $\geq$8.46 reported by Harris *et al*. (1998) to cause a 5% growth reduction (EC_5) in juvenile *H. rubra* of 22.9 ± 2.9 mm SL (mean ± s.e.). Some question however surrounds the significance of this particular EC_5 for FAN in that specific shell length growth rate of control stock, maintained in flowing seawater at a mean temperature of 19.0 ± 0.0ºC, was only 0.15 ± 0.02% per day. This equates to 34.5 µm/day, which is substantially lower than an average rate of 62.5 µm/day exhibited by equivalent size juveniles in flow-through raceway culture and also below a rate of about 45 µm/day, achieved by comparable size class 3 juveniles in Experiment 1.

During Experiment 1, mean ± s.e free ammonium nitrogen (FAN) concentrations immediately prior to water changes across all treatments were maintained within the range 0.0024 ± 0.0004 mg/L at 12ºC rising to 0.0067 ± 0.0013 mg/L at 21ºC. This trend would almost certainly have continued through to the highest test temperature of 23ºC, had all four size classes been retained in a common seawater bath. Indeed the size class 4 juveniles at 23ºC were deployed to their own separate water baths to counteract unacceptably high FAN concentrations in the lead up to Experiment 1.

In overview, FAN concentrations in Experiment 1 were one sixth or less chronic FAN levels of 0.041 mg/L determined by Harris *et al*. (1999) as causing a 5% growth reduction (EC_5) in greenlip abalone *H. laevigata* (mean ± s.e. shell length 31.8 ± 0.1 mm) but above a much lower EC_5 rate of 0.004 mg/L reported for 30-50 mm *H. rubra* juveniles recently reported by Huchette *et al.* (2003). As illustrated by data presented in Figure 22, the highest growth rate of equivalent size class 3 and 4 juveniles in Experiment 1 was only 40 and 22 µm/day respectively. This constituted growth rate reductions of about 40% and 72% when compared to raceway-reared counterparts.

It would thus appear that the threshold of tolerance of *H. rubra* to FAN is either much lower than even that reported by Huchette *et al.* (2003), or that other unspecified water quality factors such as reduced pH or elevated bacterial numbers have confounded results in the current study. What is unequivocal is that water quality thresholds for optimum growth and survival decline profoundly with increasing age and size in juvenile *H. rubra*. Under the particular set of rearing conditions provided in Experiment 1, apparent optimum growth temperatures for size class 1 to 4 juvenile *H. rubra* followed a progressive decline as illustrated in Figure 23. While best fit regression equations fitted to temperature growth data of size class 1 and 2 juveniles were simple and symmetrical

(Figure 13 & 14), those fitted to data of size class 3 and 4 juveniles (Figures 15 to 18) were more complex and asymmetrical.

These results raise the possibility that one or a combination of deleterious water quality factors correlated to temperature exerted a downward bias on optimum rearing temperatures for these two size classes. This possibility is supported by the findings of Gilroy & Edwards (1998) that the preferred temperature of large (mean shell length ± s.d., 74.5 ± 11.2 mm) juvenile *H. rubra* collected in winter and spring on the north and northeast coasts of Tasmania, was 16.9ºC. This is very similar to optimum temperature determined for size class 2 juveniles in the current study.

Findings of the present study for *H. rubra* were similar to those of Uki *et al*. (1981) for four size/age classes of juvenile *H. discus hannai* that ranged from early post-larvae to advanced juveniles. They were also similar to those of Leighton (1974) for three abalone species from southern California, optimum temperatures of which all exhibited a strong downward trend with increasing age and size and even within the brief larval and early post-larval development phases. In the most extreme case of *H. rufescens,* optimum temperature fell from 22°C for 2-day-old larvae to 17°C for 24-day-old post-larvae, i.e. a downward shift of 5°C in only 3 weeks.

Effects of temperature and progressive size and age on survival over the term of Experiment 1 followed the same general trend as growth, with size classes 1 and 2 suffering no mortality even at the highest test temperature of 23ºC. In contrast, size class 3 juveniles sustained high losses of 20% and 25% at rearing temperatures of 21ºC and 23ºC respectively. Size class 4 juveniles also suffered high losses of 25% at 21ºC and catastrophic losses of 83% at 23ºC. These results were consistent with losses of all four size class juveniles experienced during the preliminary phase of Experiment 1 whilst attempting to acclimate them at the rate of +1ºC/day to an intended top test temperature of 27ºC.

These results and observations are consistent with upper thermal tolerance limits identified in an earlier experiment (Section 4.7) that investigated the effects of temperature on settlement and metamorphosis and subsequent post-larval growth in *H. rubra*. As previously discussed, such thermal responses pose serious risks to abalone reared in shallow raceways of the type used by many abalone farms in southern Australian. These farms operate under ambient conditions at sites subject to summer source water temperatures of at least 20°C, and summer air temperatures in the range 30 to 40°C. High temperature stress induced disease, especially *Vibriosis*, has been reported in farmed *H. rubra* in Tasmania, (Handlinger, pers. comm.) and in other farmed species such as *Haliotis laevigata* in South Australia, (Reuter & McOrist 1999) and *H. rufescens* in California (Elston & Lockwood 1983). Likewise, high (above 20°C) temperature stress has been shown to induced the intracellular protozoan disease *Perkinsus* in *H. rubra* and responsible for catastrophic losses of wild stocks in higher temperature regions of *H. rubra's* distribution within both NSW (Dr Craig Hayward, pers.com 2003) and South Australia (Goggin and Lester, 1995).

Table 3. Summary of pH data for Experiment 1.

	12° C	15° C	18° C	21° C	23° C (Class 1-3)	23° C (Class -4)
Overall Mean ± s.e.	7.98±0.01	7.98±0.01	7.96±0.01	8.07±0.01	7.94±0.06	7.99±0.02
Range (all replicates)	7.72–8.13	7.75–0.13	7.82–0.09	7.76–8.09	7.82–8.24	7.73–8.19

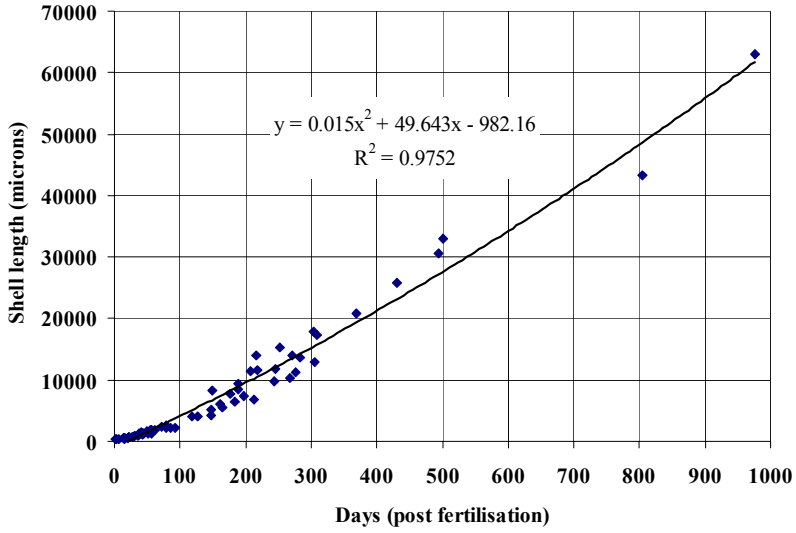


Figure 21. Juvenile *H. rubra* growth rates at the Tomaree Head facility. Data represents 13 different batches reared between July 1998 and June 2001.

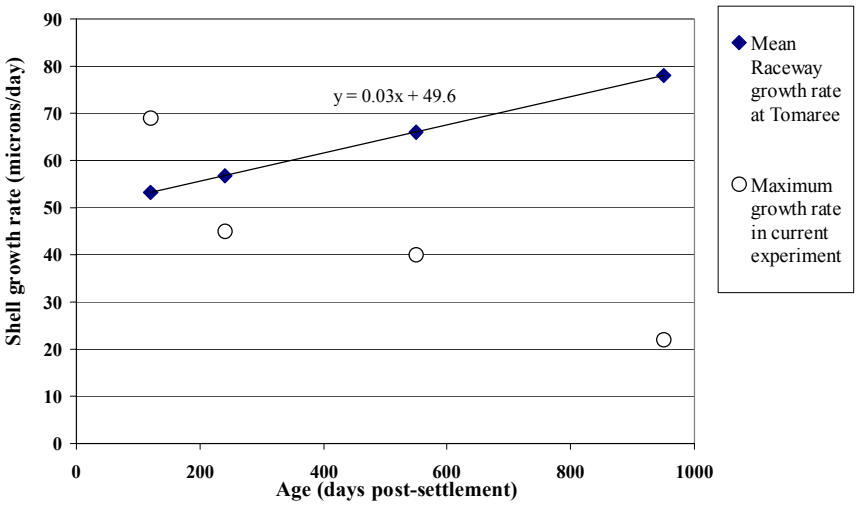


Figure 22. Comparison of maximum growth rate of juvenile *H. rubra* in the current laboratory experiment and in raceways at the Tomaree facility (open circles from left to right are size classes 1 to 4 respectively).

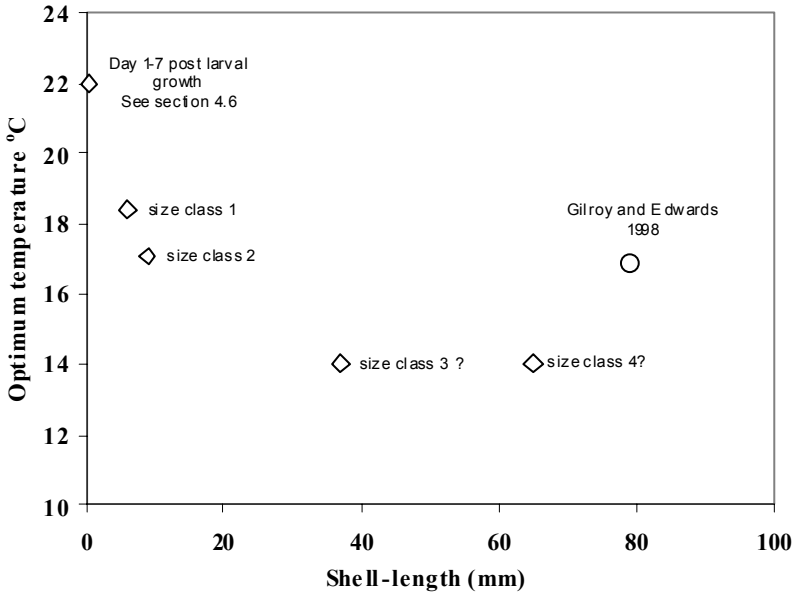


Figure 23. Variation in optimum growth temperature of juvenile *H. rubra* with size/age.

5. INVESTIGATION OF OPTIMAL CONDITIONS FOR TRANSPORTING JUVENILE *H. RUBRA*

M.P. Heasman, N. Savva, C. Brand & J. Diemar

5.1. Introduction and aims

The need to transport and deploy batches of up to several hundred thousand 6 to 9 month old button size (5 to 15 mm) juvenile *H. rubra* over distances up to 500 km from a hatchery at Port Stephens prompted investigation of suitable methods of transportation. The aim of the following investigations was to determine whether stock should be transported wet or damp, what was the optimum temperature for transportation and the maximum duration over which juvenile *H. rubra* can be safely transported without compromising post release vigour and survival.

5.2. Methods

The effect of storage temperature on the subsequent survival of juvenile abalone was investigated using a thermal gradient block (TGB) (Leighton 1972; Thomas *et al*. 1963) constructed from a solid 700 x 300 x 100 mm block of marine grade aluminium alloy (Tomago Aluminium P/L). The TGB accommodated a maximum of 78 (120 mL) plastic screw top vials that fitted snugly within cylindrical wells machined into the block. The 78 wells were arranged as an array of 13 equally spaced parallel rows of 6, oriented perpendicular to the long axis of the block. One end of the block comprised an integrated heated water manifold and the opposite, a chilled water manifold. The 13 rows of wells thus spanned the full temperature gradient between hot and cold water manifolds thereby enabling up to 13 different temperatures (with 6 replicates per temperature) to be investigated at one time.

Hatchery produced juvenile *H. rubra* were 266 days old and averaged about 14mm (range 9-18 mm) shell length. These juveniles were removed from nursery raceway tanks after anaesthesia with benzocaine (applied at 0.5 mL/L for 15 minutes at 19-20ºC). They were then thoroughly rinsed in flowing ambient seawater for a further 60 minutes before being transferred in groups of 10 into 78 polyester 500 μm mesh envelopes, (75x40 mm). The envelopes of abalone were held for a further 30 minutes in vigorously aerated seawater at 18ºC before being transferred to the TGB.

The TGB was set up 12 h before commencing the experiment to ensure stabilization of the temperature gradient and for equilibration of temperatures within each row of 6 wells. The screw top vials were placed in the wells without seawater and the air within displaced with oxygen before and immediately following insertion of abalone contained within mesh envelopes. For wet storage treatments, juveniles also in envelopes were maintained in about 60mL of seawater within the vials. Approximately 1L of medical grade oxygen was bubbled through each vial over a period of 20 seconds using a pasteur pipette inserted through a 4 mm hole in the lid. The hole was then sealed with a plug of “blue-tac” to ensure oxygen did not escape during the course of the experiment. Temperatures of seawater in each vial were recorded twice daily using a digital stab probe thermometer inserted through the same hole.

5.2.1. Experimental design

The TGB was used to accommodate two companion experiments simultaneously, one to evaluate damp storage in an atmosphere of oxygen and the other, wet (in seawater) storage under an atmosphere of oxygen. The damp storage treatment was allocated to seven alternate rows of the TGB (rows 1, 3, 5, 7, 9, 11 and 13). The wet storage treatment was allocated to remaining six alternate rows of wells (rows 2, 4, 6, 8 10 and 12). For both experiments, 3 replicates within each row of 6 were selected at random and sampled after 24 h of storage and the remainder after 48 h. Abalone used in the control treatment were subjected to the same handling and containment procedures as their counterparts, except that they were continuously maintained in flowing ambient seawater.

In the case of wet storage treatments, dissolved oxygen, pH and ammonium levels were recorded at the start and finish of the storage period. Dissolved oxygen and pH were measured using a water quality meter (TPS Australia P/L). Ammonium levels were assessed using colorimetric test kit, (Merck 1.08024.0001).

At the time of sampling, replicate lots of abalone retained in the envelopes were transferred to larger 250mL plastic jars supplied with continuously flowing ambient seawater. As with controls, these stock were monitored daily for a full week to detect and remove dead animals. Minimum and maximum ambient water temperatures were recorded daily.

5.3. Results

The temperature gradient for the 6 replicates within each of the 13 rows of the TGB (Figure 1) was linear. Mean ambient seawater temperature during days 1 and 2 of storage and for the seven day recovery period after storage varied from 19.0 to 20.2ºC. The mean ±S.E survival rate of 95 ± 3% was achieved by the control stock continuously maintained in ambient flowing seawater.

5.3.1. Damp storage

Best-fit symmetrical second order polynomial equations (Figure 2) were found to adequately describe the relationship between storage temperature and survival rate after both 24 and 48 h of damp storage (R^2 = 0.7366 and 0.9933, respectively). Survival after 24 h of damp storage remained within a designated acceptable range above 80% over a wide temperature range of 8 to 18ºC. Extension of storage time to 48 h narrowed this range to 13 to 17ºC with a peak survival of 95% (equal to that of the controls) occurring at 14ºC. Relatively poor survival of *H. rubra* juveniles stored at 15ºC for 24 h was probably an aberration, given that survival was higher for counterparts stored at the same temperature for 48 h.

5.3.2. Wet storage

A best-fit symmetrical second order polynomial equation (Figure 3) was found to adequately describe (R^2 = 0.8122) the relationship between temperature and survival rate after 24 h of wet storage. Acceptably high survival rates above 80% were achieved over a narrower temperature range of 10 to 16ºC than in the case of damp storage. Highest survival rates of *H. rubra* juveniles stored wet for 24 h occurred at 13.0ºC. However when storage time was extended to 48 h, the relationship between survival and temperature became asymmetrical. Highest survival rates of 70 to 79% were achieved at 10 and 12ºC but a steady decline to 0% survival occurred with increasing storage temperature over the range 13 to 20ºC.

Total ammonium nitrogen, dissolved oxygen and pH data for wet storage treatments are presented in Figures 4, 5 & 6 respectively. Prior to storage, total ammonium concentrations were less than the minimum detectable level of 0.1 mg/L. Dissolved oxygen was at super saturation levels of about

200%, while pH was 8.1. After 24 h of storage, total ammonium nitrogen concentrations remained at or slightly above the minimum detectable level of 0.1 mg/L at temperatures up to 16ºC and increased marginally to concentrations in the range 0.3 to 0.6 mg/L at the highest experimental temperatures of 18 and 21ºC. However with extended storage to 48 h, total ammonium levels increased moderately from about 0.1 to 0.5 mg/L with increasing temperature over the range 10 to 16ºC and then steeply to levels in the range 1.5 to 2.0 mg/L at the highest test temperatures of temperatures of 18 and 21ºC. At all test temperature storage time combinations pH fell to the low narrow range of 6.6 to 7.1 at which 0.2-0.08% of total ammonium nitrogen exists as toxic free ammonium nitrogen (FAN) (Liao & Mayo 1972). Accordingly, FAN levels remained below 0.002 mg/L, a harmless level being half that of the EC_5, the chronic exposure concentration causing a 5% reduction in growth (Huchette *et al.* 2003).

Dissolved oxygen levels after 24 h storage declined progressively with increasing temperature from about 95% saturation at 10ºC, down to about 30% at 21ºC. Corresponding dissolved oxygen levels after 48 h of storage were much lower ranging from about 50% saturation at 10ºC, to below 10% at 21ºC. These trends (Figure 5) of decreasing residual oxygen saturation with rising temperature after 24 and 48 h of storage, were adequately described by best fit power equations (R^2 = 0.8024 and 0.8664 respectively). pH values after both 24 and 48 h storage exhibited very similar trends declining progressively with increasing temperature from 7.0 at 10ºC to 6.6 at 21ºC. These trends were well described by best-fit second order polynomial equations (R^2 = 0.9881 and 0.9754, respectively).

5.4. Discussion

Juvenile *H. rubra* previously held at ambient temperatures of 18 to 22ºC survived at acceptable rates above 80% for up to 48 h when stored damp in an atmosphere of pure oxygen at temperatures of between 13 and 16ºC. The best 48 hour survival rate of 95% was achieved by juveniles stored damp at 14ºC. Generally poorer rates of survival exhibited by counterparts stored wet are most probably attributable to declining physiochemical conditions, especially DO concentrations that rapidly fell below ≥95% saturation, considered essential to continued good health and vigour of juvenile abalone (Fleming 2000). Indeed, DO concentrations as low as about 30% saturation developed within 24 h and as low as 10% within 48 h. Best water quality conditions after wet storage were recorded for the coolest temperatures. The decrease in dissolved oxygen levels and pH along with the increase in ammonium levels with increased temperature (and to a lesser extent storage time) were expected. Low DO and pH explain the decreased survival after storage at higher temperatures. The extent of the pH decline from 8.1 to 7.0 and less was surprising. Presumably the build-up of carbonic acid from carbon dioxide was sufficient to overcome the buffering capacity of the small volume of seawater. This in turn suggests that use of physiologically benign buffers such as TRIS or sodium bicarbonate could be of significant benefit to wet transportation of juveniles. Wet storage could also probably be improved by providing a greater air space charged with oxygen, and acclimation to cooler temperatures.

Damp storage during transport is preferable over wet storage due to the better subsequent survival and avoids the inconvenience, cost and hazards associated with transporting significant volumes of seawater. Accordingly, future studies on transport should concentrate on improvements to damp methods. The mortalities recorded amongst the controls (although low) suggest that some of the handling procedures were injurious. Placement of the juveniles into the envelopes was a protracted process and may explain these mortalities. More recent methods we have developed to transport large numbers of juveniles allow the abalone to attach firmly to a substrate prior to transport. When using these methods, mortalities have been routinely negligible. Following this experiment transport has been conducted in a damp environment at 14-15ºC over storage periods up to 12 h (Section 5.2). As we sometimes have cause to move abalone from ambient temperatures of up to 25ºC, further investigation of the following are warranted:

1. The relationship between the initial ambient temperature and the ideal storage/transport temperature.
2. Acclimation of abalone to lower temperatures prior to storage.
3. Attachment to a firm substrate prior to storage.

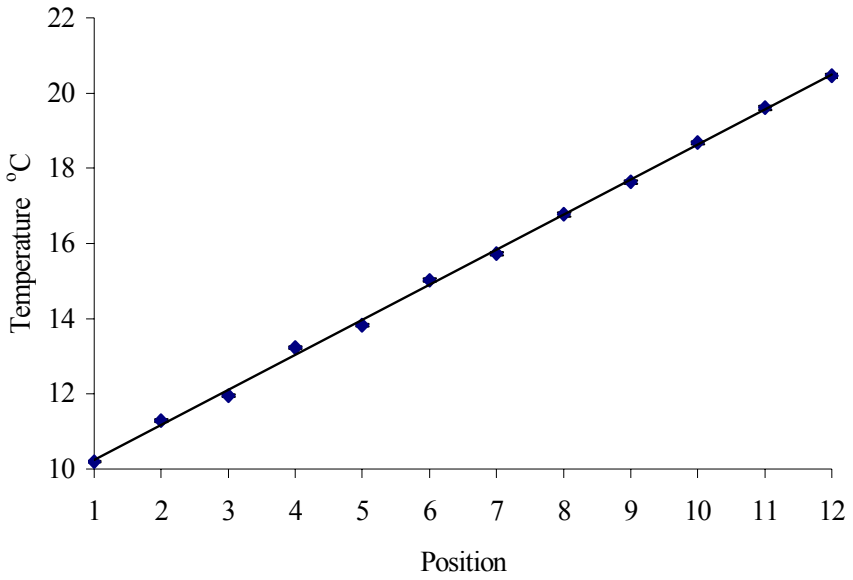


Figure 1. Temperature along block (mean± s.e.).

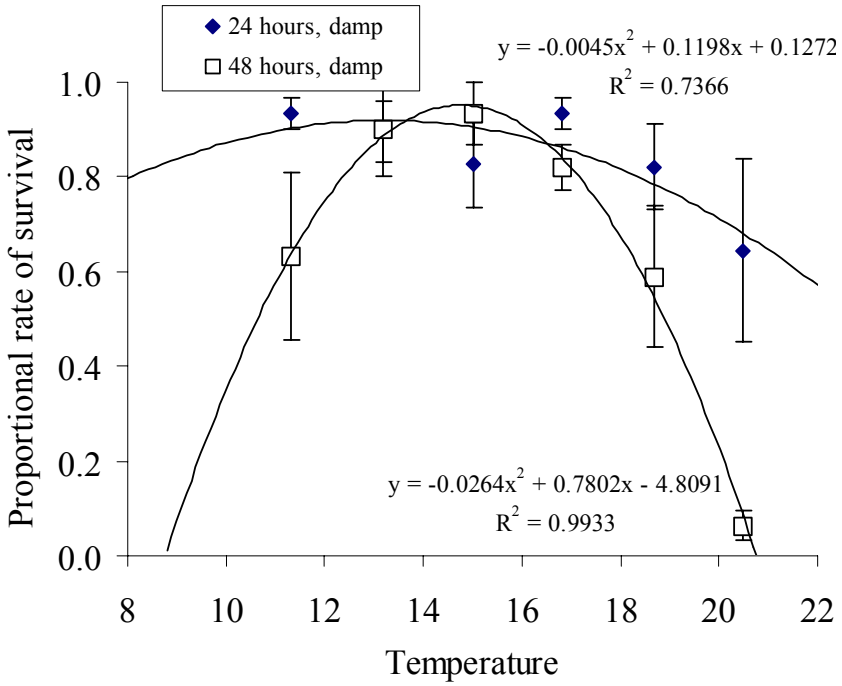


Figure 2. Survival after damp storage (mean ± s.e.).

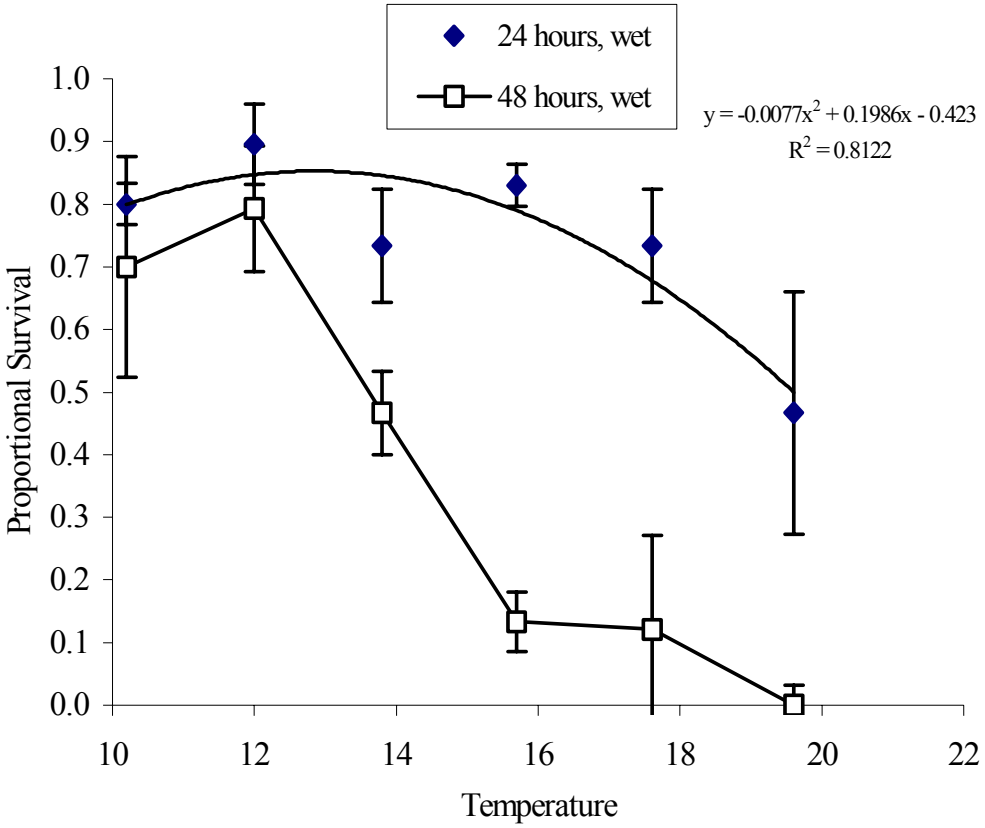


Figure 3. Survival after wet storage (mean ± s.e.).

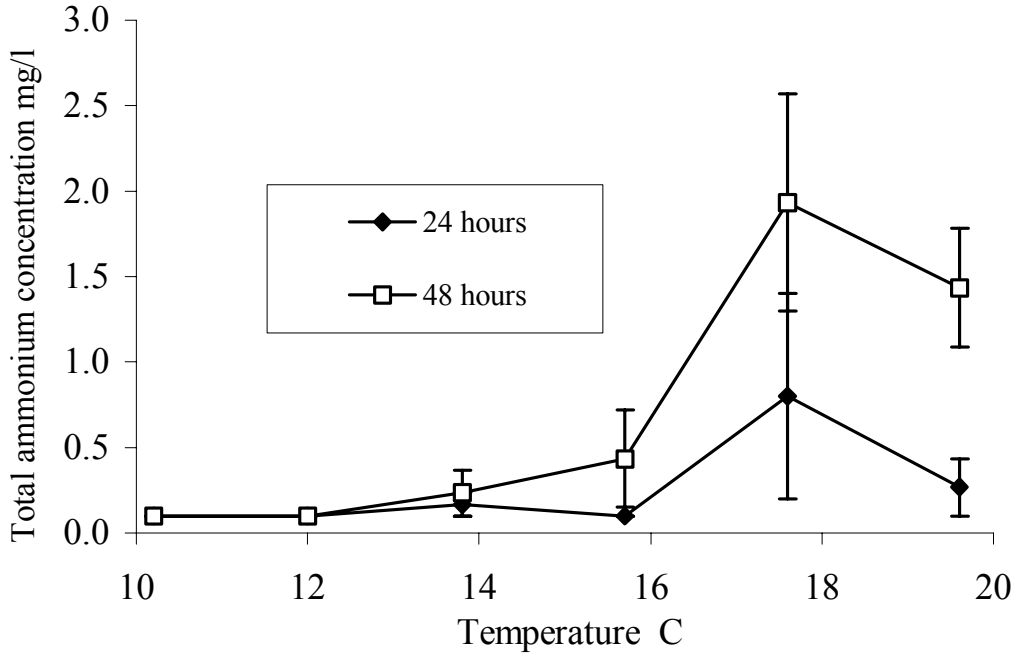


Figure 4. Ammonium concentration after wet storage (mean ± s.e.).

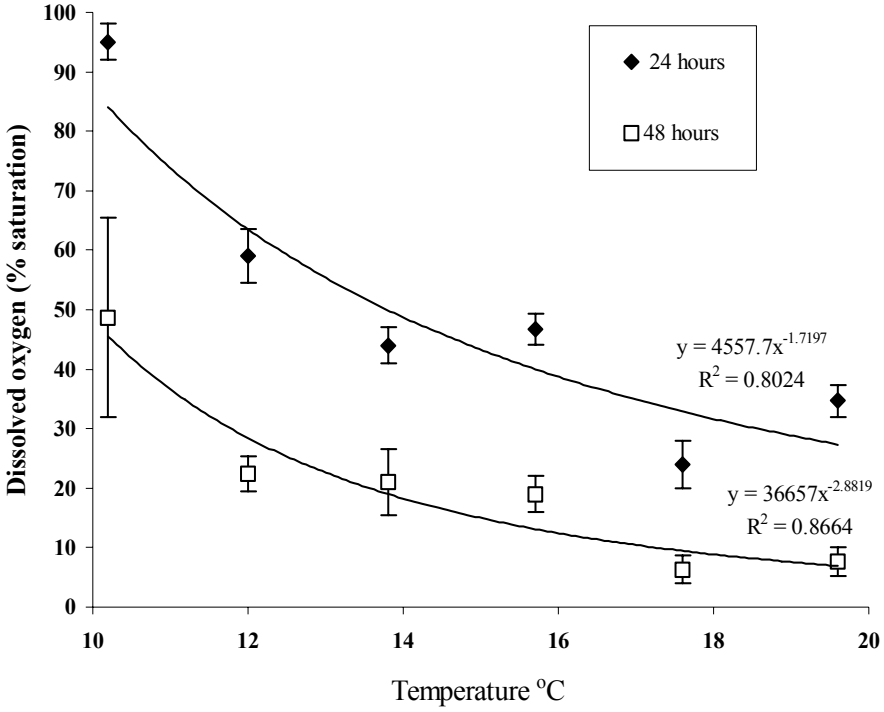


Figure 5. Dissolved oxygen after wet storage (mean ± s.e.).

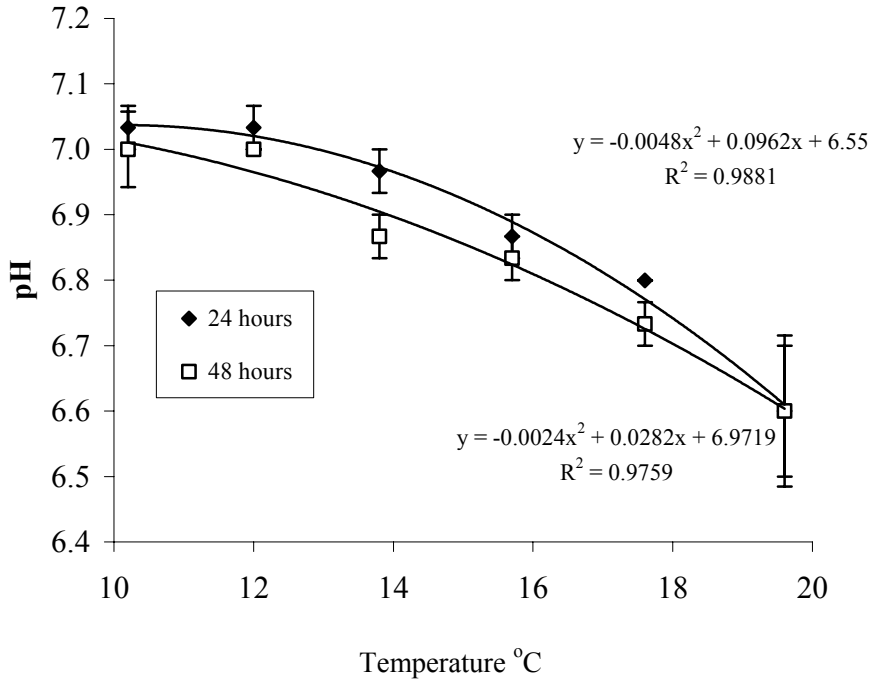


Figure 6. pH after wet storage (mean ± s.e.).

6. FACTORS AFFECTING THE SETTLEMENT OF H. RUBRA IN THE FIELD

R.C. Chick, D.G. Worthington, P.T. Gibson, M.P. Heasman, N. Savva & C. Brand

6.1. Introduction

Literature investigating the settlement of abalone larvae has primarily focused on the identification of settlement cues, the magnitude of their effect and techniques to maximise settlement and metamorphosis within a controlled, hatchery-like environment. Studies have emphasised the application to aquaculture industries, with some studies outlining the potential role some of these factors play in determining patterns of wild recruitment (see reviews McShane 1996; Roberts 2001). Comparatively little is known about the response of abalone larvae at settlement to the variable conditions they are exposed in the field (also see Naylor & McShane 1997; 2001). Similarly, little is known about settlement responses of abalone larvae following exposure to the range of conditions imposed during handling, transport and release activities associated with enhancement operations.

The release of hatchery-reared larvae to investigate their potential to enhance local wild populations requires their exposure to a range of handling, transport and release conditions prior to settlement on the reef. These can include the addition of settlement inducing chemicals (e.g. gamma amino butyric acid [GABA]), prolonged or abrupt changes in temperature during transport and release in the field, and rates of water movement following release. Little is known about the ability of abalone larvae to withstand these conditions and successfully settle and metamorphose on the reef. If the larvae are exposed to conditions that compromise their ability to settle and metamorphose, this could seriously compromise the success of enhancement projects. As a consequence, conditions that impair settlement and metamorphosis need to be identified, and their effects minimised. Alternatively, any conditions that increase the settlement and metamorphosis of larvae could be adopted to increase the success of an enhancement project.

6.2. Aims

The aim of this study was to investigate the effects of several conditions imposed during handling, transportation and release on the settlement and metamorphosis of hatchery-reared *H. rubra* larvae. First, we describe a miniature down-welling system designed to allow the exposure of small batches of abalone larvae to replicated treatments of different experimental conditions. Next, we use this down-weller to compare rates of settlement and metamorphosis on crustose coralline algae (CCA) coated rocks and conditioned microscope slides. In subsequent experiments, settlement to the conditioned microscope slide was compared among a range of experimental treatments. These treatments included exposure to different concentrations of the settlement inducing chemical GABA, refrigeration of the larvae (a common, simple and effective storage method), an abrupt temperature increase prior to settlement (common during the storage, transportation and release of larvae from the hatchery to field sites) and water flow across the settlement substrate (to simulate water movement in the field, common during the release of larvae). A series of experiments were designed to investigate the effects of these factors and their interactions on the rate and absolute number of abalone larvae that settle and metamorphose. Several experiments were also repeated with different batches of larvae to investigate variation in the effects of different treatments among batches.

6.3. Methods

6.3.1. Miniature down-welling system

A method of assessing the rate and absolute number of abalone larvae that settle and metamorphose in response to a number of replicated experimental treatments was required. To achieve this, a miniature down-welling system was designed (Plate 1). The design chosen was modular, with each module or bath capable of holding 6 rows of 4 down-welling units or replicates. Each bath had dimensions of 360x250x150 mm, holding approximately 12 L of water, with an overflow outlet set approximately 10 mm below the rim of the bath. Along both long sides of the bath were six equally spaced grooves. Each groove held an end of a 4 mm diameter rod, which supported 4 replicate down-wellers. Each replicate down-weller consisted of an inverted 120 mL, screw top plastic jar (~40 x 100 mm) with the base cut off and a 35 mm hole cut out of the lid. The inside of each lid was lined with a sheet of 80μm polyester mesh, which was secured by screwing the lid back onto the tube. In addition, two 5mm holes were drilled into opposite sides of the open end of the tube, approximately 7 mm from the top lip. These holes allowed the tube to be threaded onto the rod and suspended in the water with the open end (and the holes) above the water level.

Seawater inflow to each replicate in the bath came via a manifold comprising of a central, 20mm diameter PVC pipe with six pairs of 3 mm taps spaced equally along opposite sides of the pipe concurrent with the 6 rows of down-wellers in the bath. Secured to each pair of taps was a loop of 3 mm plastic tubing that was secured to the top of the lid on the bath and ran parallel with the rows of down-wellers. The 6 loops each supplied seawater to the groups of 4 down-wellers via four "T" fittings, the stems of which were pierced through the lid of the bath at points directly above each down-weller. The continuous connection of pipe and tubing provided an even pressure of water to each down-weller in a bath. In addition, a 3 mm tap was fitted to the central pipe beyond the last of the 6 loops that, when opened, allowed air in the system to be bled. Seawater supplied to this system was from a reservoir containing 5 μm filtered, UV treated seawater cooled to a temperature of 18^{o}C.

Three modules or baths were used to allow 72 replicated, experimental down-wellers. Where experimental designs required more than 72 replicates, treatments with short settlement times (i.e. 30 or 60 seconds) were completed first, and replaced with clean down-wellers for additional replicates. Due to constraints of this down-weller system, replicates were grouped into sets of four to allow the introduction of larvae and subsequent sampling, whilst not interfering with replicates from other groups. Groups of 4 replicates for each treatment in each experiment were randomly assigned to one of the 3 baths (unless otherwise described in specific experiments below).

Plate 1. One module of the miniature down-welling system showing 6 rows of down-welling units each with its own supply of water.

6.3.2. Standard experimental protocol

Competent larvae were sourced from captive conditioned broodstock and reared through to settlement (7-9 days post-fertilization) in accordance with methods described in Section 4. For each experimental treatment, separate groups of approximately 20,000 larvae were held in 1 L containers and exposed to the required level of the treatment. These groups of larvae were handled identically prior to and after their exposure to the treatment. Once treated, the larvae were mixed thoroughly but gently in the container and a minimum of four, 1.00 mL sub-samples were taken. The number of larvae in each sub-sample was counted, and the mean used to estimate the volume of the sample required to be taken from the larval suspension to yield 200 individuals. Aliquots of the required volume were then removed from the container, using an automatic pipette with an enlarged tip, to minimise any physical damage to the larvae, and placed in the designated replicate down-weller. Prior to larvae being added, the required settlement substrate was fully immersed in each replicate down-weller.

Two types of settlement substrate were used. The first type of substrate consisted of a 75 x 25 mm glass microscope slide, carrying a biofilm of adventitious diatoms. The biofilm on this substrate was created by conditioning the slides in aerated, 10 μm filtered ambient seawater for 4 to 6 weeks prior to the experiment being done. The second type of substrate consisted of 2 small CCA coated rocks, with a combined displacement volume of approximately 70 mL. After larvae were exposed to the settlement substrate for the required period of time, the substrate was removed from the down-weller using fine forceps and preserved in a jar containing 70% alcohol. The remaining contents of that replicate (larvae that did not settle on the substrate), hereafter termed rinse, were

washed out and preserved separately in the same manner as the substrate. The number of larvae or post-larvae in substrate and rinse samples was then counted with the aid of a binocular microscope.

Throughout the series of experiments, larvae were exposed to the settlement substrate for a range of times. Several standard times between 60 sec and 48 hrs were used in all experiments, and all were independent. Short periods of time (30 and 60 seconds) were chosen to determine if larvae could settle quickly, as would be required when released to remain within the release site. Longer periods of time (48 and 96 h) were chosen to investigate settlement and metamorphosis.

6.3.3. Experimental treatments

A series of experiments were designed to investigate the effects of several factors, and their interactions, on the settlement and metamorphosis of larvae (Table 1). The treatments investigated included factors required or imposed during the handling and transport of larvae to field sites (e.g. refrigeration and temperature shock), or imposed after release in the field (e.g. exposure time and water flow). All of the experiments consisted of an orthogonal design investigating the effects of replicated treatments. Each experiment was designed within the logistical constraints of the miniature down-weller system, and using information from previous experiments to determine appropriate levels and replication of treatments. The data analysed consisted of the proportion of larvae on the settlement substrate after the designated exposure time. This data was transformed using the function arcsine-square root, and Cochran's test was used to confirm the homogeneity of variances (Winer 1971). Variance components were calculated to assist in the interpretation of the relative importance of factors and their interactions. Negative variance components were set to zero prior to calculation of their relative size (i.e. percent of total variance for all factors and interactions). Interpretation of the results from this method of calculating variance components does however require caution, particularly for fixed factors (Underwood 1997).

Standard levels of each experimental treatment were used throughout the series of experiments. Following the comparison of conditioned slides with CCA-coated rocks collected from natural reefs, conditioned slides were used in all subsequent experiments. Standard GABA treatments were chosen to compare the commonly used concentration (i.e. 1×10^{-6} M) with two alternatives (0×10^{-6} and 2×10^{-6} M). GABA treatments were achieved by adding the required concentration to the 1 L container of larvae and stirring for 15 min. Following addition of the larvae to the down-weller, water flow to each replicate down-weller was turned off for the first 24 h, but water was allowed to flow through the bath. Each GABA treatment was assigned to a separate bath, to minimise the chances of any contamination among the different treatments.

Two standard rates of water flow were investigated. The first had no water flow through the down-weller (-Flow), whilst the other had a flow of 250 mL per minute (+Flow). These were chosen to investigate the effects of different rates of water flow on the ability of larvae to settle and the rate of settlement, as levels of flow in the field were known to be highly variable. Three standard levels of refrigerated storage were investigated. The first, a control had no refrigerated storage, whilst the other two levels consisted of refrigeration for 24 h and 48 h. These durations were chosen because they reflected the likely times required to transport and deploy larvae from the hatchery to sites in southern NSW. To achieve the treatments, larvae were transferred to a 1 L container of seawater, through which oxygen was bubbled for between 3-5 minutes prior to the container being sealed in a plastic bag and placed in a domestic refrigerator for the required time. Prior to their addition to the down-wellers, larvae that had been stored were acclimated to ambient temperatures over a period of approximately 3 h. Larvae for the treatment control (i.e. without refrigerated storage) were maintained in the conventional larval rearing system described in Section 4 for the same period of time as the refrigerated storage treatment.

Three standard water temperature treatments (i.e. temperature shock) were investigated. The first, a control, maintained larvae at ambient water temperature, whilst the others involved temperature increases of 5^{o}C and 10^{o}C applied over ~30 min followed by their immediate return to the ambient

water temperature of 18°C. These treatments were chosen to simulate the temperature fluctuations either inadvertently imposed during the processes of handling and transportation of larvae or the potential temperature shock experienced at the time of release to field sites at depths of 5-10 m (i.e. often 5°C cooler than surface water). To achieve the required treatments, larvae in three 1 L containers of seawater were transferred to water baths of 18, 25 and 32°C respectively, and held for ~30 min prior to exposure to the settlement substrate in seawater at the original temperature of 18°C.

The use of constant factors was designed to provide a standard set of data to be used to investigate the variation in the patterns of settlement of larvae of the same age, among different batches. This was done using larvae of two ages (7 and 8 days) from three different batches. An age treatment was not included in these analyses as the source of larvae for each age (7 and 8 days) was from the same batch for two of the three batches analysed. The common treatments used were, Substrate [Slides], Flow [-], GABA [1], Refrigeration [0], Temperature shock [0] and Exposure times of 60 sec and 24 hr. Three replicates from each of the experiments 4c, 5d and 10e and 2b, 7c, and 8e were used to investigate variability in the mean number of larvae settling on a standard settlement slide with regard for the factors, batch and exposure time for 7 and 8-day-old larvae respectively.

Table 1. Design of experiments investigating the settlement and metamorphosis of *H. rubra*. Levels for each treatment are shown in brackets and described in the text. Common letters attached to experiment numbers denote the use of the same batch of larvae.

	Expt. No.	Factors [and levels]	Age (days)	Replicates	Exposure time					
					sec.			min.	hr.	
	1a	Substrate [Slides, Rocks]	8	4		60	30	24		96
*	2b	Substrate[Slides,Rocks] Flow [+, -]	8	3	30	60	30	24		
	3b	Substrate [Slides, Rocks] Flow [+, -] Refrigeration [0, 24]	9	3		60	30	24		
*	4c	GABA [0, 1, 2]	7	8		60		24	48	
*	5d	GABA[0,1] Flow [+, -]	7	3	30	60	30	24		
	6d	GABA [0, 1] Flow [+, -] Refrigeration [24]	8	3	30	60	30	24		
*	7c	Refrigeration [0, 24]	8	4		60		24	48	
*	8e	Refrigeration[0,24,48] Flow [+, -]	8	3		60	30	24	48	
	9a	Temperature shock [0, 5, 10]	7	4		60	30	24		96
*	10e	Temperature shock [0 ,5] Flow [+, -]	7	3		60	30	24	48	
	11	Batch [c, d, e]	7	4		60		24		
	12	Batch [b, c, e]	8	4		60		24		

* Experiments used to investigate effects of batch and exposure time to standard settlement substrates on mean settlement rates.

6.4. Results and discussion

There were large and significant differences in the attachment, settlement and metamorphosis of abalone larvae in response to the experimental treatments, which provide important information about appropriate methods of handling and release of larvae in the field. For example, the experimental treatments included several factors required or imposed during the handling and transport of larvae to field sites (e.g. refrigeration and temperature shock), or imposed after release in the field (e.g. exposure time and water flow). Below we describe the effects of each treatment across several experiments and batches, and attempt to generalise about their potential effects on the release of hatchery-reared abalone (in order of the approximate magnitude of their effect).

6.4.1. Effects of settlement substrate

There were large and consistent differences in the settlement of larvae among the two settlement substrates (Figure 1). The rates of settlement on the CCA-coated rocks were generally more than double those on conditioned slides. In Experiment 1, differences in the rate of settlement among substrates changed significantly among the exposure times (Appendix 6, Table A6.1). Despite this, the interaction between substrate and exposure time only explained 3% of the total variation in the rate of settlement, and consistent differences among the substrates explained 68% of the total variation. Similarly, in Experiment 2, differences in the rate of settlement among substrates changed significantly among the exposure times, but consistent differences in the rate of settlement among the substrates explained 56% of the total variation (Figure 2 and Table A6.2). In contrast, in Experiment 3 differences among the substrates in the rate of settlement through time changed with different levels of water flow and refrigeration, but again consistent differences in the rate of settlement among the substrates explained a high proportion (25%) of the total variation (Figure 3 and Table A6.3).

Despite the significant differences in the rate of settlement among the substrates in all three experiments, the general response of larvae to the two substrates was very similar when exposed to a range of other treatments. For example, in experiments 2 and 3 the effects of water flow and refrigeration on differences in settlement among the substrates were dominated by consistent differences in settlement among the substrates. This implies that despite large absolute differences in settlement among the substrates, the general response of larvae to several experimental treatments was relatively similar for both conditioned slides and CCA-coated rocks.

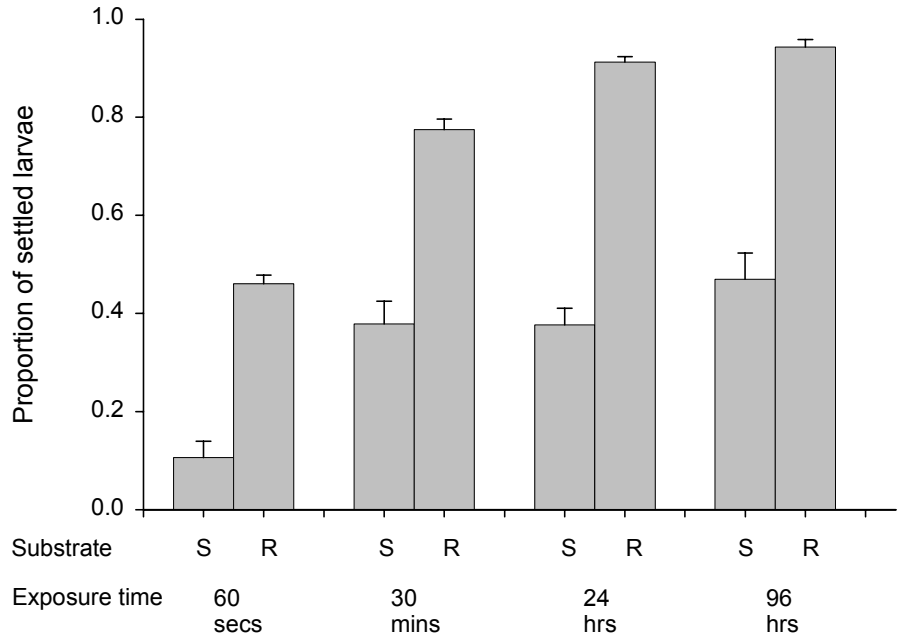


Figure 1. Mean proportion of larvae (± SE) on a standard settlement substrate (S) and CCA rocks R for each of 4 exposure times (n = 4).

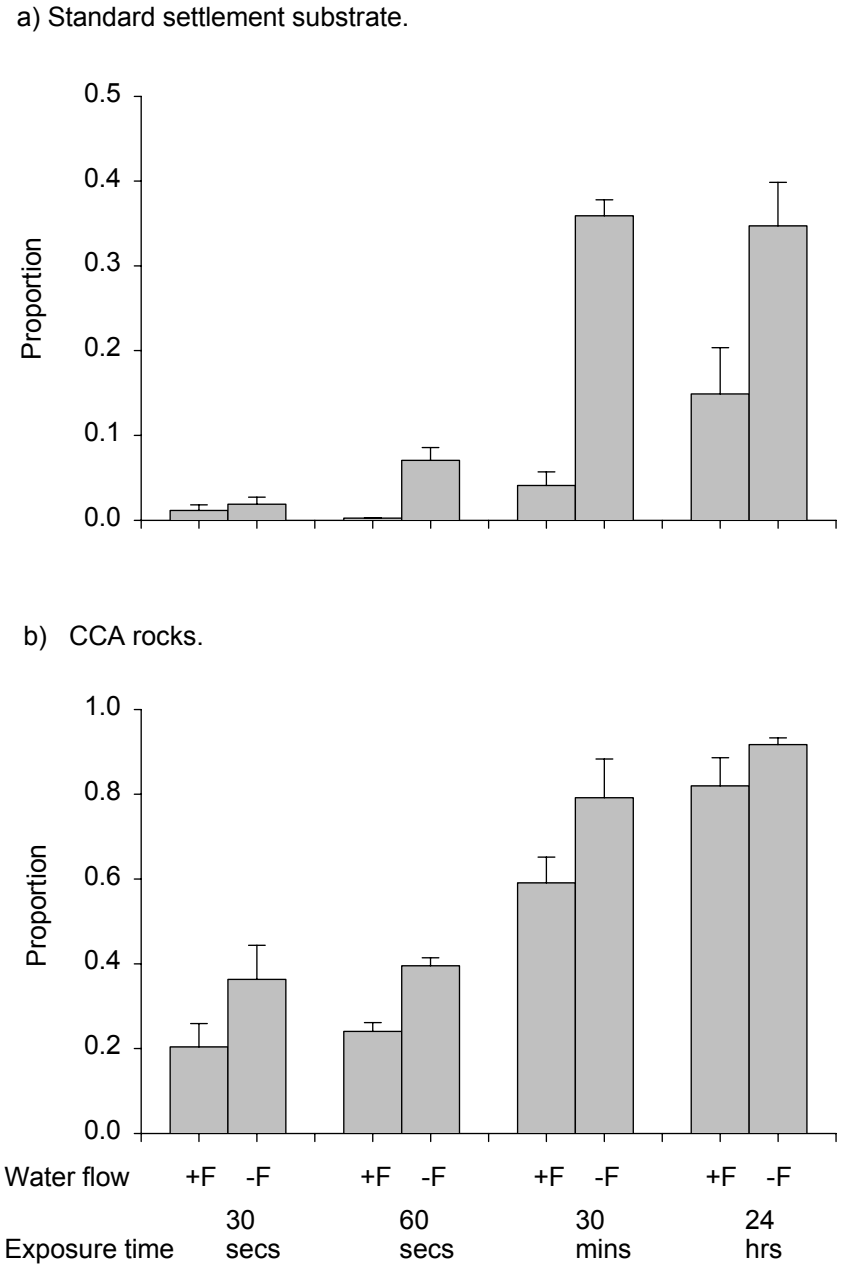


Figure 2. Mean proportion of larvae (± SE) on a standard settlement substrate and CCA rocks with and without water flow (F) for 4 exposure times (n = 3).

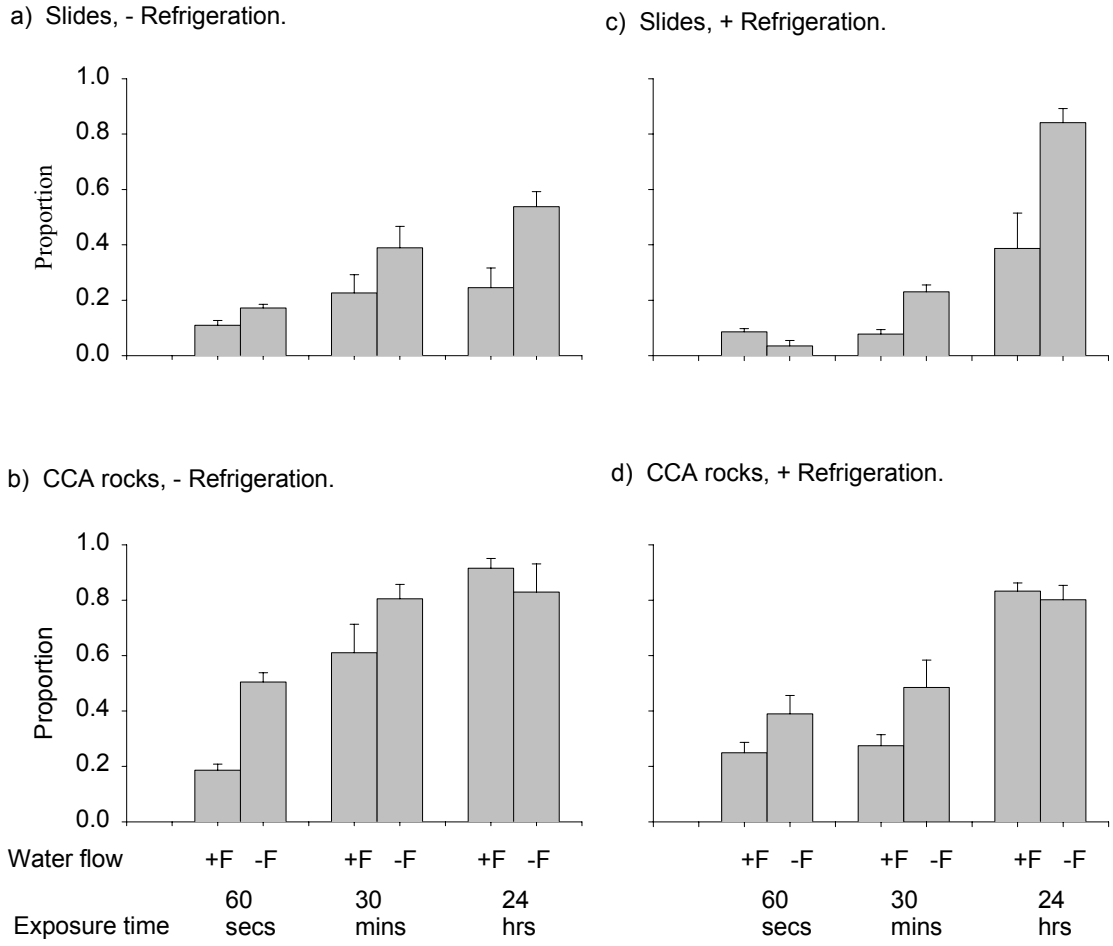


Figure 3. Mean proportion of larvae (± SE) on a standard settlement substrate and CCA rocks with and without water flow (F) and refrigeration for 3 exposure times (n = 3).

6.4.2. Factors related to handling and transport

Different concentrations of GABA resulted in only minor differences in the settlement of larvae to conditioned slides. In Experiment 4 the different concentrations of GABA significantly changed the rate of settlement of larvae through time, but over 90% of the variation in settlement was related to differences through time that were consistent across the different concentrations (Figure 4 and Table A6.4). Similarly, in Experiment 5 the effects of GABA on the rate of settlement of larvae through time differed significantly with water flow, but most variation in settlement was not related to the effects of GABA (Figure 5 and Table A6.5). In Experiment 6, GABA did not significantly change the rate of settlement of the 8-day-old larvae (Figure 6 and Table A6.6).

Despite the significant differences in the rate of settlement among the different concentrations of GABA, the general effects of GABA were small compared to the other treatments investigated. Where significant differences occurred, higher rates of settlement over short time periods (i.e. 30-60 sec) were associated with higher concentrations of GABA in each experiment. Further, larvae appeared more vulnerable to the effects of water flow, particularly over longer time periods (i.e. 24 hr) in the absence of GABA. This implies that despite variation in the effects of GABA among the other experimental treatments, most of the significant increases in settlement were related to greater concentrations of GABA. Further research is needed to investigate differences in the response of larvae to GABA at different ages, among batches and when exposed to CCA-coated rocks.

The effect of the refrigerated storage of larvae on their subsequent ability to settle was variable across three experiments. In Experiments 3, 7 and 8, there were significant differences in the settlement of larvae related to refrigeration that changed through time (Table A6.7 and A6.8), and in Experiment 3 these changes also differed among substrates (Table A6.3). Despite that, in Experiments 7 and 8 increasing the time larvae were refrigerated resulted in significantly higher rates of settlement over short time periods (Figures 7 & 8), whilst this did not occur in Experiment 3 (Figure 3).

There was no significant difference in the settlement of larvae related to the temperature shocks investigated (Table A6.9 and A6.10). These included increases in water temperature of up to 10^{o}C over ~30 min, followed by immediate exposure to the settlement substrate in seawater of the original temperature. Rates of settlement in some treatment combinations were, in fact, higher following a temperature shock (Figures 9 & 10).

Prior to the release of larvae at field sites, it is unavoidable that they will be exposed to a variety of treatments required for handling and transport. With a single hatchery at Port Stephens in the central north of NSW and release sites as far as 500 km south, this situation clearly required treatments of storage and transport, as well as routine handling methods to deploy larvae at various field sites. The techniques for storage and transport developed in this project were designed for the times and distances required for areas of release in southern NSW. Similarly, the experimental treatments investigating the effects of temperature shock were also designed to reflect realistic changes encountered during field releases, particularly during summer. The relatively minor effect of these treatments on the successful settlement of larvae was surprising, particularly the increased settlement in some treatment combinations. We had expected decreased rates of settlement, particularly in response to factors such as short-term temperature shocks.

