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Early life history of abalone (*Haliotis rubra, H. laevigata*): settlement, survival and early growth

Final Report for FRDC Project 1998/306

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1998/306 Early life history of abalone (*Haliotis rubra*, *H. laevigata*): settlement, survival and early growth

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Objectives

1. To test performance of different settlement inducers at both the laboratory and commercial scale.
2. To improve the rate and consistency of abalone larval settlement.
3. To compare different microalgal diets for their nutritional value in terms of digestibility and growth.
4. To quantify the suitability of different microalgae for their ease of use, both for maintaining mass cultures and ability to recolonise settlement surfaces post grazing.
5. To increase growth and survival for the first two months of post-larval life at a commercial scale.

Non-technical summary

Outcome achieved

1.0 Settlement: Laboratory experiments

1.1 Algal species: Results of laboratory experiments demonstrated that the macroalga *Ulvella lens* is a suitable settlement inducer for larvae of blacklip and greenlip abalone (*Haliotis rubra* and *Haliotis laevigata* respectively). Settlement rates were improved from around 1-10% to 30-60%. Settlement was significantly lower on plates with biofilm dominated by diatom species.

1.2 Age and developmental stage of settlement substrate: Settlement of *H. rubra* was higher on older compared to younger thalli of *U. lens* and diatom films. Settlement on *U. lens* appears to be reduced when previously inoculated with a diatom species. However inoculation with diatoms has advantages for the subsequent growth of the post-larvae.

1.3 Pregrazed substrates: Settlement plates conditioned with algae (*U. lens* or diatoms) that were pre-grazed by juvenile abalone induced higher larval settlement for *H. rubra* than plates that were not grazed prior to larval settlement.

1.4 Abalone species differences: Greenlip (*H. laevigata*) larvae show less species-specific response. The difference between the algal species and developmental stages tested were not as pronounced than with blacklip (*H. rubra*) larvae.

1.5 Batch differences and use of antibiotics: Marked differences in overall settlement rates were found between larval batches. Antibiotic treatment enhanced the survival of *H. rubra* larvae in experimental containers but did not enhance the settlement rate nor did it change settlement preferences. The results indicate that unfit larvae may survive if treated with antibiotics but they do not settle successfully.

2.0 Growth and survival: Laboratory experiments

2.1 Diatoms and *U. lens*: Growth-rates reached 30-40 μm day^{-1} when feeding on the cultured diatom *Navicula* sp. during the first two months of rearing. The green alga *U. lens* is not sufficient for the initial growth of the abalone *H. rubra*. When single species diets were compared to a combination of the macroalga *U. lens* and the diatom *Navicula* sp., growth-rates reached 42 μm day^{-1} on the combined diet but only 35 μm day^{-1} on single species diets. Survival up to 2 months post settlement was highest on the combination (70%) and lowest on the diatom only.

2.2 Feeding capacity:

2.2.1 Post-larvae 0-4 weeks: Post-larvae rely on yolk reserves during the first 2 weeks of development but can ingest small diatoms cells (<17 μm in valve length). Most of the nutrition must be derived from the biofilm components such as extracellular material produced by the diatoms and associated bacteria, because diatom cells were ingested but not digested and cells remained alive in faeces samples.

2.2.2 Juveniles 1-3 month: During the first months of post-larval life *H. rubra* grows best with the highest survival when feeding on diatom species like *Navicula* sp., which is well attached to the settlement substrate. *U. lens* is suitable as a food source once juveniles reach 3 mm in shell length (ca. 2 month post-settlement) when growth rates become comparable to those juveniles feeding on diatoms.

3.0 Algal culture and biochemical composition of algae

3.1 Suitable diatom species for commercial culture on vertical plates: Only *Navicula* sp. developed on vertical settlement plates in a satisfactory time frame. Other species either did not attach well or grew very slowly.

3.2 Recommended commercial protocol for algal culture: Methods were developed for large-scale culture and succession of algal species. Plates were initially colonized with *U. lens* for settlement and after settlement inoculated with the cultured diatom *Navicula* sp. for post-larval growth. Plates with recently settled larvae were inoculated without any harm to the animals.

3.3 Nitrogen level for algal culture: 10-fold increase in nitrate used in growth media resulted in higher total amino acid levels in the diatom feed species and an increase

in growth-rates of *H. rubra* juveniles. We suggest, in order to achieve uniformity in nutritional quality of feed species in abalone nurseries, that the nitrogen level should be monitored and supplied at an optimal rate between 2 and 12 mg NO_3-N L^{-1}.

3.4 Nutritional quality of *Navicula* sp. and *U. lens* *Navicula* sp. is rich in 20:5n-3 whereas the major fatty acids in *U. lens* include 18:2n-6 and 18:3n-3. *U. lens* shows a high protein content of 31-38% which is comparable to microalgae like *Navicula* sp. but not to other macroalgae.

4.0 Settlement: commercial scale

Settlement rates in large-scale experiments in the nursery were amongst the highest recorded in commercial nurseries suggesting that the use of the macroalga *U. lens* for conditioning the settlement plates was efficient and reliable.

4.1 Larval release density: Larval release density was not detected as a significant effect on settlement rates of *H. rubra*.

4.2 *U. lens* age, cover and developmental stage: The settlement of *H. rubra* and *H. laevigata* larvae was higher on older *U. lens* plates with less percentage cover indicating that the developmental stage of *U. lens* and associated biofilm species and not the cover per se are important. Larvae of two abalone species (*H. rubra, H. laevigata*) can distinguish between different developmental stages of the alga.

5.0 Growth and survival: commercial scale

5.1 *U. lens* and *Navicula* sp.: Growth rates of *H. rubra* on a combination of *U. lens* and *Navicula* sp. were improved at commercial scale to 35 μm day^{-1} during the first month, 50 μm day^{-1} during the second month and 100 μm day^{-1} during the third month of rearing.

5.2 Animal density and light: Post-larval density and light (which affects the food density) have very remarkable effects on early growth (first month of post-larval development) of *H. rubra*. Interestingly, this difference in early growth remained and was amplified until the end of the experiment, suggesting that early growth is important in determining later performances. The survival was strongly density-dependent about 2 months after settlement when the food started to become limiting. Growth of juveniles increased by 3 fold in a few days when the density was reduced to less than 50 juveniles per plate ca. 2 months after settlement.

5.3 Formulated feed: The formulated feed used might not be well matched for the nutritional requirements of small *H. laevigata* juveniles. Feed might be suitable for juveniles larger than 4 mm in shell length.

5.4 Feeding capacity:

5.4.1 Post-larvae 0-4 weeks: During early development post-larvae rely on lipid reserves of the larval yolk for their nutrition. The yolk reserves are exhausted approximately 2 weeks after settlement when growth rates decreased presumably because the post-larvae started feeding inefficiently on the diatoms.

5.4.2 Post-larvae 1-3 months old: When animals reached ca. 2 mm in shell length the growth-rates improved substantially indicating that juveniles can fully access the diatom feed provided. This is also the time when the first respiratory pore is formed. Juveniles started to remove parts of *U. lens* about 2 months after settlement, when they reached 3 mm in shell length, which was evident by a sharp decline in percentage cover of *U. lens* and growth-rates increased substantially (*H. rubra, H. laevigata*).

6.0 Recommendations to industry

6.1 Settlement protocol: We recommend that plates colonised with young germlings of *U. lens,* grown for a period of 2 weeks, so that algae cover ca. 30-40% of the plate, should be used for the settlement induction of abalone larvae (*H. rubra, H. laevigata)*.

6.2 Recommended feeds for juveniles

6.2.1 Juveniles 1-3 months: Plates inoculated with the cultured diatom *Navicula* sp. will provide suitable food and rapid growth of young post-larvae. This species produces extracellular mucus and is therefore suitable for early post-larval growth (first 4 weeks post-settlement). This species will sustain good growth until juveniles reach about 3 mm in shell length (*H. rubra, H. laevigata*).

6.2.2 Juveniles 3 months + : We suggest that plates covered with the green alga *U. lens* should be used as a food source for juvenile abalone larger than 3 mm in shell length. New plates covered by the algae should be introduced every 3-4 weeks and the stocking density should be reduced to 50 juveniles per plate (*H. rubra, H. laevigata*). That way high growth rates of 80-100 µm day^{-1} can be maintained on this feed until juveniles reach 8-10 mm in shell length (*H. laevigata*).

KEYWORDS: Abalone, aquaculture, nursery production, algal culture, settlement, growth, *Haliotis rubra, Haliotis laevigata*.

List of publications

Daume, S., Krsinich, A., Farrell, S. and Gervis, M. 2000. Settlement, early growth and survival of *Haliotis rubra* in response to different algal species. Journal of Applied Phycology 12: 479-488.

Daume, S., Long, B. M. and Crouch, P. 2003. Changes in amino acid content of an algal feed species (*Navicula* sp.) and their effect on growth and survival of juvenile abalone (*Haliotis rubra*). Journal of Applied Phycology 15: 201-207.

Daume, S., Huchette, S., Ryan,S. and Day, R. W. In print. Nursery culture of *Haliotis rubra*: The effect of cultured algae and larval density on settlement and juvenile production. Aquaculture.

Daume, S. and Ryan, S. submitted. Nursery culture of the abalone *Haliotis laevigata*: Larval settlement and juvenile production using cultured algae or formulated feed. Journal of Shellfish Research.

A Masters thesis was produced during this project. A summary paper is attached in Appendix II. Krsinich, Anton, 2000. Effects of seeding with the macroalga (*Ulvella lens*) and inoculation with a benthic diatom (*Navicula* sp.) on the settlement, growth and recruitment of abalone (*Haliotis rubra*) under commercial conditions. Master's dissertation (Applied Science in Aquaculture), University of Tasmania, Australia. A copy of the thesis can be downloaded from the FRDC Abalone Aquaculture Subprogram's website www.frdc.com.au/research/programs/aas.

A CD was developed to demonstrate the algal culture methods for commercial abalone nurseries. Copies of the CD: Abalone Nursery Manual: Algal culture methods for abalone nurseries, can be purchased through the FRDC Abalone Aquaculture Subprogram's website at www.frdc.com.au/research/programs/aas.

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The Editor (Dr Sabine Daume) acknowledges the contributions made by the individual chapter authors.

1.0 General Introduction

1.1 Background

Australian abalone fisheries produce a high value, export-orientated products that provided 60% of the world supply in 1994 (Fleming, 2000). With wild abalone fisheries declining in countries worldwide, the interest in aquaculture products has increased substantially. The world production of abalone from aquaculture in 1999 was approximately 7,775 tonnes with only 89 tonnes (ca. 1%) being produced in Australia (Fleming, 2000). Abalone farming is expected to develop into a major industry and is a significant investment opportunity (McNamara, Victorian Aquaculture Strategy, May 1997). Future production from the numerous farms and sites either established, under construction or approved in Australia, could be substantial.

In Australia abalone larvae are produced by spawning recently collected wild broodstock, or wild or farmed abalone broodstock that has been held in conditioning systems for extended periods. The non-feeding larvae have a short larval phase e.g. 7 days at 17° C for *Haliotis rubra*. When larvae are ready for settlement they actively seek a suitable surface. In the natural environment abalone larvae settle on coralline red algae (Shepherd & Daume, 1996); however on farms the surface is typically vertical, spaced plastic plates that have been colonised by a variety of different microalgal species. When algal supplies decline, the juveniles may be weaned onto formulated feeds. They can be transferred to a variety of land- based tanks or sea- based systems (Freeman, 2001).

Abalone are slow growing, therefore any increased growth-rates resulting in a reduction in production time, have large cost benefits to the farmer. While some information is known about the early life history of abalone, very little of this information can be applied directly in commercial nurseries. The main areas of research to increase growth rates for juvenile abalone are in the following:

1. settlement systems design
2. dietary requirements for settled abalone
3. pre-settlement larvae quality
4. optimum water quality and other environment conditions for growth promotion
5. genetic improvement of abalone

1. Settlement

Benthic biofilm consisting of bacteria and mixed species of diatoms growing on PVC settlement plates have traditionally been used as a settlement substrate in abalone nurseries worldwide. This process is unpredictable and larval settlement rates can be very low (1-10% of larvae). Enhanced settlement up to 80% has been obtained in small scale experiments through the use of the non-geniculate coralline red alga, *Sporolithon durum* (Daume et al., 1999b). In a previous study *Haliotis rubra* did not respond to films of any diatom species tested, but settled on the non-geniculate coralline red alga *Phymatolithon repandum* (Daume et al., 1999a). In contrast *Haliotis laevigata* settled particularly well on the diatom *Navicula ramosissima* (Daume et al., 1999b). Other diatom species, however, sustained poor settlement. Despite this result naturally developing diatom films are readily used as a settlement substrate for *H. rubra* and *H. laevigata* larvae in southern Australia. Without much control over composition and density of the biofilm species settlement rates are very inconsistent and often very poor (1-10%). Greater control is achieved by isolating particular diatom species and growing them in monoculture

before inoculating settlement tanks in the nursery. This however has not been embraced by the industry because they feel that the gain does not justify the extra costs involved for the scale-up diatom culture on site. Further investigations are needed to assess the effectiveness in larger scale culture systems for diatoms in the nursery environment.

Hatcheries in Japan culture the macroalga *Ulvella lens* to improve settlement of the Japanese abalone *Haliotis discus hannai* (Takahashi & Koganezawa, 1988). Settlement rates of 67% where reported on *U. lens* which was not previously grazed by juvenile abalone by Takahashi and Koganezawa (1988). Pregrazed *U. lens* yield a settlement rate of 93-100% (Takahashi & Koganezawa, 1988; Seki, 1997). The settlement on *U. lens* had never been investigated with Australian species of abalone.

2. Dietary requirements

Post-larval abalone feed on benthic diatoms (Kawamura et al., 1995), so the diatom film on which animals settle also provides the feed for the growing post-larvae. The film is maintained through passive seeding (new cells are brought in with the incoming seawater), adding nutrients and manipulating the light intensity through shading. However, a significant bottleneck experienced by industry is the inability to maintain adequate feed (both quantity and quality) on the plates particularly at later stages of the nursery phase. Commercial operations have traditionally relied on mixed species of diatoms as an initial food source throughout the nursery period (settled larvae to 8-10 mm). Worldwide, only a few studies have examined post-larval feeding and growth on different algal species and little is known about initial growth-rates of recently settled post-larvae (Ohgai et al., 1991; Ishida et al., 1995; Kawamura et al., 1998a; Roberts et al., 1999). Feeding experiments of post-larval abalone on species of benthic diatoms (Kawamura & Kikuchi, 1992; Kawamura & Takami, 1995; Kawamura et al., 1995; Matthews & Cook, 1995; Kawamura, 1996) have shown that feed requirements may change as abalone grow. Two to three weeks after settlement post-larvae become responsive to the "digestibility" of the diatom strains and grow more rapidly on effectively digested strains (Kawamura et al., 1998a). Post-larvae 0.8-2 mm in shell length grow ca 40-60 μm day^{-1} on "digestible" diatoms and only ca 15-30 μm day^{-1} on "indigestible" diatoms (Kawamura et al., 1998b). In addition, the diatom cell size, attachment strength, frustule's strength and post-larval size can influence digestion. In a feeding trial covering the whole post-larval period, Roberts et al. (1999) showed that different diatom feed species affected both survival and growth. After day 17, post-larvae grew faster on *Cocconeis scutellum* and *Cylindrotheca closterium*. Both species were most efficiently digested. Seki (1997) reported that growth rates of post-larvae on *U. lens* were improved by the inoculation of cultured diatoms. However, research on post-larval feeding preferences has not been undertaken on Australian species of abalone. It is well known that adults of Australasian species of abalone vary in feeding preference and favour red macroalgae (Rhodophyta) compared to species from America, South Africa and Japan, which prefer brown algae (Phaeophyta) (McShane et al., 1994, Fleming, 1995). The food requirements of post-larvae and juveniles may also differ. In addition, feeding experiments were mainly done on monocultures of diatoms rather than on mixed cultures developing naturally on settlement plates in a hatchery situation. Post-larvae have not been offered a choice between different diatom strains and the change in feed requirements during the first weeks of post-larval life has not been investigated.

The aims of this project are to:

- determine and quantify the performance of different settlement inducers, both separately and in combination within the laboratory and at a commercial scale, in order to develop a practical settlement protocol for abalone farms.
- improve the settlement rate and consistency of abalone larval settlement.
- compare different microalgal diets for their nutritional value in terms of digestibility and growth.
- determine the suitability of different microalgae for their ease of use, both for maintaining mass cultures and ability to recolonise settlement surfaces.
- develop a succession of algal food species for post-larvae that will sustain maximum growth and survival for the first two months of post-larval life at a commercial scale.

1.2 Need

Abalone farming can be improved in Australia. The profit will be increased and the cost and risk of production reduced. Key strategies in this project have been:

- increased knowledge of the settlement process and development of a better settlement system resulting in more reliable production and lower cost juvenile abalone which is essential for both integrated (hatchery and growout) abalone farms and for the supply of seed to growout farms;
- the development of a practical settlement protocol for abalone requires a greater understanding of microalgae requirements and their culture and should reduce the cost for juvenile abalone as well as reducing the risk of not meeting annual seed production for the start of the growout stage;
- the project will be integrated into the Abalone Aquaculture Subprogram and has extensive industry involvement in developing the practical applications;
- the success of the Australian abalone aquaculture industry is dependent on developing methods that reduce costs in labour and growout time;
- the project will deliver information that can result in technically advanced culture methods that will maintain a competitive edge over countries with low labour costs.

1.3 Objectives

1. To test performance of different settlement inducers at both the laboratory and commercial scale.
2. To improve the rate and consistency of abalone larval settlement.
3. To compare different microalgal diets for their nutritional value in terms of digestibility and growth.
4. To quantify the suitability of different microalgae for their ease of use, both for maintaining mass cultures and their ability to recolonise settlement surfaces post grazing.
5. To increase growth and survival for the first two months of post-larval life on a commercial scale.

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2.0 Laboratory scale settlement experiments

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2.1 Aims

Experiments were designed to investigate if settlement is species specific for a variety of algal species tested and if differences in percentage cover, age and/or developmental stage of the algae influence the settlement rate (Table 1). To achieve similar percentage cover of different developmental stages, reducing or adding more spores to the culture manipulated the number of spores per area. In addition, the antibiotics Ampicilin and Kanamycin (50 µg mL^{-1}) were used in one experiment with *H. rubra* larvae to investigate the influence of the antibiotics on the settlement substrata (algae), larvae and overall settlement rates (Table 1).

2.2 Methods

2.2.1 Algal cultures

Five diatom species, *Navicula* sp., *Navicula jeffreyi* Hallegraeff et Burford, *Cylindrotheca closterium* (Ehrenberg) Reimann et Lewin, *Amphora* sp. and *Cocconeis* sp., were isolated from settlement plates at Southern Ocean Mariculture in Victoria, Australia and maintained in culture (Plate 1). Diatom species were cultured in f/2 medium (Guillard & Ryther, 1962) and maintained at 17 ± 2° C, with a 12 : 12 hours L : D photo cycle. The cultures were not axenic. For the settlement experiments, all diatom species were cultured for 1-2 weeks and allowed to attach to small plastic squares (2 cm^2) until they reached a density of approximately 10^5 cells cm^{-2}.

Thalli of *Sporolithon durum* (Foslie) Townsend et Woelkerling with patches of Sori (reproductive structures) were selected and placed in small aquaria with a 12 : 12 hours L:D photo cycle. The water temperature was raised to 2-4° C above ambient temperature to induce the release of spores. Spores are negatively buoyant, hence small plastic squares (2 cm^2) were placed onto the bottom of the tank to collect them.

Spores attached to the squares and germinated during the next 24 hours. Plastic squares with more than 50 germlings were selected and used in settlement experiments. The germlings were grown for 5 and 10 days and reached a diameter of 10-100 and 100-150 µm respectively.

Settlement plates with large patches of *Ulvella lens* Crouan were collected from the commercial nursery. Some of the plates had been previously grazed by juvenile abalone (2-3 mm shell length). Plates of both treatments (with or without pregrazing) were cut into 2 cm^2 squares.

In other settlement experiments germlings and mature (spore producing) *U. lens* were compared. Methods for spore collection were adapted from Takahshi & Koganezawa (1988) (see below for large scale culture methods). After 1-2 weeks germlings reached a diameter of 100-300 µm. Germlings were grown for an additional 2 weeks to develop sporangia (mature plant). Six small squares (2 cm^2) were cut from a randomly selected position of six randomly taken settlement plates.

Some squares with germlings of *S. durum* and *U. lens* were inoculated with the diatom *Navicula* sp. for the fourth and sixth experiment and cultured in f/2 medium until densities of 10^4 cells cm^{-2} were reached.

2.2.2 Experimental protocol

All batches of *Haliotis rubra* Leach larvae (1-13), batch 1 and 3 of *Haliotis laevigata* Donovan larvae and one batch of hybrids (*Haliotis rubra* ♀ x *H. laevigata* ♂) were reared at Southern Ocean Mariculture (SOM) in Victoria, Australia (Table 1).

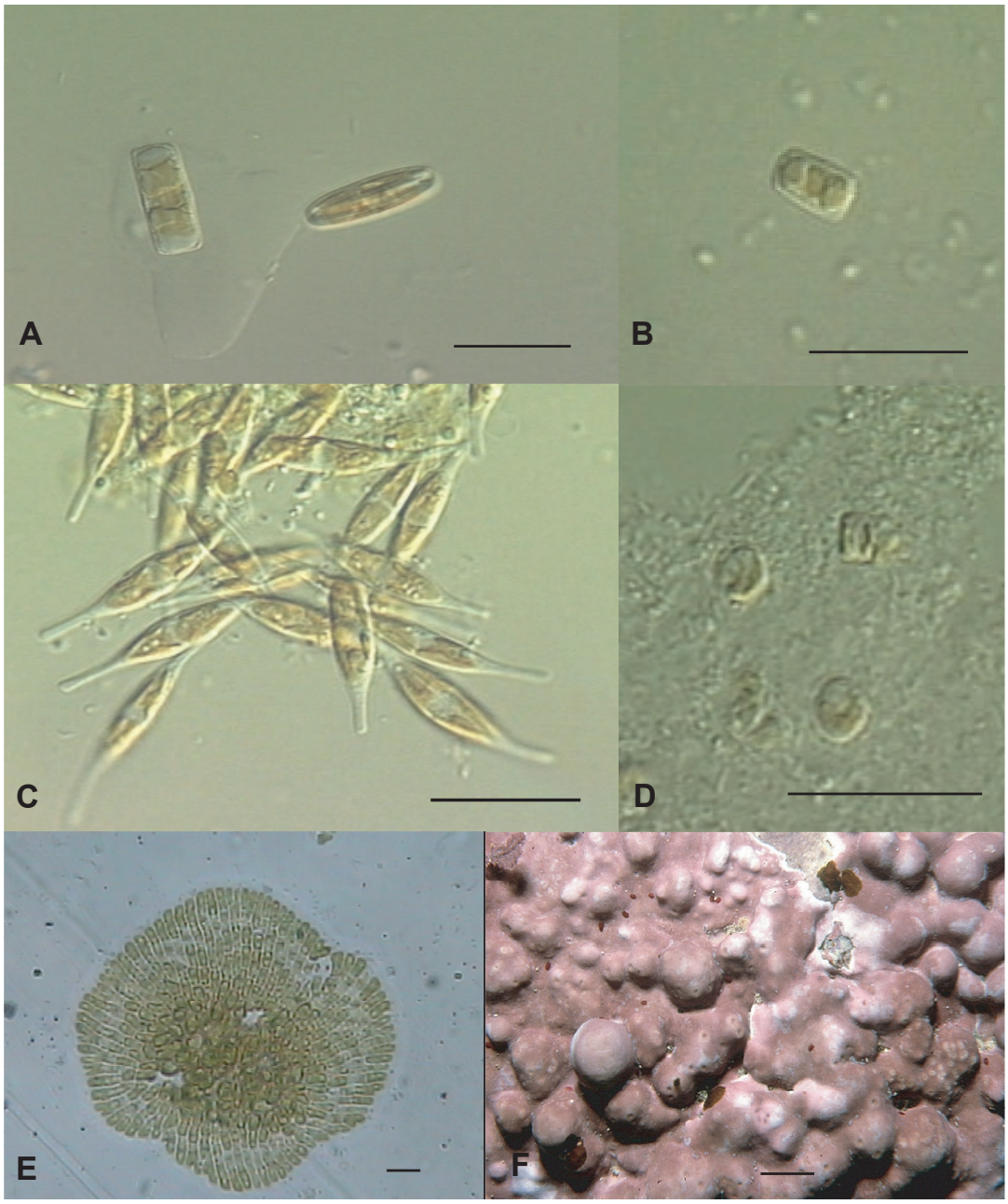

Plate 1. Four diatom species (A: *Navicula* sp., B: *Navicula jeffryi*, C: *Cylindrotheca closterium*, D: *Cocconeis* sp.) and two macroalgae (E: *Ulvella lens*, F: *Sporolithon durum*). A-E: scalebar indicates 10 µm; F: scalebar indicates 1 cm.

Table 1. Results summary of laboratory settlement experiments.

SETTLEMENT EXPERIMENTS (*Haliotis rubra*)			
Batch	**Experiment**	**Settlement Date**	**% Settlement**
1	1: No-choice, 4 diatom species (*Navicula* sp., *Cylindrotheca closterium, Amphora* sp., *Cocconeis* sp.)	06.11.98	1%/ -6% Negative control: 0.5 % Positive control: 30%
1	2: No-choice, plates unused, 1 year old, 2 year old with film of *Navicula* sp., *Cylindrotheca closterium* (unused, 1 year, 2 year with *Navicula* sp.), (unused, 1 year, 2 year with *Cylindrotheca closterium*)	06.11.98	(6%/ 6%/ 2%), (1%/1%/2%) Negative control: 0.5 % Positive control: 30%
1	3: Choice between single species diatom and *Sporolithon durum*	06.11.98	(5%+15%) Negative control: 0 %
1	4: Choice between single diatom species (*Navicula* sp. + slurry mix), (*C. closterium* + slurry mix)	06.11.98	(5% + 3%) (1% + 2%) Negative control: 0 %
2	1: Choice between 4d, 6d *Amphora* sp., *Navicula* sp., *C. closterium* (10^3-10^4 cells cm^{-2}) (4d +6d Am.), (4d +6d Nav.), (4d +6d C.clost.).	18.11.98	(3+5%) (3+4%) (1+1%) Negative control: 0 %
2	2: Choice between single species (*Amphora*, *Navicula*) and natural mix (Am + natural mix), (Nav + natural mix)	18.11.98	(6%+6%) (2%+8%) Negative control: 1 %
2	3: Choice between grazed and ungrazed substrate	18.11.98	(10% + 5%) Negative control: 0 %
2	4: Choice between *S. durum* treated with and without CO_2	18.11.98	(7% + 4%) Negative control: 0 %
3	1: No-choice, *S. durum* with and without diatom *Amphora* sp.	01.12.98	10%/ 36% Negative control: 1 %
3	2: Choice between single species (*Amphora* sp.) and 2 species mix (*Navicula* sp./ *Amphora* sp., *Cocconeis* sp./ *Amphora* sp.)	01.12.98	(7%+12%), (13%+8%) Negative control: 1 % Positive control: 36%
3	3: Settlement on mixed diatom film when larvae were introduced at different developmental stage (development of their 3rd vs 4th tubule on their cephalic tentacle)	01.12.98	19%/ 15% Negative control: 1 % Positive control: 36%
4	1: No-choice, vertical vs horizontal diatom plates	17.12.98	1%/ 12% Negative control: 1.5 % Positive control: 36%
4	2: Choice between 1-week-old and 2-week-old spores of *Sporolithon durum*	17.12.98	(22%+13%) *) Negative control: 1.5 % Positive control: 49%

Negative control: 1 cm^2 piece of settlement plate without diatoms or other algae.
Positive control: ca. 1 cm^2 piece of the non-geneculate corralline red alga *Sporolithon durum.*

4	3: Choice between 1-week-old and 2-week-old films of single and mix species (10^3-10^4 cells cm^{-2}) (1 week + 2 week of single species), (1 week + 2 week of mixed species).	17.12.98	(5%+8%) (8%+13%) *) Negative control: 1.5 % Positive control: 36%
4	4: Settlement on mixed diatom film and *S. durum* when larvae were introduced at different developmental stage (development of their 3rd vs 4th tubule on their cephalic tentacle)	17.12.98	3%, 77% / 5%, 36% Negative control: 1.5 %
5	1: No-choice, single diatom species (*Navicula* sp., *C. closterium, Cocconeis* sp.) compared to mixed species (*Navicula* sp., *C. closterium*)	24.01.99	1%/ 6% Negative control: 0.3 %
6	1: No-choice, single diatom species (*Navicula* sp., *C. closterium, Amphora* sp., *Cocconeis* sp.)	17.03.99	1-6% Negative: 0.3 % Positive control: 14%
6	2: No-choice, mix of *U. lens* + *S. durum*, grazed and ungrazed *U. lens* (mix, grazed U.l., ungrazed U.l.)	17.03.99	10%/ 23%/ 12% Negative control: 0.3 % Positive control: 14%
6	3: No-choice, *S. durum* germlings with *Navicula* sp. Film and *Navicula* sp. film only	17.03.99	13%/ 5% Negative control: 0.3 % Positive control: 14%
7	1: No-choice, diatom mix, *U. lens* grazed/ ungrazed germlings of *U. lens* (diatom mix, grazed U.l., ungrazed U.l.)	21.04.99	1%/16%/12% Negative control: 0.3 % Positive control: 15%
8	1: No-choice, three new diatom isolates compared to old *Navicula* sp. isolate	30.10.99	1-7%/ 2% Negative control: 0.3 %
8	2: No-choice, *U. lens* compared to *Navicula* sp. both from inoculated nursery tanks	30.10.99	16%/ 2% Negative control: 0.3 %
9	1: No-choice, single diatom species compared to mixed species (*Navicula* sp. - *Amphora* sp.)/ (*Navicula* sp.+ *Amphora* sp.- *Cocconeis* sp. + *Amphora* sp.)	12.11.99	1-3%/ 2-17% Negative control: 0% Positive control: 21%
9	2: No-choice, single species compared to mixed species (*Navicula* sp./ *U. lens*/ *U. lens* + *Navicula* sp.	12.11.99	3%/ 52%/ 9% Negative control: 0% Positive control: 21%
9	3: No-choice, clippings from nursery tanks, tanks were inoculated with *Navicula* sp. 1 and 2 weeks before settlement	12.11.99	6%/ 1% Negative control: 0% Positive control: 21%
10	1: No-choice single diatom species compared to mixed species (*Navicula* sp. - *C. closterium, Cocconeis* sp.)/ *U. lens* /(*U. lens* + *Navicula* sp.)	19.01.00	3-14%/ 21%/ 19% Negative control: 1% Positive control: 41%
11	1: No-choice 2 and 4 weeks old *U. lens* with high % cover (85-90%) and *U. lens* spores (4-5%)	30.03.00	32%/ 27%/ 12% Negative control: 2% Positive control: 49%

Negative control: 1 cm^2 piece of settlement plate without diatoms or other algae.
Positive control: ca. 1 cm^2 piece of the non-geneculate corralline red alga *Sporolithon durum.*

12	1: No-choice 3, 17, 37 days old *U. lens* with ca. 55%, 36% and 32% cover respectively	17.11.00	13%/ 36%/ 49% Negative control: 0% Positive control: 35%
13	1: No-choice mature *U. lens* and *Navicula* sp. both with and without antibiotics (Ampicilin, Kanamycin both at 50 µg/ mL) (*U. lens* + AB/*U. lens* / Nav + AB / Nav)	09.12.00	22%/ 30%/ 0.3%/ 5% Negative control: 0% (+, - antibiotics) Positive control: 39%
SETTLEMENT EXPERIMENTS (*Haliotis laevigata*)			
Batch	**Experiment**	**Settlement Date**	**% Settlement**
1	1: No-choice, mixed species (*Navicula* sp./*C. closterium*) compared to *S. durum* spores 2: No-choice, *Cylindrotheca closterium*, *Navicula* sp. compared to mixed species (*Navicula* sp./*C. closterium*)	22.01.99 22.01.99	6%/ 5% Negative control: 0% Positive control: 14% 3%/ 6%/ 11% Negative control: 0% Positive control: 14%
2	1: No-choice, single diatom species (*Navicula* sp. - *C. closterium* - *Amphora* sp. - *Cocconeis* sp.) compared mixed species (*Navicula* sp.+ *C. closterium*)	12.12.99	6-20%/ 11% Negative control: 2.5%
2	2: No-choice, *U. lens* compared to *U. lens* + *Navicula* sp.	12.12.99	20%/ 10% Negative control: 2.5%
2	3: No-choice, 3 days- compared to 10-days-old films of *Navicula* sp. and *Cylindrotheca closterium* (10^3-10^4 cells cm^{-2}) (3d Nav/10d Nav, 3d Cy/10d Cy)	12.12.99	14%/ 6%, 20%/ 2% Negative control: 2.5%
2	4: No-choice, clippings taken from nursery tanks inoculated with *Navicula* sp. compared to natural developed films	12.12.99	13-15%/ 11% Negative control: 2.5%
3	1: No-choice, *U. lens* of different maturity and % cover (69%, 25%, 10%, 8%)	12.04.00	17%/ 16%/ 9%/ 7% Negative control: 0.5% Positive control: 34%

Negative control: 1 cm^2 piece of settlement plate without diatoms or other algae.
Positive control: ca. 1 cm^2 piece of the non-geneculate corralline red alga *Sporolithon durum.*

All experiments conducted with these larvae were run in the laboratory at SOM. Batch 2 of *Haliotis laevigata* larvae were obtained from South Australian Abalone Development in South Australia. Settlement experiments with this batch were conducted at the Lincoln Marine Science Centre in South Australia.

Only larvae from the same batch were used in each experiment. Larvae were judged to be competent for settlement when the third tubule of the cephalic tentacle was well developed and larvae began exploring the surface (Hahn, 1989). The density of abalone larvae was estimated by counting the larvae in ten 1 mL sub samples drawn from the whole sample of the batch.

Settlement substrata (approximately 2 cm^2 plastic squares and pieces of the non-geniculate coralline red alga *Sporolithon durum*) were placed in 250 mL jars with 100 ± 5 larvae each. For each experiment, each replicate piece of *S. durum* or square of settlement plate was obtained from a different plant or plate to ensure independence.

Both no-choice and choice experiments are used in this study. In a no-choice experiment, all levels of the experimental factor are tested separately in contrast to the choice experiment (block design) where the levels of the one factor are tested together to offer larvae a choice between the different substrata. The block design was chosen because we considered it to be closer to the natural situation. Larvae experience some degree of choice between different substrata and are unlikely to only encounter one possible substratum.

Larval behaviour was observed under a dissecting microscope. The term "settlement" describes the permanent attachment of the larvae to the substrate after shedding of the velum to complete metamorphosis. Both initial shell-growth (visible after 24 hours) and ciliary processes in the mantle cavity indicated that metamorphosis was completed (Hahn, 1989).

Jars with plastic squares without diatoms or other algae were used as controls for each experiment (negative control). In most experiments positive controls were used and consisted of jars with a piece of the non-geniculate coralline red alga *Sporolithon durum*. Jars from all experiments were kept at 17 ± 2° C, with a 12 : 12 hours L : D photo cycle. Settled larvae were counted under a dissecting microscope 24 and 48 or 72 or 96 hours after the larvae were added.

2.2.3 Data analysis

Statistical analyses were carried out using the STATISTICA computer package. Numbers of settled larvae after 24 hours (not percentages) were analysed. Assumption of normality and homogeneity of variance was checked graphically for each data set. One-way ANOVAs with Tukey HSD tests were used for all "no-choice" experiments. Data of experiment 3-6 were analysed as repeated measure ANOVAs to assess the change over time. Substrata were not independent in "choice" experiments and consequently, data from these experiments were analysed using paired *t*-tests.

2.3 Results

A summary of results for laboratory scale settlement experiments is given in Table 1. Details of specific results follow below. Results of experiments conducted with larvae of the abalone *Haliotis rubra* are presented first followed by experiments with *Haliotis laevigata* and one experiment with a batch of hybrids (*Haliotis rubra* x *Haliotis laevigata*). Not all batches are discussed in the following section, however an overview of main findings is given. Further details can be found in Table 1.

2.3.1 Settlement experiments with *Haliotis rubra* larvae

The settlement rate was very low on all single species diatom films and ranged between 1%-6% (Figure 1A, Table 1: Batch 1, Exp. 1). Only the settlement rates on films of *Cylindrotheca closterium* and *Amphora* sp. were significantly different to the negative control ($p < 0.05$). Overall a settlement rate of 3% was calculated after 24 hours, which did not increase between 24 and 48 hours. The hierarchy in settlement response changed when the experiment was repeated in different month (Figure 1B, Table 1: Batch 6, Exp. 1). There was a significant difference between films of *Navicula* sp. and the control ($p < 0.05$) and between films of *Cocconeis* sp. and the control ($p < 0.001$). However, the overall settlement was still very low and ranged between 1%-6%.

New isolates of diatoms were tested in experiments with batch number 10. The settlement rates were slightly higher and varied between 3-14%, however, settlement was higher on *Ulvella lens* than any of the monospecific diatom films.

Significantly more larvae of batch 3 (Table 1: Batch 3, Exp. 2) settled on a mixed film of *Navicula* sp. and *Amphora* sp. than on a film of *Amphora* sp. alone (*t*-test, $p < 0.001$) (Table 2). In contrast, significantly fewer larvae settled on a mixed film of *Cocconeis* sp. and *Amphora* sp. than on a film of *Amphora* sp. alone (*t*-test, $p < 0.001$).

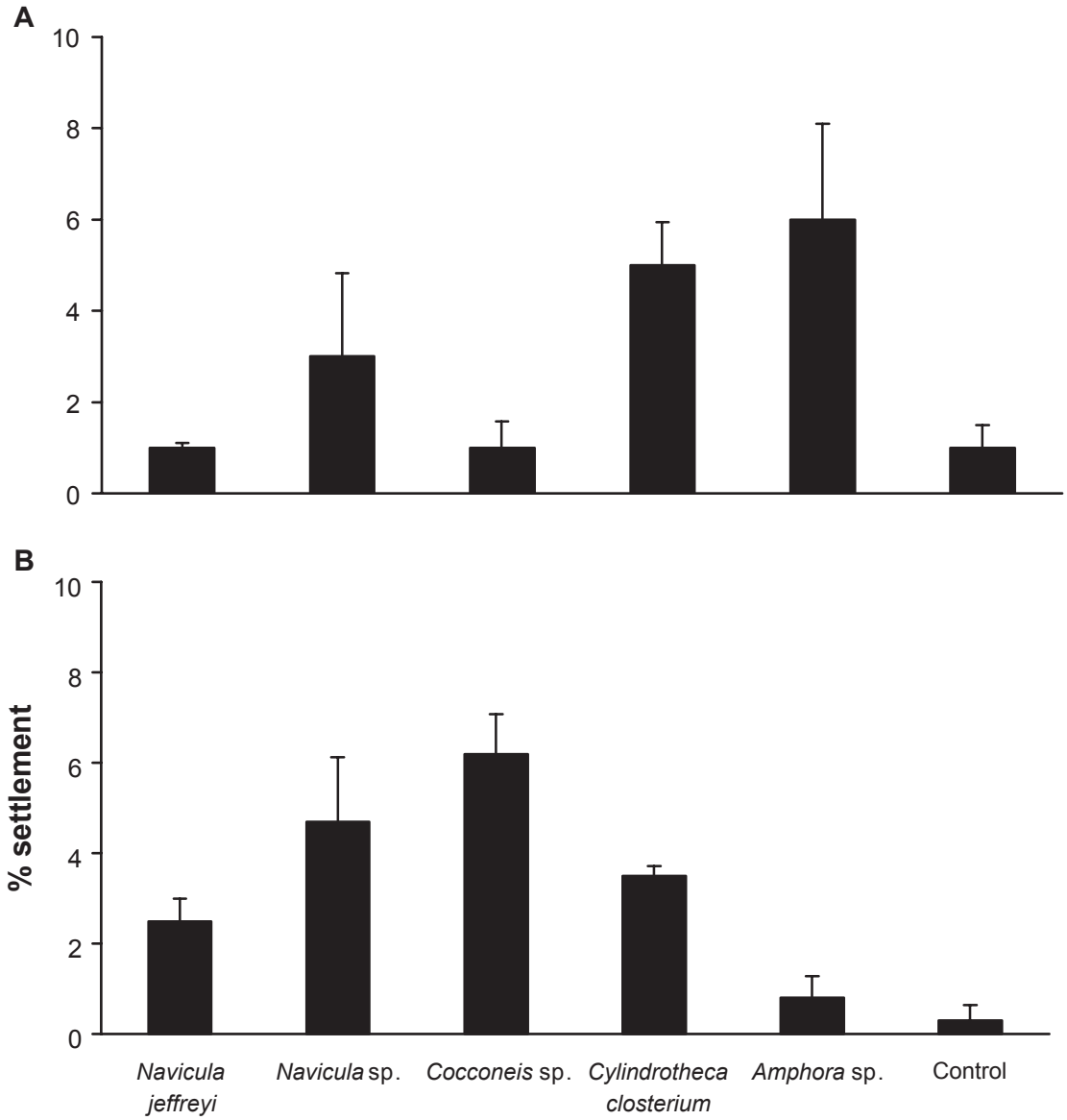

Figure 1. Percentage settlement of *Haliotis rubra* on monospecific diatom films after 24 hours. A. At the beginning of the spawning season (Table 1: November '98, Batch 1, Exp. 1). B. At the end of the spawning season (Table 1: March '99, Batch 6, Exp.1). Vertical bars indicate the standard error (n = 6).

Table 2. Percentage settlement of *Haliotis rubra* (Table 1: Batch 3, Exp. 2) when given a choice between a monospecific diatom film and a mixed film of two diatom species (n = 6 ± S.E.).

Species	% settlement after 24 hours
Amphora sp.	7 ± 1.1
Navicula sp. & *Amphora* sp.	12 ± 2.9
Amphora sp.	13 ± 1.9
Cocconeis sp. & *Amphora* sp.	8 ± 2.0
Control	1 ± 0.2

The settlement rate was 19% (7% + 12%) in the first set comparing *Amphora* sp. and a mix of *Navicula* sp./*Amphora* and 21% (13% + 8%) in the second set comparing *Amphora* sp. and a mix of *Cocconeis* sp./*Amphora* sp.

Significantly higher settlement was achieved on mature thalli of *S. durum* compared to plates with *S. durum* germlings and the negative control ($p < 0.001$) (Figure 2, Table 1: Batch 4, Exp. 2). No significant difference was found between the two treatments of the *S. durum* germlings ($p > 0.05$). There was a significant difference between the 24 and 48 hours count ($p < 0.05$). The settlement increased on the whole thallus of *S. durum* between 24 and 48 hours (Figure 2). However, no difference was found on squares with 10-day-old germlings and the number of settled larvae decreased on squares with 5-day-old germlings after 48 hours.

Significantly higher settlement occurred on *S. durum* germlings inoculated with the diatom *Navicula* sp. compared to plates with a film of *Navicula* sp. alone ($p < 0.05$) (Figure 3, Table 1: Batch 6, Exp. 3). There was no significant difference between the diatom film and the negative control ($p > 0.05$). No significant difference between the 24 and 48 hours count was found ($p > 0.05$). All treatments increased slightly between 24 and 48 hours.

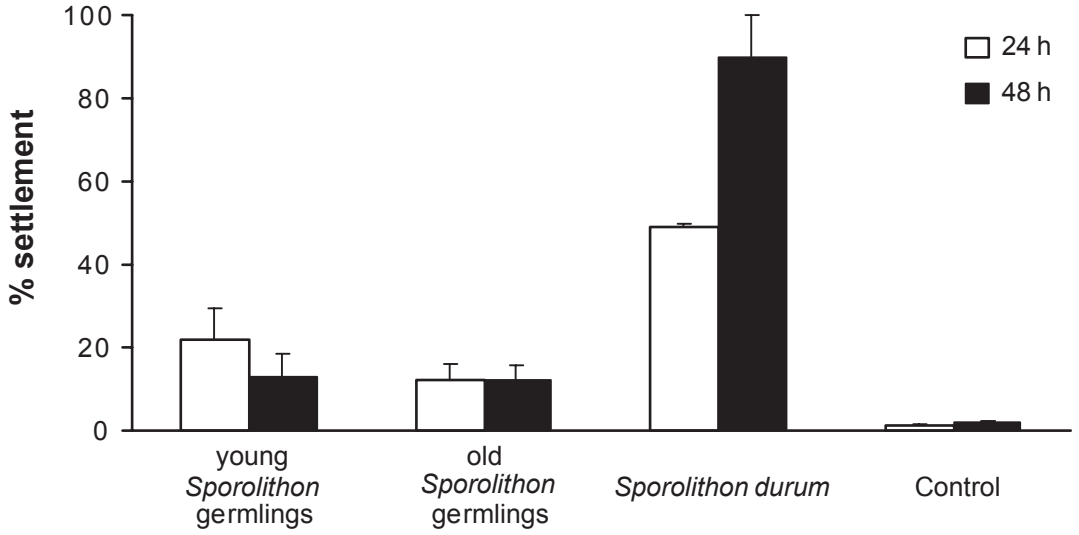

Figure 2. Percentage settlement of *Haliotis rubra* on young and old germlings of the non-geniculate coralline red alga *Sporolithon durum* in comparison to mature thalli of *Sporolithon durum* after 24 hours and 48 hours (Table 1: Batch 4, Exp. 2). Vertical bars indicate the standard error (n = 4).

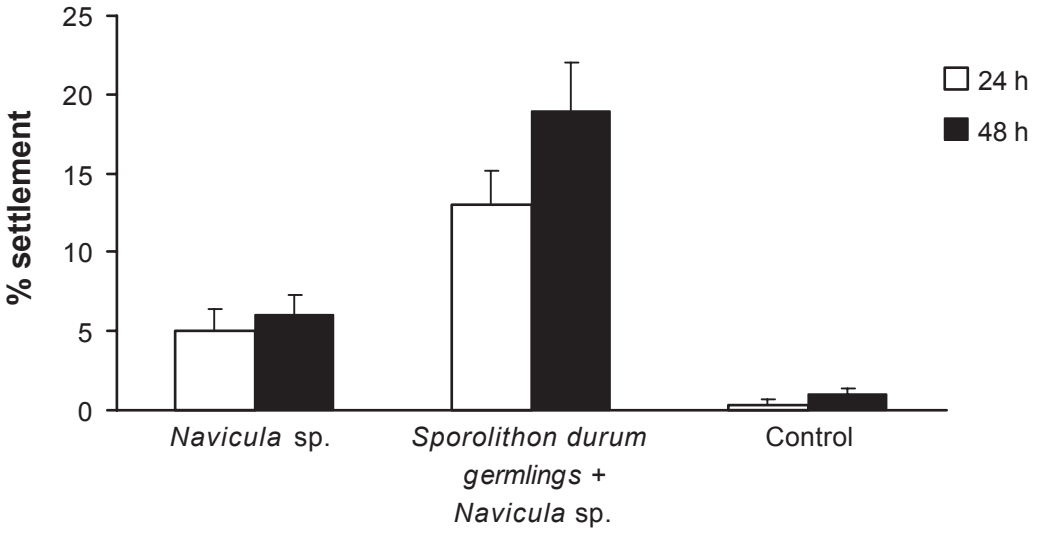

Figure 3. Percentage settlement of *Haliotis rubra* after 24 hours and 48 hours on a diatom film of *Navicula* sp. and germlings of *Sporolithon durum* inoculated with the diatom *Navicula* sp. (Table 1: Batch 6, Exp. 3). Vertical bars indicate the standard error (n = 6).

Significantly more larvae settled on the grazed squares of *U. lens* compared to the ungrazed squares ($p < 0.05$) and both were significantly different to the negative control ($p < 0.05$ and $p < 0.001$ respectively) (Figure 4, Table 1: Batch 6, Exp. 2). There was a significant difference between the 24 and 48 hours count ($p < 0.05$). The settlement rate was 12% on ungrazed plates and 23% on grazed plates of *U. lens* after 24 hours and increased to 20% and 36% respectively after 48 hours.

Settlement was significantly higher on plates with *U. lens* compared to the diatom film of *Navicula* sp. and plates with *U. lens* inoculated with the diatom *Navicula* sp. ($p < 0.001$) (Figure 5, Table 1: Batch 9, Exp. 2). Settlement was higher on plates with *U. lens* inoculated with *Navicula* sp. than on films of *Navicula* sp. alone but this difference was not significant ($p > 0.05$). Overall a settlement rate of 3% on the diatom film, 9% on *U. lens* inoculated with the diatom and 52% on pure *U. lens* was calculate after 24 hours. Between 24 and 72 hours the settlement rate increased to 5%, 12% in the first two treatments but decreased to 44% in the last treatment.

The settlement rate was significantly higher on 2 and 4-week-old *U. lens* both with high percentage cover (85-100% cover) compared to *U. lens* spores covering 3-5% of the plates ($p < 0.05$) (Table 3) (Table 1: Batch 11, Exp.1). There was however no significant difference between 2 and 4 week old *U. lens* ($p > 0.05$). After 24 hours a settlement rate of 27% was found on the older and 32% on the younger *U. lens*, whereas only 12% of the larvae settled on *U. lens* spores.

Table 3. Percentage settlement of *Haliotis rubra* on 2 week and 4-week-old *Ulvella lens* and *U. lens* spores compared to positive *(Sporolithon durum)* and negative control (n = 6 ± S.E.) (Table 1: Batch 11, Exp.1).

Species	% Settlement after 24 hours
Ulvella lens 2 week old (86-94% cover)	32 ± 4.7
Ulvella lens 4 week old (85-100% cover)	27 ± 3.7
Ulvella lens spores (4-5% cover)	12 ± 2.2
Sporolithon durum	49 ± 9.8
Control	2 ± 0

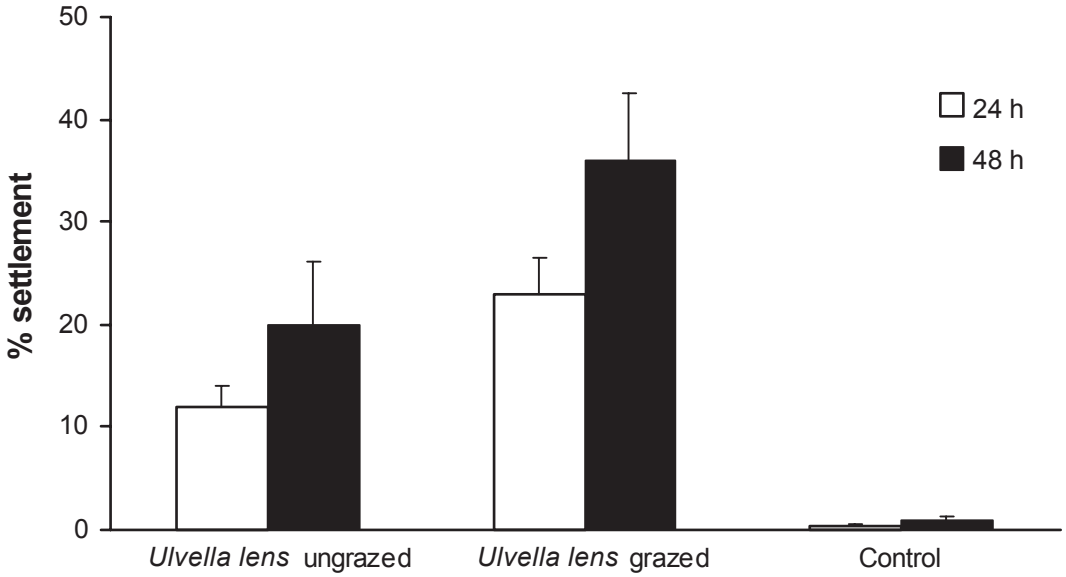

Figure 4. Percentage settlement of *Haliotis rubra* after 24 hours and 48 hours on grazed and ungrazed plates with *Ulvella lens* (Table 1: Batch 7, Exp. 1). Vertical bars indicate the standard error (n = 6).

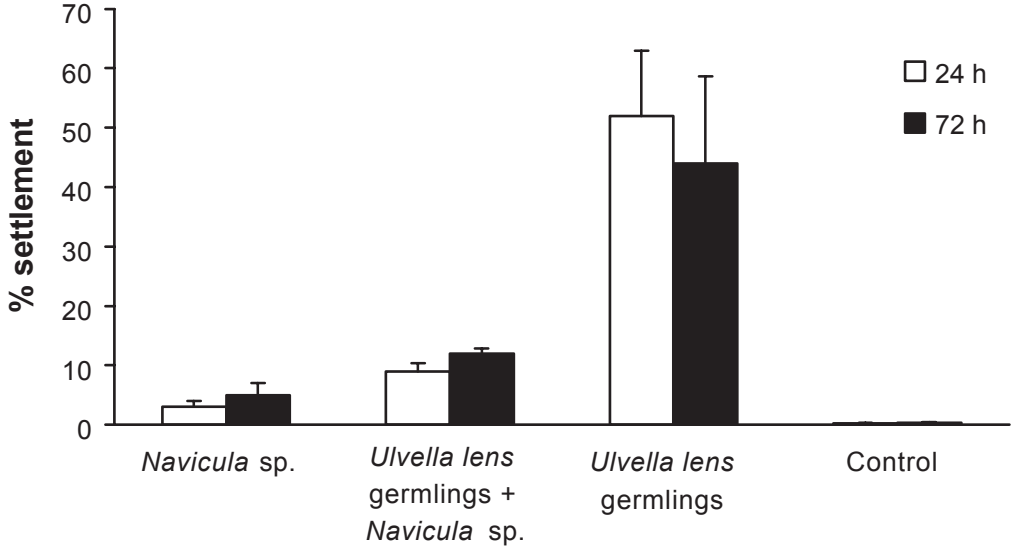

Figure 5. Percentage settlement of *Haliotis rubra* after 24 hours and 72 hours on a diatom film of *Navicula* sp., *Ulvella lens* inoculated with the diatom *Navicula* sp. and *U. lens* without a diatom (Table 1: Batch 10, Exp. 1). Vertical bars indicate the standard error (n = 6).

Significantly higher settlement occurred on *U. lens* grown for an additional 37 days after germination compared to 3-day-old germlings ($p < 0.05$) (Figure 6, Table 1: Batch 12, Exp. 1). The 3-day-old germling showed the highest cover (55%) but were not significantly different to the negative control ($p > 0.05$). The highest settlement (49%) occurred on the oldest culture of *U. lens*, with a cover of 32% it was comparable to the 17-day-old culture with 36% cover and a settlement rate of 36%. There was no significant difference between the two treatments ($p > 0.05$).

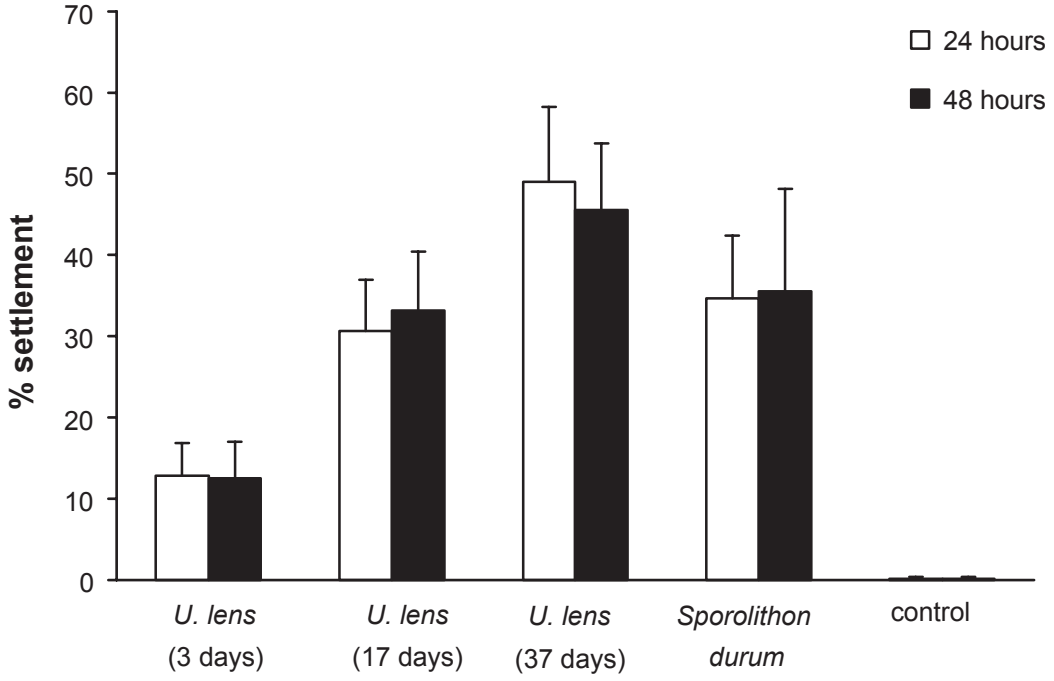

Figure 6. Percentage settlement of *Haliotis rubra* after 24 hours and 96 hours on 3, 17 and 37 day old *Ulvella lens* compared to positive (*Sporolithon durum*) and negative control (Table 1: Batch 12, Exp. 1). Vertical bars indicate the standard error (n = 6).

No significant difference was found between any treatment combination with and without the antibiotics Ampicilin and Kanamycin ($p > 0.05$) (Table 4, Table 1: Batch 13, Exp.1). In all cases the settlement rate after 24 and 48 hours was lower or similar on the substrate treated with antibiotics compared to untreated substrate of the same species. The settlement was significantly higher on both macro-algae *U. lens* and *S. durum* (positive control) compared to the diatom film of *Navicula* sp. and the negative control ($p < 0.05$). One week after the initial count, 54% of larvae were still swimming in control jars with antibiotic treatment (Table 4). Significantly fewer larvae (3%) were still active in the control jars without antibiotics ($p < 0.001$). More larvae survived up to 1 week on *U. lens* treated with antibiotics and fewer larvae survived on the treated *Navicula* sp. film compared to the same untreated species, but this difference was not significant.

Table 4. Percentage settlement of *Haliotis rubra* on *Ulvella lens* and *Navicula* sp. both with and without antibiotics after 24, 48 hours, % settled and survived up to 1 week and % of larvae in water column after 1 week (n = 6 ± S.E.) (Table 1: Batch 13, Exp.1).

Species	% settlement 24 hours	% settlement 48 hours	% survival to 1 week	% in water column after 1 week
Ulvella lens without antibiotics	30 ± 8.1	35 ± 7.6	12 ± 1.5	0 ± 0
Ulvella lens with antibiotics	22 ± 4.4	36 ± 5.3	17 ± 1.1	5 ± 1.6
Navicula sp. without antibiotics	5 ± 1.4	3 ± 2.0	4 ± 1.2	8 ± 3.5
Navicula sp. with antibiotics	0.3 ± 0.3	1 ± 0.3	2 ± 0.3	30 ± 4.2
Control without antibiotics	0 ± 0	1 ± 0.6	0.5 ± 0.3	3 ± 1.1
Control with antibiotics	0 ± 0	0.3 ± 0.3	0.6 ± 0.3	54 ± 6.7

2.3.2 Settlement experiments with *Haliotis laevigata* larvae

Settlement rates were highly variable on single species diatom films and ranged between 2%-20% (Figure 7, Table 1: Batch 2, Exp. 1). Films of *Cylindrotheca closterium, Navicula* sp. and *Amphora* sp. were significantly different to the negative control after 24 hours ($p < 0.05$). However no difference was found between *Cocconeis* sp. and the negative control. Settlement was highest on *C. closterium* (20%) followed by *Navicula* sp. and *Amphora* sp. and lowest on *Cocconeis* sp. (6%). The settlement rate did not increase after 48 hours and is therefore not presented here.

Settlement was significantly higher on younger than older films of *C. closterium* ($p < 0.001$) but not difference was found between the two *Navicula* sp. treatments ($p > 0.05$) (Table 5, Table 1: Batch 2, Exp. 3). Only younger films of both species were significantly different to the negative control ($p < 0.001$). The cell density ranged between 10^3 to 10^4 cells per cm^2 (Table 6). No significant difference in cell density was found between the young and old film of *C. closterium*. However old films of *Navicula* sp. showed significantly higher cell density than young films (t-test, $p < 0.05$).

There was no significant difference between single species (*Navicula* sp., *Cylindrotheca closterium*) and mixed species film (*Navicula* sp. and *Cylindrotheca closterium*) ($p > 0.05$) (Figure 8, Table 1: Batch 1, Exp. 2). The settlement ranged from 3-11%.

The settlement was highest on mature *Ulvella lens* and ranged from 16-17% (Figure 9, Table 1: Batch 3, Exp. 1). Mature *U. lens* induced more larvae to settle than young germlings of *U. lens* (Figure 9). However, settlement was significantly higher on the positive control (*Sporolithon durum*) compared to all *U. lens* treatments ($p < 0.001$). Only *S. durum* and two *U. lens* treatments with the highest percentage cover were significantly different to the negative control ($p < 0.05$).

Settlement was reduced if plates with *U. lens* were inoculated with the diatom cultures *Navicula* sp. before larval settlement (Figure 10, Table 1: Batch 2, Exp. 2). Both *U. lens* treatments (with and without diatoms) were significantly different to the negative control ($p < 0.05$).

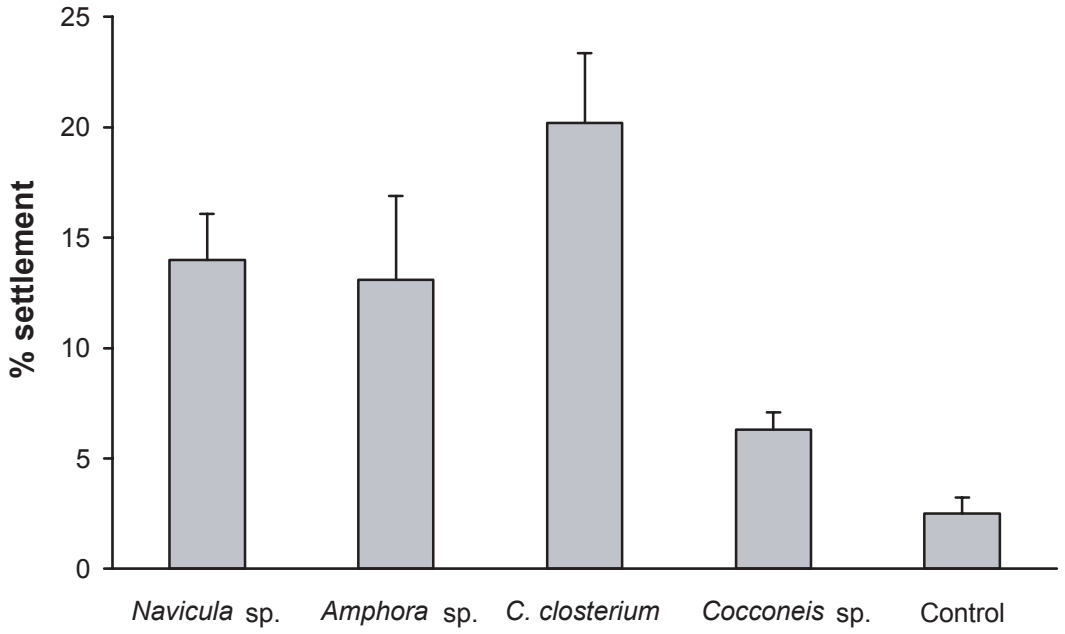

Figure 7. Percentage settlement of *Haliotis laevigata* on monospecific diatom films after 24 hours (Table 1: Batch 2, Exp. 1). Vertical bars indicate the standard error (n = 6).

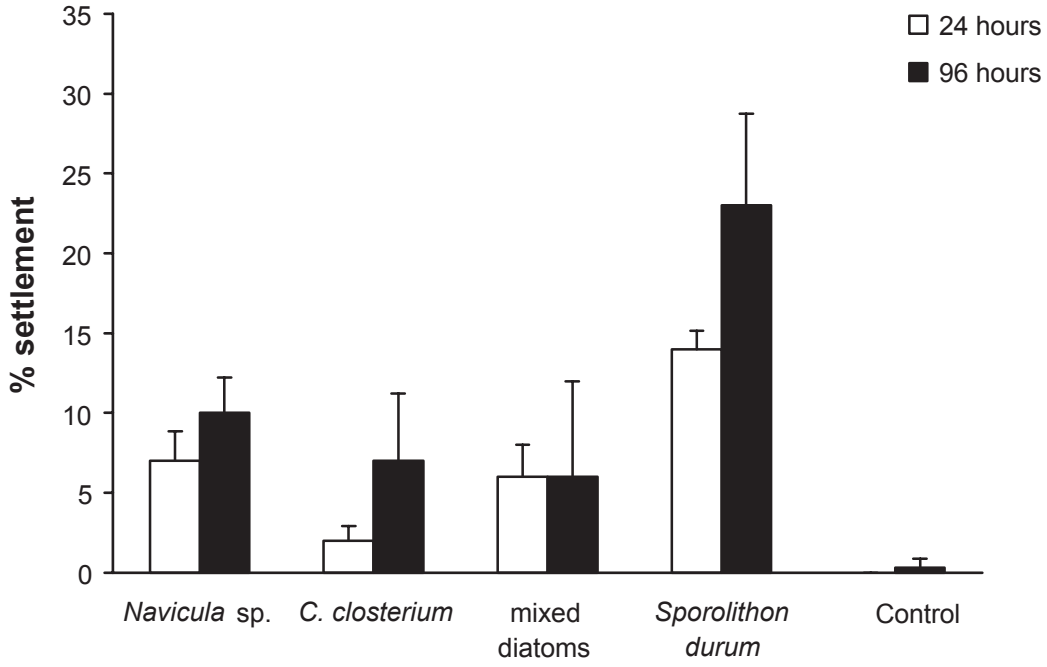

Figure 8. Percentage settlement of *Haliotis laevigata* after 24 hours and 96 hours on diatom films of *Navicula* sp., *Cylindrotheca closterium* and mixed species film compared to positive (*Sporolithon durum*) and negative control. Vertical bars indicate the standard error (n = 6).

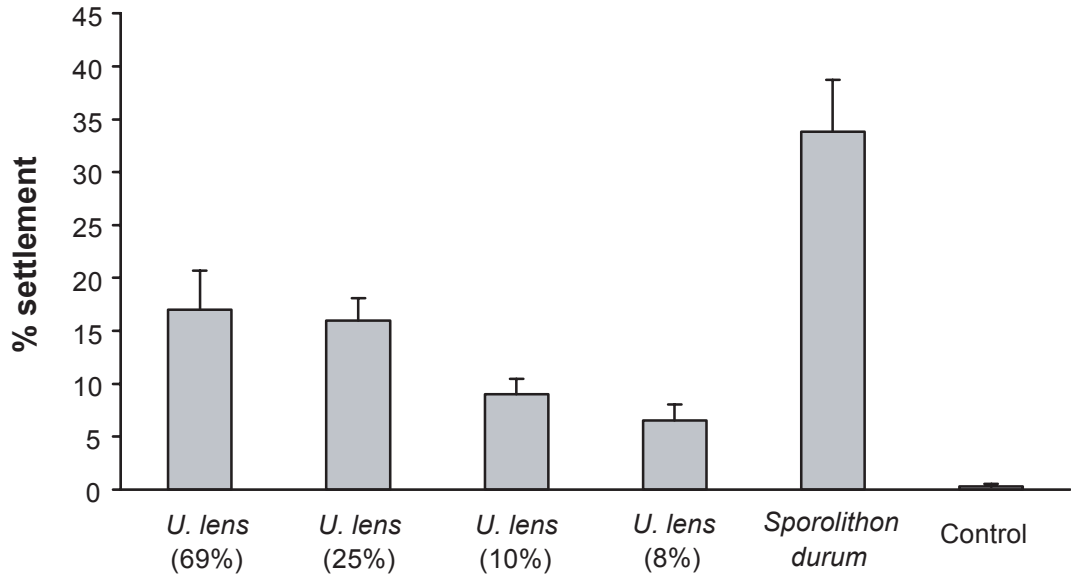

Figure 9. Percentage settlement of *Haliotis laevigata* after 24 hours on *Ulvella lens* of different maturity and % cover compared to the positive control (*Sporolithon durum*) and negative control (Table 1: Batch 3, Exp. 1). Vertical bars indicate the standard error (n = 6).

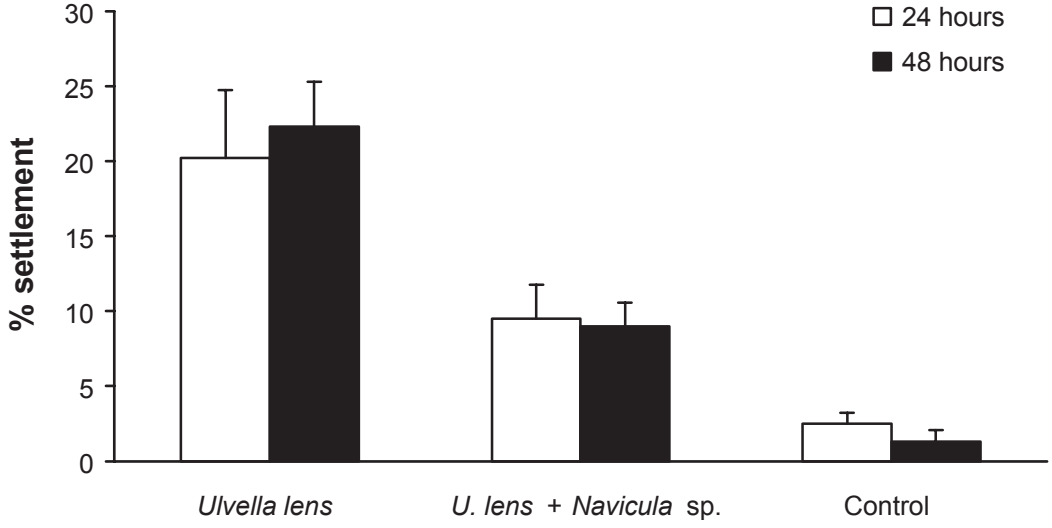

Figure 10. Percentage settlement of *Haliotis laevigata* after 24 hours and 48 hours on *Ulvella lens* compared to *Ulvella lens* inoculated with the diatom *Navicula* sp. and the negative control (Table 1: Batch 2, Exp. 2). Vertical bars indicate the standard error (n = 6).

2.4 Discussion

A small experimental system (250 mL jars with 100 competent larvae in each) was chosen for the first stage of this project, to allow screening a variety of different settlement cues and maintaining a high level of replication. The system was static (not slow flowing like in the nursery) and the substrate was presented horizontally (not vertically like the settlement plates in the nursery tanks). However, the purpose here was to test for difference in settlement induction between different substrates within one batch of larvae and select for promising candidates for the larger scale experiments in the nursery. The results of the settlement experiments cannot be compared across batches because the quality of the larvae can strongly influence

the settlement rate. Results across treatments and experiments, with larvae of the same batch, are not influenced by the larval quality and can thus indicate the strength of a settlement cue. The very high settlement rates on the positive control (*Sporolithon durum*) showed that the system is suitable and also served as a quality control of the batches i.e. if settlement is poor on *S. durum*, then larval quality and/ or environmental conditions are probably infavourable. The negative control provides an indication of random settlement and results show that larvae are very selective.