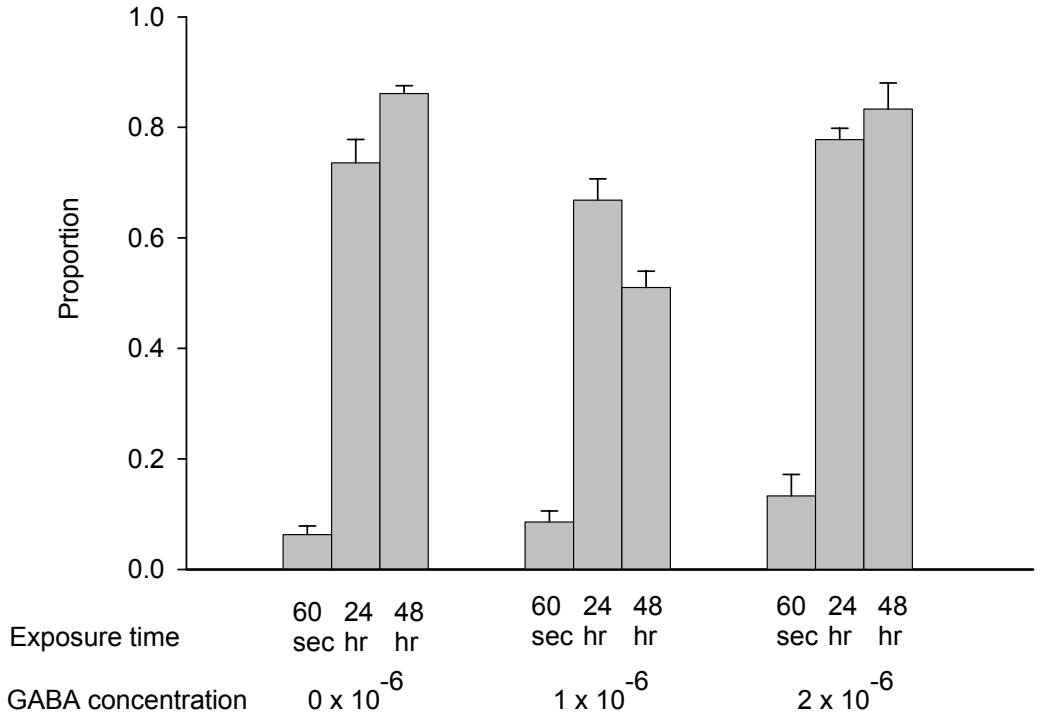


Figure 4. Mean proportion of larvae (± SE) on a standard settlement substrate in response to a control and two concentrations of GABA for 3 exposure times (n = 8).

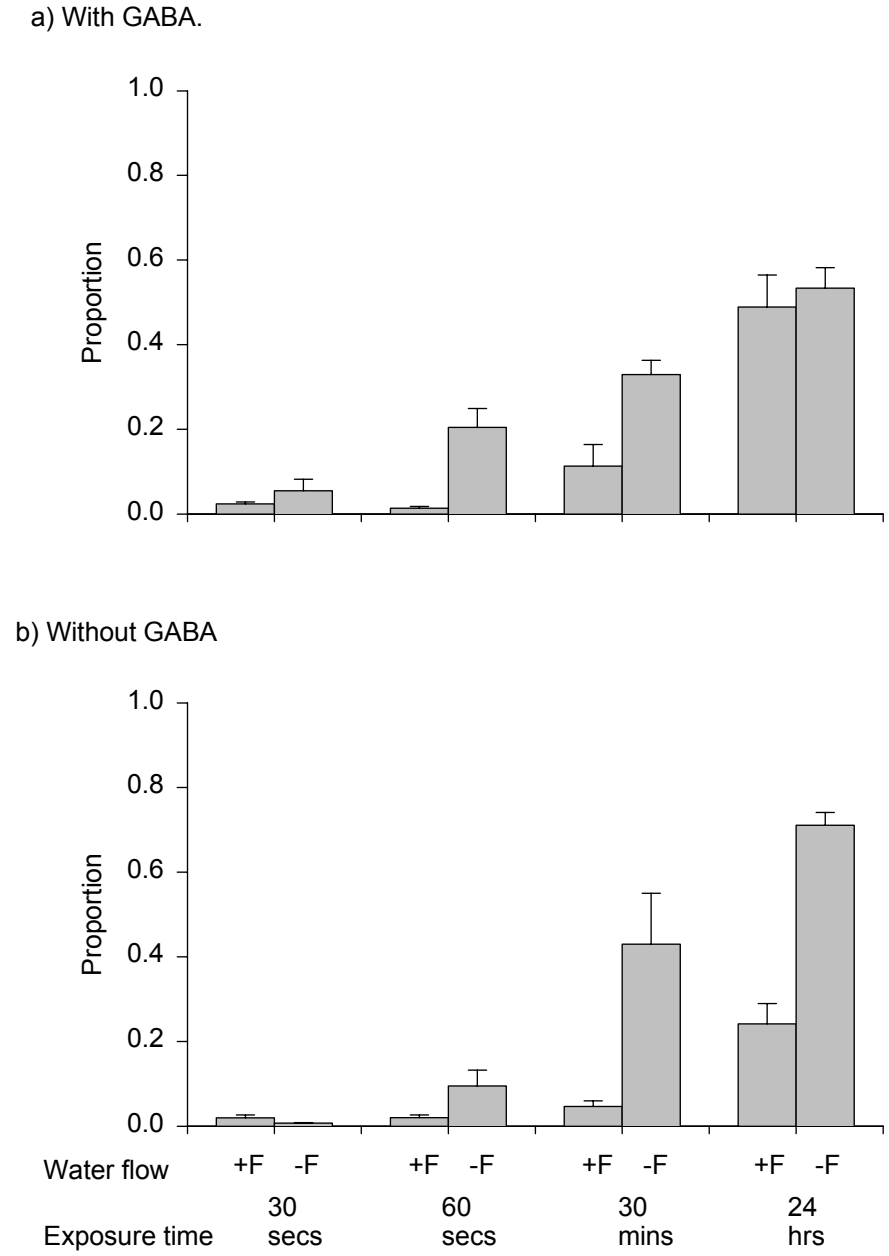


Figure 5. Mean proportion of larvae (± SE) on a standard settlement substrate with a) and without b) GABA and water flow (F) for 4 exposure times (n = 3).

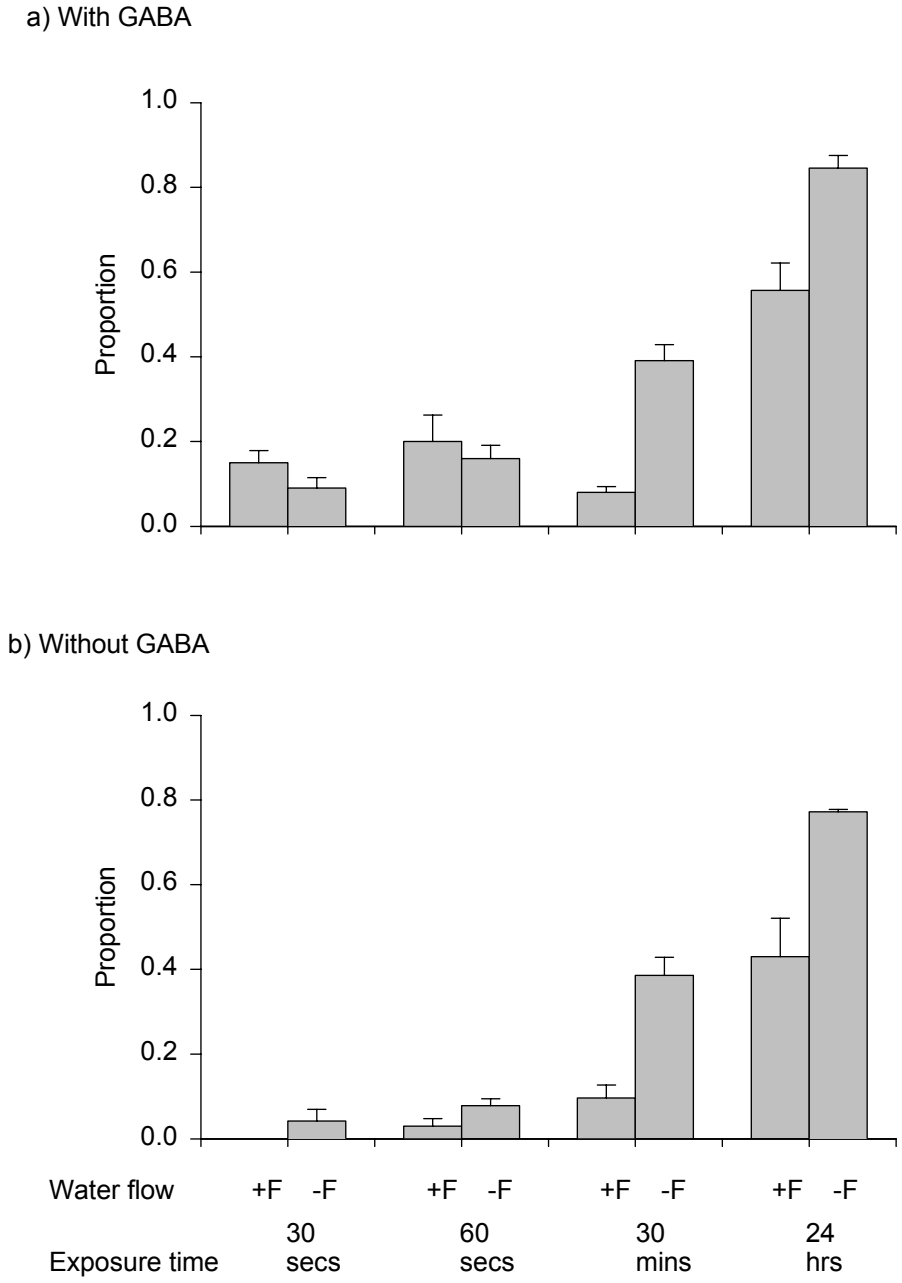


Figure 6. Mean proportion of larvae (± SE) on a standard settlement substrate after 24 h of refrigeration with a) and without b) GABA and water flow (F) for 4 exposure times (n = 3).

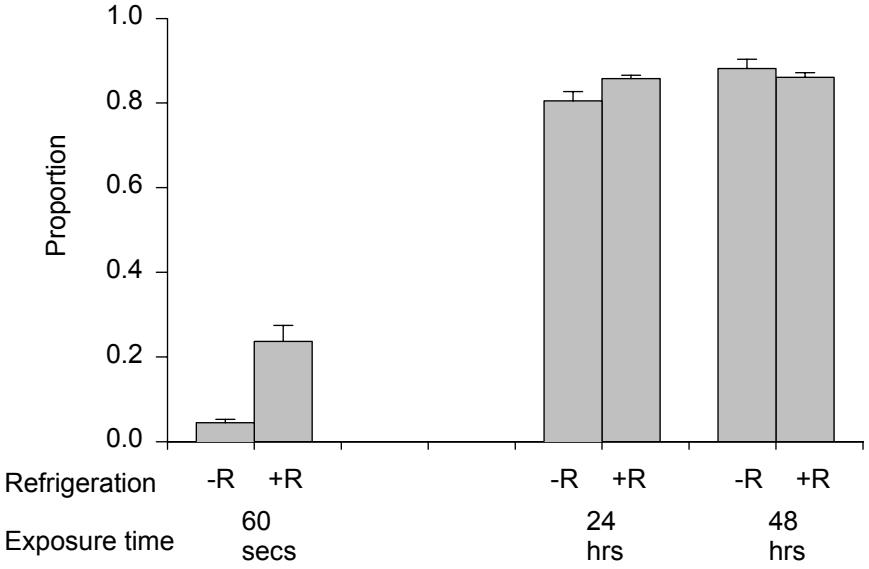


Figure 7. Mean proportion of larvae (± SE) on a standard settlement substrate after 0 and 24 h of refrigeration without water flow (F) for 3 exposure times (n = 3).

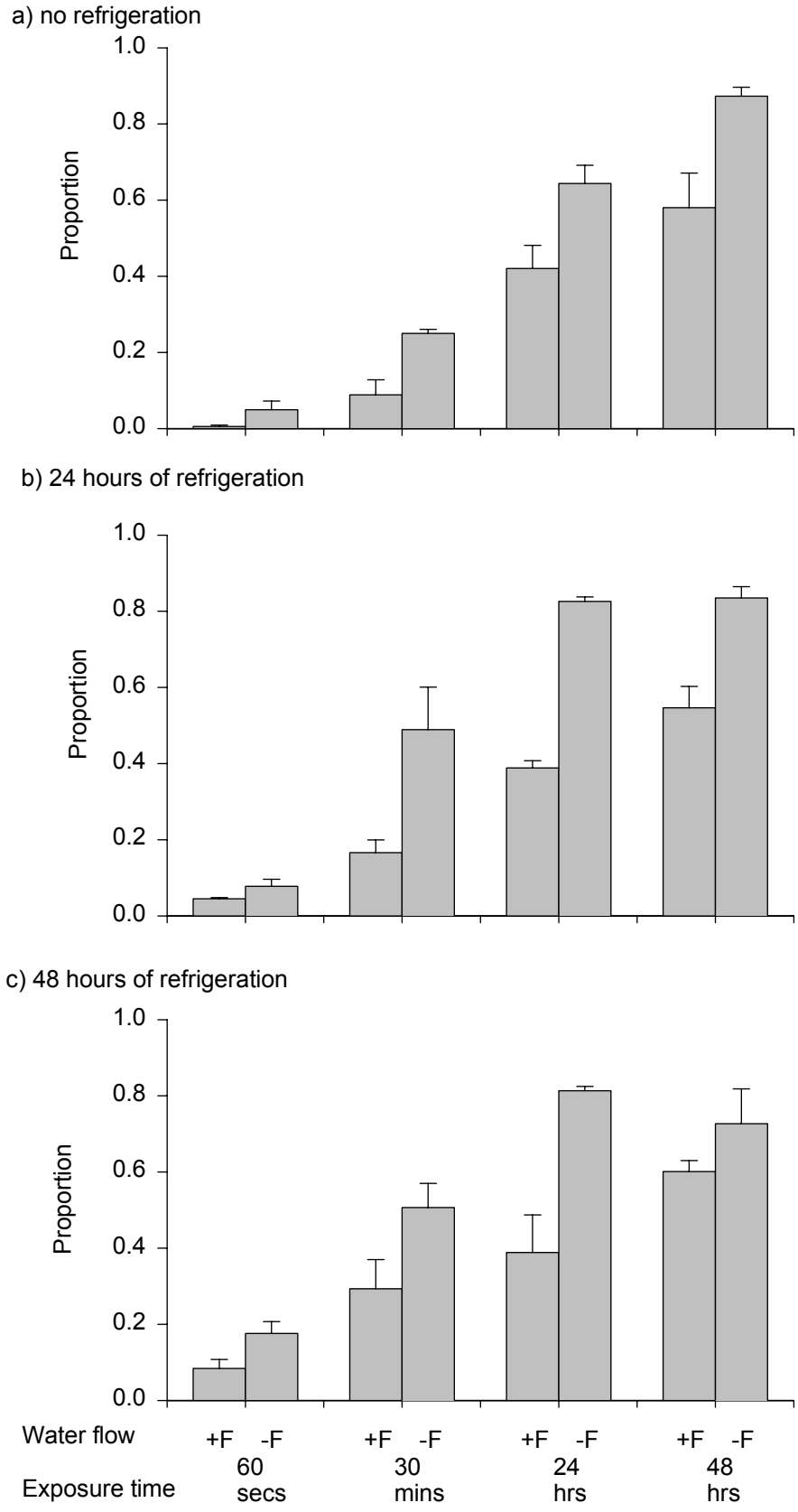


Figure 8. Mean proportion of larvae (± SE) on a standard settlement substrate after 0, 24 and 48 h of refrigeration with and without water flow (F) for 4 exposure times (n = 3).

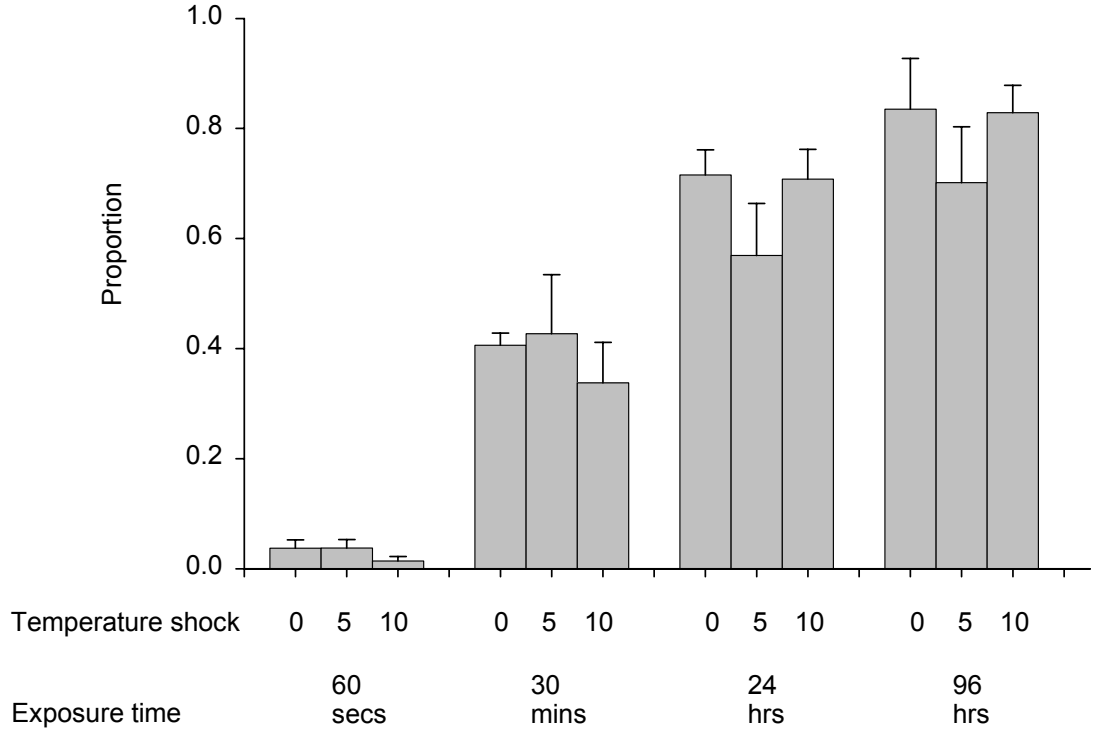


Figure 9. Mean proportion of larvae (± SE) on a standard settlement substrate after a temperature shock of 0, 5 or 10°C for 4 exposure times (n = 4).

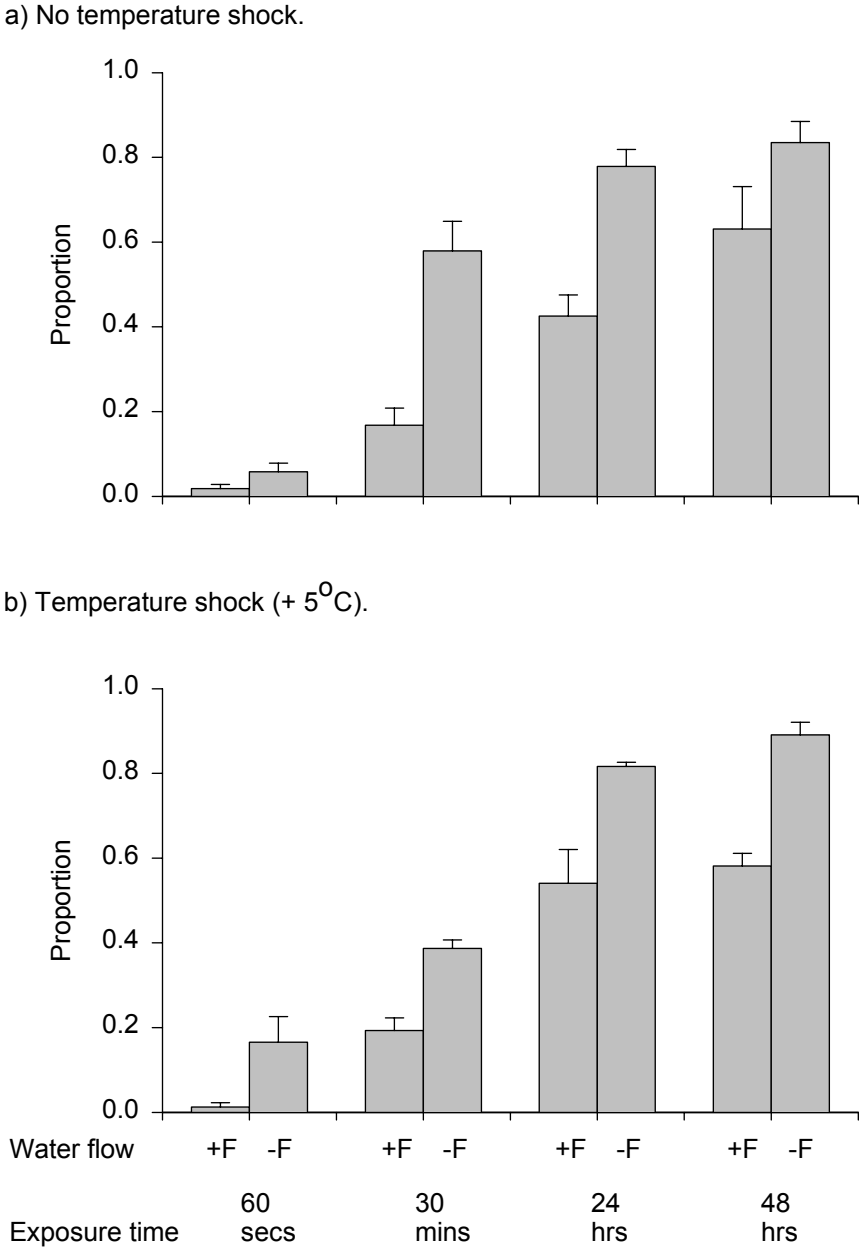


Figure 10. Mean proportion of larvae (± SE) on a standard settlement substrate with and without a temperature shock and water flow (F) for 4 exposure times (n = 3).

6.4.3. Factors imposed following release

There were large and consistent differences in the settlement of larvae through time (Figure 2). Rates of settlement were often 4-10 times higher over long time periods (30 sec v 24 hr). For example, in experiments 2 and 3 changes in the rate of settlement through time differed among substrate and refrigeration, but consistent differences in settlement through time explained 25% and 30% of the total variation, respectively (Tables A6.2 and A6.3). In Experiments 2 and 3, rates of settlement approached 90% for CCA-coated rocks exposed to water flow after 24 hr, whilst only about 20% of individuals had settled after 30 sec (Figures 2 & 3). Again in both experiments, rates of settlement over 30 sec to CCA-coated rocks approached those for conditioned slides over 24 hr.

There were also large and consistent differences in the settlement of larvae related to water flow. Rates of settlement over short time periods (30-60 sec) were often about halved in the presence of a water flow. For example in Experiments 2 and 3, rates of settlement to CCA-coated rocks were approximately halved by water flow over short time periods, and were not significantly affected over 24 h (Figure 2). Effects of water flow on settlement to conditioned slides were more consistent through time with settlement reduced by water flow even after 24 h in both experiments (Figures 2 & 3).

Following the release of larvae at field sites, they are exposed to a variety of potentially interacting factors that may compromise their ability to settle, metamorphose and grow. Most importantly, the experiments here have demonstrated the requirement of larvae to be exposed to suitable settlement substrates for extended periods of time, and their vulnerability to even minor water flow across that substrate. Further, even minor water flow has the potential to move larvae out of appropriate habitats for settlement. These vulnerabilities emphasize the importance of deployment methods that might be able to place larvae in areas of entrained or minimal water flow, to allow prolonged exposure to suitable substrates for settlement. Such areas would include those with limited water flow beneath and between rocks and boulders, and within small cracks, crevices and caves.

6.4.4. Contributing factors

Several confounding factors may have influenced the results presented here. These include the relative importance of stages of larval recruitment from the water column to the substrate i.e. attachment, settlement and metamorphosis. Only some experiments were continued for 48 and 96 h to investigate the effects on metamorphosis and early shell growth. Patterns in the abundance of larvae over shorter time periods could reflect only brief attachment or settlement and may not relate to subsequent metamorphosis. Despite that, patterns of attachment and settlement may still be particularly important in the field to ensure larvae remain close to the substrate in appropriate habitat. Similarly, patterns in the settlement or metamorphosis of larvae to adventitiously conditioned slides, with their variable but limited cover of algae may also not relate to patterns under more complex conditions in the field.

Rates of settlement also differed quite extensively between batches (Figure 6.11 & 6.12 and Tables A6.11 and A6.12). Variability in the rate of settlement and variation in the strength of the response of different batches of larvae to settlement cues has been observed by other authors (Trapido-Rosenthal & Morse 1986; Roberts & Nicholson 1997). Further, the interaction between these factors may also change with the age of the larvae, as rate of larval settlement in response to cues has been documented to be affected by the age of larvae (Roberts 2001). With a limited ability to replicate treatments within and among batches, some confounding of treatment effects related to batch quality is unavoidable. Nevertheless, variation in rates of settlement between replicates of the same treatment within batches was generally low (rarely more than 5% of the total variation in settlement remained in the error term of the model). This implies variation between batches, and potentially between settlement substrates for different batches, are the more likely source of confounding.

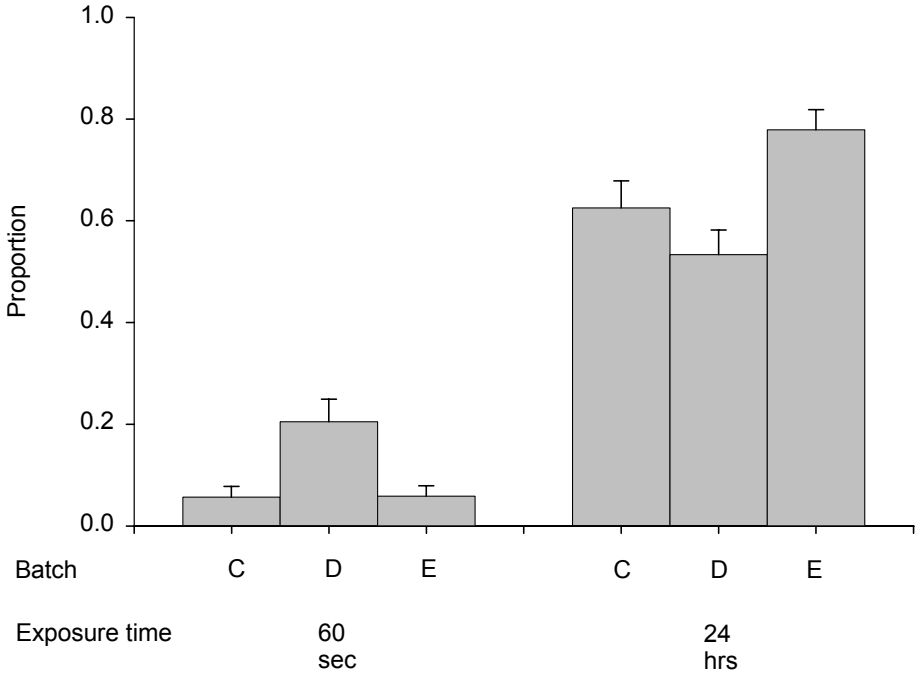


Figure 11. Mean proportion of 7-day-old larvae from 3 separate batches on a standard settlement substrate for 2 exposure times (n = 4).

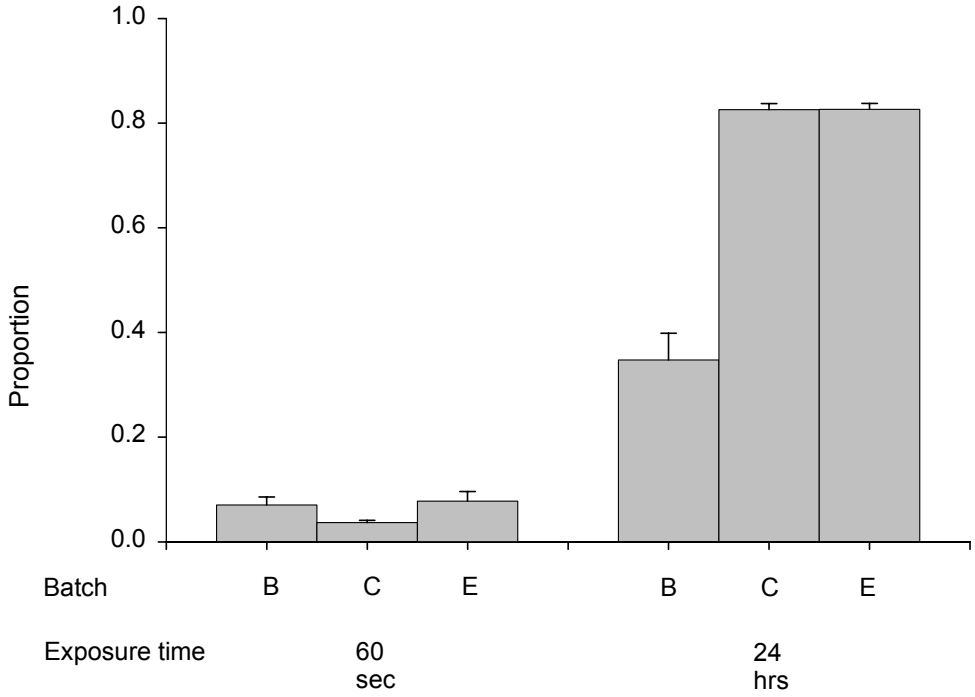


Figure 12. Mean proportion of 8-day-old larvae from 3 separate batches on a standard settlement substrate for 2 exposure times (n = 4).

6.5. Conclusions

It is clear from the experiments that several factors have the potential to influence the ability of *H. rubra* larvae to settle and metamorphose in the field. Perhaps most importantly, the remarkably low rates of settlement over short time periods in the presence of relatively low water flow (~20% over 30-60 sec.) suggest settlement in the field will be limited unless larvae can be positioned in areas that maximise the duration of their exposure to appropriate habitat. Appropriate habitats in the field are discrete and restricted, being interspersed with areas where survival and growth is likely to be low. Unless larvae can be released into areas with limited water flow few will be able to settle prior to being transported into inappropriate habitat. This emphasizes the importance of release methods such as the larval pump (see next chapter), which allows the relatively controlled delivery of larvae into small areas with limited water movement, within larger suitable habitats. Finally, the limited potential for settlement in the actual area of release complicates the design of experiments investigating the release of larvae in the field, because of uncertainty related to the number of larvae released that were actually able to settle within the area proposed for future sampling. Mesh tents (see next chapter) can be used to restrict the inadvertent movement of larvae out of release areas, although logistics restricts their use to reasonably small-scales.

The techniques of handling and transportation investigated in these experiments had relatively minor effects on the successful settlement of larvae. Indeed some combinations of treatments, expected to compromise the ability of larvae to settle, resulted in increased rates of settlement. Regardless, the results emphasize the resilience of *H. rubra* larvae to the handling and transportation techniques developed here. Unfortunately, these techniques can only maintain the larvae for periods of several days, and will not enable the holding of larvae for longer periods prior to settlement. This might be desirable during periods of bad weather and rough seas, where the release of larvae into areas of limited water flow becomes much more difficult.

6.6. Appendix – Section 6

Table A6.1. Summary of analysis of variance in the number of larvae on settlement substrates, at different times in Experiment 1a (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
Substrate	1	2.07	97.16		68
Time	3	0.40	71.31		27
S*T	3	0.02	3.75	**0.024**	3
Error	24	0.01			3
Total	31				

Table A6.2. Summary of analysis of variance in the proportion of larvae attached to a standard settlement substrate with and without water flow, at different times in Experiment 2b (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
Flow	1	0.53	18.38	**0.023**	8
Substrate	1	3.53	71.82		56
F*S	1	0.01	0.58	0.502	0
Time	3	0.79	74.91		25
F*T	3	0.03	2.72	0.061	2
S*T	3	0.05	4.64	**0.008**	3
F*S*T	3	0.01	1.41	0.258	2
Error	32	0.01			4
Total	47				

Table A6.3. Summary of analysis of variance in the proportion of larvae attached to a standard settlement substrate with and without water flow and 24 hrs refrigeration, at different times in Experiment 3b (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
Flow	1	0.48	31.92		5
Substrate	1	2.20	196.50		25
F*S	1	0.02	0.09		0
Refrigeration	1	0.16	0.88		0
F*R	1	0.00	0.04	0.852	0
S*R	1	0.07	0.80		0
F*S*R	1	0.00	0.55	0.535	0
Time	2	1.73	114.20		30
F*T	2	0.02	0.99		1
S*T	2	0.01	0.74		0
F*S*T	2	0.22	14.81	**0.000**	16
R*T	2	0.18	11.94		6
F*R*T	2	0.05	3.10	0.054	3
S*R*T	2	0.09	5.95	**0.005**	6
F*S*R*T	2	0.00	0.15	0.864	0
Error	48	0.02			6
Total	71				

Table A6.4. Summary of analysis of variance in the proportion of larvae attached to a standard settlement substrate after exposure to different concentrations of GABA, at different times in Experiment 4c (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
GABA	2	0.23	2.12		2
Time	2	4.48	344.35		90
G*T	4	0.11	8.46	**0.000**	4
Error	63	0.01			4
Total	71				

Table A6.5. Summary of analysis of variance in the proportion of larvae attached to a standard settlement substrate with and without exposure to GABA and water flow, at different times in Experiment 5d (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
Flow	1	0.65	8.13		16
GABA	1	0.03	7.14		1
F*G	1	0.02	0.33		0
Time	3	0.92	93.19		52
F*T	3	0.08	8.07		9
G*T	3	0.00	0.38		0
F*G*T	3	0.07	6.74	**0.001**	15
Error	32	0.01			7
Total	47				

Table A6.6. Summary of analysis of variance in the proportion of larvae attached to a standard settlement substrate with and without exposure to GABA and water flow, at different times after 24 hrs refrigeration in Experiment 6d (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
Flow	1	0.29	1.62		3
GABA	1	0.15	6.74	0.081	4
F*G	1	0.00	0.35	0.596	0
Time	3	1.12	143.48		64
F*T	3	0.18	22.6	**0.000**	20
G*T	3	0.02	2.76	0.058	2
F*G*	3	0.00	0.46	0.710	1
Error	32	0.01			5
Total	47				

Table A6.7. Summary of analysis of variance in the proportion of larvae attached to a standard settlement substrate after 24 hrs refrigeration, at different times in Experiment 7c (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
Refrigeration	1	0.07	1.27		1
Time	2	1.80	600.92		92
R*T	2	0.06	18.96	**0.000**	6
Error	18	0.00			1
Total	23				

Table A6.8. Summary of analysis of variance in the proportion of larvae attached to a standard settlement substrate after different periods of refrigeration, with and without water flow, at different times in Experiment 8e (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
Flow	1	1.23	22.62		19
Refrigeration	2	0.10	2.45		1
F*R	2	0.01	0.52	0.620	0
Time	3	2.04	192.08		67
F*T	3	0.05	5.09	**0.004**	4
R*T	6	0.04	3.77	**0.004**	3
F*R*T	6	0.02	1.51	0.194	2
Error	48	0.01			4
Total	71				

Table A6.9. Summary of analysis of variance in the proportion of larvae attached to a standard settlement substrate after exposure to different temperature gradients, at different times in Experiment 9a (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
Temperature	2	0.03	1.50	0.297	0
Time	3	2.21	79.08	**0.000**	85
Tmp*T	6	0.02	0.74	0.619	2
Error	36	0.03			13
Total	47				

Table A6.10. Summary of analysis of variance in the proportion of larvae attached to a standard settlement substrate, with and without exposure to a temperature gradient and water flow, at different times in Experiment 10e (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
Flow	1	1.07	121.80	**0.002**	21
Temperature	1	0.01	0.34	0.600	0
F*Tmp	1	0.00	0.00	0.961	0
Time	3	1.70	162.11	**0.000**	67
F*T	3	0.01	0.84	0.483	1
Tmp*T	3	0.02	1.59	0.210	1
F*Tmp*T	3	0.03	2.70	0.062	4
Error	32	0.01			5
Total	47				

Table A6.11. Summary of analysis of variance in the proportion of 7-day-old larvae from 3 separate batches attached to a standard settlement substrate, at different times in Experiment 11 (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
Batch	2	0.01	0.14		0
Time	1	1.77	18.72		82
B*T	2	0.09	13.09	**0.001**	14
Error	12	0.01			4
Total	17				

Table A6.12. Summary of analysis of variance in the proportion of 8-day-old larvae from 3 separate batches attached to a standard settlement substrate, at different times in Experiment 12 (Table 1). Significant effects are shown in bold (i.e. $P < 0.05$).

Source	df	MS	F	P	%V
Batch	2	0.12	0.79		0
Time	1	2.36	15.69		82
B*T	2	0.15	52.09	**0.000**	17
Error	12	0.00			1
Total	17				

7. DEPLOYMENT AND EARLY SURVIVAL OF *H. RUBRA* LARVAE AND JUVENILES

R.C. Chick, D.G. Worthington, P.T. Gibson, M.P. Heasman, N. Savva & C. Brand

7.1. Introduction

Releasing hatchery-reared larvae to enhance local populations of abalone has been investigated previously with variable results (Tong *et al*. 1987; review by McCormick *et al*. 1994; Schiel 1992, Preece *et al*. 1997). Success appears to have been complicated by a variety of factors. First, there is evidence that the method of deploying larvae may compromise settlement or early survival (Schiel 1992, Preece *et al* 1997). Next, there are great difficulties related to the methods of collecting a representative and reliable sample of the number of small abalone in an area (Schiel 1992, McShane & Smith 1988). Finally, there are difficulties in distinguishing between juveniles that were initially released as larvae and those that recruited naturally (Preece *et al.* 1997). Only once these difficulties have been addressed can a study attempt to consider the effects of additional processes influencing settlement and survival (e.g. density, predation and competition), or estimate the success of an enhancement project.

Releasing hatchery-reared juveniles to enhance local populations of abalone has been investigated in various countries throughout the world (Tegner & Butler 1985; Schiel 1992; 1993; review by McCormick *et al*. 1994; Kojima 1995; de Waal & Cook 2001). The regular release of juveniles to enhance natural abalone populations is only carried out in Japan (review by Masuda & Tsukamoto 1998). Several of these studies have emphasized the importance of the relationship between the size of juveniles released and their survival (Mgaya & Mercer 1995; Inoue 1976, in Masuda & Tsukamoto 1998). Similarly, several studies have emphasized the importance of methods for releasing juvenile abalone. For example, the use of shelters can reduce mortality compared to release with no shelter (McCormick *et al*. 1994). It appears that early mortality, particularly due to predation, can be minimised by the provision of some shelter. Despite this, difficulties associated with methods used to estimate the abundance of released juveniles complicate this comparison. Settlement and the first few weeks of juvenile life appear to be associated with CCA, whilst latter in their life history they become more cryptic and inhabit the undersides of boulders and fissures in solid substrata. Consequently, a combination of sampling methods will be required to adequately sample abundance, and hence attempt to estimate the effects of processes influencing survival, or to estimate the early success of an enhancement project.

A variety of methods of deploying hatchery-reared larvae have been developed (Tong *et al.* 1987; Schiel 1992; Preece *et al.* 1997). Preece *et al*. (1997) developed a method of deploying larvae involving minimal handling, while evenly distributing and directing larvae around rocks, minimising the number of larvae that might be lost from the site due to advection. Similarly, Preece *et al.* (1997) used this method to deploy larvae to small distinct areas, restricting the dispersal of larvae from these areas using small tents.

A number of sampling methods have been used in previous studies to estimate the abundance of juvenile abalone. These include the use of a venturi lift (McShane & Smith 1988), the collection and washing of small boulders (Prince & Ford 1985; Schiel 1992) and visual inspection of the substrate, with or without disturbance by divers (Prince & Ford 1985; Shepherd & Turner 1985; Shepherd 1998). Each of these methods has limitations that restrict their ability to reliably estimate the abundance of juveniles. For example, it is very difficult to ensure samples in different habitats

are representative of the abundance of juveniles, or to maintain reliable estimates of abundance through time as the juveniles grow and move among habitats. This has major implications for estimates of the absolute abundance of juveniles, and consequently for attempts to gauge the ongoing success of an enhancement project.

7.2. Aims

The aim of this study is to investigate the potential of a deployment method to deliver known numbers of larvae, and to determine if this process affects the competency of larvae to settle. In addition, we investigate the use of similar structures to those described by Preece *et al.* (1997) and Schiel (1992) to maintain larvae within a defined area. We compare a range of both destructive and non-destructive sampling methods, with the aim of developing a strategy for combining sampling methods to produce the most reliable and representative estimate of the abundance of juveniles through time and among habitats.

Fluorochrome dyes can be used to bind to calcium that is incorporated into newly mineralised shell providing a mark that is visible under UV light (Day *et. al.* 1995). Here we investigate the potential of calcein to mark hatchery-reared larvae for subsequent identification as juveniles after release. Perhaps the most important factor determining the value of this method is the length of time the fluorescent mark remains visible in the shell of juveniles. Here we investigate the method of marking larvae, and the length of time this mark can be positively identified in the juvenile shell. This was done for larvae and juveniles grown in the hatchery, and larvae released to the natural reefs and recaptured up to 15 months later.

7.3. Deployment and settlement of larvae

7.3.1. Methods

A variety of methods were investigated for the storage, transportation and deployment of larvae at field sites. The preferred method of storage and deployment was dependent on the objectives of the release. For example, the release of small, but accurately known, numbers of larvae was required in some small-scale experiments, whilst methods that might be used in the commercial-scale deployment of large numbers of larvae were also required. For experiments requiring small, but accurately known numbers of larvae, techniques were developed that enabled the transport of larvae in small plankton mesh bags, stored in seawater in small jars and/or on damp sponges. This enabled the rapid deployment of known numbers of larvae direct to the substrate within defined areas of reef. These techniques were however replaced by alternative techniques more appropriate for directing the release of greater numbers of larvae evenly across larger areas, and which also required fewer levels of handling the larvae prior to their transportation and deployment (see below).

7.3.1.1. Deployment pump

We used a modified version of the deployment pump developed by Preece *et al.* (1997) to enable the release of large numbers of larvae in preferred habitats across a large area (Plate 1). The deployment pump consisted of a sealed 35 L drum with several additional fittings. The lid of the drum was fitted with a hose coupling and a pressure relief valve to enable the supply of compressed air from a SCUBA bottle into the drum to a pressure of approximately 42 Kpa (6 psi). A hose coupling was also inserted near the base of the drum to allow the connection of a 20 m or 40 m (12 mm diameter) hose to deliver the larvae from the drum on a boat to the reef, under the control of a SCUBA diver. Larvae in seawater are added to seawater in the drum, the lid sealed, secured and the drum then pressurized. A constant supply of compressed air delivered through an air-stone, fitted to the end of a hose located on the floor of the drum, was used in an attempt to maintain an even distribution of larvae in the seawater in the drum. Pressurization of the drum enabled the seawater

and larvae suspension to be pumped down the hose and for this suspension to be pumped evenly over the substrate within the release site, subject to flow from the hose being controlled and directed by a diver.

Plate 1. The deployment pump consisting of a 35 L drum with hose, the lid with in coming (short hose, without air stone attached) and out going (hose connected and fitted with pressure relief valve) couplings and SCUBA tank with high pressure (in coming) hose.

A series of experiments were designed to address concerns regarding the possible uneven distribution of larvae within the deployment drum and the effects of the deployment process on the larvae (Table 1). The first experiment was designed to investigate the effect of several treatments, required during handling or release of larvae, on the rate of supply of larvae through the deployment drum and hose. Replicated samples of larvae from a single batch were exposed to two orthogonal treatments prior to their introduction to the deployment drum. The rate of supply of larvae was then measured at the start [S] and end [Ed] of the deployment hose when the drum was full [F], half full [½] and almost empty [E]. This rate of supply was measured as the number of larvae in replicate 50 mL samples, taken at each level of the treatments. The two treatments and controls included exposure to GABA at concentrations of 0 and 1 x 10^{-6} M and refrigerated storage at 0 and 24 hr (see methods in Chapter 6). This experimental design was repeated for larvae from the same batch at 7 and 8 days after fertilization. The comparison of refrigerated and non-refrigerated larvae that were 7 and 8 days old required samples of larvae from the same batch to be placed in the refrigerator at 6 and 7 days old respectively. Prior to these refrigerated larvae being used in the experiment they were removed from the refrigerator to acclimate to the ambient seawater temperature of 18^{o}C.

A second experiment was designed to investigate the effects of the deployment pump on the ability of larvae to settle (Table 1). Replicate, 50 mL samples of seawater containing larvae were collected from the start and end of the deployment hose when the drum was full, half full and almost empty. Larvae from these samples were then exposed to a standard settlement substrate (a conditioned microscope slide) for 30 min without any water flow across the substrate. The slide was then removed and stored in 70% alcohol. Larvae remaining in the replicate were collected using a 90 µm sieve and separately stored in 70% alcohol. The number of larvae that had been removed from

the replicate, attached to the slide and the number of larvae remaining in the replicate were counted. Additional experiments using similar methods were done to investigate the competency of larvae to settle prior to and during their actual deployment to field sites (Table 1).

Table 1. Design of experiments investigating the rate of supply of larvae through the deployment pump and the effect of the deployment pump and other treatments on the rate of settlement of larvae. Levels for each treatment are shown in brackets and described in the text. Common letters are used to indicate experiments completed with the same batch of larvae.

Expt. No.	Factors [and levels]	Age (days)	Field release locations	Replicates	Response variable
1a	GABA [0,1]				
	Refrigeration [0,24]				
	Drum [F, ½, E]				
	Hose [S, Ed]	7		4	Number of larvae supplied
1a	GABA [0,1]				
	Refrigeration [0,24]				
	Drum [F, ½, E]				
	Hose [S, Ed]	8		4	Number of larvae supplied
2b	GABA [0, 1]				
	Drum [F, ½, E]				
	Hose [S, Ed]	7		3	Settlement after 30 min
3c	GABA [1]				
	Drum [F, E]				
	Hose [S]	7	Port Stephens	5	Settlement after 24 hrs
4d	GABA [1]				
	Drum [F, E]				
	Hose [S]	7	Sydney	3	Settlement after 24 hrs

7.3.1.2. Deployment shelters

An experiment was designed to investigate the potential for physical shelters to restrict larvae to a small area of reef and consequently increase the number of larvae that settle within that area. In restricting movement of the larvae, these shelters may also have reduced the flow of water across the substrate and restricted the access of large predators, such as fish. Shelters consisted of open based cone shaped canopies made of fine mesh cloth weighted with chain around the skirt with a basal area of 1 m^2 (Plate 2). A batch of 2.2 million, 7-day-old larvae were deployed using the deployment pump previously described. These larvae were evenly dispersed across a defined 100 m^2 area of boulder field that contained five shelters. Larvae were pumped under the skirt of each shelter for 12 sec, allowing approximately 22,000 larvae to be pumped into each shelter. The remaining larvae were pumped over the remainder of the defined boulder field at the same density. After 24 hr, the shelters were removed and 5 small (maximum diameter 300 mm) boulders were sampled from within the area previously covered by each shelter, and from within 5 equivalent 1 m^2 areas within the boulder field that were not sheltered. The boulders were collected by divers and were carefully placed in plastic bags and sealed before being returned to the boat. Within 4 h each boulder was soaked in 70% alcohol for 5 min and gently brushed to remove any larvae. The samples from each boulder were sieved through an 80 µm mesh and the particulate matter was stored in 70% alcohol. This material was sorted and the number of abalone larvae was counted using a binocular microscope.

Plate 2. Physical shelters positioned on the reef prior to larval deployment.

7.3.2. Results and discussion

7.3.2.1. Deployment pump

There was significant variation in average densities at which larvae were delivered by the deployment pump (Tables A1a & b) with densities for various treatment combinations ranging from less than 20 to more than 100 larvae per 50 mL sample. If all larvae loaded into the drum of the pump had been evenly dispersed and delivered an average of 71 larvae per 50 mL sample, across all treatments in the experiment, would have been expected. The actual average number of larvae per 50 mL sample was only 54. This implies that only 76% of the larvae placed in the drum were released, with the remainder presumably having attached to surfaces within the drum and hose. The use of GABA and the refrigeration of larvae both significantly affected the supply of larvae from the drum (Figures 1a & b; Tables A1a & b). Effects of GABA on the density of larvae delivered varied with refrigeration and with different volumes of the drum, whilst the effects of refrigeration were also varied over the levels of the hose treatment. For both 7- and 8-day-old larvae, effects of GABA and refrigeration at different levels of the drum explained the largest amount of variation in the densities of larvae delivered per sample (Tables A1a & b). These results imply that the deployment pump, particularly in conjunction with some necessary handling procedures, cannot be used to deliver consistent densities of larvae to the reef required for small-scale experiments. This inconsistent supply is probably caused by a variety of factors including larval behaviour and the inconsistent mixing of water in the drum. Despite this variation in the supply of larvae by the deployment pump at the scale of 50 mL samples, variation among larger samples is likely to be lower, allowing the more reliable supply of larger numbers of larvae.

There was also significant variation in the settlement of larvae related to use of the deployment pump (Table A2). For some treatment combinations average settlement rate was less than 10%, whilst in others it was above 30%. The effects of GABA varied with the position of delivery from the hose, and the differences among positions on the hose changed with the level of the drum (Figure 7.2; Table A2). This variation in the settlement of larvae related to the deployment pump, further complicates its use for delivering small numbers of larvae ready for settlement in small-scale experiments.

There was no significant variation in the settlement of larvae related to the level of the pump when larvae were deployed at field sites (Tables A3a & b). The average settlement rate was significantly higher at Port Stephens than Sydney (59% v 45%, Figure 3). These differences in settlement between larvae released at Sydney and Port Stephens were very similar to those found in the hatchery, and suggest that methods of transportation and handling do not adversely affect the competency of larvae to settle.

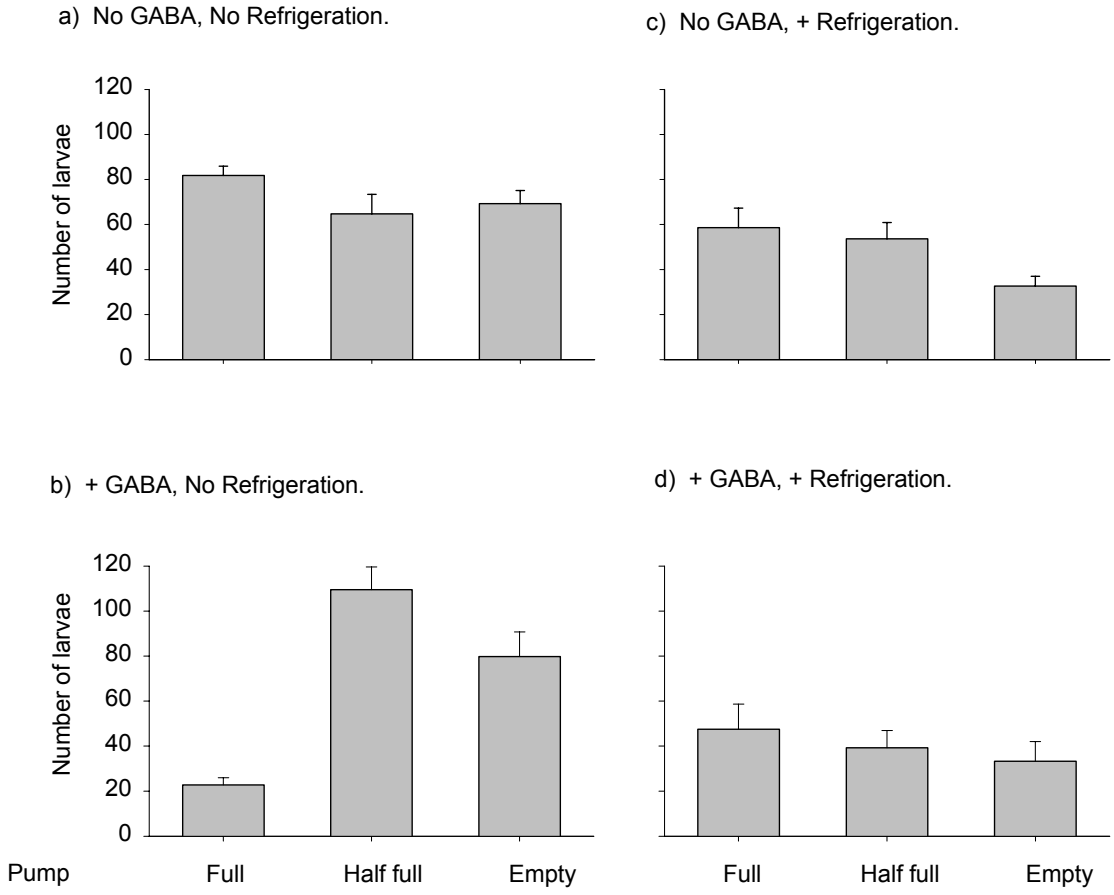


Figure 1a. Mean number of larvae, 7 days post-fertilization (± SE) pooled from 50 mL samples from the deployment pump at the start and end of the hose, with and without the addition of GABA and with and without 24 hr of refrigeration (n = 4).

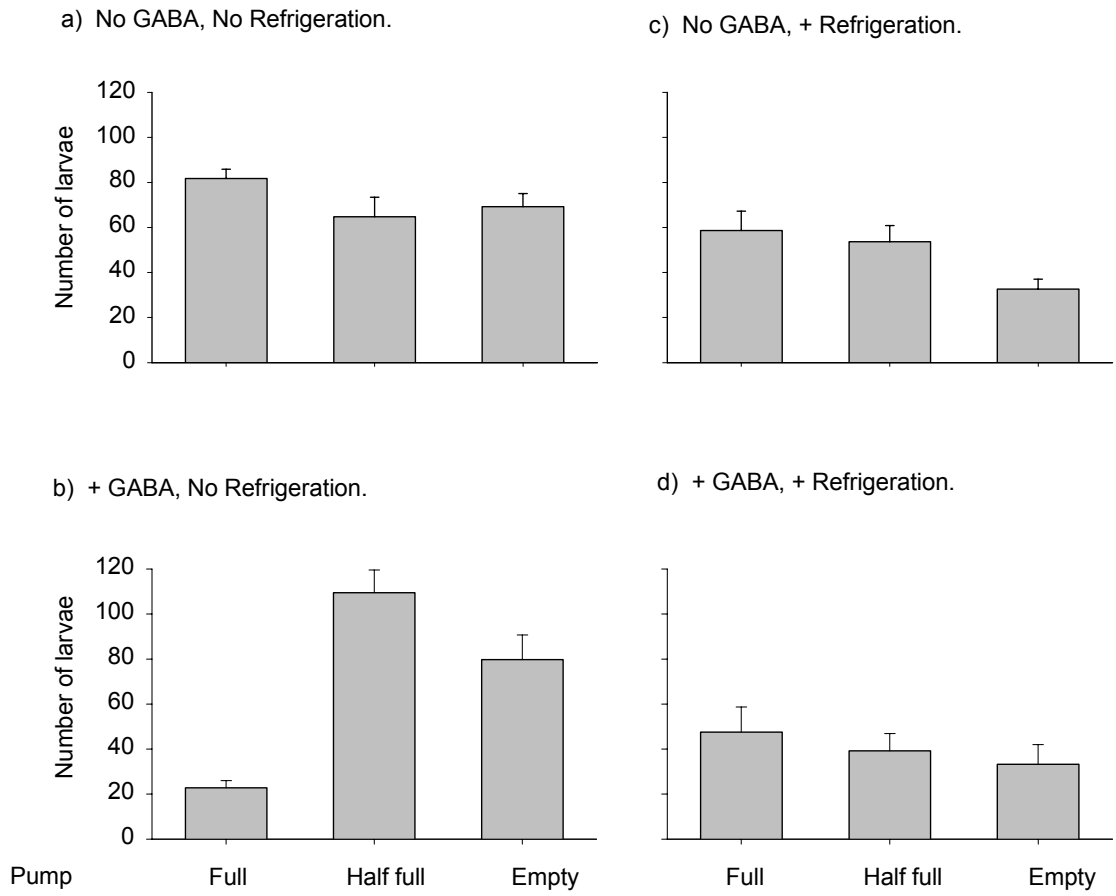


Figure 1b. Mean number of larvae, 8 days post-fertilization (± SE) pooled from 50 mL samples from the deployment drum at the start and end of the hose, with and without the addition of GABA and with and without 24 h of refrigeration (n = 4).

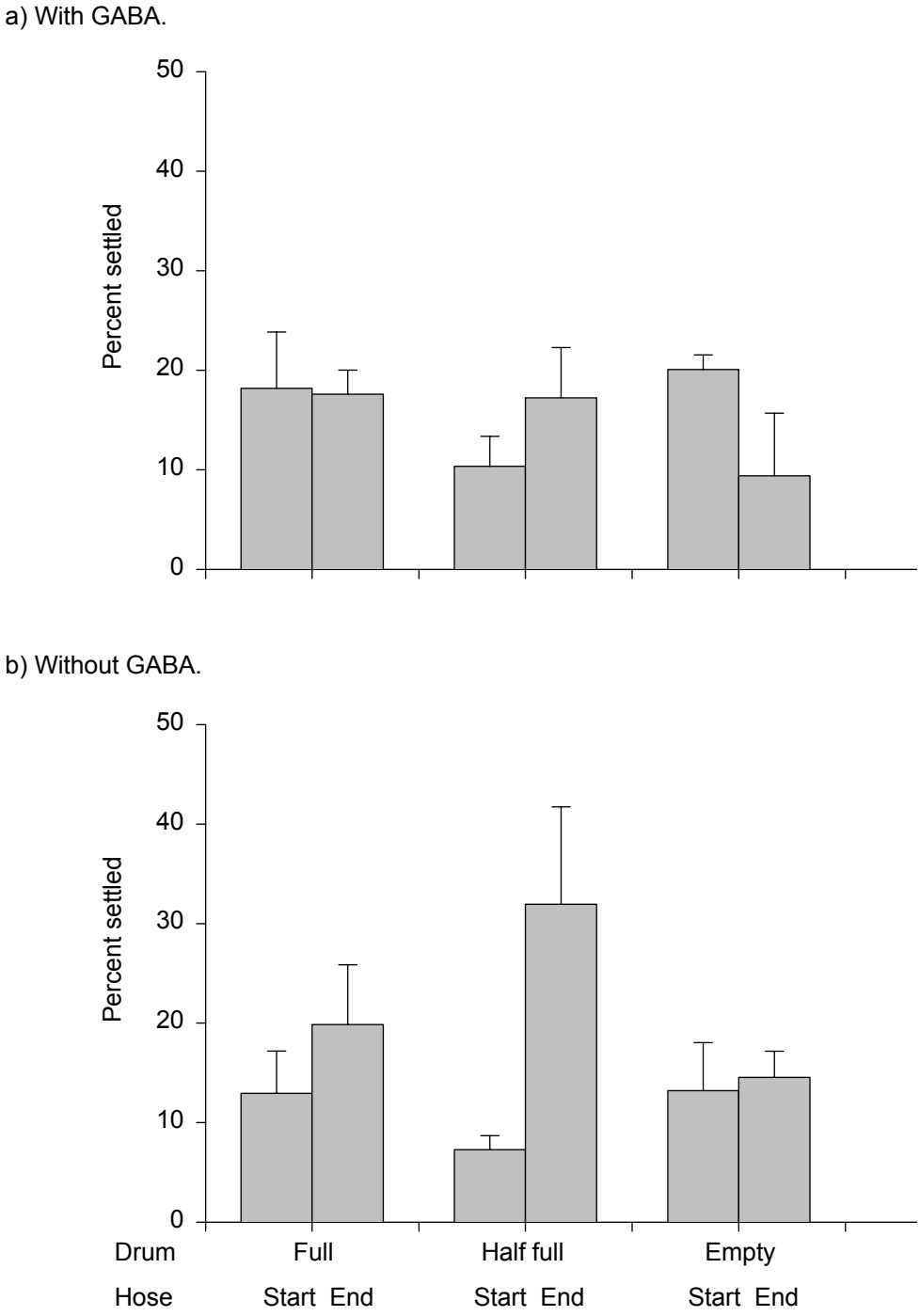


Figure 2. Mean percent of larvae (± SE) on a standard settlement substrate after an exposure time of 30 mins when sampled from the start and end of the 20 m length of hose, at 3 levels of the deployment drum with (a) and without (b) the addition of GABA (*n* = 3).