2.4.1 Settlement of *H. rubra*

Larvae of the abalone *H. rubra* did not respond well to any of the diatom species tested (Figure 1A, B) but settled well in response to the positive control, the non-geniculate coralline red alga *Sporolithon durum* (see Table 1, Batch 1). This is in agreement with a previous study, which showed that *H. rubra* did not respond to any of the diatom films tested but settled on the non-geniculate coralline red alga, *Phymatolithon repandum* (Daume et al., 1999a). The inconsistency in the hierarchy of diatom species in terms of settlement rates might have been caused by differences in culture conditions and production of extra cellular material by the algae at the time of larval settlement. The diatom strains in the previous study were obtained from a culture collection in New Zealand but belong to the same genera than the ones tested in this study. Both studies indicate that monospecific diatom strains are not particularly useful for the settlement of *H. rubra*. In contrast, Roberts and Nicholson (1997) and Kawamura and Kikuchi (1992) showed that *Cocconeis scutellum* induced high settlement of the abalone *H. virginea* and *H. discus hannai* respectively. Other diatoms species such as *Navicula ramosissima* also induced high settlement rates of *H. discus hannai* (Kawamura & Kikuchi, 1992) and *H. laevigata* (Daume et al., 1999a).

2.4.2 Settlement of *H. laevigata*

In this study, *H. laevigata* showed variable responses on mono-specific diatom films (Figure 7). Species of the same genus *Navicula* sp. and *Cylindrotheca* sp. sustained good settlement (up to 80-90%) in a previous study conducted with *H. laevigata* larvae (Daume et al. 1999a). In addition, also *Amphora* sp. showed a significant response in this study but other species like *Cocconeis* sp. did not sustain good settlement. Overall settlement rates cannot be compared across studies because batch quality can influence the results as explained above. The results however indicate that the settlement response to monospecific diatom films is species specific and diatoms may be useful for the settlement of some abalone species.

Only younger films with a lower cell density showed significant settlement when compared to the negative control (Table 5). In a previous study younger films (3 and 5 days old) showed significantly lower settlement that older films (14 and 19 days old) (Daume et al. 1999a). However when cell densities are compared, at least the cell density of the *Navicula* sp. was well below the cell density of the best performing films in the previous study. In addition, a strong correlation between cell density and larval settlement was found with these treatments in the previous study. This suggests that cell densities tested here might have been too low and films should have been cultured for a longer period of time to achieve higher cell densities before settlement induction.

Settlement on mixed cultures of diatoms achieved higher settlement but this depended on the species combination. If only naturally developing diatom films are used in an abalone nursery, the species combination on the settlement plate is very unpredictable and varies among

and between plates and seasons. The result from the second settlement experiment indicate that settlement plates in the nursery can produce inconsistent and variable settlement rates if species combinations are not monitored and controlled. The occurrence of diatoms is also greatly dependent on the season, a factor that needs to be considered further because abalone hatcheries in Australia are currently moving towards broodstock conditioning and off-season spawning.

Even small germlings of *S. durum* are sufficient for the settlement induction, but the settlement rate is lower than on pieces of the whole thallus of the alga. The germlings used might have been too young and need to be cultured over a longer period of time to improve settlement rates. The settlement on germlings inoculated with the diatom *Navicula* sp. was however higher than on the diatom alone, indicating that most of the settlement response can be associated with the germlings. The use of *S. durum* germlings for the settlement induction in abalone nurseries has not been fully explored. However, coralline red algae are slow growing in temperate waters and seasonal in tetraspore production. Culturing on a large scale to induce abalone larval settlement in the nursery would need appropriate time with an estimate of at least 3-4 weeks to inoculate settlement plates and several months to establish the next spore producing generation.

2.4.3 Settlement on *Ulvella lens*

In the present study, we showed for the first time that larvae of the Australian abalone *H. rubra* settle on the encrusting, green alga *U. lens*. The settlement can be enhanced if *U. lens* is pregrazed by conspecific abalone. Similarly, Takahashi and Koganezawa (1988) reported that *U. lens*, which had been pregrazed by conspecific juveniles, induced higher settlement than the ungrazed alga. Settlement induced by conspecific grazing mucus has been described for other abalone species (Seki & Kan-no, 1981; Slattery, 1992). In this study we showed that the settlement of the abalone *H. rubra* is higher on the macroalga *U. lens* than on diatom films. Naturally developing diatom films on plastic plates are currently predominantly used as the settlement cue in abalone nurseries in Australia. This study suggests that settlement plates seeded with *U. lens* could induce high and consistent settlement of *H. rubra* (see below).

In addition, *H. laevigata* settled well on *U. lens*, however the difference between the settlement on *U. lens* compared to some diatom films was not as pronounced as in experiments conducted with *H. rubra* larvae. The response to diatoms was, however, variable and depended on species and age of the film. This indicates that diatoms might be useful for the settlement of *H. laevigata* but more experiments are needed to determine differences between diatom species and cell density as well as large-scale inoculation and management of films dominated by other diatom species.

The results however, warrant the use of *U. lens* in large-scale trials in the nursery (see Chapter 5).

2.4.4 Settlement assays with antibiotics

No differences were found between the settlement rates on substrates treated with and without antibiotics (Table 4). The ratios between treated and untreated substrates did not change over time. The difference between specific substrates stayed the same regardless if antibiotics were used or not. The antibiotics were most likely effective, which was indicated by the higher survival in control jars treated with antibiotics. This indicates that even if more larvae survive in jars treated with antibiotics, those larvae do not influence the overall result e.g. no more

larvae choose to settle on diatom films treated with antibiotics. In this study we showed that when the antibiotic combination Kanamycin and Ampicillin is used at 50 µg mL^{-1}, the larval survival is higher than on untreated films. However, the settlement rate was not higher in the antibiotic treatment, indicating that unfit larvae might survive if treated with antibiotic but they do not settle successfully.

2.5 References

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3.0 Laboratory scale growth experiments

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3.1 Aim

The aim of the following experiments has been to assess the growth and survival of *H. rubra* and *H. laevigata* post-larvae feeding on different algal species.

3.2 Methods

3.2.1 Static system

Larvae were settled onto small plastic sheets with algal feed species and maintained in glass jars (250 mL) without water flow. The seawater was filtered to 1 μm and replaced every 3-4 days. The shell length of post-larvae was measured before and every week up to 4 weeks after the experiment started.

3.2.2 Flow through system

A flow through system, utilizing large petri dishes of ca. 250 mL capacity, with a constant flow of 20 mL min^{-1} of 1 μm filtered seawater in each, was installed (Figure 11). Dishes with algal feed were rinsed in flow for 1 hour to remove medium. Plates with *U. lens* and pieces of *S. durum* were collected from the nursery and placed into petri dishes in the flow. Six settled larvae were transferred into each of 4 replicate dishes of the same algal species. If not noted otherwise in Table 7, post-larvae were ca. 300 μm in shell length at the start of the experiments.

All post-larvae used within one experiment were derived from the same batch. Post-larvae were fed every 2-3 days with 30-50 mL of diatom cultures per dish to maintain a cell density of approximately 10^5 cells cm^{-2}. Post-larvae fed *U. lens* and *S. durum* did not receive any diatoms. Animals feeding on formulated food were fed every 2-3 days with 1 mL of feed suspension per dish. The formulated feed was previously suspended at a ratio of 5 g 100 mL^{-1}. Food (formulated or cultured algae) of all trials were never consumed completely and always offered in excess.

The shell length of post-larvae were measured before being added to the dishes at weekly intervals. Growth rates of post-larvae were calculated. Faecal samples and the bottom of the grazed dishes were examined for broken valves to investigate if diatom cell contents are available to post-larvae as a food source.

3.2.3 Algal culture

Diatom species were cultured in f/2 medium over 1-2 weeks at 17 ± 2° C with a 12 L: 12 D photo cycle and allowed to attach to small plastic squares (2 cm^2) (static system) or the bottom of the petri dishes (flow through system) until they reached a density of approximately 10^5 cells cm^{-2}.

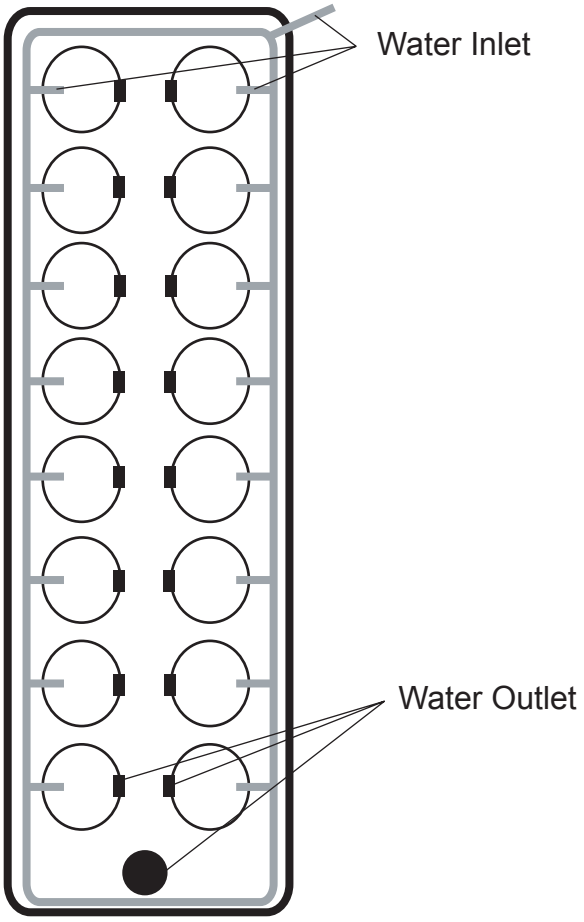

Figure 11. Diagram of flow through system showing 16 petri dishes, water inlet and outlet.

Methods for *U. lens* spore collection were adapted from Takahshi & Koganezawa (1988) (see chapter 4 for large scale culture methods). Spores were collected on plastic squares (static system) or the bottom of the petri dishes (flow through system). After 1-2 weeks, germlings reached a diameter of 100-300 µm. Germlings were grown for an additional 2 weeks to reach maturity. Dishes or plastic squares were inoculated with the diatom *Navicula* sp. for the combination treatment and cultured for an additional 2 weeks until a density of approximately 10^5 cells cm^{-2} was reached.

3.2.4 Estimates of cell density of diatoms

Sub samples of specific and species combinations were taken and the number of cells was recorded in 15 randomly chosen fields of view at a magnification of 400 x. The number of cells cm^{-2} was calculated.

3.2.5 Data analysis

Data of abalone growth rates were analysed as a repeated measure analyses of variance. Data were also analysed separately for each measurement using one-way ANOVAs with Tukey HSD tests.

3.3 Results

A summary of all results for the laboratory scale settlement experiments are given in Table 7. Results of experiments conducted with larvae of *H. rubra* are presented first followed by experiments with *H. laevigata* and one experiment with a batch of hybrids (*H. rubra* x *H. laevigata*). Results of experiments of each species are presented in chronological order starting with the spawning season 1998/99. Details of specific results follow below. If not indicated, experiments were started 1 week after settlement when post-larvae reached 300-400 μm in shell length.

3.3.1 Growth experiments with *Haliotis rubra*

Experiment 1 and 2

These experiments can be regarded as one long experiment stretching over a 2-month period. Post-larvae were fed with a small variety of *Navicula jeffryi* (ca. 5 μm valve length). This diatom species grow well in suspension but does not attach well to the substrate and gets easily dislodged in flow. The average growth was 25 ± 5 μm day^{-1} for recently settled post-larvae (Table 7: Exp. 1) and 35 ± 6 μm day^{-1} for 1 month old post-larvae (Exp. 2). The survival was only 17% during the first month. Dead animals were replaced with post-larvae of similar shell length and feeding history and the survival increased to 58% during the second month (Exp. 2).

Experiment 3

At the end of the 11-week experiment, films of the larger variety of *Navicula* sp. (13 μm in valve length) produced the largest post-larvae with an average of 3200 μm in shell length (Figure 12, Table 7: Exp. 3). Post-larvae feeding on the macroalgae *U. lens* were the smallest with an average of 1.2 mm in shell length. Post-larvae feeding on *Navicula* sp. showed the highest growth-rate (Table 8).

Table 7. Result summary of laboratory feeding experiments. If not noted otherwise, post-larvae were 300 µm in shell length at the start of the experiment.

Feeding experiments with *Haliotis rubra*						
Experiment	**Set up**	**Feed species**	**1 – 4 weeks**		**5 – 8 weeks**	
			Growth (µm day^{-1})	Survival (%)	Growth (µm day^{-1})	Survival (%)
1	28.01.99 Flow	*Navicula jeffryi*	25	17	–	–
2	09.02.99 Flow	*Navicula jeffryi*	–	–	35	38
3	22.03.99 Flow (run for 11 weeks in total)	*Navicula* sp.	35	79	39	58
		Navicula jeffryi	31	75	43	63
		Cocconeis sp.	29	71	27	71
		C. closterium	26	25	37	17
		Sporolithon durum	14	100	15	58
		Ulvella lens	6	29	16	29
4	22.04.99 Flow	*Navicula* sp.	25	68	–	–
		Amphora sp.	13	32	–	–
5	08.06.99 Flow (1.3 mm shell length)	*Navicula* sp.	28	50	49	42
		Ulvella lens	29	80	46	60
		Sporolithon durum	14	63	19	37
		Formulated food	24	42	19	42
6	Flow (3.2 mm shell length)	Formulated food	32	80	39	73
		Ulvella lens	46	80	28	60
7	15.11.99 Static	*Ulvella lens*	17	23	–	–
		U. lens + *Navicula* sp.	23	25	–	–
		Cocconeis sp. + *Amphora* sp.	26	29	–	–
8	07.04.00 Flow (489 µm shell length)	*Ulvella lens*	24	57	34	52
		U. lens + *Navicula* sp.	29	82	42	70
		Navicula sp.	29	36	35	21
9	01.06.00 Flow (2.3 mm shell length)	*Ulvella lens*	48	72	42	61
		U. lens + *Navicula* sp.	51	73	54	61
		Navicula sp.	41	92	55	90
		C. closterium	39	76	48	76
10	20.07.00 Flow (6.8 mm shell length)	*Ulvella lens*	17	71	45	54
		U. lens + *Navicula* sp.	16	25	47	21
		Navicula sp.	18	58	22	54
		C. closterium	28	50	20	21
11	17.11.00 Static	*Ulvella lens* (55% cover)	22	25	–	–
		Ulvella lens (36% cover)	20	6	–	–
		Ulvella lens (32% cover)	25	12	–	–

Feeding experiments with *Haliotis laevigata*						
Experiment	**Set up**	**Feed species**	**1 – 4 weeks**		**5 – 8 weeks**	
			Growth (µm day^{-1})	Survival (%)	Growth (µm day^{-1})	Survival (%)
1	12.02.99	*Navicula* sp.	20	42	35	25
	Flow	*Navicula jeffryi*	17	33	24	21
2	21.12.99	*Navicula* sp.	23	32	18	13
	Static	*Navicula* sp. + *C. closterium*	27	39	40	23
		Ulvella lens	19	20	16	5
		U. lens + *Navicula* sp.	22	60	18	40
Feeding experiments with *Haliotis rubra x Haliotis laevigata*						
Experiment	**Set up**	**Feed species**	**1 – 4 weeks**		**5 – 8 weeks**	
			Growth (µm day^{-1})	Survival (%)	Growth (µm day^{-1})	Survival (%)
1	06.12.99	*Ulvella lens*	22	42	–	–
	Static	*U. lens* + *Navicula* sp.	24	29	–	–
		Cocconeis sp.	28	21	–	–
		Cocconeis sp. + *Navicula* sp.	38	33	–	–

-

Table 8. Mean daily growth rates and survival rates of *Haliotis rubra* post-larvae on different algal species (n = 4) over the entire 11 week period.

Species	**Daily growth rate (µm day^{-1}, mean ± S.E.)**	**Survival (%)**
Navicula sp.	39 ± 3.5	75
Navicula jeffreyi	35 ± 3.6	63
Cylindrotheca closterium	33 ± 3.8	17
Cocconeis sp.	27 ± 3.0	71
Sporolithon durum	15 ± 1.8	58
Ulvella lens	13 ± 2.6	29

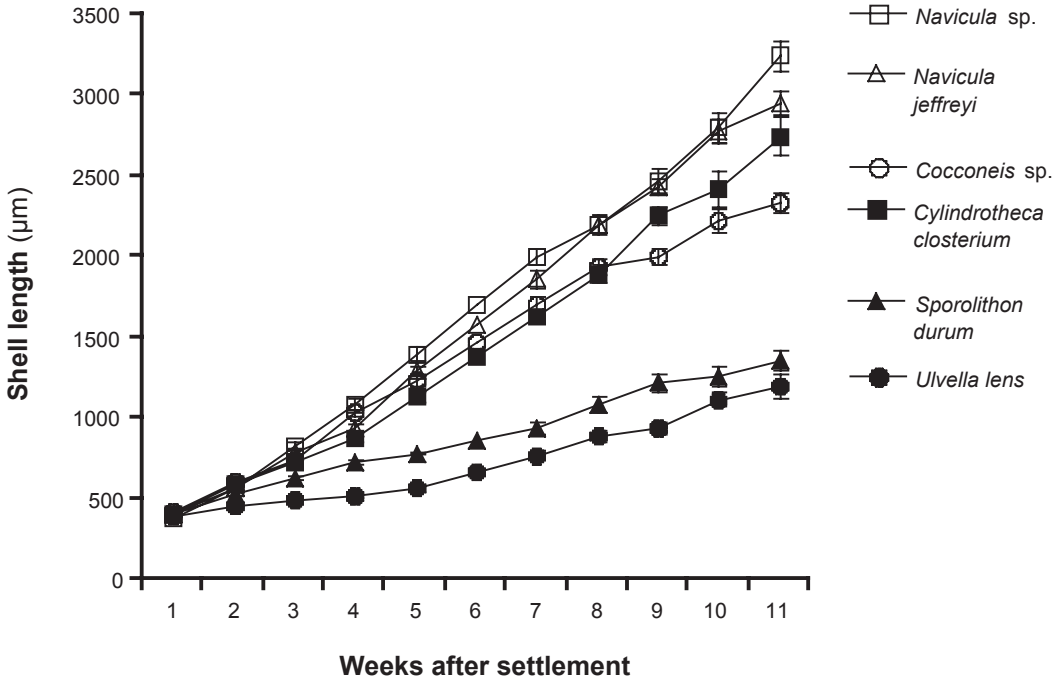

Figure 12. Early growth of *Haliotis rubra* post-larvae feeding on different algal species. Vertical bars indicate the standard error; n = 4 replicated dishes with 6 post-larvae each.

The growth-rates of post-larvae grazing on any of the four diatom species were significant higher than on the macroalgae *U. lens* and *S. durum* ($p < 0.05$). Post-larvae survived best on the diatom *Navicula* sp. Survival was low on the diatom *C. closterium* and the macroalga *U. lens*. When weekly growth rates were analysed separately, there was no significant difference during the first week after settlement ($p > 0.05$) (Figure 12). Two weeks after settlement the difference between *U. lens* and the diatom species was established ($p < 0.05$). By week 3, post-larvae growing on *U. lens* or *S. durum* showed significantly lower growth-rates to those feeding on the diatom species ($p < 0.05$). In addition, differences among the diatom species first became apparent three weeks after settlement. Post-larvae feeding on *Navicula* spp. showed higher growth-rates three weeks after settlement compared to *Cocconeis* sp. and *C. closterium*.

Diatom cells of all but one diatom species appeared in faecal samples from the beginning of the trial, indicating that cells of all diatom species were ingested. However, valves of *C. closterium*, as opposed to whole cells, were not detected before 3 weeks after settlement. Most diatom valves of all other diatom species were still intact and unbroken in faecal samples up to 3 weeks after settlement. Broken valves without cellular content of the *Navicula* spp. appeared in faecal samples three weeks after settlement. The mean cell size of *Cocconeis* sp. decreased during the trial and most cells of *Cocconeis* sp. measured only 1 µm in diameter at the end of the trial.

Experiment 4

Two different feed species, a larger size *Navicula* sp. (13 µm in length) and an *Amphora* sp., were compared in this trial (Table 7: Exp. 4). The *Navicula* sp. attaches well to the substrate, even in flow, but does not grow to high densities when using conventional methods of diatom

mass cultures. Post-larvae showed a significantly better growth rate on *Navicula* sp. (25 ± 4 μm day^{-1}) compared to *Amphora* sp. (13 ± 3 μm day^{-1}) ($p < 0.05$) and higher survival (68 and 32% respectively).

Experiment 5

Three different algal species (the diatom *Navicula* sp., *U. lens* and *S. durum*) were tested against formulated feed (nursery powder; Adam & Amos). Animals were 1.3 mm in shell length at the start of the experiment. The growth rates were significantly lower on *S. durum* compared to the other two algal species and the formulated food during the first month of the experiment ($p < 0.05$). Fewer animals survived when feeding on artificial food. During the second month animals feeding on the two algal species *Navicula* sp. and *U. lens* showed significantly higher growth-rates than animals feeding on *S. durum* and formulated food. ($p < 0.05$). The survival at the end of the trial was highest in treatment with *U. lens* and lowest on *S. durum*.

Experiment 6

In this experiment larger juvenile were fed with a formulated feed in comparison to *U. lens*. Juveniles averaged 3.2 mm in shell length at the start of the experiment. There was a significant difference in the first month. Juveniles grow better on *U. lens* with an average of 46 ± 2 μm day^{-1} compared to 32 ± 4 μm day^{-1} on artificial food ($p < 0.05$). No significant difference was found during the second month ($p > 0.05$). Both treatments sustained high survival rates (Table 7: Exp. 6). Both growth and survival of juveniles feeding on formulated feed was higher in this experiment compared to the previous experiment were smaller juveniles were tested.

Experiment 7

Growth-rates of post-larvae were significantly lower on *U. lens* compared to any other treatment combination ($p < 0.05$). Growth and survival rates were highest on the combination of the two diatoms *Cocconeis* sp. and *Amphora* sp.

Experiment 8

Post-larvae averaged 489 μm in shell length at the start of the experiment and reached 2,280 μm in shell length after 8 weeks in the combined diet treatment (Table 7: Exp. 8). During the first month there was no significant difference in growth between the treatments ($p > 0.05$). However post-larvae survived best on the combination diet. Significantly higher growth and survival occurred on the combination diet during the second month ($p < 0.05$). Post-larvae survived best on the combination diet followed by *U. lens*. The survival was poor on *Navicula* sp.

Experiment 9, 10

At the start of experiment 9 and 10 dead animals were replaced with post-larvae of similar size and feed history. In addition, a new treatment was added at the start of experiment 9 (Table 7: Exp. 9,10). The combination diet of *U. lens* and *Navicula* sp. was tested against monospecific diets (*U. lens, Navicula* sp. and *Cylindrotheca closterium*).

In experiment 9 post-larvae feeding on the combination diet showed higher growth-rates than on *Navicula* sp. and *C. closterium* during the first month and higher than on *U. lens* and *C. closterium* during the second month but these differences were not significant ($p > 0.05$). In experiment 10, when juveniles were about 6-7 mm in shell length, growth-rates were low in all treatments during the first month and increased on the combination diet and *U. lens* in the second month. However, overall survival on the combination diet in experiment 10 was poor.

Experiment 11

No significant difference was found between the growth rates of post-larvae provided with *U. lens* at different percentage cover ($p > 0.05$). Post-larvae however survived best on the highest cover of *U. lens.*

3.3.2 Growth experiments with *Haliotis laevigata*

Experiment 1

This trial was conducted over a two-month period to compare the growth and survival on the two different *Navicula* spp. The growth-rate and survival was higher on the larger size *Navicula* sp. however the differences were not significant (Table 7: Exp. 1; $p > 0.05$).

Experiment 2

The diatom *Navicula* sp., a combination of the two species *Navicula* sp. and *Cylindrotheca closterium*, the macroalga *U. lens* and a *U. lens* and *Navicula* sp. combination were compared in a two month growth-trial. Growth rates were highest on the combination of the two diatom species *Navicula* sp. and *Cylindrotheca closterium* throughout the trial (Table 7: Exp. 2). However the difference was only statistically significant during the second month ($p < 0.05$). Survival was highest on the *U. lens* and *Navicula* sp. combination throughout the trial ($p < 0.05$).

3.3.3 Growth experiment with hybrids (*Haliotis rubra* ♀ x *H. laevigata* ♂)

Experiment 1

The diatom *Cocconeis* sp., a combination of the two species *Cocconeis* sp. and *Navicula* sp., the macroalga *U. lens* and a *U. lens* and *Navicula* sp. combination were compared in a one month growth-trial (Table 7). The growth-rate was significantly higher on the *Cocconeis* sp. and *Navicula* sp. combination compared to any other treatment ($p < 0.05$). Survival was highest on the macroalga *U. lens* ($p < 0.05$).

3.4 Discussion

Kawamura et al. (1998a) suggested that nutrition from yolk reserves are still present in recently settled post-larvae and for that reason differences between feed species cannot be detected during the first 2 weeks post-settlement. Alternatively, the development of the digestive system is not completed and post-larvae lack the capability to fully utilise the different feeds (Roberts et al., 1999). In experiment 3, during the first week of rearing, there was no significant difference in growth between post-larvae feeding on five algal species. It is conceivable that after 1-2 weeks the yolk reserves are exhausted and differences between feed species then became apparent.

In our study post-larvae of the abalone *H. rubra* were 0.7-0.8 mm in shell length when differences between diatom species became apparent. Similarly, post-larvae of the abalone *H. iris* were 0.8-1 mm in shell length when differences were detected (Kawamura et al., 1998a). These researchers suggested that dietary benefits that relate to diatom feed species depend on the size of the abalone.

Kawamura et al. (1995) found that some diatom species are ingested more easily because of their cell size or lower attachment strength. It is unlikely that the observed differences between diatom diets in this study are related to size because all diatoms tested were small (< 17 μm in length, < 4 μm in width). The mouth is 30 μm in diameter just 2 days after settlement (Seki & Kan-no, 1981). In addition, diatom cells of all but one species were found in faecal samples right from the beginning of the experiment.

Martinez-Ponce and Searcy-Bernal (1998) reported that grazing rates increase rapidly during the first month of rearing. Similarly, in our experiment, feeding had to be more frequent towards the end of the experiment to maintain a high cell density in the dishes. This indicates that more biomass is needed to maintain rapid growth of the post-larvae. The *Cocconeis* sp., which was smaller in diameter at the end of the experiment, might be less suitable as a food source for older post-larvae because not enough biomass can be provided with this species.

Most of the nutrition for post-larvae during the first 2-3 weeks after settlement must be derived from the biofilm components such as extracellular material produced by the diatoms and associated bacteria, as cells were not digested and remained alive in faecal samples. It has been suggested that extracellular polysaccharides produced by diatoms are responsible for the attachment of the cells (Hoagland et al., 1993) and well-attached *Navicula* sp. produced the largest post-larvae with the highest survival.

In experiment 3 we showed that the macroalgae *U. lens* and *S. durum* are not sufficient for the initial growth of *H. rubra* post-larvae. The growth rates of post-larvae feeding on *U. lens* or *S. durum* were significantly lower than on any diatom species throughout the experiment. Similarly, Seki (1997) reported that *U. lens* produced slower growth of the abalone *H. discus hannai* than *U. lens* with an additional diatom film of *C. closterium*. Similarly, we showed in experiment 7 that growth-rates were higher on the *U. lens* and *Navicula* sp. combination compared to *U. lens* alone. However, growth rates were highest on diatoms only during the first month of post-larval development. Growth rates of post-larvae feeding on *U. lens* alone were very low during the first month of development (Experiment 3, 7) and were similar once juveniles reached about 1-2 mm in shell length (see experiment 5 and 8-9). The survival of juveniles growing on *U. lens* was high except for Experiment 3 and 5. These results warrant large-scale trials in the nursery to investigate the suitability of *U. lens* and cultured diatoms to enhance growth and survival and different stages of juvenile development (see Chapter 5).

Experiment 5 showed that formulated food (nursery powder; Adam & Amos) is not suitable for post-larvae smaller than 2 mm in shell length. Growth-rates were higher when juveniles reached ca. 3 mm in shell length (Experiment 6) indicating a potential for early weaning. For further details see large-scale trial in Chapter 5. However, growth rates with 3.2 mm juveniles were much lower than for even smaller animals on a range of different algal diets (Experiment 9).

Similar to *H. rubra,* growth rates of *H. laevigata* post-larvae feeding on *U. lens* were lower than on any diatom species tested. They were also lower on a combined diet of *U. lens* and *Navicula* sp. as well as a mixed diatom diet. However the only significant difference was found during the second month when post-larvae grew best on the combined diet of *Navicula* sp. and *Cylindrotheca closterium*. These results indicate that *H. laevigata* post-larvae do not show a strong preference for any of the species and may benefit from a mixed species diet. The experiment conducted with hybrid post-larvae showed similar results to *H. laevigata* post-larvae with a mixed diatom diet (*Cocconeis* sp., *Navicula* sp.) showing the best results in growth rate.

3.5 References

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4.0 Algal culture and succession of species on substrate

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4.1 Aim

The aim of this part of the study has been to identify suitable microalgae which can be easily used in abalone nursery, in terms of maintaining mass cultures, ability to colonise settlement surfaces and recolonise surfaces post grazing.

4.2 Introduction

Preliminary laboratory experiments and nursery trials were conducted to identify the suitable diatom species, size of the inoculum, nutrient level and time of incubation in static seawater (Table 9; June 1999). Only *Navicula* sp. developed on vertical settlement plates. Other diatom species tested did not attach well to the plates (*Cylindrotheca closterium*) or did not develop a dense biofilm during the experimental period of 1-2 weeks (*Cocconeis* sp.). Therefore *Navicula* sp. was used for all large-scale inoculations. In addition, post-larvae feeding on *Navicula* sp. showed the highest growth-rates (see Chapter 3). Experiments were run at two different farms in Victoria and at one farm in South Australia (Table 9).

Laboratory experiments have shown that the macroalgae *U. lens* is a suitable settlement inducer for larvae of the abalone *Haliotis rubra* (see Chapter 2). However, the settlement rate of abalone larvae on *U. lens* appears to be reduced if the surface had previously been inoculated with a diatom species. In contrast, inoculation with the diatom *Navicula* sp. enhanced the growth of post-larvae (see Chapter 3). Consequently methods were developed for a succession of algal species (initial development of *U. lens* for settlement followed by inoculation of *Navicula* sp. for post-larval growth). In addition, plates with recently settled larvae were inoculated with no ill effect on the post-larvae (Table 9, October/November 2000).

4.3 Developed methods for algal culture and inoculation

4.3.1 Large scale algal sterilization and nutrient addition

Tanks and plates were scrubbed clean, filled with 1 μm filtered seawater and chlorinated at 10-ppm overnight (160 mL 2,000 L^{-1}). 2M Sodium thiosulphate and heavy aeration was used for dechlorination for 4 hours (50 ml 2,000 L^{-1}). Nutrients were prepared as f_2 (Guillard & Ryther, 1962): Nitrate ($NaNO_3$) 150g 2,000 L^{-1}; Phosphate (NaH_2PO_4) 10g 2,000 L^{-1}; Ironcitrate as Fe (III) citrate trihydrate + Citric acid 9g 2,000 L^{-1} each (was dissolved in 1L before adding into the tank) and Metasilicate as Na_2SiO_3 23 g 2,000 L^{-1} (only for diatom inoculation). Alternatively a complete f-mix was sometimes used and applied at 40g 1,000 L^{-1} (Microalgae Food, Manutech, Port Lincoln). All nutrients were dissolved before adding them to the tanks. The tanks remained static with low aeration.

Table 9. Inoculation and succession of algal species.

Date	Location	Treatments
June 1999	Southern Ocean Mariculture, Port Fairy, Victoria	*Navicula* spp., *Cylindrotheca closterium*, *Cocconeis* sp. inoculation in 20 L tanks in the laboratory
July–August 1999	Southern Ocean Mariculture, Port Fairy, Victoria	*Navicula* sp. large-scale inoculation into nursery tanks (2,000 L)
December 1999	South Australian Mariculture, Port Lincoln, South Australia	*Navicula* sp. large-scale inoculation into nursery tanks (2,000 L)
July/ August 2000	Southern Ocean Mariculture, Port Fairy, Victoria	*Ulvella lens* mass spore release + *Navicula* sp. large-scale inoculation into nursery tanks (6,000 L)
August 2000	Ocean Wave Seafoods, Lara, Victoria	*Ulvella lens* mass spore release into nursery tanks (6,000 L)
September 2000	Ocean Wave Seafoods, Lara, Victoria	*Navicula* sp. large-scale inoculation (6,000 L)
October/ November 2000	Southern Ocean Mariculture, Port Fairy, Victoria	*Navicula* sp. large-scale inoculation 1-2 weeks after abalone larval settlement (2,000 L)
January 2001	Southern Ocean Mariculture, Port Fairy, Victoria	*Navicula* sp. large-scale inoculation 4 and 8 weeks after abalone larval settlement (2,000 L)
January 2001	Southern Ocean Mariculture, Port Fairy, Victoria	*Ulvella lens* mass spore release + *Navicula* sp. large-scale inoculation to split plates after 10 weeks (2,000 L)
February 2001	Ocean Wave Seafoods, Lara, Victoria	*Ulvella lens* mass spore release into nursery tanks (6,000 L)

4.3.1.1 *Ulvella lens* seeding

Seed plates with large mature patches of *U. lens* and well-developed sporangia were selected, wiped clean with a sponge to remove the diatom film and stored in a dark container or under double shading cloth, in 1 μm filtered seawater. One *U. lens* seed plate was placed between each pair of baskets (8 seed plates per 2,000 L tank). The tanks remained static with low aeration and without shading. The release of zoospores was triggered by the increase in water temperature and light. The spore release started at sunrise. The largest release occurred 4-5 days after the introduction of seed plates. The seed plates were removed after a mass release of zoospores.

4.3.1.2 Diatom inoculation

The diatom *Navicula* sp. was cultured on increasing larger, horizontally laid, algal bags up to commercial size bags of ca. 1 x 2 m. Three to four steps were required in this scaling up process. The diatom culture was harvested during the exponential phase (4-6 days after inoculation) and mixed into suspension. The diatom density was determined before tanks were inoculated. A 12 L inoculum (10^5-10^6 cells mL^{-1}) was used in 2,000 L tanks. Within 24 hours the diatoms attached and continued to divide. The tanks were allowed to remain static for at least 48 hours; tanks with post-larvae receive low water flow with light aeration for 2-3 days.

4.3.1.3 Extra plates to reduce juvenile density or supplement feeding by inoculation

A larger inoculum of 20-25 L 2,000 L^{-1} tanks was used to obtain higher diatom densities on the plates and tanks. Tanks remained static with light aeration for 3 days. Diatom density on the plates reached 10^4 cells cm^{-2} after 2 days and increased to 10^5 cells cm^{-2} after 4-5 days. The diatom density was then be manipulated by shading. Plates remained conditioned (with high diatom density) for at least 1 month under high grazing pressure.

4.4 References

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5.0 Commercial scale settlement and feeding experiments

5.1 Introduction and aims

Laboratory experiments have shown that the macroalgae *Ulvella lens* is a suitable settlement inducer for larvae of *H. rubra* and *H. laevigata* (Chapter 2). We achieved high settlement with *U. lens* of different age and developmental stages. Settlement was significantly lower on plates with biofilms dominated by diatoms. Settlement was higher on older compared to younger thalli of *U. lens* and biofilms but settlement on *U. lens* appears to be reduced when previously inoculated with a diatom species. However inoculation with diatoms has some advantages for the subsequent growth of the post-larvae (Chapter 3). During the first two months of rearing growth-rates reached 30-40 µm day^{-1} when feeding on the cultured diatom *Navicula* sp. When single species diets were compared to a combination of the macroalga *U. lens* and the diatom *Navicula* sp., growth-rates reached 42 µm day^{-1} on the combined diet but only 35 µm day^{-1} on single species diets. Survival up to 2 months post settlement was highest on the combination (70%) and lowest on the diatom only.