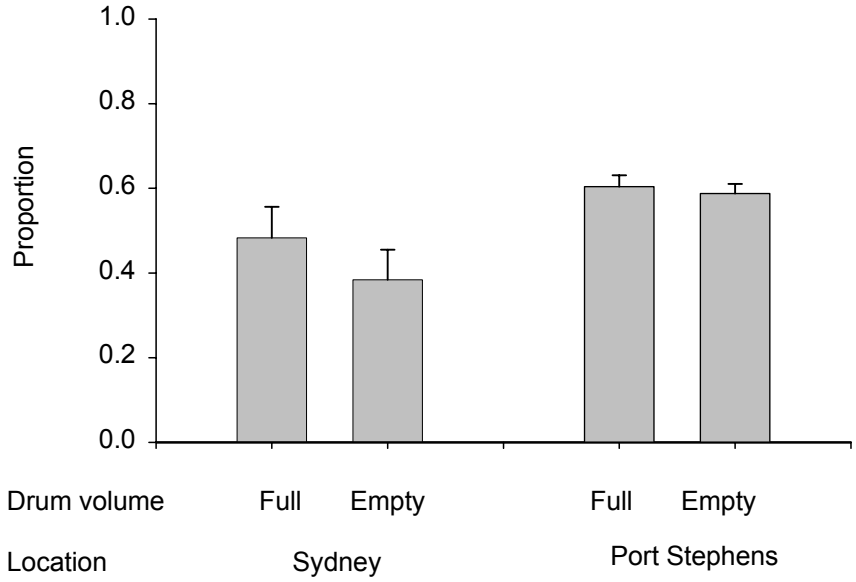


Figure 3. Mean proportion of larvae (± SE) on a standard settlement slide after an exposure time of 24 h, after being pumped from the start of the hose of the deployment pump at two levels of the drum when being released at field sites at Sydney (n = 3) and Port Stephens (n = 5).

7.3.2.2. Deployment shelters

The use of a physical shelter to restrict larvae resulted in a significantly higher abundance of recently settled larvae (Table A4). On average, areas of reef where larvae were released under physical shelters had approximately 100 settled larvae, which was more than 10 times that in areas where larvae were released without physical shelters (Figure 4). A variety of processes could have contributed to this difference including the retention of the larvae, reduced water flow across the substrate and reduced predation by the exclusion of fish. Whilst such physical shelters can be used to maximize the settlement of larvae within small areas, their use for commercial-scale releases to large areas will be limited by the logistical difficulties related to sheltering larger areas of reef. For example, to avoid density-dependent reductions in growth and mortality, it may be necessary to release larvae at densities less than 1500 m^{-2} therefore a batch of 2 million larvae might need to be dispersed over more than 1300 m^{-2} area of reef. As a consequence retention of larvae at this spatial scale could be more feasibly achieved by the release of larvae in to areas of natural shelter (e.g. using the deployment pump). Further, despite the reduced density of larvae settling in areas without shelter, this does not imply that these individuals died. Larvae not retained within a physical shelter may simply disperse to alternative appropriate habitats for settlement.

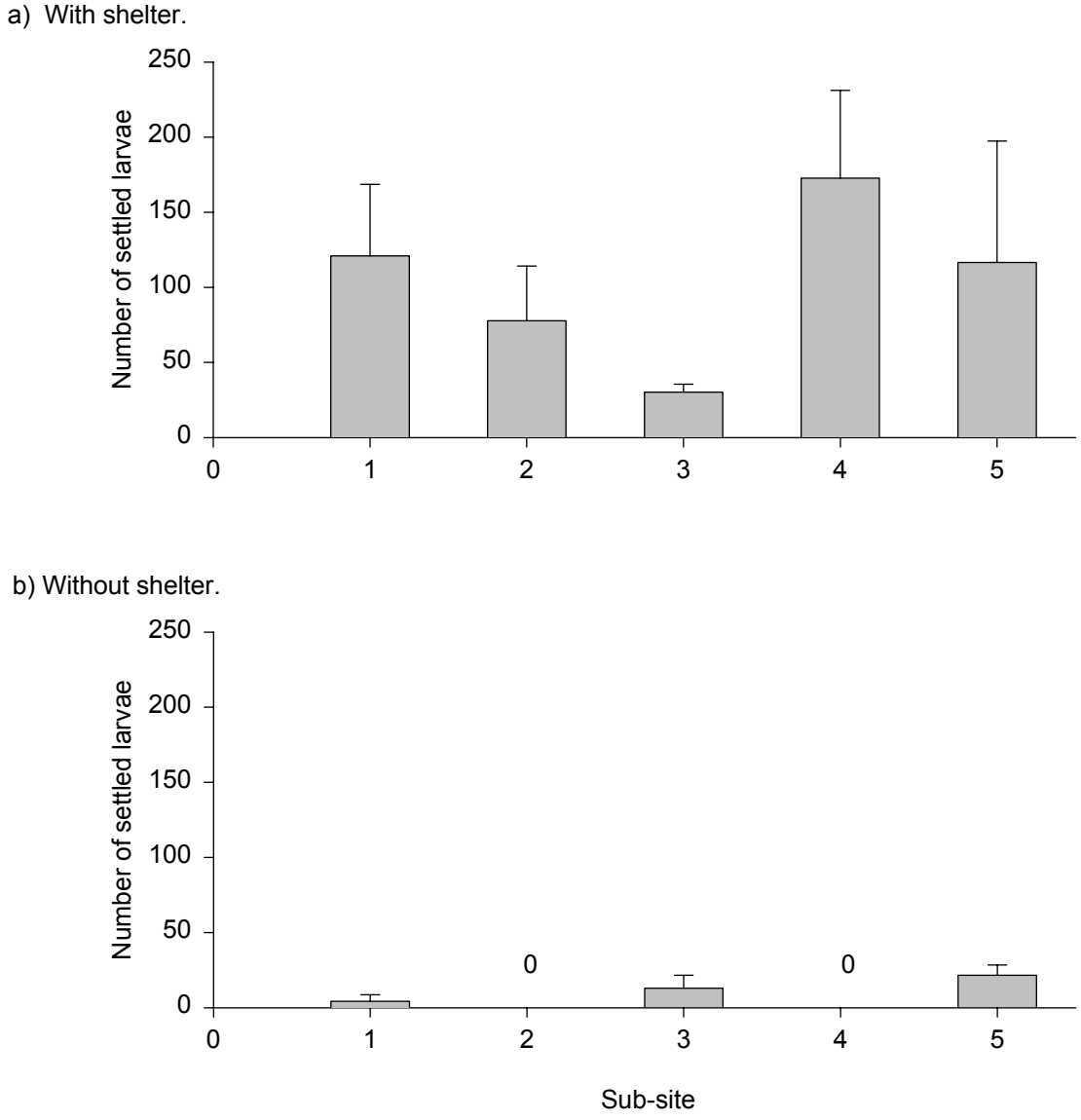


Figure 4. Mean number of abalone larvae (± SE) m^{-2} sampled 24 h after release from 5 sub-sites at Jibbon Point-south, Sydney, a) with and b) without shelter (n = 5).

7.4. Handling, transport and deployment of juvenile abalone

7.4.1. Introduction and methods

7.4.1.1. Removal of juvenile abalone from settlement plates

Methods to remove juveniles from settlement plates concentrated on the use of benzocaine. An initial trial of benzocaine at 0.5 mL of stock solution (1 g 10 mL^{-1} ethanol) per L of seawater indicated the method could efficiently remove >90% of juveniles from settlement plates within 15 min without affecting survival after 24 h. As a consequence, an experiment was designed to investigate the effect of different concentrations of benzocaine on the rate of removal of juveniles from settlement plates and their subsequent recovery. The experiment compared the effects of 3 concentrations of benzocaine (0.5, 1.0 & 2.0 mL stock solution l^{-1} seawater) and a seawater control on the numbers of juveniles (<2 mm) removed from settlement plates after 5, 10 and 20 min exposure. For each treatment combination the number of juvenile abalone on each one of three replicate settlement plates were counted prior to the plate being immersed in 5 l of benzocaine and seawater, at the required concentration, for the required time. The plate was then gently hosed with the juveniles removed from the plate collected on a sieve, rinsed, and placed in seawater to recover. Both the number of juveniles removed from the plate and those that remained on the plate were counted. The recovery of juveniles from each of three replicate groups of juveniles was recorded after 30 min and 18 h. Juveniles were classified as recovered if they were able to maintain their hold on the surface of the recovery bath after gently rocking it for 10 sec. Benzocaine solutions and the seawater in the recovery baths was kept within about a degree of initial ambient temperature, 19^{o}C throughout the experiment.

7.4.2. Results and discussion

The removal of small juvenile abalone from standard settlement plates differed with dose and was consistent among exposure times (Table A5). And the removal of juveniles was greater than 90% for benzocaine concentrations of 1mgl^{-1} and greater (Figure 5). Recovery after 30 min differed with exposure times for different doses (Table A6), with the highest dose of benzocaine indicating the slowest recovery periods when juveniles were exposed for 10 or 20 min (Figure 6). Recovery after 18 h differed with dose but not exposure time (Table A7). More than 90% of individuals all treatments had recovered after 18 h (Figure 7). Recovery of individuals from the 1 mg l^{-1} treatment was greater than that for the control or high dose treatment (Figure 7) and this treatment was also as effective as higher concentrations in removing individuals over short periods of time (Figure 5). This implies that this treatment is the most appropriate for removing small (1-2 mm) juveniles from settlement plates.

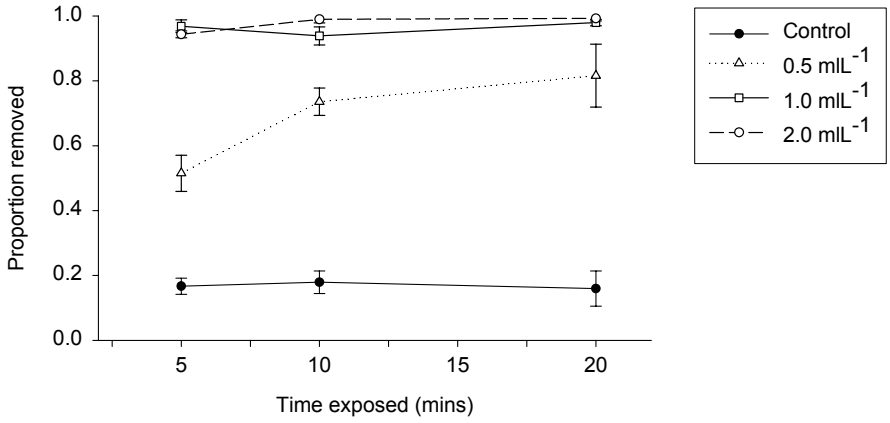


Figure 5. Mean proportion of abalone (± SE) removed from settlement plates for 3 concentrations of benzocaine and a control (seawater), for 3 exposure times (n = 3).

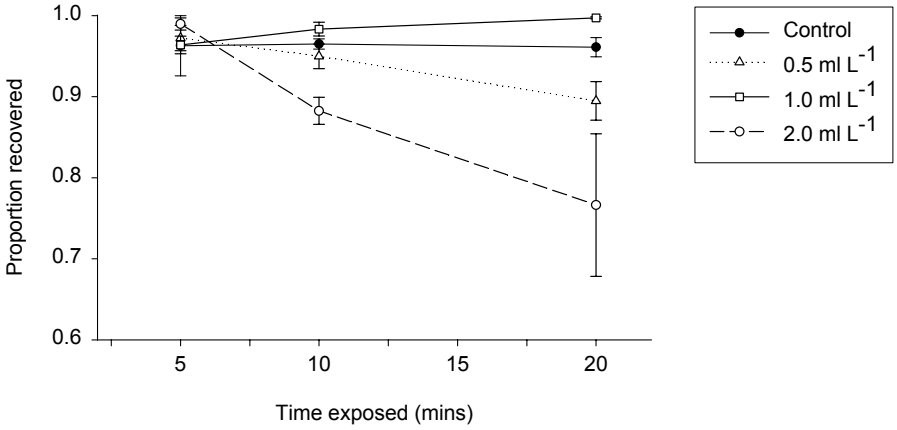


Figure 6. Mean proportion of abalone (± SE) recovered after 30 min after removal from settlement plates using 3 concentrations of benzocaine and a control (seawater) at 3 exposure times (n = 3).

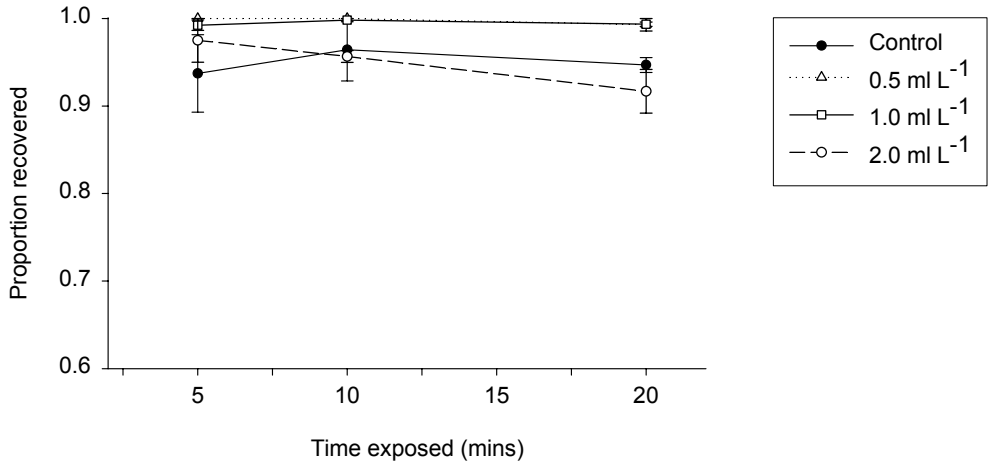


Figure 7. Mean proportion of abalone (± SE) recovered after 18 h after removal from settlement plates using 3 concentrations of benzocaine and a control (seawater) at 3 exposure times (n = 3).

7.4.2.1. Deployment of juvenile abalone

A variety of methods were investigated for the deployment of juveniles in the field. The preferred method for deployment, as with storage and transportation discussed above, was dependent on the size of the juveniles at release. For the release of small (<8 mm) juveniles that might benefit from a food source or extra shelter associated with CCA-coated rocks the preferred deployment device was groups of CCA-coated rocks enclosed within wire mesh tubes. The preferred deployment device for larger juveniles was more easily constructed from unconditioned PVC piping to encourage their rapid movement out of the device into natural habitats.

The deployment device for small juveniles (<8 mm) consisted of 300 mm long, 120 mm diameter, 10 mm square mesh wire tubes filled with CCA-coated rocks (<40 mm diameter) clamped at each end. These devices could support up to 2000 small juveniles during deployment to natural reef. Juveniles could be placed on the devices by hand and they could redistribute themselves within the shelter of the rocks. The CCA-coated rocks provided the juveniles with a suitable substrate on which they might feed and shelter after release. Experiments were designed to investigate the time over which these small juveniles would move off this type of device and into the surrounding habitat. An experiment was designed to investigate the rate that 600 small (~ 2 mm) juveniles left the deployment device and moved into surrounding habitat. A second experiment was designed to investigate the rate that 1800 larger (~7 mm) juveniles left the deployment device and moved into surrounding habitat. In each experiment the number of juveniles on replicate devices was sampled prior to the deployment to natural reefs, and at several times after deployment. At each time, devices were collected from the reefs in plastic bags, then soaked in 70% alcohol for 5 min and searched for all remaining juveniles.

The numbers of juvenile abalone on the deployment devices steadily declined over periods of up to 60 days. Within 1 day of deployment, the number of juveniles on devices declined 40-60% (Figures 8 & 9). In the first experiment the number of juveniles remaining on a device after 10 days was on average 3% of the original 600 (Figure 8). In the second experiment, approximately 20% of the original 1800 juveniles remained on each deployment device after 10 days (Figure 9). Reductions in the number of juveniles on each device are a combination of movement off the device to surrounding habitat and any mortality on the devices. Regardless, many juveniles were observed in surrounding habitats, suggesting that these devices can deploy large numbers of small juveniles to natural reefs. Despite the potential benefits of this type of deployment device there were several logistical constraints to its use. The device required juveniles to be extensively handled during transport in mesh bags and introduction to the device. Further, by using natural CCA-coated rocks in these devices there is the potential to expose juveniles to associated predators and translocate additional flora and fauna to release sites. Finally, extensive effort is required to collect, handle and transport the large weight of CCA-coated rocks required to deploy large batches of juveniles.

An alternative deployment device was developed that was lighter in weight, could be recovered and reused, and could hold considerably greater numbers and larger sizes of juveniles. This device consisted of a 300 mm length of 150 mm x 65 mm PVC tubing with three 30 mm holes cut into the upper surface and 2 mm square mesh panels secured over the open ends. These holes and covered ends allow the flow of water through the device to facilitate recovery of juveniles after anaesthesia, and control the movement of juveniles from the device. The movement of abalone from these devices during recovery from anaesthesia and storage prior to deployment was restricted by the use of bright lights above the devices. The open structure and wide flat base allowed the introduction of juveniles with minimal handling. Over 1000, 10 mm juvenile, per device were successfully stored, transported and released using this method. The device is relatively light and easily stacked for transport, and slightly malleable, allowing it to be securely wedged into cracks or under boulders within a release site.

The simple PVC design and the methods of handling for storage and transport of juveniles resulted in negligible rates of mortality and very low rates of movement from the device prior to deployment. Results presented below (see Sampling boulders and Visual surveys) indicate that juvenile *H. rubra* were able to rapidly disperse from these devices into the surrounding habitat. Factors to be considered using these devices include their potential to be transported from sites and lost if they are not adequately secured and the necessity to retrieve them after use.

7.4.2.2. Transport and storage of juvenile abalone

The preferred method for the storage and transport of juveniles was related to the method of deployment, which in turn depended on the size of the juveniles at release. For storage and transport of small (<8 mm) juveniles the use of fine mesh bags on damp sponge in a cool (14°C) insulated container was preferred (Section 5). This method was chosen because of the potential for mortality if they were transported on the deployment devices used for larger juveniles (CCA-coated rocks within mesh tubes, see below). For large (>8 mm) juveniles storage and transportation was done within the deployment device consisting of a PVC tube (see below) that minimised handling, including their removal from mesh bags after storage and transport that can result in injury to the foot and shell (pers. observation).

Juvenile *H. rubra* produced in the hatchery at Port Stephens needed to be stored and transported for release on reefs in southern NSW. This process is likely to require at least one day of travel and holding overnight prior to release. It may also be necessary to hold juveniles for longer periods if weather conditions do not allow immediate deployment. An experiment was designed to investigate two methods of storing juveniles and their effects on subsequent survival. Juveniles (>8 mm) were anaesthetised, weighed and divided into equal groups, enabling three replicates of each treatment with ~1000 individuals in each. The two treatments consisted of anaesthetised juveniles either being placed directly into a replicate plastic deployment device or fine mesh bag (see below). Juveniles within these treatments were then held in a flow through seawater tank for 12 h, at ambient temperature (18-20°C), for them to recover from anaesthesia. After this recovery time, replicates from both treatments were relocated to a seawater tank at 16°C for 45 min. Replicates were then placed between wet foam sheets in a plastic bag held in a 25 L insulated container containing an ice brick. The plastic bag was then filled with oxygen and sealed, then the lid on the container was sealed and the container stored at 18-20°C for 10 h. After this storage time the replicate groups of juveniles were removed and those in mesh bags were placed in plastic deployment devices. Deployment devices from both treatments were then placed into seawater tanks and held for an additional 12 h. The number of juveniles in each replicate that were not attached to the surface of the devices after 12 and 48 h was recorded. Results from this trial indicated that >95% of the juvenile abalone treated using either of these storage methods survived.

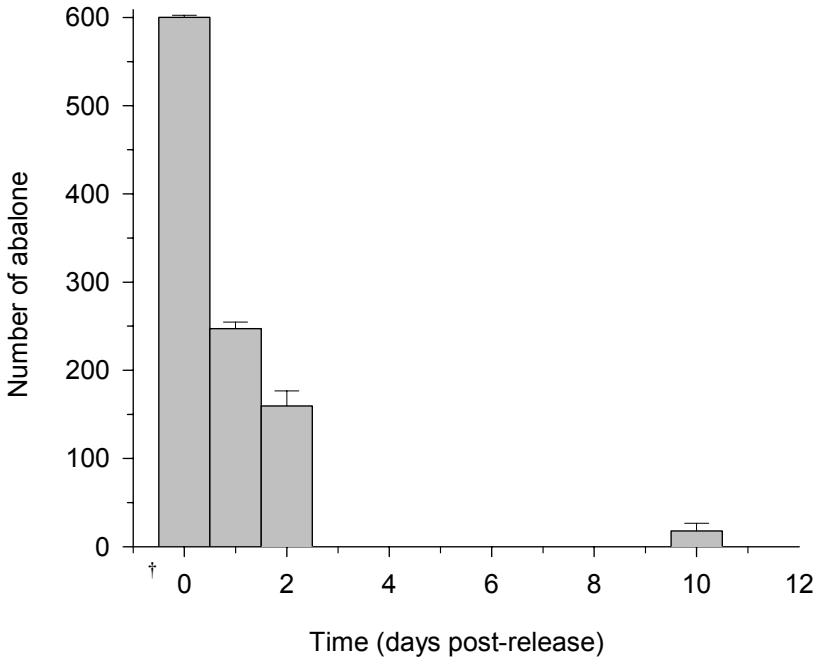


Figure 8. Mean number of 2 mm juvenile abalone (± SE) on deployment devices through time. † 5 devices sampled in the laboratory prior to deployment otherwise $n = 3$.

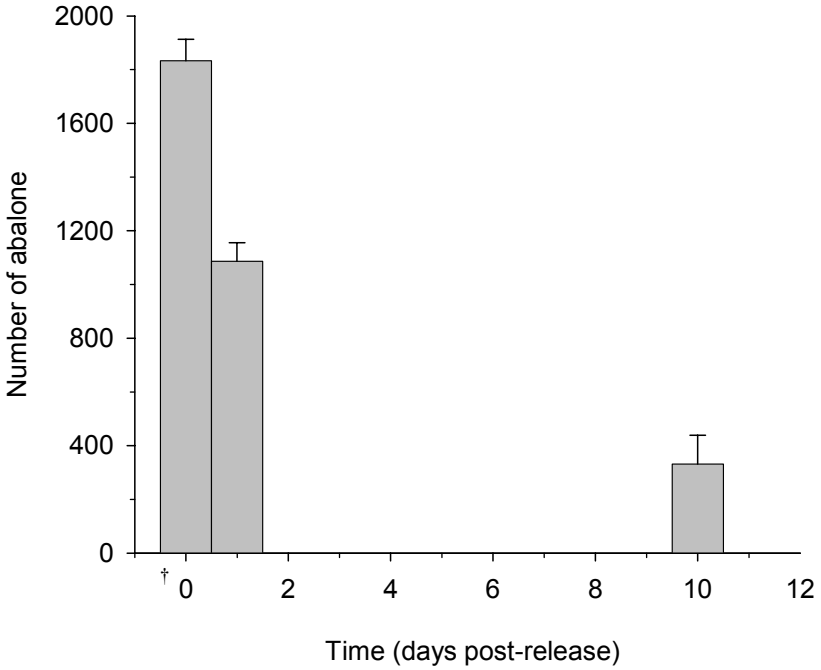


Figure 9. Mean number of 7 mm juvenile abalone (± SE) on deployment devices through time. † 5 devices sampled in the laboratory prior to deployment otherwise $n = 5$.

7.5. Improved laboratory methodology to assess survival on small juveniles in the field

Sampling methods used to assess the settlement of larvae or survival of newly settled or small juveniles produced large quantities of material to be sorted in the laboratory. All samples collected at field sites by either settlement plates, the venturi suction sampler or the collection of boulders (see below, for newly settled larvae or small juveniles) was sorted under a binocular microscope. Excess alcohol in the samples was poured off through an 80 µm sieve with any contents added to the sorting tray. The sample, or small volumes of the sample until it was completed, were successively emptied into a glass dish, scored with a 10 mm grid, and meticulously sorted through using fine dissection equipment. All larvae or similar items were removed from the sample and stored separately in a glass vial for confirmation of identification as an abalone larvae or early juvenile. All samples were stored in 70% alcohol. The complete contents of the sorted sample was stored in the original, labelled jar and the corresponding vial containing the larvae and or juvenile abalone, in 70% alcohol was sealed and stored in the original jar. The separation and storage of potential abalone larvae and or juveniles into the vial allowed confirmation of the contents of the vial and re-sampling of the whole sample by more experienced sorters, if necessary.

Samples collected using either the venturi suction sampler or by the collection and washing of boulders (see below) contained up to 200 g of sediment and other material. These samples could take between 6 and 10 h to sort using the methods described above. The majority of the samples consisted of very fine material, sand and fragments of algae and other detritus, from which newly settled larvae had to be sorted and counted. In an attempt to reduce this excess material, without compromising the integrity of the samples, a method of elutriation was tested using samples containing sand and fine material, spiked with known numbers of newly settled larval and early juvenile abalone.

Samples containing 15 g of sand and finer sediment were made up with known numbers of one of two alternative size classes of early juveniles i.e. 0.5<1 mm and 2<4 mm. Two treatments were made using the smaller size class. The first had a known high (~ 100) number of small juveniles and the second a known low (~ 20) number. One treatment was made up using the larger size class namely a known number of approximately 50. Three replicates were used in each treatment. Each replicate was elutriated prior to sorting. The treatment type and exact numbers of abalone in each replicate were unknown by the sorter. The method of elutriation notably reduced the time to sort samples in the laboratory. The mean ($\pm$ SE) percent of the small abalone recovered in low abundance treatment was 83 ($\pm$ 2), whilst the recovery from the high abundance treatment was >96 ($\pm$ 2). Results of the mean ($\pm$ SE) percent of larger sized individuals recovered, where a known number of ~ 50 individuals were placed in each replicate was 99 ($\pm$ 1).

Elutriation of these experimental samples prior to sorting removed a great deal of grit and sediment whilst not substantially reducing the numbers of abalone. Sorting time was reduced from between 6-10 h to 2.5-3.5 h per sample. The elutriation of samples collected using either the suction sampler or from boulders that contained large quantities of sediment (as determined by the sorter) was adopted as part of the standard laboratory sampling protocol.

7.6. Identification of released larvae and juveniles

7.6.1. Introduction and methods

To assess methods of releasing and monitoring released larval and juvenile abalone and to accurately estimate their survival through time it was necessary to tag each individual released from the hatchery. Positive identification of individuals released from the hatchery was required so as not to confound estimates of survival of those released through natural recruits or existing abalone from the wild population. A simple and reliable method of marking hatchery larvae prior to release was therefore developed and routinely applied. The need to develop a similar method for hatchery reared juveniles was unnecessary as the use of an artificial diet used in the hatchery from the point of weaning resulted in the development of a distinct blue green coloured shell that readily distinguished hatchery reared juveniles from wild counterparts.

An experiment was done to investigate the effect of immersing 7-day-old larvae in two concentrations of the epi-fluorescent dye calcein for two periods of time on the overall competency of larvae to survive and on the durability of this tagging technique. This technique was also repeated on the same individuals to investigate its potential for multiple marking of larvae and juveniles. Seven-day-old larvae were immersed in seawater with calcein at concentrations of either 50 or 100 mg l^{-1} for periods of either 24 or 48 h. In addition, 112 days later sub-samples of juveniles from these treatments were subjected to the same marking procedure for a second time. In all cases seawater was buffered with sodium bicarbonate at 100 mg l^{-1} prior to the addition of calcein and the temperature of the seawater baths used to treat larvae and juveniles was maintained at approximately 19°C.

Once the two larval baths were set up, each with the appropriate calcein concentration, larvae were added at densities of 100 mL^{-1}. After 24 and 48 h exposure, approximately 3000 larvae were added to one of each of the 4 settlement tubs containing 12 settlement plates, from each of the corresponding tagging treatments. After 75 days juvenile abalone remaining on the settlement plates in each treatment tub were removed using benzocaine and placed back in the respective tubs and weaned onto formulated food. At each of the 15, 23, 30, 37, 56, 75, 97, 120 and 260 days after larval labelling and the 8 and 148 days after re-labelling as juveniles approximately 50 abalone were removed from each of the four tubs and stored in 70% alcohol for subsequent assessment of the fluorescent mark in the shell of a proportion of those abalone removed. All samples were assessed 329 days after larval labelling. Assessment was made on whole preserved abalone under a binocular microscope using a filtered UV light source. The level of irradiance of the larval shell in the spire of 20 juveniles removed from each replicate of each calcein treatment at each sampling time was scored on a visual index from 0 to 4. This index was objective with a score of 0 indicating little or no discernable irradiance, without a positive indication of a larval shell, and a score of 4 indicating bright and obvious irradiance with clear definition of the larval shell in the spire.

7.6.2. Results and discussion

The calcein mark in the larval shell was visible under binocular microscope, using a filtered UV light source, in all juveniles examined over the time of the experiment (Figure 1). Although the larval shell could be easily distinguished in most of the abalone treated at the lowest concentration of calcein i.e. 50 mg/l and shortest soak time i.e. 24 hrs, occasional examples were faint (scoring only 1). The calcein marks in larval shells treated at the highest concentration, 100 mg/l for the same period of time were more obvious. Larval shells treated at either the low or high concentration for 48 h were more clearly marked again than those treated at 100 mg/l for 24 h. The marked larval shell of individuals treated at either concentration for 48 h did not provide any reliable or consistent difference in the level of irradiance (Figure 1).

Additional calcein marks were clearly visible in the shells of all treated juveniles at both 8 and 148 days after the secondary treatment regardless of the dose or duration of exposure to calcein. The growing edge of all shells were clearly marked, as were other areas of shell growth such as around the respiratory pores, and this irradiance could be clearly seen without the aid of a microscope. Occasionally individuals also had higher levels of irradiance in the spire, potentially affecting the readings of irradiance from the initial labelling as larvae.

A reliable mark in the shell of recaptured, newly settled larvae or juveniles is required to positively determine the origin of the individual i.e. that individual was initially released from the hatchery. That marks could be seen in 100% of abalone treated both as larvae and juveniles makes calcein labelling a potentially valuable tool for reseeding. A consistent mark in the spire of shells of juvenile abalone recaptured after more than 540 days provides additional support to this marking procedure (Plates 1a & b). Moreover, with regard to identifying released batches of juveniles, there is the likelihood that this labelling method provides a means of distinctively marking individual groups of juvenile abalone, potentially with multiple marks, prior to their release, allowing different release groups to be monitored within the same site.

It is likely that live juveniles could be identified in the field, with the use of an appropriate UV source as samples require very little preparation and the level of irradiance seems to be high up to 196 days after marking in the laboratory and supporting evidence providing the identification of the larval shell in the spire of juveniles after over 500 days of being released.

Juveniles that had been weaned and reared for a short period of time on a formulated diet in the hatchery were clearly identifiable from abalone of wild origin through the development of a distinctive blue/green shell (Plate 2). It is possible that the development of this conspicuous shell colour could reduce predator avoidance or increase their susceptibility to visual predators, which could have significant implications on the use of this method to enhance local abalone populations. It is also possible however that this distinctive identification could have little effect on the survival of juveniles if they are deployed to areas of reef and their exposure to visual predators is limited until they disperse to more cryptic habitats. It is also possible that such conspicuous colouration could be minimised or eliminated by supplementing artificial diets with natural food sources or with specific additions to this artificial diet.

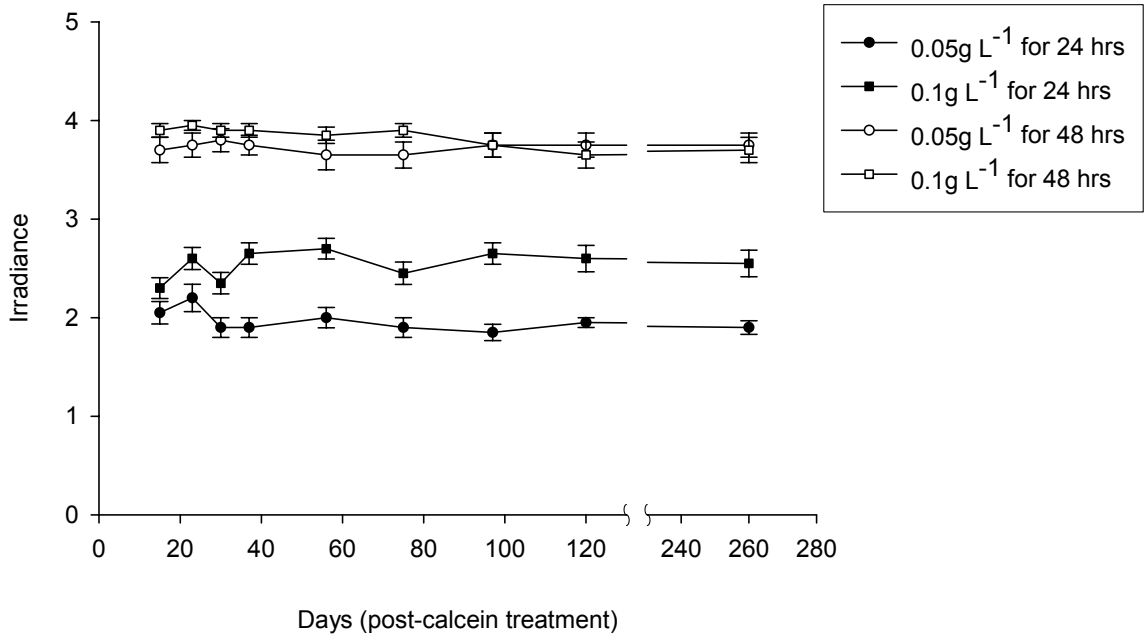


Figure 1. Mean level of irradiance of the larval shell (± SE) in the spire of juvenile abalone for each calcein treatment at periods of time after being labelled as larvae (n = 20).

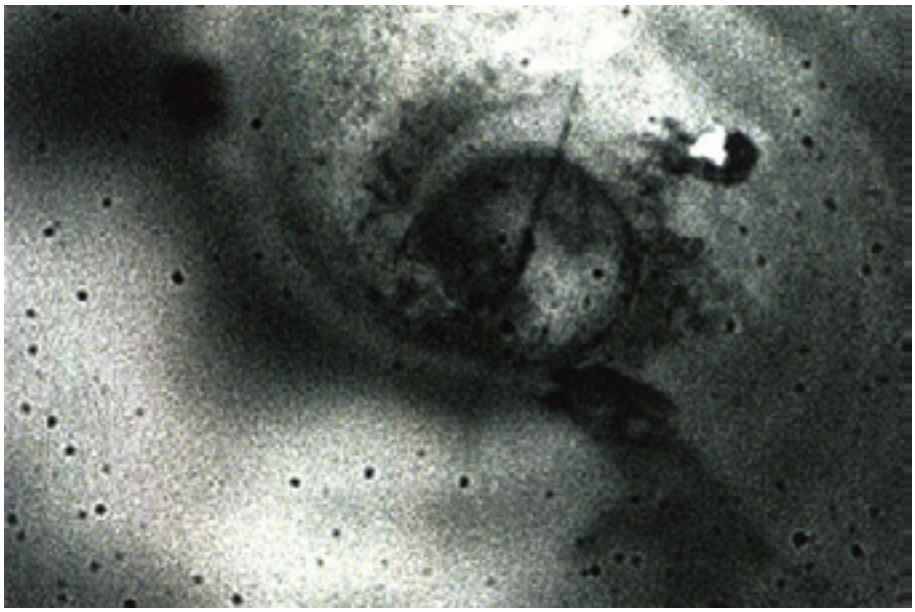


Plate 1a. View of the spire of an abalone, under incident light (40x), recaptured 540 days after release as a larva.

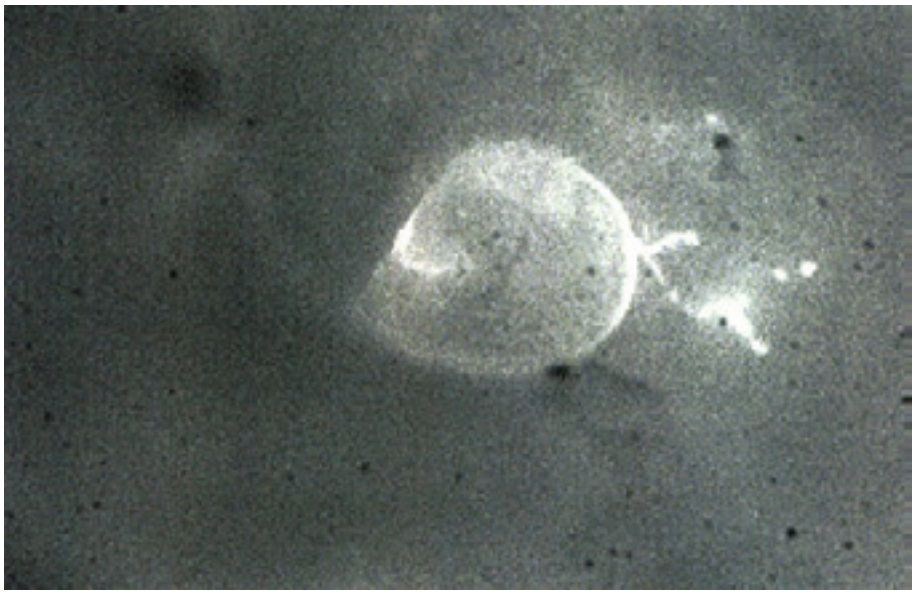


Plate 1b. View of the spire of the abalone shown in Plate 1a, under filtered UV light (40x), showing the calcein labelled larval shell in the spire of the juvenile shell.

Plate 2. Selection of juvenile abalone recaptured after 463 days showing the distinctive blue/green coloured shell, a consequence of artificial diet in the hatchery, and natural shell growth after release.

7.7. Monitoring settlement and early survival of abalone larvae and juveniles

7.7.1. Methods (also see Table 1)

7.7.1.1. Settlement plates

The settlement of released larvae can be assessed in the field by the use of standard conditioned settlement plates. We used plastic sheets (350 x 250 x 3 mm) that were held in aerated, flow through seawater for a period of approximately 4-6 weeks. These plates had small holes drilled in them and were weighted and evenly dispersed within the release site by divers. Following release of the larvae, plates were placed into a plastic bag, then both were rinsed and gently brushed in 70% alcohol. The sample was then washed through a 180 µm sieve, and stored in 70% alcohol prior to identification and counting of settled larvae with a binocular microscope.

The use of settlement plates as a tool to indicate larval settlement and early survival at field release sites was initially tested at one site and subsequently used during the release of a batch of larvae at a following 3 sites. This subsequent release used standard settlement plates and plates that had been conditioned and then grazed by juvenile abalone prior to their dispersal at the release sites.

7.7.1.2. Venturi sampler

A venturi sampler can be used to estimate the abundance of newly settled larvae and juveniles (e.g. <1 mm; McShane & Smith 1988). The venturi sampler used here (Plate 1) consisted of a 1500 mm length of 40 mm diameter PVC tube. A high pressure hose fitting with a ball valve was fitted near the lower end of the tube to allow the introduction of compressed air to generate airlift suction. A length of 45 mm diameter flexible hose with brush attachment was fitted over the lower end of the tube. At the opposite end, a mesh (200 µm) bag was used to retain the material lifted after gently brushing the substrate with the air rising through the tube. Replicate, 250 x 250 mm quadrats were haphazardly placed within the release site and sampled using the venturi lift. A quadrat was not sampled if the area lying within it contained more than a sparse, single layer of sediment or if it contained foliose or geniculate alga. Each quadrat was sampled for 30 seconds and within that time the quadrat was covered with ~2 passes of the brush. Following sampling, the mesh bag was removed from the tube and placed inside a labelled plastic jar. Each mesh bag and jar was then rinsed with 70% alcohol. The sample was washed through a 180 µm sieve, and stored in 70% alcohol prior to identification and counting of newly settled larvae and or small juveniles with a binocular microscope.

An experiment was designed to investigate the sampling selectivity of the venturi sampler at different depths. Approximately thirty small (~2 mm) juveniles were placed on each of 15 replicate standard substrates (i.e. large concrete pavers) and left to recover for a period of 4 h. These pavers were then transported to the field, and groups of five were placed at depths of 2.5, 5 and 8 m. A diver counted the number of juveniles on each substrate prior to sampling with the venturi sampler for 30 sec. Following sampling, the number of juveniles remaining on each substrate was again counted. In addition, abalone within each of the mesh sampling bags associated with each replicate from each depth were counted to assure no abalone were lost from the replicate. Samples taken from replicates at each depth were grouped through time (i.e. 2 from each depth then 3) to reduce any chance of time confounding the effects of depth.

An experiment was also designed to investigate the use of the venturi sampler to estimate the abundance small (<2 mm) juveniles through time and to determine the rate at which these small juveniles disperse from their wire mesh and CCA-coated rock deployment devices over bedrock consisting of large expanses of flat reef interspersed with small fissures. Twenty deployment

devices were made up and each had approximately 600 individual abalone, at an average length of 1.5 mm placed on it. After an 18 hour period, to allow the small juveniles to recover from anaesthetic and to disperse through the devices, the devices were placed in cracks and fissures on an area of reef consisting of flat bedrock interspersed with small fissures and a uniform coverage of large brown foliose algae (*Eklonia radiata*), at a depth of approximately 6 m. Each device was separated by approximately 5 m. Sampling was done 1, 2, 10 and 40 days after their deployment. At each sampling time, two areas of reef surrounding each of 3 deployment devices were sampled with the venturi sampler. The first area of reef was within a 300 mm radius of the device and the second area was between 300-600 mm from the device. At each sampling time three replicate quadrats were sampled in each of the inner and outer areas surrounding each of three replicate deployment devices.

This venturi sampler method was also investigated to determine the abundance of newly settled larvae and early juveniles and to compare these estimates with an alternative method of boulder collection (see below, Sampling boulders - Boulder collection).

Plate 1. The venturi sampler consisting of a 1500 mm length of 40 mm diameter PVC tube (orange tube), high pressure hose fitting with ball valve switch near the lower end of the tube, length of 45 mm diameter, flexible hose (grey hose) with brush attachment and at the opposite end of the orange tube, the mesh (180 µm) sample bag with velcro strap.

7.7.1.3. Sampling boulders

A variety of methods of sampling boulders have been used to estimate the abundance of abalone from newly settled larvae through to large juveniles (e.g. Prince & Ford 1985). These include the collection and washing of individual boulders, the non-destructive visual inspection of individual boulders *in situ* by gently lifting them to sample underneath, and the destructive sampling of fixed areas within boulder habitats. Each of these methods were used to address different questions and for development of a strategy for estimating the abundance of abalone at a variety of sizes from settlement onwards. A number of experiments were done to investigate the use of these boulder sampling methods to estimate the numbers of newly settled and small juvenile abalone and the efficiently of visually inspecting boulders *in situ*, as a means of estimating the abundance of large juveniles. Boulders sampled within each of either the collection or non-destructive methods had a maximum diameter of 300mm.

Boulder collection

The collection and washing of small boulders can be used as a method of estimating the abundance of newly settled larvae and small juveniles. Boulders were collected from within the top layer of boulders only, for habitats containing more than a single layer, and placed in plastic bags by divers, and both the bag and boulder were rinsed and gently brushed in 70% alcohol. The sample was then washed through a 180 μm sieve, and all particulate matter caught on the sieve was stored in 70% alcohol prior to identification and counting of larvae and/or juveniles with the use of a binocular microscope.

In the first experiment designed to compare estimates of abundance of newly settled larvae and early juveniles using the boulder collection method with the venturi sampler, approximately 1.1 million larvae were released to 3 sites each approximately 375 m^{-2}, primarily consisting of boulder habitat. Boulders were collected and venturi samples were done 1, 2, 14 and 56 days after the release of larvae. The samples were sorted as described previously, and the number of newly settled larvae or small juveniles counted.

Non-destructive boulder sampling

The use of non-destructive sampling of small boulders within field sites was also assessed as a method for estimating survival and growth of large juveniles. This method of non-destructively sampling juvenile abalone involved the visual inspection of boulders *in situ*, from within the top layer of boulders only, for habitats containing more than a single layer.

An experiment was done to investigate the efficiency of the *in situ*, non-destructive boulder sampling method on 3 size classes of juvenile abalone. The release of 3 different sizes of juvenile abalone to similar boulder field habitat was done at 5 sites for each size. The experiment was done with the assumption that juvenile abalone of these size classes experienced no mortality after being released for only 2 h. Moreover, for the 2 smaller sizes this assumption was extended for an additional 16 h with the added assumption that the disturbance to the habitat (not the individuals) during original sampling time did not effect their dispersal. Each size class of juvenile abalone was divided into 5 equal portions in the laboratory and held in mesh bags in seawater. At each of 5 sites, for each size class of juvenile, one of each of the mesh bags were emptied within an area of 1m from a central release point. Eight hundred 7.5 mm juveniles and seven hundred 11.5 mm and 13.5 mm juveniles were used at each of the 5 sites.

An experiment also investigated the use of this non-destructive boulder sampling method to assess the rate of dispersal of small juveniles (<8 mm) from wire mesh and CCA-coated rock deployment devices. Approximately 1800 juveniles were settled on each of 40 CCA rock deployment devices in the laboratory. After a period of 18 h, to allow the juveniles to recover from anaesthesia and to disperse through the devices, these devices were deployed to a field site

where the primary habitat was boulders. Each device, separated by at least 8 m, was placed by divers within the defined site. At each of the first 2 sampling times (1 and 10 days post-release) 3 boulders were collected from an area within 0-1 m, and 1-3 m of each of 5 devices. For successive sampling periods (>10 days) divers counted the number of released abalone on each of the 3 boulders sampled within each area before replacing it. Total numbers of abalone were calculated based on the mean number of boulders counted per m^2 and multiplying the mean number of juveniles per boulder to get a mean density within the area sampled.

Destructive habitat sampling

The destructive sampling of boulder habitats was done to provide estimates of the absolute abundance of juveniles. This method targeted large juvenile abalone and consisted of the systematic removal and inspection of each of the boulders within a designated area within the release site. Abalone within the designated area were identified as of hatchery origin or wild (see below - Identifying released larvae and juveniles) and the number per area counted.

7.7.1.4. Visual surveys

Visual surveys are commonly used to assess the relative abundance of wild abalone populations (Worthington *et. al.* 2001). And visual surveys have been used as a means of estimating juvenile abundance, although limitations of these methods are well publicised (Prince & Ford 1985; McShane & Smith 1988; Shepherd 1998). These survey methods, despite their limitations, do provide a means of obtaining repeatable measures of the abundance of abalone, particularly those greater than 20 mm in length (Prince & Ford 1985) without disturbing the habitat or the abalone.

Visual transects were used at field sites after the release of larvae and juveniles in an attempt to obtain estimates of the abundance of abalone estimated to be greater than 40 mm in length. Transects within release sites consisted of a diver intensively searching the habitat within a 5 x 0.25 m area without disturbing any of the habitat.

An experiment was designed to investigate the efficiency of two visual survey methods to assess the abundance and distribution of large (11 mm) juvenile abalone. This experiment particularly focused on investigating the rate and maximum dispersal of these abalone from a central release point, over a very short period of time compared with the time scales over which most juvenile releases would be assessed. Moreover, the sampling strategy identified a number of spatial scales within sites to gain resolution on the rate of dispersal from the release point. Approximately 700, 11 mm juvenile abalone were placed in each of 24 plastic deployment devices and allowed to recover prior to being deployed to field sites. At each of 3 sites, separated by approximately 150 m, 8 devices were placed within a central area of 1 m^2, and marked with a submerged float. The sampling methods investigated included incremented spoke-transects and multiple small quadrats within increments from the release point. At each sampling time, at each site, four 10 x 0.5 m long transects were run from the centre of the release point. Along each of these transects, for successive increments of 0-2, 2-3, 3-5 and 5-10 m the number of released abalone were counted. At each site and for each sampling time the number of juveniles were counted if less than 100, or estimated to the nearest 50 individuals if more than 100. In addition, five 0.25 m^2 quadrats were sampled within each increment of each transect with the exception of the first increment, where only 5 quadrats were sampled per time. Quadrats were randomly placed within increments along the transect line, within 0.5m of the line. Sampling was done 1, 4 and 8 days after release.

Table 1. Outline of field experiments to investigate sampling methods and juvenile dispersal.

Sample method	Size at release (mm)	Habitat	Times sampled	Days after release	Sites	Replicates	Distances sampled from release point (m) 0 0.3 0.6 1 2 3 5 10
Venturi-lift							
Venturi-lift	2	Pavers	1	-	1	5	
Venturi-lift	1.5	Bedrock	4	1, 2, 10, 40	1	3	
Venturi-lift & boulder coll.	see below						
Sampling boulders							
Boulder coll. & Venturi-lift	Larvae	Boulders	4	1, 2, 14, 56	3	5	
Non-destructive	2	Boulders	2	2hr, 18hr	1	5	
Non-destructive	7.5	Boulders	2	2hr, 18hr	1	5	
Non-destructive	12	Boulders	2	2hr	1	5	
Non-destructive	7.5	Boulders	4	1, 10, 30, 60	1	5	
Visual surveys							
Transects	11	Solid boulders	3	1, 4, 8	3	4	
Quadrats	11	Solid boulders	3	1, 4, 8	3	5, 20	

7.7.2. Results and discussion

7.7.2.1. Settlement plates

Settlement plates used at field sites showed low and highly variable settlement within the short sampling periods after the release of larvae. This variation in settlement is likely to be significantly influenced by the variation in the assemblages of diatoms and algae on the settlement plates. It is quite likely that the composition of diatoms on these plates was not the same at the time of each of the larval releases. However, a comparison between and among sites where larvae were released for the second experiment would not have been confounded as the plates were all conditioned at the same time. It is unfortunate however that there was such low settlement of larvae on these plates across each of the treatments in each of the sites.

Settlement plates within release sites do have the potential to provide a clean, quick method of assessing settlement success. However, it is likely that this method of assessing early settlement and survival within and among sites would require a high level of replication to obtain meaningful results. Also, the variation between plates would likely be reduced if they could be conditioned with a consistent and reliable assemblage of algae known to induce the settlement of *H. rubra* larvae, prior to their deployment within release sites.

7.7.2.2. Venturi sampler

Depth does significantly affect the reliability of this sampling method (Table A8). At 2.5 m an average of only 68% of juveniles were removed from the substrate compared with an average of 92% at 8.5 m (Figure 1). It is likely that using this method in relatively shallow water will result in the under sampling of individuals from the substrate by 25-30% with this percentage decreasing with depth. It does seem likely however that this dramatic effect of depth would not be as pronounced if attempting to sample smaller animals as they have a reduced physical ability to maintain a hold on the substrate.

It is also evident that small juveniles can rapidly disperse from CCA-coated rock deployment devices into the surrounding habitat, with small juveniles moving between 300-600 mm from devices within the first 24 h (Figure 2a). Although numbers of juvenile abalone are relatively low, there is an indication that movement from these devices may occur within the first 10 days and that juveniles either immigrate from the sampling area within 40 days of being deployed, suffer high mortality or this particular method of sampling these juveniles is less effective as they grow and move into more cryptic habitat (Figure 2). The venturi sampler provides a method of sampling newly settled and small juvenile abalone (<2 mm). In addition, this method is more reliable at providing accurate estimates of the abundance of these sizes of abalone on habitat that would otherwise be very difficult to reliably sample (McShane & Smith 1988, this study). It is however necessary to understand the limitations of this method and it does have the potential to under estimate the numbers of small juveniles either due to its reduced effectiveness in shallow water or as a consequence of the size of the juveniles sampled.

7.7.2.3. Sampling boulders

Boulder collection

The highest numbers of settled larvae peaked 2 days after their release for each of the boulder collection and venturi sampler method at each site, with the exception of the venturi sampler method at site 3 (Figure 3). Higher numbers of larvae were collected from boulders compared with samples taken using the venturi lift for sampling times within the first 14 days after the release of larvae, with the exception of the first sampling time at site 3 (Figure 3). The number of newly settled juveniles rapidly declined 2 days after their release at all three sites. Despite the relatively high variation associated with these sampling methods they do provide a means of determining early settlement and survival of released larvae. Boulder collection provides a more reliable method of assessing early settlement and survival compared with the venturi sampler, when either method may be used. It is likely that the complex three dimensional boulder habitat and shallow –3 - 4 m) depth at these sites could have limited the efficiency of these methods to assess the abundance of early juvenile abundance more than 56 days after being released as larvae.

Non-destructive boulder sampling

The non-destructive sampling of small boulders in situ does provide a means of assessing the abundance of large juveniles (>8 mm) and larger size abalone without destructively sampling the habitat.

Results of the census of the area within 1 m, and between 1-2 m of the release point of these juveniles 2 h after release, indicates that juveniles ≤8 mm do not disperse beyond 1m, with the exception of 5% of juveniles at distance 2, site 3 (Figure 4a & c). Moreover, ≥95% of the total number of the two size classes ≤8 mm were accounted for using this sampling method, in consideration of the assumptions stated earlier (Methods, Sampling boulders - Non-destructive boulder sampling). After a period of 18 h however, on average only 52% of juveniles ≤8 mm could be accounted for within 2 m of the central release point (Figure 4b & d). Larger (12mm) juvenile abalone did move beyond the initial release area within a 2 h period with an average of only 81% of these juveniles accounted for within 2 m of the central release point (Figure 4e). These results indicate that this sampling method is capable of assessing the abundance of juvenile abalone.

Movement of small (≤8 mm) juveniles from devices to the surrounding areas of between 0-1 m and 1-3 m can be very quick (1 day) (Figure 5). Moreover, this rate of dispersal continues as can be seen by the numbers of released juveniles increasing in the 1-3 m area within 1-9 days (Figure 5). Numbers of juveniles within each of the 2 areas sampled around the devices decreased through time as either actual numbers of the juveniles decrease due to processes affecting survival or as these juveniles move beyond the sampling area. It is likely that the decrease in numbers over these time periods is a factor of both of these scenarios. Unfortunately, the relatively small spatial scales applied to sampling in each of these experiments to assess efficacy of these survey methods were not adequate to determine rates of dispersal over periods of days for juveniles of this size.

7.7.2.4. Visual surveys

Visual surveys provide a means of assessing the relative abundance of early juvenile abalone through to adult size whilst obtaining information on the distance these juveniles can move without disturbing either the individual or habitat. The largest estimate of abundance was provided by the radial transect method 1 day post-release at site 1 and was slightly more than 3000 individuals i.e. 26.3% of the total number released (Figure 6a). The highest estimate of abundance provided by the small quadrats was made 1 day post-release, again at site 1 and was just over 1300 individuals i.e. 19.3% of the total number released (Figure 7a). Juveniles released at each site had dispersed up to 5 m from the release point after 4 days (Figures 6 & 7), with the only exception of site 1 where

juveniles were detected only as far as 3 m from the release point (Figure 7b). After 8 days the numbers of abalone assessed using the radial transect method, within each increment, became more consistent, however the highest numbers were still recorded within 2 m of the release point.

Results of this experiment provide strong evidence that these visual survey methods can detect juvenile abalone in complex habitat, unsuited to alternative sampling methods discussed above, and provide relative measures of abundance. They also show that juveniles of this size class can disperse over 5-10 m in complex habitat within short periods of time (Figures 6c & 7c). Moreover, results from this experiment imply that both of these visual sampling methods under estimate the total abundance of juvenile abalone within this complex habitat (i.e. a habitat where large boulders are piled upon one-another and cemented together) or, perhaps a less likely scenario, that these juveniles experienced very high mortality within the first 24 h of release. If it can be assumed that there is negligible mortality within 24 h of release it is likely that these visual assessment methods underestimate the actual total abundance by up to 80%. Using information from earlier experiments investigating the efficiency of alternative sampling methods in conjunction with additional experiments aimed at validating specific assumptions, it may be possible to develop an index based on these relatively quick and simple visual assessment methods, to reliably estimate the total abundance of abalone in this type of habitat.

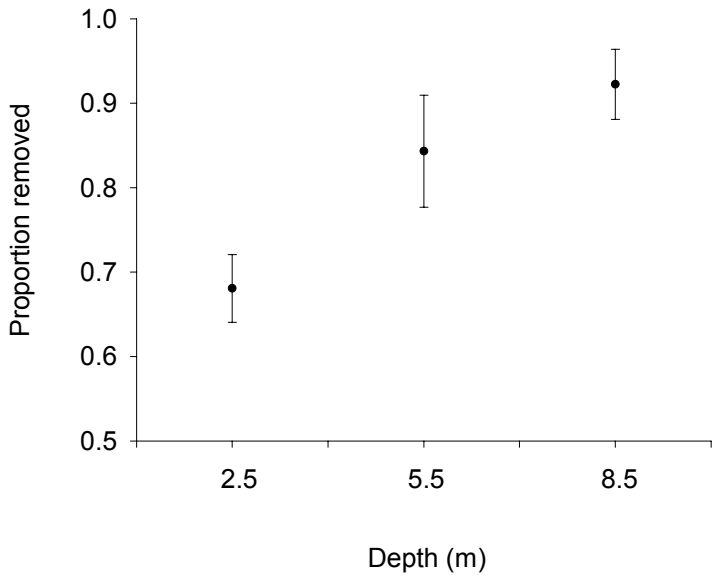


Figure 1. Mean proportion of abalone (± SE) sampled from a standard substrate at 3 depths using the venturi lift (n = 5).

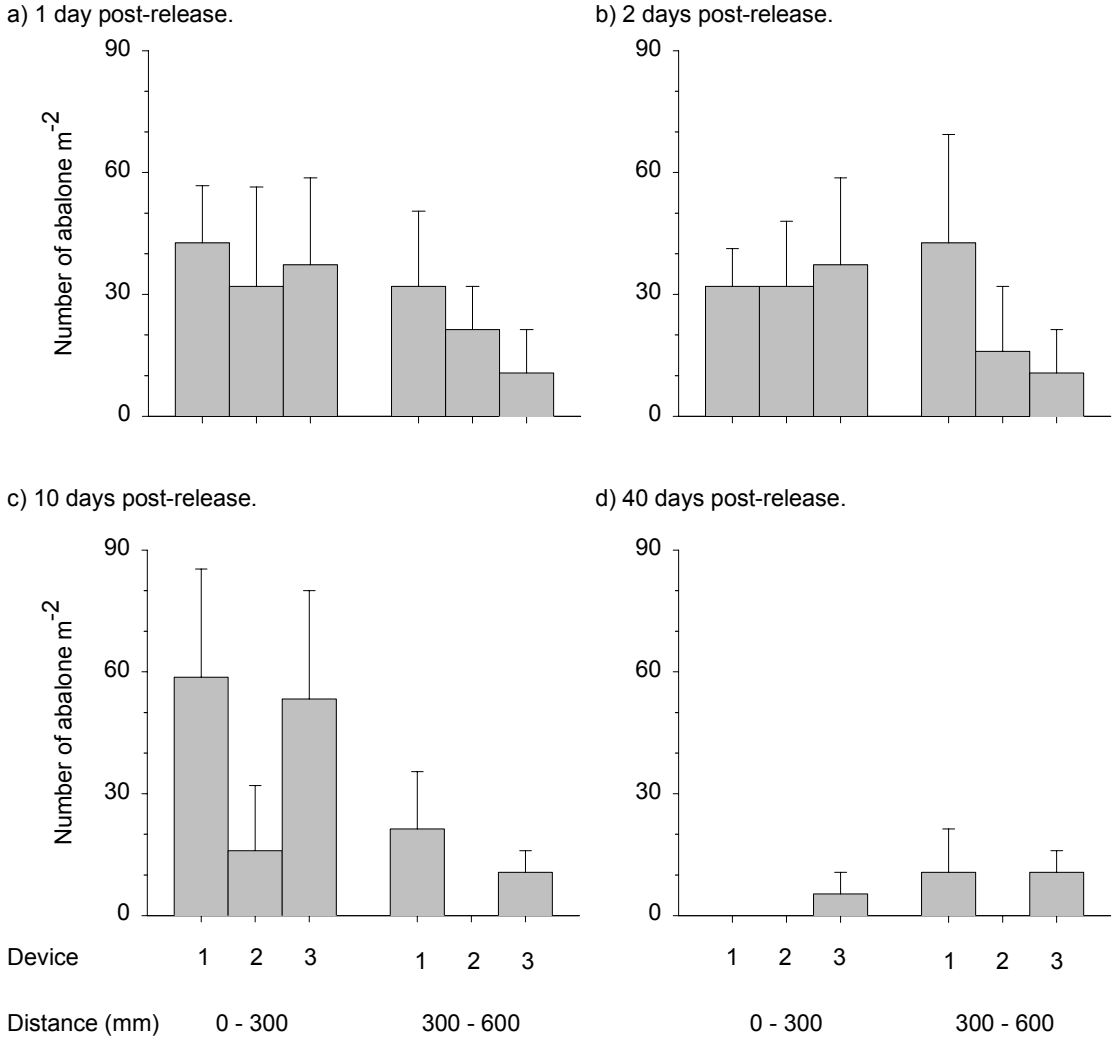


Figure 2. Mean number of juvenile abalone m^{-2} ($\pm$ SE) sampled by the venturi sampler within 300 mm, and between 300-600 mm of deployment devices (n = 3).

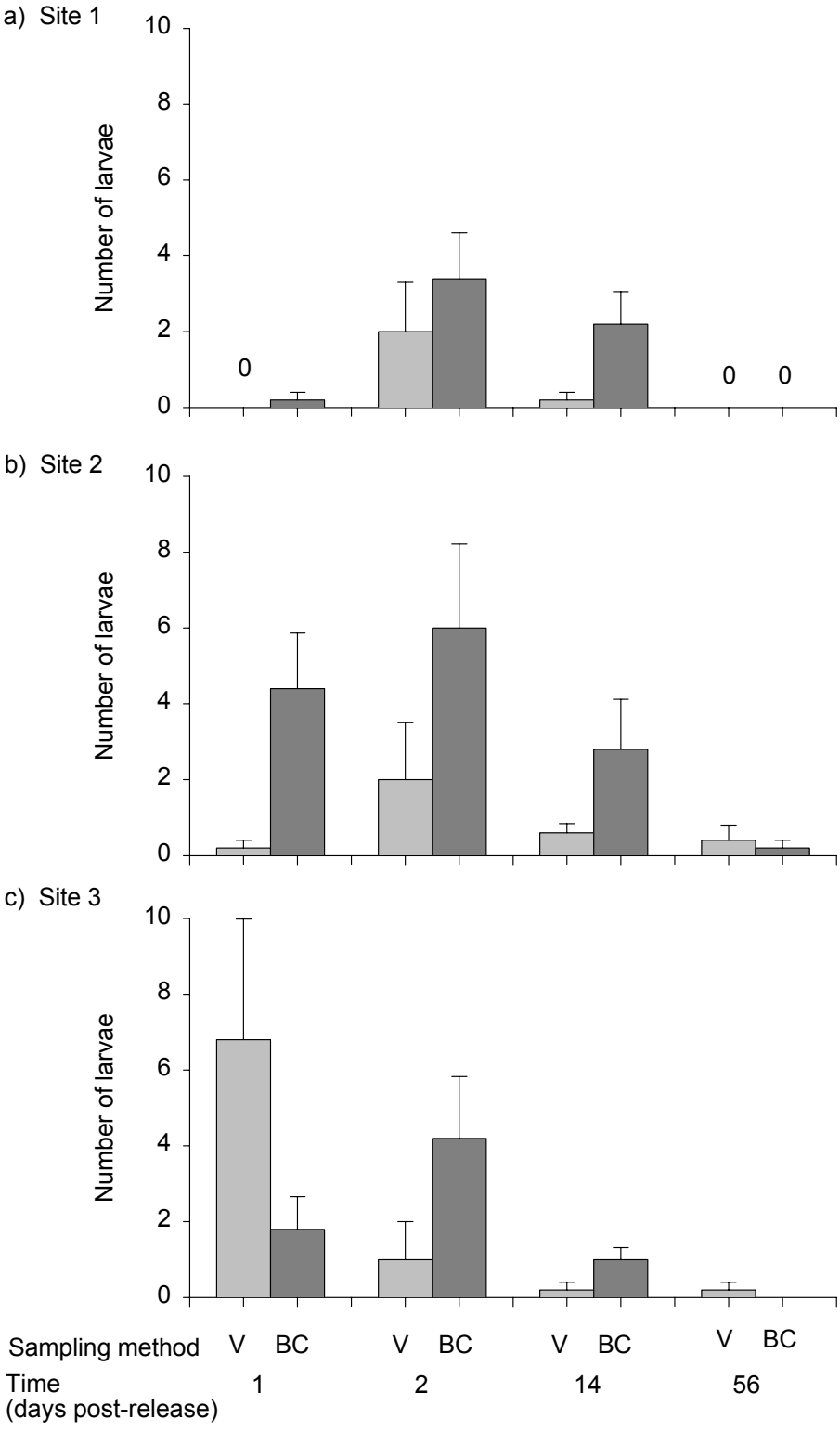


Figure 3. Mean number of abalone (± SE) per sampling method (V, samples from the venturi sampler and BC, collection of small boulders) at each of three larval release sites through time (n = 5).

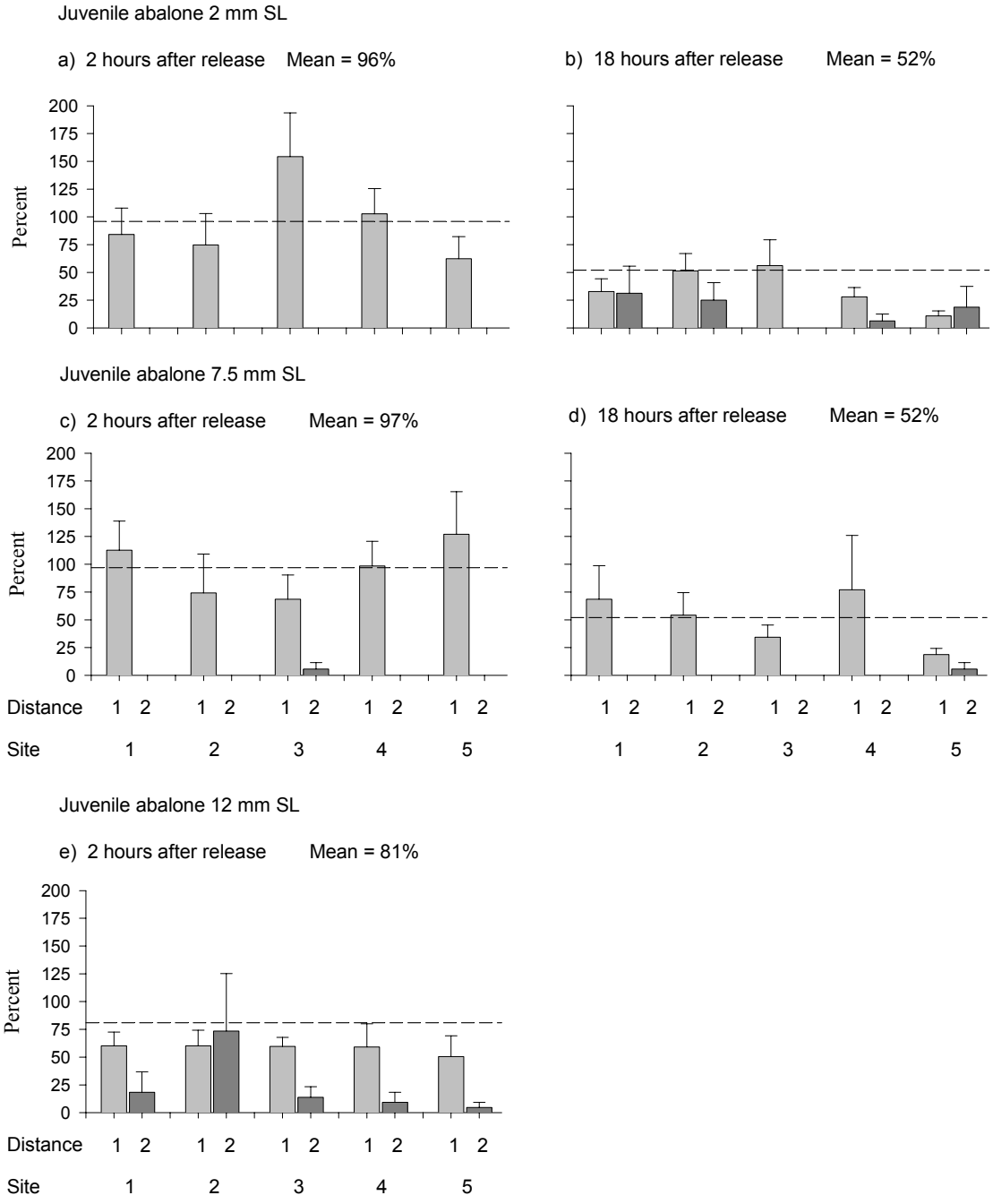


Figure 4. Mean percent recapture (± SE) of 3 different sizes of juvenile abalone after 2 h and after 18 h (for 2 sizes of juveniles) within 1 m (1) and between 1-2 m (2) of the release point at five sites (n = 5). Dashed lines represent the average percent of juveniles surveyed.

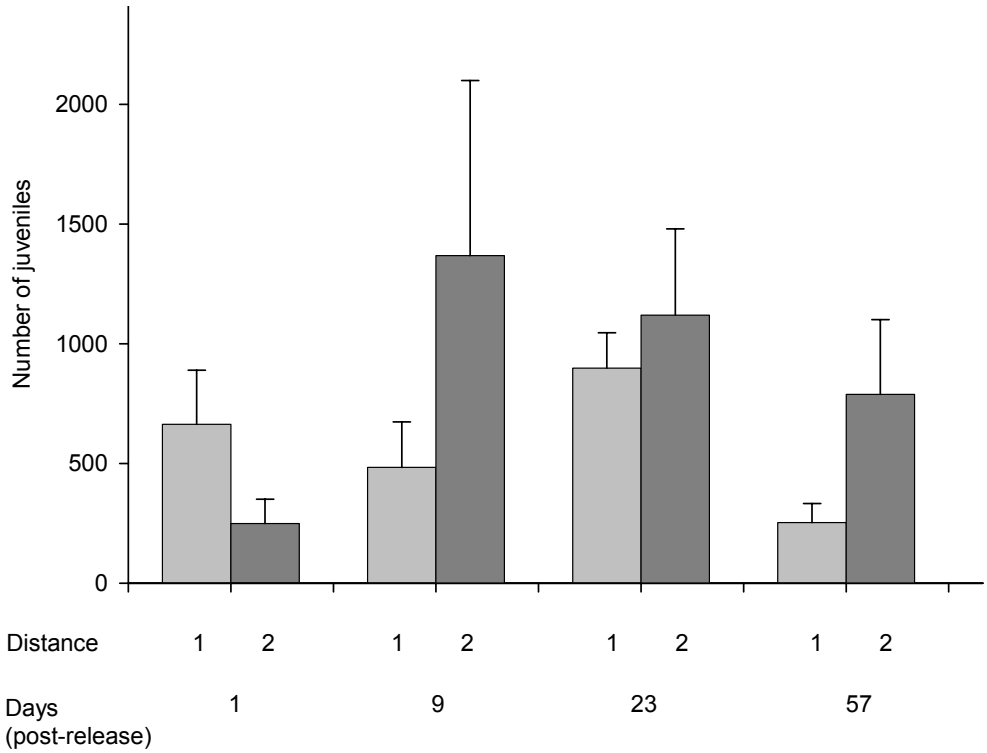


Figure 5. Mean number of juveniles (± SE) within 1 m (1) and between 1-3 m (2) of three deployment devices through time (n = 3).

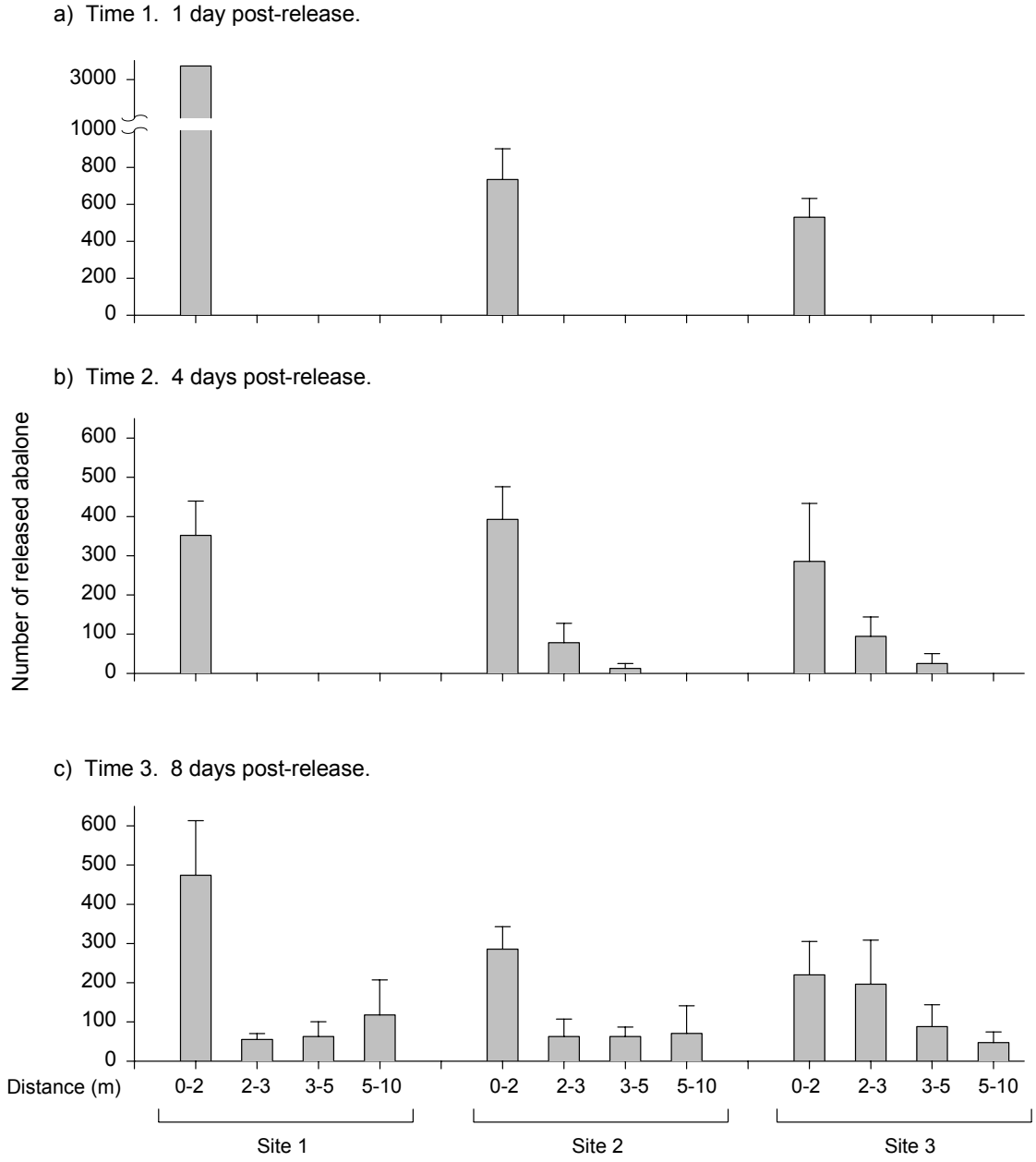


Figure 6. Mean total number of released abalone (± SE) in the seeding area based on abundance estimates from radial transect data (n = 4).

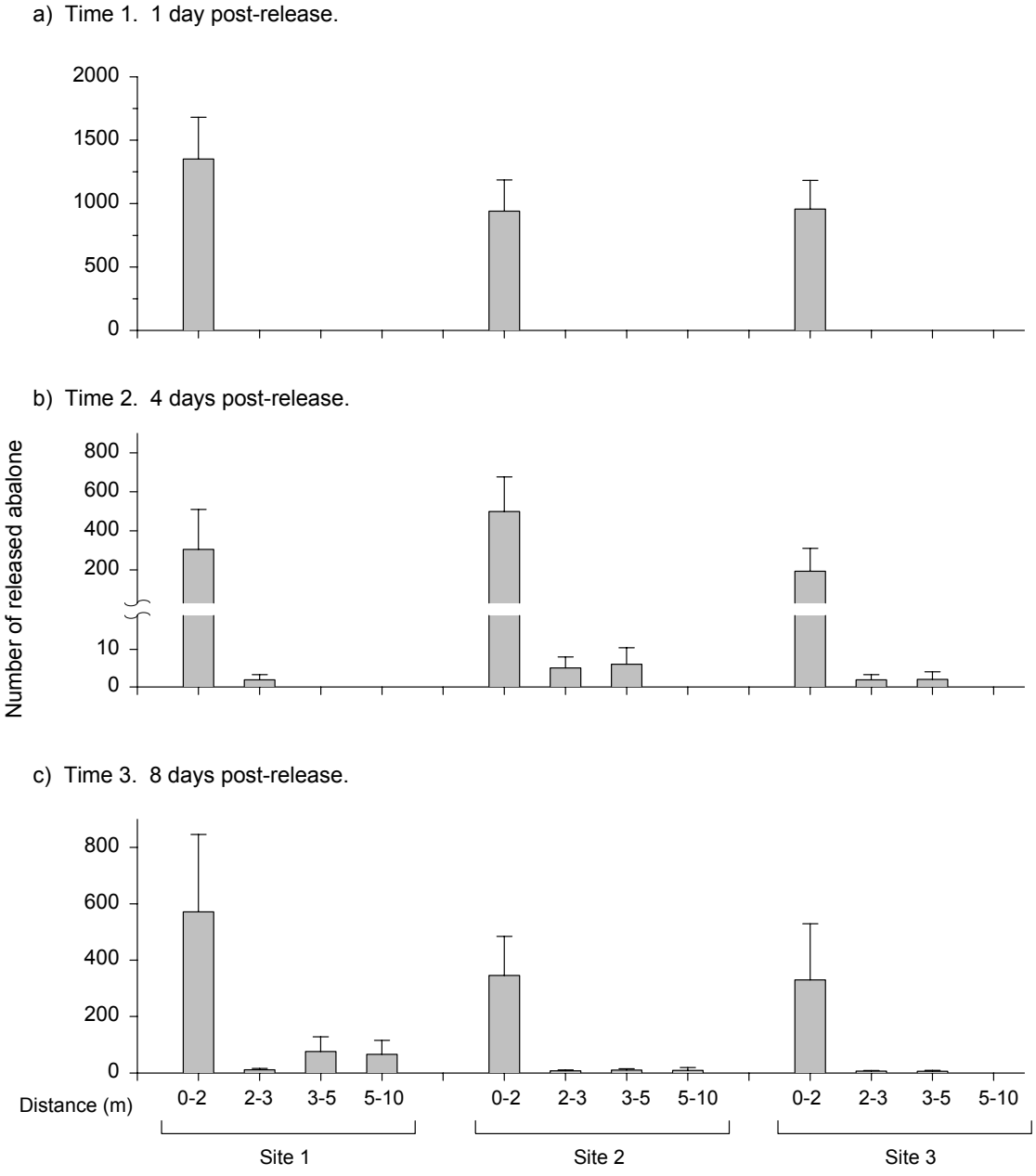


Figure 7. Mean total number of released abalone (± SE) in the seeding area based on abundance estimates from quadrats within radial transects. For distance 0-2m $n = 5$ otherwise $n = 20$.

7.7.3. Discussion

Results from a number of field experiments testing methods for sampling juvenile abalone over short temporal scales indicate that the methods are adequate to detect them. Sampling juvenile abalone between the period relatively shortly after release (<2 months) up until when they become emergent has proven to be very difficult within the constraints of not wanting to destructively sample the habitat. Short-term sampling methods indicate that the methods of releasing small juveniles do allow for their dispersal from the deployment device and that this dispersal varies from tens of centimetres to metres. This rate of dispersal is likely to be a function of habitat complexity and release size.

Concern over the most appropriate method for continued sampling of juvenile releases, after the initial short term period (<2 months), and the inability of initial methods to detect migration from the survey sites prompted the development of surveys that had minimal disturbance of the substrate and that covered a greater area (visual surveys). The destructive sampling of the habitat and the disturbance of recaptured juveniles (for assessment of growth) have had to be accepted as a necessary component of this work. Results of juvenile survival and growth are required for assessment of the methods used not only for the deployment of the juveniles but also the methods used to prepare the juveniles for deployment and to assess spatial variation in survival and growth.

The potential for medium (and larger) size juvenile abalone to move beyond the short distances sampled within many experiments after periods of less than 1 day and less than 1 week highlights the need to expand sampling strategies beyond distances of 1-2 m or potentially even 5-10 m. Extending sampling effort beyond the immediate seeded area was identified as a low priority within the constraints of this investigation. Initial methods were focused on determining the best methods to deploy juvenile abalone to reefs and to estimate survival and growth of seeded juveniles within the seeded area. The results from these experiments have provided information that has to be considered when attempting to determine long-term survival estimates of these releases. The means of sampling large areas outside of seeded reef will require a stratified sampling approach, whereby areas are targeted to provide estimates beyond the immediate area. In conjunction with this stratified sampling, there will have to be quantitative measures of target and non-target habitat so that appropriate estimates of abundance and associated error can be determined.

7.8. Appendix - Section 7

Table A1a. Summary of analysis of variance in the number of 7-day-old larvae delivered from the deployment drum in experiment 1a (see Section 7.2, Table 1). Significant effects ($P < 0.05$) are shown in bold.

Source	df	MS	F	P	%V
GABA	1	3863.34	6.81		4
Refrigeration	1	4200.26	7.40		4
G*R	1	1100.26	1.94		2
Drum	2	3161.17	5.57		3
G*D	2	942.88	1.66		2
R*D	2	33.17	0.06		0
G*R*D	2	2987.79	5.27	**0.007**	12
Hose	1	189.84	0.33	0.565	0
G*H	1	0.09	0.00	0.990	0
R*H	1	2762.76	4.87	**0.031**	8
G*R*H	1	1283.34	2.26	0.137	8
D*H	2	3238.63	5.71	**0.005**	10
G*D*H	2	1001.63	1.77	0.178	6
R*D*H	2	670.29	1.18	0.313	4
G*R*D*H	2	646.13	1.14	0.326	8
Error	72	567.27			28
Total	95				

Table A1b. Summary of analysis of variance in the number of 8-day-old larvae delivered from the deployment drum in experiment 1a (see Section 7.2, Table 1). Significant effects ($P < 0.05$) are shown in bold.

Source	df	MS	F	P	%V
GABA	1	546.26	2.21		1
Refrigeration	1	17685.50	71.64		21
G*R	1	297.51	1.21		1
Drum	2	1980.13	8.02		2
G*D	2	5690.79	23.05		14
R*D	2	4691.54	19.01		11
G*R*D	2	5736.54	23.24	**0.000**	27
Hose	1	906.51	3.67	0.059	2
G*H	1	304.59	1.23	0.270	1
R*H	1	677.34	2.74	0.102	2
G*R*H	1	0.09	0.00	0.985	0
D*H	2	630.17	2.55	0.085	2
G*D*H	2	39.50	0.16	0.852	0
R*D*H	2	166.50	0.67	0.513	1
G*R*D*H	2	18.50	0.07	0.928	0
Error	72	246.87			14
Total	95				

Table A2. Summary of analysis of variance in the proportion of larvae on a standard settlement substrate in experiment 2b (see Section 7.2, Table 1). Significant effects ($P < 0.05$) are shown in bold.

Source	df	MS	F	P	%V
GABA	1	0.00	0.11		0
Drum	2	0.01	0.44		1
G*D	2	0.01	0.41	0.666	3
Hose	1	0.03	2.42		5
G*H	1	0.07	5.08	**0.034**	21
D*H	2	0.06	4.59	**0.021**	29
G*D*H	2	0.00	0.27	0.766	3
Error	24	0.01			38
Total	35				

Table A3a. Summary of analysis of variance in the proportion of larvae on a standard settlement substrate in experiment 3c (see Section 7.2, Table 1). Significant effects ($P < 0.05$) are shown in bold.

Source	df	MS	F	P
Treatment	1	0.01	10.74	0.082
Replicate	2	0.04		
T*R	2	0.00		
Error	0			
Total	5			

Table A3b. Summary of analysis of variance in the proportion of larvae on a standard settlement substrate in experiment 4d (see Section 7.2, Table 1). Significant effects ($P < 0.05$) are shown in bold.

Source	df	MS	F	P
Treatment	1	0.00	0.56	0.531
Replicate	2	0.00		
T*R	2	0.00		
Error	0			
Total	5			

Table A4. Summary of analysis of variance in the number of larvae on natural substrate with and without physical shelter at 5 sites, 24 h after deployment (n = 5). Significant effects ($P < 0.05$) are shown in bold.

Source	df	MS	F	P	%V
Tent	1	3.10	17.27	**0.014**	56
Site	4	0.06	0.73	0.575	3
T*S	4	0.18	2.16	0.091	17
Error	40	0.08			24
Total	49				

Table A5. Summary of analysis of variance in the number of juveniles removed from settlement plates for 3 concentrations of benzocaine and a control at 3 exposure times (n = 3). Significant effects ($P < 0.05$) are shown in bold.

Source	df	MS	F	P
Dose	3	2.06	72.47	**0.000**
Exposure	2	0.05	1.90	0.230
D*E	6	0.03	2.37	0.061
Error	24	0.01		
Total	35			

Table A6. Summary of analysis of variance in the number of juveniles recovered after 30 mins from exposure to 3 concentrations of benzocaine and a control at 3 exposure times (n = 3). Significant effects ($P < 0.05$) are shown in bold.

Source	df	MS	F	P
Dose	3	0.06	1.51	
Exposure	2	0.06	1.35	
D*E	6	0.04	4.24	**0.005**
Error	24	0.01		
Total	35			

Table A7. Summary of analysis of variance in the number of juveniles recovered after 18 hrs from exposure to 3 concentrations of benzocaine and a control at 3 exposure times (n = 3). Significant effects ($P < 0.05$) are shown in bold.

Source	df	MS	F	P
Dose	3	0.07	9.31	**0.011**
Exposure	2	0.02	2.77	0.140
D*E	6	0.01	0.73	0.628
Error	24	0.01		
Total	35			

Table A8. Summary of analysis of variance in the number of juveniles removed from a standard substrate at 3 depths using the venturi-lift (n = 5). Significant effects ($P < 0.05$) are shown in bold.

Source	df	MS	F	P
Depth	2	0.19	4.72	**0.031**
Error	12	0.04		
Total	14			

8. LONG-TERM SURVIVAL AND GROWTH OF RELEASED *H. RUBRA* LARVAE AND JUVENILES

R.C. Chick, D.G. Worthington, P.T. Gibson, M.P. Heasman, N. Savva & C. Brand

8.1. Introduction

Research into the use of hatchery-reared larval or juvenile abalone to re-establish or increase local populations has primarily focused on short-term settlement, survival and growth (Schiel 1992; Preece *et. al.* 1997; Sweijd *et al.* 1998; de Waal & Cook 2001 and Len & Lee 2001). The only regular use of releasing hatchery produced juvenile abalone as a means of increasing natural populations to sustain and support a commercial fishery is done in Japan; where in excess of 80% of the large juveniles released are recaptured in the commercial fishery (review by Masuda & Tsukamoto 1998). There has been no long-term assessment of the efficacy of releasing larvae to enhance local populations of abalone. Investigation into the use of larvae to seed reefs began in Australia during the mid 1990s with the work of Preece *et al*. (1997) in South Australia. This work developed a method of deploying larvae to the reef and further tested methods developed by Schiel (1992) to maximise larval settlement and/or retention within the release area. There has also been some use of miscellaneous data from Australia to support the investigation of releasing hatchery reared juvenile abalone (Shepherd 2000). Currently, there are a number of small research studies underway in Australia investigating the survival and growth of hatchery produced abalone released onto natural and artificial reefs (R. Day, C. Dixon & P. Hanna, pers. comm.). These studies are focusing on the growth and survival of relatively large (>30 mm) juveniles in either manipulated rocky reef habitats or artificial reefs. The research described here attempts to investigate the potential of releasing hatchery-reared larval and juvenile *H. rubra* at a range of sizes to natural reefs.

Estimation of the survival and growth of released abalone is complicated by several factors (see also Shepherd *et al.* 2000). As soon as larval or juvenile abalone are released they can move away from the point of release and hide within complex rocky habitats. Further, both the distance they move and our ability to find the abalone in complex habitat changes with time as the abalone grow and change their behaviour and habitat preference (Section 7). Much of this spatial and temporal variation could be avoided by simply releasing the abalone and delaying sampling until they have reached maturity or the minimum legal length. Because of the large number of releases, this strategy of delayed sampling has been followed for some of the releases (Tables 1 & 2). In contrast, regular sampling as the abalone grow can be used to infer expected future rates of survival and growth and investigate other specific questions. Because few of the abalone released during this project would have even matured, rates of survival and growth to maturity or entering the fishery need to be predicted from shorter time periods. Regardless, the ongoing monitoring of the releases should enable these predictions to be tested.

8.2. Aims

The final two objectives of this project were to release larval and juvenile *H. rubra* at several locations on the NSW coast and attempt to quantify their survival and growth for at least 18 months. Here, we report on the survival and growth of released *H. rubra* at 57 sites within 6 locations over a period up to 30 months or more after release (from March 2000). Early releases were designed to test and develop techniques to successfully deploy *H. rubra* to natural reefs. Subsequent releases have investigated techniques to maximise the settlement, survival and growth of released *H. rubra*. During all these releases, short-term sampling has also been designed to investigate associated questions, such as the movement of juvenile *H. rubra* and the comparison of various sampling techniques. This section describes the standard methods developed to release and resample hatchery-reared *H. rubra* to provide long-term estimates of survival and growth.

8.3. Methods

8.3.1. Standard methods and experimental design

8.3.1.1. Release of larvae and juveniles

A series of standard methods were used in all releases for long-term monitoring to facilitate their comparison. These methods included those for deployment, sampling and the overall experimental design. Despite that, there were some significant improvements in methods of release and sampling that may confound the long-term comparison of releases. Further, some releases were used for short-term experiments (Section 7) and, because of the similarity of their methods of release and sampling, are also being used for long-term monitoring. Most releases were completed in 2000 and 2001, so at the time of this report had only been at sea for 1-2 years. Monitoring of all releases will continue. As a consequence, we will delay the presentation of a formal assessment of the significance of any changes in abundance caused by the release method of *H. rubra* and only report on estimated rates of survival and growth in line with the final objective of the project.

The standard methods of deploying larvae and juveniles to reefs and for the subsequent monitoring of their survival and growth follows those outlined in Section 7. Briefly, competent to settle larvae (7-9 days post-fertilization), reared in the hatchery (Sections 4.1 to 4.3) were transferred and held in a 20 L container. Replicate samples of 2 mL were taken from this container, after the contents were agitated to provide a homogeneous suspension, and the number of larvae in each sample counted to estimate their density and hence the total number of larvae in the container. The contents of the container were then concentrated further into 1 L containers for deployment at each site. Once at the release site, seawater was added to the drum of the deployment pump and the contents of the 1 L container were acclimatised and slowly added to the drum. The drum was then sealed and pressurized, and the contents delivered down a 20 m or 40 m hose to a diver on the reef who directed the flow over the substrate.

The deployment of juveniles was dependent on their size at release (Section 7). Briefly, small (<8 mm) juveniles were deployed using wire tubes filled with CCA-coated rocks, with the exception of 2 releases where large numbers of small animals were released directly from mesh bags at Boulder Bay Pt – North, and South and Twofold Bay – North. Larger juveniles or higher numbers of juveniles than could be deployed with rock devices were deployed using rectangular PVC tube deployment devices, with the exception of 2 releases of very large (>40 mm) juveniles, which were individually placed by hand. The total number of juveniles that were deployed to a site was determined by anaesthetising the juveniles in the hatchery and estimating their total weight after draining them in a coarse sieve for approximately 30 seconds. From these juveniles 5 replicate samples were taken, weighed and the number of individuals counted to estimate an average weight

per individual. Specific weights of abalone were then calculated to place the desired number of juveniles into each deployment device. Once loaded with juveniles, devices were transported to release sites. Once on site they were placed in a plastic tub and swum to a central location of the site from where divers distributed the devices through the site and secured them within the habitat.

8.3.1.2. Sampling larvae and juveniles

The standard methods of sampling larvae and juveniles were dependent on the type of habitat and the size of the abalone (Section 7). Sampling within 3 months of release of larvae was either by venturi sampler or collection of small boulders and again depended on the type of habitat in the release site. Sampling 3-12 months after the release of larvae, or immediately after the release of juveniles, was either by the collection of small boulders (i.e. destructive) or the non-destructive visual inspection of boulders. Non-destructive methods of sampling could be used as the released *H. rubra* were visible on close inspection of the reef and the disturbed boulder. However, the collection of small boulders and the non-destructive visual inspection of boulders generally provided lower estimates of abundance than more destructive methods (i.e. biased low because of crypsis and complexity of the habitat). At all other sample times, the most reliable sampling method for estimates of abundance was used, which involved the deconstruction and inspection of habitat within a defined area. Where destruction of the habitat was not possible, such as in areas with solid bedrock or cemented substrate, visual surveys in randomly placed quadrats or transects (Fingal Island - South East 1-3) or fixed areas (Boulder Bay Pt - North and South) were used despite the possible biases related to crypsis.

Most releases were undertaken in areas with partially cemented or loose rock substrates or small boulder fields. This enabled more reliable estimates of abundance using destructive sampling methods that are only possible in such habitats. Nevertheless, some releases were also completed in habitats where destructive sampling was not practical or possible, including multi-layered (>3 layers) small boulder fields, fields of larger boulders and areas of solid bedrock with crevices. Sampling in such areas was limited to visual surveys with their associated biases related to the crypsis and habitat preference of *H. rubra* of different sizes. Clearly, any differences in the survival of released *H. rubra* among the habitats are confounded by differences in the efficiency of sampling. As a result, we only present formal results for releases completed within partially cemented or loose rock substrates or small boulder fields. The interpretation and comparison of results are further complicated by a number of factors such as the strong possibility of differences in mortality among the habitats and the likelihood that habitats chosen because of their amenability to the sampling method may also provide lower levels of protection to juvenile abalone from boulder disturbance during rough seas.

Despite the careful selection of release sites, there was often variation in the type of habitat available for abalone within the sites. For example, while most release sites were dominated by partially cemented or loose, small boulder fields, they also contained small areas of flat bedrock with crevices or larger boulders that could not be sampled. As a result, at each release site a quantitative estimate of the area of habitat that could not be sampled was made. This involved recording the number of haphazardly placed quadrats that fell in habitats that could not be sampled. To offset these sampling constraints and despite them, estimates of the total number of *H. rubra* in each site were made under the assumption that densities of *H. rubra* in areas that could not be sampled were the same as in sampled areas. As a result, estimates of the total number of *H. rubra* within a site were calculated by combining an estimate of the density of *H. rubra* in the area sampled with an estimate of the total area of the site.

Several natural events may have contributed to the mortality of abalone. In particular, the protozoan, *Perkinsus olseni*, was infecting up to 50% of *H. rubra* populations in the Port Stephens location during early 2002 and appears to have killed >90% of these stocks between 1998 and 2002. It is therefore likely that this disease and the associated high rates of mortality also affected the survival of all hatchery-reared *H. rubra* released in the area. For example, destructive sampling

of the release site at Fingal Island - SSE 407 days after release provided an estimate of 2500 surviving juveniles. By contrast, sampling in early 2002, 603 days after release, found no juveniles suggesting high mortality coincident with large declines in wild populations in the area. Moreover, in an independent study done during mid 2002, *H. rubra* infected by *Perkinsus* were found from Port Stephens to Jervis Bay, and all releases in this area could have been affected. It is likely that this disease had a significant impact on the numbers of *H. rubra* surviving at release sites particularly those more southern sites within the Port Stephens location.

Several large storm events occurred during the time of the releases and significantly impacted the habitat available to abalone at several sites. For example, habitat at the 2 sites at Jibbon Point - South and Red Point - South were substantially damaged due to storm events. At each of the sites at Jibbon Point - South the habitat was significantly disturbed including the loss of all macroalgal cover only 1-2 days after release. Red Point - South was affected by a storm only 2-3 days after release, resulting in a loss of approximately 40% of the available habitat due to destruction of the boulder field and inundation by sand.

The deployment of *H. rubra* to locations remote from the hatchery often required more than 10 h of transportation followed by over night storage. The effect of the handling, transportation and storage compromised the survival of at least one batch of larvae and juveniles released in Eden. The batch of larvae released to Yullumgo Cove was exposed to very low temperatures (3-4^{o}C) with short-term fluctuations of 5-8^{o}C during the storage and transport. Subsequent settlement on conditioned glass slides before release was very low, suggesting low rates of settlement were also likely in the field. The batch of juveniles released to Fisho Cull Bay received extended exposure to anaesthetic following transport because of problems with the supply of water in the holding facility prior to release. As a result of these handling, transport and storage methods affecting the survival of larvae and juveniles in these specific releases, results are not included in the long-term assessment of survival.

Quantitative estimates of the number of released *H. rubra* surviving outside each site were not practicable and therefore not attempted. To minimise emigration, all release sites were selected with some boundaries of inappropriate or less preferred abalone habitat (e.g. sand, barren rock or intertidal areas), and abalone were not released adjacent to boundaries of the site where they could easily emigrate. Nevertheless, some emigration of released abalone is likely to have occurred at all sites, particularly in view of the rates of movement of juveniles described in Section 7. Similarly, this is likely to be particularly the case for larval releases where larvae could have been transported large distances from the release site prior to settlement. Estimates of the abundance of released *H. rubra* outside all sites were completed, but here we do not attempt to estimate the area over which they were distributed and how that changed through time. As a result, estimates of the survival or mortality of released *H. rubra* are based only on those individuals that remained within the release site, and hence are likely to be conservative. For example, rates of instantaneous mortality per year was estimated from the difference between the number of individuals released, and those estimated to have survived within the release site until each sampling time:
(= proportion surviving/days since release x days in a year).

Several strategies were determined for the temporal sampling of release sites. The first involved standard times for sampling the release site within 3 months, between 3-12 and 12-18 months and after 18 months to assess long-term survival and growth. The second strategy involved release sites that were not disturbed until after 18 months of release. Finally, when releases were also used for short-term experiments, additional sampling times were included to assess long-term survival. To date, *H. rubra* found in release sites can be identified as originating from the hatchery. This was possible through either the marking of the larval shell or the conspicuous blue/green colour of the spire of the shell a consequence of the artificial diet used in the hatchery (Plate 1 and Plate 2, Section 7). The blue/green colour of the spire also enabled an estimate of the possible size of the juvenile at release. As a result, growth of each individual could be estimated from the difference between the length of the blue/green shell and the subsequent shell. In addition, the average length

of individuals within each batch at release was measured allowing an estimate of the average growth of individuals that remained.

8.3.1.3. Experimental design

A formal experimental design to assess the significance of changes in abundance of abalone caused by release related factors would ideally involve the replicated release of abalone across any potentially confounding factors, such as batch, release method, time and space. Several factors combined to complicate the general experimental design of the releases. These included the variability in the size and timing (seasonal availability) of batches of *H. rubra* larvae and juveniles generated from broodstock from different regions in the state, difficulties of finding significant numbers of *H. rubra* from small-scale releases, and progressive development of improved methods of release and sampling through the project. In an attempt to minimise such confounding effects, releases of all *H. rubra* for long-term monitoring were done within a staircase experimental design (Walters *et al*. 1989) and changes to methods of release and sampling were minimised. The staircase experimental design allows for the release of batches as they become available, but assumes that any interactions between time and the experimental treatments are not significant. This assumption is essential if results from all releases are to be combined into a formal assessment of the significance of any changes in abundance caused by the release of abalone. No formal analyses of the significance of changes in abundance relative to controls are presented here, as most releases occurred within the last year of the project and ongoing sampling and analysis will occur. Despite that, the very low abundance of wild *H. rubra* in release areas and the lack of increases in abundance in control areas suggest significant increases in abundance will be detected.

Tables 1 and 2 provide a summary of the details of each release of hatchery-reared larvae and juveniles to be used in the long-term assessment of survival. The information in the tables includes the general location, site name, area of the site, the size and number of individuals released, and an estimate of the density of hatchery-reared *H. rubra* and their survival. Additional information including the general habitat type and the sampling methods used at each sample time are included in Tables A1 and A2 in the Appendix. Thorough site descriptions, maps of individual sites and technical detail related to each release are available as an unpublished technical report through NSW Fisheries.

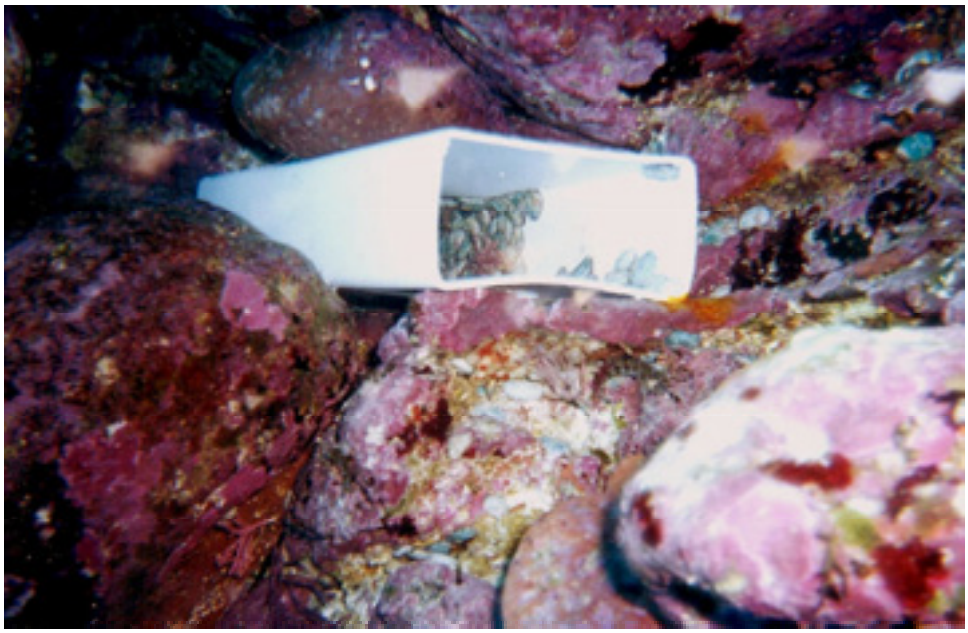


Plate 1. Released juvenile abalone in a PVC deployment device and in the surrounding habitat 18 h after release.

8.4. Results

8.4.1. Long-term survival and growth of released larvae

More than 24 million larvae were released within 10 sites from Port Stephens to Eden (Table 1). Of these 10 sites, 9 consisted of boulder habitat that enabled a number of alternative destructive sampling techniques to be used to assess survival and growth, and 6 had larvae released for over 18 months (Table 1). The survival of larvae at 2 of these 6 sites (Jibbon Point and Yullumgo Cove) were compromised by either external factors or irregularities in handling and transport protocols. Results from the 4 sites where larvae have been released for over 18 months and have been sampled using destructive sampling techniques are presented.

Greater than of 3 million larvae were released into each of 4 replicate sites on Fingal Island (Table 1). Sampling after the initial releases provided estimates of about 10,000 individuals settled within the sites. Subsequent sampling found numbers generally declining, although this comparison is confounded by the different sampling techniques required to estimate the abundance of *H. rubra* of different sizes (Figures 1 & 2). At 531 and 553 days after release no *H. rubra* were found at two of the sites, while only 1 and 4 juveniles found during sampling were positively identified as originating from the larval release at the other two sites. These numbers provide estimates of the total number of *H. rubra* surviving from the larvae originally released at 75 and 300 individuals at each of the two sites.

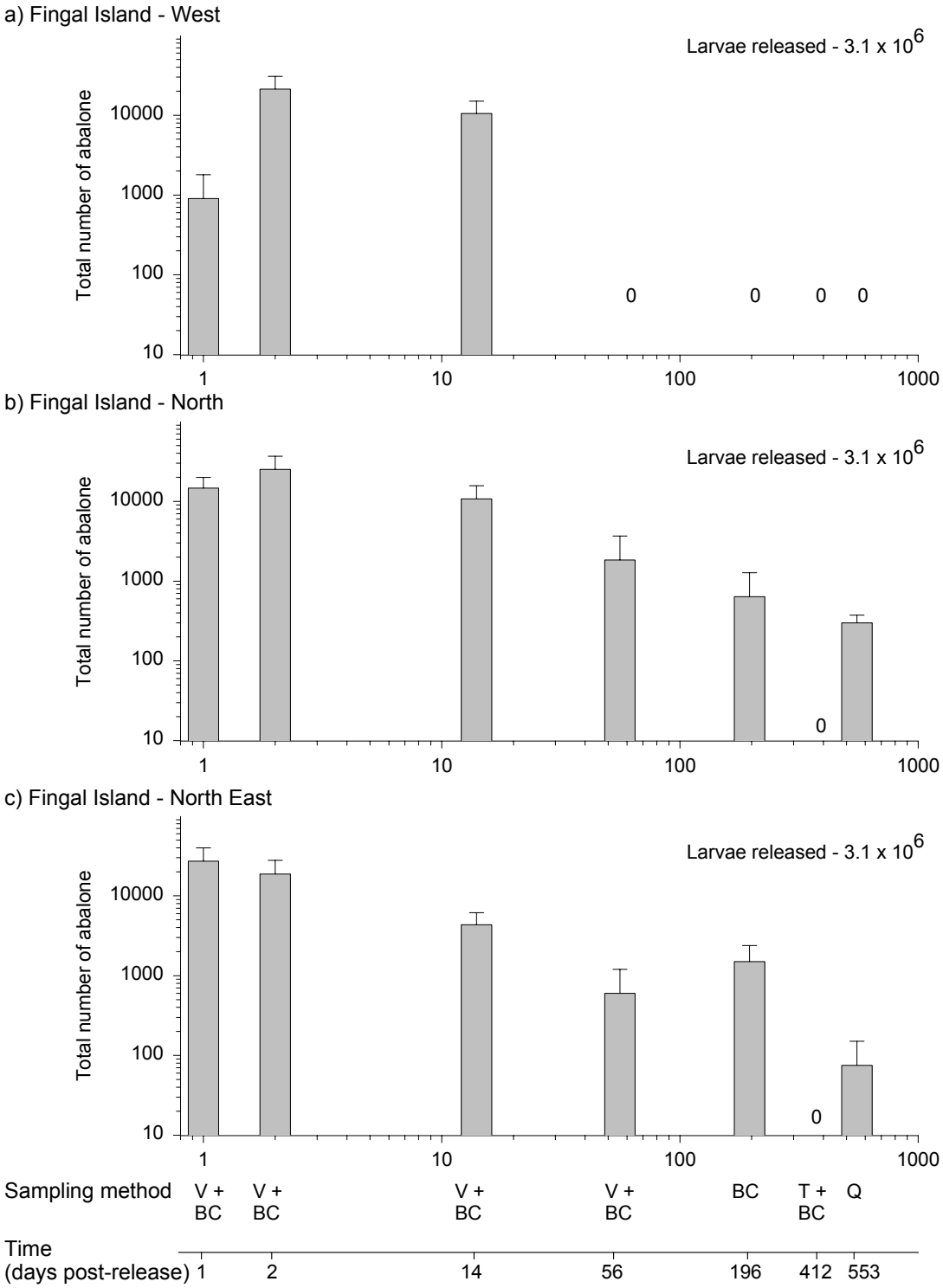


Figure 1. Estimated mean total numbers of released *H. rubra* (± SE) at each of the 3 larval release sites through time at Fingal Is. - North, Port Stephens. Four sampling methods were used to estimate abundance, Venturi sampler (V) (n = 5 for days 1-56), boulder collections (BC) (n = 5, 20 and 10 for days 1-56, 196 and 412 respectively), visual transects (T) (5 x 0.25 m) (n = 5), and 1 m^2 destructive quadrat (Q) (n = 5).

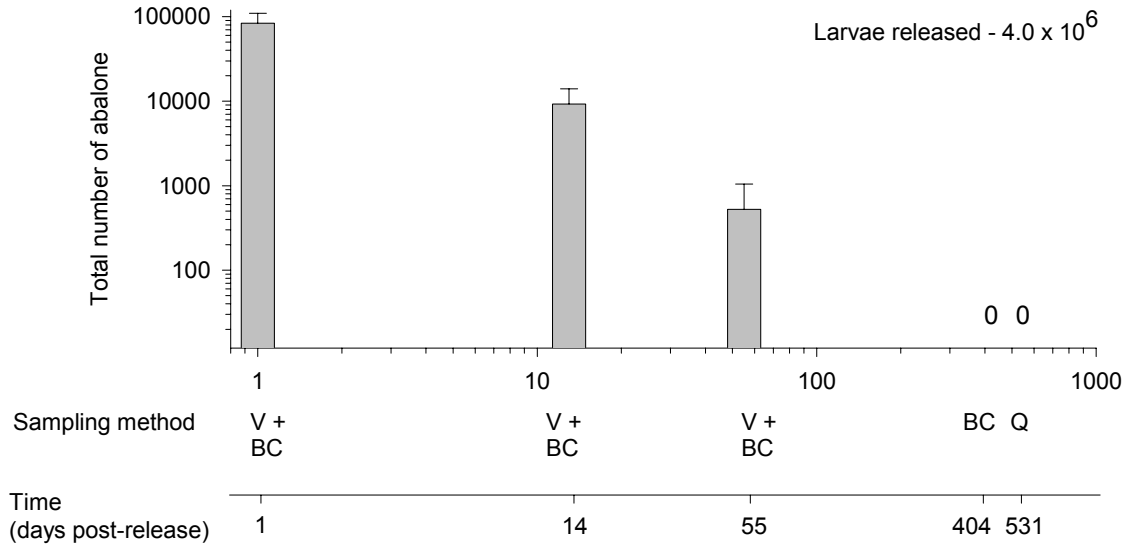


Figure 2. Estimated mean total number of released *H. rubra* (± SE) at Fingal Is. - NNE, Port Stephens through time. Three sampling methods were used to estimate abundance, Venturi sampler (V) (n = 5), boulder collections (BC) (n = 5 and 20 for days 1-55 and 404 respectively) and 1 m^2 destructive quadrat (Q) (n = 5).

8.4.2. Long-term survival and growth of released juveniles

More than 875,000 juvenile *H. rubra*, ranging in standard length (SL) from 1.3 to 43 mm, were released at 47 sites along the NSW coast at locations including Port Stephens, Sydney, Kiama, Ulladulla, and Eden (Table 2). Of these 47 sites only 3 comprised of a boulder habitat and had juveniles released for over 18 months (Table 2). Survival of juveniles at each of these sites may have been compromised by extraneous factors. For example, it is likely that *Perkinsus* infected abalone at Fingal Island - SSE, a large storm destroyed habitat at Red Point - South shortly after release, and incorrect handling may have compromised individuals at Fisho Cull Bay, Eden. Also, by the end of the reporting period 18 sites had juveniles that had been released for over 12 months, and sampled using destructive methods (Table 2).

8.4.2.1. Broughton Island

Approximately 40,000, 10 mm mean SL juvenile *H. rubra* were released as two separate batches, 273 days apart, into each of 4 sites off Broughton Island (Table 2). Visual transects completed 5 days after the initial release estimated only 17-31% of those released remained in the sites (Figure 3). After 378 days, no released abalone were found at site 3, and only 6, 1 and 4 were found at the other 3 sites. These data provide estimates of a total of 360, 60 and 240 released abalone surviving at the three sites (Figure 3). Growth of the juveniles recaptured suggested most individuals were from the second release, 105 days prior to sampling (Figure 3). It is likely that infection by *Perkinsus* may have adversely effected survival in these sites.

8.4.2.2. Cabbage Tree Island and Fingal Island

Approximately 690, 43 mm mean SL, juvenile *H. rubra* were released into 4 sites around Cabbage Tree and Fingal Islands, Port Stephens (Table 2). After 119 days, 8, 4, 17 and 0 surviving abalone were found at each of the 4 sites respectively. These data provide estimates of a total of 300, 80, 425 and 0 released abalone were surviving at these sites (Figure 4).

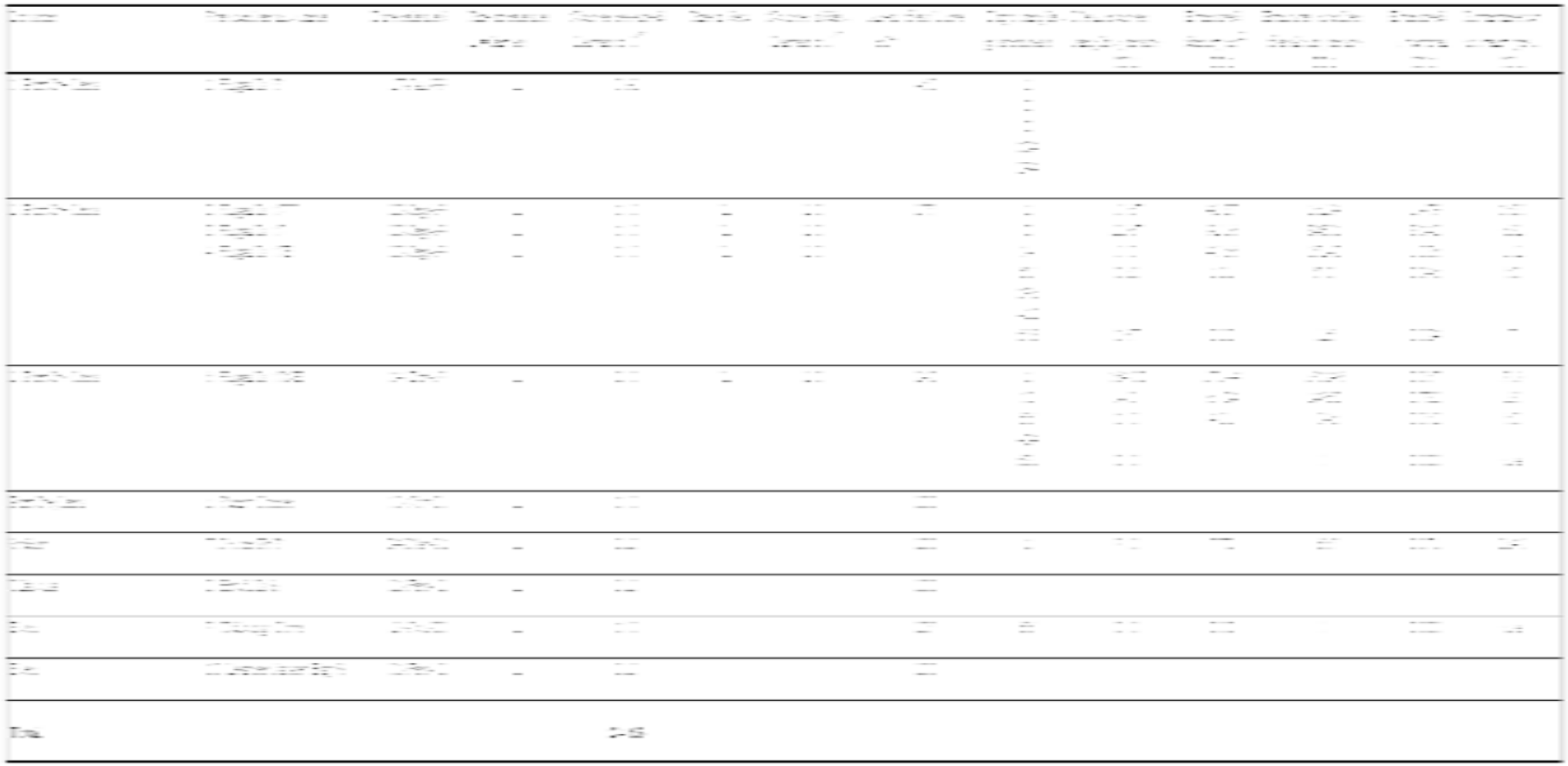
8.4.2.3. Fingal Island

Approximately 80,000, 7 mm mean SL juvenile *H. rubra* were released at one site on Fingal Island (Table 2). Destructive sampling completed within a month of release estimated 89-111% of the individuals released remained within the site. After 407 days, 25 abalone were found at the site providing an estimate of the total number of abalone surviving at the site over 2,500 (Figure 5). The mean length at release of individuals that were recaptured after 407 days was 6.6 mm ($\pm$0.3 SE), with subsequent mean growth of 25.4 mm ($\pm$0.7 SE, Figure 6). Growth varied among individuals by up to 10.8 mm, with a maximum growth of 28.9 mm (i.e. 6.1-35.0 mm). After 794 days, no released abalone were found at the site. Again, it is likely that infection by *Perkinsus* may have adversely affected survival in these sites.

8.4.2.4. Jibbon Point

Approximately 15,000, 15 mm mean SL juvenile *H. rubra* were released into one site on Jibbon Point (Table 2). Destructive sampling completed within a month of release estimated 45% of the individuals released remained within the site. After 43 days, 3 abalone were found at the site, suggesting a total of over 1,500 released abalone were at the site (Figure 7). From the major change in habitat at the site, it was clear the site had been dramatically disturbed by a severe storm.

Table 1. Summary of information for each of the sites where larvae were released. All numbers are calculated from the numbers of hatchery abalone averaged across sites for each sample time. Method and site details are presented in Appendix, Table A1.