The objectives of the following commercial scale experiments (Table 10) were to test:

1. If the technology from the laboratory can be transferred into the nursery; and
2. whether the results would be similar in commercial scale experiments.

The aims for individual experiments are given in Table 10.

5.2 Methods

All commercial scale experiments are listed in Table 10. The experiment number, the duration, location, species and the aims of the experiment are presented.

5.2.1 Experiment 1: The effect of cultured algae and larval density on settlement and early growth of the abalone *Haliotis rubra*

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5.2.1.1 Experimental design

An overview of the experimental design is given in Figure 13. Initially, four combinations of two larval densities and two developmental stages of *U. lens* were evaluated for abalone larval settlement. Subsequently, after settlement, all plates were systematically redistributed across the tanks and these four combinations were all subjected to inoculation with the cultured benthic diatom *Navicula* sp. or natural colonization with a mix of diatom species and the growth and survival of the abalone were measured.

5.2.1.2 Preparation of tanks

Each of 4 tanks (2,000 L) was divided into two parts with a separate inlet and outlet (= 8 tanks). The arrangements of tanks in the nursery can be seen in Figure 14. All 8 tanks contain 4 baskets with 14 plates each (Figure 13, Table 11). Airlines were installed along each side of the baskets (4 airlines per tank).

Table 10. List of commercial scale experiments.

No	Date and Duration	Location	Species	Aims
1	11/2000 – 4/2001	Southern Ocean Mariculture	*Haliotis rubra*	1. To assess settlement rates on *U. lens* of different age and/ or developmental stages. 2. To test if different larval density influences overall settlement rates. 3. To investigate if inoculation with *Navicula* sp. will improve growth and/ or survival.
2	11/2000 – 3/2001	Southern Ocean Mariculture	*Haliotis laevigata*	1. To assess settlement rates on *U. lens* of different developmental stages. 2. To investigate growth and survival on a combined diet of *U. lens* with *Navicula* sp.
3	2/2001 – 6/2001	Ocean Wave Seafoods	*Haliotis rubra*	1. To investigate if differences in light intensity (shading/ no shading) influences algal and post-larval growth.
4	11/2001 – 11/2001	Great Southern Marine Hatcheries	*Haliotis laevigata*	1. To assess settlement rates on different developmental stages of *U. lens* in comparison to the diatoms *Cocconeis* sp. and *Navicula* sp.
5	12/2001 – 06/2002	Great Southern Marine Hatcheries	*Haliotis laevigata*	1. To assess growth and survival of juveniles < 3 mm on a combined diet of *U. lens* and *Navicula* sp. 2. To investigate if *U. lens* or *Navicula* sp. is a better diet for juveniles > 3 mm in shell length.

Figure 13. Flow chart of the experiment design depicting the 2 distinct phases of the experiment and treatments T1-3, with 2 levels each (T1. high and low larval density, T2. young and old *U. lens*, T3. inoculation or no inoculation of diatoms) resulting in 8 different types of plates for the second phase of the experiment.

5.2.1.3 Preparation of plates

A removable platelet (12 cm x 17 cm) was bolted to the centre of sample plates in each basket prior to seeding and inoculation. At the time of settlement 2 sample plates, of each treatment in each basket (= 4, ∑ 128), received a platelet. Platelets are used to monitor algal species, density and cover as well as the settlement and growth of post-larvae.

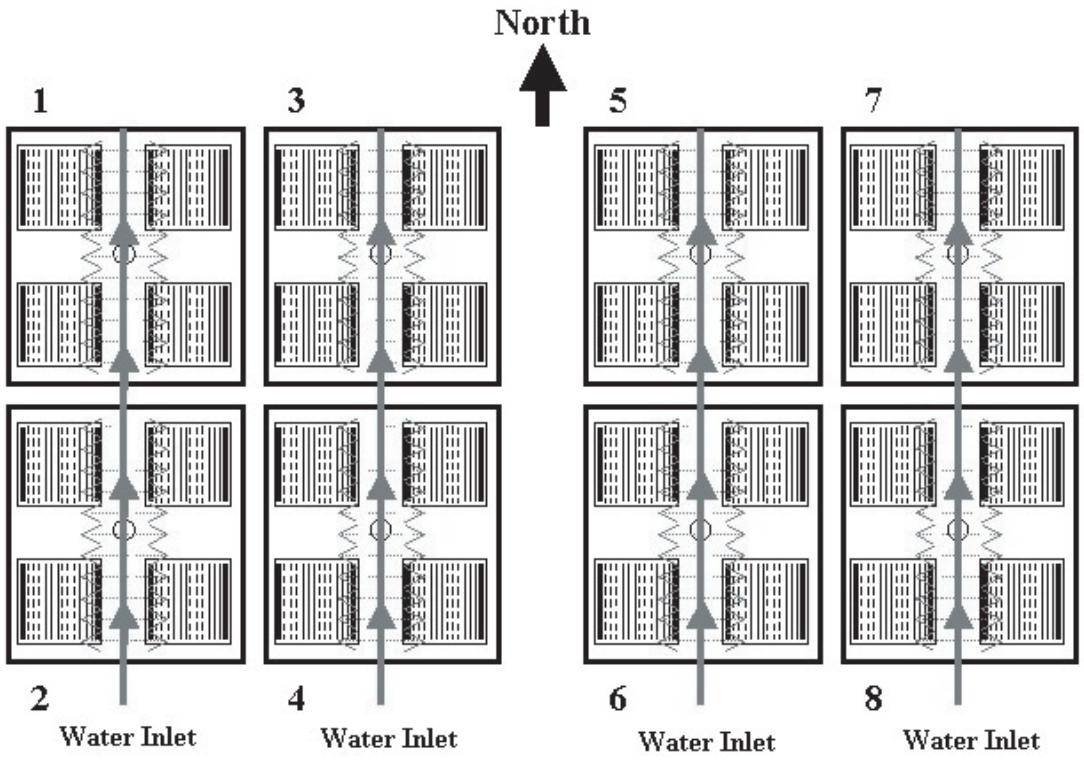

Figure 14. Diagram of tank arrangement in the nursery. Each of 8 tanks was set up with a central outlet (circle) and 14 plates in each of 4 baskets. A spray bar was arranged above two tanks (grey lines, with arrows). The plates were arranged in groups of 3 (old and young *U. lens*, dotted and continuous line respectively), with 2 edge plates (thick black line) on the side of each of the basket.

One plate of the same treatment was placed on each side of each of the sample plate (Table 11). In addition two extra plates were used to reduce the edge effect in each basket. The two plates were placed at either end of the basket (Table 11).

For algal culture methods see chapter 4.

Table 11. Design in each tank at settlement (x4).

Baskets	½ tank (1,000 L) High density of larvae (100,000)	½ tank (1,000 L) Low density of larvae (50,000)
1 (14 plates)	1, 3OU, 3YU, 3OU, 3YU, 1	1, 3OU, 3YU, 3OU, 3YU, 1
2 (14 plates)	1, 3OU, 3YU, 3OU, 3YU, 1	1, 3OU, 3YU, 3OU, 3YU, 1
3 (14 plates)	1, 3OU, 3YU, 3OU, 3YU, 1	1, 3OU, 3YU, 3OU, 3YU, 1
4 (14 plates)	1, 3OU, 3YU, 3OU, 3YU, 1	1, 3OU, 3YU, 3OU, 3YU, 1
∑	56 plates	56 plates

OU = old *Ulvella lens* (18 days after germination)
YU = young *Ulvella lens* (4 days after germination)

5.2.1.4 Larval settlement

Larvae were released into each tank at different densities (Figure 13, Table 11) and left with low water flow (1% exchange rate per hour) and low aeration for 48 hours. Banjo sieves (118 μm) were fitted onto each standpipe for the time of settlement to prevent the escape of larvae. Platelets were removed after 4 days to estimate the percentage settlement of larvae, to monitor algal density and to estimate the cover of *Ulvella lens* at settlement. Platelets were kept submerged at all times and replaced immediately after measurements. Settled larvae were counted in 6 small squares (2 x 2 cm) on each platelet (32 per treatment), the number was averaged and the total per settlement plate and tank was estimated.

5.2.1.5 Post-larval growth and survival

After settlement plates were randomized, each basket in each tank received 3 plates of each treatment combination and 1 plate on each side of the basket to minimize the edge effect (Figure 14, Table 12, 13). The seawater flow was increased to 12 % exchange rate per hour and run for 1 week. Nutrients were added to all tanks 1 week after settlement. Four of the eight tanks will be inoculated with the larger species of *Navicula* sp. (see chapter 4). Counts and measurements of post-larvae/ juveniles as well as presence and absence of algal species, estimates of algal species density and cover were undertaken every two weeks.

5.2.1.6 Growth on platelet

The growth of post-larvae was followed for 10 weeks. Four of the 8 tanks were inoculated with a supplement of cultured diatoms (*Navicula* sp.) ca. 2, 4 and 8 weeks after settlement. All tanks received 40 g of Manutec Microalgae Food 1,000 L^{-1} immediately before inoculation. Water was filtered at 1 μm during the first 8 weeks and at 5 μm until the end of the trial.

5.2.1.7 Growth and survival on the plate

Animals were observed to move off the platelets 3-4 weeks after settlement. Consequently the growth and survival of juveniles was also monitored on whole plates 22, 64, 94 and 114

days after settlement. New plates colonised by *U. lens* and inoculated with the cultured diatom *Navicula* sp. were introduced after 64 days to provide extra food and to reduce the juvenile density. Survival here describes the minimum survival on the plates without accounting for juveniles that have moved off the plates onto the tank wall or bottom and are still alive. Thus survival is likely to be underestimated.

5.2.1.8 Grading of juveniles

A sub sample of animals was graded 64 days after settlement. Two cohorts, 2.2 ± 0.11 mm and 3.1 ± 0.07 mm in shell length, were placed on new plates (*Ulvella lens* + *Navicula* sp.) at a low density of less than 50 juveniles per plate. The growth was followed for an additional 3 months (up to 141 days after settlement) to establish if the size frequency distribution of the two cohorts changes over time and whether smaller animals reach the size of larger ones providing the food is not limiting. Juveniles were measured and counted 94 and 114 days after settlement.

5.2.1.9 Light measurements

The greenhouse where the tanks were located had clear plastic walls and roof with a vent along one side of the greenhouse. A 70% shade cloth was covering the roof but not the vent. The vent was open for most of the experimental trial and light measurement were taken to confirm the visual difference in light intensity across the tanks. The light intensity in and outside each tank was measured with a Kahlsico Irradiameter Model 268-WA-310. Measurements (µw cm^{-2}) were taken on two average days (1 overcast 21/02/01, 1 sunny 22/02/01), three times a day (9 am, 12 am, 3 pm). The greenhouse were the tanks are located has clear plastic walls and a 70% shade cloth was covering the plastic roof. Each time the light was measured at the top and bottom of the tank, without a shading cloth and with 1 and 2 covers (1 cloth = 70% shading). Measurements of tanks top and bottom and over the whole day (3 times) were averaged. The appropriate measurements e.g. level of shading and weather condition (sunny or overcast), were chosen for analysis of covariance.

5.2.2 Experiment 2: Use of cultured *Ulvella lens* and diatoms to improve the settlement, growth and survival of *H. laevigata*

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This experiment with greenlip larvae was run concurrently with the blacklip trial. Animals were spawned on the same day. Larvae were reared at the same ambient temperature and were released into the tank on the same day than the blacklip larvae.

5.2.2.1 Preparation of tanks and plates

Two 2000 L tanks were set up with 8 baskets each. Two treatments were tested: 4-day young patches and 38-day-old *Ulvella lens*. Two sample plates of each treatment in each basket received a platelet (see above). One plate of the same treatment was placed on each side of the sample plate. Two plates, one at each end of the basket, were used to reduce an edge effect.

For algal culture methods see chapter 4.

5.2.2.2 Larval settlement

200,000 larvae were released into each of the two tanks and left with very low water flow and low aeration for 48 hours. Banjo sieves (118 μm) were fitted onto each standpipe for the time of settlement to prevent the escape of larvae. Platelets were removed after 3 days to estimate the percentage settlement of larvae, to monitor algal density and to estimate the cover of *Ulvella lens* at settlement. Platelets were kept submerged at all times and replaced immediately after measurements. Settled larvae were counted in 6 small squares (2 x 2 cm) on each platelet (x 24 per treatment), the number was averaged and the total per settlement plate and tank was estimated.

5.2.2.3 Growth and survival

The growth of post-larvae was followed for 10 weeks. Both tanks were inoculated with of cultured diatoms (*Navicula* sp.) as a feed supplement ca. 1, 4 and 8 weeks after settlement. Both tanks received the same dose of nutrients immediately before inoculation (40 g Manutec Microalgae Food 1,000 L^{-1}). Water was filtered at 1 μm during the first 8 weeks and at 5 μm until the end of the trial. Survival here describes the minimum survival on the plates without accounting for juveniles that have moved off the plates onto the tank wall or bottom and are still alive. Thus survival is likely to be underestimated.

5.2.3 Experiment 3: Light management (shading vs. no shading) to improve the growth and survival of *H. rubra* post-larvae

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5.2.3.1 Tank preparation

One 6,000 L tank was divided into 8 sections with separate in- and outlet (= 8 tanks). Each tank was set up with 3 baskets. Two airlines were installed, one on each side of the baskets. All plates (20 per basket) were inoculated with *Ulvella lens* for 2 weeks before larval settlement. Larvae were released into each section at the same density (38,000 per section). All tanks remained static with low aeration for 3 days.

For algal culture methods see chapter 4.

5.2.3.2 Growth and survival

The growth of post-larvae was followed for 13 weeks. Tanks were inoculated with the cultured diatom *Navicula* sp. 1, 4 and 8 weeks after settlement. Throughout the trial four of the eight tanks were shaded (shade) with double shading cloth, four tanks were kept unshaded (light). Survival here describes the minimum survival on the plates without accounting for juveniles that have moved off the plates onto the tank wall or bottom and are still alive. Thus survival is likely to be underestimated.

5.2.3.3 Light measurements

The light intensity in and outside each tank was measured with a Kahlsico Irradiameter Model 268-WA-310. Measurements (μw cm^{-2}) were taken on the 04/04/2001 at 12 pm. The tanks are

located outdoors without a shade cloth. The light was measured at the top and bottom of each tank, half of the tanks were shaded with 1 shading cloth (1 cloth = 70% shading) the other half was unshaded. Measurements of tanks top and bottom and were averaged. Light measurements were analysed in an analyses of covariance.

5.2.4 Experiment 4: The settlement of *H. laevigata* on cultured *Ulvella lens* and selected diatoms (*Cocconeis* sp. and *Navicula* sp.)

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5.2.4.1 Preparation of tanks

Three 490 L tanks were set up with 3 baskets each. Four treatments were tested: 4-day and 38-day-old *Ulvella lens* and the diatom species *Cocconeis* sp. and *Navicula* sp. Five plates of each treatment were alternated so that each tank received 15 plates of each treatment in total.

For algal culture methods see chapter 4.

5.2.4.2 Larval settlement

100,000 larvae were released into each tank and left with very low water flow and low aeration for 3 days. Banjo sieves (118 µm) were fitted onto each standpipe for the time of settlement to prevent the escape of larvae. The settlement rates of larvae were estimate on whole plates after 3 days. Plates were kept submerged in seawater during counts and replaced into tanks immediately after measurements. A grid with equal size squares was placed inside the tray and a stereomicroscope was mounted above the tray to view and assess the settled larvae.

The algal density and cover was monitored on sub samples of six plates per tank at the time of settlement.

5.2.5 Experiment 5: Settlement, growth and survival of the abalone *Haliotis laevigata* on the cultured algae (*Ulvella lens, Navicula* sp.) and the effect of early weaning onto formulated feed in comparison to live algae, on growth and survival of the juveniles

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5.2.5.1 Preparation of tanks

Three 490 L tanks were set up with 3 baskets and 20 settlement plates in each. Airlines were installed along each side and through the middle of the baskets (3 airlines per tank). Plates were conditioned with *Ulvella lens* 7 days before larval settlement and then inoculated with 10 L of cultured *Navicula* sp. per tank, 4 days before settlement.

5.2.5.2 Larval settlement

100,000 larvae were released into each tank and left with very low water flow and low aeration for 3 days. Banjo sieves (118 μm) were fitted onto each standpipe for the time of settlement to prevent the escape of larvae. The settlement rates of larvae were estimate on whole plates after 3 days (see Exp. 4).

5.2.5.3 Growth and survival

Tanks were inoculated with 10 L of *Navicula* sp. culture, 30 days after larval settlement and every 2 weeks thereafter until the end of the trial. Juveniles (< 3 mm in shell length) were reared on a combined diet of *Ulvella lens* and diatoms. Counts and measurements of algal species, density and cover as well as growth rate of the abalone were undertaken at approximately two-week intervals. Survival here describes the minimum survival on the plates without accounting for juveniles that have moved off the plates onto the tank wall or bottom and are still alive. Thus survival is likely to be underestimated.

5.2.5.4 Early weaning

After 10 weeks, when juveniles were > 3 mm in shell length in the proceeding trial, all animals were taken off the plates. Three experimental raceways (1,000 mm x 300 mm) were set up with a fixed stocking density of 2,500 animals (75% cover). Abalone were weighed and fed 24 hours later. Animals were fed to excess with a commercial weaner diet at 1% of their body weight every day for the duration of the trial. All tanks had separate inlets and outlets. Six 490 L tanks were set up with new plates (3 tanks with *U. lens* and 3 tanks with *Navicula* sp. plates). The abalone were placed onto one horizontal settlement plate on top of each basket and left for 24 hours allowing them to reattach before turning the plates vertically into the tanks. Animals were maintained on natural food and stocked at ca. 50 animals per plates (ca. 3,000 per tank).

Growth-rates of abalone were determined by measuring the size of 72 juveniles per tank or raceway every 2 weeks. The numbers of juveniles per plate and per tank/raceway were estimated in all treatments. After 10 weeks, when the natural food became limiting, animals were taken off the old plates and moved onto a new set of plates (both treatments). The trial was terminated in week 26 when animals reached 10 mm in shell length in one of the treatments.

5.2.5.5 Data analyses for all experiments

Statistical analyses were carried out using the STATISTICA computer package. Assumption of normality and homogeneity of variance were checked graphically for each data set using boxplots (means vs. variances, residuals vs. means). Numbers of settled larvae (not percentages) were analysed. Substrata were not independent at settlement in experiments 1, 2 and 4. Consequently, data from these experiments were analysed using paired *t*-tests. Total settlement rates per tank were analysed using a two-way ANOVA (experiment 1 and 4).

Shell length or growth-rates of post-larvae of each measurement was analysed separately as a one-way ANOVA (Experiment 2, 3, 5). The size of the post-larvae at 10, 22, 38 and 52 days after settlement in experiment 1 was corrected for the light gradient between tanks using a regression. The residuals of these regression and the daily mortality rate between 0-22 and 22-64 days were analysed using a 2-way ANCOVA, with *U. lens* and diatom treatment as factors (Experiment 1). Tank was used as an additional factor nested within the diatom

treatment. The initial count of post-larvae on the plates was used as covariate to account for the difference in number of post-larvae on the plate at settlement (Experiment 1). *U. lens* cover and diatom densities were analysed in a two-way ANCOVA using and light intensity as a covariate with repeated measures (Experiment 1). The average proportion of light transmitted in each treatment in Experiment 3 was compared with a t-test.

5.3 Results

5.3.1 Experiment 1: The effect of cultured algae and larval density on settlement and early growth of the abalone *Haliotis rubra*

5.3.1.1 Settlement

An average of 62% of the larvae release into the tanks settled successfully (Table 14). The higher density of larvae (100,000 per tank) resulted in 54% settlement with an estimate of ca. 1,133 larvae per plate. In the lower density tanks (50,000 per tank) an average of 70% settled which equalled ca. 739 larvae per plate.

Out of the 62% larvae settled, an average of 38% chose to settle on the plates covered with older patches of *Ulvella lens* (33% in high density tanks, 44% in low density tanks) whereas 24% were estimated on the younger *U. lens* (21% in high density tanks, 26% in low density tanks). Therefore significantly more larvae settled on the older compared to the younger *U. lens* (paired $t = 3.14$, $df = 7$, $p = 0.016$). A parallel trial running in the laboratory supported this result but gave a lower overall settlement rate (see Chapter 2; Table 1, Exp. 12). There was a significant negative correlation between the number of larvae settled and the % cover of *U. lens* ($ß = -0.80$, $p < 0.05$) but no significant correlation between the number of larvae settled and the diatom counts ($ß = -0.58$, $p > 0.05$).

Table 14. Percentage settlement of *Haliotis rubra* in the nursery 3 days after larval release (n = 4 replicated tanks, 8 plates per treatment).

Larval density	Ulvella lens	% Settlement	S.E.	% Settlement	S.E.
High	old	33.01	7.49	54.31	5.85
High	young	21.31	6.82		
Low	old	43.81	7.33	70.30	8.66
Low	young	26.49	7.59		
Average				62.31	

5.3.1.2 Growth and survival on the platelets

Post-larvae of all treatments reached 2 mm in shell length 52 days after settlement (Table 15). The highest growth-rate was measured on old *U. lens* platelets with a low density of larvae, inoculated with the cultured diatom *Navicula* sp. (Table 15). These platelets showed the highest density of diatoms (Table 16).

Table 15. Growth-rates (µm day^{-1}) of *Haliotis rubra* post-larvae and final size (mm ± standard error) on the platelets 10, 22, 38 and 52 days after settlement.

Diatoms	*U. lens*	Density of larvae	0-10	10-22	22-38	38-52	Size at 52 days
Mixed species	young	high	36.66	29.56	56.13	43.70	2.15 ± 0.08
	old	high	39.43	32.28	53.93	52.36	2.06 ± 0.12
	young	low	39.84	33.40	52.25	40.30	2.25 ± 0.09
	old	low	39.34	28.38	50.66	49.88	2.17 ± 0.10
***Navicula* sp.**	young	high	39.16	30.82	24.41	41.66	2.01 ± 0.11
	old	high	37.35	26.23	24.14	46.48	2.02 ± 0.11
	young	low	38.38	26.16	27.43	41.01	1.99 ± 0.09
	old	low	36.78	28.15	26.44	60.32	2.22 ± 0.13

The size of the post-larvae was significantly affected by the light gradient in the hatchery (Average size at 52 days (µm) = 932.8 + 108.8 Light (µW cm^{-2}), $R^2 = 0.23$, $F_{1,122} = 35.7$, $p < 0.001$). There was a highly significant impact of initial density on the growth of the post-larvae (ANCOVA, F = 48.3, df = 1, $p < 0.001$), a significant difference in the size of post-larvae growing on old and young *U. lens* (F = 7.23, df = 1, $p < 0.05$) and significant differences between tanks inoculated with diatom and the others (F = 6.92, df = 6, $p < 0.05$).

Table 16. Average *Ulvella lens* cover and diatom density in four treatments, 10, 22, 38 and 52 days after settlement.

Diatoms	*U. lens*	10	10	22	22	38	38	52	52
		% cover	cells cm^{-2}	% cover	cells cm^{-2}	% cover	cells cm^{-2}	% cover	cells cm^{-2}
Mixed species	young	70	23,567	85	1,933	89	750	87	1,900
	old	32	4,850	63	18,775	77	7,150	68	8,900
***Navicula* sp.**	young	68	10,413	81	4,850	85	7,350	91	800
	old	42	22,805	53	22,900	64	24,458	59	11,750

Early growth between 0 and 10 days after settlement was even across treatment and remained between 36 and 40 µm day^{-1} (Table 15). Growth rates then decreased significantly until 22 days after settlement after which they increased again. Growth rates then remained over 40 µm day^{-1} in all treatments. During the initial 52 days, growth was generally higher on old *U. lens* plates (Table 15).

Across all treatments, 52 days after settlement, post-larvae in tanks closest to a vent (Tank D 1,2) were larger than post larvae in tanks furthest away from the vent (Table 17). The difference was more pronounced in tanks inoculated with *Navicula* sp. Platelets with *Navicula* sp. in tanks closest to the vent supported a higher diatom density than those in tanks furthest away from the vent (Table 18). The smallest difference occurred between the 4 tanks furthest away from the vent (Tanks A1-2, B1-2).

Table 17. Average shell length (µm) of *Haliotis rubra* 52 days after settlement in tanks 1 - 7; tanks 7 and 8 were closest to the vent.

Treatment	Tanks	1-2	3-4	5-6	7-8
Mixed species	1	1,998	1,954	2,343	2,331
***Navicula* sp.**	2	1,717	1,936	2,060	2,492

Table 18. Average diatom density (cells cm^2) in tanks 1 - 7; tanks 7 and 8 were closest to the vent.

Diatoms	*U. lens*	Tanks	1-2	3-4	5-6	7-8
Mixed species	young	1	1,750	0	4,883	9,633
	old		20,350	12,150	36,100	21,533
***Navicula* sp.**	young	2	2,500	2,550	2,200	10,767
	old		10,700	17,992	28,450	35,167
Average			8,825	8,173	17,908	19,275

5.3.1.3 Growth and survival on the plate

Across all treatments post-larvae on plates (1,082 ± 14.79 µm) were on average slightly larger than those on platelets (1,001 ± 6.10 µm) 22 days after settlement but the estimated number of post-larvae per plate were slightly lower on whole plate than on platelet (354 ± 56.58 and 403 ± 42.73 respectively). However, none of the differences were significant (ANOVA, $p > 0.05$). Post-larvae were observed to move off the platelets 4-5 weeks after settlement and consequently measurements were measured on whole plates. Growth-rates on the plates averaged 37 µm day^{-1} up to 22 days after settlement and 41 µm day^{-1} between 22 and 64 days after settlement (Table 19).

Table 19. Shell length (µm) and growth-rates (µm day^{-1}) of *Haliotis rubra* on the plates 22 and 64 days after settlement.

Diatoms	*U. lens*	Density	22		64	
			shell length	Growth-rates*	shell length	Growth-rates*
Mixed species	young	high	1,090.77	37.53	2,745.38	39.40
	old	high	990.51	32.98	2,854.30	44.38
	young	low	1,143.24	39.92	2,843.17	40.47
	old	low	1,064.06	36.32	3,070.85	47.78
***Navicula* sp.**	young	high	1,146.85	40.08	2,751.49	38.21
	old	high	1,067.26	36.47	2,725.54	39.48
	young	low	1,088.02	37.41	2,828.56	41.44
	old	low	1,024.96	34.54	2,922.95	45.19

* Between consecutive sampling times.

Table 20. Shell length (µm) and growth-rates (µm day^{-1}) of *Haliotis rubra* on plates 94 and 114 days after settlement.

U. lens	Density of larvae	94		114	
		Shell length	Growth rates	Shell length	Growth rates
Young	high	5,885	104.65	7,135	62.5
Old	high	5,280	80.86	6,455	58.75
Young	low	5,920	102.56	7,695	88.75
Old	low	5,760	89.64	7,083	66.15
Average		5,711	94.43	7,092	69.04

We estimated 22% survival across all treatments 64 days after settlement. No significant difference in survival was found between the treatments ($p > 0.05$).

Juveniles reached 7 mm in shell length 114 days after settlement (Table 20). There was a significant difference between juveniles growing on old and young *U. lens* ($p < 0.001$), low and high densities of larvae ($p < 0.05$) and a significant *U. lens* and density interaction ($p < 0.001$). Juveniles on old *U. lens* with a high original larval density were significantly smaller than juveniles in all other treatment combinations ($p < 0.001$, post-hoc). The highest growth-rates were measured 94 days after settlement, after new plates had been introduced into the tanks and the number of juveniles was reduced to 160 animals per plate (Table 20).

5.3.1.4 Grading of smallest and largest juveniles

At the end of the trial the small cohort of the juveniles (2.2 ± 0.11 mm at the start) reached 9.8 mm (± 0.09) in shell length whereas the larger cohort (3.1 ± 0.07 mm at the start) averaged 10.9 mm (± 0.12) in shell length. The difference between the population means increased to 1.4 mm 93 days after settlement and to 1.8 mm 113 days after settlement (Figure 15).

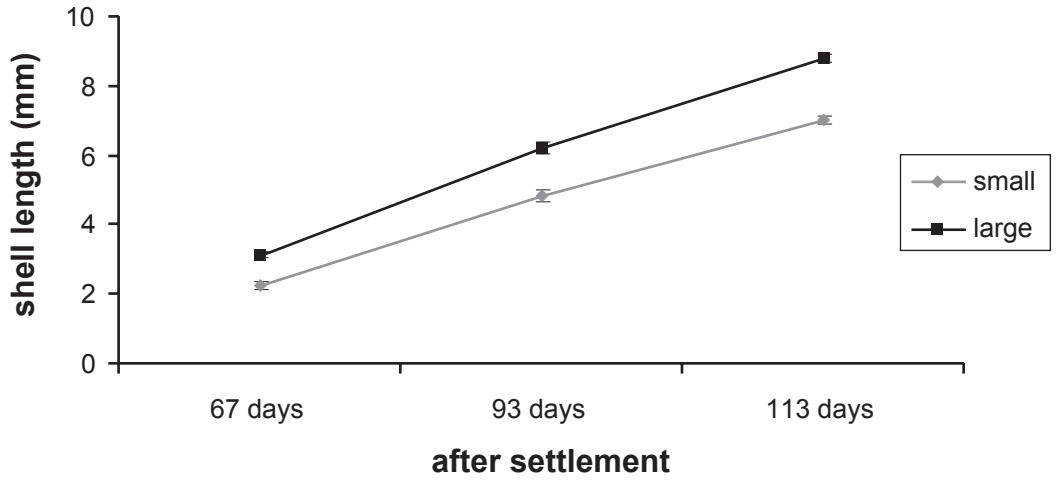

Figure 15. Shell length of two cohorts of *Haliotis rubra* post-larvae (small, large) 67, 93 and 113 days after settlement. Vertical bars indicate the standard error; n = 2 replicated tanks, 4 plates of each treatment, 20 animals per plate.

Growth-rates of larger cohort averaged 108 µm day^{-1} whereas smaller cohort grew 103 µm day^{-1}. There was no significant difference in growth rates between graded and ungraded stock (Table 20, 21; $p > 0.05$). Cohorts of graded animals became more variable in size especially in the larger cohort (Figure 16). Throughout the experiment there was a significant difference between the two cohorts ($p < 0.05$).

Table 21. Growth-rates (µm day^{-1}) of graded *Haliotis rubra* on plates 93 and 113 days after settlement.

U. lens	Density of larvae	93	113
Young	high	103.55	62.48
Old	high	86.94	51.56
Young	low	102.80	61.44
Old	low	91.44	66.14
Average		96.18	60.41

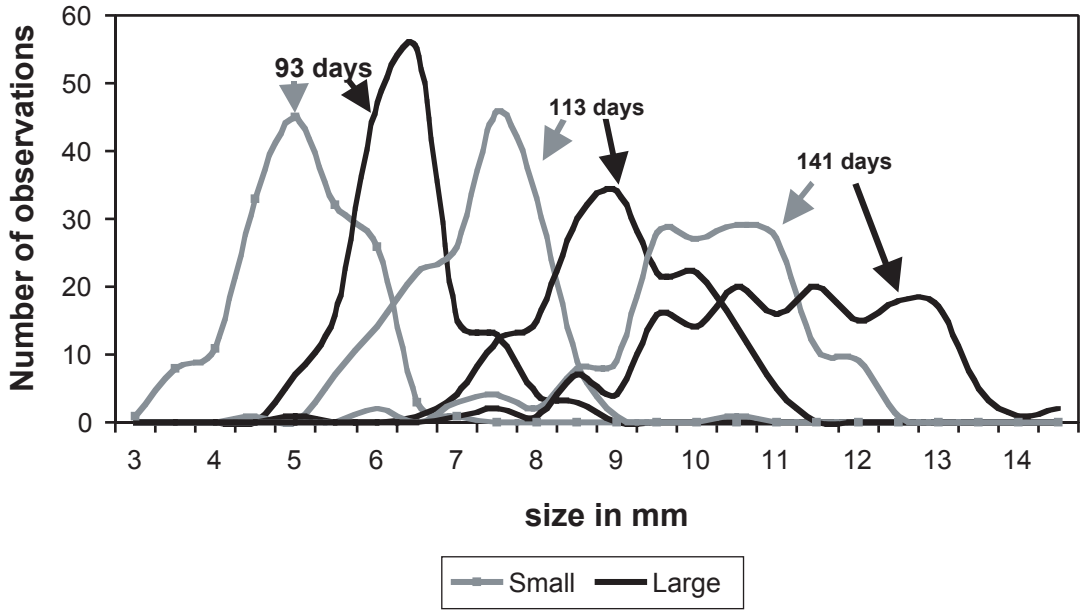

Figure 16. Change in size frequency distribution of two cohorts of *Haliotis rubra* (small, large) 93, 113 and 141 days after settlement.

5.3.2 Experiment 2: Use of cultured *Ulvella lens* and diatoms to improve the settlement, growth and survival of *H. laevigata* post-larvae

5.3.2.1 Settlement

An average of 26% of the released greenlip larvae (200,000 per tank) settled successfully (Table 22). There was no significant difference in larval settlement between old (38 days) and young (4 days) *U. lens* plates ($p > 0.05$). However, an average of 417 larvae per plate settled successfully on older plates, whereby ca. 310 larvae were estimated on each younger plate of *U. lens* (Table 22).

Table 22. Percentage settlement of *Haliotis laevigata* in the nursery 3 days after larval release (n = 24 platelets).

U. lens	% Settlement	S.E.	Number per plate
Old (38 days)	14.46	2.29	417
Young (4 days)	10.99	2.99	310
Total	25.45		

5.3.2.2 Growth and survival

Post-larvae averaged 2.7 mm in shell length 66 days after settlement (Table 23). Growth-rates were low between 9 and 21 days post settlement. Greenlips only reached ca. 0.7 mm in shell length 21 days after settlement. At the end of the experiment growth-rates were significantly higher on older compared to younger plates of *U. lens* ($p < 0.05$).

Table 23. Shell length (µm) and growth-rates (µm day^{-1}) of *Haliotis laevigata* 9, 21, 51 and 66 days after settlement

			9	21	51	66
U. lens	Young	Shell length	613.15	744.41	2,075.39	2,699.69
U. lens	Young	Growth-rates	40.91	10.94	44.37	41.62
U. lens	Old	Shell length	611.91	744.43	2,097.03	2,856.69
U. lens	Old	Growth-rates	40.77	11.04	45.09	50.64

On average we estimated 61 juveniles per plate at the end of the experiment. There was no difference between the treatments ($p > 0.05$). Consequently plates in the greenlip trial did not need to be split at the end of the experiment. More juveniles survived on younger than on older plates of *U. lens* throughout the trial (Table 24), settlement however was higher on older than on younger plates (Table 22, see above).

Table 24. Percent survival of *Haliotis laevigata* on young and old *U. lens* 21, 51 and 66 days after settlement.

Time	21		51		66	
U. lens	young	old	young	old	young	old
Survival	59.13	27.06	38.70	15.73	20.50	10.83

5.3.2.3 Algal density and cover

The percentage cover of *U. lens* was higher in younger compared to older plates throughout the trial (Table 25). The cover increased in both treatments as the experiment progressed. Significant numbers of diatom cells were only found at the start of the experiment (10 days after settlement). A higher density of diatom cells were found on the older plates which coincided with lower % cover of *U. lens*.