8.4.2.5. Kiama

Approximately 4,000, 7 mm mean SL and 5,000, 14 mm mean SL juvenile *H. rubra* were released at 4 sites at Kiama (Table 2). Destructive sampling completed after 108 and 280 days provided estimates of between 2-31% and 9-18% of those abalone released remained within the sites respectively (Figure 8). After 280 days, 27, 43, 66 and 45 abalone were found at each of the 4 sites, respectively. These data provide estimates of the total number of abalone surviving at over 800, 1,200, 1,600 and 1,000 at these sites (Figure 8). The mean length at release of abalone that were recaptured after 108 and 280 days was 14 and 12.4 mm (each $\pm$0.3 SE, Figures 9a & c) respectively, with subsequent growth of 7.5 and 16.9 mm ($\pm$0.2 and 0.4 SE, Figures 9b & d). After 108 days growth did not vary greatly, with the mean length of abalone at each of the 4 sites being within 1 mm of 21.5 mm (Figure 10a-d). After 280 days there were greater differences in mean growth at the 4 sites with mean growth increments of between 12.2 and 20.6 mm (Figure 11a-d). Results of the mean length at release of abalone that were recaptured suggest that a disproportionately low number of the larger size abalone released to these sites (i.e. 5,000, 14 mm abalone, see Table 2) were being recaptured, indicating that they may have experienced higher rates of mortality than the smaller size abalone.

8.4.2.6. Red Point

Approximately 32,000, 7 mm mean SL juvenile *H. rubra* were released into one site south of Eden (Table 2). Destructive and non-destructive boulder sampling completed within 6 months provided estimates of 17-9.6% of those abalone released remaining within the site. After 463 and 916 days, 16 and 5 released abalone were found within the site respectively, providing estimates of the total number of abalone surviving within the site at over 200 and 20 respectively (Figure 12). The mean length of released abalone that were recaptured after 463 and 916 days was 10.0 and 8.4 mm ($\pm$0.5 and 0.3 SE) respectively, with subsequent mean growth increments of 36.1 and 74.5 mm ($\pm$2.1 and 4.5 SE, Figure 13a-b). At each of the 2 sampling times growth varied among individuals by up to 25 and 28 mm (Figure 13a-b).

8.4.2.7. Steamtrawler

Approximately 25,500, 14 mm mean SL juvenile *H. rubra* were released at one site south of Eden (Table 2). Destructive sampling 93 days after release provided an estimate of 47% of the abalone released remaining within the site (Figure 14). After 274 and 455 days, 25 and 6 abalone were found at the site, providing estimates of the total number of abalone surviving within the site at over 1,600 and 250 respectively (Figure 14). The mean length at release of abalone that were recaptured after 455 days was 12.2 mm ($\pm$ 1.1 SE), with a subsequent mean growth increment of 22.2 mm ($\pm$1.0 SE). Growth varied among individuals by only 6 mm, with a maximum growth increment of 25 mm (i.e. 13.0-38.0 mm).

8.4.2.8. Disaster Bay

Approximately 1,000, 15 mm mean SL juvenile *H. rubra* were released into 6 sites at Disaster Bay, south of Eden (Table 2). Destructive sampling completed after 363 days provided estimates of between 0 - 23% of the abalone released remain within the sites (Figure 15a-f). After 363 days 7, 19, 4, 4, 5 and 0 abalone were found at each of the 6 sites respectively. These data provide estimates of the total number of abalone surviving at over 90, 220, 50, and 50, 60 and 0 at the six sites (Figure 15).

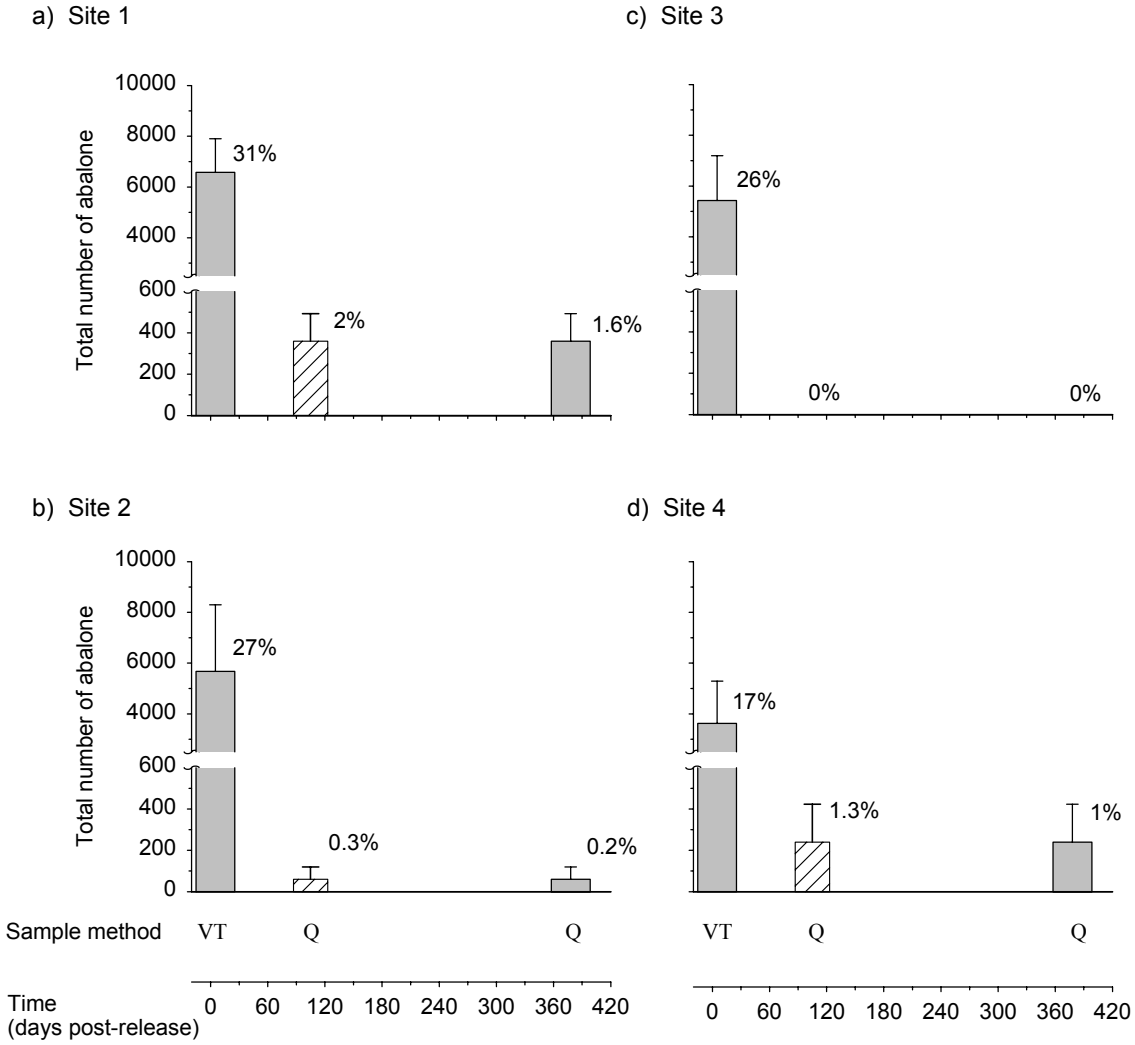


Figure 3. Total number of released *H. rubra* (± SE), 5 and 378 days after release, at each of the 4 sites at Broughton Is., Port Stephens. Sampling consisted of visual radial transects (2 x 0.5 m) from devices (VT) (n = 5), and sampling 1 m^2 destructive quadrats (Q) (n = 10). Numbers indicate the percent of juveniles surviving at each time.

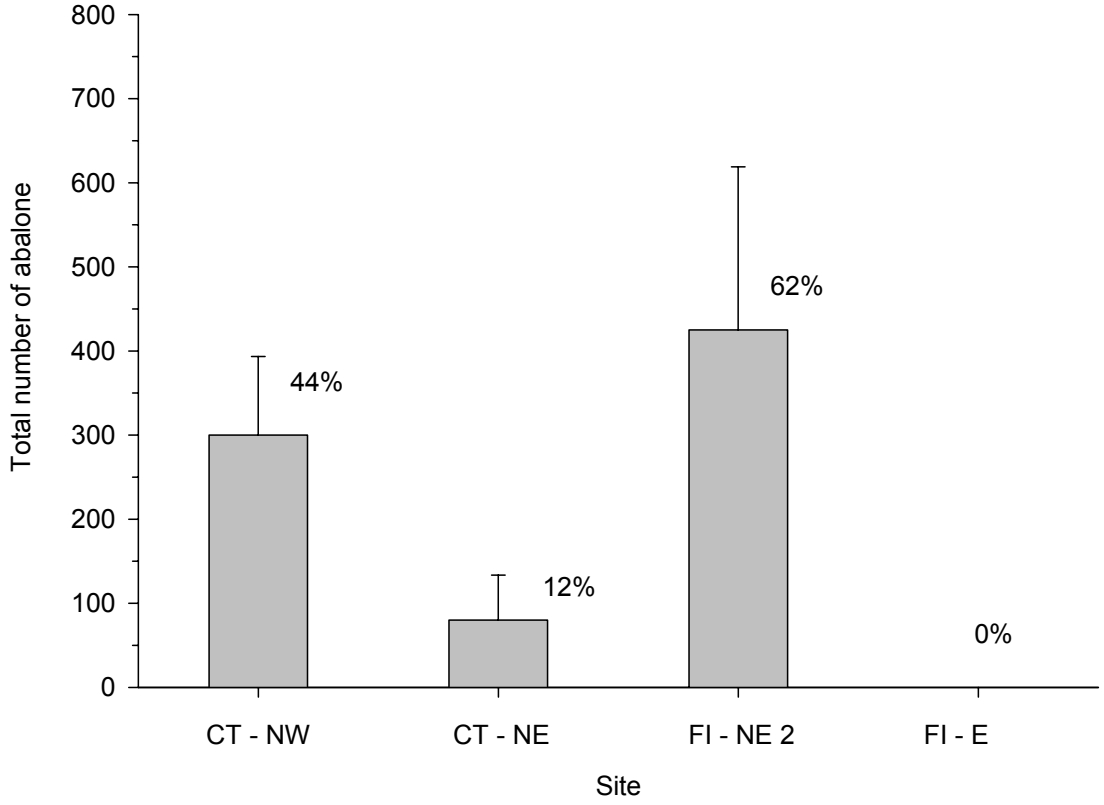


Figure 4. Total number of released abalone (± SE) at each of the four sites at Cabbage Tree (CT) and Fingal Islands (FI), Port Stephens after 119 days, sampled using 1 m^2 destructive quadrats (n = 10). Numbers indicate the percent of juveniles surviving.

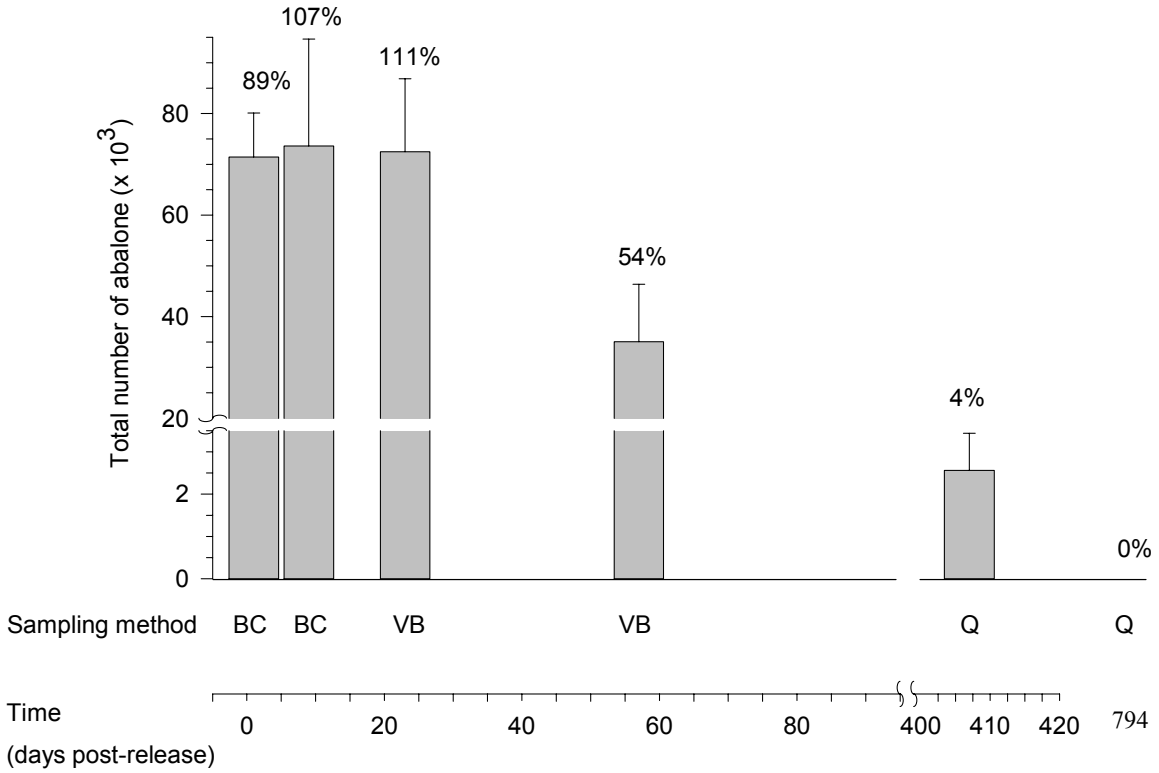


Figure 5. Total number of released abalone (± SE) at Fingal Is. - SSE (Gibbo Bay), Port Stephens through time. Three sampling methods were used to estimate abundance; boulder collections around devices (BC) (n = 6 for days 1 and 9), visual inspection of boulders (VB) (n = 6 for days 23 and 57) and 1 m^2 destructive quadrat (Q) (n = 10 for days 407 and 794). Numbers indicate the percent of juveniles surviving at each time. Note; numbers of juveniles on devices within stratified sampling areas were included in the calculation of the average number of abalone per sample for days 1-57.

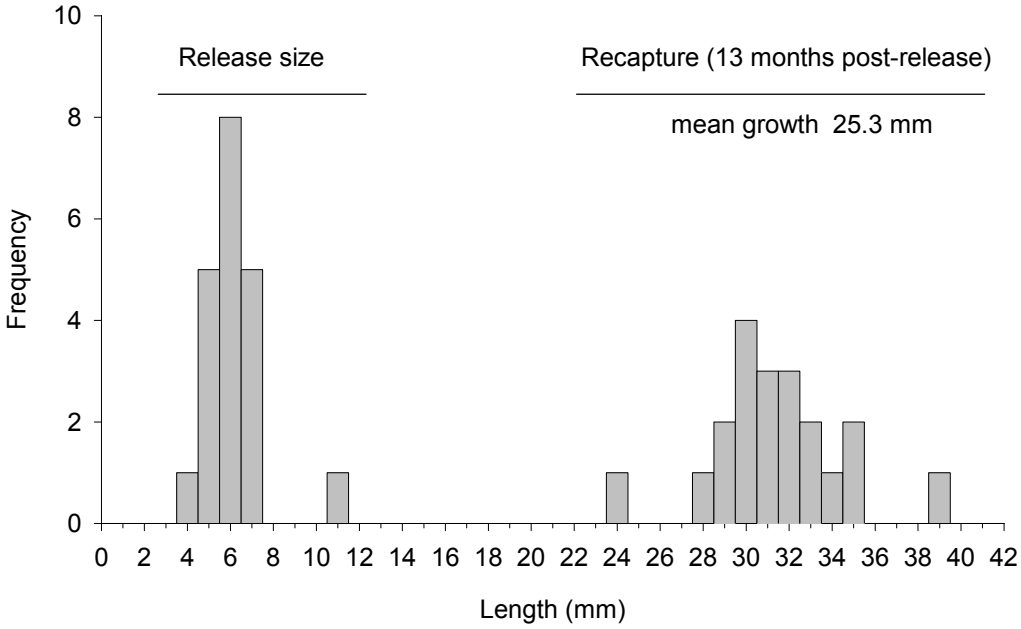


Figure 6. Length frequency of juveniles recaptured from Fingal Is. - SSE (Gibbo Bay), Port Stephens, and the length of the hatchery shell in the spire of those juveniles recaptured, (n = 20).

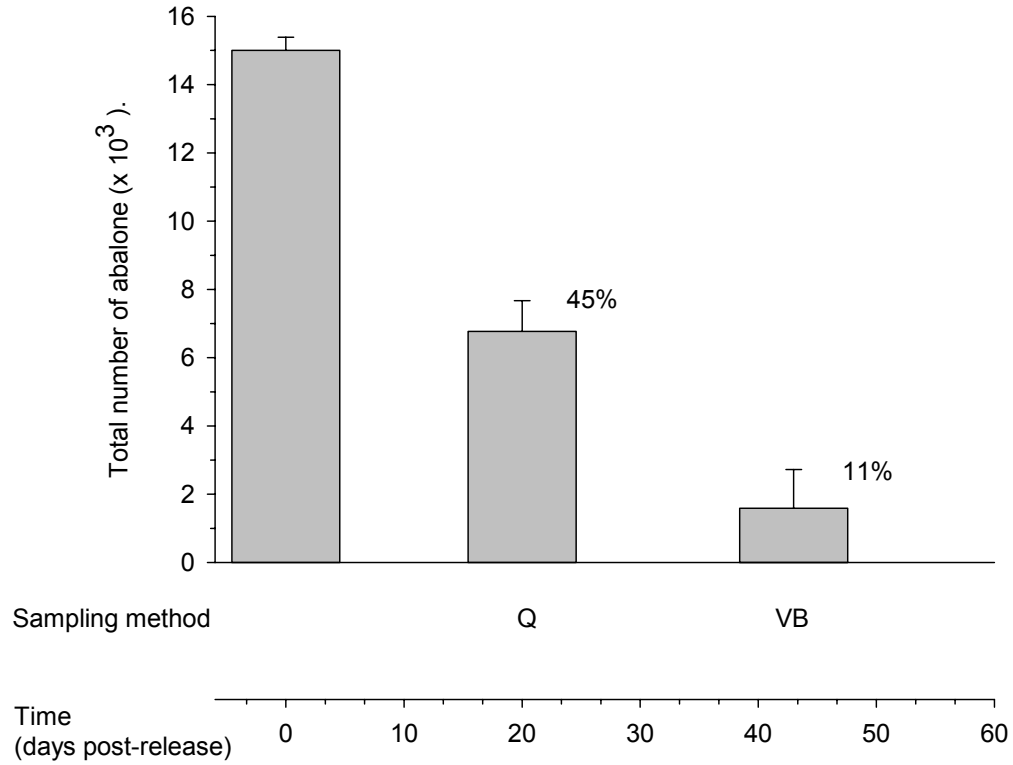


Figure 7. Total number of released abalone (± SE) at Jibbon Point - South, Sydney through time. Sampling at 20 and 43 days post-release was by 1 m^2 destructive quadrats (Q) (n = 6) and the visual inspection of boulders (VB) (n = 10) respectively. Numbers indicate the percent of juveniles surviving at each time.

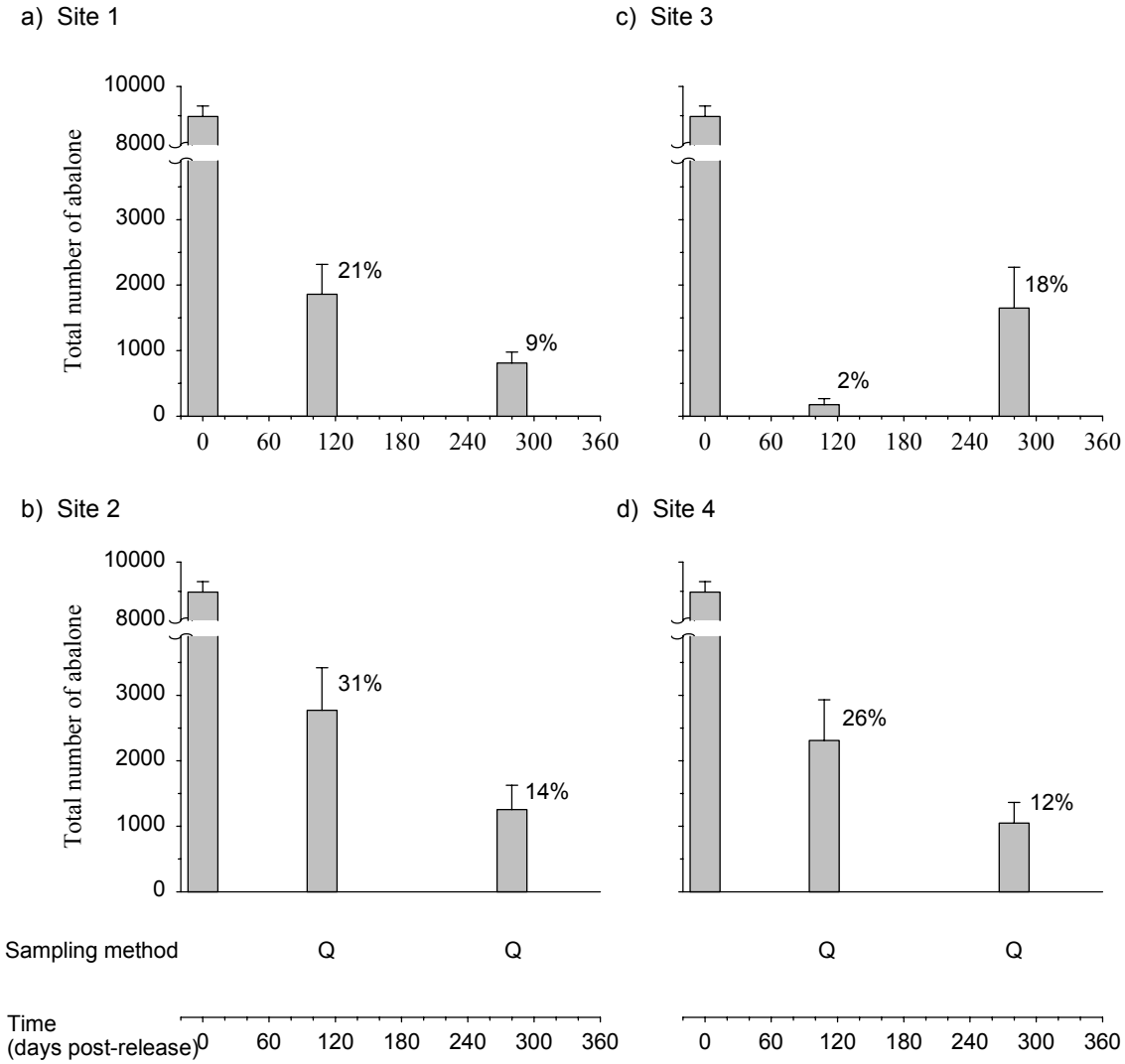


Figure 8. Total number of abalone (± SE) at the 4 sites off Blowhole and Easts Beach, at Kiama. Sampling was done by 0.36 m^2 destructive quadrat (Q) (n = 10). Numbers indicate the percent of juveniles surviving.

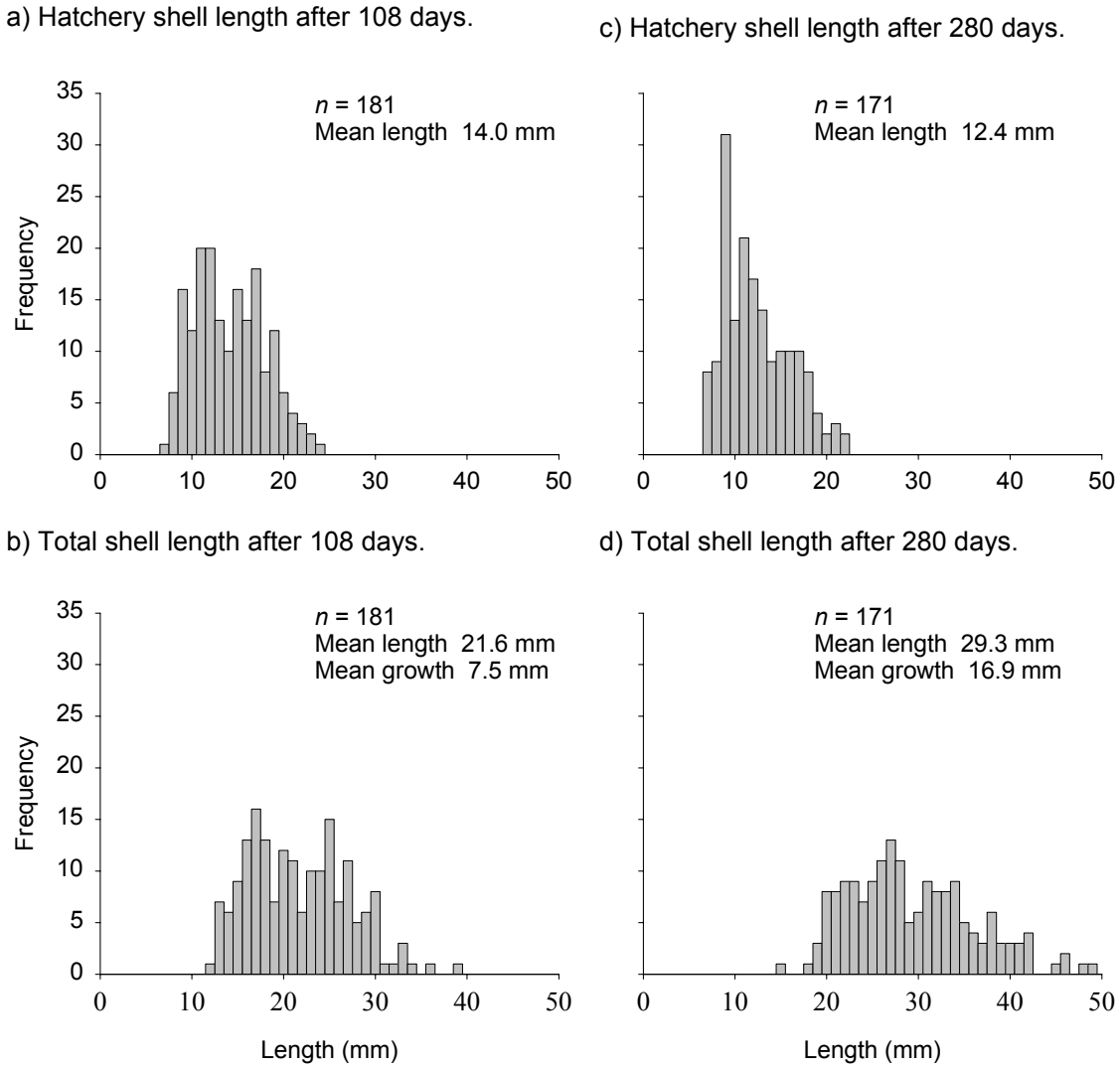


Figure 9. Length frequency distribution of the hatchery shell (measured from the spire of recaptured *H. rubra*) and the total shell length of *H. rubra* recaptured after 108 and 280 days from the 4 sites at Kiama.

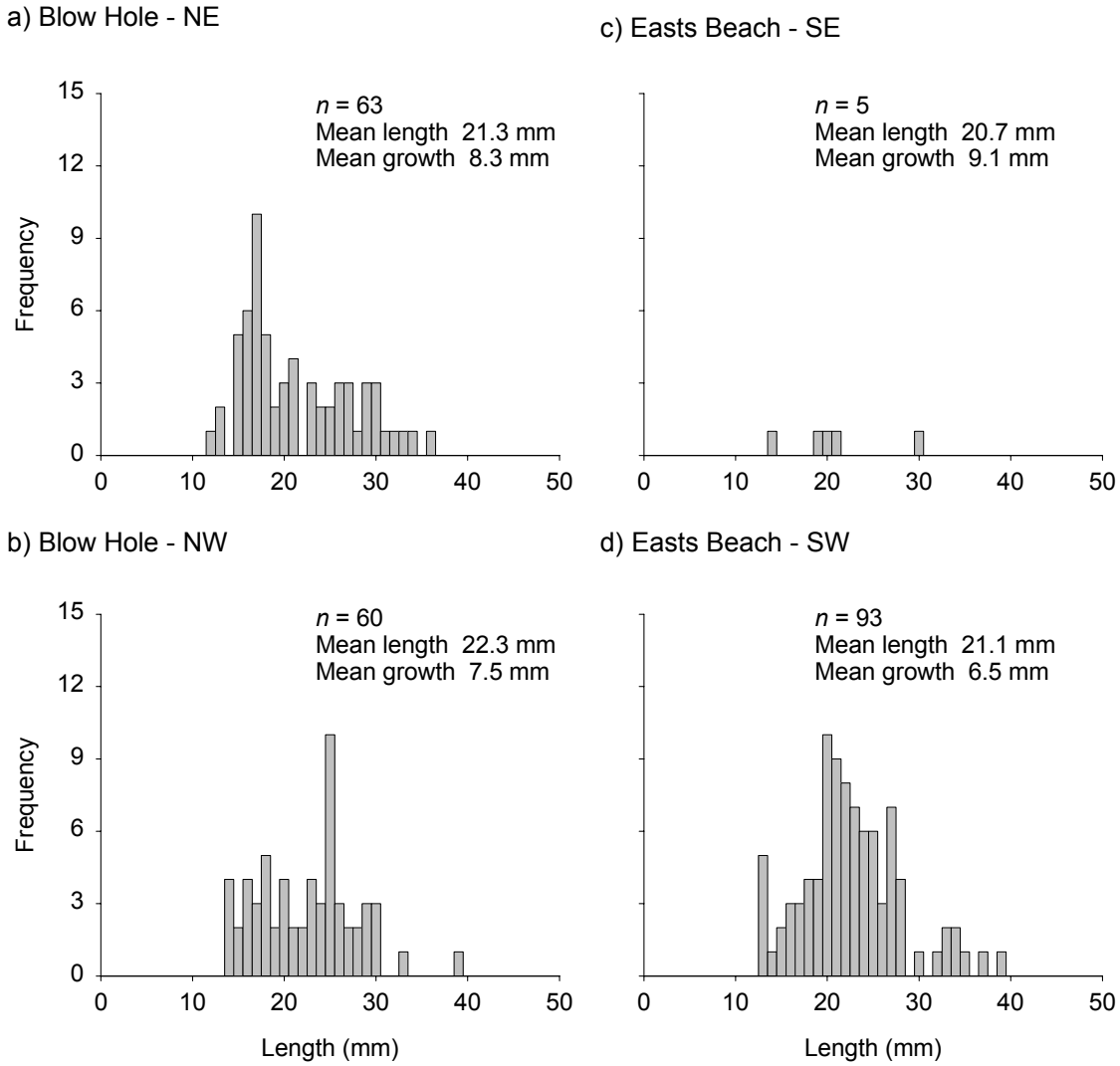


Figure 10. Length frequency distribution of *H. rubra* recaptured 108 days after release at each of the 4 sites at Kiama.

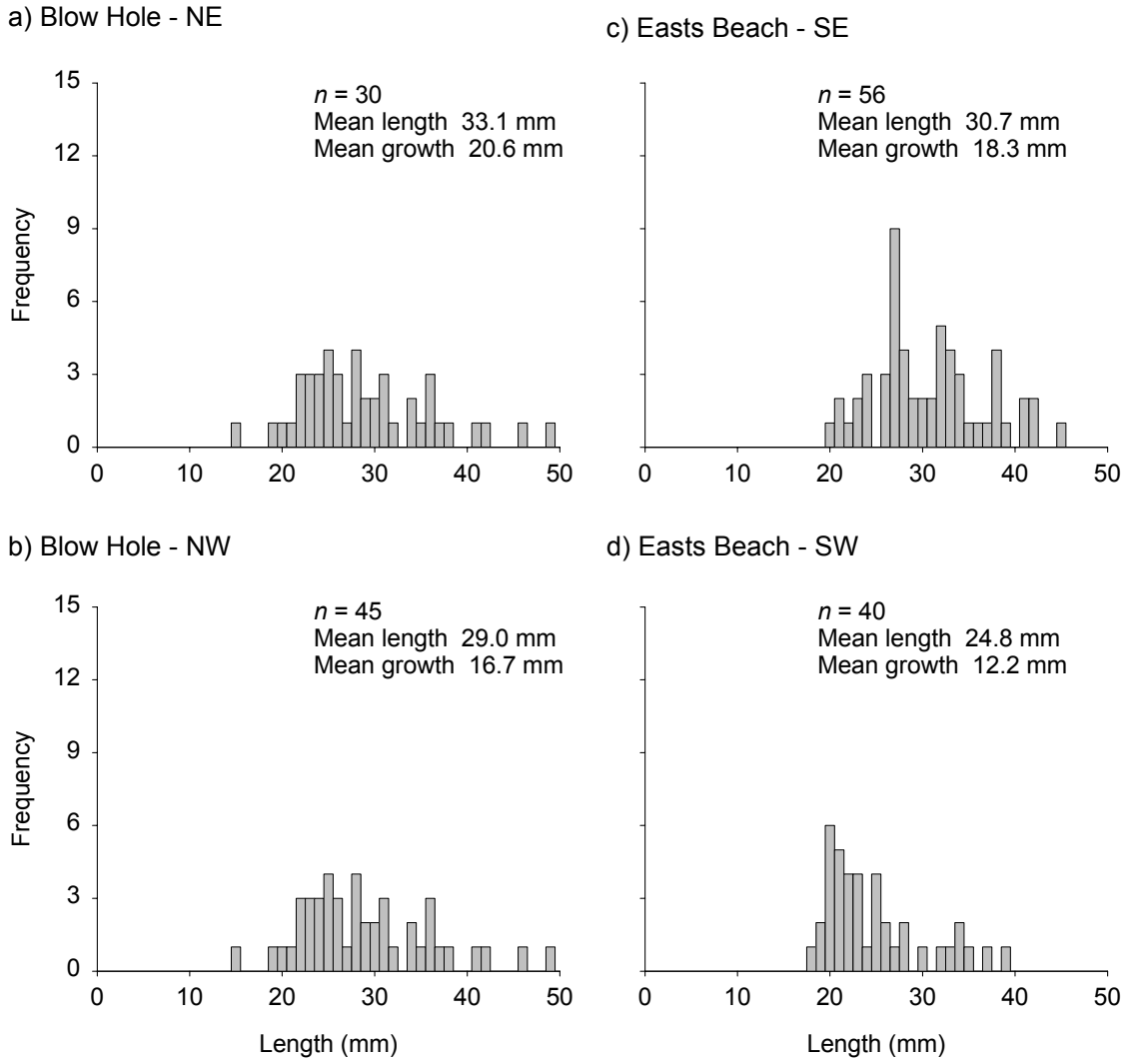


Figure 11. Length frequency distribution of *H. rubra* recaptured 280 days after release at each of the 4 sites at Kiama.

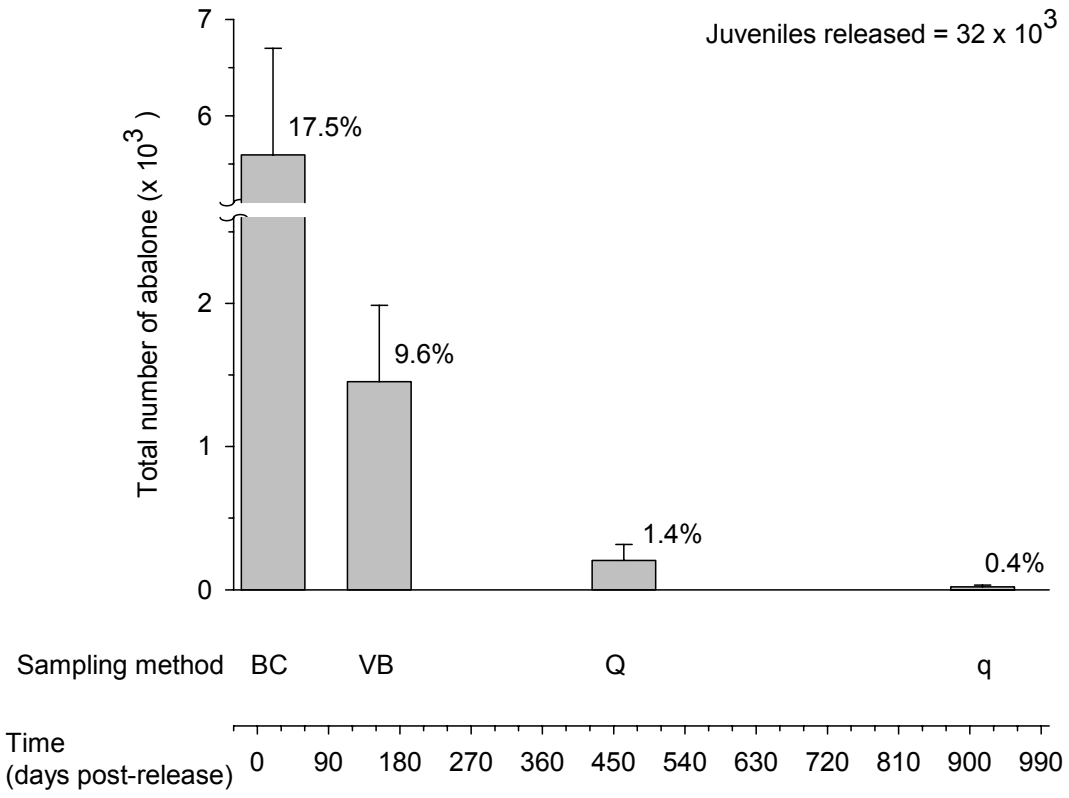


Figure 12. Total number of released *H. rubra* (± SE) at Red Point - South, Eden through time. Three sampling methods were used to estimate abundance; boulder collections around devices (BC) (n = 6), visual inspection of boulders (VB) (n = 6), 1m^2 destructive quadrats (Q) (n = 5) and 0.36m^2 destructive quadrat (q) (n = 15). Numbers indicate the percent of juveniles surviving at each time. Note; numbers of juveniles on devices within stratified sampling areas were included in the calculation of the average number of *H. rubra* per sample for days 20 and 154.

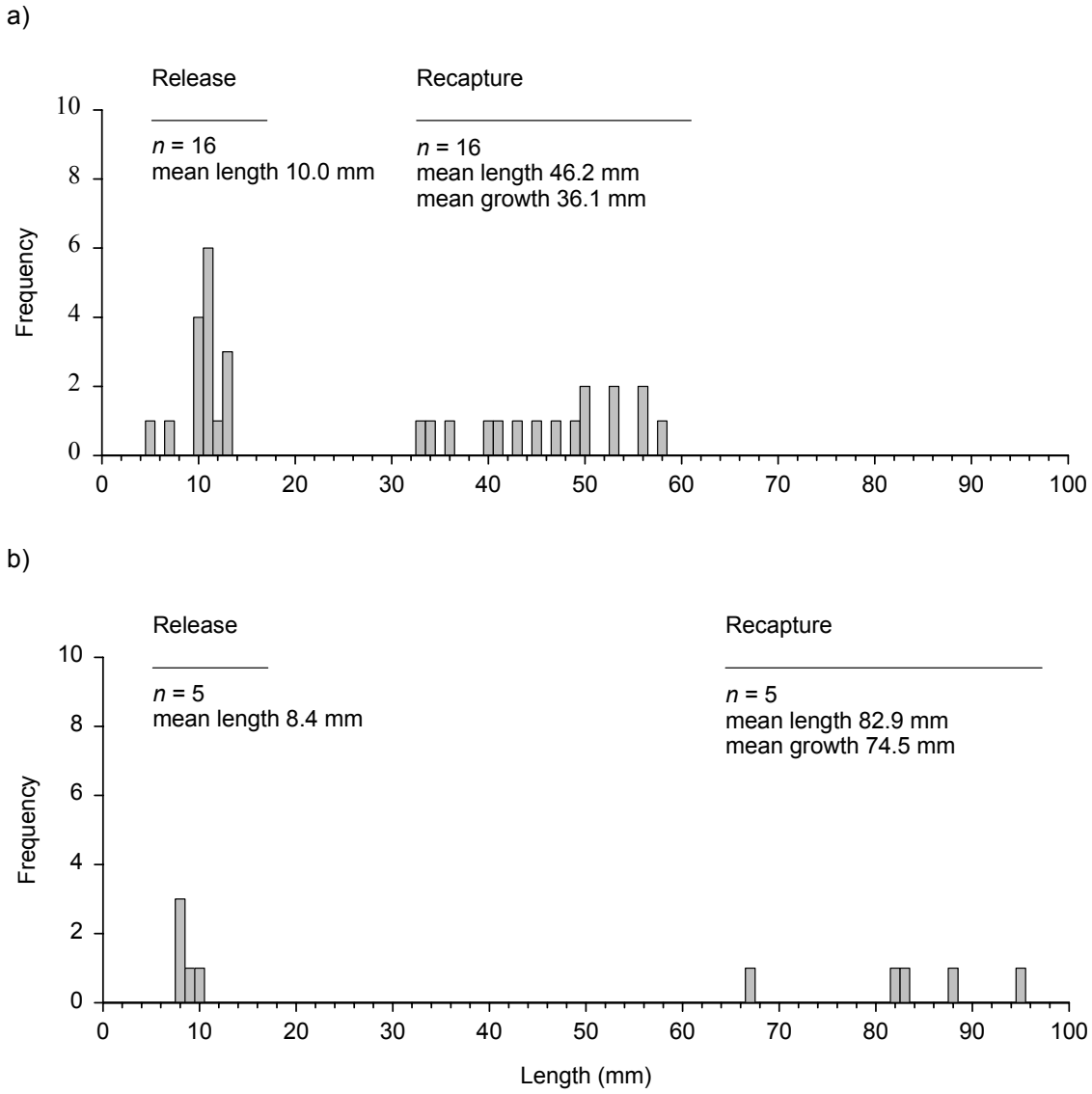


Figure 13. Length frequency distribution of the hatchery shell (measured from the spire of recaptured *H. rubra*) and the total length of *H. rubra* recaptured after (a) 463 and (b) 916 days at Red Point - South, Eden.

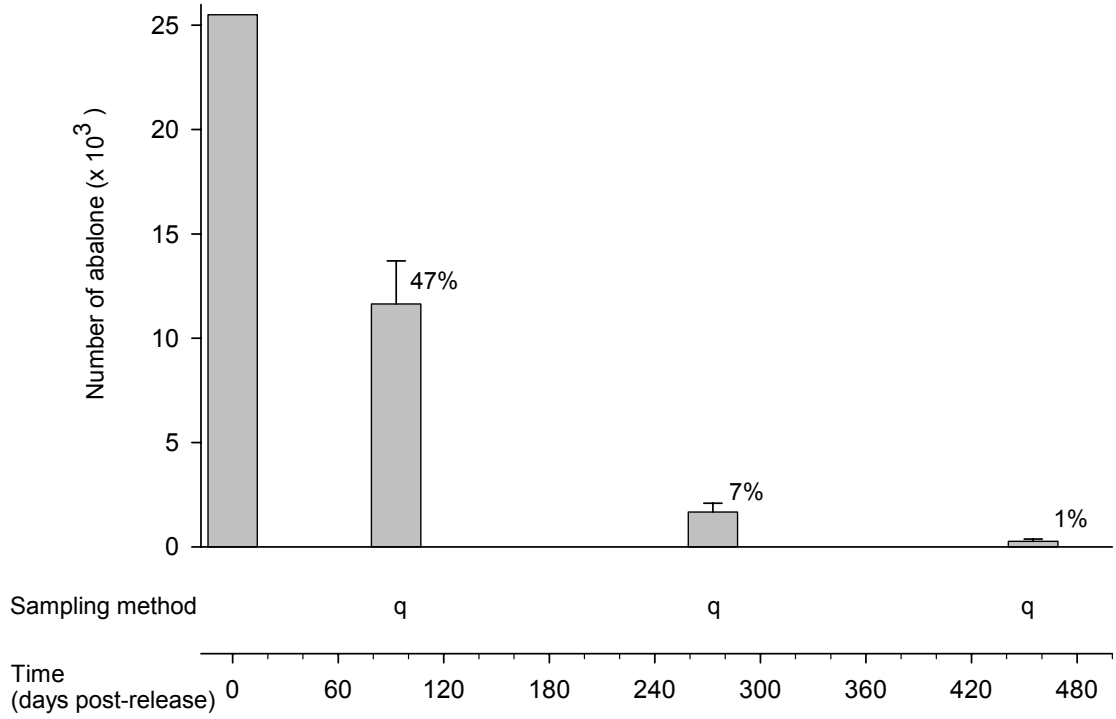


Figure 14. Total number of released *H. rubra* (± SE) at Steamtrawler, Eden, through time. Sampling was done using 0.36m^2 destructive quadrats (q) (n = 15, 10 and 15 for each sampling time respectively). Numbers indicate the percent of juveniles surviving at each time.

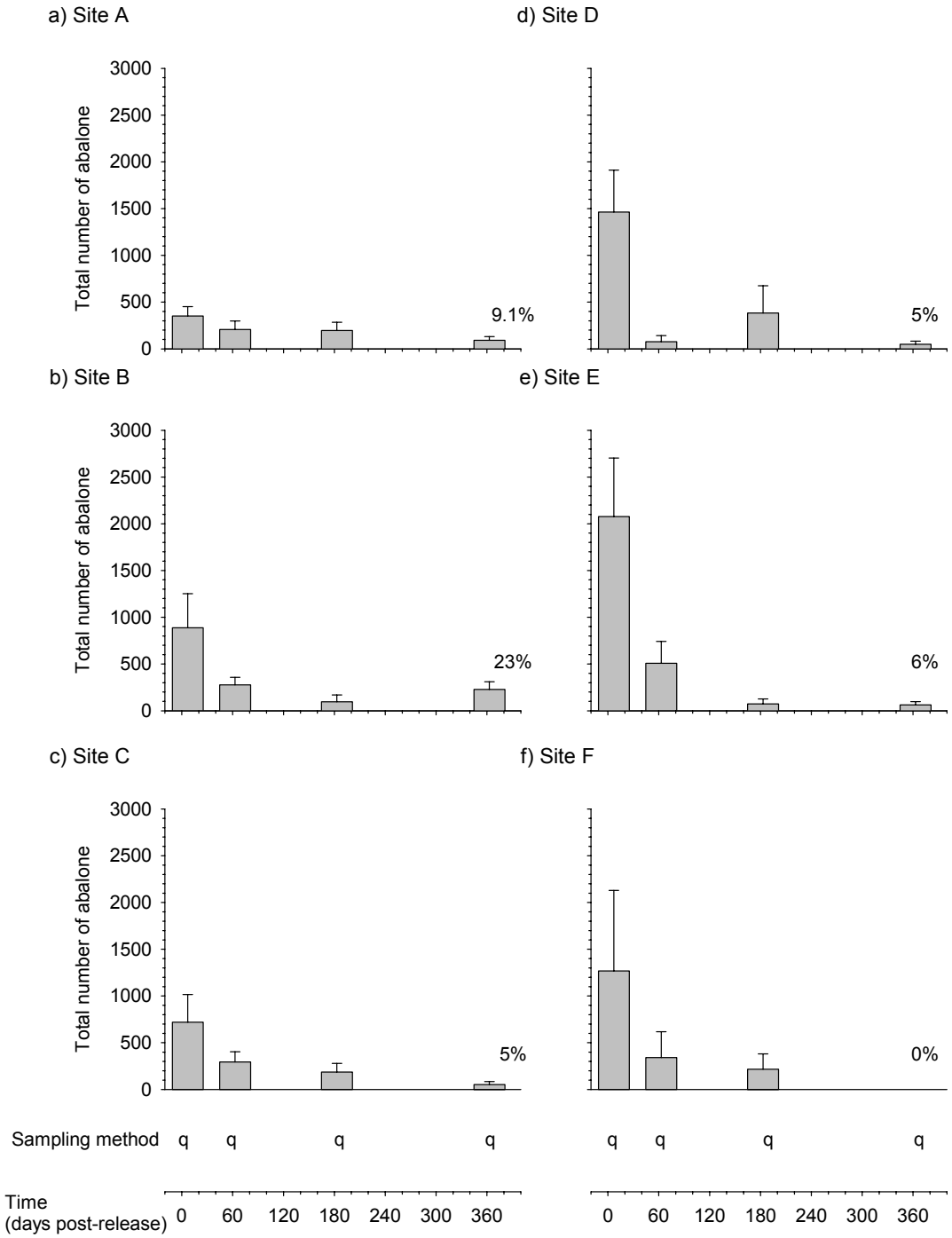


Figure 15. Total number of released *H. rubra* (± SE) at each of the release sites at Disaster Bay - Fenceline, Eden through time. Sampling at all sites at each time was by 0.36m^2 destructive quadrat (q) (n = 25). Sites A-C had 10 PVC devices each holding 100 juveniles evenly placed throughout each site. Sites D-F each had one PVC device holding 1,000 juveniles placed in the centre of the site.

Table 2. Summary of information for each of the sites where juvenile *H. rubra* were released. All numbers are calculated from the numbers of recaptured abalone averaged across sites for each sampling time. * *H. rubra* collected from deployment devices have been included in the calculation of the mean number per sample to represent the true number present within the sample area. See Appendix Table A2 for method and site detail.

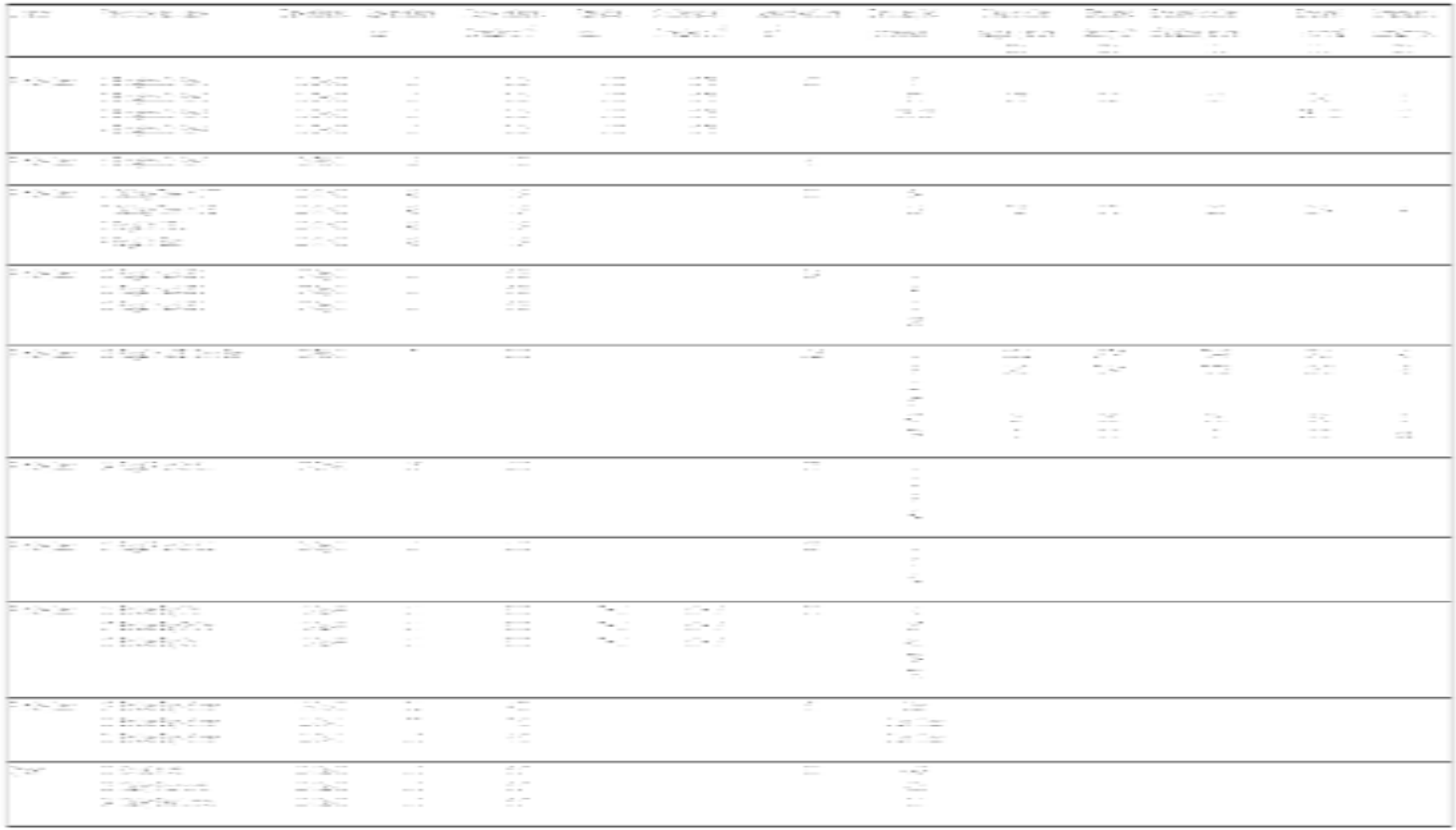
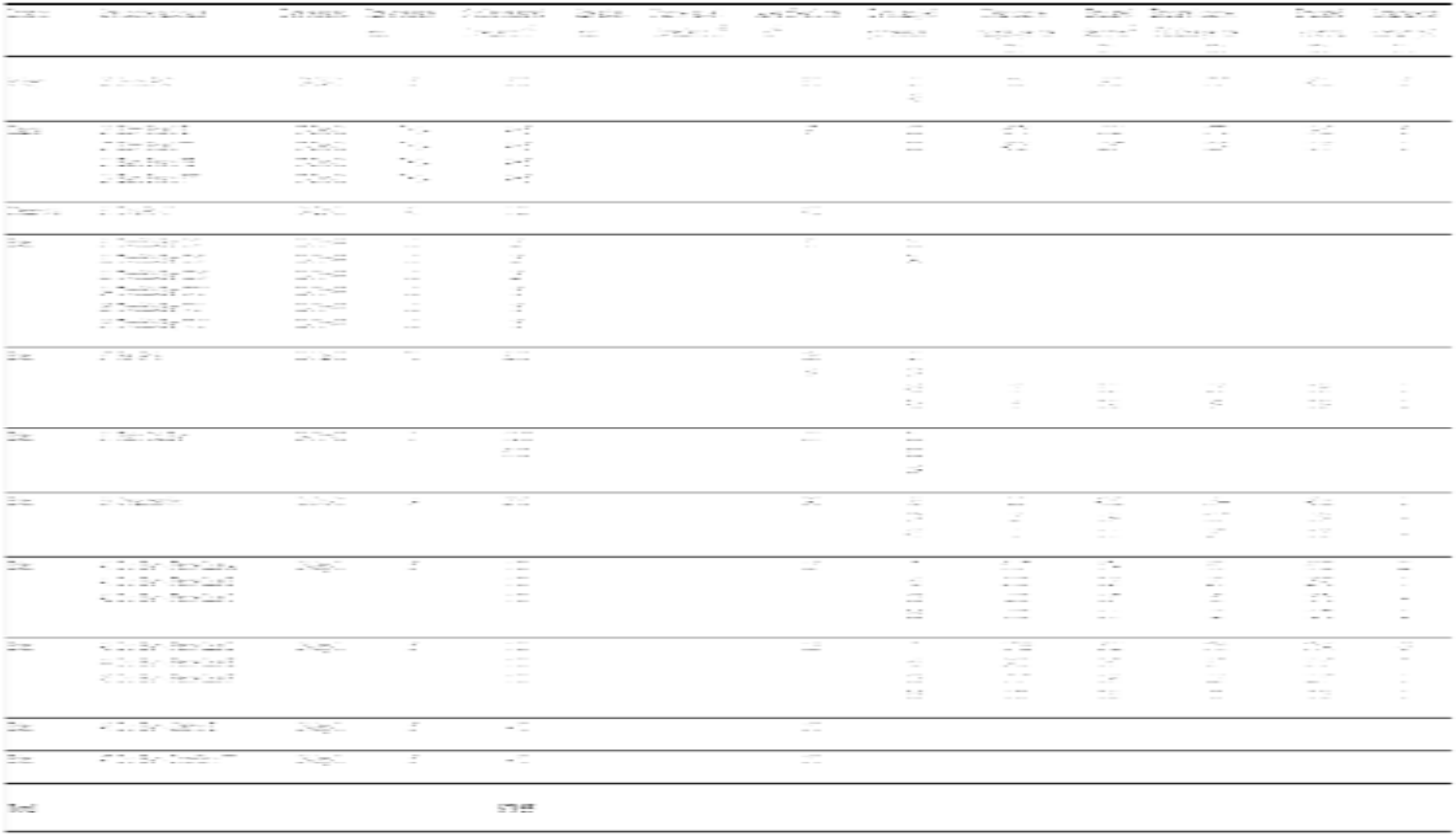


Table 2. Continued.



8.5. Discussion

The results of experiments where hatchery reared *H. rubra* were released and sampled using the most accurate techniques developed in this study have provided estimates of the survival and growth of these abalone in natural habitat for up to 916 days. Future sampling of the release sites will provide further estimates of these parameters through time. In general, only a small proportion (0-23%, Figure 16) of the abalone released within a site could be found after a year or more. Several factors could have contributed to this loss of individuals including emigration from the site, sampling inefficiency within the site and mortality. Rates of emigration were minimised by the selection of sites with borders of mostly inappropriate or potentially undesirable habitat for abalone. The efficiency of sampling the experiments presented in this section was maximised by using destructive methods that allowed the searching of most cryptic habitat. Despite attempting to minimise emigration and maximise sampling efficiency, estimates of survival have been confounded, to some extent, by these factors. Further, estimates of survival are also likely to have been affected by mortalities caused during the handling, transportation and release of these abalone. Despite these confounding factors, the natural mortality of released *H. rubra* is likely to have a major effect on the estimated rates of survival.

Estimates of the survival of released *H. rubra* can be compared to those for wild abalone (Figure 16). As with the estimates of survival of released abalone, the survival of wild abalone appears to vary among sites and through time as the abalone grow. Shepherd and Breen (1992) presented an estimated average rate of survival through time based on published data for wild *H. rubra*. In that study mortality of larval and small juvenile *H. rubra* was very high (i.e. $M > 5$), and declined from approximately 2.5 at 6 months of age to 0.3 after several years (see solid reference line, Figure 16). In this study most estimates of the survival of released *H. rubra* were lower than the expected estimates for wild abalone. Some estimates of the survival of released *H. rubra* suggested mortality rates more than twice that expected for wild counterparts. For example, survival of released *H. rubra* at Jibbon Point was many times lower than expected for wild abalone, and this was probably related to the major habitat disturbance caused by a storm 1-2 days after their release. Three sites, Easts Beach SE, and Disaster Bay sites B and D, show rates of survival higher than expected for wild abalone (Figure 16).

In this study, estimated rates of survival varied greatly among release sites and through time. For example, following the survival of an estimated 2500 juvenile *H. rubra* at Fingal Island - SSE after 407 days, most of the remaining individuals died rapidly, presumably in response to the *Perkinsus* outbreak in the area. Similarly, the major storm that disturbed habitat at Jibbon Point – S. presumably caused intense mortality over a very short time period. Spatial variation in survival was also high, even between apparently similar sites separated by short distances. For example, estimates of survival among the sites within Disaster Bay after 363 days ranged from 0-23%. Any variation in survival related to the size of *H. rubra* at release was swamped by the large spatial and temporal variation in survival. Furthermore, in the sites at Kiama where individuals from two batches of different sized *H. rubra* were released, there was some indication that juveniles of a smaller size had a higher rate of survival than larger juveniles released with them.