Table 25. Average *Ulvella lens* cover (%) and diatom density (cells cm^{-2}) on old and young plates 10, 21, and 51 days after settlement

	10	10	21	51
Treatments	***U. lens***	**Diatom**	***U. lens***	***U. lens***
Young	67.04	11680	70.08	84.69
Old	31.95	40556	34.00	56.46

5.3.3 Experiment 3: Effect of light (shading vs. no shading) on the growth and survival of *H. rubra* post-larvae

5.3.3.1 Growth and survival

Post-larvae reached 2.85 mm in shell length in the unshaded tanks and 2.20 mm in the shaded tanks 84 days after settlement (Figure 17). Post-larvae were significantly larger in unshaded tanks at the end of the experiment ($p < 0.05$). The growth-rates were low in April, 33-61 days after settlement and increased in May when juveniles reached 1.5 mm in shell length (Table 26). During April and May the water temperatures did not exceed 16º C. There was no significant difference in survival between the treatments ($p > 0.05$). Up to 70% of the post-larvae survived the first 3 weeks and 35% were still present at the end of the experiment.

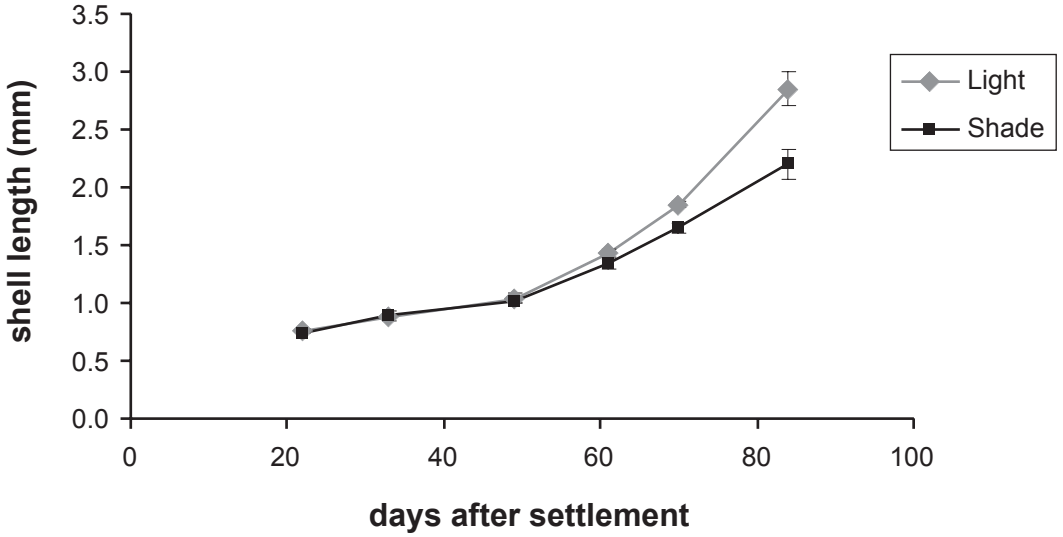

Figure 17. Shell length of *Haliotis rubra* growing in tanks without shading cloth (light) or with 2 shading cloth (shade) up to 84 days after settlement. Vertical bars indicate the standard error; n = 4 replicated tanks, 2 plates of each treatment, 10 animals per plate.

Table 26. Growth-rates (μm day^{-1}) of *Haliotis rubra* in shaded (shade) and unshaded (light) tanks up to 84 days after settlement.

	March	April	April	April	May	May
Days after settlement	**0-22**	**22-33**	**33-49**	**49-61**	**61-70**	**70-84**
Light	25.50	11.41	9.40	17.80	30.38	28.51
Shade	25.00	12.72	7.90	14.71	22.27	15.78

5.3.3.2 Light measurements

The proportion of light transmitted was significantly lower in the shaded (1.9 ± 0.2%) than in the unshaded tanks (8.7 ± 1.8 %) ($t = 3.6$, $df = 7$, $p < 0.01$). The bottom of the plates received less light (5.2 ± 1.5%) than the top (12.2 ± 2.0%) in the light treatment (paired $t = 3.04$, $df = 3$, $p = 0.056$). It was not the case in the shaded tanks where the proportion of light transmitted was similar between the top (1.6 ± 0.1%) and the bottom (2.2 ± 0.3%) of the tanks (paired $t = 2.00$, $df = 3$, $p = 0.139$).

5.3.3.3 Algal density and cover

The average cover of *U. lens* increased from ca. 55 to 75% in the shade treatment and from 68 to 91% in the light treatment during the experimental period. *U. lens* cover was significantly affected by the light treatment ($p < 0.001$) (Figure 18). The cultured diatom *Navicula* sp. dominated in unshaded tanks, however a species of *Cocconeis* sp. became dominant in shaded tanks towards the end of the experiment (Figure 18).

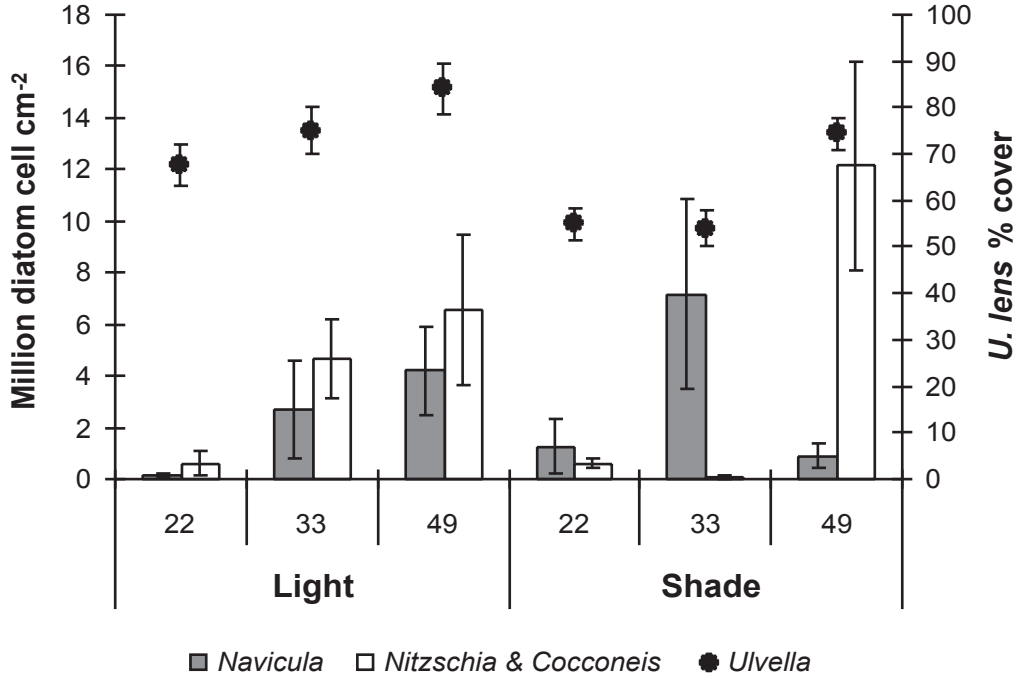

Figure 18. *Ulvella lens* percentage cover and diatom cell count on the plates at 22, 33 and 49 days after settlement.

5.3.4 Experiment 4: The settlement of *H. laevigata* on cultured *Ulvella lens* and selected diatoms (*Cocconeis* sp. and *Navicula* sp.)

5.3.4.1 Settlement

The percentage of settled larvae in all treatments is displayed in Table 27. On average we estimated ca. 1,000 larvae on old *U. lens*, 233 on young *U. lens*, 83 on *Cocconeis* sp. and 117 on *Navicula* sp. Settlement was significantly higher on old *U. lens* than on younger *U. lens* ($t = 7.19$, $p < 0.001$) and higher than on the two diatom species ($t = 8.67$, $p < 0.001$). No significant difference was found between the two diatom species ($t = 1.81$, $p = 0.07$).

There was a significant effect of tank and the position of plates in the tank ($F = 3.70$, $p = 0.03$; $F = 8.69$, $p < 0.001$ respectively). Settlement was higher in the tank closest to a clear Perspex wall (more light) compared to the tank furthest away from the wall (post-hoc $p = 0.025$). Plates closer to the outlet of the tank had higher settlement than plates in the middle or close to the other end of the tank (post-hoc $p < 0.001$).

Table 27. Percentage settlement (± S.E.) of *Haliotis laevigata* after 3 days (n = 3).

Treatments	Old *U. lens*	Young *U. lens*	*Cocconeis* sp.	*Navicula* sp.
% Settlement	61 ± 14	14 ± 1	5 ± 0.5	7 ± 0.3

5.3.4.2 Algal density and cover

The estimated percentage cover of the old *U. lens* was 97% and 82% on young *U. lens* at the time of settlement. A cell density of 4,978-cells cm^{-2} were estimated on plates in the *Cocconeis* sp. treatment and 1.5×10^5 cells cm^{-2} on plates with *Navicula* sp.

5.3.5 Experiment 5: Settlement, growth and survival of the abalone *Haliotis laevigata* on the cultured algae (*Ulvella lens, Navicula* sp.) and the effect of early weaning onto formulated feed in comparison to live algae, on growth and survival of the juveniles

5.3.5.1 Settlement, growth and survival up to 4 mm in shell length

A settlement rate of 36% ± 7.9 was estimated 3 days after larvae release. No significant difference was found between tanks or the position of the plates in the tank ($p > 0.05$).

This initial growth trial was run from November 2001 to February 2002. The water temperature ranged between 17º C and 24º C; with an average of 20º C. Water temperatures were highest during the last 4 weeks of the trial.

Juveniles growing on a mixed diet of cultured algae (*Ulvella lens*, *Navicula* sp.) reached 2.9 mm in shell length about 9 weeks after settlement. Juveniles averaged 3.9 mm in size at the end of the trial (Table 28). The growth-rates were low during the first three days after larval release, averaged about 40 µm day^{-1} during the following 41 days but were very promising at 69 µm day^{-1} during the last 4 weeks of the trial. Post-settlement survival was estimated at 46%, 16 days after settlement, and this decreased to 27% at the end of the trial.

Table 28. Shell length (µm) and growth-rates (µm day^{-1}) and survival (%) of *Haliotis laevigata* post-larvae at 3, 16, 30, 44, 58 and 72 days after larval release and fed a mixed diet of cultured algae (*Ulvella lens, Navicula* sp.).

	3	16	30	44	58	72
Shell length	338.36	749.48	1,356.48	1,921.58	2,890.33	3,850.22
Growth-rates *	26.12	37.37	43.36	40.36	69.20	68.56
Survival		46	37	29	28	27

* Between consecutive sampling times.

5.3.5.2 Algal density and cover

Ulvella lens covered between 50 and 70% of the plates for most of the trial but around 58 days after settlement the cover decreased substantially (Figure 19). Initially, *Nitzschia* sp. was the dominant species in the diatom assemblage on the plates. This species developed naturally on the plates. Tanks were first inoculated with the cultured diatom *Navicula* sp. 4 days before and than 30 days after settlement.

The cell densities of *Navicula* sp. increased 3.5 times and dominated the plates from 44 days until the end of the trial (Figure 19).

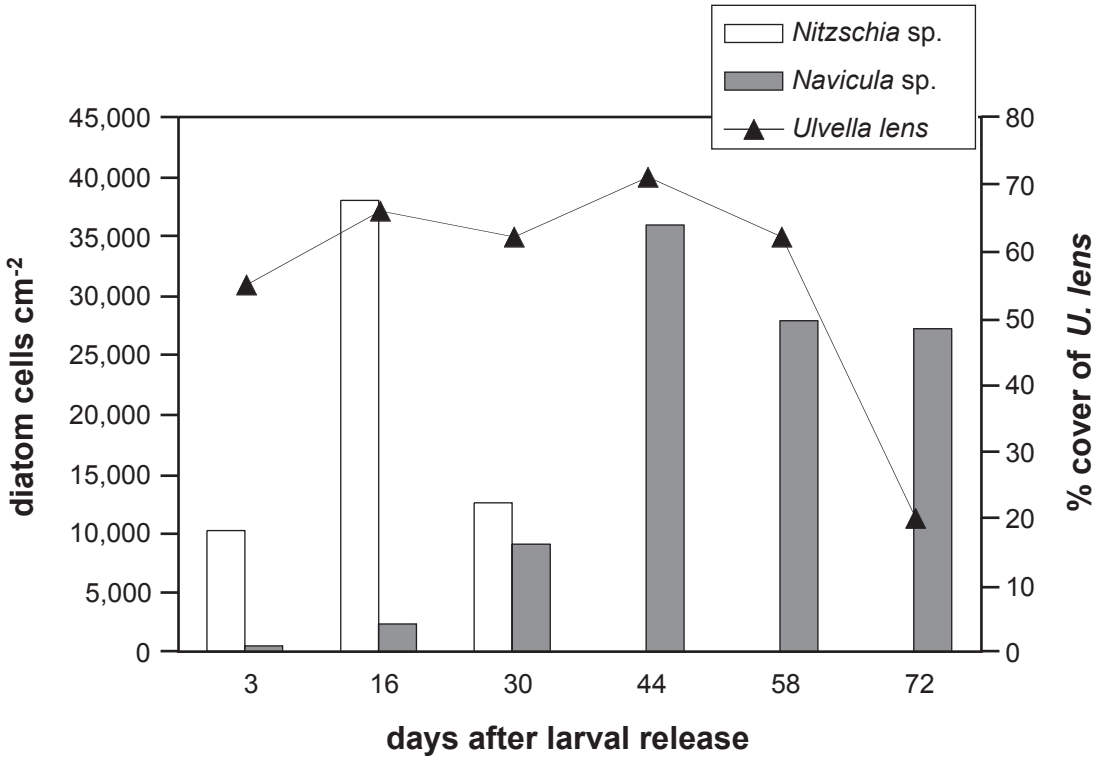

Figure 19. Cell density (cells cm^{-2}) of diatoms and cover (%) of *Ulvella lens* 3, 16, 30, 44, 58 and 72 days after larval release.

5.3.5.3 Growth and survival in early weaning trial

At the start of the weaning trial abalone averaged 3.79 ± 0.06 mm in shell length. Juveniles grew much on *Ulvella lens* than in the other two treatments (Figure 20).

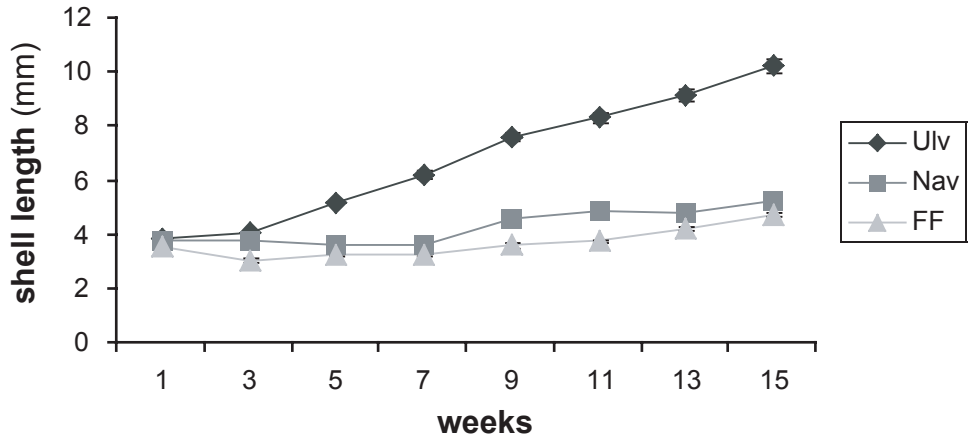

Figure 20. Shell length (µm) of *Haliotis laevigata* juveniles feeding on natural (*Ulvella lens* - Ulv, *Navicula* sp.- Nav) in comparison to formulated feed – FF.

Juveniles feeding on *U. lens* reached 10 mm in shell length in less than 15 weeks. Juveniles feeding on *U. lens* were significantly larger at the end of the trial, than juveniles feeding on the diatom *Navicula* sp. or the formulated feed ($p < 0.001$).

Growth rates were highest on *U. lens* and averaged 84 µm day^{-1} during the first 8 weeks and 63 µm day^{-1} during the following weeks after new plates had been introduced (Table 29). The growth-rates of abalone juveniles increased when the % cover of the alga decreased (Figure 21).

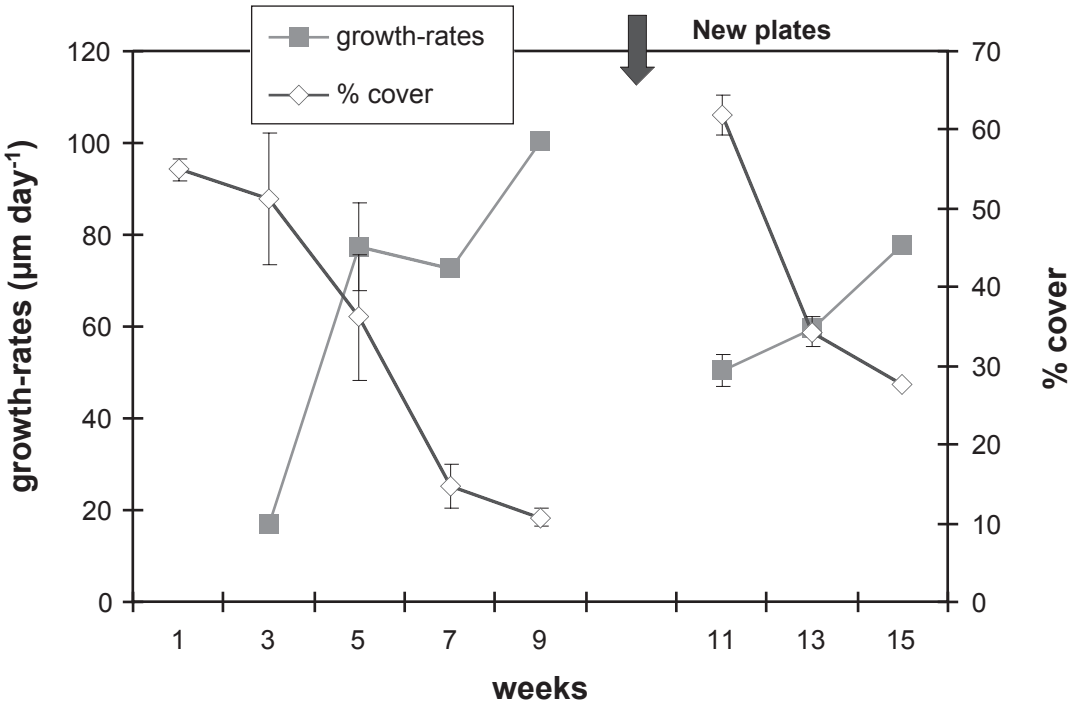

Figure 21. Relationship of the *Haliotis laevigata* growth-rates (µm day^{-1}) on *Ulvella lens* and cover (%) of *Ulvella lens* over the trial period.

After 9 weeks juveniles were moved onto new plates with higher % cover and their growth rate declined substantially possibly due to the handling stress. Growth-rates did not recover before the end of the trial. Juveniles survived best on *U. lens* followed by juveniles feeding on *Navicula* sp. Growth-rates were lowest on the formulated feed (Table 29).

Table 29. Growth-rates (µm day^{-1}) and survival (%) of *Haliotis laevigata* juveniles feeding on natural (*Ulvella lens, Navicula* sp.) in comparison to formulated feed.

	Growth-rates Week 3-9	**Growth-rates Week 9-15**	**Survival to week 15**
Ulvella lens	83.56	62.67	82.96
Navicula sp.	20.44	16.05	76.16
Formulated feed	13.54	26.63	67.06

5.3.5.4 Algal density and cover

At the start of the weaning trial, *Ulvella lens* covered about 55 % of the plates. The cover decreased continuously until only 11 % of the plates were covered after 9 weeks (Table 30).

Table 30. Average cover of *Ulvella lens* (%) and cell density of *Navicula* sp. (cells cm^{-2}) after 1, 3, 5, 7, 9, 11, 13 and 15 weeks.

	1	**3**	**5**	**7**	**9**
Ulvella lens	55	51	36	15	11
Navicula sp.	297,890	110,443	72,957	20,596	11,783
	New plates				
	11	**13**	**15**		
Ulvella lens	62	34	28		
Navicula sp.	485,126	57,549	117,549		

The cell density on the plates in the *Navicula* sp. treatment followed a similar trend. New plates were introduced after 9 weeks with even higher % cover in the *U. lens* treatment and higher cell densities in the *Navicula* sp. treatment than at the start of the trial. Both *U. lens* cover and *Navicula* sp. cell density declined rapidly.

5.4 Discussion

5.4.1 Settlement

Settlement in commercial abalone farms in Australia is unreliable and usually yields a 5-10% settlement rate (pers. obs.). Settlement rates obtained during these experiments (up to 62%) are the highest recorded in commercial facilities in Australia suggesting that the settlement induction using plates conditioned with *U. lens* was efficient. The level of settlement was close to those reported in studies on smaller scale (Chapter 2). The present study indicates that the conditioning techniques developed at smaller scale can be successfully transferred into the nursery and will greatly improve the reliability of settlement in commercial abalone farms.

Initial larval density in the first experiment with blacklip larvae did not significantly affect settlement rates. This may be an artefact of our complex experiment design, which would have required more replication at the tank level. However, the observed lower settlement rate at high larval density (even though not statistically significant) may be the result of competition for suitable settlement substrate, as larvae are known to actively explore the substrate before attachment (Harvey & Bourget, 1997; Boxshall, 2000).

In experiment 1, larvae showed a clear preference for the substrate on which they settled and older plates with a lower cover of *U. lens* were preferred. *U. lens* seems to provide a much better cue for settlement than diatom biofilm (Chapter 2); however, settlement was reduced when the cover was higher (Chapter 2, Table 1, Exp.12). In the first experiment significant more larvae settled on older compared to younger *U. lens* plates. The older plates supported lower % cover of *U. lens* than the younger plates at the time of settlement. However in the greenlip experiment this difference was not significant. The statistical power may have been too low to detect any significant difference because fewer replicates were used in the greenlip experiment. However, even in small-scale experiments the difference between some *U. lens* treatments (above 25% cover) was not as pronounced in a greenlip experiment compared to a blacklip experiment (Chapter 2, Table 1) suggesting a less specific response in greenlip larvae.

The developmental stage of *U. lens* rather than the actual cover might be important and older plants may provide a better or more pronounce cue for settlement. Alternatively, the associated biofilm, including diatoms bacteria and mucus might have been more attractive on older *U. lens* when the cover of *U. lens* is lower.

In experiment 1 plates with younger thalli of *U. lens* showed a higher overall percentage cover because more spores originally attached and germinated on those plates. These plates had more but smaller individual patches of *U. lens*. Perhaps the smaller patches are less inductive than larger but fewer patches on older *U. lens* plates. In addition, at about 2-3 weeks of development sporangia are formed and these or associated mucus could have contributed to the higher settlement rate on older plates. Settlement of *H. rubra* had been observed on the sori and conceptacles of specific coralline red algae (S. Daume, unpublished observations).

The first experiment with blacklip larvae showed very high settlement on *U. lens*. Settlement of greenlips in parallel running trial was substantially lower. Similarly, in laboratory experiments, settlement rates of the greenlip larvae on *U. lens* were much lower than rates of blacklip larvae (Chapter 2). Settlement substrates in both experiments were of similar age and percentage cover when tested. Differences in settlement response between the two species have been reported previously (Daume et al., 1999a) however differences in larval quality between the batches could have contributed. Experiment 4 and 5 were conducted with greenlip (*H. laevigata*) larvae and the settlement rate especially in experiment 4 on older *U. lens* was comparable to the first experiment with blacklip larvae indicating that batch differences may play a major role.

Experiment 4 and 5 clearly demonstrates, that *U. lens* is a suitable settlement inducer for greenlip larvae on a commercial scale. It also indicates, that larvae can distinguish between different developmental stages of the alga. As with larvae of the abalone *H. rubra*, settlement of greenlip larvae was higher on older compared to younger *U. lens* (Experiment 4). In experiment 1 the cover of *U. lens* was lower on the older plates compared to the younger plates. In experiment 4 *U. lens* covered the plates almost completely in both treatments (97%

and 82% respectively). The overall settlement rate in experiment 5 was lower than on the older *U. lens* treatment but higher than in the younger *U. lens* treatment of experiment 4, possibly because the alga was not as developed (younger) and plates had a biofilm dominated by the diatom species *Nitzschia* sp. and *Navicula* sp. at the time of settlement. Settlement experiments in the laboratory showed that settlement was reduced if plates with *U. lens* were previously inoculated with a diatom species (Chapter 2).

5.4.2 Growth

Experiment 1 and 2 were conducted between November 2000 and April 2001, taking advantage of the high water temperatures over the summer months. Factors that are likely to play a major role in the population dynamic of these early stages are competition, which is determined by the density of the animals and the food availability, which is dependent on the light because light controls the growth of the feed species.

In experiment 1 we showed that the type of substrate on which the larvae settled influences the settlement densities; and density and light subsequently affected early growth. Because of the distribution of the tanks in the nursery, the tanks supporting the fastest initial growth with a lower settlement densities, received a higher light intensity, resulting in a denser diatom biofilm. This was supported by experiment 3 where animals in tanks without shading cloth were significantly larger than animals in tanks with double shading cloth. Other parameters such as temperature did not appear to vary between tanks as a result of the constant water flow. Our results suggest that animal density and light intensity had the greatest impact on early growth through effects on food availability. The higher diatom density may have provided the post-larvae with more extracellular mucus for their early growth. In addition, bacteria and mucus associated with older *U. lens* plates may have played an important role in the early growth (Garland et al., 1985). Interestingly, this difference in early growth remained and was amplified until the end of the experiment, suggesting that early growth is important in determining later performances.

Early growth is thought to be affected by the lipid reserves of the larval yolk in the first two to three weeks after settlement (Kawamura & Takami, 1995; Roberts et al., 1999; Chapter 3). The absorption of the yolk reserves must have ended approximately 2 weeks after settlement and the growth decreased presumably as the post-larvae started feeding inefficiently on the diatoms (Kawamura et al., 1998). In this second phase, growth was best where the diatoms abundance was high and the associated intraspecific competition was low. When animals reached ca. 2 mm in shell length the growth-rates improved substantially indicating that juveniles can fully access the feed provided. This is also the time when the first respiratory pore was formed (pers. obs.). In addition, we demonstrated in experiment 5 that juveniles started to remove parts of *U. lens* about 58 days after larval release, when they reached 3 mm in shell length, which was evident by a sharp decline in percentage cover of *U. lens* and growth-rates increased substantially. The cell density of the cultured diatom *Navicula* sp. was low at the start of the trial, which could have contributed to the lower growth-rate at the earlier stages.

The survival was higher in tanks inoculated with the cultured diatom than in tanks with mixed naturally developing species 22 days after settlement. At that time tanks had been recently inoculated and the cell density of *Navicula* sp. was higher in these treatments. Naturally developing diatoms (mixed species) might have been compromised because of the high filtration at the beginning of the trial. It was mainly on plates with low *U. lens* cover that

a high cell density could be maintained through the inoculation of cultured diatoms. This explains why the *U. lens* treatment was more significantly affecting growth than the diatom treatment. This emphasises the importance of space availability for the attachment of cells and the competitive advantage for the species colonising the space early. In experiment 5 it took about 1 month for the cultured, inoculated diatom *Navicula* sp. to out-compete the naturally developed diatom species *Nitzschia* sp., which was dominant at the start of the experiment. This demonstrates that the species composition of a biofilm can be altered by inoculation of a cultured diatom. However, if competitive species are present at the time of inoculation this process might take some time.

The use of new plates covered with a good diatom biofilm and *U. lens* allowed efficient thinning of the juveniles, preventing handling stress and high labour costs. As a result, competition for space and food was reduced efficiently and growth improved significantly. Juvenile growth increased by 3 fold in a few days when the density was reduced to less than 50 juveniles per plate ca. 2 months after settlement. Hence introducing new plates have significant advantage. Our results also show the importance of the preparation of the substrate for nursery performance. Initial diatom cover had a positive impact on the growth even at later stages. Older *U. lens* plates had a lower cover allowing more diatom cells to develop. When juveniles are large enough to move readily between plates, this technique should be preferred to diatom inoculation. However cohorts of graded animals became more variable in size as the experiment progressed indicating that some of the variability in growth may not be genetic. The grading at this stage did not improve the growth-rate of the juveniles, questioning the need of the labour intensive exercise in a commercial farm situation.

U. lens was not affected as much by the light intensity as diatoms, indicating that light is not limiting for *U. lens* in shaded tanks during the summer month. However, when tanks were shaded with two shading cloths in Experiment 3, which was run over the winter period, *U. lens* cover was reduced in the shaded tanks. In addition, the light treatment appeared to affect the algal community composition on the plates in this experiment even in the first 49 days. The dominant diatom species at 49 days after settlement were *Navicula* sp. and *Cocconeis* sp. in the unshaded tanks and it was *Cocconeis* sp. only in the shaded tanks.

We successfully used shading to prevent the formation of filamentous, chain forming diatoms at the early phase of the experiment 1 and 2 without reducing the cover of *U. lens*. It is very important to maintain a low density of diatoms on the plates at the early stages of post-larval life because a thick film of fast growing diatoms can smother young animals.

Juvenile survival was higher on younger *U. lens* plates whereby settlement was higher on older plates indicating that juveniles prefer a different substrate for feeding than larvae do for settlement. Migration of the juveniles off the plates could have confounded the survival results in all experiment but could also indicate a less attractive grazing substrate.

Comparing experiment 1 and 2, growth rates and shell length of juveniles were similar at the end of the two experiments. However greenlips grew much slower at the start of the experiment and growth rates were particularly low between 9 and 21 days post settlement. Survival was lower in the greenlip trial particularly on older than younger plates of *U. lens* plates. The diatom cell density was undetectable for most of the greenlip trial and is unlikely to have contributed to the observed difference in this case.

Greenlips show higher optimal temperature requirements than blacklips (12-22° C and 8-22° C respectively) (Gilroy & Edwards, 1998). At the start of the experiments the ambient water temperature was low (ca. 16° C) and post-larvae of the greenlips might have been compromised. The third experiment was conducted from February to May 2001 with blacklip abalone and growth rates were low throughout the trial particularly in the shaded treatment. Seawater temperatures never exceeded 16° C during the time of the experiment. The low growth rate in the third experiment may partially be explained by differences in batches, lower water temperature and or feed availability during the trial.

We showed in chapter 3 that the green alga *U. lens* is not sufficient for the initial growth of the abalone *H. rubra*. However, when animals reached ca. 2 mm in shell length the growth-rates improved and were in some cases comparable to growth-rates of post-larvae feeding on diatoms. Experiment 5 clearly demonstrated that *U. lens* is a suitable food source for juveniles > 3 mm. Juveniles started to remove parts of *U. lens* ca. 58 days after larval release (about 3 mm in shell length), which was evident by a sharp decline in percentage cover of *U. lens* and resulted in an increase in growth-rates. Juveniles feeding on *U. lens* grow significantly faster and survived better than on the diatom *Navicula* sp. or the formulated feed. The formulated feed might not be well matched for the nutritional requirements of small juveniles and other formulae should be tried. The growth-rates of juveniles feeding on formulated feed improved towards the end of the trial indicating that the feed might be suitable for larger juveniles > 4 mm in shell length. Unless a suitable formulated feed is found, which can match growth-rates achieved with the macroalga *U. lens* we suggest keeping animals on plates colonised by *U. lens* as long as possible.

The potential of diatoms as a food source for juvenile abalone might be underestimated in experiment 5 because cell densities were low at times. However, we regard *U. lens* as the preferred food species for juveniles >3 mm in shell length. As demonstrated, high growth-rates can be achieved and cultures of *U. lens* can be easily maintained in the nursery.

5.4.3 Recommendations for industry

We suggest that plates with a low cover of young germlings of *U. lens* could be used for high settlement induction and followed with an inoculation with *Navicula* sp. to ensure sufficient food for the growing post-larvae during the first 1-2 month of rearing. During summer, stipe (hair-like structures) production and mass spore release of *U. lens* can cause high mortality of recently settled post-larvae if not properly managed (A. Krisinich pers. comm.). Over summer two shading cloths can be used to reduce the light intensity, which in turn reduces stipe production and spore release of *U. lens* without compromising its growth. The shading will also inhibit growth of filamentous, chain forming diatoms, which can be detrimental during the early stages of the abalone post-larval life. Over the winter period older plates could be used to induce settlement because both stipe production and spore release is hindered through lower light intensity and water temperature. New plates colonised with cultured diatoms could be introduced into the tanks for the following 2 months of post-larval development. Alternatively, settlement plates with developing *U. lens* germlings leave adequate space on the plates for a cultured diatom film to develop. After 2 months *U. lens* will provide sufficient food for the animals and plates can be re-seeded with spores or additional plates colonised with *U. lens* can be added every month to ensure sufficient food for larger juveniles.

5.5 References

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6.0 Changes in amino acid content by alteration of nitrogen levels in growth media of an algal feed species (*Navicula sp.*) and their effect on growth and survival of juvenile abalone (*Haliotis rubra*)

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6.1 Introduction and aim

Abalone, like other herbivores, grow under conditions leading potentially to nitrogen limitation (White, 1978; Mattson, 1980) because the ratio of nitrogen to fibre and carbohydrates is low in plants. Fleming (1995) found that nitrogen is a limiting nutrient for the growth of the abalone *Haliotis rubra* when feeding on seaweeds, because the intake of digestible nitrogen directly influences their growth rates. Post-larval abalone are known to feed mainly on benthic diatoms (Kawamura et al., 1998) until animals reach 7 to 8 mm in shell length and switch to eating macroalgae (Hahn, 1989). In abalone nurseries diatoms are cultured on vertical PVC plates and fed to juveniles up ca 10-12 mm in shell length. Growth rates are therefore influenced by the availability, digestibility and nutritional composition of the diatoms, which can vary substantially between species, season, tanks and even plates (Kawamurra et al., 1998, Roberts et al., 1999, Daume et al., 2000). Once juveniles reach 12-15 mm in shell length they are transferred to a different growout system where they are fed formulated feed.

Previous research has shown that the biochemical composition of microalgae varies between species (Brown et al., 1996) and is greatly affected by harvest stage, light intensity (Thompson et al., 1993; Brown et al., 1996) and culture methods (Otero & Fábregas, 1997).

Thus the biochemical composition of microalgae can be influenced by the nutrient concentration of the growth media (Fabregas et al. 1996, D`Souza & Kelly 2000) and in turn the animals fed on these algae may show changes in growth parameters (D`Souza and Kelly 2000).

There are a number of studies examining the changes in biochemical composition by changing the growing conditions of the algae (e.g. Otero and Fábregas, 1997, Thompson et al., 1993; Brown et al., 1996). However, only a few studies have investigated the effect of varied biochemical composition of the feed on the growth and survival of the animals (Fabregas et al. 1996, D`Souza and Kelly 2000).

The aim of these experiments was to determine the effect on biological performance (growth rate and survival) of juvenile abalone (1-3 mm in shell length) feeding on a single species of diatom grown under different nitrogen regimes. This size range of the animal was chosen because we established in previous experiments that post-larvae at this stage are able to ingest the diatom cells and most of their valves in faeces samples are broken. The content of cells is therefore considered to be available to the animal as a food source.

6.2 Methods

6.2.1 Experiment conducted at Southern Ocean Mariculture

6.2.1.1 Location

This experiment was conducted in May-June 2000 at Southern Ocean Mariculture (SOM), Port Fairy, Victoria, Australia. The farm provided the juvenile abalone (*Haliotis rubra*). Diatoms and juveniles were grown in an air-conditioned room at 17 ± 3° C with a 12 L: 12 D photo cycle.

6.2.1.2 Algal culture

The diatom species *Navicula* sp. was cultured in f/2 medium with varying nitrogen levels. Three treatments were tested, high nitrogen = 24.71 mg NO_3-N L^{-1}, normal = 12.35 mg NO_3-N L^{-1} and low = 2.47 mg NO_3-N L^{-1}. Cultures were allowed to adjust for 2 weeks to the new nutrient levels. Cells were grown attached to the bottom of the petri dishes until they reached a density of approximately 1 x 10^5 cells cm^{-2}. Four extra dishes of each treatment were set up for biochemical analysis and harvested at the end of the trial.

6.2.1.2 Juvenile culture

Larvae were settled in a commercial abalone farm (SOM) and grown until they reached ca. 1 mm in shell length. Seven juveniles were placed into each of 3 replicated dishes of each of the three treatments (high, normal and low nitrogen). The media was replaced every 3-4 days with the respective, fresh sterilised media. The shell length of post-larvae was measured at the beginning of the experiment and every week of the four-week trial. Dead juveniles were replaced with animals of the same feeding history.

6.2.1.3 Sample preparation and amino acid analysis

Diatom samples were collected at the end of the experiment and analysed for amino acid composition. The amino acid composition of all of the 12 samples (3 treatments x 4 replicates) was analysed. Samples were filtered onto pre-weighed glass fibre filters (Whatman GF/C, 4.7 cm diameter). Each filter was washed with 0.5 M ammonium formate to remove residual salts from the seawater medium. The filters were freeze-dried and weighed again. The samples were hydrolysed for 24 h at 100° C with 6N HCl in sealed glass tubes replaced with nitrogen. An aliquot of an appropriate amount of the hydrolysate was taken, diluted with 0.25 M borate buffer, pH adjusted to 8.5, and was filtered through a 25 μm membrane filter.

The pH-adjusted samples were reacted with 9-fluroenylmethyl chloroformate (FMOC) to form amino acid FMOC derivatives using an automated GBC LC 1610 Autosampler, with a Hypersil column (150 mm L x 4.6 mm internal diameter). L-hydroxyproline was used as an internal standard and was analysed by the precolumn fluorescence derivative method using a fully automated GBC LC 1150 HPLC (GBC Scientific Equipment, Victoria, Australia). Resulting peaks were analysed using a Winchrom software package (GBC scientific Equipment, Victoria, Australia).

6.2.2 Experiment conducted at La Trobe University

6.2.2.1 Location

This experiment was conducted in May-June 2001 at La Trobe University, Department of Botany, Melbourne, Australia. The juveniles were provided by Ocean Wave Seafoods, Lara, Victoria, Australia. Diatoms and juveniles were grown in a constant temperature room at 17 ± 2° C with a 12 L: 12 D photo cycle.

6.2.2.2 Algal culture

The diatom species *Navicula* sp. was cultured in f/2 medium with varying nitrogen levels. Three treatments were tested, high nitrogen = 24.71 mg NO_3-N L^{-1}, normal = 12.35 mg NO_3-N L^{-1} and low = 2.47 mg NO_3-N L^{-1}. Cells were grown attached to the bottom of the petri dishes until they reached a density of approximately 10^5 cells cm^{-2}.

6.2.2.3 Juvenile culture

H. rubra larvae were settled on a commercial farm and grown until they reached ca. 1 mm in shell length. Ten juveniles were placed into each of 4 replicated dishes of each of the three treatments (high, normal and low nitrogen). The media was replaced every 3-4 days with the respective, fresh autoclaved media. After 2 and 4 weeks juveniles were transferred into dishes with fresh cultures. The diatoms in previous dishes were harvested and processed for biochemical analysis. The shell length of post-larvae was measured initially and every 6-8 days for the 32-day duration of the experiment started.

6.2.2.4 Sample preparation and amino acid analysis

Diatom samples were collected at week two, four and at the end of the experiment and analysed for amino acid composition. The amino acid composition of all of the 36 samples (3 times x 3 treatments x 4 replicates) were analysed using a HPLC (GBC Scientific Equipment).

The samples were washed with 0.5 M ammonium formate to remove residual salts from the seawater medium and freeze-dried. Approximately 3 mg of each of the freeze-dried material was transferred to glass hydrolysis tubes. 0.5 mL of 6M HCL containing 1 mM phenol was added. The tubes were purged with nitrogen gas and then flame sealed. Sealed tubes were incubated at 110° C for approximately 12 hours. Hydrolysates were transferred to 1.5 mL microfuge tubes and centrifuged to remove cell debris. 50 mL of the hydrolysates were dried in a speedy-vac (Savant). The dried samples were resuspended with 100 mL of 250 mM borate buffer. The samples were derivatised for amino acid analyses using FMOC with a total derivatised sample volume of 400 mL according to the method of Ou et al. (1996).

6.2.2.5 Data analysis

Statistical analyses were carried out using the STATISTICA computer package. Assumption of normality and homogeneity of variance were checked graphically for each data set using boxplots (means vs. variances, residuals vs. means). Data of abalone shell length were analysed as a repeated measure analyses of variance with Turkeys HSD post-hoc test. Two-way analyses of variance (ANOVA) were used to test whether the dry weight of the residual algae differed among algal harvest times and between the treatments in the La Trobe experiment. Relationships between the shell length, growth and survival of the juveniles and the total

amino acid composition of the feed species at the end of the experiment were explored with simple regression analyses.

6.3 Results

6.3.1 Experiment conducted at Southern Ocean Mariculture

6.3.1.1 Amino acid composition of algae and abalone

The mean amounts of individual and total essential amino acids (TEAA), and individual and total non-essential amino acids (TNEAA) in the total amino acid (TAA) pool for algae are given in Table 31. Nine EAA and seven NEAA were quantified. The TAA content was lowest in the low nitrogen treatment (604 μmol g^{-1} dry sample) followed by the high nitrogen treatment (1134 μmol g^{-1} dry sample) and highest in normal nitrogen treatment (1526 μmol g^{-1} dry sample). Samples in low nitrogen treatment (LN) showed lower values in all amino acids except of the EAA lysine. Several essential AA (arginine, histidine and methionine) and the non-essential AA ornithine/proline were substantially lower in low nitrogen treatments. On average EAA contributed 37% of the TAA in low nitrogen treatment, 42% in normal nitrogen treatment and 46% in high nitrogen treatment. There was no significant difference in sample dry weight between the treatments (one way ANOVA, $F = 1.03$, $df = 2$, $p = 0.41$).

The mean amounts of individual and total essential amino acids (TEAA), and individual and total non-essential amino acids (TNEAA) in the total amino acid (TAA) pool, for abalone, is listed in Table 32. Both individual and total amino acids were very similar across the treatments. Approximately 31-32% of TAA were essential AA whereas the remaining 68-69% belong to the non-essential AA. The non-essential AA proline was the most dominant amino acid in pre-settled larvae and young juveniles feeding on *Navicula* sp. but not in adult *Haliotis rufescens*. Lysine contributed only 2% of TAA in animals of all treatments whereas about 6-7% of TAA are reported to be lysine in samples of abalone of other developmental stages (Table 32).

Table 31. Mean amino acid composition of algal diet *Navicula* sp. (µmol g-1 dry weight) cultured in high (HN = 24.71 mg NO_3-N L^{-1}), normal (NN = 12.35 mg NO_3-N L^{-1}) and low (LN = 2.47 mg NO_3-N L^{-1}) nitrogen f/2 growth media.

				% of TAA		
Essential	HN	NN	LN	HN	NN	LN
Arginine	69.59	131.50	25.73	8.77	8.62	4.26
Histidine	14.67	32.16	1.50	2.32	2.11	0.25
Isoleucine	34.03	68.75	28.26	4.65	4.50	4.68
Leucine	65.29	124.60	44.58	8.64	8.16	7.38
Lysine	21.40	23.22	22.23	2.53	1.52	3.68
Methionine	10.76	18.11	2.91	2.15	1.19	0.48
Phenylalanine	29.08	61.61	20.63	4.50	4.04	3.42
Threonine	68.40	97.98	45.94	6.69	6.42	7.61
Tryptophan	0.00	0.00	0.00	0.00	0.00	0.00
Valine	44.69	80.18	32.57	5.33	5.25	5.39
TEAA	357.91	638.10	224.37	45.58	41.80	37.15
Non-essential						
Alanine	86.17	154.46	65.73	7.65	10.12	10.88
Aspartate	91.38	131.94	49.67	8.47	8.64	8.22
Cystine	0.00	0.00	0.00	0.00	0.00	0.00
Glutamate	104.64	164.81	59.98	10.70	10.80	9.93
Glycine	143.28	183.79	85.03	12.21	12.04	14.08
Ornithine/Proline	0.00	0.00	0.00	0.00	0.00	0.00
Proline	223.66	83.25	38.53	5.96	5.45	6.38
Serine	90.98	113.62	54.41	7.68	7.44	9.01
Tyrosine	35.54	56.42	26.26	3.61	3.70	4.35
TNEAA	775.65	888.28	379.60	56.29	58.20	62.85
Total AA	1,133.56	1,526.39	603.96			

TEAA, total essential amino acids; **TNEAA**, total non-essential amino acids;
TAA, total amino acids

Table 32. Mean amino acid composition of *Haliotis rubra* (µmol g^{-1} dry weight) feeding on *Navicula* sp. cultured in high (HN = 24.71 mg NO_3-N L^{-1}), normal (NN = 12.35 mg NO_3-N L^{-1}) and low (LN = 2.47 mg NO_3-N L^{-1}) nitrogen f/2 growth media.

				% of TAA				
Essential	HN	NN	LN	HN	NN	LN	***H. rufescens*** [a]	***H. rubra*** [b]
Arginine	69.59	57.77	64.29	6.14	5.64	5.80	7.3	5.1
Histidine	14.67	6.46	13.63	1.29	0.63	1.23	2.0	1.5
Isoleucine	34.03	35.73	38.88	3.00	3.49	3.51	3.8	3.9
Leucine	65.29	58.64	69.73	5.76	5.72	6.29	7.8	7.3
Lysine	21.40	18.01	18.69	1.89	1.76	1.69	6.0	7.4
Methionine	10.76	19.11	0.00	0.95	1.86	0.00	2.6	2.8
Phenylalanine	29.08	26.01	30.87	2.57	2.54	2.78	4.1	3.7
Threonine	68.40	61.29	64.66	6.03	5.98	5.83	4.6	6.0
Tryptophan	0.00	0.00	0.00	0.00	0.00	0.00	0.4	0.0
Valine	44.69	42.07	45.49	3.94	4.10	4.10	4.7	4.4
TEAA	357.91	325.09	346.24	31.57	31.72	31.22	43.3	42.2
Non-essential								
Alanine	86.17	73.39	82.65	7.60	7.16	7.45	5.5	7.9
Aspartate	91.38	73.50	82.99	8.06	7.17	7.48	11.2	8.2
Cystine	0.00	0.00	0.00	0.00	0.00	0.00	0.7	11.0
Glutamate	104.64	86.87	94.50	9.23	8.48	8.52	13.5	10.6
Glycine	143.28	127.33	134.70	12.64	12.42	12.15	5.0	8.6
Ornithine/Pro	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0
Proline	223.66	214.36	239.02	19.73	20.91	21.55	2.7	12.9
Serine	90.98	84.66	92.20	8.03	8.26	8.31	4.3	7.0
Tyrosine	35.54	39.71	36.79	3.14	3.87	3.32	3.8	2.7
TNEAA	775.65	699.83	762.85	68.43	68.28	68.78	46.6	57.8
Total AA	1,133.56	1,024.92	1109.09					

TEAA, total essential amino acids; **TNEAA**, total non-essential amino acids; **TAA**, total amino acids
[a] Allen and Kilgore (1975), 2-3 g soft body, [b] Litaay et al. (2001), presettled larvae whole.

6.3.1.2 Growth and survival of juvenile abalone

At the start of the experiment, juveniles averaged 1.2 mm in shell length and reached 2 mm in both the high and normal nitrogen treatment (Figure 22). Juveniles in low nitrogen treatment however, averaged only 1.7 mm in shell length. There was a significant difference between the treatments ($F = 6.52$, $df = 2$, $p = 0.04$) and between the different measurement times ($F = 84.9$, $df = 3$, $p < 0.001$). In addition, the interaction between the treatments and time was highly significant ($F = 7.0$, $df = 6$, $p < 0.001$). The post-hoc test revealed a significant difference between low and high nitrogen (Tukeys HSD, $p = 0.04$). The growth rates of juveniles were highest in the high nitrogen treatment (42 ± 10.1 µm day^{-1}) followed by the normal nitrogen treatment (30 ± 5.5 µm day^{-1}). Growth rates were lowest in the low nitrogen treatment (21 ± 8.7 µm day^{-1}). Juveniles survived best in high nitrogen treatment (81%) followed by the normal nitrogen treatment (76%) and the low nitrogen treatment (48%).

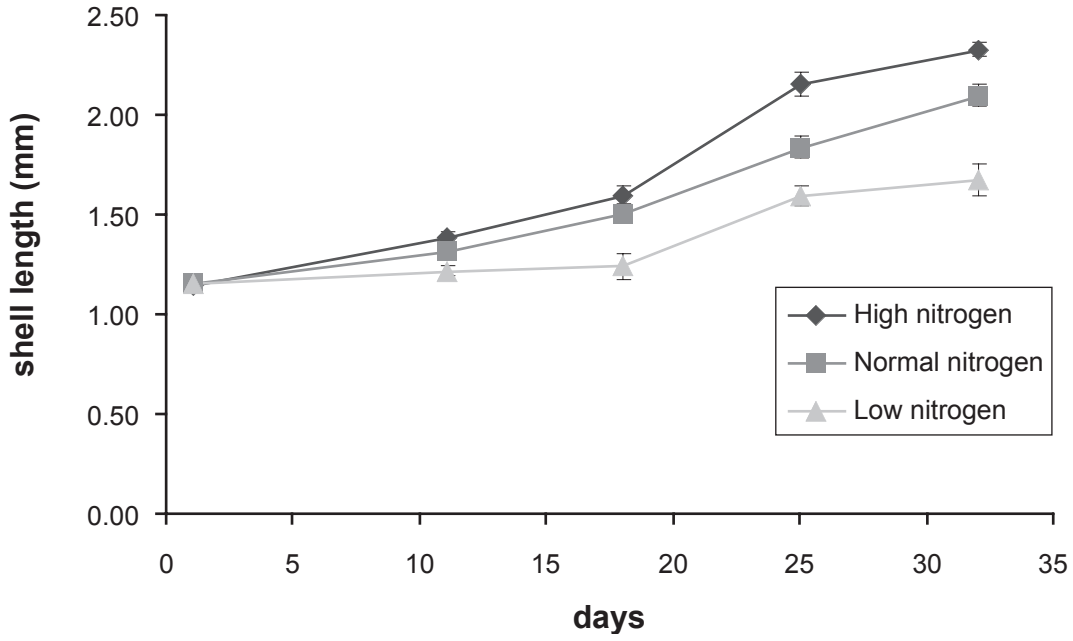

Figure 22. Shell length of *Haliotis rubra* post-larvae feeding on *Navicula* sp. cultured in high (24.71 mg NO_3-N L^{-1}), normal (12.35 mg NO_3-N L^{-1}) and low (2.47 mg NO_3-N L^{-1}) nitrogen f/2 growth media. Vertical bars indicate the standard error; n = 3 replicated dishes, 7 animals per dish.

6.3.2 Experiment conducted at La Trobe University

6.3.2.1 Amino acid composition of algae

The dry weight of algal samples did differ significantly between harvests (2 way ANOVA, $F = 44.94$, $df = 2$, $p < 0.001$). The second harvest yielded more biomass than harvest 1 and 3 resulting in less total amino acid content (Table 33). However no significant difference was observed in dry weight between treatments ($F = 0.78$, $df = 2$, $p = 0.47$).

Table 33. Mean (n = 4) total cell dry weight (mg per culture plate) and total amino acid content (µmol g^{-1} dry weight) of algal diet *Navicula* sp. cultured in high (HN = 24.71 mg NO_3-N L^{-1}), normal (NN = 12.35 mg NO_3-N L^{-1}) and low (LN = 2.47 mg NO_3-N L^{-1}) nitrogen f/2 growth media.

Harvest	Treatment	Total cell dry weight	TAA per g cell dry weight
1	LN	35.23	477.69
1	NN	31.44	695.57
1	HN	27.97	686.36
2	LN	115.12	174.71
2	NN	94.43	367.01
2	HN	88.78	307.00
3	LN	24.39	510.15
3	NN	24.65	859.19
3	HN	27.00	858.85

The mean amount of individual and total essential amino acids (TEAA), and individual and total non-essential amino acids (TNEAA) in the total amino acid (TAA) pool for each harvests is given in Table 34. Nine EAA and eight NEAA were quantified. In low nitrogen treatments (LN) all EAA and NEAA decreased. The decrease was particularly high in the essential EAA arginine (about 50%). Individual amino acids in samples from the high and normal nitrogen treatments were very similar. In the low nitrogen treatment the total amino acid content (TAA = 407 μmol g^{-1} dry sample) was reduced by ca. 37% compared to the high and normal nitrogen treatments (TAA = 643 μmol g^{-1} dry sample, 641 μmol g^{-1} dry sample respectively) (Table 34). The reduction was slightly higher in essential amino acids (EAA = 40%) than in non-essential amino acids (NEAA = 35%). On average EAA contributed 47% of the TAA in low nitrogen treatments, whereas an average of 49% EAA where estimated in both high and normal nitrogen treatments.

Table 34. Mean amino acid composition (n = 4) and % TAA of *Navicula* sp. (μmol g^{-1} dry weight) and cultured in high (HN = 24.71 mg NO_3-N L^{-1}), normal (NN = 12.35 mg NO_3-N L^{-1}) and low (LN = 2.47 mg NO_3-N L^{-1}) nitrogen f/2 growth media and % TAA of abalone species.

Harvest	**Harvest 1**			**Harvest 2**			**Harvest 3**			**% TAA**		
Essential	HN	NN	LN	HN	NN	LN	HN	NN	LN	HN	NN	LN
Arginine	61.0	66.8	33.1	28.7	38.6	12.9	58.8	54.2	27.8	7.7	8.3	6.0
Histidine	15.0	15.3	10.7	8.2	8.2	4.5	21.1	21.5	10.6	2.3	2.3	2.1
Isoleucine	31.6	30.7	21.5	21.0	18.4	12.4	45.9	45.5	25.2	5.1	4.9	4.8
Leucine	59.1	58.7	41.5	34.3	30.2	20.4	82.0	83.1	47.7	9.1	8.9	9.0
Lysine	28.5	30.3	18.9	17.4	17.3	10.1	39.4	39.3	23.6	4.4	4.5	4.3
Methionine	18.9	18.4	13.3	10.7	10.4	6.3	22.3	22.5	14.4	2.7	2.7	2.8
Phenylalanine	24.4	24.0	17.6	14.1	12.4	8.6	33.3	33.7	20.1	3.7	3.6	3.8
Threonine	44.2	45.1	33.0	26.4	23.6	17.6	58.6	58.6	35.5	6.7	6.6	7.0
Tryptophan	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Valine	45.9	45.7	32.8	26.2	23.6	15.5	66.4	67.5	38.7	7.2	7.1	7.1
TEAA	328.6	335.0	222.4	187.0	182.7	108.3	427.8	425.9	243.6	48.9	49.1	47.0
Non-essential												
Alanine	67.5	67.5	48.1	37.3	34.2	22.8	87.4	89.6	53.8	10.0	10.0	10.2
Aspartate	51.2	51.3	39.5	30.2	28.9	18.5	42.6	45.2	33.1	6.4	6.5	7.5
Cystine	6.4	6.3	4.5	3.5	3.4	1.9	7.6	7.6	5.2	0.9	0.9	0.9
Glutamate	45.7	50.3	33.5	25.6	26.7	14.7	46.4	42.1	28.1	6.1	6.2	6.2
Glycine	80.5	81.5	56.6	44.0	38.9	25.6	112.8	113.0	64.0	12.3	12.1	12.0
Ornithine/Pro	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Proline	38.4	38.6	27.4	21.5	19.6	13.2	49.6	50.3	30.4	5.7	5.6	5.8
Serine	45.7	45.6	33.1	24.6	22.0	15.1	60.9	61.1	37.9	6.8	6.7	7.0
Tyrosine	20.1	19.5	12.6	11.7	10.7	13.8	23.8	24.4	14.0	2.9	2.8	3.3
TNEAA	355.5	360.6	255.3	198.4	184.4	125.6	431.1	433.3	266.5	51.1	50.9	53.0
TAA	684.1	696	477.7	385.4	367.1	233.9	858.9	859.2	510.1			

6.3.2.2 Growth and survival of juvenile abalone

Juveniles averaged 1.8 mm in shell length at the start of the experiment. Animals feeding on the diatoms grown in high and normal nitrogen media reached 3 mm in shell length after 33 days (Figure 23). Juveniles in low nitrogen treatment were on average 0.3 mm smaller

at the end of the trial. There was a significant difference between the treatments ($F = 12.2$, $df = 2$, $p = 0.005$) and between the different measurement times ($F = 387.1$, $df = 5$, $p < 0.0001$). In addition the interaction between the treatments and time was highly significant ($F = 8.7$, $df = 10$, $p < 0.001$). The post-hoc test revealed a significant difference between low and high nitrogen (Tukeys HSD, $p = 0.007$) and between low and normal nitrogen (Tukeys HSD, $p = 0.018$).

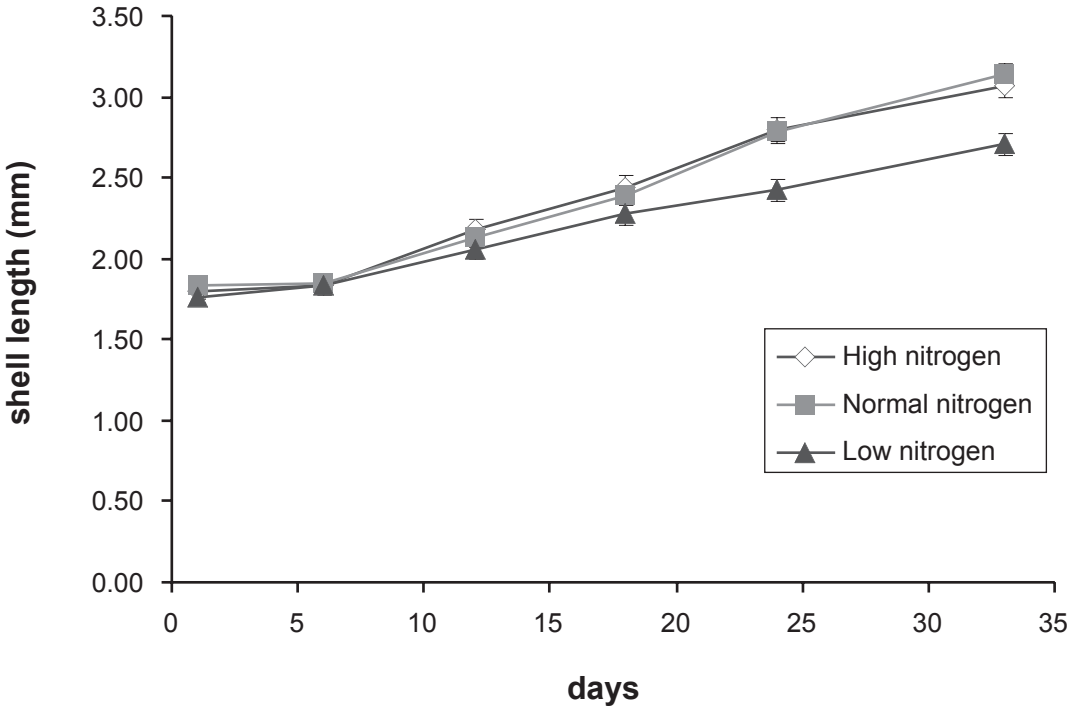

Figure 23. Shell length of *Haliotis rubra* post-larvae feeding on *Navicula* sp. cultured in high (24.71 mg NO_3-N L^{-1}), normal (12.35 mg NO_3-N L^{-1}) and low (2.47 mg NO_3-N L^{-1}) nitrogen f/2 growth media. Vertical bars indicate the standard error; n = 4 replicated dishes, 10 animals per dish.

The growth rates of juveniles were highest in the high nitrogen treatment (43 ± 6.5 μm day^{-1}) followed by the normal nitrogen treatment (40 ± 9.3 μm day^{-1}). Growth rates were lowest in the low nitrogen treatment (31 ± 4.4 μm day^{-1}). Juveniles survived best in high and normal nitrogen treatment (88%). The survival was lower in low nitrogen treatment (75%).

Final length and growth rates were significantly positively correlated with the total amino acid content at the end of the experiment ($F = 9.40$, $df = 1$, $p = 0.01$), ($F = 8.71$, $df = 1$, $p = 0.014$). The correlation between survival and total amino acid content was not significant ($F = 1.03$, $df = 1$, $p = 0.33$). There was no significant correlation between the total dry weight and the shell growth ($F = 2.57$, $df = 1$, $p = 0.14$) or between the total dry weight and the survival ($F = 1.27$, $df = 1$, $p = 0.27$) at the end of the experiment.

6.4 Discussion

Juveniles of the abalone *Haliotis rubra* feeding on the diatom *Navicula* sp. grown in limited nitrogen media grow slower than when feeding on the same species of diatom grown in normal

or higher nitrogen media. Shell length and growth rates were affected by the diet and, as evident from the significant correlation, the differences were most likely related to the total amino acid contents of the diets. In addition, survival was lower in low nitrogen treatment. However, no significant correlation could be found between survival and the total amino acid contents of the diets.

It is uncertain which other factors related to the diets might have affected survival. We observed that cells in all treatments were readily ingested so it is unlikely that diatoms in the LN treatment produce a feeding deterrent. No significant differences in biomass yield of residual food were found between the treatments. The growth rate of the feed species however, could have varied under different nitrogen regimes, providing juveniles with different amounts of feed. These however, must have been compensated by different grazing rates of the animals to result in similar residual biomass between the treatments. Food was always offered in access and is thus unlikely to cause differences in survival.

Brown (1991) reported that aspartate and glutamate were generally the highest components in amino acid profiles of 16 species of microalgae. In contrast, we document that alanine and glycine were in highest concentration (> 10% of TAA) of the diatom *Navicula* sp. In agreement with Brown (1991) we found arginine and leucine to be the highest components in the pool of EAA.

The nutritional value of a feed species is considered to be high if its essential amino-acid composition is closely matched to that in the feeding animal. However, this does not suggest that absolute levels of amino acid in the muscle are reflections of the absolute requirements for that amino acid (Fleming et al., 1996). Adult abalones are reported to be high in aspartate and glutamate whereas arginine, leucine and lysine are the most common ones in the pool of essential amino acid (Allen & Kilgore, 1975). The relative proportions of individual essential amino acids in the algal diets in this study were in most cases greater or similar than in the abalone of two different species and different developmental stages (see Table 32). The proportion of lysine, however, was less indicating that lysine might be limiting. Similarly, Brown (1991) found a lower proportion of lysine in all microalgal species investigated compared to 10-day old oyster larvae. They suggested that this amino acid could be limiting in normal algal diets of the animal. Lysine is reported to be the major limiting amino acid in pigs feeding on cereal-based diets (Batterham, 1992). Similar to adult abalone, in our study lysine was high in larvae but substantially lower in juveniles only feeding on *Navicula* sp., as well as the diet. However, the differences between larvae, juvenile and adult abalone displayed in Table 32 have to be treated carefully because different studies and species of abalone were compared.

Proline was the most predominant NEAA followed by glutamate and glycine in pre-settled larvae and young juveniles feeding on *Navicula* sp. only, whereas leucine, threonine and arginine were found in high quantity in the pool of EAA (Litaay et al., 2001; Daume unpublished data; Table 32). Proline is considered to be an essential amino acid for molluscs by Harrison (1975) but this was not confirmed for abalone (Allen and Kilgore, 1975).

Despite the similarity in amino acid composition of the treatments, the total amounts varied significantly between the treatments. TAA was lower in the low nitrogen treatment at all three times of harvest and TAA was correlated with the growth of the juveniles. Similarly, Fleming (1995) found a significant relationship between the intakes of digestible nitrogen and the growth rates of adult *H. rubra* when feeding on seaweeds, suggesting that nitrogen is a limiting

nutrient for abalone growth. Juvenile abalone are likely to require more protein and energy per unit of body weight because their rate of growth is higher than that of adults. Thus, it is likely that any small increase in nitrogen and TAA in the feed can result in an increase in growth-rate even over a short period of time.

At abalone farms in Australia the seawater is enriched with a mixed fertiliser (Aquasol, Hortico Ltd and Sodium metasilicate) at approximately 3-4 mg L^{-1} equivalent to 0.7-0.9 mg NO_3-N L^{-1} to enhance diatom growth. This dose is well below the level in our low nitrogen treatment and our result suggests that algae grown in the nursery might be nitrogen limited. In addition, Henley et al. (1991) showed that N-deficiency causes greater sensitivity to bright light in algae and prevented recovery of photoinhibition. This indicates that a too low dose of nitrogen in the high light nursery environment can also influence the quantity (growth rate) of the algae.

We suggest, in order to achieve uniformity in nutritional quality of feed species in abalone nurseries, the nitrogen level should be monitored and supplied at an optimal rate between 2-12 mg NO_3-N L^{-1}. A higher nitrogen level would be wasteful and uneconomic because the TAA did not further increase and did not result in higher growth-rates of the abalone. This indicates that the TAA level in the NN treatment was sufficient to maintain high growth-rates of the abalone. Pulse feeding could be considered where nutrients are only added 1-2 times a week and the water flow is turned off for a period of 24 hours. Ryther et al. (1981) showed that nitrogen-starved algae could take up nitrogen very fast and double their total nitrogen content in less than 8 hours. Algae returned to not enriched flowing seawater were able to maintain the higher growth for up to 2 weeks.

In this study we demonstrated the importance of maintaining the right level of nitrogen to achieve a high value feed for optimal abalone juvenile growth. Further studies are needed to determine the feeding regime of nutrient addition to the tanks to maintain high quality and quantity of feed under nursery conditions and to increase the cost efficiency of algal production in abalone nurseries.

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7.0 Benefits

This project has made significant improvements in nursery management strategies for both blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*) abalone. The aim of the project has been to determine suitable algal species for improving settlement and growth-rates of abalone during the nursery phase. We identified the green alga *Ulvella lens* as a suitable settlement inducer for both *Haliotis rubra* and *H. laevigata* larvae. Settlement rates have been improved from 0-10% to 40-60%, which is well beyond the 20-30% set as a performance indicator in the original application. The surface area needed for settlement (settlement plates and tanks) will be reduced, which will result in lower capital and labour costs through weaning or re-settlement. Methods have been developed for commercial scale algal culture. By the end of this project, the culture methods for *Ulvella lens* had been adopted at most abalone farms in Australia. The industry will benefit by high and consistent larval settlement. Beyond the scope of this project *U. lens* proved to be a good food source for juveniles larger than 3 mm in shell length. This will help to overcome problems of shortage in adequate feed (both quantity and quality) on the plates particularly at later stages of the nursery phase. However, *U. lens* has limited value as a feed for growing post-larvae. Instead, a cultured diatom species can be added after the settlement phase.

During this project we identified a suitable diatom species (*Navicula* sp.) that can be cultured on a commercial scale. Promising growth-rates of 30-40 μm day^{-1} were achieved with this diatom species during the first 2 months of post-larval development. Methods for large-scale culture were refined to allow the culture of this attaching, benthic diatom species. However, only batch cultures were used in this project. The mass culture of diatoms has not been adopted at farms in southern Australia as yet because abalone farmers regard diatom production as being too costly. Better control and intensification of the system has been achieved through the mass culture of selected diatom species. The survival of juvenile abalone was significantly higher and the feed on the plate was improved in both quality and quantity. As a result, higher densities of juveniles were maintained per plate throughout the nursery phase. Growth-rates of post-larvae were improved by about 30-50%, and hence the cost for animals up to 10 mm will decrease and seed can be produced cheaper. Since the initial results of this project were conveyed to and adopted by industry, juvenile abalone prices have in fact declined.

8.0 Further development

The industry is currently experiencing high juvenile mortalities at weaning and food shortage at the later stages of the nursery phase. Further research is needed to improve formulated feeds for younger juveniles. Alternatively, a suitable algal species could be sought to improve growth-rates and harvest size of juveniles at the end of the nursery phase (juveniles 5 mm+ in shell length. In addition, the mass culture of diatoms and other algal species needs to be optimised to increase the cost efficiency of algal production. An interactive CD has been produced to extend the presently used methods for algal culture to industry in a user-friendly form. This CD could be extended to include a cost-benefit analysis of management strategies, which address issues such as stocking density and food management on the plate. This would provide a decision making tool for farmers, to select a nursery system that meets their needs.

9.0 Conclusion

9.1 Settlement – laboratory

Laboratory experiments in Chapter 1 and 2 have shown that the macroalga *Ulvella lens* is a suitable settlement inducer for larvae of the abalone *Haliotis rubra* and *Haliotis laevigata.* Settlement rates have been improved from around 1-10% to 30-60%. Settlement was significantly lower on plates with biofilms dominated by diatoms. Settlement was higher on older compared to younger plants and biofilms but settlement on *U. lens* appears to be reduced when previously inoculated with a diatom species. However, inoculation with diatoms has advantages for the subsequent growth of the post-larvae. Settlement plates conditioned with algae (*U. lens* or diatoms) that were pre-grazed by juvenile abalone induced higher larval settlement than plates that were not grazed prior to larval settlement.

Greenlip (*H. laevigata*) larvae show less species-specific response. The difference between the algal species and developmental stages tested were not as pronounced than with blacklip (*H. rubra*) larvae.

Marked differences in overall settlement rates were found between larval batches. Antibiotic treatment enhanced the survival of the larvae in experimental containers but did not enhance the settlement rate nor did it change settlement preferences. The results indicate that unfit larval may survive if treated with antibiotics but they do not settle successfully.

9.2 Growth and survival – laboratory

Growth-rates reached 30-40 µm day^{-1} when feeding on the cultured diatom *Navicula* sp. during the first two months of rearing. Growth-rates were significantly lower on the macroalga *U. lens*. The green alga *U. lens* is not sufficient for the initial growth of the abalone *H. rubra*. However, when animals reached ca. 2 mm in shell length the growth-rates improved and were in some cases comparable to growth-rates of juveniles feeding on diatoms. When single species diets were compared to a combination of the macroalga *U. lens* and the diatom *Navicula* sp., growth-rates reached 42 µm day^{-1} on the combined diet but only 35 µm day^{-1} on single species diets. Survival up to 2 months post settlement was highest on the combination (70%) and lowest on the diatom only.

9.3 Settlement – commercial scale

Settlement rates in large-scale experiments in the nursery were amongst the highest recorded in commercial nurseries suggesting that the conditioning technique of the settlement plate using the macroalgae *U. lens* was efficient and reliable. Larval release density was not detected as a significant effect on settlement rates of *H. rubra*. Larvae showed a strong preference for substrates covered with older *U. lens*. The settlement was higher on older *U. lens* plates with less percentage cover.

9.4 Growth and survival – commercial scale

Growth rates were improved to 35 µm day^{-1} during the first month, 50 µm day^{-1} during the second month and 100 µm day^{-1} during the third month of rearing. The experiments demonstrated,

that animal density and light (which affects the food density) have very remarkable effects on growth. The survival was strongly density-dependent after 64 days when the food started to become limiting.

Experiment 5 in Chapter 5 clearly demonstrated that *U. lens* is a suitable food source for juveniles larger than 3 mm. Juveniles started to remove parts of *U. lens* when they reached 3 mm in shell length. Juveniles feeding on *U. lens* grow significantly faster and survived better than on the diatom *Navicula* sp. or a formulated feed. The formulated feed used might not be well matched for the nutritional requirements of small juveniles and other formulae should be tried. The growth-rates of juveniles feeding on formulated feed improved towards the end of the trial indicating that the feed might be suitable for juveniles larger than 4 mm in shell length. Unless a suitable formulated feed is found, which can match growth-rates achieved with the macroalga *U. lens* we suggest keeping animals on plates colonised by *U. lens* as long as possible.

9.5 Algal culture and biochemical composition of algae

Methods were developed for large-scale culture and succession of algal species and are outlined in chapter 4. Plates are initially colonised with *U. lens* for settlement and after settlement inoculated with the cultured diatom *Navicula* sp. for post-larval growth. Plates with recently settled larvae were inoculated without any harm to the animals.