Rates of growth of *H. rubra* were highly variable, both prior to and following release. At release, sizes of juvenile *H. rubra* within a batch frequently varied by over 50%, and the mean length of *H. rubra* within the batch was often associated with a CV of 20%. For example, the mean ($\pm$SE) length of *H. rubra* released to Broughton Island, Port Stephens was 9.8 mm ($\pm$0.2, $n = 150$) (a CV of 28%). Following release, rates of growth among juvenile *H. rubra* from a single batch within a site were also highly variable, with a tendency for juveniles below the average size at release to grow disproportionately faster than animals above the average size at release. Average rates of growth of juveniles from the same batch at different sites also varied, for example mean growth increments ranged from 12.2-20.6 mm over 9 months at the sites at Kiama. At these average rates of growth, abalone released at 5-10 mm SL would be expected to reach maturity (e.g. >90 mm, see

Worthington & Andrew 1997) after 2.5-3.5 years, and the minimum legal length of 115 mm after >4 years. This suggests that juvenile *H. rubra* most recently released during this project are expected to enter the fishery on or after 2005.

During the project, techniques of handling, transport and release were developed and improved in an attempt to maximise the survival of released *H. rubra*. Several developments, in particular, may have contributed to higher rates of survival. For example, reduced handling and increased time for abalone to recover from anaesthesia used for later releases may have been particularly beneficial. Whilst these improvements confound the comparison of rates of survival among sites, they demonstrate the potential of continued research to further improve survival of released abalone. In particular, investigation of several factors should provide beneficial information to further improve survival. For example, investigation of ways to reduce the naivety of released abalone, potentially through modifications to behaviour from the use of different tanking systems prior to release (Osumi 1999). Further, the rapid grow-out techniques developed in this project need to be investigated to determine if they affect the physiological development (digestive tract) and subsequent survival of released *H. rubra*.

Several methods to sample the abundance of settling larvae and juvenile *H. rubra* were developed during this project. Because of the cryptic behaviour of juvenile *H. rubra*, and apparent ontogenetic changes in their preferred micro-habitat, a range of methods needed to be developed to estimate abundance through time. The most reliable, and least biased method of estimating abundance and hence survival involved the destructive sampling of small areas of reef. Estimates of density within sampled areas were then used to estimate the total abundance of *H. rubra* within the release sites. Despite the advantages of these methods, sampling and extrapolation of density is still complicated by several factors. For example, habitat within sites was not homogeneous and not all areas within sites were able to be sampled. Whilst estimates of the area of habitat that could be sampled within sites were made, habitat unable to be sampled may contain different densities of *H. rubra* and could experience different rates of survival. Another difficulty was the compromise between sampling a large fraction of the release site as frequently as possible, whilst trying to minimise disturbance to the habitat and inhabiting abalone. Finally, as described above, quantitative sampling was only completed within the release site. *H. rubra* released as larvae probably settle and survive outside the immediate release site, and once released, juvenile *H. rubra* could also move rapidly outside the release site. These sampling difficulties lead mostly to conservative estimates of survival.

Releases described in this section have provided estimates of survival and growth over periods of up to 916 days in unmodified natural habitat. Estimates of survival were confounded by several factors including development of methods of handling and releases, unusual natural occurrences such as *Perkinsus* and storms, and difficulties of sampling. Techniques of handling, transportation, release and sampling have been greatly developed to improve the chances of survival of released *H. rubra*. Survival for some releases was estimated to be above that which might be expected for wild populations of abalone. With additional investigation of several factors that might compromise the survival of released abalone (see further discussion in Section 9), further improvements in long-term survival and growth should be possible.

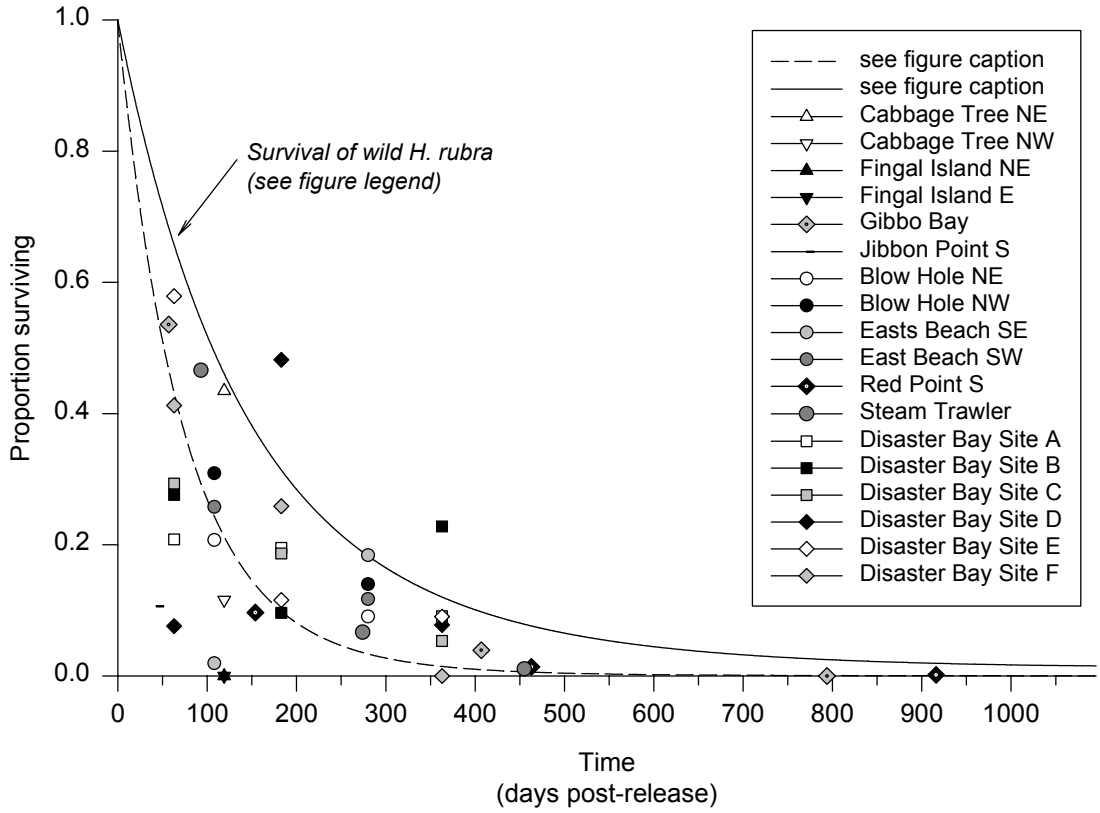


Figure 16. Proportion of *H. rubra* surviving at each site through time, where sampling using destructive methods has been done. Methods of sampling through time at sites vary (see Table 2). Solid line provides a reference of a linear decline in M (mortality) from 2.5 to 0.3 from 0 to 1095 days, as suggested by Shepherd & Breen (1992) for wild *H. rubra.* Dashed line provides a reference of twice the M of the solid line. Note, the curved lines represent linear declines in M, but are plotted as the proportion of abalone surviving through time.

8.6. Appendix - Section 8

Table A1. Comprehensive information for each of the sites where *H. rubra* larvae were released. All numbers are calculated from the numbers of recaptured abalone averaged across sites for each sample time.

Location	Site number and name	GPS - MapInfo.exe East	GPS - MapInfo.exe South	Date released	Size at release L = larvae	Number released (Larvae x 10^6)	Date of addition of abalone	Size added	Number added (Larvae x 10^6)	Area of each site (m^2)	Habitat type	Dates each site sampled	Days sampled post release	Sample method Method 1 (M 1)	Sample method Method 2 (M 2)	Mean number sampled per site M 1	Estimated density m^{-2} M 1	Estimated number of abalone per site M 1	Estimated % survival M 1	Instantaneous mortality yr-1 M 1
1. Port Stephens	1. Fingal Is. S	152°11'10.92"	32°44'59.29"	27-Jan-99	L	0.93				450	Bedrock	28-Jan-99	1	V-lift	Settlement plate					
												29-Jan-99	2	V-lift	Settlement plate					
												04-Feb-99	8	V-lift						
												11-May-99	104	V-lift						
												17-Nov-99	294	Random visual t'sect						
2. Port Stephens	2. Fingal Is. NW	152°11'41.00"	32°44'25.76"	22-Sep-99	L	1.10	16-May-00	L	2.00	375	Lg. Bould.	23-Sep-99	1	Bould.-coll.	V-lift	10.67	43.37	16264	1.479	1538
	3. Fingal Is. N	152°11'51.49"	32°44'25.76"	22-Sep-99	L	1.10	16-May-00	L	2.00		Lg. Bould.	24-Sep-99	2	Bould.-coll.	V-lift	22.67	92.16	34561	3.142	632
	4. Fingal Is. NE	152°12'07.56"	32°44'31.65"	22-Sep-99	L	1.10	16-May-00	L	2.00		Lg. Bould.	06-Oct-99	14	Bould.-coll.	V-lift	10.00	40.66	15248	1.386	112
												17-Nov-99	56	Bould.-coll.	V-lift	0.33	1.36	508	0.046	50
												05-Apr-00	196	Bould. non-destructive						
												07-Nov-00	412	Bould. non-destructive	Visual t'sect					
												28-Mar-01	553	Destructive Quadrats		1.67	0.33	125	0.004	7
2. Port Stephens	5. Fingal Is. NNE	152°12'02.68"	32°44'28.12"	14-Oct-99	L	2.00	16-May-00	L	2.00	240	Sm. Bould.	15-Oct-99	1	Bould.-coll.	V-lift	154.00	671.44	161146	8.057	919
												27-Oct-99	13	Bould.-coll.	V-lift	14.00	61.04	14650	0.732	138
												08-Dec-99	55	Bould.-coll.	V-lift	1.00	4.36	1046	0.052	50
												21-Nov-00	404	Bould. non-destructive	Visual t'sect					
												28-Mar-01	531	Destructive Quadrats		0.00		0	0.000	n/a
Port Stephens	6. Sunny Corner	152°07'14.29"	32°46'54.57"	10-Nov-01	L	1.00				300	Lg. Bould.									
Sydney	7. Jibbon Pt S	152°10'18.86"	34°05'01.73"	24-May-01	L	2.20				200	Sm. Bould.	25-May-01	1	Bould.-coll.		9.00	7.78	1555	0.071	2648
Ulladulla	8. Brush Is S	150°25'05.28"	35°31'50.38"	21-Feb-02	L	3.20				300	Sm. Bould.									
Eden	9. Yullumgo Cove	149°54'47.52"	37°04'09.29"	29-Jun-00	L	1.80				225	Sm. Bould.	23-Aug-00	55	Bould.-coll.		0.00	0.00	0	0.000	n/a
Eden	10. Leather Jacket Bay S	149°58'24.22"	37°07'37.07"	01-Feb-02	L	2.20				200	Lg. Bould.									
Total						**24.63**														

Table A2. Comprehensive information for each of the sites where juvenile *H. rubra* were released. All numbers are calculated from the numbers of recaptured abalone averaged across sites for each sample time. * *H. rubra* collected from deployment devices have been included in the calculation of the mean number per sample to represent the true number present within the sample area.

Location	Site number and name	GPS - MapInfo.exe East	GPS - MapInfo.exe South	Date released	Size at release (mm)	Number released (Juveniles x 10^3)	Date of addition of abalone	Size added	Number added (Juveniles x 10^3)	Area of each site (m^2)	Habitat type	Dates each site sampled	Days sampled post release	Sample method Method 1 (M 1)	Sample method Method 2 (M 2)	Mean number sampled per site M 1	Estimated density m^{-2} M 1	Estimated number of abalone per site M 1	Estimated % survival M 1	Instantaneous mortality yr-1 M 1
Port Stephens	1. Broughton Is. Site 1	152°19'23.07"	32°37'15.24"	06-Dec-00	10	21.25	05-Sep-01	10.00	18.75	600	Lg. Bould.	11-Dec-00	5	Visual radial t'sect						
	2. Broughton Is. Site 2	152°19'49.64"	32°37'07.00"	06-Dec-00	10	21.25	05-Sep-01	10.00	18.75		Lg. Bould.	19-Dec-01	378	Destructive Quadrats		2.75	0.28	165	0.41	5
	3. Broughton Is. Site 3	152°19'20.97"	32°36'48.18"	06-Dec-00	10	21.25	05-Sep-01	10.00	18.75		Lg. Bould.		OR 105						OR 0.88	16
	4. Broughton Is. Site 4	152°19'04.89"	32°37'27.59"	06-Dec-00	10	21.25	05-Sep-01	10.00	18.75		Lg. Bould.									
Port Stephens	5. Broughton Is. Site 5	152°19'18.18"	32°37'17.59"	06-Feb-01	13	8.00				60	Lg. Bould.									
Port Stephens	6. Cabbage Tree Is. NW	152°13'24.51"	32°4'1'01.48"	22-Nov-00	43	0.69				300	Lg. Bould.	15-Jan-01	54	Random visual t'sect						
	7. Cabbage Tree Is. NE	152°13'34.30"	32°4'1'06.77"	22-Nov-00	43	0.69					Lg. Bould.	21-Mar-01	119	Destructive Quadrats	Ramdom visual t'sect	7.25	0.73	218	31.64	4
	8. Fingal Is. NE 2	152°12'04.78"	32°44'30.47"	22-Nov-00	43	0.69					Lg. Bould.									
	9. Fingal Is. East	152°12'08.97"	32°44'44.58"	22-Nov-00	43	0.69					Lg. Bould.									
Port Stephens	10. Fingal Island SE 1	152°12'08.97"	32°44'42.23"	07-Sep-00	11	5.33				314	Lg. Bould.	08-Sep-00	1	Visual radial transect	Visual quadrat					
	11. Fingal Island SE 2	152°12'10.38"	32°44'46.35"	07-Sep-00	11	5.33					Lg. Bould.	11-Sep-00	4	Visual radial transect	Visual quadrat					
	12. Fingal Island SE 3	152°12'11.77"	32°44'48.12"	07-Sep-00	11	5.33					Lg. Bould.	15-Sep-00	8	Visual radial transect	Visual quadrat					
												21-Mar-01	195	Visual radial transect	Visual quadrat					
Port Stephens	13. Fingal Is. SSE (Gibbo Bay)	152°11'47.96"	32°44'48.70"	08-Feb-00	7	80.00				1025	Sm. Bould.	09-Feb-00	1	Bould.-coll.		11012	69.74*	71449	89.31	41
												17-Feb-00	9	Bould.-coll.		3450	71.94*	73703	106.85	-3
												02-Mar-00	23	Bould. non-destructive						
												05-Apr-00	57	Bould. non-destructive						
												21-Mar-01	407	Destruct. quadrat		25	2.50	2561	3.91	3
												12-Apr-02	794	Destruct. quadrat		0	0.00	0	0.00	n/a
Port Stephens	14. Fingal Point South 1	152°10'19.64"	32°45'26.15"	27-Oct-98	1.5	12.00				875	Bedrock	28-Oct-98	1	V-lift						
												29-Oct-98	2	V-lift						
												05-Nov-98	9	V-lift						
												08-Dec-98	42	V-lift						
Port Stephens	15. Fingal Point South 2	152°10'13.34"	32°45'25.56"	06-Sep-00	18	11.00				125	Bedrock	07-Sep-00	1	Fixed areas						
												11-Sep-00	5	Fixed areas						
												07-Nov-00	62	Fixed areas						
Port Stephens	16. Boulder Bay Nth	152°10'02.85"	32°45'40.26"	13-Jan-99	1.8	30.00	31-Jan-01	7 + 10	6.8 + 16	250	Bedrock	12-Jan-99	-1	Fixed areas						
	17. Boulder Bay Pt Nth	152°09'57.95"	32°45'39.09"	13-Jan-99	1.8	30.00	31-Jan-01	7 + 10	6.8 + 16		Bedrock	29-Jun-99	167	Fixed areas						
	18. Boulder Bay Sth	152°09'36.97"	32°45'50.85"	13-Jan-99	1.8	30.00	31-Jan-01	7 + 10	6.8 + 16		Bedrock	18-Apr-00	461	Fixed areas						
												16-Jan-01	734	Fixed areas						
												21-Mar-01	798	Fixed areas						
Port Stephens	19. Boulder Bay - 5 sites	152°09'42.57"	32°45'37.32"	09-Jun-00	2.2	4.00				50	Sm. Bould.	09-Jun-00	2 hrs	Bould. non-destructive						
	20. Boulder Bay - 5 sites	152°09'42.57"	32°45'37.32"	26-Jul-00	7.7	7.50					Sm. Bould.	26-Jul-00	2 hrs, 18 hrs	Bould. non-destructive						
	21. Boulder Bay - 5 sites	152°09'42.57"	32°45'37.32"	26-Jul-00	11.8	6.50					Sm. Bould.	26-Jul-00	2 hrs, 18 hrs	Bould. non-destructive						
Sydney	22. Kurnell south	151°13'51.48"	34°01'06.27"	20-Mar-02	11.8	5.67				300	Bedrock	21-Apr-98	-1429	Fixed areas						
	23. Marley Point north	151°08'39.54"	34°07'02.18"	20-Mar-02	11.8	5.67					Bedrock	27-Feb-01	-386	Fixed areas						
	24. Marley Beach south	151°08'04.57"	34°07'34.60"	20-Mar-02	11.8	5.67					Bedrock	16-Oct-02	210	Fixed areas						

Table A2. Continued.

Location	Site number and name	GPS - MapInfo.exe East	GPS - MapInfo.exe South	Date released	Size at release (mm)	Number released (Juveniles x 10^3)	Date of addition of abalone	Size added	Number added (Juveniles x 10^3)	Area of each site (m^2)	Habitat type	Dates each site sampled	Days sampled post release	Sample method Method 1 (M 1)	Sample method Method 2 (M 2)	Mean number sampled per site M 1	Estimated density m^{-2} M 1	Estimated number of abalone per site M 1	Estimated % survival M 1	Instantaneous mortality yr-1 M 1
Sydney	25. Jibbon Pt S	151°10'18.86"	34°05'01.73"	04-Jul-01	15	15.00				350	Sm. Bould.	24-Jul-01 16-Aug-01	20 43	Destruct. quadrat Bould. non-destructive		116	19.33	6767	45.11	15
Kiama	26. Blow Hole NE	150°53'30.32"	34°35'58.72"	07-Dec-01	7 + 14	4 + 5				97	Sm. Bould.	25-Mar-02	108	Destruct. quadrat		65.75	18.26	1772	19.68	5
	27. Blow Hole NW	150°53'24.72"	34°35'56.42"	07-Dec-01	7 + 14	4 + 5					Sm. Bould.	13-Sep-02	280	Destruct. quadrat		45.25	12.57	1219	13.55	3
	28. Easts Beach SE	150°53'04.44"	34°36'17.14"	07-Dec-01	7 + 14	4 + 5					Sm. Bould.									
	29. Easts Beach SW	150°53'01.64"	34°36'15.86"	07-Dec-01	7 + 14	4 + 5					Sm. Bould.									
Merimbula	30. Tura Pt - N	149°56'23.81"	36°50'48.74"	04-Oct-01	40	0.80				450	Bedrock									
Eden	31. Twofold Bay I N	149°56'12.85"	37°03'00.71"	02-Nov-99	1.3	25				50	Bedrock	11-Apr-00	161	Random visual t'sect						
	32. Twofold Bay II N	149°56'12.85"	37°03'00.71"	02-Nov-99	1.3	25					Bedrock	30-Jun-00	241	Random visual t'sect						
	33. Twofold Bay III N	149°56'12.85"	37°03'00.71"	02-Nov-99	1.3	25					Bedrock									
	34. Twofold Bay IV N	149°56'12.85"	37°03'00.71"	02-Nov-99	1.3	15					Bedrock									
	35. Twofold Bay V N	149°56'12.85"	37°03'00.71"	02-Nov-99	1.3	15					Bedrock									
	36. Twofold Bay VI N	149°56'12.85"	37°03'00.71"	02-Nov-99	1.3	15					Bedrock									
Eden	37. Red Pt S	149°57'16.97"	37°06'29.26"	22-Mar-00	7.3	32.00				136 64	Sm. Bould.	11-Apr-00 23-Aug-00 28-Jun-01 24-Sep-02	20 154 463 916	Bould. non-destructive Bould. non-destructive Destructive Quadrat Destructive Quadrat		16 5	3.20 0.93	205 59	1.36 0.39	3 2
Eden	38. Fisho Cull Bay	149°57'42.84"	37°06'57.50"	29-Nov-00	6	102.00 50.00				1000	Sm. Bould.	28-Jun-01 27-Sep-01 24-Sep-02	211 302 664	Bould. non-destructive Destructive Quadrat Destructive Quadrat						
Eden	39. Steamtrawler	150°00'23.62"	37°09'03.90"	26-Jun-01	14	25.50				240	Sm. Bould.	27-Sep-01 27-Mar-02 24-Sep-02	93 274 455	Destructive Quadrat Destructive Quadrat Destructive Quadrat	Visual quadrat	262 25 6	48.52 6.94 1.11	11644 1667 267	45.66 6.54 1.05	3 4 4
Eden	40. Dis. Bay - Fence Line A	150°02'52.56"	37°15'45.19"	26-Sep-01	15	1.00				115	Sm. Bould.	03-Oct-01	7	Destructive Quadrat		51.67	5.74	660	66.02	22
	41. Dis. Bay - Fence Line B					1.00					Sm. Bould.	28-Nov-01	63	Destructive Quadrat		20.33	2.26	260	25.98	8
	42. Dis. Bay - Fence Line C					1.00					Sm. Bould.	28-Mar-02	183	Destructive Quadrat		12.33	1.37	158	15.76	4
												24-Sep-02	363	Destructive Quadrat		10.00	1.11	128	12.78	2
Eden	43. Dis. Bay - Fence Line D	150°02'52.56"	37°15'45.19"	26-Sep-01	15	1.00				113	Sm. Bould.	03-Oct-01	7	Destructive Quadrat		137.33	15.26	1755	175.48	-29
	44. Dis. Bay - Fence Line E					1.00					Sm. Bould.	28-Nov-01	63	Destructive Quadrat		24.00	2.67	307	30.67	7
	45. Dis. Bay - Fence Line F					1.00					Sm. Bould.	28-Mar-02	183	Destructive Quadrat		17.67	1.96	226	22.57	3
												24-Sep-02	363	Destructive Quadrat		3.00	0.33	38	3.83	3
Eden	46. Dis. Bay - Skelton E	150°01'38.08"	37°15'33.21"	26-Sep-01	15	4.50				150	Lg. Bould.									
Eden	47. Dis. Bay - DoorKnob W	150°02'45.54"	37°15'38.51"	26-Sep-01	15	4.50				150	Lg. Bould.									
Total						**879.55**														

9. INVESTIGATION OF EARLY *H. RUBRA* POST-LARVAE AS AN ALTERNATIVE TO COMPETENT LARVAE FOR SEEDING OPERATIONS

M.P. Heasman, N. Savva, C. Brand & J. Diemar

9.1. Introduction, aims and overview

Seeding of hatchery produced larval or juvenile abalone has often been proposed as a panacea for enhancing production of otherwise healthy or rehabilitating depleted wild stocks, (Shepherd *et al*. 2000). Seeding of hatchery-produced juveniles has been practised with some success for almost four decades in Japan (Saito 1984; Kojima 1995; Masuda & Tsukamoto 1998) and experimentally with mixed results in several other countries. Low survival rates of 0 to 4% after 1 to 2 years were achieved in various studies with juvenile *H. rufescens, H. fulgens* and *H. corrugata* in the USA (Tegner 2000; McCormick *et al*. 1994). Reviewing results of 12 seeding studies involving 21 case histories with abalone over 6 months to 3 years, Shepherd (1998a) reported a mean ± s.e. instantaneous mortality per year (M/year) of 1.8 ± 0.5, representing a mean annual survival rate of 16.5%. These rates of survival were about half those of about 30% achieved with *H. midae* seeded at several sites in South Africa by Sweijd *et al*. (1998) and about a third those of 40 to 50 % (M/year values of 0.7 to 0.9) reported by Prince *et al.* (1988) and Day & Leorke (1986) for wild *H. rubra.*

Large scale seeding with competent larvae rather than juveniles has been practised sporadically in Mexico since the 1960's, but its effectiveness has never been formally evaluated (Ortiz-Quintanilla 1980). Experimental larval seeding onto coastal reefs in the Chatham Islands east of New Zealand by Schiel (1992), resulted in mean ± s.e. 24 h post-settlement yields of 10.0± 2.5% but equivalent yields for 4 to 5 month post settlement of only 0.056 ± 0.024%. This constitutes a survival rate of only one 5 mm juvenile for every 1800 larvae seeded. In Australia, a small number of releases of blacklip abalone *H. rubra* larvae at sites in Twofold Bay were undertaken in 1990 but the results were not formally evaluated (Keesing *et al.* 1994). As discussed below, this cursory investigation was succeeded by a comprehensive seeding project near Port Lincoln in South Australia by Preece *et al*. (1998). The authors tracked growth and survival of post-larval and juvenile *H. rubra* and *H laevigata* for more than a year after seeding them as competent larvae into boulder zone habitats at densities in the range 1,000 to 80,000/m^2.

A summary of published natural mortality data for *H. rubra* is presented in Table 1. These data illustrate that extremely high rates of mortality sustained by demersal embryos and planktonic larvae during their precarious first week of life continues during the subsequent days and weeks that span settlement, metamorphosis and early post-larval growth and development. The highly specialized juvenile habitat of *H. rubra* is crustose (non geniculate) coralline red algae (CCA) coated boulders and fissured rock within turbulent shallow sub-tidal waters of exposed coastal headlands, and adjacent islands and reefs, (McShane 1992 & 1996; Shepherd & Daume 1996). In the above cited study of Preece *et al*. (1998), survival rates of *H. rubra* post-larvae over the first 49 days averaged about 0.5%. The authors estimated that if seeded at densities of 1,000-4,000/m^2, which they deemed as low', and if protected from being dispersed by wave action and tidal currents, survival rates after one year would be in the order of 0.03% (3 in 10,000). The authors also estimated yields of legal size *H. laevigata* after six years as 0.0074%. The latter represents 1

harvestable abalone from 13500 seeded larvae or 74 harvestable abalone from a million seeded larvae.

In the current study (Section 8), 10 releases totalling 24 million competent *H. rubra* larvae were undertaken at locations between Port Stephens to Eden. Although survival rates for most releases are still to be evaluated, a notable exception was the seeding of 3 replicate sites on Fingal Island, Port Stephens. Each of the three 500m^2 boulder zone sites were seeded with 1.1 million (2,200/m^2) competent calcein labelled larvae then over-seeded 8 months later with a second batch of 2.0 million larvae (4,000/m^2). The three sites yielded an estimated average of 125 juveniles of hatchery origin when sampled 316 days after the second seeding. The weighted mean age of surviving seeded stock at the time of sampling was 400 days and the average yield of 42 juveniles per million larvae seeded (0.0042%) constituted one survivor per 24,000 released larvae.

Such extraordinarily low yields, particularly within the first week of settlement, when considered in conjunction with high costs of hatchery production, prompted Schiel (1992) to conclude that transplantation of larvae was not economically viable. This view was shared by Shepherd *et al*. (2000) in relation to seeding of *H. rubra* and *H. laevigata* in southern Australia and prompted us to investigate the potential use of post-larvae as an alternative to competent larvae.

Additional advantages of post-larvae over larvae recognised from the outset of this study was their greater potential, if pre-settled onto small crustose coralline algae coated rocks, to be evenly spread at low density to match the low carrying capacity of juvenile habitat estimated empirically by Shepherd *et al*. (2000) as <100/m^2. Another disadvantage of *H. rubra* larvae is that once they achieve competence to settle and metamorphose 5 to 7 days after fertilisation, seeding cannot be postponed more than a few (1 to 3) days.

A preliminary trial to investigate settlement inducing characteristics and carrying capacity of CCA coated rocks generated some important and unanticipated results. These results prompted a succession of three laboratory experiments each building on results of its predecessor.

The first experiment was to validate (or refute) results of the preliminary CCA rock seeding trial that had potentially profound practical implications for intermediate use of small CCA rocks as vectors for dispersing post-larvae. The second experiment investigated the upper short-term carrying capacity of CCA coated rock. Of particular interest was effects of larval seeding density on the subsequent growth and survival of post-larvae and juveniles up to 56 days later. It was anticipated that this knowledge could be used to determine the size/age and density at which post-larvae, initially settled on small CCA vector rocks, can be most efficiently seeded into boulder field habitats of juvenile *H. rubra*. The aim of the third and final experiment was to evaluate the ability and efficiency of *H. rubra* post-larvae, densely seeded on small CCA vector rocks, to disperse off these rocks into simulated boulder field habitats.

Table 1. Published survival data for backlip abalone *H. rubra.*

Mean Age (age span)	Survival for period or per year	Source
(0-49 Days)	0.5%	Preece *et al*. (1998)
(1 week to 5 months)	3%	McShane (1991)
(1 week to 8 months)	5.5%	Prince *et al.* 1988
1.25 years (6months-2 years)	20%	Prince *et al.* 1988
2 years (1.5-2.5 years)	44% per year	Day & Leorke (1986)
2.25years (2 to 2.5 years)	41% per year	Prince *et al.* 1988
2.5years (2 to 3 years)	64% per year	Hamer 1982
3 years (2-4 years)	66% per year	Shepherd & Breen (1992), based on Shepherd & Hearn (1983)
3.3years (3 to 3.5 years)	45% per year	Prince *et al.* 1988
3.5 years (2-5years)	70% per year	Shepherd *et al*. (1982)
4 years (2-6years)	81% per year	Shepherd *et al.* (1982)
4.3years (4 to 4.5 years)	74% per year	Prince *et al.* 1988
4.5years (4 to 5 years)	76% per year	Hamer 1982
(5+years)	82% per year	Beinssen & Powell (1979)
5.3years (5 to 5.5years)	90% per year	Prince *et al.* 1988
6.3years (6 to 6.5 years)	78% per year	Prince *et al.* 1988

9.2. Common methods for preliminary trial and experiments 1 to 3

9.2.1. Broodstock acquisition, conditioning, spawning induction and hatchery rearing of larvae

Larvae were sourced from captive conditioned broodstock collected from reefs of the Tomaree Peninsula and adjacent Islands at Port Stephens, NSW (32°45'S, 152°10'E). Broodstock collection, conditioning, induced spawning, fertilisation and larval rearing protocols were as described in Section 4.2.

9.2.1.1. Collection and handling of CCA rock used in experiments

Unless otherwise stated, all volumes of CCA rock cited hereafter refer to displacement volume that constituted about half the volume actually occupied i.e. interstitial volume made up of spaces between the rocks occupied about the same effective volume as the rocks themselves.

Two days before each of the experiments, 100 to 250 L of small CCA rocks were collected by SCUBA divers from a local shallow (3 to 5 m) boulder field at Boulder Bay on the Tomaree Peninsula. These rocks were representative of a smaller cohort of CCA rocks interspersed among a much larger boulders ranging in volume from about 20 to 2000 L. The former ranged from pebbles of 1-15 mL to grapefruit size up to about 1l but mainly below 500 mL. These were collected over a periods of 45 to 90 minutes and kept continually moist in polyethylene fish bins (Nally Plastics P/L) prior to being transported in an air-condition vehicle to the hatchery. At the hatchery, the CCA rocks were immediately re-immersed in seawater in a shallow 2.7 x 1.2 x 0.2 m fibreglass trough that served as a water bath. The troughs were supplied with ambient (18 to 22ºC) coastal seawater filtered to 10 µm (nominal) at a flow rate of ~ 20 L/min.

For each experiment, 4 lots of either 0.5l or ~1L of assorted CCA rocks were sampled the following morning to determine background numbers and sizes of wild *H. rubra* juveniles. To do this, replicate lots of CCA rocks were fully immersed in 70% ethanol. From 1 to 7 days later, the rocks were stripped of adhering abalone and other errant epi-fauna by vigorously shaking the rocks in 70% ethanol within sealed plastic containers for 10 to 20 seconds. The surfaces of each individual rock was then systematically brushed with 25 mm medium bristle paint brushes and rinsed with 70% ethanol dispensed from a 500 mL squeeze bottle. Finally, all resultant organic and inorganic particulate matter was captured on a 160 µm nylon filter screen then stored in 70% ethanol within 70 or 120 mL sterile screw cap specimen bottles (Disposable Products, P/L).

Following removal of samples of CCA rock to determine background numbers and sizes of wild abalone, the troughs were drained and the rocks systematically cleansed using high pressure seawater of potential predators and competitors, including polychaete worms, small crabs, limpets, chitons and other gastropods plus other loosely adhering epi-fauna. The CCA rocks were divided into lots representative of the overall mix of sizes. These lots, as well as those sampled for background numbers and sizes of wild post-larval and juvenile abalone, ranged in volume from ~0.5 L in (Experiment 2) up to ~5.0 L (preliminary trial and Experiment 3). Individual lots that served as experimental replicates were stocked either into 4 L plastic ice-cream tubs (Experiments 1 and 2) or 12 L capacity prawn IQF trays (Preliminary Trial and Experiment 3).

9.2.1.2. Seeding, harvesting, counting and measurement of post-larvae

Larvae were stocked at prescribed densities into the replicate seawater filled vessels loaded with prescribed volumes of CCA rocks. To stock experimental replicates at prescribed densities, competent larvae were wet screen harvested from rearing vessels and transferred to a calibrated 20 L bucket. Their density was determined from the mean of counts in each three 1.00 mL aliquots sampled with an automatic pipette from a homogenised suspension of the larvae. Volumes of these suspensions, sufficient to yield specific numbers of larvae required for particular experimental replicates, were then calculated. Individual replicates were then stocked by collecting and dispensing appropriate volumes of homogenised larval suspension using the automatic pipette.

After the addition of larvae, trickle aeration and cessation of seawater flow to replicate vessels was imposed for 6 to 24 h to facilitate settlement. At all but the highest stocking densities of 16,000 or 32,000/L CCA rock used throughout these experiments, no actively swimming larvae were observed beyond about 6 h after seeding. Even in the latter cases, numbers of swimming larvae were so low (<100 per replicate) as to be considered inconsequential. Following settlement, seawater supply to each replicate vessel was restored at regulated rates of 5 to 10 exchanges per hour using drip irrigation valves. The same type of irrigation valve was used to apply moderate aeration (1 L/min) with one aerator per replicate where 4 L tubs were used and 4 aerators per replicate where 15 L IQF baskets were used. This level of aeration ensured continuous thorough mixing and circulation of aerated seawater. Where replicate vessels comprised 4 L plastic ice-cream tubs, discharging seawater was vented as a "telltale" through a simple 4 mm diameter overflow port located just below the rim. The telltales aided equilibration of seawater flow to all tubs and enabled immediate detection of low or failed seawater supply. Where 15 L IQF baskets were used, seawater supplied via 4 irrigation drip valves per basket was checked twice daily.

At each prescribed sampling time for each experiment, methods used to collect and preserve surviving juveniles were the same as those already described above for determining background levels of wild abalone. Samples were examined either as 1.0 mL aliquots pipetted onto a Sedgewick rafter slide, or for larger volumes, as 5 to 6 mL aliquots pipetted into the 6 mm wide 4mm deep peripheral trough of a 200 mm diameter black plastic plankton sorting disc. Sub-samples were systematically examined at 5 to 15X using a stereo microscope and a fibre optics light source. Juveniles that were alive or dead at the time of sampling were separately counted. The shell length of juveniles alive at the time (generally 30 per sample) of sampling was measured at either 40 or 100X using a binocular microscope fitted with a calibrated eyepiece micrometer.

9.3. Preliminary trial: Assessment of suitability of CCA rocks as a settlement medium for post-larvae

9.3.1. Aims

This trial was to assess the general suitability of CCA rocks as a medium for attracting high-density settlement and metamorphosis of competent *H. rubra* larvae and thence as vectors for large scale cost effective seeding of depleted reefs with post-larvae. Key information sought was short to medium term post-larval carrying capacity of CCA rock including the influence of individual CCA rock size.

9.3.2. Methods

9.3.2.1. Experimental system and design

On the 22/09/1999, CCA rocks totalling about 17 L in volume were collected and cleansed of potential predators and competitors in accordance with generalized procedures described above. The CCA rocks were then roughly apportioned into eight plastic mesh prawn IQF trays, positioned in a 2.7 x 1.2 x 0.2 m fibreglass nursery raceway.

A sub-batch of 100,000 six-day-old competent larvae, sourced from locally collected and conditioned broodstock that were induced to spawn on 14/9/99, were seeded into 8 CCA rock filled IQF trays on the same day. The effective seeding rate of the larvae was about 6,000/L CCA rock. The larvae were allowed to settle in the absence of seawater flow and aeration for several hours until numbers of free-swimming larvae had fallen to insignificant levels. Flow-through of seawater, pre-filtered to 10 µm (nominal), was then resumed at the rate of about 20 L/hour, as was turbulent aeration. The entire array was maintained beneath a 70% shade-cloth canopy to prevent sunlight bleaching the CCA.

On each of three occasions, 2, 9 and 29 days after stocking, a single IQF tray of mixed grade CCA rock was removed from the raceway. All CCA rocks within the trays were allocated into one of five size grades. Demarcation of grades was made on the basis that the average volume of individual rocks within successive grades was approximately half that of rocks in the preceding grade. Nominal ranges of volume for individual rocks within each successive grade were: Grade 1, 150 to 500 mL; Grade 2, 80 to 150 mL; Grade 3, 50 to 70 mL; Grade 4, 15 to 45 mL and Grade 5 1 to 14 mL. Sub-samples of pre-graded CCA rocks, comprising lots in the range 200 to 500 mL, were separately preserved using generalized procedures described above. At the time that post-larvae in ethanol fixed sub-samples were counted, numbers of CCA rocks within each sub-sample were also counted and their combined volume determined by displacement to the nearest 10 mL.

9.3.3. Results

Seawater temperatures remained within the range 16 to 21ºC, and salinity within the range of 34 to 36g/kg. Mean volumes of individual CCA rocks allocated to size grades 1 to 5 (Figure 1) were: 250; 96; 52; 27 and 13 mL respectively. Regardless of size grade, volume of water required to just cover the rocks, i.e. occupying the interstitial spaces, was very close to the volume of the rock. Surface area (cm^2) to volume (mL) ratios of size grades 1 to 5 were estimated as 0.77; 1.1; 1.3; 1.6 and 2.1 respectively. In practical terms, the smallest grade (Grade 5) pebble size rocks provided 2.7 times more surface area per volume than the largest (Grade 1) rocks.

The overall mean volumetric contributions of the 5 size grades of CCA rock used as settlement substrate in this trial are presented in Figure 2. The largest Grade 1 CCA rocks contributed more

than a third (37.3%) of total volume and more than twice the volume of any other grade. Relative volumes of size grades 2 to 5 were relatively uniform, namely 18.2; 17.5; 12.9 and 14.0 % of the total CCA rock volume respectively.

It was anticipated that carrying capacity of post-larvae per unit volume of CCA rock would reflect the relative surface area provided by the different size grades i.e. that density of post-larvae /L CCA rock on the various size grades would follow the sequence Grade 5 > Grade 4 > Grade 3 > Grade 2 > Grade 1. However, post-larvae density distribution data presented in Figure 3 showed that this was not the case. To the contrary, these data showed that post-larvae /L of CCA rock on day 2 after seeding was highest (1320/L) on the largest Grade 1 CCA rocks, relatively uniform for grades 2, 3 and 4 (533, 528 and 593/L respectively) but very low (56/L) in the case of smallest Grade 5 pebbles. The post-larvae density distribution pattern shifted towards intermediate CCA rock grades on subsequent sampling occasions 9 and 29 days after seeding.

Non-replicated mean post-larvae density data, pooled across all rock size grades, are presented in Figure 4. These show that post-larvae density steadily declined from 942/L on day 2 to 718 on day 9 and down to 228/L at the conclusion of the trial on day 29 after seeding.

9.3.4. Conclusions and discussion

This preliminary trial showed that it is possible to achieve good settlement of *H. rubra* (between about 10 and 20%, Figure 3) on CCA rocks ranging in individual volume from 1 to 500 mL. Surprisingly, the rates of settlement appeared not to be surface area dependent. Moreover, the density of post-larvae on rocks was not stable over time. These results raised several issues that needed to be clarified to assess and maximise the usefulness of CCA rocks as vectors for seeding reefs with post-larvae, namely the their ability to support normal growth and survival and to enable rapid dispersion of post-larvae into juvenile habitats.

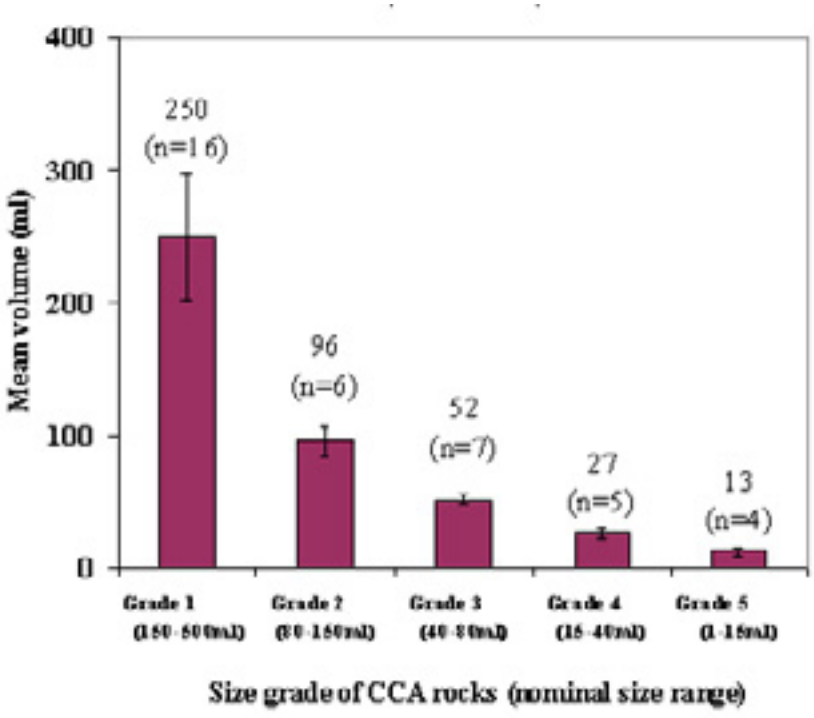


Figure 1. Volume of individual rocks within each grade of CCA rock (means ± s.e.).

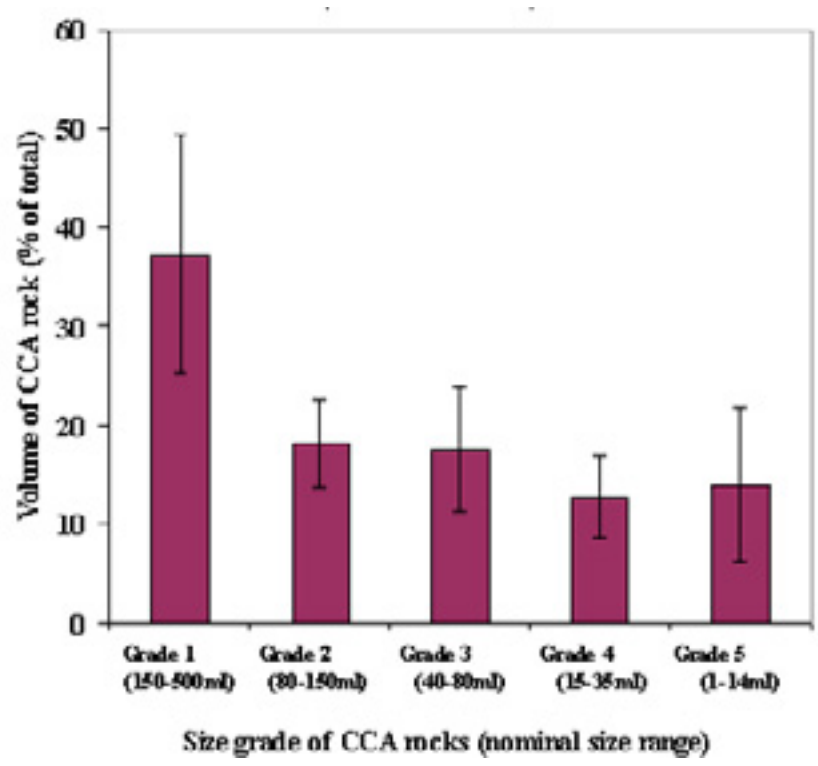


Figure 2. Contribution of each size class to the total volume of CCA rock (mean of five size grades (means ± s.d.; $n = 3$).

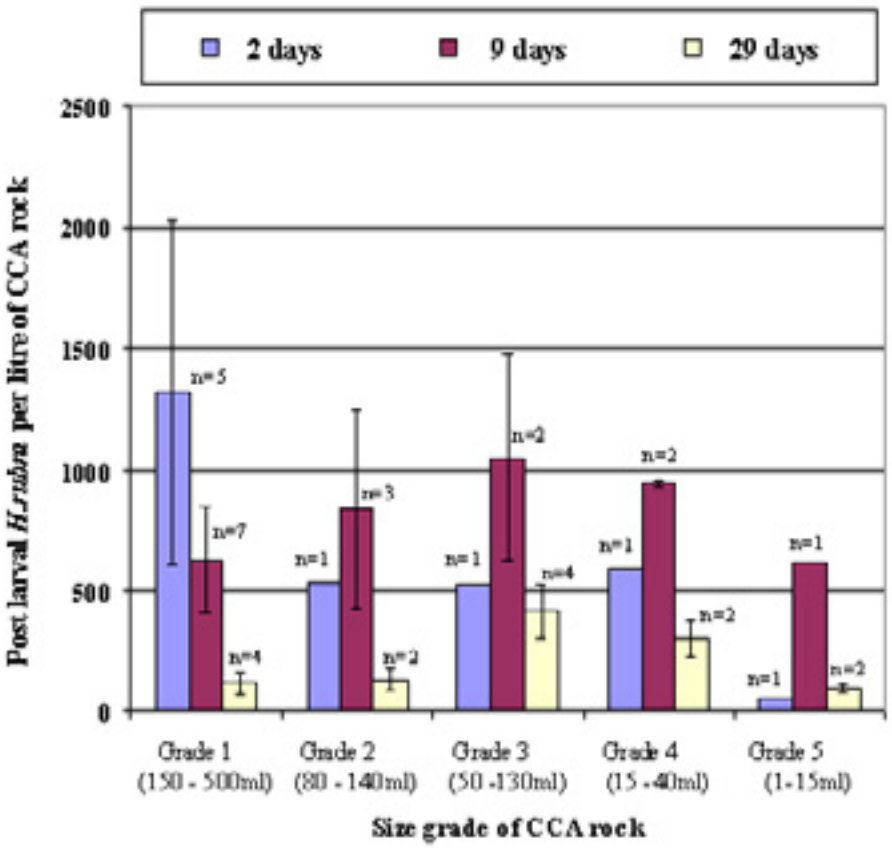


Figure 3. Density of *H. rubra* post larvae on CCA rock (means ± s.e.).

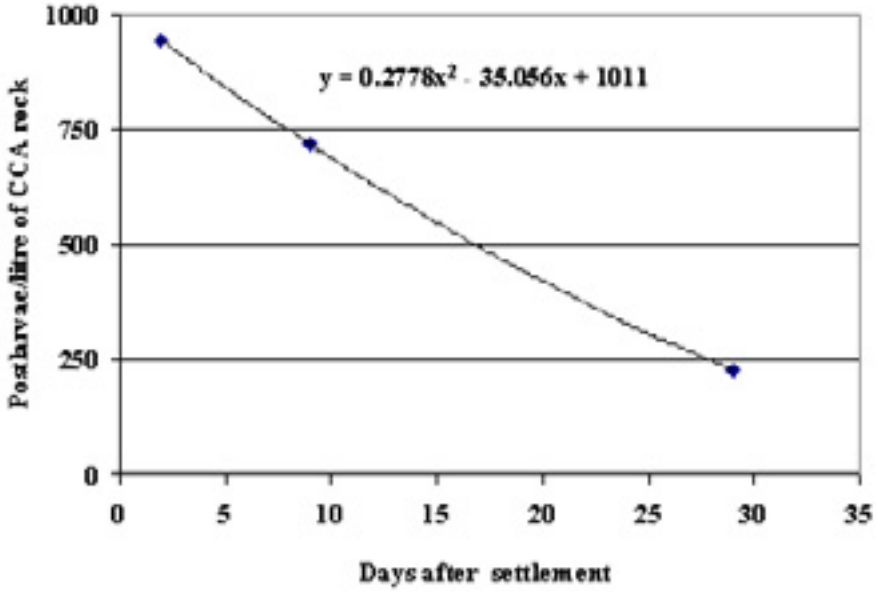


Figure 4. Yields of *H. rubra* post larvae 2, 9 and 29 days after seeding on mixed grade CCA rock.

9.4. Experiment 1: Effects of larval seeding density and rock size on settlement success and on subsequent growth and survival of post-larvae

9.4.1. Aims

This experiment investigated the effects of larval seeding density on subsequent settlement success, growth and survival of *H. rubra* post-larvae seeded onto natural settlement substrate comprising mixed grade CCA rock. Specifically, it investigated whether initial settlement success and subsequent growth and survival were correlated with surface area of CCA rock or some other relationship existed as suggested by the results of the preliminary trial.

9.4.2. Methods

9.4.2.1. Experimental system and design

CCA rock of mixed sizes were stocked with competent *H. rubra* larvae at densities of 1,000, 2,000, 4,000, 8,000 and 16,000/L rock. Mixed grade CCA rock, seeded at densities up to 8,000 larvae/L were sampled 2, 14, 28 and 56 days after seeding. An additional treatment, involving 4 replicate 4L ice-cream tubs, stocked at the highest seeding density of 16,000 larvae/L of CCA rock, was run for 2 days in an attempt to identify an upper density limit for successful seeding. Four replicates were established for each of the 17 combinations of stocking density and sampling time and for an additional non-stocked control. As described in the general procedures section above, the latter were immediately fixed in 70% ethanol to determine background numbers and sizes of any residual wild post-larvae or juveniles at the commencement of the experiment.

Each replicate comprised a 4 L food quality plastic tub containing 0.89 ± 0.10 L of mixed grade CCA rock. Four replicate tubs for each of the 17 density x duration treatment combinations were randomly positioned within a shallow fibre-glass raceway. The raceway was fitted with a simple standpipe overflow to maintain water level 2 cm below the rim of the tubs. In this way the troughs served as temperature buffering water jackets that enabled CCA rocks and adhering abalone to be maintained within ± 0.2ºC of the temperature of incoming seawater (18 to 20ºC). This was in spite of wide variation of 16 to 30ºC in air temperature experienced over the 56 day duration of the experiment. Seawater flow rates to individual tubs were regulated within the range 20 to 40 L (5 to 10 exchanges) per hour, using drip irrigation valves attached to a common seawater supply manifold that served both troughs.

9.4.2.2. Seeding, harvesting, counting and measurement of post-larvae

On the first day of the experiment, larvae were stocked at variously prescribed densities into each of 68 replicate tubs in accordance with generalised methods described in Section 9.2 and the tubs of seawater and CCA rock, variously seeded with prescribed numbers of larvae.

Trickle aeration without seawater flow to tubs was imposed for approximately 6 hours after seeding. By this time no actively swimming larvae could be observed in any of the tubs except those stocked at the highest density (16,000 larvae/tub). Even in the latter case, numbers of swimming larvae were so low (<100 per tub) as to be considered inconsequential. Seawater supply and elevated aeration rate to each tub was then restored. Exhalent seawater was discharged as a telltale through a simple 4mm diameter over-flow port located just below the rim of each tub as already described in Section 9.2. As also previously described, seawater flow rates to individual tubs were regulated within the range 20 to 40 L (5 to 10 exchanges) per hour using drip irrigation valves. The same type of valve was used to apply a moderate level about 1 L/min of bubbled air to each tub.

At each of the four prescribed sampling times of 2, 14, 28 and 56 days after stocking, 4 replicate tubs stocked at each of the seeding densities were harvested. Methods used to remove and preserve post-larvae adhering to CCA rock were the same as described for determining background levels of wild abalone in the generalized procedures section 9.2. As with the preliminary trial, all CCA rocks were separated into one of 5 prescribed size grades. This was to further investigate the effect of individual rock volume and surface area on settlement success and carrying capacity, as indicated by subsequent growth and survival of post-larvae. Rocks from the different size classes were separately preserved in of 70% ethanol in screw cap 500 or 1000 mL plastic jars until being stripped of adhering abalone and other epi-fauna. Once harvested, post-larvae were archived as already described for the preliminary trial in Section 9.2. Methods used to count and measure of post-larvae, which were identified as being alive or dead at the time of sampling and preservation, were also as described in the generalized procedures (Section 9.2).

9.4.3. Results

9.4.3.1. Temperature salinity

Temperatures remained within the range 18 to 22ºC and salinity within the range 34 to 36g/kg.

9.4.3.2. Size frequency characteristics of CCA rock substrate

Mean volumes of individual CCA rocks in grades 1 to 5 (Figure 5) were 271, 94, 51, 27 and 8 mL respectively. These were similar to those in the preliminary trial, namely 250, 96, 52, 27 and 13 mL respectively. Corresponding mean surface areas of the five rock grades were 203, 100, 67 44 33 cm^2 respectively while corresponding surface area to volume ratios were 0.75, 1.1, 1.3, 1.6 and 1.8 cm^2/mL. The smallest (pebble size) grade 5 rocks were therefore presenting about 2.5 times the surface area per unit volume than the largest (lemon to orange size) grade 1 rocks.

The overall representation of size grades of CCA rocks used as settlement substrate in this experiment is presented in Figure 6. These data show that the majority (83%) of CCA rock comprised Grades 1 (21%), 2 (36%) and 3 (26%) with grades 4 and 5 accounting for only 13% and 5% of total volume respectively. This representation of CCA rock grades varied substantially from that of the preliminary trial where Grade 1 rocks made up more than a third (37%) of volume and remaining size grades 2, 3, 4 and 5 were equally represented, (13 to 18%; of the total CCA rock volume).

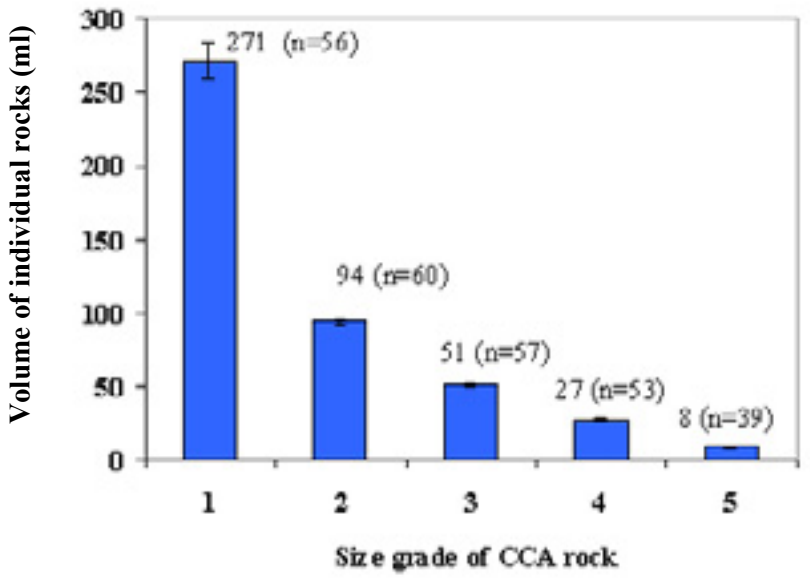


Figure 5. Volume of individual rocks within each size grade of CCA rock (means ± s.e.).

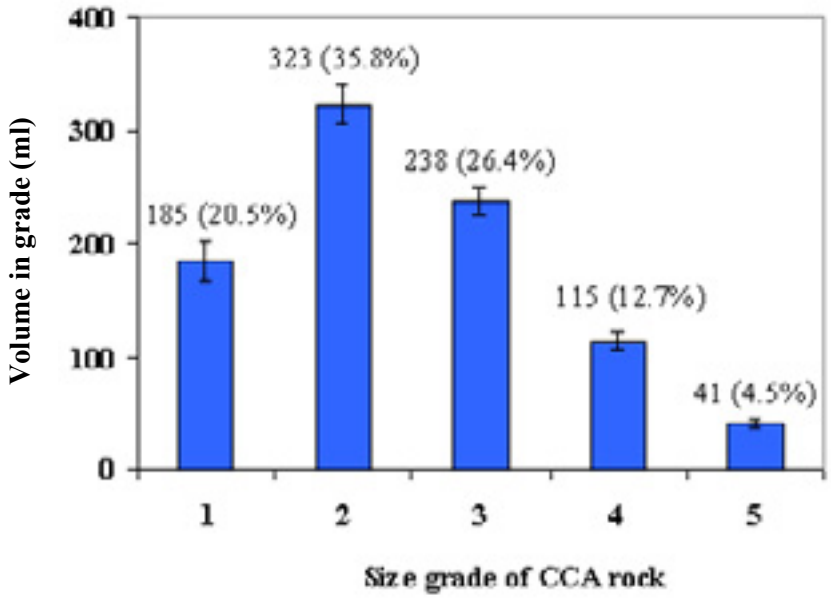


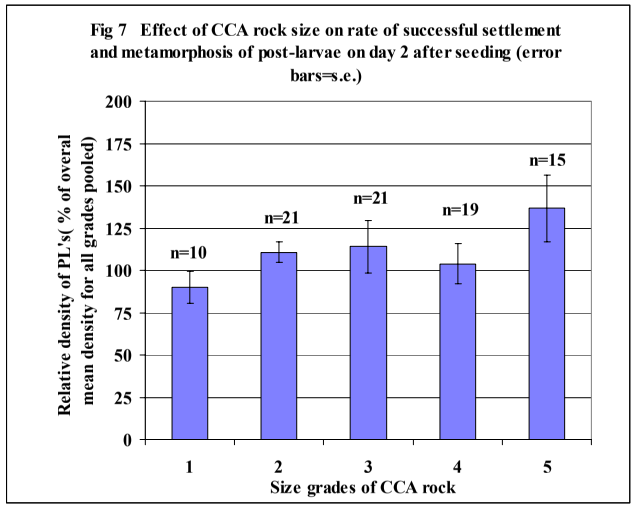
Figure 6. Absolute and relative volumes of grades within mixed grade CCA rock (n = 64) (means ± s.e.).

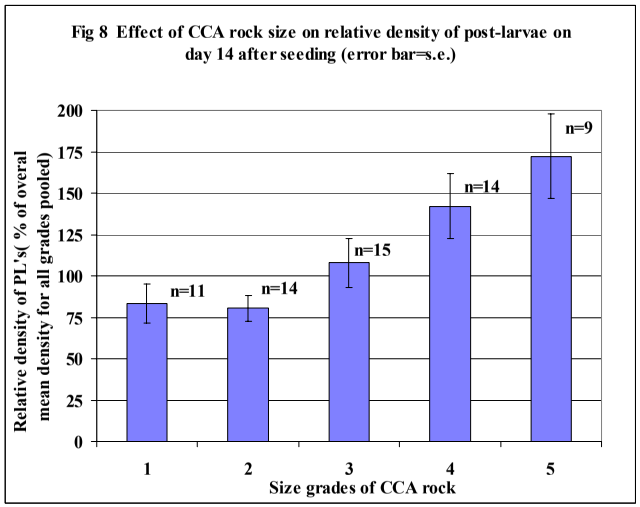
9.4.3.3. Relationship of CCA rock size with initial settlement success and subsequent carrying capacity

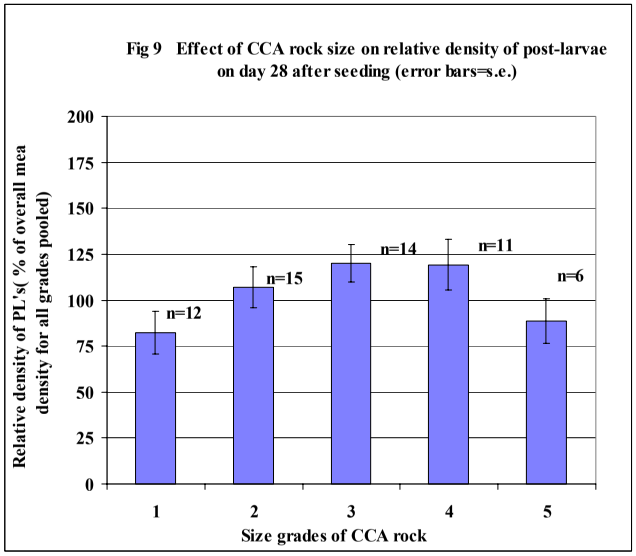
The density of newly settled post-larvae (day 2) and the subsequent density on days 14, 28 and 56 after seeding across the five size classes of CCA rock are presented in Figures 7 to 10. This was a preference experiment allowing larvae to choose to settle among the different sized CCA rocks. In analysing this experiment the density of larvae on the different sized rocks is not independent of each other because if a larva chooses to settle on one rock it cannot also settle on another at the same time. This lack of independence between treatments limits the utility of ANOVA in analysing such data. There have been methods proposed to analyse preference experiment data by ANOVA (Peterson & Renaud 1989) however, the number of replicates needed to perform this procedure made it impractical to use in this case. The data were thus not formally analysed but presented with standard errors to give an indication of the variation in the means.