Nitrogen is regarded as a limiting nutrient for abalone growth (Fleming, 1995) and we established in Chapter 6 that a 10-fold increase in nitrogen resulted in higher total amino acid levels in the diatom feed species and an increase in growth-rates of the juveniles. We suggest, in order to achieve uniformity in nutritional quality of feed species in abalone nurseries, the nitrogen level should be monitored and supplied at an optimal rate between 2 and 12 mg NO_3-L^{-1}. A higher nitrogen level would be wasteful and uneconomic, while lower nitrogen levels would result in lower growth rates of the juveniles.

In collaboration with CSIRO we established, that there is a difference in fatty acid compositions between the diatom and the macroalgal species used in this study. *Navicula* sp. is rich in 20:5n-3 whereas the major fatty acids in *U. lens* include 18:2n-6 and 18:3n-3 (see Appendix 1 for details). In contrast to other macroalgae, *U. lens* had a relatively high protein content of 31-38 %, which is comparable to that of diatom species like *Navicula* sp. This could explain why juveniles larger than 3 mm in shell length grew very well on *U. lens*.

9.6 Recommendations to industry

We suggest that plates with a low cover of young germlings of *U. lens* could be used for settlement induction and followed with an inoculation with *Navicula* sp. to ensure sufficient food for the growing post-larvae during the first 1-2 months of rearing. Alternatively, new plates colonised with cultured diatoms only could be introduced into the tanks if older plates with very high cover of *U. lens* are used for settlement. Settlement plates with developing *U. lens* germlings leave adequate space on the plates for a cultured diatom film to develop but will result in a lower settlement density. After 2 months *U. lens* will provide sufficient food for the animals and plates can be re-seeded with spores or alternatively additional plates colonised with *U. lens* can be added every month. In addition the stocking density should be reduced to 50 juveniles per plate before the food becomes limiting.

10.0 Appendices

Appendix 1 Analysis of algal feeds for abalone culture: Biochemical assessment and abalone growth trials

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Analysis of algal feeds for abalone culture: Biochemical assessment and abalone growth trials

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SECTION 1: Biochemical Analysis of Algae used for Abalone Feed

Outline

As the first part of the contract, CSIRO Marine Research analysed the proximate composition of samples of 29 algae used as diatom feed. They were supplied by Dr Sabine Daume, Deakin University.

Sample history and processing (prior to analysis)

Samples were dispatched to CSIRO on two occasions:

1. On the 10/10/2000, CSIRO received filtered, frozen samples of both *Cylindrotheca closterium* and *Navicula* sp. These were obtained from log and stationary phase from three replicate cultures, provided as 2 x 47 mm filters per culture – thus making a total of 24 filters. The samples had been filtered through pre-weighed filters, washed with ammonium formate (done at Deakin) and upon arrival to CSIRO were freeze-dried to estimate the material available per filter for analyses.

   At the same time, additional cultures were received and filtered separately (250 to 400 mL culture) through pre-weighed, precombusted filters, washed with ammonium formate, dried (100° C, 24 h), then ashed (450º C, 24 h) for the determination of the % ash weight.

2. On the 14/11/00, a second batch of samples were received, as frozen samples (harvested at Deakin and washed with ammonium formate) in glass vials. These were from *Ulvella* lens, *Sporolithon* sp, and *Cocconeis* sp. Samples were also immediately freeze dried, and weighed to estimate the material available for analysis.

Sample codes, and weight available for the analyses are shown in Tables 1 and 2.

Table 1. Filter samples supplied on 10/10, and the dry weight available for analysis.

Vial id	Sample	Filter wt (g)	Filter + sample wt (g)	sample supplied (mg)
1	*Cylindrotheca* (stat)	0.0861	0.1214	35.3
2	*Cylindrotheca* (stat)	0.0867	0.1268	40.1
3	*Cylindrotheca* (stat)	0.0875	0.1279	40.4
4	*Cylindrotheca* (stat)	0.0869	0.1281	41.2
5	*Cylindrotheca* (stat)	0.0868	0.1273	40.5
6	*Cylindrotheca* (stat)	0.0861	0.0995	13.4
7	*Cylindrotheca* (log)	0.0872	0.1129	25.7
8	*Cylindrotheca* (log)	0.0849	0.1151	30.2
9	*Cylindrotheca* (log)	0.0859	0.1225	36.6
10	*Cylindrotheca* (log)	0.0861	0.1124	26.3
11	*Cylindrotheca* (log)	0.0862	0.1079	21.7
12	*Cylindrotheca* (log)	0.085	0.0927	7.7
13	*Navicula* (stat)	0.085	0.1174	32.4
14	*Navicula* (stat)	0.0853	0.1241	38.8
15	*Navicula* (stat)	0.0842	0.1138	29.6
16	*Navicula* (stat)	0.0846	0.1178	33.2
17	*Navicula* (stat)	0.0842	0.1344	50.2
18	*Navicula* (stat)	0.0845	0.1179	33.4
19	*Navicula* (log)	0.0836	0.1067	23.1
20	*Navicula* (log)	0.0835	0.1028	19.3
21	*Navicula* (log)	0.0843	0.1215	37.2
22	*Navicula* (log)	0.0849	0.1212	36.3
23	*Navicula* (log)	0.0844	0.1141	29.7
24	*Navicula* (log)	0.0846	0.0904	5.8

Table 2. Samples supplied on 14/11/00 and the dry weight available for analysis.

Sample i.d.	Sample name	wt of sample (mg)
1	*Cocconeis* - 4 weeks	6
2	*Cocconeis* - 4 weeks	227
3	*Cocconeis* - 4 weeks	144
4	*Cocconeis* - 2 weeks	12
5	*Cocconeis* - 2 weeks	10
6	*Cocconeis* - 2 weeks	13
7	*Cocconeis* sp.	13
8	*Cocconeis* sp.	12
9	*Cocconeis* sp.	8
I	*Ulvella lens*	180
II	*Ulvella lens*	120
III	*Ulvella lens*	244
IV	*Ulvella lens*	168
V	*Sporolithon durum*	1,318
VI	*Sporolithon durum*	1,445
VII	*Sporolithon durum*	2,022
VIII	*Sporolithon durum*	1,735

Methods:

Because the samples from the different batches were received in different forms, this necessitated different analytical approaches.

Batch 1 (filter samples from 10/10):

Lipid: Lipid was extracted from the filters (all odd numbers filters in Table 1, i.e. 1, 3, 5, etc) by the Bligh and Dyer method, as outlined in Dunstan et al. (1992). Solvent was removed from the extracts, and the % lipid was determined gravimetrically.

Protein and carbohydrate: Because only one, 47 mm filter sample was available for both carbohydrate and protein, we first had to homogenize the filter to sub-sample. Hence each filter was added to a glass beaker with 10 ml of water then homogenized to a fine pulp by using a tissue homogeniser for 30 secs. 1 ml aliquots were then removed, and analysed for both protein and carbohydrate. Protein was analysed colorimetrically using the method of Clayton et al. (1988). Carbohydate was determined after hydrolysis of the samples by the method of Dubois et al. (1956).

Extracellular (culture filtrate) carbohydrate: Samples were filtered through GF/C filters, and filtrates were analysed for carbohydrate using a colorimetric assay (Mykelstad et al., 1997).

Ash was determined from the % weight loss of the dried material on glass-fibre filters (prepared by filtering culture samples at CSIRO, and subsequent washing with ammonium formate, and

drying) after combusting at 450° C for 24 h.

Batch 2 (frozen samples from 14/11/00):

Samples had first been freeze-dried, and the total amounts available for all analyses are specified in Table 2.

Where possible, we used the following amounts for the various analyses, as we consider these as optimal required for the analysis.

lipid – 100 mg

protein, carbohydrate – 5 mg each

ash – 50 mg

The biochemical analyses used were otherwise the same as those cited above, for batch 1.

Results and Discussion:

Results for the proximate analysis of samples provided in Batches 1 and 2 are given in Tables 3 and 4. The discussion of the results from the different batches is given separately.

Batch 1:

There appears to be a major discrepancy with the results, as for most of the samples, the sum of ash, lipid, protein and CHO adds up to <50% (Table 3). Whilst there can be some minor inaccuracies with the methodologies for some specific samples (esp. CHO where, if the sample/alga contains unique sugars that give different colour reactions to glucose), nevertheless our previous experience is that sum values should add to between 75-105% of the dry weight.

The most likely factor that could account for this was an inefficient washing of the filters using ammonium formate. For example, if only 50% of the seawater had been removed (i.e. replaced with ammonium formate) this would be sufficient to add between 20 to 40 mg of "apparent" dry weight, thus leading to significant underestimations of lipid, carbohydrate and protein (by ½ or more).

There are three reasons that suggest inefficient washing as the cause for these apparent discrepancies:

1. Separate culture samples filtered at CSIRO for ash determination using approx. the same volumes as filtered for the other (lipid, protein, CHO) filters, contained between 4 to 20 mg (average ≈ 10 mg). These samples were all washed with 15-20 ml of ammonium formate. This compared to average values of algal biomass retained on the other (lipid, protein, CHO) filters, of ≈ 30 mg (see Table 1).
2. Usually filters will clog up once 10 to 20 mg of algal biomass is retained
3. Filter samples 6, 12 and 24 had by far the lowest "apparent biomass" (i.e. 13.4, 7.7 and 5.8 mg respectively; Table 1). These samples also had the highest estimated % protein and % CHO, and also typical of values we would expect. This suggests that the reason these samples had the lowest biomass (and correspondingly highest % protein and % CHO) was because these were washed efficiently with ammonium formate, whereas other samples were not.

Concentrations of total extracellular CHO in the *Cylindrotheca* and *Navicula* samples ranged from 27 to 80 μg mL^{-1} of culture, with average values of *Cylindrotheca* and *Navicula* similar. Typically, 60 to 80% of the total CHO was present as polysaccharide (Table 5).

Batch 2:

Sporolithon was very high in ash and contained very little lipid. At the low levels of detected lipid, the analyses can become inaccurate, but nevertheless it would be accurate to quote *Sporolithon* as containing < 0.4% of lipid.

As seen from Table 2, the low amounts of *Cocconeis* samples compromised our ability to obtain accurate analyses for most samples. However, for samples 2 and 3 we had sufficient samples, yet the sum of ash, lipid, protein and CHO adds up to <20%. The following reasons are suggested to account for the remaining ≈ 80%:

- some 5 to 10% of the freeze-dried sample could be moisture
- if the sugar composition of polysaccharides is unique (e.g. low glucose) then there is the potential to underestimate CHO. Normally this is not a problem as most microalgae contain > 50% glucose. However, for example, pentoses, methylpentoses and uronic acids are inert and do not give a positive colour reaction.
- "fibre" is not detected by the assay (but usually it is < 5 to 10% of algal biomass).
- protein could be underestimated, if there was a problem with efficiently extracting it from the sample
- similarly, lipid could be underestimated, if there was a problem with efficiently extracting it from the sample (this can be an issue with certain algae).

Table 3. Analysis of samples; Batch 1.

Code	Sample i.d.	% ash	% lipid	% protein	% CHO
1,2	*Cylindrotheca* (stat)	6.20	5.67	14.11	10.44
3,4	*Cylindrotheca* (stat)	8.16	7.16	12.09	9.32
5,6	*Cylindrotheca* (stat)	8.95	6.67	35.70	22.94
7,8	*Cylindrotheca* (log)	9.86	5.84	14.66	7.05
9,10	*Cylindrotheca* (log)	21.51	2.19	11.23	6.88
11,12	*Cylindrotheca* (log)	13.66	3.69	47.30	16.02
13,14	*Navicula* (stat)	10.82	8.33	9.39	17.13
15,16	*Navicula* (stat)	10.13	7.09	12.76	15.76
17,18	*Navicula* (stat)	11.77	3.19	15.02	25.96
19,20	*Navicula* (stat)	7.39	3.88	12.92	9.91
21,22	*Navicula* (stat)	19.30	2.69	13.45	4.68
23,24	*Navicula* (stat)	4.74	3.70	45.44	17.42

Samples with odd number (1,3,5 etc.) were analysed for lipid;
Samples with even number analysed for protein and CHO;
Ash was determined from a separate culture sample

Table 4. Analysis of samples; Batch 2.

Code	Sample i.d.	% ash	% lipid	% protein	% CHO
1	*Cocconeis*-4 wk	n.a.	n.a.	29.82	4.88
2	*Cocconeis*-4 wk	8.20	1.85	3.81	0.97
3	*Cocconeis*-4 wk	9.61	1.50	7.50	0.93
4	*Cocconeis*-2 wk	n.a.	n.d	25.52	1.05
5	*Cocconeis*-2 wk	n.a.	n.d	5.47	n.a.
6	*Cocconeis*-2 wk	n.a.	n.d	17.48	1.11
7	*Cocconeis sp.*	n.a.	n.d	13.80	1.48
8	*Cocconeis sp.*	n.a.	n.d	16.73	1.69
9	*Cocconeis sp.*	n.a.	n.d	n.a	n.a.
I	*Ulvella lens*	6.29	3.53	50.99	12.17
ii	*Ulvella lens*	4.68	4.97	35.76	18.32
iii	*Ulvella lens*	1.88	8.30	34.02	18.40
iv	*Ulvella lens*	1.17	2.68	33.18	14.11
v	*Sporolithon durum*	83.25	0.12	5.75	4.31
vi	*Sporolithon durum*	84.58	0.00	5.31	2.31
vii	*Sporolithon durum*	85.87	0.37	3.49	2.44
viii	*Sporolithon durum*	84.51	0.07	4.05	2.54

n.a. = not able to be assayed because of insufficient sample

n.d. = not detected; i.e.< 5%

Table 5. Extracellular carbohydrate (CHO) of *Cylindrotheca* and *Navicula* samples.

	µg CHO / ml of culture		µg ext CHO/ mg biomass	
	Non PS	Total	Non PS	Total
Cylindrotheca (lag)	16.6	52.1	1.37	4.30
Cylindrotheca (lag)	15.2	62.5	1.41	5.79
Cylindrotheca (lag)	15.5	35.4	1.29	2.95
Cylindrotheca (log)	12.5	33.6	1.35	3.61
Cylindrotheca (log)	11.7	30.3	2.35	6.05
Cylindrotheca (log)	9.5	26.7	2.10	5.93
Navicula (lag)	11.7	61.8	0.95	4.98
Navicula (lag)	11.2	54.2	1.10	5.32
Navicula (lag)	16.9	79.6	0.82	3.85
Navicula (log)	13.4	47.5	3.83	13.58
Navicula (log)	5.6	49.5	0.93	8.25
Navicula (log)	12.9	46.2	3.08	11.01

Non PS = non polysaccharide, i.e. mono-, di-, oligo-saccharides.

SECTION 2: Nutritional assessment of feeds for juvenile abalone

Background to study and objectives:

The biochemical composition of algae changes according to the environmental conditions to which it is subjected, and potentially this can impact on the growth rate of abalone feeding on such algal diets. Abalone farmers culture algae for feeding to newly settled abalone, and manipulate algal growth by varying light and nutrient levels.

Our objective was to measure the biological performance (growth rates, % survival) of newly settled abalone, fed one diatom (*Navicula* sp.) and one macroalgae (*Ulvella lens*) grown under two light and two nitrogen regimes, and a formulated diet (control).

Methods:

- Newly-set juvenile abalone were obtained from Tas. Tiger Abalone, and cultured for 6 weeks. The shell length of post-larval abalone were measured every second week using Leica stereo- microscope (x 16 eyepiece, 3.2 x zoom).
- The growth assay comprised twelve treatments [3 (diets) x 2 (sodium nitrogen concentrations) x 2 (light intensities) factorial], each with four replicates (Table 6).

Table 6. Treatments used in the abalone experiment.

Species ➜	*Navicula* sp.				*Ulvella lens*				Powdered formulated diet (Control)			
Nitrogen	LN		HN		LN		HN		LN		HN	
Light	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL	LL	HL
Replicates ∑ 48	4	4	4	4	4	4	4	4	4	4	4	4

Abbreviations:
LN LL = low nitrogen, low light LN HL = low nitrogen, high light
HN LL = high nitrogen, low light HN HL = high nitrogen, high light

- There were eight abalone per replicate culture vessel (250 ml petri dish).
- An isothermal growth room (17° C) with shelves lit by independent light sources were set up to be radiated at a high and low intensity (120 μE m^{-2} sec^{-1}, 40 μE m^{-2} sec^{-1}) on a 18:6 light regime, each shelf held half the culture vessels. The replicates within each light regime were randomly assigned the treatment diets (Table 6), which were:
  - *Navicula* sp. cultured with low nitrogen medium (15 mg/l)
  - *Naviculs* sp. cultured with high nitrogen medium (150 mg/l)
  - *Ulvella* lens cultured with low nitrogen medium (15 mg/l)
  - *Ulvella* lens cultured with high nitrogen medium (150 mg/l)

- powdered formulated diet with low nitrogen medium (15 mg/l)
- powdered formulated diet with high nitrogen medium (150 mg/l)

The procedures for maintaining algal cultures were as following:

Navicula sp.

Week 1: set up four small petri dishes for stock cultures, one of each treatment combination (two with low nitrogen medium- one low light LNLL, one high light LNHL, two with high nitrogen medium- one low light HNLL, one high light HNHL)

Week 2: transfer once a week 1 ml from each small petri dishes into four new small dishes, from each small dish set up four large petri dishes grow under same conditions, grow for 5-7 days, repeat 2. task every second week.

Ulvella lens

Week 1: of 32 large petri dishes containing disks (sent from Sabine) covered with *Ulvella lens* (+two spare sets) place 16 in the dark, 8 with low nitrogen medium, 8 with high nitrogen medium, place 4 dishes LN LL, 4 dishes HN LL and 4 dishes LN HL, 4 dishes HN HL.

- Abalone in control vessels with powdered formulated diet were grown in the same light and nutrient regimes as the living diets (thus emulating abalone farms) to examine if these regimes have an effect on abalone growth.
- Culture vessels were not aerated but media was changed every Monday, Wednesday and Friday.
- Dead/missing abalone were replaced with abalone of similar treatment history.
- Every second week the abalone from the *Navicula* sp., treatments were gently transferred to fresh culture vessels containing logarithmic cultures of *Navicula* sp., and the appropriate culture media and light history. Negligible "crawl-out" and mortality occurred with abalone from the *Navicula* sp. treatments. But between weeks 4 and 6 the HNHL plates were significantly contaminated with a *Tetraselmis*-like flagellate.
- It was intended that the abalone from the *Ulvella lens* treatments be transferred to fresh culture vessels every second week. Due to the high degree of abalone "crawl-out" (when abalone climb up the wall of the vessel and sit above the water level, desiccating) and resultant high mortalities, by the end of week 2 these treatments had to be discontinued.
- The abalone from the formulated feed treatments were fed every two days. Due to heavy fouling and high mortalities, (but a low incidence of "crawl out"), the abalone were gently transferred to fresh culture vessels at the beginning of week 2. By the end of week 2 these treatments also had to be discontinued due to high mortalities (Oxygen problem??).
- Diets were collected at week two and week six and analysed for proximate (% protein, % CHO, % lipid) and fatty acid composition, where enough sample was available. After removal of the abalone, the entire algal film was removed using a spatula from each culture vessel and placed on foil and immediately frozen in liquid nitrogen.

Results and Discussion:

Composition of diets:

There were some major differences in the proximate composition (% of organic weight, OW) of *Navicula* samples collected on the 29/12/00 and on the 26/01/01, respectively and *Ulvella* samples (Table 7). Within the 2 groups of *Navicula* samples, there was no significant difference in composition attributable to the different light x nitrogen combinations, though it appeared higher nitrogen cultures had slightly higher % protein. *Ulvella* grown under high nitrogen had more protein but less carbohydrate than *Ulvella* grown under low nitrogen (Table 7).

For *Navicula* samples, a higher total biomass (mg OW/plate) was produced in samples grown under a higher light intensity, irrespective of nitrogen concentration (Table 7).

There were no significant differences in the percentages of fatty acids in *Navicula* that could be attributed to the different light x nitrogen treatments. All samples were rich in 20:5n-3 (18 to 23%) and had low amounts of 22:6n-3 (1.6 to 2.4%) (Table 8 and Appendix 1). In contrast, major fatty acids in *Ulvella* samples included 18:2n-6 (17 to 18%) and 18:3n-3 (23 to 25%) (Table 8).

Growth rates of abalone:

The survival of abalone fed the different *Navicula* diets were all high (87 to 96%) and not significantly different (Table 9). However, the diets of *Ulvella* and formulated feed (FF) both gave poor survival (3% and 23%), and were therefore not effective in these culture systems. We also found that "crawl out" of abalone (i.e. attempting to escape from plates) was significant in the *Ulvella* lens and FF plates. These latter treatments were discontinued after 2 weeks.

Abalone fed *Navicula* diets showed similar growth rates between treatments measured over various time periods, except:

- from 2 to 4 weeks, the Lo-N, Hi-light treatment produced a lower growth rate than all other treatments
- over the whole experiment, the Lo-N, Hi-light treatment produced a lower growth rate than the Hi-N, Hi-light treatment (Table 9).

There were several factors that contributed to our inability to see differences in abalone growth associated to the difference in *Navicula* treatments:

1) there were no significant different in the biochemical composition of *Navicula* grown under the different treatments – despite quite different environmental culture conditions.
2) despite 4 replicates per treatment, the growth of microalgae was not always consistent between replicates (patchy in distribution, and differing in total biomass). For example, the co-efficient of variation of OW biomass/plate ranged from 15 to 80% within treatments.

Table 7. Proximate composition (expressed as % of organic weight (OW) and biomass/ plate of algal samples used in the abalone feeding experiment. For CHO and protein, n = 4; for lipid n = 0 (insufficient material to analyse) to 4.

	Expressed as % of organic weight (OW)							Biomass/plate (mg)						
Sample code/harvest date	% lipid		% CHO		% protein		OW	lipid		CHO		protein		
Navicula samples 29/12/00														
Hi-N,Hi-light	8.9	± 3.8	38.3	± 4.1	19.7	± 0.6	10.6	± 3.4	1.1	± 0.6	4.1	± 1.7	2.1	± 0.7
Hi-N,Lo-light	12.1		36.7	± 3.0	19.9	± 1.1	7.1	± 2.5	1.2		2.6	± 0.9	1.6	± 0.4
Lo-N,Hi-light	15.9	± 2.0	38.7	± 4.8	16.6	± 1.1	13.3	± 4.3	2.4	± 0.6	5.1	± 1.5	2.2	± 0.7
Lo-N,Lo-light			39.4	± 5.0	15.8	± 12.2	6.1	± 2.2			2.4	± 0.8	1.5	± 0.4
Navicula samples 26/01/01														
Hi-N,Hi-light	11.0	± 4.5	50.5	± 26.7	67.7	± 15.3	17.6	± 10.4	2.1	± 0.2	8.0	± 3.8		± 6.0
Hi-N,Lo-light	3.1		47.9	± 16.7	52.3	± 7.0	5.9	± 4.6	0.4		4.0	± 3.2	3.8	± 1.6
Lo-N,Hi-light	11.9	± 1.7	50.4	± 2.8	46.9	± 14.7	19.9	± 2.9	2.4	± 0.5	10.1	± 2.0	9.5	± 3.7
Lo-N,Lo-light	10.7	± 1.9	61.9	± 35.1	37.9	± 7.2	15.0	± 4.5	1.6	± 0.7	9.3	± 5.2	5.6	± 1.6
Ulvella samples 29/12/00														
Hi-N,Hi-light			28.8	± 3.5	34.4	± 5.7	22.3	± 7.2			6.6	± 2.5	7.8	± 3.0
Hi-N,Lo-light	10		27.9	± 0.8	35.6	± 3.1	41.8	± 28.0	7.8		11.6	± 7.5		± 11.6
Lo-N,Hi-light	8.2		47.1	± 5.4	25.0	± 4.2		± 44.8	11.0		50.0	± 24.8		± 9.2
Lo-N,Lo-light			41.0	± 4.5	26.2	± 4.6	30.3	± 12.6			12.7	± 6.1	7.8	± 3.3

Table 8. Fatty acids of importance (% of total composition) in algal samples used in the abalone feeding experiment.

***Navicula* samples, 29/12/00**							***Navicula* samples, 26/01/01**								***Ulvella* samples, 29/12/00**	
Fatty acid	Hi-N, Hi-light (n = 3)		Hi-N, Lo-light (n = 1)	Lo-N, Hi-light (n = 3)			Hi-N, Hi-light (n = 3)		Hi-N, Lo-light (n = 1)	Lo-N Hi-light (n = 4)		Lo-N, Lo-light (n = 4)			Hi-N, Lo-light (n = 1)	Lo-N, Hi-light (n = 1)
14:0	1.7	± 0.2	2.1	2.3	± 0.1		1.8	± 0.6	2.4	2.2	± 0.1	2.5	± 0.3		5.0	5.3
16:0	21.7	± 1.8	21.8	25.7	± 0.9		21.1	± 1.9	25.3	28.2	± 0.9	27.1	± 1.2		14.5	17.0
18:0	0.7	± 0.1	0.7	0.6	± 0.1		6.8	± 3.8	0.7	0.5	± 0.1	0.7	± 0.3		6.8	4.9
18:1(n-9)	0.9	± 0.3	1.1	0.9	± 0.1		2.6	± 1.2	0.7	0.8	± 0.3	1.1	± 0.4		0.4	0.6
18:1(n-7)	1.1	± 0.7	0.7	0.7	± 0.1		1.5	± 0.3	1.0	0.9	± 0.4	1.0	± 0.3		3.3	4.3
18:2(n-6)	0.2	± 0.0	0.2	0.2	± 0.1		1.1	± 0.5	0.2	0.2	± 0.0	0.2	± 0.1		17.7	17.0
18:3(n-6)	0.2	± 0.1	0.2	0.2	± 0.0		0.2	± 0.0	0.2	0.1	± 0.0	0.2	± 0.1		0.9	1.0
18:3(n-3)	0.2	± 0.0	0.2	0.2	± 0.1		6.7	± 3.9	0.2	0.2	± 0.0	0.2	± 0.0		23.0	24.5
20:4(n-6)	0.2	± 0.1	0.0	0.3	± 0.3		0.3	± 0.1	0.4	0.2	± 0.0	0.3	± 0.1		2.0	1.6
20:5(n-3)	22.8	± 2.0	19.8	18.8	± 1.2		17.9	± 2.0	22.1	19.7	± 1.2	19.7	± 0.9		1.9	1.0
22:5(n-3)	0.4	± 0.1	0.3	0.1	± 0.0		0.2	± 0.0	0.2	0.2	± 0.1	0.2	± 0.1		0.8	1.3
22:6(n-3)	2.4	± 0.3	1.6	1.7	± 0.2		1.9	± 0.2	2.4	2.1	± 0.5	2.1	± 0.4		1.5	1.8

Table 9. Growth rates and survival of *Haliotis rubra* fed various diet combination over a 6 week period.

Diet/treatment	0 to 2 weeks		2 to 4 weeks		4 to 6 weeks		Whole expt.	
Growth rate (µm day^{-1}):								
Navicula/ Hi-N, Hi-light*	31.5	± 3.8	23.4	± 3.9	32.2	± 21.9	29.4	± 7.8
Navicula/ Hi-N, Lo-light	26.3	± 4.9	20.9	± 4.6	26.6	± 11.1	23.6	± 4.0
Navicula/ Lo-N, Hi-light	24.3	± 3.6	10.6	± 1.4	13.1	± 4.4	16.4	± 1.8
Navicula/ Lo-N, Lo-light	29.0	± 4.3	22.6	± 6.2	17.8	± 4.1	22.7	± 4.7
Ulvella/ ALL treatments	n.d		n.d		n.d		n.d	
FF/ ALL treatments	n.d		n.d		n.d		n.d	
Survival (%):								
Navicula/ Hi-N, Hi-light*	93.8	± 7.2	96.9	± 6.3	96.9	± 6.3	95.8	± 3.4
Navicula/ Hi-N, Lo-light	90.6	± 12.0	87.5	± 25.0	100.0	± 0.0	92.7	± 7.1
Navicula/ Lo-N, Hi-light	84.4	± 6.3	100.0	± 0.0	90.6	± 12.0	91.7	± 5.9
Navicula/ Lo-N, Lo-light	68.8	± 23.9	100.0	± 0.0	93.8	± 12.5	86.9	± 7.6
Ulvella/ ALL treatments	3.1	± 7.2	n.d		n.d		n.d	
FF/ ALL treatments	22.7	± 25.5	n.d		n.d		n.d	

* cultures contaminated with a Tetraselmis-like flagellate.

It was also noted that between weeks 4 to 6 the *Navicula* plates grown under Hi-N, Hi-light treatment developed significant amounts of a flagellate resembling *Tetraselmis*. This confounded the interpretation of the efficacy of this diet treatment from week 4 to week 6, and over the whole experiment.

As we did not see differences in the biochemical composition of the *Navicula* cultures, we undertook additional analyses to see whether there might be a relationship between the OW/plate and the growth of abalone within culture units. Because of the contamination of flagellates within the Hi-N, Hi-light treatments, we excluded this data from the analysis. We found a negative correlation between the amount of food on plates, and the growth of abalone ($p < 0.02$; adj. $r^2 = 0.43$) (see scatter chart; Fig 1). This suggested that abalone were satiated at lower algal concentrations, and increased food densities were having a negative impact on growth.

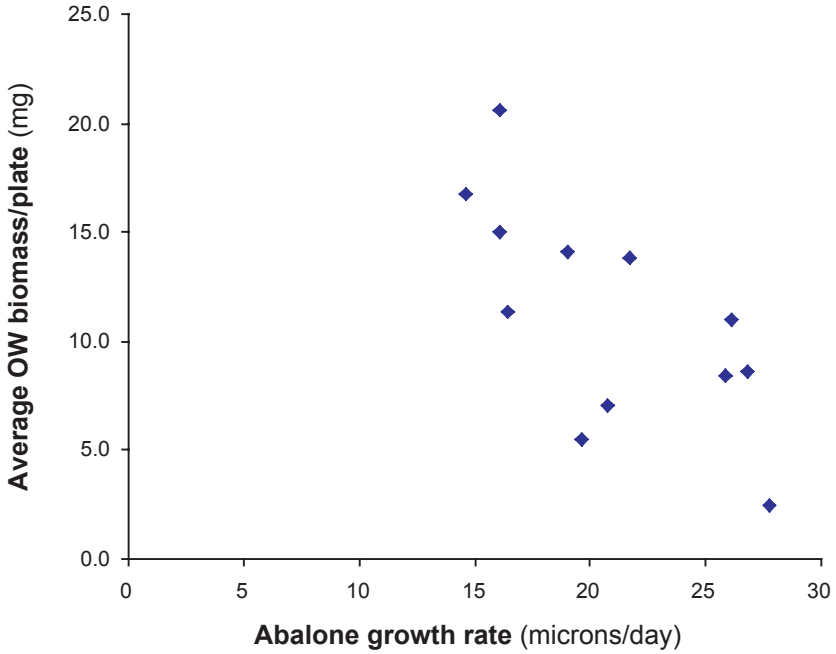

Figure 1. Scatter plot comparing average OW (organic weight) biomass from the *Navicula* plates (across all N x light combinations) and total *Haliotis rubra* growth rate over 6 weeks.

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Appendix 2 Effects of seeding with the macroalga (Ulvella lens) and inoculation with a benthic diatom (Navicula sp.) on the settlement, growth and recruitment of abalone (Haliotis rubra) under commercial conditions

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Abstract

The settlement, shell growth and recruitment of abalone *Haliotis rubra* on plates seeded with the macroalga *Ulvella lens* and inoculated with the microalga *Navicula* sp. were examined in 6 commercial nursery tanks. *U. lens* produced a greater settlement rate of larvae at 34% ± 2.28 (± S.E., n = 3) than *Navicula* sp. at 18% ± 6.83 (± S.E., n = 3) however it was not statistically significant ($p > 0.05$). There was no significant added variance from among tanks within treatments. A simultaneous settlement trial conducted in the laboratory exposed the same cohort of larvae to 2 macroalgae (*Sporolithon durum* and *U. lens*) and 3 benthic diatom films (*Navicula* sp., *Cocconeis* sp. and *Cylindrotheca closterium*). Excluding the positive control (*S. durum*), *U. lens* produced the greatest settlement rate at 20% ± 4.8 (± S.E., n = 6), however it was not significantly greater than the *Navicula* sp. (13.8% ± 0.8), or the *Cocconeis* sp treatments (10.0% ± 1.3) ($p > 0.05$). *C. closterium* resulted in the lowest settlement rate at 2.8% ± 1.3 (± S.E., n = 6) which was not significantly different to the negative control (clean plastic). Post-larvae grazing on *Navicula* sp. had a significantly greater shell length than those grazing on *U. lens*, over the 35 day commercial scale nursery feeding trial ($p < 0.05$). The shell length curves diverged between day 21 and 28, as post-larvae grazing on *Navicula* sp. had a peak in the growth rate (64 μm day^{-1}) between these days. At day 35, *Navicula* sp. and *U. lens* produced mean shell lengths of 1.82 mm ± 0.43 and 1.76 mm ± 0.46 (± S.E., n = 3). At 42 days post-settlement, the recruitment (juveniles/side of a nursery plate) for the *U. lens* and the *Navicula* sp. treatments were 226 ± 16 (± S.E., n = 3) and 73 ± 5 (± S.E., n = 3) respectively, which was significantly different ($p < 0.05$). There was no significant effect of tank-to-tank variation within the *U. lens* treatment ($p > 0.05$), in contrast, the *Navicula* sp. treatment resulted in tank-to-tank variation ($p < 0.05$). This commercial scale nursery trial suggests that the macroalgae *U. lens* can result in greater recruitment of juvenile abalone than the cultured *Navicula* sp. and can sustain only slightly reduced growth despite a much higher density of abalone on plates than the *Navicula* sp.

1.0 Introduction

Abalone nursery operations continue to experience inconsistent and inefficient seed production (Roberts et al., 1998). In Australia juvenile abalone production relies predominantly on naturally developing biofilms as a settlement cue and food source (Daume et al., in print). Benthic diatoms with their extracellular secretions can be the dominant component in a naturally developing biofilm. In addition bacteria, fungi, other microbes and organic molecules will be present in a biofilm (Roberts, in print). In extensive nursery operations there is limited control of naturally developing biofilms.

In a previous laboratory study Daume et al. (2000) showed that settlement of blacklip abalone, *Haliotis rubra* on *Ulvella lens* was higher than on monospecific benthic diatom films. *U. lens* is a crustose green macroalga that grows as prostrate rosettes on hard surfaces (Roberts, in print). Settlement of up to 52% was achieved with germlings of *U. lens*. This is supported by the study by Takahashi and Koganezawa (1988), that found *U. lens* resulted in 60-70% settlement of *H. discus hannai* larvae.

Benthic diatoms are considered to be the primary food for abalone prior to consuming macroalgae (Tomita and Tazawa, 1971; Kawamura, 1996; Kawamura et al., 1998). A diatom strain with the attribute of being tightly-attached to a substrate can be beneficial for post-larvae >1,000 μm, as it results in the cell wall being ruptured during grazing allowing post-larvae access to the cell contents improving digestion (Kawamura et al., 1995). In a study with four benthic diatom strains and two macroalgae, Daume et al. (2000) found that films of a tightly attached *Navicula* sp. produced significantly larger post-larvae with greater survival from 1 week post-settlement to week 11 (Daume et al., in print).

The attribute of being tightly attached is also critical in obtaining and maintaining a suitable diatom film in an extensive nursery environment. In Australia culturing of diatoms for abalone nursery operations has had little success because "surface oriented" diatoms species with unknown nutritional value have been used. These diatoms can be cultured in suspension but do not attach and divide well on the surface of vertical nursery plates (Krsinich, A., unpubl. data; Farrell, S., pers. obs.). As a consequence, these cultured diatoms are not good "space competitors" on the surface of a nursery plate, which results in a wild biofilm out-competing the cultured diatom. The *Navicula* sp. (isolated for Daume et al., in print) is a "true" benthic diatom and thus cannot be successfully cultured in suspension like other planktonic diatom species. However larger-scale culture of this isolate can be achieved by using progressively larger surface areas.

In Japan *U. lens* plates in the nursery environment have been used as a diet for juveniles resulting in increased production with larger juveniles (Takahashi and Koganezawa, 1988). It has been reported that the *U. lens* sustained growth of post-larvae after settlement, however, the initial growth rate of post-larvae on *U. lens* was lower compared to diatom films (Seki, 1997 cited in Kawamura, et al., 1998; Daume et al., 2000). It has been shown that the initial growth rates of post-larvae on *U. lens* have been improved by the inoculation with cultured *Nitzschia closterium* (Seki, 1997 cited in Kawamura, et al., 1998). Furthermore it was been suggested that the transition from a benthic diatom diet to a macroalgae-dominated diet (such as *U. lens*) occurs when juveniles have a shell length of about 5,000 μm (Tomita and Tazawa, 1971, cited in Kawamura, 1996). The presence of a macroalga such as *U. lens* on nursery plates may help to alleviate the reported problem of maintaining adequate food when there is grazing pressure from larger juveniles and/or copepods (Farrell, S., pers. comm.).

Intensification of an abalone nursery operation can be achieved by providing an appropriate larval settlement clue to improve the rate and consistency of settlement. In addition if algal food is supplied for post-larvae that sustain maximum growth and survival, and increases the carrying capacity of the nursery this will further intensify the nursery operation.

The focus of this study was to intensify a commercial nursery operation for *H. rubra* by seeding with zoospores from the macroalga *U. lens* and inoculating with a "true" benthic diatom *Navicula* sp.

2.0 Materials and Methods

2.1 Nursery Trial: settlement, growth and survival

Preparation of nursery tanks and plates

Six 2000L tanks randomly chosen from Southern Ocean Mariculture (SOM), Port Fairy, Victoria were located in a plastic greenhouse covered with 70% shade cloth. Each tank was constructed of a galvanised steel frame with a PVC liner and consisted of a flow-through system, 50 cm high PVC standpipe and two air-lines along its length. Seawater was pumped from an adjacent bay and filtered through two sand filters (~70 μm filtration). Further filtration to the intake of each tank was possible with cartridge filters (1 or 5 μm nominal).

Twelve baskets, each containing 15 clear PVC plates (30 cm x 60 cm x 1.5 mm), were placed in each tank (12 x 15 = 180 plates/tank). The sampling unit was a removable platelet (12 cm x 17 cm) bolted to the centre of one plate and placed in the middle of each basket (Figure 1). The platelets' dimensions allowed them to be easily observed under a stereo microscope. Each platelet had a grid of 16 rectangles (4.25 cm x 3 cm), to allow further division of the platelets to assess settlement. The microscope had a graticule fitted to the eyepiece to measure the shell length of the post-larvae.

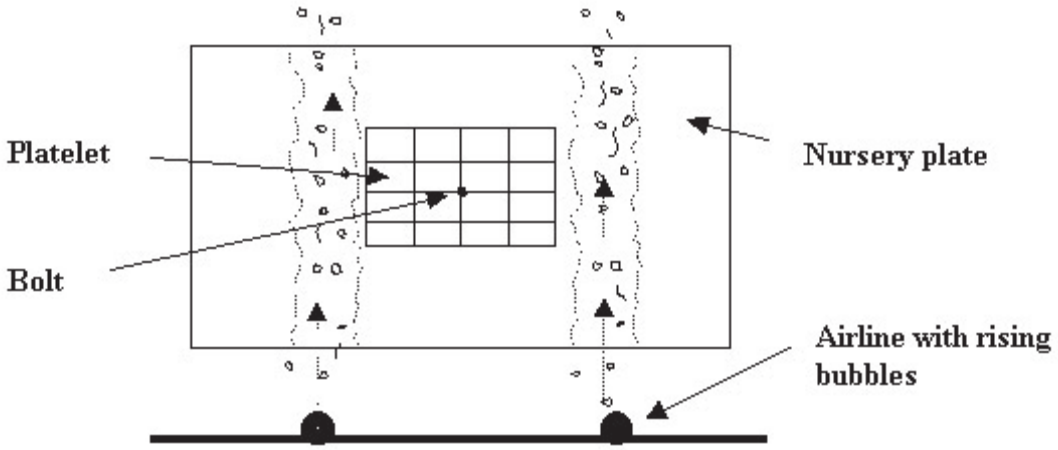

Fig. 1: Nursery plate with platelet bolted to the centre.

Protocol to securing *U. lens* seed plates

The protocol to secure adult sporophyte seed plates begin with wild *U. lens* zoospores, which are present in the incoming nursery seawater. Two grams of young juvenile abalone with a shell length >5 mm are transferred to nursery plates, that have a wild biofilm (Farrell, S., pers. comm.). This grazing pressure with the introduction of unfiltered seawater results in a prostrate algal community, which can include the macroalga *U. lens*. Plate clippings are monitored under the microscope and plates dominated with *U. lens* are selected. To the naked eye these plates are pale green.

The selected plates are transferred into a static tub to allow the *U. lens* to develop with Aquasol (Hortico Ltd) added at 80 mg/L. Each week there is a complete water exchange with 1 μm

filtered seawater with the addition of new nutrients. These plates with developing *U. lens* need to be spawned onto fresh plates to increase the percentage cover of *U. lens* and to remove other completive algal communities such as *Myrionema* sp.

To synchronise a mass release of *U. lens* zoospores, adult sporophyte seed plates are placed in total darkness for two weeks prior to being used (adapted from Takahashi & Koganezawa, 1988). To further aid the mass release of zoospores, the seed plates are desiccated for 3 minutes before being transferred into a static tank with clean plates with full daylight.

The seed plates spawn 3 to 5 days after the transfer to light and these "spent" seed plates should be immediately removed after spawning to reduce competitive algae on the old seed plates from contaminating the next generation of *U. lens* plates.

Seeding of nursery plates with U. lens

Three tanks with plates were chlorinated at10 ppm for four hours. Dechlorination was achieved with sodium thiosulphate and heavy aeration a days prior to the introduction of with *U. lens*. Aquasol was added at 80 mg/L. Trace metals and iron stock solutions were prepared as for f/2 medium (Guillard and Ryther, 1962) and added at 1 ml/L. Sodium metasilicate (Laboratory Supply, cat. no. 26186) was added to tanks at 22.7 mg/L.

Ten *U. lens* seed plates, which were 2 months old with ~60% cover, were placed between the baskets in 3 tanks. Four days later there was a mass release of zoospores in all 3 tanks. Aeration was maintained to break up and disperse the aggregation of zoospores on the surface of the water. The *U. lens* seed plates were removed from the tanks after 8 days. After 10 days of the tanks remaining static, 1 μm filtered seawater, at a 20% per hour exchange rate was introduced to the tanks. Aquasol was constantly pumped into the inflowing seawater to give a constant rate of Aquasol in a tank of 3.47 mg/L. Abalone larvae were added to the tanks 5 days after the introduction of fresh seawater.

Isolation and culturing of Navicula sp.

A benthic diatom, *Navicula* sp. was isolated from a SOM nursery plate (diatom isolated for Daume et al., 2000). The Diatoms were cultured at 17 ± 2° C in f/2 medium, on a 12:12 L:D photo cycle from daylight fluorescent lamps. A stock culture was maintained in an exponential phase on 25 cm^2 tissue culture plates (Becton Dickinson Labware, Falcon 3014).

The culture was non-axenic. The species could not be successfully cultured in suspension. However large-scale culture of this isolate was achieved by using progressively larger surface areas. Three mls of the benthic culture was transferred from the bottom of a tissue culture flask into a Petri dish (150 cm^2) containing 100 ml of f/2 using a pipette. Four days later the Petri dish culture was brushed into a 1218 cm^2 tray (Nally Ltd, IH009) with a clear fitted PVC lid. After a further 4 days the culture was brushed into a 1.3 m^2 (30 L capacity) plastic sealed bag, which was laid horizontally. The bag culture was harvested into a bucket after 5 days while the culture was in an exponential phase. The harvested culture in the bucket was suspended by stirring.

Inoculation of nursery plates with Navicula sp.

Three tanks with plates were chlorinated, dechlorination and nutrients added (see method above for *U. lens* tanks).

Ten L of the culture, with a density of 10^4 cells mL^{-1}, was used to inoculate each of the three nursery tanks with plates. The tanks remained static with low aeration for five days, followed by the introduction of 1 μm filtered seawater at a 20% per hour exchange rate. Aquasol and sodium metasilicate pentahydrate were constantly pumped into the inflowing seawater (see method above for *U. lens* tanks). Abalone larvae were added to the tanks 7 days after the introduction of the seawater.

Abalone larvae

Abalone larvae were obtained from the hatchery at SOM. Competence of the larvae to settle was defined by the development of the third tubule of the cephalic tentacle (Hahn, 1989; Seki & Kan-no; 1981a) and the observation that larvae started to explore the surface of the larval tank (Ebert & Houk, 1984).

345,000 competent larvae were transferred to each of the 6 nursery tanks. For the first 3 days the nursery tanks had 118 μm banjo sieves connected to the outlets and an exchange of 1.5% per hour of 1 μm filtered seawater with low aeration. After the third day the water exchange rate was increased to a 20% per hour exchange rate with moderate aeration. Seven days after the introduction of larvae the filtration was increased from 1μm to 5μm. After two weeks the filtration was increased from 5μm to only sand filtration (~70 μm filtration).

Nursery larval settlement

The term "settlement" describes the permanent attachment of the larvae to the substrate after shedding of the velum to initiate metamorphosis. Furthermore, both peristomal shell-growth and ciliary processes in the mantle cavity indicated the initiation of metamorphosis (Hahn, 1989).

Seventy-two hours after the larvae were introduced to the tanks, three platelets from each tank were observed under a dissecting microscope. Six rectangles were randomly chosen from the grid of each platelet, and all settled larvae within the rectangles or overlapping on the bottom and left-hand lines were counted.

Post-larvae growth

At the end of week 1 after the introduction of larvae, the number of post-larvae was reduced to 6 on 3 platelets in each tank. Those platelets were repeatedly used to measure the shell length of individual post-larvae at weekly intervals for 5 weeks. The platelets remained submerged while being viewed under the microscope.

Monitoring the biofilm

The same 3 platelets used in the growth trial were also used to record the density of *Navicula* sp., percentage coverage of *U. lens* and the succession of the wild biofilm. The biofilm on the platelets were sampled in 5 randomly chosen fields of view, at a magnification of 400x, at weekly intervals for 5 weeks.

Recruitment of post-larvae on the nursery plates

At week 6 after the introduction of larvae, the recruitment in each tank was estimated by counting the number of juvenile abalone on 4 randomly chosen plates in 4 randomly chosen baskets (a total of 16 plates/tank).

2.2 Laboratory trial: settlement

Four treatments, *Navicula* sp., *U. lens* + wild biofilm, *Cylindrotheca closterium* and *Cocconeis* sp. were used in a laboratory settlement trial (diatom isolated for Daume et al., in print). Six settlement substrata for each treatment were cut from clear PVC plates into 2 cm^2 pieces. Each of these plate pieces were placed into a 250 mL jar with 100 ± 5 larvae (same cohort as the nursery trial). *Navicula* sp. and *U. lens* + wild biofilm substrata were cut from spare platelets from the nursery trial. *C. closterium* and *Cocconeis* sp. were cultured in the laboratory for 5 days on 6 plate pieces each. Six jars each with one plate piece, without a biofilm (clean plastic) were used as negative controls. Six 1 cm^2 pieces *S. durum* were used as a positive control. Settled larvae were counted under a dissecting microscope 24 hours after the larvae were added to the jars.

2.3 Data Analysis

ANOVA was used to test differences in the mean rate of settlement, shell growth and recruitment. Homogeneity of variance and normality were assessed by Cochran and Shapiro-Wilkes tests, respectively. Nursery settlement data had one outlier replaced (1 rectangle out of 6 rectangles on one platelet, 3 platelets per tank) with the overall mean to achieve homogeneity of variance. A nested ANOVA (4 level) was performed where species represented fixed treatment effects and tanks, platelets and rectangles on the platelets random effects (chart 1). This gave the estimates of the variance between post-larvae settled per rectangle among species, among tanks within species, among platelets within tanks and within platelets. Similarly growth data for week 4 and 5 (average SL of the platelet) were tested using a nested ANOVA (3 level). Recruitment data required a logarithmic transformation to achieve homogeneity of variance before a nested ANOVA (3 level) was performed. Laboratory settlement data required a logarithmic transformation to achieve homogeneity of variance before an ANOVA was undertaken.

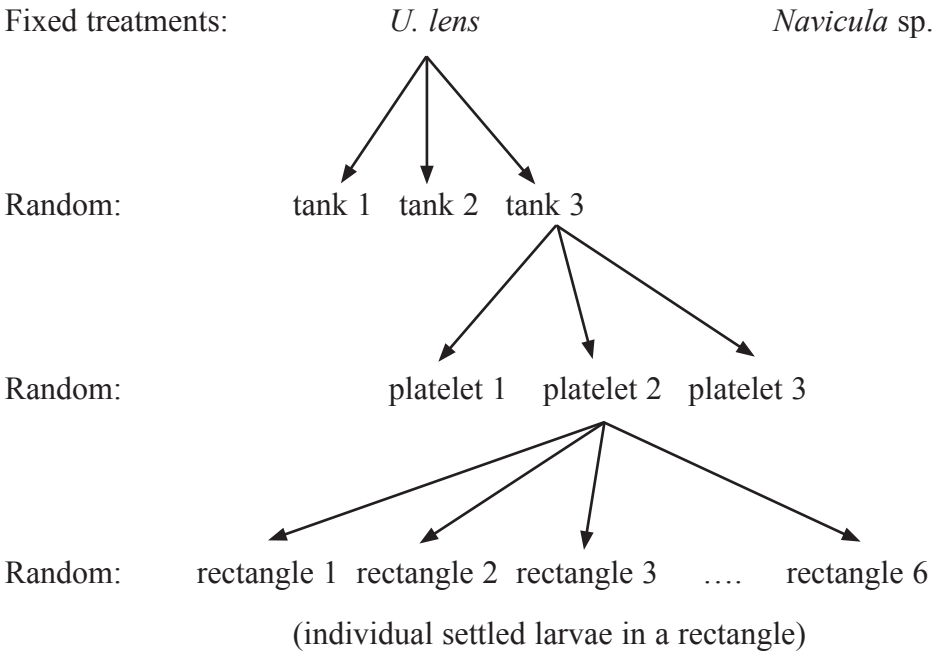

Chart 1. Nursery settlement nested ANOVA design.

3.0 Results

3.1 Nursery Trial

Larvae settlement in the nursery

Seventy-two hours following the introduction of larvae to the nursery tanks, the *U. lens* + wild algae treatment had a greater settlement rate of abalone larvae at 34% ± 3.27 than *Navicula* sp. at 18% ± 2.54 (Figure 2). However it was not statistically significant ($p > 0.05$, table 1) and there was no significant added variance from among tanks within treatments ($p > 0.05$). There was significant platelet-to-platelet variation within tanks ($p < 0.01$). In the *U. lens* tanks the coefficient of variation (CV) for the settlement response was 71%, whereas in the *Navicula* sp. tanks the CV was 103 %. This indicates that the *Navicula* sp. tanks had the greatest variation in the number of settlers on the platelets.

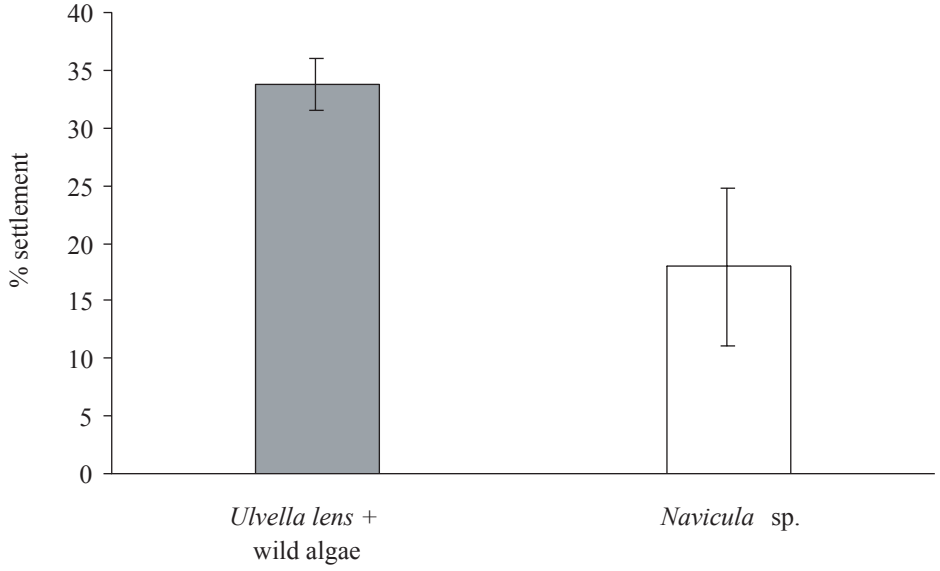

Fig. 2: Nursery settlement response of *Haliotis rubra* larvae after 72 hours on platelets inoculated with *Navicula* sp. or seeded with *U. lens.*

Post-larval growth

The growth rates from day 7 to day 21 were similar on the two treatments (Figure 3). At day 21 the mean shell length for the post-larvae feeding on *U. lens* + wild algae and *Navicula* sp. was 987 μm ± 13.42 and 993 μm ± 10.93 (± S. E.) respectively. Both treatments had a growth rate of 47 μm day^{-1} over the first 21 days.

The growth curves diverged between day 21 and day 28, as the post-larvae grazing on *Navicula* sp. had a peak in the growth rate to 64 μm day^{-1} between these days. While those grazing on *U. lens* + wild algae also had an increase in growth rate to 56 μm day^{-1} between days 21 day 28. The net result was that the post-larvae grazing on *Navicula* sp. had a significantly greater shell length at day 28 ($p < 0.05$). There was no significant added variance among tanks within treatments ($p > 0.05$).

At day 35, *U. lens* + wild algae and *Navicula* sp. treatments had mean shell lengths of 1.76 mm ± 0.46 and 1.82 mm ± 0.43 (± S. E.) respectively, which was not significantly different ($p > 0.05$). Furthermore there was no significant effect of tank-to-tank variation within treatments ($p > 0.05$) with respect to shell length. From day 28 to day 35 both treatments had a growth rate of 55 μm day^{-1}. The growth trial was terminated at day 35 as the post-larvae started to migrate off the platelets at this stage of development.

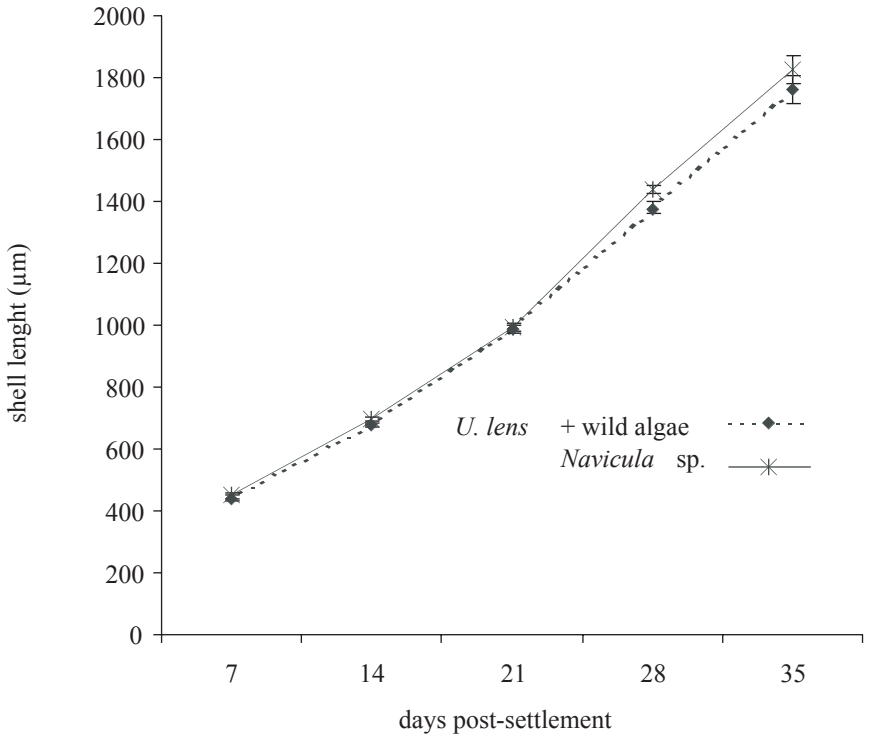

Fig. 3: Shell length of *Haliotis rubra* post-larvae on nursery platelets inoculated with *Navicula* sp. or seeded with *Ulvella lens.*

Recruitment on plates at week 6

At week 6, the overall means for the number of post-larvae per side of a nursery plate for the *U. lens* + wild algae and the *Navicula* sp. treatments were 226±16 (± S.E.) and 73 ± 5 respectively, which was significantly different ($p < 0.01$) (Figure 4).

There was a significant effect of tank-to-tank variation within treatments ($p < 0.05$, table 4). The *U. lens* + wild algae tanks 1, 2 and 3, resulted the number of post-larvae per side of a plate of 205 ± 24 (± S.E.), 185 ± 23, and 290 ± 32 respectively, which was not significantly different ($p > 0.05$, table 5). While the *Navicula* sp. tanks 4, 5 and 6, resulted in means of 88 ± 7, 84 ± 10 and 49 ± 7 respectively. The *Navicula* sp. tank 6 was statistically significantly lower than both *Navicula* sp. tanks 4 and 5 ($p < 0.05$).

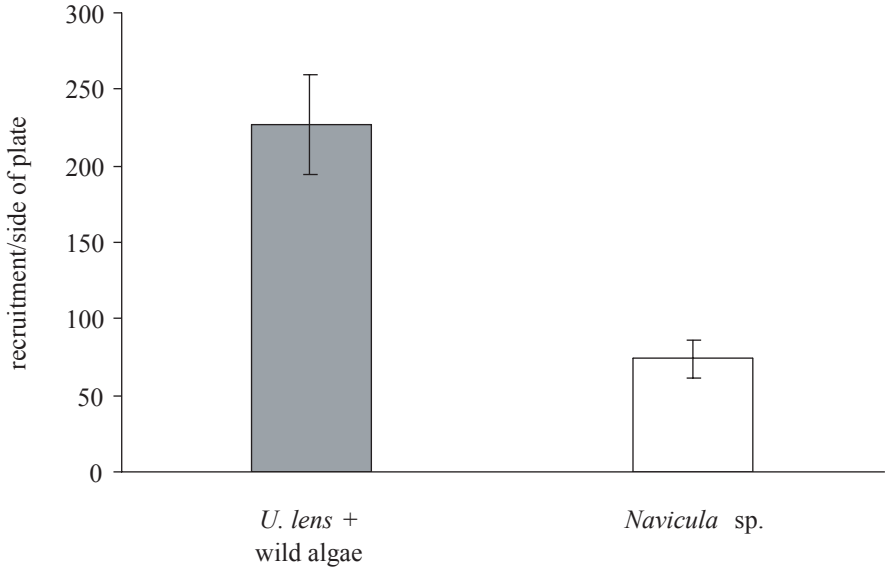

Fig. 4: Recruitment at week 6 post-settlement of *Haliotis rubra* post-larvae on nursery plates inoculated with *Navicula* sp. or seeded with *U. lens*.

U. lens coverage and succession of wild algae

The day prior to the introduction of larvae, each *U. lens* nursery plate exhibited two, 5 cm wide, clear streaks where the zoospores were unable to attach due to the aeration disturbance. Thus there was an uneven coverage of *U. lens* over the entire plate, which included the platelet bolted to the centre. The platelets appeared to have a lower coverage of *U. lens* compared to the entire plate as they were in the centre of the plates between the air-lines.

The day prior to the introduction of larvae, the *U. lens* tanks 1, 2, and 3 had an overall mean *U. lens* coverage of 13.8 ± 1.1 % (± S. E.) on the platelets. The water exchange and the wild algae on the *U. lens* seed plates also resulted in the introduction of wild algae to the tanks. A small "needle" *Nitzschia*, colonies of cells attached with a mucous thread and a *Navicula* type diatom dominated the wild algae on the *U. lens* platelets. The mean number of wild algae cells on the platelets in the *U. lens* tanks was 3.9 $x10^3/cm^2$ ± 8.5 $x10^2/cm^2$ (± S. E.).

The coverage of *U. lens* increased over the surface of the plates from 16% at week 1 to 22% at week 5 (Table 6). *U. lens* began to spawn in all three tanks between weeks 2 and 3. A succession of wild algae occurred around and over the developing *U. lens*.

Navicula sp. density and succession of wild algae

The day prior to the introduction of larvae, the three *Navicula* sp. tanks 4, 5 and 6 appeared to have an even diatom film over the plates. The overall mean number of *Navicula* sp. cells on the platelets was 3.7 x $10^5/cm^2$ ± 2.3 x $10^4/cm^2$ (± S. E.). *Navicula* sp. was dominant in the biofilm and no other wild algae were apparent at sampling.

From week 1 to week 5, the *Navicula* sp. film in tanks 4 and 5, remained dominant over wild algae, as it was a very good "space competitor" (indicated by the high density of cells/cm^2 above). However a wild alga contaminated the *Navicula* sp. tank 6. The wild alga had the

growth form of colonies of cells attached with a mucous thread to the platelets and was probably contaminated from an adjacent tank. It appeared that this wild alga required relatively little space to attach to a plate, as only a mucous thread was required for attachment as opposed to solitary cells with a prostrate growth form.

Copepods

The density of *Navicula* sp. decreased between week 4 and week 5 from $x10^5$ cells/cm^2 to $x10^2$ cells/cm^2. The wild algae on the *U. lens* platelets also decreased in similar magnitude whereas the coverage of *U. lens* increased. It was observed that the decrease in cell density coincided with a sharp increase in copepod numbers in all tanks. *U. lens* was apparently resistant to grazing by copepods.

3.2 Laboratory larval settlement

Navicula sp., *C. closterium* and *Cocconeis* sp. treatments had mean cell densities of $1.4 \times 10^5/cm^2 \pm 4.2 \times 10^3$, $1.1 \times 10^5/cm^2 \pm 3.8 \times 10^4$ and $7.6 \times 10^4/cm^2 \pm 2.9 \times 10^4$ (± S. E.) respectively. The laboratory *U. lens* treatment had a mean coverage of 19 ± 3% (± S. E.).

There was a significant difference between the numbers of settled post-larvae after 24 hours on the different treatments ($p < 0.001$) (Figure 5). The two macroalgae treatments, *U. lens* + wild algae and *S. durum* resulted in the greatest settlement rates, which were not significant different from each other ($p > 0.05$). A significantly greater number of larvae settled on *S. durum* compared to any of the diatom films ($p < 0.05$). Excluding the positive control (*S. durum*), the *U. lens* + wild algae nursery treatment resulted in the greatest settlement rate at 21%, however it was not significantly greater than *Navicula* sp. nursery treatment or the *Cocconeis* sp. ($p > 0.05$). *C. closterium* resulted in the lowest settlement rate among the diatom films ($p < 0.01$), which was not significantly different to the negative control ($p > 0.05$). The coefficient of variation for the two nursery conditioned treatments of *Navicula* sp. and *U. lens*+wild algae was 6% and 24% respectively.

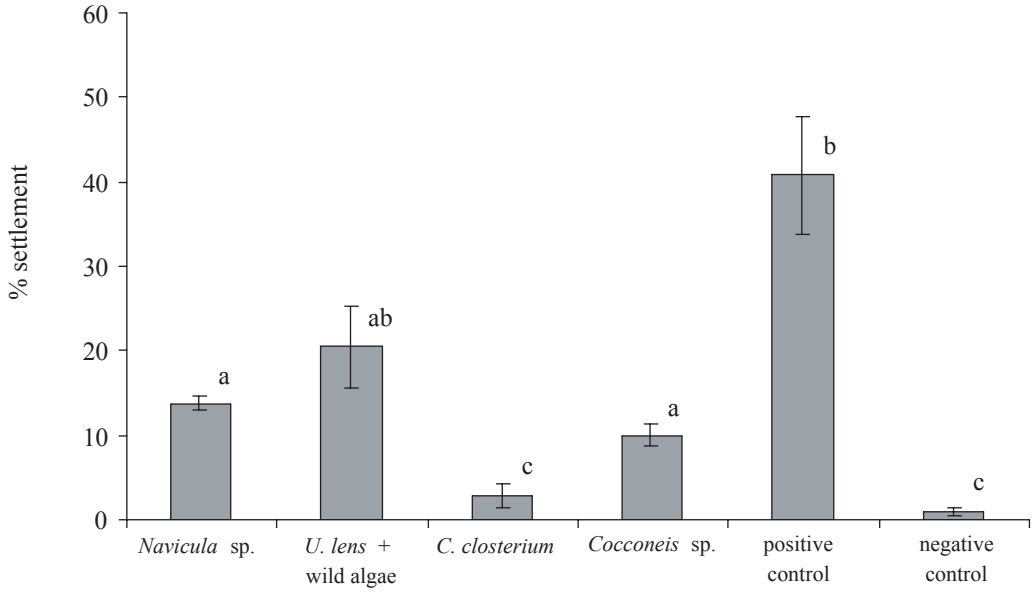

Fig. 5: Laboratory settlement response of *Haliotis rubra* after 24 hours. Positive control: *Sporolithon durum*. Negative control: clean plastic. Same superscript indicates no significant difference ($p>0.05$).

4.0 Discussion

4.1 Larval settlement

This study used two treatments to condition nursery settlement plates, which reduced the reliance upon natural biofilms. The laboratory and nursery settlement trials clearly demonstrated that plates seeded with *U. lens* or inoculated with *Navicula* sp. in a nursery environment possess positive inducer(s) for larvae settlement. In the laboratory trial, *C. closterium* produced lower settlement than *U. lens* + wild algae or *Navicula* sp. treatments. Clean plastic was a valid negative control.

In both the laboratory and nursery trials *U. lens* + wild algae had a greater settlement rate of abalone larvae than *Navicula* sp. however it was not statistically significant in either trial. This statistically non-significant result may have been caused by the low statistical power (low platelet replication) and the large variability from platelet-to-platelet with a tank. Daume et al. (in print) demonstrated that settlement of larvae was significantly greater on *U. lens* compared to *Navicula* sp. but that there was no significant difference between *Navicula* sp. and the combination of *U. lens*/*Navicula* sp. These results suggest that the presence of diatoms on an *U. lens* substrate may reduce settlement. Furthermore these results may explain why there was no significant difference in settlement between *U. lens* + wild algae and *Navicula* sp. in this study, as the *U. lens* platelets had the addition of a wild algae, which included diatoms. The wild algae on the *U. lens* platelets included a motile "needle" *Nitzschia*, *Navicula* type diatom and a three-dimensional growth form (colonies connected together by mucous thread). Excessive motility in a diatom hinders larvae settlement (Roberts, in print) and a high density of three-dimensional growth form results in larvae being unable to attach effectively to a substrate causing mortality prior to metamorphosis (Kawamura, 1996).

The development of the wild algae can be reduced by keeping the water exchange to a minimum and removing the wild algae from the *U. lens* seed plates. However the initial post-larvae growth and survival on *U. lens* with reduced diatoms at this initial stage in the nursery environment must be investigated. The degree of coverage by *U. lens* on the plates for high consistent larvae settlement also needs further investigation.

4.2 Abalone Growth rates

At day 28 a significant difference in shell length between the treatments was recorded, although the actual difference was small. The diet of *Navicula* sp. produced the greatest shell length of 1,439 μm ± 14 at day 28, with a growth rate of 64 μm day^{-1} between day 21 to day 28. Kawamura et al. (1998a) review of the feeding and growth of post-larval abalone generalised that post-larvae with shell length of 800-2,000 μm, that graze on highly digestible diatoms films, grow at about ~40-60 μm day^{-1}. Post-larvae grazing, however, on a poor diatom film (low "digestion efficiency") will generally grow at ~15-30 μm day^{-1} (Kawamura et al., 1998a).

At day 35 the mean abalone shell lengths for those on diets of *U. lens* + wild algae and *Navicula* sp. was 1,760 μm ± 46 and 1,820 μm ± 43 respectively, which was not significantly different. At day 35 there was no tank-to-tank variation with respect to the post-larvae shell length within either treatment. Roberts et al. (1999) laboratory study found that *H. iris* post-larvae grew fastest on a diets of *Cocconeis scutellum*, *Navicula ramosissima* and *Cylindrotheca closterium* compared to diets of *N. britannica* and *Pleurosigma* sp. The three diets that produced the fastest growth achieved ~1,500 μm at day 35 post-settlement. In Daume et al. (in print) the diatom diet that resulted in the greatest post-larval growth rate produced a shell length of ~1,400 μm at day 35 post-settlement. However caution is required when comparing shell length across cohorts, experimental designs and abalone species. Furthermore, growth rates in laboratory experiments may be lower than in flow through tanks (Roberts R., pers. comm.).

In this nursery study it is difficult to identify which food item(s) resulted in the high growth rates achieved on the macroalgae *U. lens* + wild algae, as *U. lens*, *U. lens* germlings and a wild algae were all present on the platelets. *U. lens* naturally spawned in all three tanks and *U. lens* germlings were first recorded at day 21. In the laboratory post-larvae at > 2 mm were observed clearing *U. lens* germlings from a substrate (pers. obser.). It is hypothesised that germlings and younger *U. lens* algae with new growth may be more suitable as a food source for post-larvae < 5 mm than well-developed *U. lens*.

4.3 Recruitment

At week 6, the *U. lens* + wild algae treatment resulted in significantly greater numbers of post-larvae on the plates compared with the *Navicula* sp. treatment. Furthermore there was no tank-to-tank variation in the number of post-larvae on the plates in each of the three *U. lens* + wild algae tanks. However across the *Navicula* sp. treatment there was tank-to-tank variation in the number of post-larvae across the three tanks.

4.4 Implications for Aquaculture

In this study the full potential of *Navicula* sp. to improve the growth rate of the post-larvae was not realised when compared to the *U. lens* + wild algae treatment, as the extensive infrastructure in the nursery lacked the ability to control the copepod populations and a developing competitive algae. These issues need to be addressed.

Seeding with *U. lens* in the nursery environment results in a settlement rate of 34 % of *H. rubra* larvae. The high growth rate on this treatment resulted in a shell length of ~1.8 mm at the end of week 5. The recruitment at week 6 was 226 post-larvae per side of a nursery plate. Furthermore the *U. lens* treatment did not result in tank-to-tank variation with respect to post-larvae settlement, shell growth or recruitment.

Once the free "swimming" zoospores from *U. lens* settle and germinate on a nursery plate, this macroalgae with a prostrate growth form increases in coverage over time. Furthermore the development of a wild algae does not appear to be inhibited around *U. lens*. *U. lens* appears to be resistant to copepod grazing and naturally re-spawns which continuously re-seeds the nursery tanks.

Maintenance of adequate food is a limiting factor in the intensification of abalone nursery production due to grazing pressure of > 3 mm post-larvae and/or copepods (Farrell, S., pers. comm.). Although > 3 mm post-larvae were outside the scope of this study, the presence of *U. lens* on a nursery plate is seen as advantageous in increasing the carrying capacity of a plate (Farrell, S., pers. comm.). The protocol to set up *U. lens* nursery tanks for settlement and feeding from dense mature seed plates requires limited labour and infrastructure. However the role and value of the additional developing wild algae around *U. lens* needs further investigation.

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