The density of *H. rubra* larvae at day 2 on different size CCA rocks at each of the seeding densities (1,000, 2,000, 4,000 and 16,000 larvae/L) showed no clear trend relating to the five size grades of CCA rock (Figure 7). That is, the larvae showed no settlement preference for different size rocks but settled evenly per unit surface area across the five size grades of rocks.

The density of post-larvae on CCA rocks at days 14, 28 and 56 at each of the seeding densities also showed no clear trend relating to the five size grades of CCA rock (Figures 8, 9 & 10). This indicates that survival on or movement to different rocks was not affected by the size of individual CCA rocks per se but was dictated by available surface area.







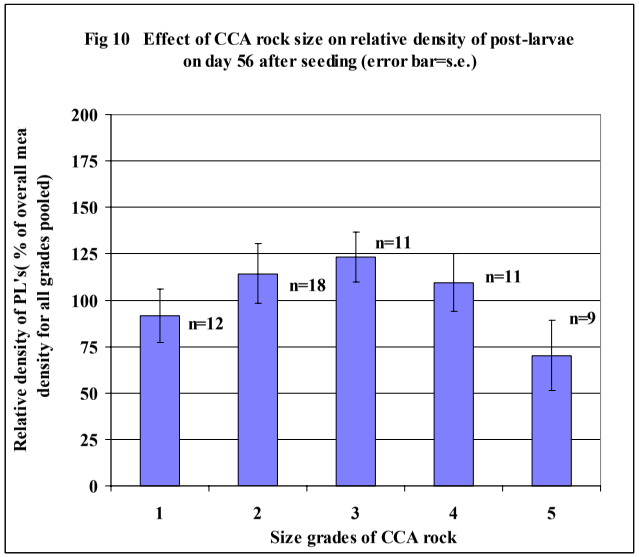


Figure 7-10. Effect of CCA rock size on post-larvae after seeding.

9.4.3.4. Effect of larval seeding density on survival

Yields of *H. rubra* post-larvae were calculated as percentages of initial number of larvae seeded for the various stocking densities at 2, 14, 28 and 56 days (Figures 11 & 12). There was a significant difference in the initial settlement success of larvae (i.e. yield at day 2) among stocking densities (Figure 11) as indicated by ANOVA (Table 1), followed by Tukey's HSD test (not shown). At the lowest stocking rate of 1,000 larvae/L of CCA rock, an average of approximately 20% of larvae were successfully settled at day 2. This was a significantly lower settlement rate than achieved at higher stocking densities of 2,000, 4,000, 8,000 and 16,000/L, which at day 2 gave average yields between 32 and 40 % (Figure 11, ANOVA Table 1, followed by Tukey's HSD test (not shown).

Over time, percentage yields of post-larvae for the four stocking densities decreased and converged so that by day 56 there was no significant difference in the yield between the four stocking densities (Figure 12**,** ANOVA). Average percentage yield at day 56 were between 3 and 12 % of the original number seeded. When expressed as a number of larvae per volume of rock, it is evident that larvae from all stocking densities tend to converge to approximately the same density of between 100 and 250/L of CCA rock (Figure 13).

Table 1. One-way ANOVA on the log-transformed yield of post-larvae at day two at five stocking densities (Density).

Source	SS	df	MS	*F*-ratio	P
Density	1.056	3	0.352	10.14	0.001
Error	0.416	12	0.034		

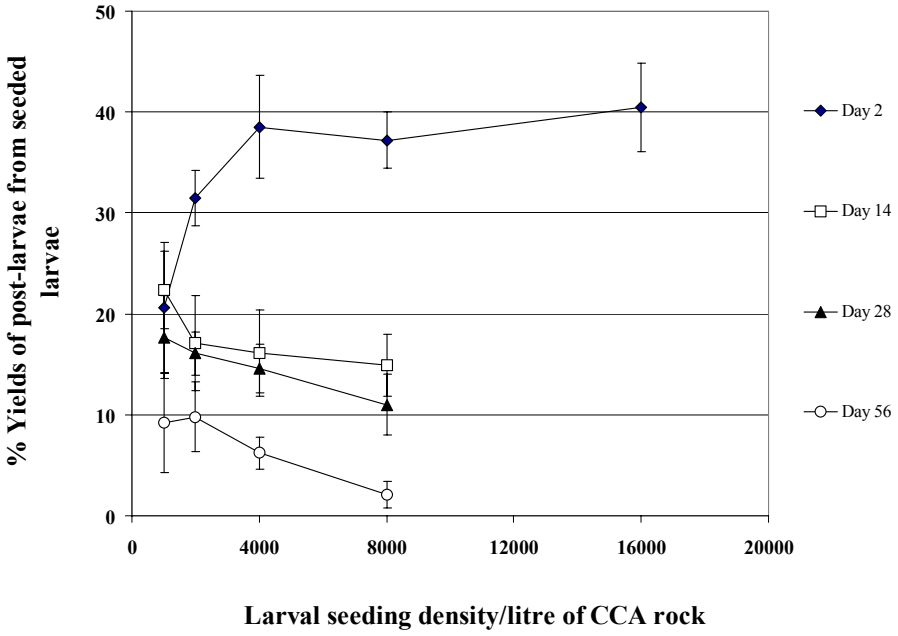


Figure 11. Effects of larval seeding density on relative yields of post-larvae (error bars = s.e.).

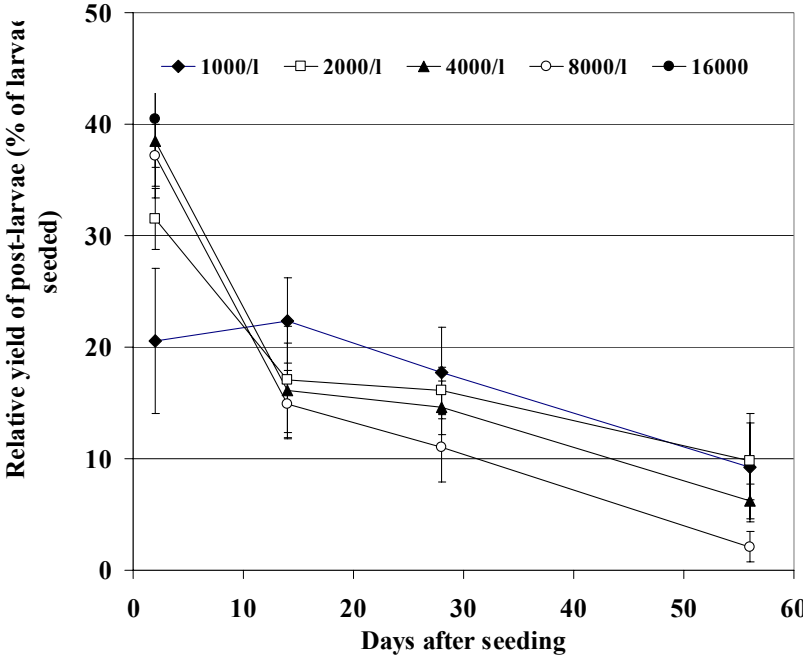


Figure 12. Relationship between initial larval seeding density and percentage yields of *H. rubra* post-larval and juveniles (error bars = s.e.).

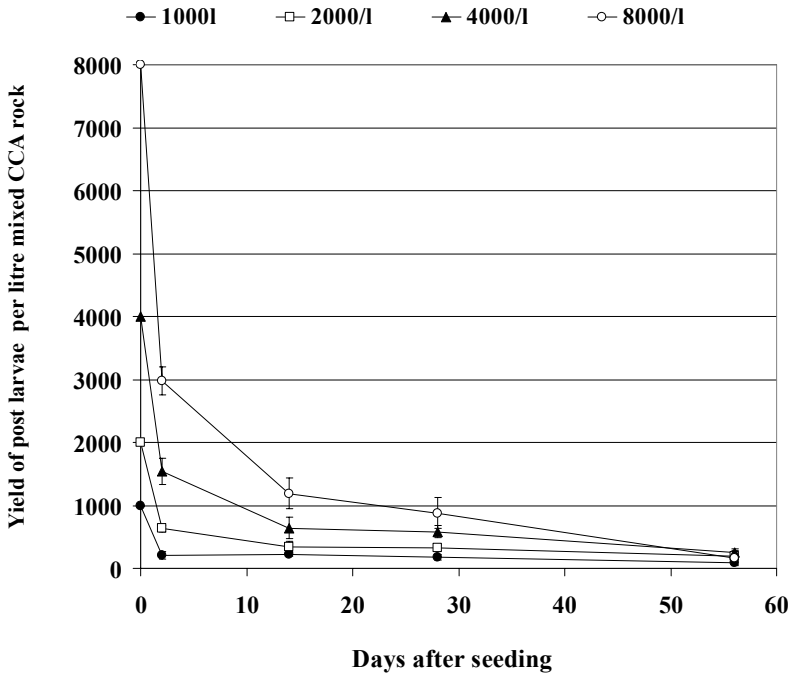


Figure 13. Effects of initial larval stocking density in the range 1000 – 1600/l on yield of H. rubra post-larvae and juveniles seeded onto mixed grade CCA rock (error bars = s.e.).

9.4.3.5. Effects of density on growth

Plots of mean (n = 30) shell length and corresponding residual density data for post-larvae on days 2, 14, 28 and 56 after seeding are presented in Figures 14 to 17. In contrast to all subsequent sampling occasions, mean shell lengths of day 2 post-larvae (Figure 14) showed an upward trend with density, however this relationship was weak with only 28% of the variation in shell length explained by the density of post-larvae (R^2 = 0.28). This result is perhaps an artefact of the settlement dynamics of larvae at different stocking densities. Seeding of *H. rubra* larvae at low densities of 1,000 larvae/L of CCA rock resulted in relatively poor and protracted rates of settlement, metamorphosis and initiation of post-larval growth and development. At the opposite extreme, seeding of *H. rubra* larvae at densities of ≥4,000 larvae/L of CCA rock promoted precocious settlement, metamorphosis and initiation of post-larval shell growth.

However, once settlement and metamorphosis was completed and post-larval shell growth initiated, subsequent growth of *H. rubra* post-larvae on CCA rock became strongly density dependent (Figures 15, 16 & 17). Mean shell lengths on days 14, 28 and 56 after seeding clearly declined with increasing number of post-larvae per unit area of CCA rock. At each of these times the relationship between density and mean size was best described by equations that ascribe 59, 75 and 60 percent (for day 14, 28 and 56 respectively) of the variation in shell length of post-larvae to density (Figures 15-17), followed by linear regression $P > 0.05$).

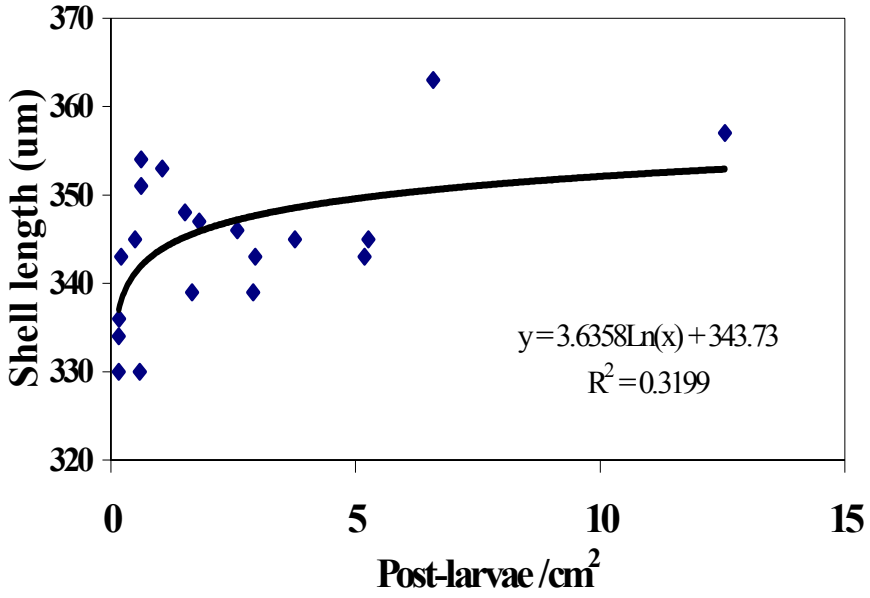


Figure 14. Relationship between density and size of post-larval on CCA rock on day 2 after seeding (means, n = 30).

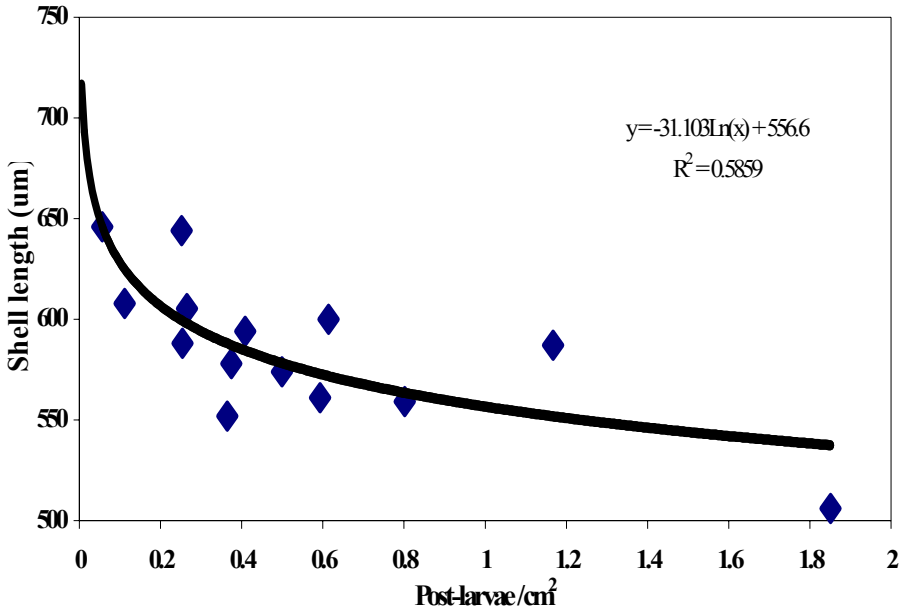


Figure 15. Relationship between density and size of post-larval on CCA rock on day 14 after seeding (means, *n* = 30.).

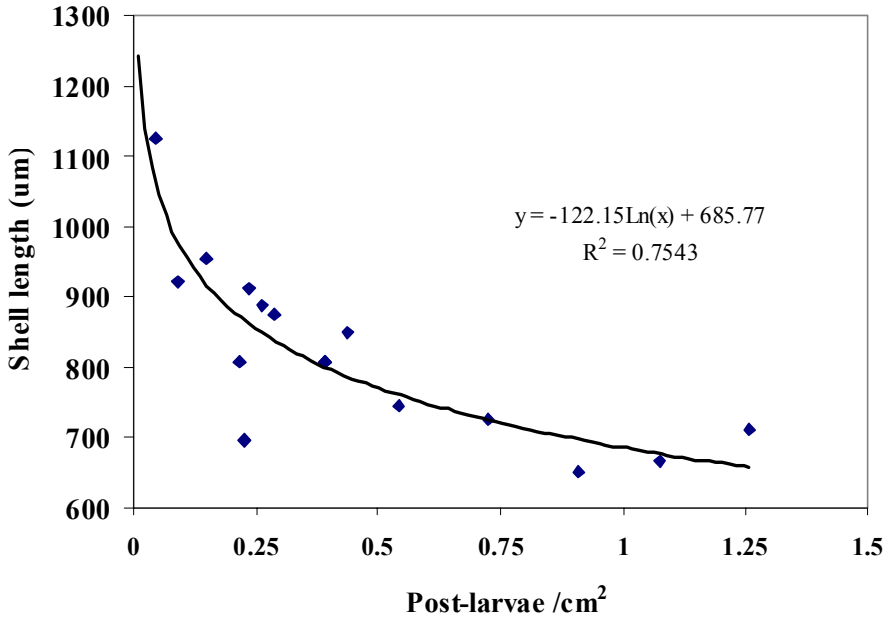


Figure 16. Relationship between density and size of post-larval on CCA rock on day 28 after seeding (means, *n* = 30.).

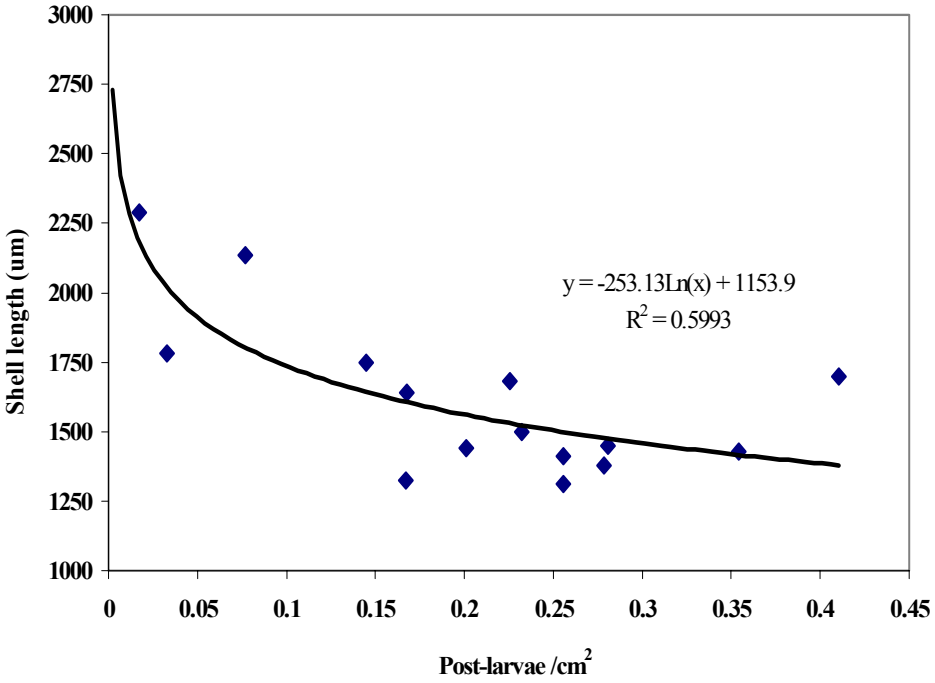


Figure 17. Relationship between density and size of post-larval on CCA rock on day 56 after seeding (means, *n* = 30.).

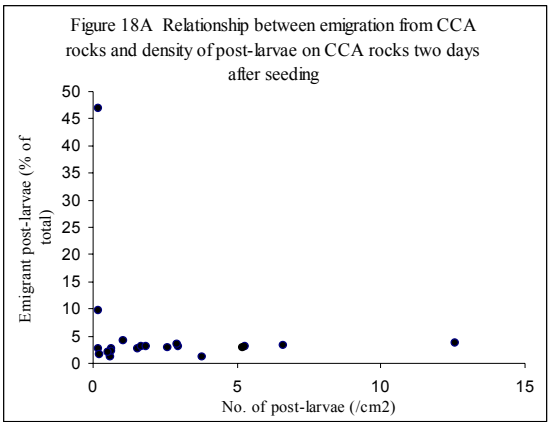
9.4.3.6. Effects of density on emigration of post-larvae from CCA to adjacent container surfaces

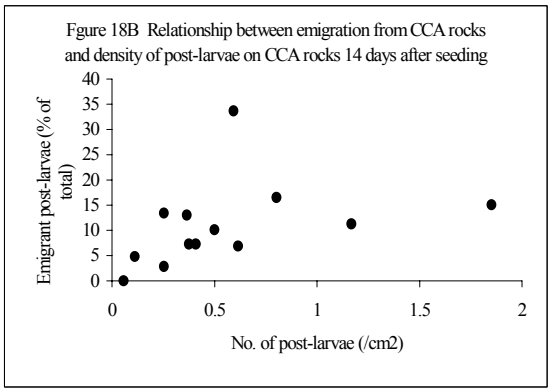
To gauge the timing and extent of emigration of post-larvae off CCA rock and onto surrounding surfaces of the plastic tubs, numbers of post-larvae adhering to these surfaces on each successive sampling occasion, expressed as a percentage of total surviving post-larvae, were determined. Data presented in Figure 18A show that in almost every case, ≥96% of initial settlement and metamorphosis of larvae across all seeding densities on Day 2 was confined to surfaces of CCA rock. The only exceptions were two replicates at the lowest seeding density 1,000 larvae/L CCA.

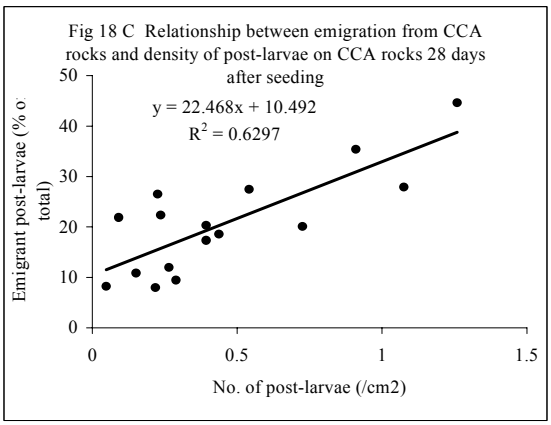
Equivalent data for day 14 after seeding presented in Figure 18B show that extensive emigration of post-larvae had already commenced in spite of their small size (mean shell length range 500 to 650 µm, Figure 15). Relative proportions of migrants ranged between 0 and 24% of total residual post-larvae. These data exhibited apparent density dependence, a best fit linear regression equation being accompanied by an R^2 value of 0.5164. However the slope of this regression did not prove significantly different from zero.

At day 28 after seeding, the proportion of post-larvae that had emigrated from CCA rocks onto surrounding surfaces of plastic tubs (Figure 18C) had increased considerably accounting for 8 to 45% of all residual post-larvae. Although the extent of emigration again appeared density dependent, the slope of a best-fit linear regression again did not prove significantly different ($P < 0.08$) from zero.

At the termination of the experiment on day 56 after seeding (Figure 18D), the relative proportion of post-larvae comprising emigrants had further increased to the range 17 to 62%.







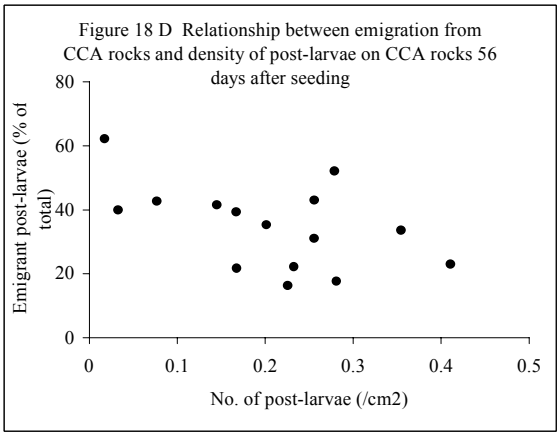


Figure 18. Relationship between emigration from CCA rocks and density of post-larvae on CCA rocks after seeding.

9.4.3.7. Growth performances of emigrants and CCA rock associated post-larvae

Mean shell length data for post-larvae that had remained attached to CCA rock through to the termination of Experiment 1 on day 56 are compared with those of post-larvae that had migrated to adjacent surfaces of 4L plastic tubs are presented in Figure 19. For all seeding density treatments, shell lengths of emigrant post-larvae were significantly greater than shell lengths of post-larvae that remained (or returned to) the CCA rocks (Table 3). The mean ± s.e. SL of emigrant post-larvae at day 56 ranged from 2,312 ± 66 to 2,833 ± 45 μm representing mean growth rates of 35 to 45 μm/day. Mean SL± s.e of migrant post-larvae were consistently 900 to 1000 μm greater than their CCA counterparts, which ranged from 1,402 ± 41 to 1,825 ± 2 27 μm representing growth rates of 20 to 27 μm/day.

While it is tempting to dismiss these differences on the basis of density, a comparison of density/size data for individual replicates of CCA rock (Figure 17) with that of migrant post-larvae (Figure 20) reveals that this size discrepancy is consistent even at the extreme low range of density for each of the two groups. Moreover the mean ± s.d. shell length of 2300 ± 600 μm recorded for the migrant post-larvae at the highest encountered density of 280 per tub was the same as the mean value of 2,300 ± 400μm recorded for CCA post-larvae at the lowest encountered density of 21/L of CCA rock. Accordingly, an alternative more likely explanation of these data is that CCA rocks support relatively poor growth rates of post-larvae as well as having low inherent carrying capacity that falls to levels of 100 to 250/L of CCA rock (Figure 13 and associated text) by the time (in this case 56 days) that *H. rubra* post-larvae attain shell lengths mainly in the range 1 to 2 mm.

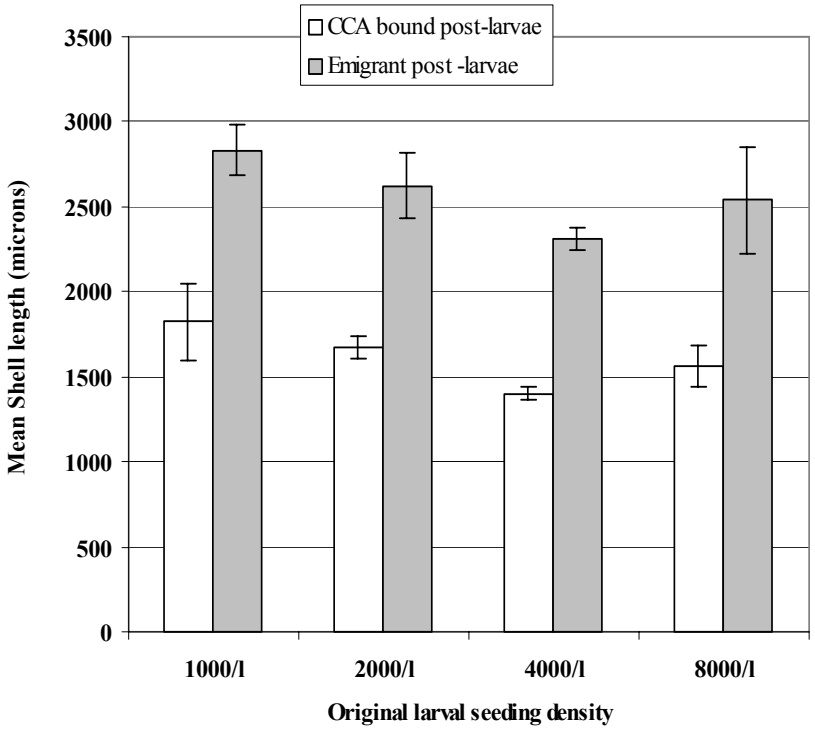


Figure 19. Mean shell lengths of CCA rock bound and emigrant post-larvae on day 56 (error bars are s.e.).

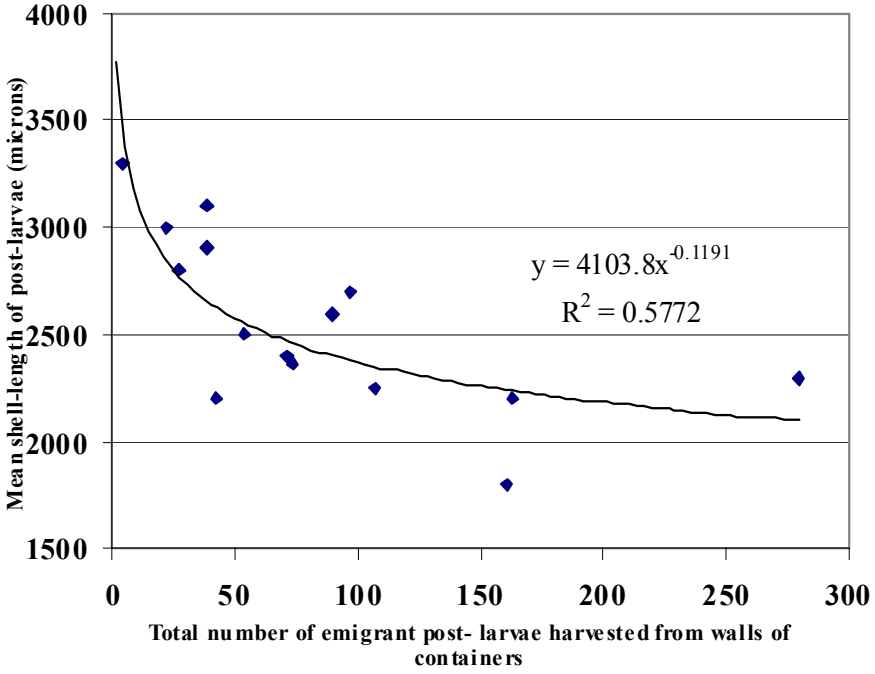


Figure 20. Relationship between numbers of emigrant *H. rubra* post-larvae and their mean shell lengths on day 56 after seeding.

9.4.4. Discussion

This was the first in a series of experiments to develop techniques for dispersing early post-larvae onto reefs at low densities over large areas to restock natural populations. The effects of rock size and stocking density on the settlement, growth, survival and retention of *H. rubra* larvae and post-larvae on CCA rocks were investigated. Previous studies have shown that CCA covered rocks are the natural settlement substrate of *H. rubra* (McShane 1992) and that CCA enhance settlement of *H. rubra* larvae in laboratory (Daume *et al*. 2000) and in hatchery scale experiments. Here too it was demonstrated that high levels of settlement of *H. rubra* larvae can be achieved on CCA rocks. Counter to the preliminary experiment, larval settlement was unrelated to the size of the rocks *per se*. That is, within individual stocking densities the density of larvae per unit area were approximately the same for different sized rocks.

Gregarious settlement of marine larvae including *Haliotis* spp. has been well documented under natural, laboratory and commercial conditions (Pawlik 1992). Larvae in this experiment also displayed gregarious settlement with stocking densities of ≥1,000 larvae/L of rock (≥1/cm^2) displaying significantly lower settlement rates than higher stocking densities. In relation to settlement success, no upper limit in the stocking density was found with all stocking densities above 1,000/L giving the same percentage yield. After settlement, however, density dependant processes strongly influenced survival, movement and growth of the post-larvae on the CCA rocks. After settlement the percentage yield of post-larvae on CCA rocks decreased at a steady rate to the point where at day 56 actual yields of all seeding densities were essentially the same. The cause of this decrease in yield was probably two fold. Most obviously, density dependant mortality had a strong effect. When total larvae on rocks and on the walls of containers were counted it was found that mortality increased with the stocking density. Movement of post-larvae from CCA rocks also was affected by the density of post-larvae, with a greater rate of emigration from rocks with higher

densities of post-larvae. The combined effect of density dependant mortality and emigration lead to the residual density of post-larvae on CCA rocks for all stocking densities converging to similar a level of 0.1-0.25 post-larvae per cm^2 on day 56 by which time they had attained a mean SL of 1 to 3mm.

Growth of post-larvae on CCA rock was inversely related to density. Early post-larvae have poorly developed radulae and digestive systems and are unable to utilise CCA (or any intact algal cells) directly as a food source (Kawamura *et al.* 1998). Instead it is thought that they rely on ingesting algal secretions especially from diatoms (Kawamura *et al.* 1998). CCA rocks have a unique system of shedding epithelial cells as an antifouling strategy designed to reduce overgrowth of their surface by epiphytes such as diatoms. These factors combine to make CCA rocks a poor substrate to sustain high densities of post-larvae. Indeed, post-larvae that had emigrated from the CCA rocks had significantly faster growth rate. This difference in size was most probably generated by the greater food resources off the CCA rocks. As a caveat to this however, there may also be an interaction between size and movement in post-larvae. If larger post-larvae are more likely to move than smaller ones this may also generate smaller animals on the CCA rock.

In conclusion, CCA rocks can accommodate large settlement densities of *H. rubra* larvae. However, an upper limit to the number of larvae that could be settled on CCA rocks was not found. Once settlement and metamorphosis had occurred, CCA rock proved to have a low carrying capacity with rates of mortality and emigration increasing with density of animals on the CCA rock. This result is consistent with low densities recorded for wild *H. rubra* post-larvae and juveniles on natural CCA rock habitats. An encouraging aspect of these results is that they have demonstrated the ability of small CCA rocks to rapidly attract high larval settlement densities but then be able to quickly shed resultant post-larvae. Consequently CCA rocks can be considered as potential vectors for seeding post larvae in the field.

9.5. Experiment 2: Further evaluation of the scope of CCA rocks as intermediate settlement and carrier substrates for seeding post-larvae onto depleted reefs

9.5.1. Introduction and aims

The upper limits of CCA rocks for attracting initial settlement and metamorphosis of *H. rubra* larvae and their subsequent short-term carrying capacity in relation to *H. rubra* post-larvae were not found in Experiment 1. The aim of this second experiment was to address these important knowledge gaps.

9.5.2. Methods

Unless otherwise stated, all methods and equipment used were as described in the general methods (Section 4.2) and/or for Experiment 1. The 6x3 factorial design of the experiment comprised six seeding rates of 1,000, 2,000, 4,000, 8,000, 16,000, 32,000 competent *H. rubra* larvae/L of mixed grade CCA rock, and 3 sampling times of 2, 8 and 16 days after seeding. Four replicates were established for each of the 18 (6 seeding rate x 3 sampling time) treatments. Each replicate was accommodated in a 4 L plastic ice-cream tub as used in Experiment 1 but employed 0.5 ± 0.1 L rather than 1.0 ± 0.2 L of mixed grade CCA rocks of individual volume in the range 5 to 200 mL. Also in contrast to Experiment 1 and the preliminary trial, mixed grade CCA rock used in each replicate was not separated into grades prior to fixation and storage in 70% ethanol. As in Experiment 1, post-larvae adhering to the floor and walls of each of the replicate plastic tubs and those adhering to CCA rock were separately harvested, counted and measured.

9.5.3. Results

9.5.3.1. Temperature salinity

Temperatures remained within the range 19.0 to 23.5ºC and salinity within the narrow range 34 to 36g/kg.

9.5.3.2. Background densities and sizes of wild post–larvae and juvenile abalone

No wild post-larvae or juvenile abalone were recovered from any of the four control non-seeded CCA rock replicates of on Day 2.

9.5.3.3. Effect of larval seeding density on survival

The combined yields of post-larvae harvested from CCA rocks and internal surfaces of the replicate plastic tubs give an indication of the survival of post-larvae among density treatments without the complication of movement between rocks and containers. As in Experiment 1, yields of post-larvae on day 2 after seeding (i.e. settlement) (Figure 1), increased with increasing initial seeding density (Figure 1, ANOVA Table 1, followed by Tukey's HSD test, not shown).

Table 1. One-way ANOVA on the log-transformed yield of post-larvae at day two at five stocking densities (Density).

Source	SS	df	MS	*F*-ratio	*P*
Density	1.056	3	0.352	10.14	0.001
Error	0.416	12	0.034		

The yields from the four lowest seeding densities from 1,000 to 8,000 larvae/L CCA rock were not significantly different at about 23-32%. A significantly higher settlement rate of about 55% was found at seeding densities of 16,000 larvae/L and a significantly higher rate again of about 80% was found for the highest seeding rate of 32,000 larvae/L CCA rock. Data presented in Figure 1 also show that subsequent rates of survival on days 8 and 16 were strongly density dependent. For example, yields of post-larvae seeded at 32,000 larvae/L CCA rock declined drastically from 80% on day 2 to less than half this rate (38%) by day 16. At the opposite extreme, post-larval yields for the lowest initial seeding rate of 1,000 larvae/L CCA rock actually increased from 23% to 43% over the same time-frame possibly reflecting protracted settlement and metamorphosis at low density. Yields of post-larvae from intermediate seeding rates of 2000 to 8000 larvae/L CCA rock, remained more or less constant between days 2 and 16. These changes in survival among densities over time were confirmed by a significant interaction between time and density (Figure 1, ANOVA Table 2).

Table 2. Two-way ANOVA on the yield of post-larvae on CCA rocks seeded at six densities (*Density*) sampled at three times (*Time*).

Source	SS	df	MS	*F*-ratio	*P*
Density	4260	5	852.0	10.48	0.000
Time	1023	2	511.9	6.3	0.004
Density X Time	3610	10	361.1	4.44	0.000
Error	4388	54	81.3		

Yields of post-larvae harvested from CCA rock and the sides of the containers are plotted separately in Figures 2 & 3 respectively. For post-larvae harvested from rocks there was a significant interaction between the effects of density of seed set and the time when post-larvae were sampled (ANOVA Table 3). This interaction was caused by the significantly higher yield of post-larvae at day 2 for the highest two seeding densities, 16,000 and 32,000 larvae/L of CCA rock and day 8 for the highest seeding density.

Table 3. Two-way ANOVA on the yield of post-larvae on CCA rocks and the walls of containers seeded at six densities (*Density*) sampled at three times (*Time*).

Source	SS	df	MS	*F*-ratio	*P*
Density	11322	5	2264	19.25	0.000
Time	219	2	109.8	0.93	0.4
Density X Time	2924	10	292.5	2.49	0.016
Error	6234	53	117.6		

All other time x density combinations were not significantly different. This result is consistent with a density dependant effect on survival. It also can be partially explained by post-larvae being more likely to move from CCA rocks when densities are high. This density dependant effect on emigration is confirmed by data presented in Figure 3 indicating that about half the observed decline in yields of CCA associated post-larvae that occurred between days 2 and 8 is attributable to corresponding increments in numbers of post-larvae on surfaces of experimental containers, i.e. by migration of post-larvae from the CCA rock. The subsequent decline in both CCA rock and container surface yields of post-larvae between days 8 and 16 can only be ascribed to mortality.

Another important result was that by day 16, yields of post-larvae from CCA rock (Figure 2), regardless of initial larval seeding rate, had converged to a common level of about 20% (also regardless of the initial three-fold variation in yields on day 2). This result was similar to the convergence of survival rates to a common level also of about 20% exhibited by post-larval *H. rubra* seeded over a wide range of densities onto standard diatom coated nursery plates (Figure 2, Section 4.5). This again raised the question of whether such patterns of survival are simply a delayed expression of inherent viability of particular broods of *H. rubra*.

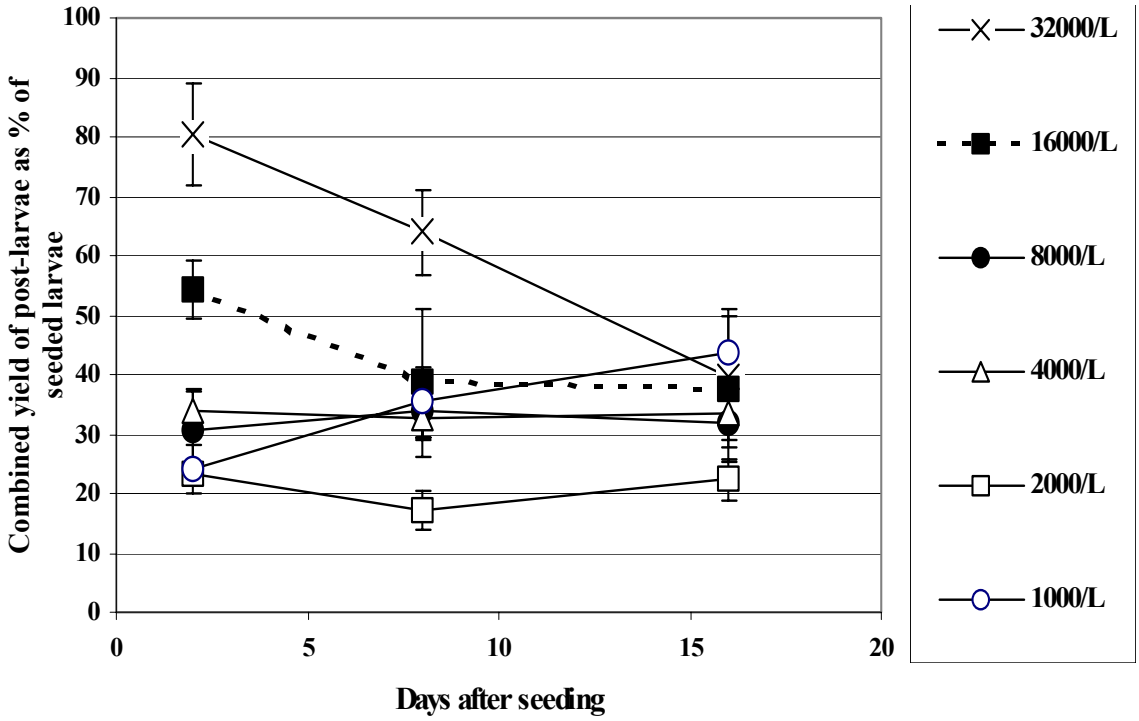


Figure 1. Effect of time from seeding on combined yield of *H. rubra* post-larvae on CCA rock and on walls of containers (error bars = s.e.).

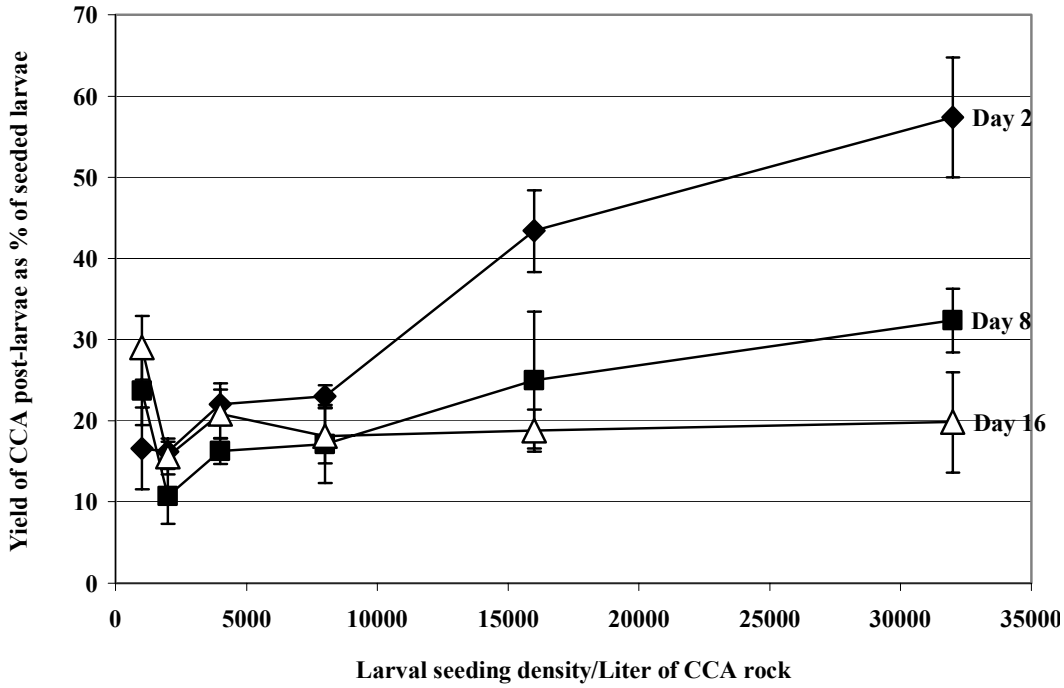


Figure 2. Effect of larval seeding density on yield of *H. rubra* post-larvae on CCA rock (error bars = s.e.).

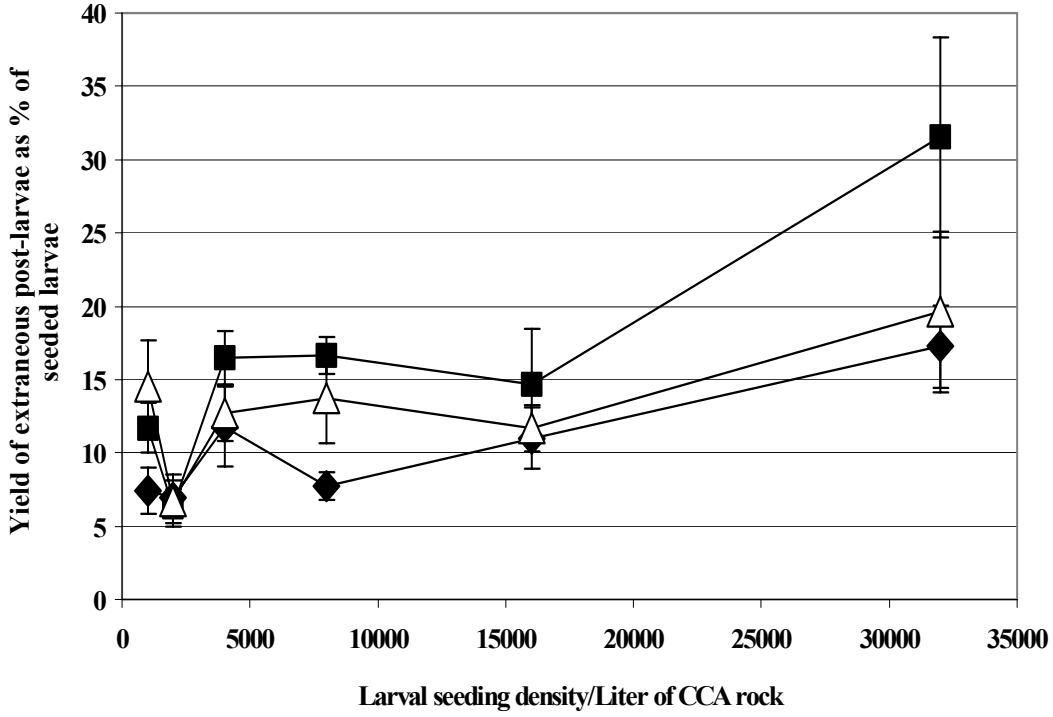


Figure 3. Effect of larval seeding density on yield of extraneous *H. rubra* post-larvae on surface of tubs (error bars = s.e.).

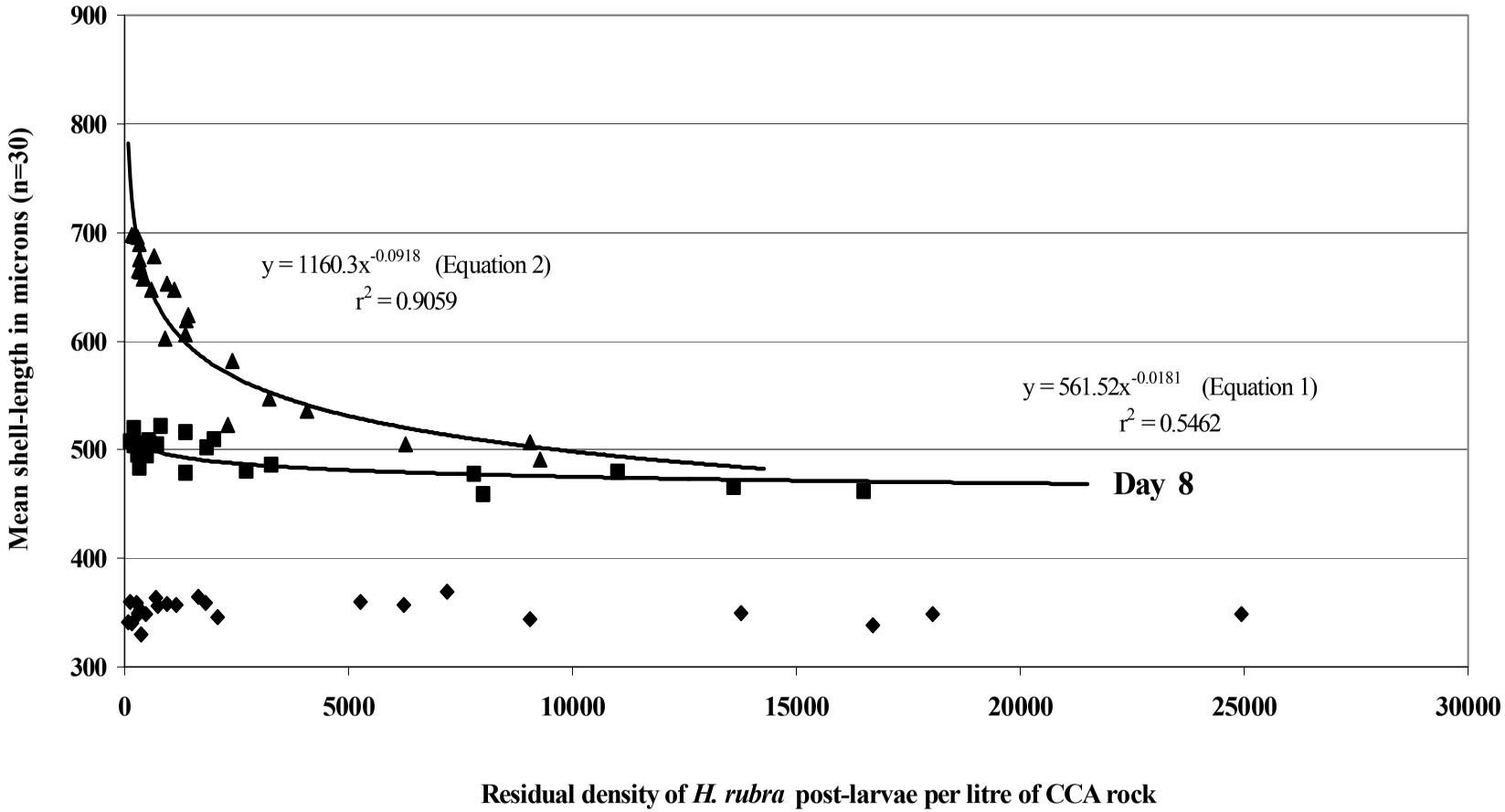


Figure 4. Effect of density on growth of *H. rubra* post-larvae on CCA rock.

9.5.3.4. Effects of density on growth

Day 2:

Plots of mean (n = 30) shell length vs residual density data for CCA associated post-larvae on days 2, 8 and 16 (Figure 4) showed that mean SL on day 2 did not vary in any clear way with density in the range 100 to 25,000 post-larvae/L CCA rock.

Day 8:

A power equation (equation 1 in Figure 4) was found to best describe (R^2 = 0.5462) density/size data for day 8 post-larvae. The mean ± s.e shell length of post-larvae at the highest recorded density of 16,500/L CCA rock was 454 ± 3.5µm. This was only 48 µm less than the corresponding mean ± s.e. shell length of 506 ± 6µm for post-larvae at the lowest recorded density of 124/L CCA rock.

Day 16:

Growth from day 8 to 16 was strongly density dependent in contrast to growth up to day 8. A best-fit power equation fitted to residual post-larval density vs mean shell length data for day 16 (equation 2 in Figure 4) was accompanied by a high R^2 value of 0.9059. The mean ± s.e. shell length of day 16 post-larvae at the highest residual density of 9,284/L CCA rock was 491 ± 5 µm. This was only 10 µm greater than a corresponding mean shell length of 48 µm estimated using equation 1 in Figure 4, for day 8 post-larvae at the same density. This indicates that there is profound suppression of growth at such high densities beyond day 8 after seeding. By contrast, the mean shell length of day 16 post-larvae at the lowest recorded density of 124/L CCA was 698 ± 15 µm which was 187 µm greater than the corresponding mean shell length of 511 µm for day 8 post-larvae at the same density estimated using equation 2 in Figure 4.

Summarizing, mean shell length of post-larvae from day 2 to 8 increased from about 350 µm up to a range of 454-506 µm. Final mean shell length of post-larvae was inversely related to residual density over the range 124 to 16,500 post-larvae/L CCA rock. This translated to moderately variable growth rates from 16 to 26 µm /day during days 2 to 8. However between days 8 and 16, growth rates of post-larvae at densities in the range 124 to 9,284/L CCA rock varied inversely with density over the very wide range of 1.5 to 23 µm/day.

9.5.4. Conclusions and discussion

As in Experiment 1, settlement of *H. rubra* larvae appears to be highly gregarious. Despite doubling the highest seeding density used in Experiment 1, rates of successful settlement again significantly increased to 80%. However, increased settlement using the higher densities of larvae appears to have very little effect on the long-term yield because of the very low carrying capacity of the CCA rock. Accordingly, the same density dependant effects on survival, growth and emigration as in Experiment 1 were evident.

These results give some guidance in using CCA rocks as vectors for seeding post-larvae. CCA rocks can be stocked with very high densities of early post-larvae, indeed the higher the stocking rate the more efficient the settlement. These stocking rates however are not sustainable. Survival and growth of the post-larvae decrease quickly in a density dependant manner. Because of this the most efficient use of these rocks as vectors of post-larvae is to deploy them within the first week after settlement before survival, growth rates and presumably vigour are significantly reduced. Fortuitously, emigration of post-larvae from CCA rocks is density dependant. Thus the high stocking rates will also facilitate good dispersal of post-larvae.

9.6. Experiment 3: Evaluation of post-larvae laden CCA rocks for dispersing post larvae into simulated habitat

9.6.1. Introduction and aims

Results of Experiments 1 and 2 demonstrated the high capacity of small CCA rocks in the range of 5 to 250 mL, to attract settlement and to induce metamorphosis of *H. rubra* larvae. The results also showed that resultant post-larvae could be sustained at very high densities for periods of up to 8 days without significantly restricting their growth or ability to rapidly disperse from the rocks onto adjacent surfaces. These encouraging results prompted this third laboratory experiment to evaluate the efficiency of different size grades of CCA rock to carry and disperse post-larvae onto simulated boulder field habitats.

9.6.2. Methods

Unless otherwise stated, all methods and equipment used were as described in the generalised methods Section 4.2 and for Experiments 1 and 2. Experiment treatments comprised 5 alternative methods of seeding 0.5 L of mixed grade CCA rocks that simulated a shallow, sub-tidal boulder field.

Four of the five alternative seeding techniques (Table 1) comprised preliminary seeding of approximately 5,000 competent *H. rubra* larvae onto 0.5 L lots of pre-graded CCA vector rocks held within individual 2 L plastic tubs. Four replicate tubs were established for each of 4 discrete grades of CCA vector rocks. Numbers and individual volumes of which are specified in Table 1. Every vector rock was individually identified using a spot of quick drying, white enamel paint. Immediately prior to seeding the larvae onto the CCA vector rocks, aeration rates within the 2 L tubs were reduced to a trickle and seawater flow stopped for 6 h to allow settlement and initiation of metamorphosis. Over the subsequent week, seawater flow to each tub of CCA vector rocks was continuously maintained at about 0.5 L/min.

On day 7 after seeding, each of the sixteen 2 L tubs containing the 0.5 L lots of seeded vector rock were randomly allocated to one of 16 plastic mesh IQF prawn trays fitted with polyethylene film liners. The trays had been stocked a week earlier with 4.5 L of random grade CCA rock. The vector rocks were spread uniformly amongst the mixed grade CCA rock to facilitate even dispersion.

The sides and end walls (but not the floors) of the liners, which extended several centimetres above water level, were lacerated every 5 cm or so with 2 to 3 cm vertical slits inserted with a scalpel blade. These slits enabled unrestricted outflow of seawater but did not allow significant escape of post-larvae from the trays. Each IQF tray was supplied with four evenly spaced 3mm airlines fed by an overhead grid manifold constructed of 20 mm PVC pipe and fittings. Flow rates of each airline were controlled using a 3 mm drip irrigation valve. Each IQF tray was served by four seawater supply lines in the same array as the seawater feed lines. Combined flow from the four seawater lines into each of the IQF trays was maintained at about 2 L/minute. Continuous high aeration was used to maintain turbulent mixing of seawater that minimized “dead spots” within the trays.

An additional four replicates of the control treatment, each comprising the same type of IQF tray, liner, air and seawater supply systems but 5.0 L rather than 4.5 L of mixed grade CCA rock, were set up and directly seeded with 5,000 competent larvae at the same time as the 16 x 0.5 L tubs of vector rock. As with the vector rocks, larvae used to directly seed mixed grade CCA rocks of the control treatments were allowed to settle and begin metamorphosis in gently aerated static seawater for 6 h before lacerating the walls of the liners and resuming seawater flow and high turbulent aeration.

9.6.2.1. Sampling of carrier and mixed grade CCA rocks

General sampling methods were the same as those described for Experiments 1 and 2. First sampling was conducted on day 6 after seeding when a quarter (1.25 L) of mixed grade CCA rock was randomly selected and removed from each of the four control treatment IQF rays to provide an estimate of initial yield of direct seeded post-larvae. Extraneous post-larvae that had settled and metamorphosed on the inner walls of each of the 16 x 2 L tubs of vector rock were also harvested and preserved immediately following removal and deployment of the vector rocks to their designated IQF trays. Numbers of extraneous 6-day-old post-larvae once determined were deducted from the original 5,000 seeded larvae to provide a more valid estimate of initial number of larvae seeded onto the CCA vector rocks.

On day 14 after seeding one quarter of the total vector and mixed grade CCA rock from was randomly sampled from replicates of all treatments, including those of the directly seeded control treatment. In all replicates, other than those of the direct larval seeded control, vector rocks readily distinguished by spots of white enamel paint, were sorted and separately preserved and archived from mixed grade CCA rock.

This procedure was repeated on days 28 and 56 after seeding. However on the final day 56 of the experiment, all residual mixed grade CCA rock was harvested from all replicates, and archived in 70% ethanol. In the case of control treatment replicates, residual mixed grade CCA rock was sampled, which constituted a quarter (1.25 L) of that originally loaded into the IQF trays. For all other treatment replicates, residual CCA rock sampled on day 56 comprised 50% (2.5 L) of that originally loaded into the IQF trays. Also on day 56, post-larvae adhering to the plastic liners of all replicate IQF trays were separately harvested, preserved in 70% ethanol and archived. As in previous experiments, mean shell lengths of post-larvae in samples were determined from 30 randomly selected individuals whenever numbers permitted. Otherwise, all available post-larvae were used to estimate mean size.

Table 1. Specifications of experimental treatments used to assess and compare the efficiency of direct seeding of competent larvae with that of seeding day 6 post-larvae pre-settled onto 4 grades of vector rocks.

Treatments		CCA vector rock size specifications (mL)			Number of individual vector rocks used per replicate	Volume (mL) of random size CCA rock provided for dispersal	Number of competent larvae initially used per replicate	No of Replicates
Number	Description	Min. size	Max size	Total Volume				
1	Post-larvae presettled on Grade 1 CCA vector rocks	50	100	500	8	4500	5000	4
2	Post-larvae presettled on Grade 2 CCA vector rocks	20	40	500	16	4500	5000 – post-larvae recovered from walls of carrier rock seeding containers on day 6	4
3	Post-larvae presettled on Grade 3 CCA vector rocks	5	15	500	40	4500	As above	4
4	Post-larvae presettled on Grade 4 CCA vector rocks	1	4	500	160	4500	As above	4
5	Control (direct seeding of competent larvae)	na	na	na	na	5000	As above	4

9.6.3. Results

9.6.3.1. Temperature salinity

Mean daily temperature over the 56-day period from 19/01/00 to 22/03/00 ranged from 20 to 23ºC while salinity remained within the narrow range 34 to 36 g/kg.

9.6.3.2. Effect of carrier rock size on dispersal efficiency

The densities of post-larvae on the four grades of vector rocks from day 0 to the end of the experiment at day 56 are shown in Figure 1a. There was no significant difference in the density of larvae settled on the four grades of rock at day 0 (Table 2). The numbers of post-larvae on the four grades decreased steadily from day 0 when there was about 5,500 larvae/L of rock until day 56 when there was approximately 140/L of rock. There was no significant difference in the density of larvae remaining on the four size grades of rocks at the end of the experiment at Day 56 (Figure 1a, Table 2).

Table 2. One-way ANOVAs on the density of post-larvae remaining on CCA vector rocks at the initial (day 0) and final (day 56) sampling times, on four different rock size classes.

Day 0

Source	SS	DF	MS	*F-ratio*	P
Vector rock size	574307	3	191436	0.83	0.504
Error	2530460	11	230041		

Day 56

Source	SS	DF	MS	*F-ratio*	P
Vector rock size	13819	3	4606	0.41	0.746
Error	133679	12	11139		

The density of post-larvae on the mixed grade rock in the IQF trays that had been seeded directly as larvae or that had immigrated onto these rocks from vector rocks over time are presented in Figure 1b. At day 14, significantly more post-larvae (Table 3, followed by Tukey's test, not shown) found on the mixed grade rock in the direct larval seeded control treatment (approximately 700/L CCA rock) compared to those that immigrated there from vector rock (approximately 230/L). By day 28 the difference between the density of post-larvae on the mixed grade rock that emigrated from different grades of vector rock was not significant. By day 56, densities of post-larvae on the mixed grade CCA rock were similar (approximate range 50-120/L) regardless of whether post-larvae were directly seeded as larvae or immigrated as early post-larvae from vector rocks.

These results indicate, as in the previous two experiments, that high initial stocking densities are negated by the inherent long-term carrying capacity limitations of CCA rock habitats. By day 56 (at a shell length of 1 to 2 mm) in this experiment, as in Experiment 1, it seems that CCA rocks could only sustain post-larvae at densities in the order of 100-200/L of CCA rock (~0.1 to 0.2 per cm^2 surface area).

Table 3. One-way ANOVAs on the density of post-larvae that immigrated to mixed grade CCA rocks from vector rocks at three times after seeding using four different size classes of vector rock and a direct larval seeded control.

Day 14

Source	SS	DF	MS	*F-ratio*	P
Method of seeding (4 Vector rock grade & direct)	4.71	4	1.18	5.90	0.005
Error	2.8	14	0.2		

Day 28 (log-transformed data)

Source	SS	DF	MS	*F-ratio*	P
Method of seeding (4 Vector rock grade & direct)	4.09	4	1.02	3.01	0.052
Error	5.1	15	0.34		

Day 56

Source	SS	DF	MS	*F-ratio*	P
Method of seeding (4 Vector rock grade & direct)	9935	4	2483	0.64	0.64
Error	57824	15	3854		

9.6.3.3. Effects of density and seeding methods on the growth of post-larvae

The growth rate data of post-larvae that remained on the four size grades of vector rock are presented in Figure 2a. By day 56, post-larvae had grown to mean shell lengths (SL) of between 1.2 and 1.4 mm and there was no significant difference in the size of post-larvae still residing on the different size grades of vector rock.

The growth rate of post-larvae seeded directly as larvae and stocked indirectly via vector rock onto mixed grade CCA rock are presented in Figure 2b. Post-larvae that were directly seeded as larvae were smaller (mean SL approximately 1.2 mm) than those seeded as post-larvae via vector rock (mean SL ≥1.4 mm). Post-larvae that had immigrated from the four size grades of vector rocks were not significantly different in size from one another (Figure 2b, Table 4, followed by Tukeys test, not shown).

Table 4. One-way ANOVAs on the mean shell lengths of post-larvae at three times after seeding that had immigrated to mixed grade CCA rocks from four grades of vector rocks or directly seeded as larvae (control).

Day 14

Source	SS	DF	MS	*F-ratio*	P
Method of seeding (4 Vector rock grades & direct)	8314.8	4	2078.96	2.49	0.09
Error	11666.9	14	833.35		

Day 28

Source	SS	DF	MS	*F-ratio*	P
Method of seeding (4 Vector rock grades & direct)	64516	4	16129	2.28	0.11
Error	105990	15	7066		

Day 56

Source	SS	DF	MS	*F-ratio*	P
Method of seeding (4 Vector rock grades & direct)	249751	4	62438	3.03	0.051
Error	308896	15	20593		

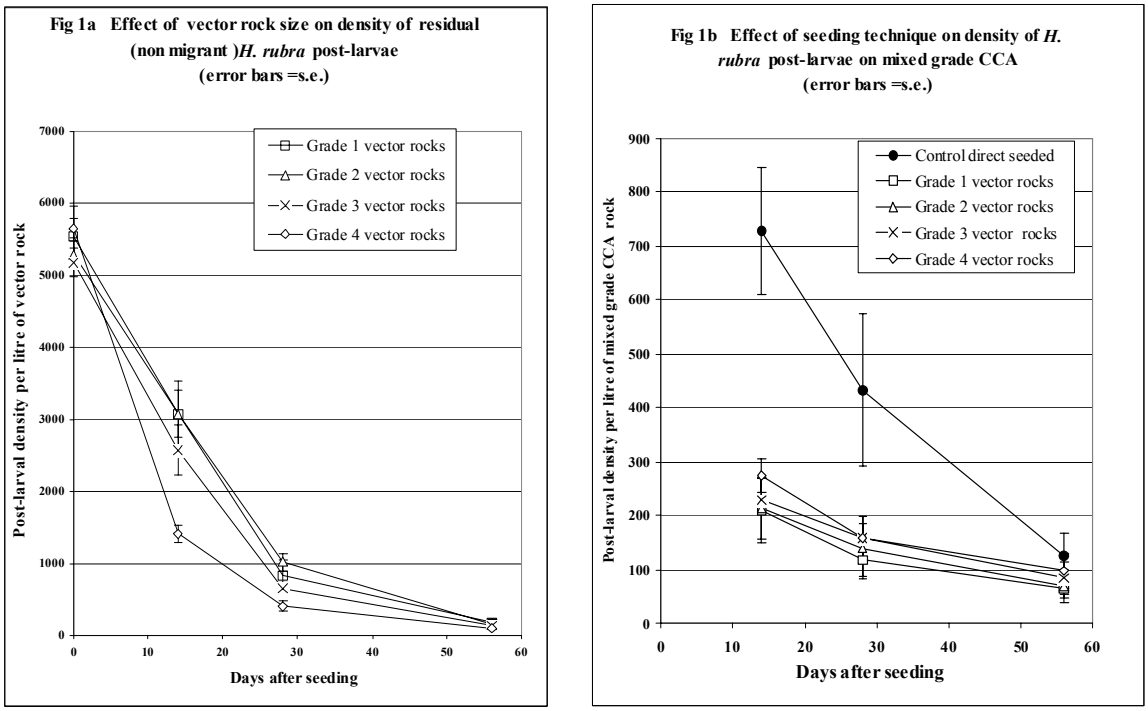


Figure 1. Effect of (a) vector rock size and (b) seeding technique on density of *H. rubra* post-larvae.

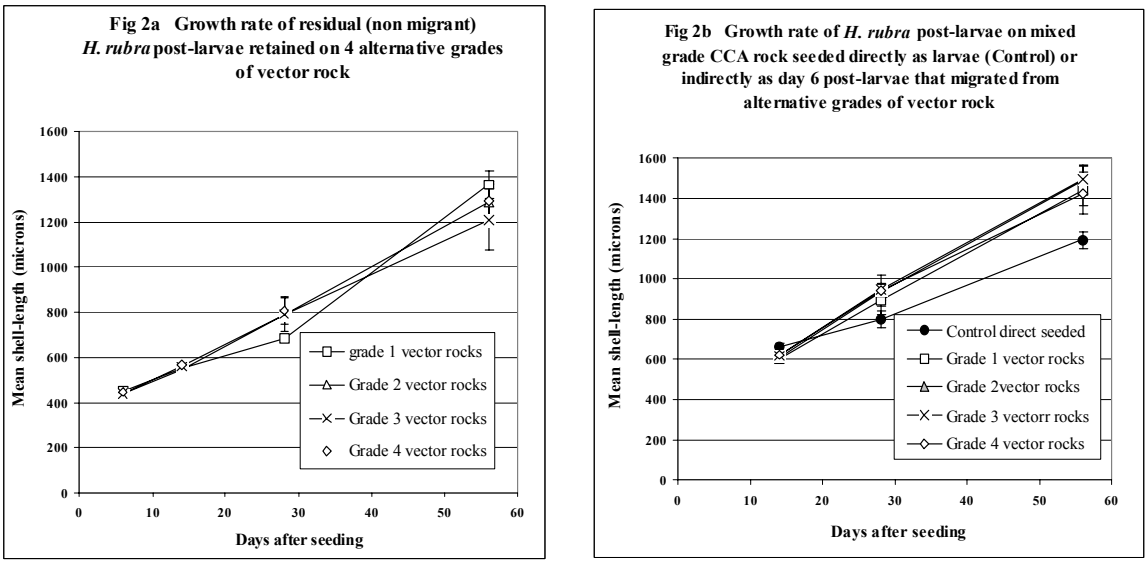


Figure 2. Growth rate of *H. rubra* post-larvae.

To examine the influence of time and density on growth and survival of post-larvae that had either remained on vector rock or migrated onto mixed grade CCA rock, on each sampling occasion, data from all replicates of all four vector rock treatments were pooled. Separate shell length and density data for post-larvae that remained on vector rock or successfully migrated across to mixed grade CCA rock, together with equivalent data for directly seeded post-larvae, are presented in Figures 3, 4 & 5. Data presented in Figures 3 & 4 show that within 8 to 22 days (i.e. between days 6 and 28) of migrating from densely laden vector rocks onto mixed grade CCA rock, post-larvae exhibited greatly enhanced growth rates that can most probably be attributed to reduced density. Day 14 data also showed that direct seeded post-larvae had achieved an initial size advantage over their migrant counterparts most probably as a consequence of having been at much lower densities up to day 6. However by day 28 (Figure 4) these differences had been totally eroded.

Mean size predictions derived from power equations fitted to size density data for non migrant post-larvae still residing on vector rocks at days 14 and 28 (Figures 3 & 4) showed that these post-larvae had grown at low to moderate density dependent rates. For example, growth rate estimates for post-larvae at lower and upper density extremes of 300 and 5,000/L of vector rock, were 17 and 5 µm/day respectively. Migrant post-larvae exhibited moderate to high growth rates in accordance with their low overall densities. Growth rates of migrant post-larvae at extreme upper and lower densities of 50 and 350/L mixed CCA rock were 27 and 17 µm/day respectively.

Best fit power equations fitted to size density data for day 56 (see equations 1 and 2, Figure 5) showed that post-larvae that had successfully migrated from vector rock onto surrounding mixed grade CCA rock had outgrown both their non migrant counterparts and directly larval seeded "controls". Large size margins gained by migrants over non migrants cannot however be attributed to density factors alone. A probable explanation is provided by the fact that equations fitted to size density data of non migrants on days 28 and 56 (equations 1 in Figures 4 & 5) are almost identical and demonstrated that growth had fallen to negligible rates of ~2 µm/day. Over the same 28 day period, densities of post-larval on vector rock fell sharply from a mean of 728/L down to 144/L, representing a catastrophic mortality rate of 80%.

In contrast to the above, mean overall densities of migrant post-larvae declined by a more moderate factor of 40% from 146/L CCA rock on day 28 down to 86/L on day 56. Shell growth of migrant post-larvae also contrasted with stalled growth exhibited by their non-migrant counterparts. Mean size predictions of best fit power regressions fitted to these two sets of data (equation 2 in Figures 4 & 5) showed that these migrant post-larvae had continued to grow at moderate rates. For example growth rates based on predictions of the regressions for post- larvae at density extremes of 20 and 300/L mixed grade CCA rock, were 18 and 13 µm/day respectively.

Mean ± s.e. shell lengths of migrants on day 56, within individual replicates of all four vector rock treatments, ranged from 1077 ± 35 µm to 1,696 ± 96 µm. Corresponding densities ranged from 22 to 203/L CCA rock. Mean ± s.e. shell lengths of post-larvae harvested from the polyethylene film liners of IQF trays of these same treatments ranged from 1322 ± 25 to 2294 ± 113 µm. These sizes were not significantly different from the above-cited sizes of migrant post-larvae. However, overall mean ±s.e. size of post-larvae removed from liners of replicates of the control (direct larval seeded) treatment were significantly smaller (1,193 ± 43 µm) than counterparts harvested from liners of treatments replicates involving indirect seeding via four grades of post-larvae laden vector rock (1,377 ± 9 µm).

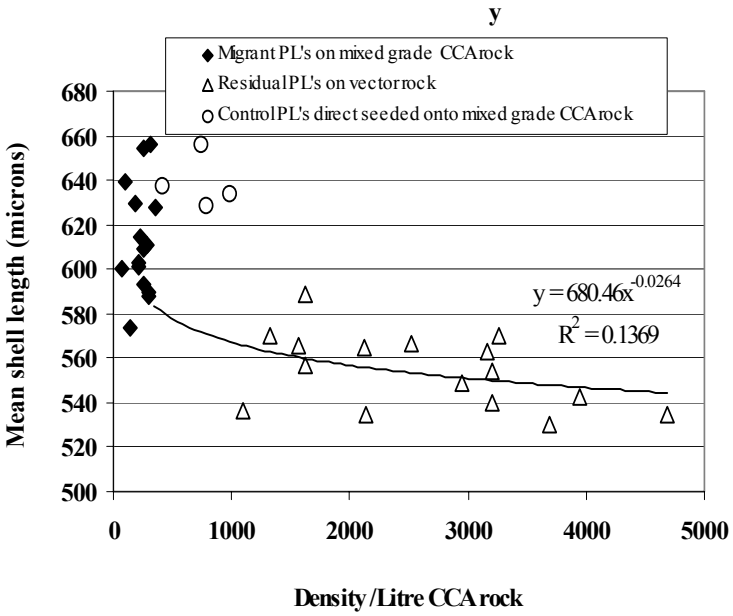


Figure 3. Relationship between actual residual density and mean sizes of abalone that had migrated onto mixed CCA rock or that remained on vector rock on day 14.

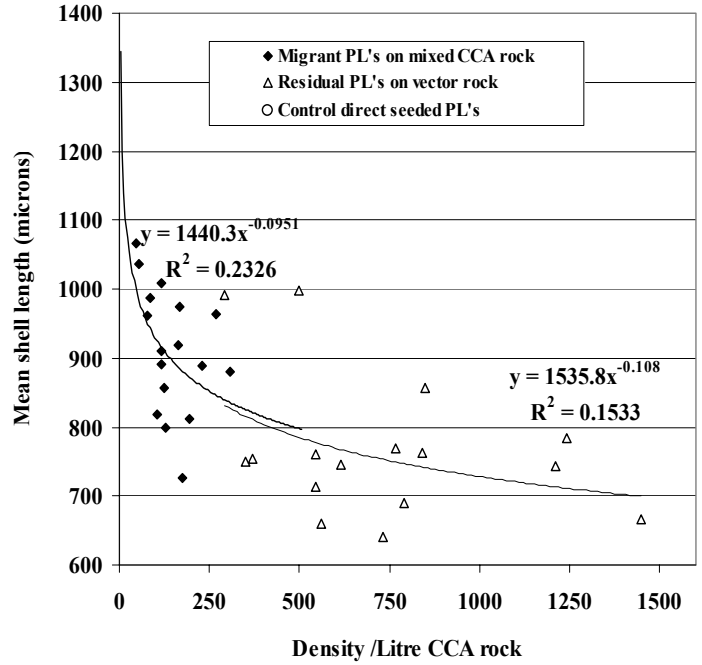


Figure 4. Relationship between actual residual density and on mean sizes of postlarve that had migrated onto mixed CCA rock or that remained on vector rock on day 28.

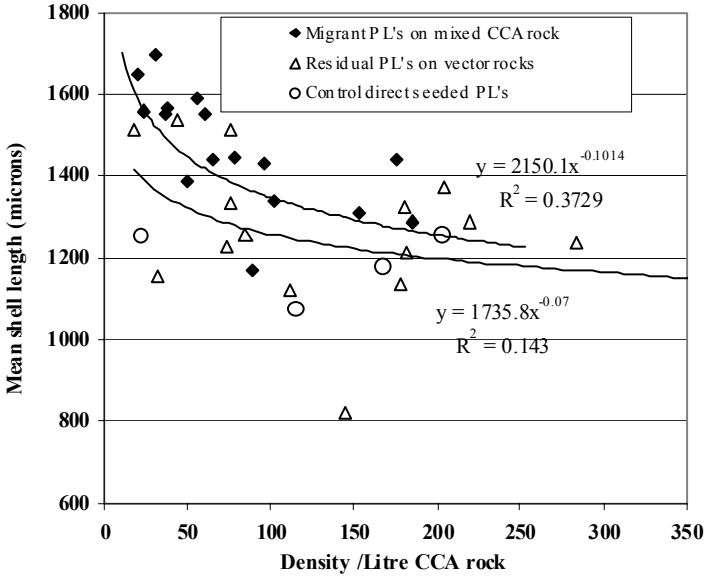


Figure 5. Relationship between actual residual density and on mean sizes of postlarvae that had migrated onto mixed CCA rock or that remained on vector rock on day 56.

A key finding of this experiment was that day 6 post-larvae densely laden onto CCA vector rocks were able to rapidly disperse into and colonise larger volumes of mixed grade CCA rock reaching low final densities in the range 20 to 350/L as early as day 14 after settlement and metamorphosis, i.e. within the 8 days of being seeded. The low densities achieved were probably a consequence of competitive exclusion exerted by earliest migrants on their successors. In spite of these low densities, successful migrants exhibited marked density dependent growth and to a lesser extent survival. Indeed results of this experiment (Figures 4 & 5) suggest that continued satisfactory growth rates above about 18µm/day cannot be sustained by *H. rubra* post-larvae on CCA rock substrates unless they are at low densities below about 500-1,000/L (0.5 to 1.0/cm^2) CCA rock at day 28 and below about 100-200/L (0.1 to 0.2/cm^2) CCA rock at day 56.

As indicated in Figure 1a, densities of post-larvae on vector rocks exhibited a continuous dramatic decline throughout the experiment, mean ± s.e densities for all the 4 grades falling from an initial range of 5,177 ± 205 to 5,655 ± 138/L to a final range of 97 ± 35 to 178 ± 50/L on day 56. Another important finding, illustrated by data presented in Figures 1a & 2a, was that seeding of 6-day-old post-larvae on the smallest pebble size (3 mL) grade 4 vector rocks facilitated both the speed and extent of their emigration off vector rock and colonisation of vacant mixed grade CCA rock.

9.6.4. Discussion

Growth rates of 18µm/day recorded by Preece *et al.* (1997) for *H. rubra* larvae seeded onto natural boulder field habitats from settlement to day 19 were very similar to rates recorded here for post-larvae from settlement to day 56. Results of the current experiment (Figures 1a,b & 2a,b) also suggest that in the absence of both competition from other species and predation, CCA rock can support satisfactory growth rates of ~15-20 µm/day for *H. rubra* post-larvae at densities of up to 500-1,000/L rock at one month (day 28) of age and mean shell length of ~0.8 mm. However carrying capacity falls to one fifth of this level (100-200 post-larvae/L) a month later (day 56), by which time post-larvae have increased in mean shell length of ~1.4 mm, i.e. by a factor of 1.75x. Knowing that biomass of *H. rubra* increases as the cube of length (Figures 5a & 5b, Section 4.5),

this observed decline in carrying capacity can be fully explained by a corresponding 1.75^3 x = 5.2x increase individual biomass over the same period.

Based on 13 years of field research and observations, Shepherd & Daume (1996) concluded that post-larvae abalone recruit onto CCA crusts on both upper and undersides of boulders and that fidelity to the CCA habitat is high for abalone up to approximately 10 mm SL. Evidence for the later is that fragments of CCA are the predominate food item in the gut in juveniles up to this size and continue to be eaten by juveniles up to at least 35 mm. Thus 6 month old juvenile *H. rubra*, having attained mean shell length in the order of 8-10 mm, or 10 - 12x that of 1 month old 0.8 mm counterparts, have also attained corresponding biomass increments of at least 1000x. Assuming that carrying capacity of CCA habitats continues to decreases inversely with biomass up to this age and size, then densities of 6 month old 8-10 mm juvenile *H. rubra* could be expected to decline to levels one thousandth those of their one month old counterparts, i.e. 0.5 - 1/L CCA rock. Using the surface area to volume conversion factor of $0.12m^2$/L for mixed grade CCA rock determined in Experiment 1, such densities translate to 4 to 8 juveniles/m^2 CCA rock surface area. Based on these arguments, carrying capacity of typical boulder habitats (characterised by surface areas of 2 m^2/m^2 planar bottom area [Preece *et al*. 2000]), for six month old 8 –10 mm juveniles are likely to be only 8 to 16/m^2.

In an assessment of the fate of both naturally recruited and artificially seeded *H. rubra* post-larvae, Shepherd *et al*. (2000) found that instantaneous mortality from ~1 month to 1 year of age (M_0) was positively correlated with the logarithm of initial post-larval density (D_0) and gave the regression, $M_0 = 1.24 + 1.47 \log D_0$ ($R^2 = 0.59$; $P < 0.01$). Densities of 1 month old wild post-larvae varied within the range of 1 to 28/m^2 with a mean ± s.e. of 10.0 ± 3.8/m^2. Subsequent densities 1 year later ranged from 0.2 to 5.5 juveniles/m^2 (mean ± s.e.; 1.25 ± 0.47/m^2). Instantaneous mortality (M) values across both wild and seeded recruits varied from 0.4/year at the lowest initial density of 1/m^2 to 4.7/year at and highest initial density 99/m^2. These extreme M values constituted highly divergent annual survival rates of 67% and 1% respectively that translated to convergent net yields of one year old juveniles of 0.7/m^2 and 1.0/m^2 respectively.

McShane (1991) also found a linear relationship between survival of 5 month old juveniles and log of the density (range 9 to 2000/m^2) of ~1 week old (mean shell length range 460 to 515 µm) post-larvae in three populations of *H. rubra* from neighbouring reefs in north-eastern Victoria. A regression ($R^2 = 0.83$; $P < 0.01$) fitted to these data predicted that mean yields from week old post-larvae densities of 1, 10, 100 and 1000/m^2 would yield 5 month old juveniles at densities of 0.06, 0.4, 2.5 and 3/m^2, respectively. Mean densities of juveniles in the range 5 to 55mm measured at the three reefs over three successive years ranged from 0.15 ± 0.08 to 2.17 ± 0.21/m^2. Prince *et al*. (1988) found that although densities of 8 to 17 mm juvenile *H. rubra* at five sites in Tasmania ranged up to 22/m^2, the great majority occurred at low densities of <2/m^2, similar to those reported by both McShane (1991) and Shepherd *et al.* (2000).

Important practical implications of this information for cost effective seeding of early post-larvae, are firstly, that regardless initial seeding density, subsequent yields of advanced post-larvae and juveniles are fixed within a low and narrow range. A second important implication, based on the above cited findings of McShane (1991), is that to restock or enhance depleted reef densities of advanced juveniles in the order of 0.2 to 2/m^2, typical of healthy productive reef, should be targeted by seeding week old post-larvae at densities in the range 10 to 100/m^2 of planar bottom area.

Results of this experiment and of Experiment 1 are in agreement with the above-cited findings of Prince *et al.* (1988), McShane (1991) and Shepherd *et al.* (2000). They collectively demonstrated that regardless of whether CCA rock habitats are directly seeded with *H. rubra* larvae or post–larvae, regardless of a wide range of initial seeding densities from 1,000 to 8,000/L (8,000 to 64,000/m^2) of CCA rock and regardless of the absence of predators and competitor species, mean yields of 56 day post–larvae varied over a relatively narrow range of ~100 to 200/L (equivalent to 800 to 1,600/m^2) CCA rock.

The smallest of 4 size grades of vector CCA rock evaluated in this experiment (Figure 1a) promoted significantly faster and more extensive transfer of day 6 post-larvae onto a simulated juvenile habitat comprising mixed grade CCA rock than three larger grades of vector rock. As indicated by data provided in Figure 1a, densities of day 6 post-larvae seeded onto vector rock averaged ~4,000 to 5,000/L. Numbers of post-larvae carried by individual grade 4 vector rocks that averaged 3mL (320/L) would therefore have ranged from about 12 to 15/rock. Accordingly, if densities of one month old post-larvae of 10 to 100/m^2 and of 5 month to 2 year old juveniles in the range 0.2 to 2/m^2, typical of normal productive reef, are to be targeted, then pebble size vector rocks each loaded with 12 to15 post-larvae should be broadcasted onto juvenile habitats of depleted reefs at rates of 1 to 10 rocks/m^2 of planar bottom. Apart from obvious advantages of ease, simplicity and cost effectiveness offered, such a seeding strategy could be applied on very large spatial scales, namely many hectares or indeed entire juvenile habitats of depleted reefs.

In summary, results of the present study provide grounds for optimism that week old *H. rubra* post-larvae pre-stocked onto CCA carrier rock for seeding depleted reef habitats could circumvent serious practical difficulties and prohibitive costs of deploying competent larvae. The cost effectiveness of seeding post-larvae may however depend on higher net yields than those achieved here. One obvious method of achieving this is simply to reduce initial seeding rates well below those used in this experiment and in line with the inherently low carrying capacity of natural CCA rock habitats discussed above.

10. BENEFITS AND ADOPTION

To what extent sustainable catches of *H. rubra* in NSW and elsewhere can be raised using hatchery produced seed can only be answered by continuing long term and large scale pilot seeding operations in each state. Preliminary cost and benefit exercises suggest that cost effective seeding can be achieved if survival rates of released 6 month old juveniles through to legal size 3-5 years later are raised to levels of 25% or greater corresponding rates for wild abalone. This represents a yield rate of about one legal abalone per 100 released seed. By raising sustainable catches by a factor of 10%, gross value of the annual commercial catch across southern Australia would rise by approximately $20 million.

Adoption of improved nursery production of technology by the abalone farming industry will be promoted by practical training programs being run in conjunction with several colleges of TAFE in NSW and by a practical user manual for intensified nursery production of *H. rubra*, due for publication and distribution by June 2004.

11. PLANNED OUTCOMES

Several critically important steps to achieving the central long-term aim of this project, namely cost effective enhancement of abalone fisheries using hatchery produced seed stock, were achieved. The first was year-round controlled temperature conditioning and spawning of captive broodstock. A flow-on effect of this achievement was that it provided impetus and guidance for practical implementation of a companion R & D project (FRDC 2000/204) to refine and improve this technology in relation to both blacklip and greenlip abalone. This controlled breeding technology is in turn providing a number of important flow-on benefits to the abalone aquaculture industry in southern Australia. Most importantly, it is assisting an industry wide selective breeding program through facilitated synchronisation of spawning that in turn enables pair crossing and identification of specific genes for faster growth.

Another important benefit for the abalone farmers has been development of technology that will enable new nursery production systems with greatly improved space efficiency and much lower initial capital costs. Uptake of this technology is expected in the next generation of land based farms that will also need to incorporate controlled reproductive conditioning systems and an additional intermediate nursery system for weaning and ongrowing of post-larvae from 1-3 mm.

Controlled year-round conditioning and spawning has also enabled initiation of research to produce and evaluate triploid abalone that offer prospects of both faster growth and near or total reproductive sterility, both of which could be of major benefit to fisheries enhancement and farming of this species.

Results of laboratory based research on use of week old post-larvae, as an alternative to competent larvae, are sufficiently encouraging to extend this research into a field evaluation phase. This technology, if successful, would largely circumvent practical problems of weather, currents and wave surge in targeted seeding of larvae onto natural CCA-coated rock habitats. In any case, results of this particular area of research also demonstrated that even if natural survival rates of one legal size abalone per 20,000 competent larvae could be achieved – costs will still be prohibitive. Hopefully this demonstrated futility of larval seeding will circumvent needless further investment in this field of research.

Relatively poor post-release survival obtained with clustered seeding of "button size" (5-15 mm) juveniles attained together with evidence that the central underlying reason is low inherent carrying capacity of juvenile CCA habitats, provides a much clearer focus for continuing research. The result of a final deployment experiment in which release of 1,000 juveniles in 10 clusters of 100 yielded 3-4 times the survival of juveniles seeded as a single cluster of 1,000, is very encouraging. This finding alone vindicates successful funding of a follow-up 12 month project (FRDC 2001/033) that will further pursue development of dispersed seeding technology matched to the low carrying capacity of CCA rock habitats for juvenile *H. rubra* up to 12 months of age and 30 mm.

12. FURTHER DEVELOPMENT

Continuing research of cost effective technology for enhancing the *H. rubra* fishery in NSW and elsewhere in southern Australia is being pursued by NSW Fisheries under FRDC project 2001/033. This research may be summarised as follows:

12.1. Seed production

- Further development of simplified high density plate production of weaning size (1-3 mm) post-larvae. This research centres on the use of gregarious settlement that regularly yields ≥80% recovery of seeded larvae as post-larvae after about one week. Gregarious settlement that is triggered by seeding threshold density in the range 1 to 2/cm^2 (3,500 to 7,000/plate) further simplifies the diatom plate nursery phase by eliminating the need of settlement inducers such as *Ulvella lens* on the plates.
- Production and evaluation of triploid *H. rubra* for both farming and fisheries enhancement.
- Production and evaluation of hybrid *H. rubra* and *H. coccordiata* for both fisheries enhancement and farming.

12.2. Deployment of seed

Continuing experimental seeding research within FRDC project 2001/033 that is being sub-contracted to a leading international coastal reef ecology research team led by Professor Tony Underwood at the University of Sydney, will test the hypothesis that dispersed seeding of 6 month old button size (5-15 mm) juveniles at densities matched to the low inherent carrying capacity (typically 1-10/m²) of natural CCA rock habitat will substantially increase post-release survival especially over the first days weeks and months after seeding.

Key elements of this project are as follows:

1. Commencing in March 2004, all field experimentation will be confined to the Port Stephens area. This is because of:
    - area translocation limitations imposed by the potentially infectious and fatal disease *Perkinsus*.
    - cash funding limited to only $100K.
    - an annual sea temperature range that is better suited to year-round nursery production of seed abalone than more northern or southern sites.

2. Results of deployments during the current project in the Port Stephens region have identified 5 sites that have supported significant and persistent (albeit low) post-release survival beyond 1 year and this can serve to evaluate and compare the efficiency and general utility of alternative seeding practices, especially clustered vs dispersed seeding.

Other deployment strategies and issues to be assessed will be:

a) Effect of seeding density *per se* on post-release survival. This question will entail use of predator proof enclosures. Natural density data of wild *H. rubra* would suggest that densities in the range 1-20/m² be compared.

b) Evaluation of urchin shadows (areas beneath the daytime sheltering sites of long spined black urchins, *Centrostephanus rodgersii*) for reducing post-release predation.

c) Evaluation of multiple lower density seeding of sites vs single higher density seeding events.

d) Comparative testing of different reef habitat types including boulder, fringe/turfing and barrens zones on survival and growth of released juveniles seeded at different densities.

e) Evaluation of growth rate and adult shell morphology (shell length/width ratios) co-variation of sub-populations of *H. rubra* (Worthington *et al.* 1995) for predicting carrying capacity of reefs in relation to larger (>50 mm) drift seaweed feeding size classes of abalone, and hence as a method of more efficient targeted seeding of more productive reefs.

Postscript: At the time of writing (April 2004) rapid progress is being made in the development of dispersed seeding technology. Predator-proof release capsules each accommodating 5 to 20 button size juvenile abalone were successfully tested in January and February 2004. The capsules and associated equipment will enable the juveniles to:

- self-load themselves into the capsules;
- be stored or bulk transported up to several weeks later in healthy relatively unstressed condition; and if warranted
- be pre-exposed to wrasse, crabs and other major predators thereby overcoming naivety to such dangers at the time of release.

13. GENERAL SUMMARY AND CONCLUSIONS

Objective 1 – Produce seed from wild collected blacklip abalone at a range of sizes and ages throughout the year.

In addressing this objective we have:

- Shown that wild *H. rubra* in NSW are a poor and unreliable source of ripe ready to spawn broodstock.
- Shown that reliable year-round spawning and hatchery production can be achieved using captive broodstock conditioned in a recirculating seawater system at constant temperature conducive to gametogenesis.
- Achieved large improvements in the reliability and efficiency of large-scale production of seed *H. rubra* can be achieved by the combined use of multiple annual batches, high-density stocking and rearing of post-larvae on conventional diatom nursery plates, and precocious weaning onto manufactured diets.
- Demonstrated that there is considerable scope for further improving production of seed *H. rubra* through:
  - use of higher larval stocking rates in the range 4,000-10,000/nursery plate (1-2.5/cm^2).
  - use of optimum temperatures that shift markedly with successive life stages.

Objective 2 – Develop techniques to enable the successful deployment of seed to coastal reefs in NSW.

Objective 3 – Develop techniques to maximise the settlement, survival and growth of seed on Coastal reefs in NSW.

Objective 4 – Reseed reefs at Port Stephens, Sydney and at least one location further south, to be Determined in conjunction with ABMAC.

Objective 5 – Quantify the survival and growth of reseeded abalone for a minimum of 18 months.

In addressing the above objectives we have:

- Optimised handling, storage, transportation and deployment methods to improve post-release survival of both larvae and juveniles.
- Developed a range of methods of post-release sampling for releases of both larvae and juveniles tailored to varying types of juvenile habitat.
- Achieved post-release survival rates of hatchery reared larvae were extremely low and similar to their wild counterparts of which about one in $\geq$13,500 are estimated to survive to commercial size of 115 mm.
- Produced and experimentally seeded almost a million mainly button size (5-15 mm) juvenile abalone in clusters ranging from 700 to 2,500 in predator protective release devices to 40 sites within six locations along the NSW coast. However, mean survival rates 1-2 years after release of 3-9 month old juveniles ranging in mean shell length from 5-10 mm, were low, generally ranging from 0-3.8%. These compared poorly with rates of 12-40% expected of their wild counterparts based on published natural mortality data.
- Demonstrated that average rates of growth of released juvenile *H. rubra* did not differ greatly between the release sites between Port Stephens and Disaster Bay. Catch release data suggests that maturity (>90 mm) is likely to be attained after 2.5-3.5 years, and the minimum legal shell length of 115 mm after 4 years.

- Showed that *H. rubra* larvae can be seeded at very high densities onto small crustose coralline algae coated pebbles (vector rocks) and retained for up to eight days as post-larvae without significant restriction of growth or ability to rapidly disperse into juvenile habitats. An important implication of results, when considered in conjunction with published age and density dependent mortality data for wild *H. rubra,* are that post-larvae pre-settled onto vector rock should be broadcast seeded over large areas of juvenile habitat at low densities of 10-100/m^2 for best effect.
- Conducted a review of literature that revealed densities of wild juvenile *H. rubra* on productive reef typica1ly fall to levels of 1-2/m^2 within their first year, regardless of initial settlement densities up to several 1,000 post-larvae/m^2. From this it was deduced that if survival rates of 1 in 20-30 exhibited by wild juveniles between button size and the legal size of 115 mm are to be achieved with seeded juveniles, the latter will also need to be broadcast seeded at very low densities of 1-10/m^2 over extensive areas of juvenile habitat (as opposed to highly clustered seeding generally practised in this and earlier studies).
- Achieved a very encouraging result in the final reef seeding experiment conducted at Disaster Bay near the Victorian border. This experiment tested a newly developed hypothesis that poor post-release survival can be overcome by dispersed rather than clustered seeding of juveniles. Two release treatments were compared, namely; 10 clusters of 100 juveniles vs one cluster of 1,000 juveniles. Thirteen months later, mean survival across the 3 replicate sites seeded with 10 x 100 juveniles was within the above cited expected range for wild stock, namely; 12.5% (range 5-23%) while that the alternative treatment was again low at 3.8% (range 0- 9%).
- Developed methods of pre-seeding larvae onto small rocks at high density and showed that resultant post-larvae up to one week old could rapidly disperse into simulated habitats thereby constituting a better alternative to direct seeding of larvae.
- Developed a simple investment model that predicts that cost-effective seeding is most likely to be achieved using either button size (5-15 mm) 6 month old juveniles and perhaps week old post-larvae pre-seeded at high densities onto small vector rocks.

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15. APPENDICES

15.1. Intellectual Property

In order that industry gains maximum immediate benefit from this research, it is recommended that all outcomes and potential associated intellectual property become public domain. It is not anticipated that any patents will arise from this project.

15.2. Staff

Michael Heasman	Principal Investigator	BAgSc BSc PhD
Duncan Worthington	Co-Investigator	BSc (Hons) PhD
Nick Savva	Scientific Officer	BSc Assoc.Dip.Appl.Sci.(Aquacult.)
Rowan Chick	Fisheries Technician	BSc (Hons)
Craig Brand	Fisheries Technician	BAppSc.
Peter Gibson	Fisheries Technician	BSc (Hons)
John Diemar	Fisheries Technician	BEd. Assoc.Dip.Aquacult.
Scott Parkinson	Fisheries Technician	Assoc.Dip.Aquacult.
Ian Diemar	Fisheries Technician	
Judy Upston	Casual assistant	BSc
Miriam Vandenberg	Casual assistant	BSc
Nicole Hill	Casual assistant	

15.3. Abalone Broodstock Conditioning System: Specifications and Operating

15.3.1. Protocol

The hatchery at Port Stephens has two essentially identical reproductive conditioning units (referred to as Brood Units 1 & 2, BU1 & BU2) for temperature controlled reproductive conditioning of abalone, *Haliotis rubra*. Each unit is housed within an insulated shipping container. The units are effectively hybrid systems incorporating flow through and recirculation. Recirculation incorporates foam fractionation, physical and biological filtration.

Holding Tank
FRP constructed rectangular tank, 1500L nominal capacity.
Dimensions: 2700 x 1000 base x 500 mm tapered wall.
BU1: Open tank with hides of fibre cement sheets.
BU2: Buckets: Abalone held in 15-18 30L drums with 5-8 abalone per drum. (The drum system follows that used by Moss (pers. comm.) Mahunga Bay, New Zealand for *H. australis*.)

Header tank: 1000L nominal capacity.

Physical filter: Dacron (Aquahort).

Biofilter: Layer of dacron over 10L of coral debris over 100L of bioballs.

Pumps: Davey XF171 and XF221.

Foam Fractionator: (Appendix 14.4)
Diameter: Down tube 150 mm PVC class 18 pressure pipe.
Height: 1890 mm.
Foam Effluent: Transparent cone with 19 mm poly waste line.
Venturi: Mazzei injector, Model 484. (20 mm).
Flow through fractionator, 1 lps.

Temperature Control
Air conditioner, Email GM155H dual cycle, 1.5 kW.
Chillers. Carrier, 30ZQ024 on header tank. Ozsea, 2hp on recirculation.
Temperature maintained @ 15.0 or 16.0^{o}C +/- 1.0°C, monitored by temperature data loggers, Hastings Data Loggers.
The header tank also supplies temperature controlled water to the spawning/Larval rearing unit.

Aeration
Gentle aeration through 3mm CVT each bucket.

Flow Rates
Throughput, 1-2 L/min. (1-2 full wc/day).
Recirculation through chiller, foam fractionator and biofilter is 1 lires/sec. (using XF 171).
Recirculation through each holding bucket is 6-8 Lpm (using XF 171).

Stocking Rate
Abalone are held in drums @ 5-8 per drum. Each system can hold 18 drums.
30kg were stocked in BU1. 16 kg were stocked in BU2.
The actual capacity of system has not been determined.

Feed rates
0.75-1% of body weight per day, formulated feed. (Adam and Amos.)

Ammonia levels: Routinely less than 0.2 mg/L, total ammonia nitrogen (TAN), pH range 7.9-8.2.

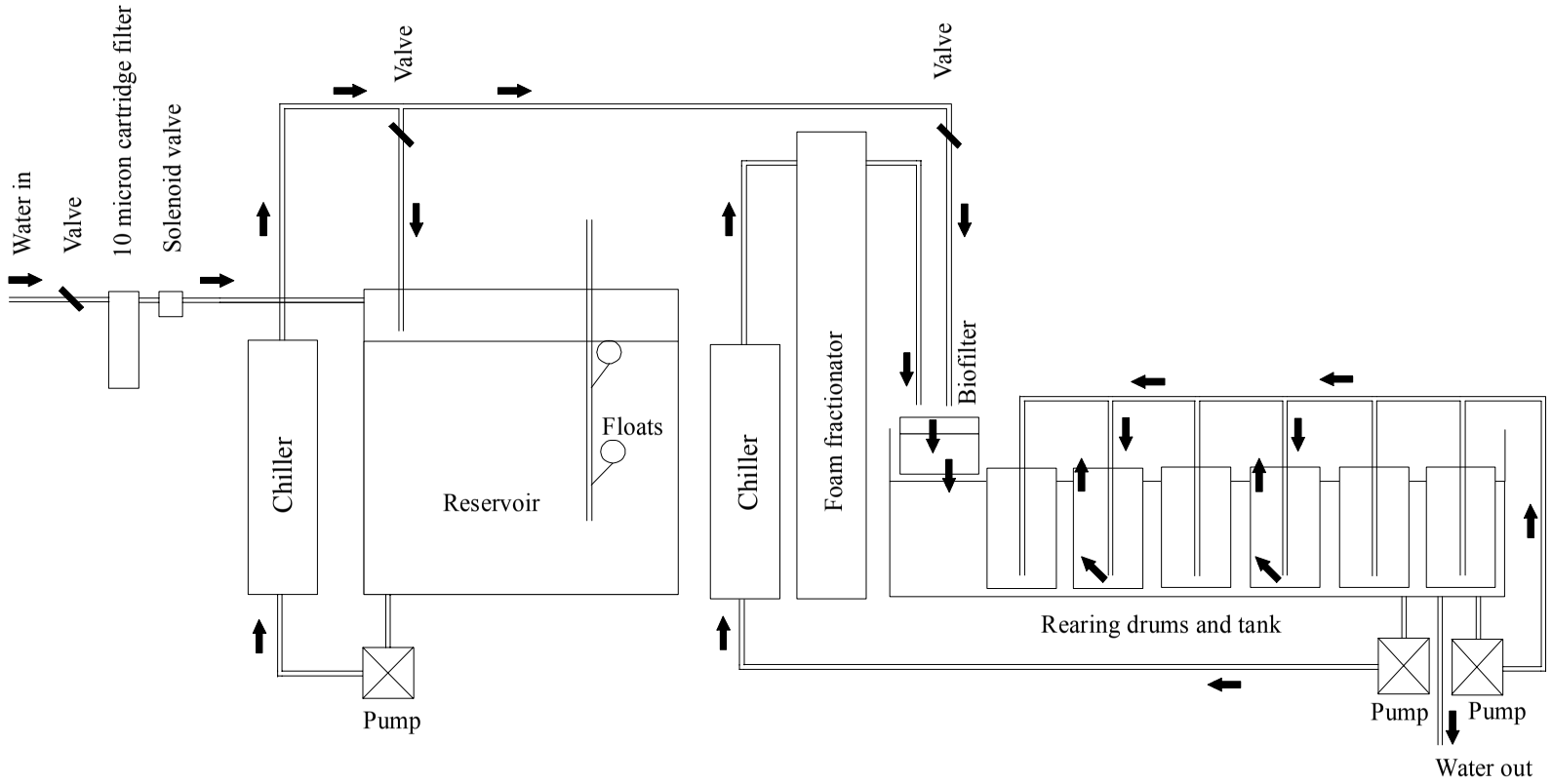
Light levels: Dim light. Constant photoperiod of 10 h light, 14 h dark.

Maintenance
Daily. Feeding, manual temperature check and visual check of system and abalone.
Each 2 days. Full water change and clean holding tank. Cartridge filter cleaned.
Each week. Holding drums cleaned. Fractionator and dacron filter cleaned.
Each month. Wash ½ of biofilter. Test for ammonium (Merck, aquamerck ammonium test).

Alarm system
Notification of system failures via phone link (Telstra SMS).
Temperature alarm, if outside +/-2.0^{o}C.
Header tank low water level alarm.

15.4. Broodstock conditioning unit – schematic design

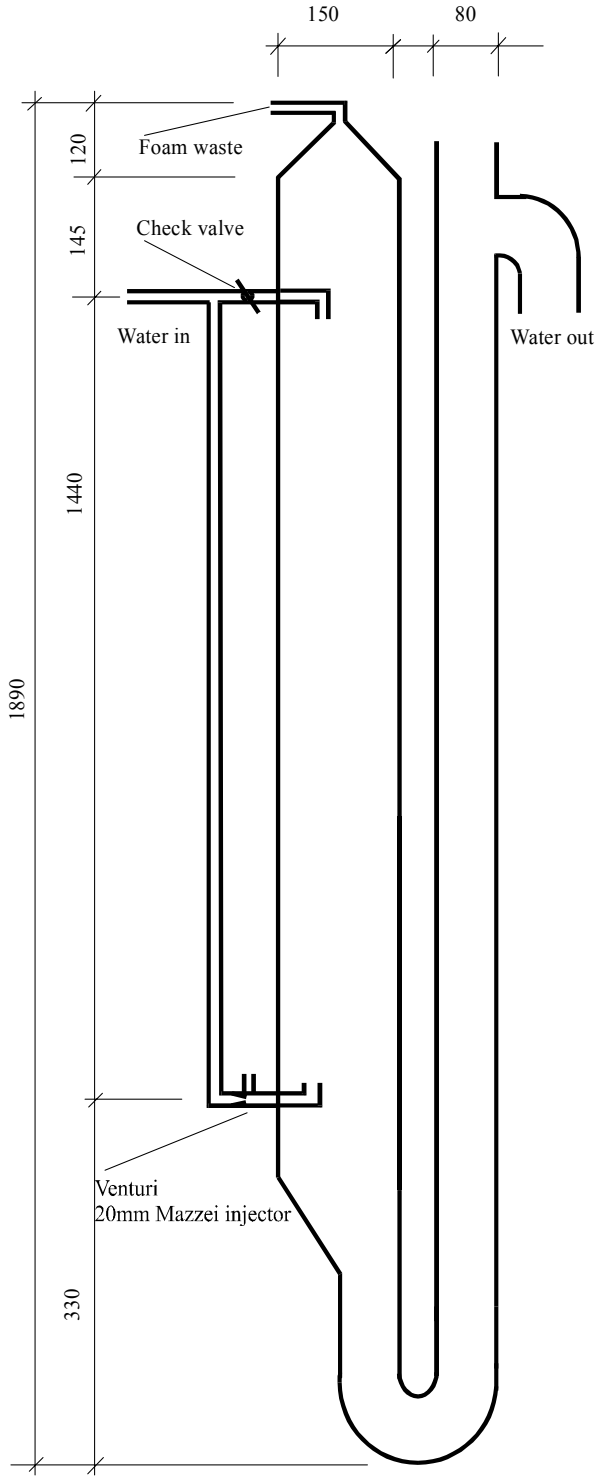
Abalone broodstock conditioning unit
NSW Fisheries / FRDC, Abalone enhancement project.



15.5. Foam Fractionator

Foam fractionator
NSW Fisheries / FRDC Abalone enhancement project.

Not to scale. All dimensions in mm. Motive flow, Davey XF 171. Flow rate, 1 l.p.s.



15.6. Technical Protocols (spawning induction, anaesthesia, calcein labelling, disinfection)

Spawning Induction using H_2O_2

Adapted from Tong *et al*. (1992), with further modifications from Morse *et al*. (1976). Spawning takes place in 14L tubs with from 1 to 6 abalone per tub.
Set up spawning tubs.
Place abalone in tubs the afternoon previous to the planned spawning.
The following morning raise the temperature 2^{o}C and leave for 2 h.
Turn off the flow to the tubs and reduce the volume of water in the tubs to 10L.
Add buffer, either TRIS or NaOH. pH should be raised to c.9.1 (Add 2M TRIS @ 66mL/10L tub.) (Add NaOH @ 10mL/tub.)
Wait 5-15 minutes before adding freshly prepared 6% H_2O_2. (Add 6% H_2O_2 @ 30mL/10L tub.)

Peroxide stock solution (H_2O_2) "Merck" or "APS" AR Grade 100 Volumes
To Break down 30% peroxide (100 Volumes) to 6%.
Use 1 part 30% H_2O_2 to 4 parts distilled water.
i.e. To make 1L mix: 200mL H_2O_2 with 800mL distilled water.
Add 6% peroxide solution at 3mL/L.

To Buffer with **Sodium Hydroxide** (NaOH mw = 40)
Make up a 1molar solution in fresh water. Use **1M. NaOH** at 1 mL/L. Wait 15 minutes before adding peroxide. When 1M sodium hydroxide is added to seawater a flocculent is formed which can harm the broodstock. Avoid this by pre-dissolving the sodium hydroxide in about 1L of seawater.
Wear eye protection when using concentrated sodium hydroxide.

To buffer with **TRIS.**
Tris-(hydroxy-methylamino) methane. (Tris-base, M.W. = 121.1.) Make up a 2 molar solution. Use **2M. TRIS** at 6.6mL/L. Wait 15 minutes before adding H_2O_2.

Anaesthesia with benzocaine

Stock Solution
100g Benzocaine dissolved in 1000 mL 95% ethyl alcohol.

Working solution
0.5 - 1.0 mL stock solution / 1.0L seawater.

Response time to benzocaine is size and temperature dependent. If a longer immersion time is expected use the minimum dose. 0.5mL/L is normally sufficient. Smaller abalone (1 to 5mm) require brushing or jetting along with benzocaine to dislodge. Avoid exposing for longer than 20 minutes, keep exposure time to a minimum and return to clean flowing seawater as soon as possible. Ensure that all anaesthetised abalone receive a direct flow. Always use the minimum dose at 20ºC and above. Wear rubber gloves when using benzocaine to avoid excessive skin exposure.

Calcein Labelling.

Buffer seawater with sodium bicarbonate (sodium hydrogen carbonate, $NaHCO_3$) @ 0.1g/L.
Wait 15 minutes before adding Calcein @ 0.05g/L. It is best to dissolve the calcein initially in a small volume of fresh water making a thin paste. Then dilute the paste and add to the treatment tank.
The treatment follows Day *et al*., (1995) adapted for adult *H. rubra.* Calcein, $C_{30}H_{26}N_2O_{13}$, FW 622.5, Sigma Chemicals.

Treat larvae or juveniles for 24-48 h.

Disinfection

Disinfection with liquid sodium hypochlorite: 10-12% free chlorine.

Nets/buckets/boots: 0.25mL/L
Transport containers: 0.9mL/L..
Rearing containers: 1.25mL/L.

15.7. Comparative bio-economic model for *H. rubra* fisheries enhancement using alternative age/size classes of seed

General assumptions and characteristics of model (also see Table 1)

- Profit = Revenue – Costs.
- Revenue = Beach price for abalone ($15ea) x additional sustainable catch generated by seeding.
- Targeted additional sustainable catch of 300 tonnes (1 million legal abalone worth ~$15 million).
- Projected costs presented in Table 1 include all elements of production, deployment but do not include those of continuous monitoring of the environmental impact of seeding (probably in the order of $200,000 pa).
- Post-release survival of seeded abalone (Table 2) matches that of equal size/age wild counterparts and that this supplementary recruitment does not significantly reduce yields of naturally recruited stock.
- Mean age and weight of abalone at harvest are 6 years and 333g respectively.
- The model is constrained by 2 conflicting elements, the smaller/younger the seed the cheaper they are to produce but on the other hand, the smaller/younger they are, the lower the survival and hence the more of them needed to do the job.
- Therefore to maximise the net returns, it is necessary to minimize the product of number of seed of particular size/age required to yield additional sustainable catch targeted, multiplied by the individual cost of that class of seed.

Table 1. Projected requirements and associated benefits and costs of producing and deploying of 5 age/size seed classes of blacklip abalone aimed at increasing the sustainable fisheries catch by 1million abalone per year.

Age & size of seed	Estimated number of seed of this age needed to yield 1 legal (115mm) abalone valued at $15M (From Table 1)	Estimated number of wild stock of this age needed to yield 1million legal abalone valued at ~$15 M	Estimated number of brood-stock spawnings needed to yield 1million legal abalone valued at ~$15M	Annual cost of production using conventional hatchery and nursery techniques	Annual cost of production of purpose built hatchery/nursery using latest production techniques developed in this study *NB Estimated capital cost of hatchery/nursery = $1-2million*
Competent larvae	20,000	20 Billion	33,000 Assumed fecundity of 1.5M eggs/spawner and 40% yield of competent larvae from eggs = 0.6M/spawner	Prohibitive	Prohibitive
1 wk old post-larvae][(0.4mm)	≤2000	≤2 Billion [a]	≤3300 Assumed 50% mean yield of PL's from larvae = 0.3M/spawner	Prohibitive	$0.5M
2 months ex plate (2mm/0.002g)	157	157 Million	3200 Assumed 20% yield to this age from 1 week post set = 0.06M/spawner	Prohibitive ($6.28M) based on 2 cents per mm = 4 cents per spat	$0.75- 1.0M
6 month old juveniles (8mm/0.1g)	26	26 Million	2600[e] Assumed 10% yield to this age from 1 week post set = 0.03M/spawner	Prohibitive ($4.16M) based on 2 cents per mm = 16 cents per spat	$0.5M
18 month old juveniles (40mm/10g)	18	18 Million	2900 Assumed 9% yield to this age from 1 week post set = 0.027M/spawner	Prohibitive facility would need to be at 2-3 times Australia's largest farm = 180 tonnes /year	Prohibitive

Table 2. Estimated survival data for blacklip abalone *H. rubra*.

Period/life stage, age (post se)t and size (mm)	Instantaneous natural mortality (M)	Estimated proportion surviving period	Cumulative proportion surviving from larvae	Number of larvae required to yield 1 abalone to this age	Proportion from this age living to legal size	Number of seed of this age needed to yield 1 legal size abalone	Number per million seed deployed at this age that will reach harvest size	Source
Spawning to competent larvae	**?**	**?**						
Competent larvae to 8 days post set (0.3 -0.4m)	0.44/day	0.03	0.0300	33	0.0000	**20704**	48	Preece *et al* 1997
8-19 days post set (0.4-0.6mm)	0.042day	0.63	0.0189	53	0.0016	**621**	1610	Preece *et al* 1997
0.5 -1.5 months (0.6-2.0 mm)	0.9/mth	0.4	0.00756	132	0.0026	**391**	2556	McShane 1991
1.5 -2.5 months (2mm - 4mm)	0.7/mth	0.5	0.00378	265	0.0064	**157**	6389	McShane 1991
2.5 -3.5 months (4mm - 6mm)	0.6/mth	0.55	0.00208	481	0.0128	**78**	12778	McShane 1991
3.5 -4.5 months (6mm - 8mm)	0.5/mth	0.61	0.00127	789	0.0232	**43**	23232	McShane 1991
5.5 -6.5 months (8mm - 10mm)	0.4/mth	0.67	0.000850	1177	0.0381	**26**	38086	McShane 1991
0.5 -1.5 years (10mm -35mm)	0.91/year	0.403	0.000342	2920	0.0568	**18**	56844	Day and Leorke (1986)
1.5 -2.5 years (35mm -60mm)	0.81/year	0.445	0.000152	6563	0.1411	**7.1**	141053	Day and Leorke (1986)
2.5 -3.5 years (60mm -85mm)	0.42/year	0.67	0.000102	9795	0.3170	**3.2**	316973	Shepherd 1992, based on Shepherd and Hearn (1983)
3.5 -4.5 years (85mm- 105mm)	0.3/year	0.74	0.0000755	13236	0.4731	**2.1**	473095	Derived from data in Figure 1
4.5 -5.5 years (105mm -115mm)	0.25/year	0.78	0.0000589	16970	0.6393	**1.6**	639317	Derived from data in Fig. 1
5.5 -6.5 years (115mm - 120mm)	0.20year	0.82	0.0000483	20695	0.8196	**1.2**	819638	Derived from data in Fig. 1

Conclusions

The model forecasts that cost effective abalone fisheries enhancement, even on such a large scale and utilizing the latest technological advances in hatchery and nursery production developed during the course of this project, will be limited to either post-larvae pre-settled onto small rocks or 5-7 month old button size (5 to 15 mm) juveniles. It should be cautioned however that encouraging results obtained in small laboratory experiments with post-larvae still need to be successfully scaled up and translated to the field.

Predictions in relation to button size juveniles are particularly encouraging in that potential margin of profit forecast is so handsome that break-even production would still be attainable even if survival of hatchery produced seed fall as low as 10% that of their wild counterparts. Alternatively, if average survival rates of say 25 to 50% those of equivalent wild stock are achievable with button size juveniles, it appears likely that cost effectiveness could be attained on much smaller scales of operation than applied to this model.

Equally clearly, the model predicts that seeding of larvae is likely to be spectacularly unprofitable and also casts doubt on the economic viability of large-scale seeding of natural reef using advanced (18 month old, 40 mm) juvenile *H rubra*.

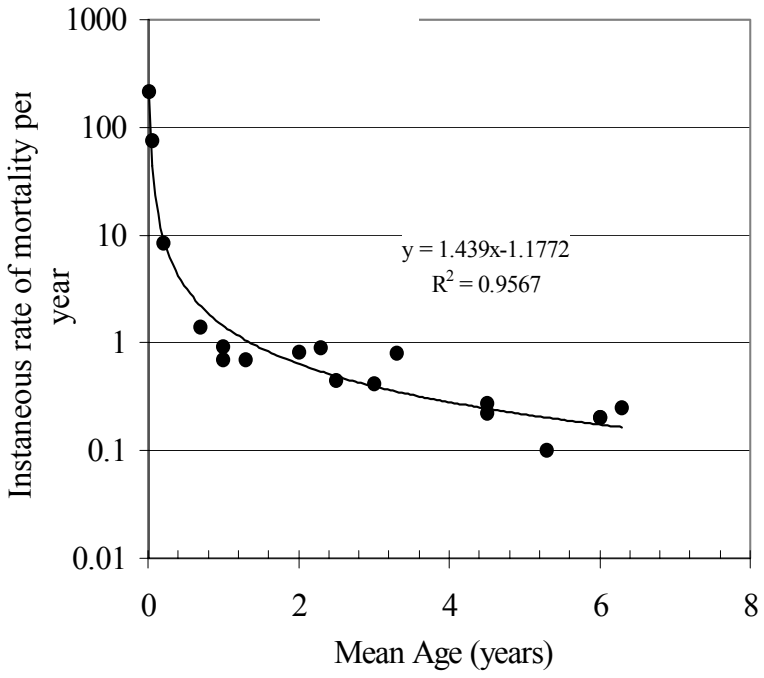


Figure 1. Published mortality data of wild blacklip abalone.

See Shepherd and Breen, (1992) for the mathematical relationship between instantaneous and proportional mortality.

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