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INVESTIGATION OF OPTIMAL TEMPERATURE AND LIGHT CONDITIONS FOR THREE BENTHIC DIATOMS AND THEIR SUITABILITY TO COMMERCIAL SCALE NURSERY CULTURE OF ABALONE (*HALIOTIS LAEVIGATA*)

FIONA PARKER,* MARK DAVIDSON, KYLIE FREEMAN, SAM HAIR AND SABINE DAUME
Department of Fisheries, Research Division, P.O. Box 20, North Beach, WA 6920, Australia

ABSTRACT A series of trials was conducted to investigate the optimal temperature and light conditions for the culture of 3 benthic diatoms (*Cocconeis sublittoralis*, *Achnanthes longipes* and *Navicula* cf. *jeffreyi*), their biochemical composition as well as their suitability as a feed source for juvenile greenlip abalone (*Haliotis laevigata*) (4.07 ± 0.08 mm shell length) in commercial scale nursery culture over 24 wk. *C. sublittoralis* can grow well in a range of light intensities (830–1,217 lux) and is thus suited for the changing light conditions in a commercial abalone nursery. *Achnanthes longipes* grows well under high light conditions (1,412–4,400 lux) at low (18°C) and high (25°C) temperatures indicating that this species is more suited to culture in unshaded tanks and higher in the water column, on plates closer to the water surface. *Cocconeis sublittoralis* and *Navicula* cf. *jeffreyi* were cultured together to investigate competitive interactions between the two species. Throughout the experiment *Navicula* cf. *jeffreyi* was significantly higher in cell density when grown separately compared with the combined culture with *C. sublittoralis*. However when considering cell volume *C. sublittoralis* is substantially larger with a cell volume of 20,183 μm^3 compared with 367 μm^3 for *N.* cf. *jeffreyi*. Thus the cell density of *N.* cf. *jeffreyi* must be around 4 times higher than *C. sublittoralis* to achieve similar biomass, which was only the case at the start of the experiment. Hence *N.* cf. *jeffreyi* is a pioneer species (early colonizer) whereas *C. sublittoralis* is likely to eventually out compete *N.* cf. *jeffreyi*. *Cocconeis sublittoralis* is a suitable diatom species for commercial abalone nurseries, particularly when larger photophobic juveniles (+5 mm shell length) are cultured and shading is often necessary. The percentage of protein was significantly higher in *N.* cf. *jeffreyi* in comparison with the other two species whereas *A. longipes* contained significantly higher percentage lipid. At a commercial scale juvenile abalone were successfully maintained on diatom diets for at least 18 wk (to ca. 8 mm in SL) after which growth slowed. All diatom species declined in density after week 18 coinciding with a drop in temperature from 20.38 ± 0.09°C at the beginning of the experiment to 16.23 ± 0.11°C. Juveniles feeding on *N.* cf. *jeffreyi* and *A. longipes* reached only 9.99 ± 3.52 and 9.49 ± 3.21 mm, respectively, in nursery tanks after 24 wk. The biomass of these later two species was lowest overall because of the small cell volume of *N.* cf. *jeffreyi* and low cell density of *A. longipes*. Shell length reached 10.71 ± 3.58 and 10.42 ± 3.71 mm in the *C. sublittoralis* and mixed diatom treatments, respectively. Specific growth rates and weight gain were highest in the mixed treatment and biomass was highest in this treatment from week four onwards. This indicates that differences in food biomass are more important for the growth of these animals than differences in biochemical composition.

KEY WORDS: abalone, *Haliotis laevigata*, diatoms, growth, light, temperature, biochemical composition

INTRODUCTION

Benthic diatoms are the major food source for juvenile abalone, up to 5 mm in shell length, after which they move onto a diet of macroalgae (Kawamura et al. 1995). During the early stages of development, juvenile abalone require high quality benthic diatoms that provide adequate nutrition for growth and survival (Wang et al. 1997, Daume & Ryan 2004).

Diatom biofilms are developed either by inoculating settlement plates with cultured strains, or by relying on the natural seawater source to provide colonizing microalgae. The latter, however, does not ensure a consistent supply of suitable diatom species for the abalone. There is a need to find alternative algal species, which can grow well under consistent grazing pressure and that provide adequate nutrition for juvenile growth and survival. Additionally, the tolerance of a diatom species to fluctuating culture conditions, including changes in temperature and light intensity will define its suitability as a food source for abalone aquaculture (Brown et al. 1997). Commercial abalone hatcheries provide natural or artificial light sources for the development and maintenance of diatom cultures (Searcy-Bernal et al. 2003). Light intensity in a hatchery can be manipulated by shading tanks, however the optimal light intensity range for maintaining an adequate food source for the growing abalone needs to be determined to maximize growth and minimize time and costs associated with the culture of the microalgal species. The conditions under which diatoms are cultured play an important role in the suitability of a diatom species as abalone food. For example, light and temperature can affect the biochemical composition of the diatoms and thus their nutritional value to juvenile abalone (Brown et al. 1997, Watson et al. 2004). The transition of feed sources, from a smaller cell size to a larger cell size, can often be marked by high mortality in culture systems. Coculture of two or more diatom species may be of benefit to early juvenile culture, providing a selection of diatom species with different cell sizes and nutritional value (biochemical composition) at the same time. They may also decrease mortality and size variability because a large size range of animals is usually present on plates at the same time, requiring different feed sizes.

The diatom *Achnanthes longipes* (Agardh) consists of a 3D growth form and may provide more biomass with a more continuous food source for the grazing juveniles. The solitary cells of *A. longipes* attach to the substratum with a mucous thread. Initial tests showed that this diatom species is fast growing and suitable for culture on plates. Kawamura et al. (1995) reported growth rates of 48-μm day^{-1} of *Haliotis discus hannai* (Ino) juveniles (1–2 mm in shell length) when fed

*Corresponding author. E-mail: fiona.parker@fish.wa.gov.au

A. longipes. More recently Takami et al. (2003) found that juveniles (2.8–2.9 mm in shell length) grew at 100-μm day^{-1} on this diatom species. However, these studies have not yet been directly compared with commercial scale abalone nursery culture.

In this study, a series of laboratory trials were conducted to investigate the optimal culture conditions of three benthic diatoms, *Cocconeis sublittoralis* Hendey, *Achnanthes longipes* and *Navicula* cf. *jeffreyi*. The growth response of *A. longipes* and *C. sublittoralis* to varied levels of light and temperature was explored. *Cocconeis sublittoralis* and *N.* cf. *jeffreyi* were cultured together and in monoculture to assess species specific interactions and their effect on cell density and biomass of each species. In addition, the biochemical composition of the diatoms was analyzed. These diatom species were assessed for their suitability as a feed source for juvenile greenlip abalone (*Haliotis laevigata*, Donovan) (4.07 ± 0.08 mm shell length) in a commercial scale nursery culture system.

MATERIAL AND METHODS

Location

Laboratory experiments were conducted at the Fremantle Maritime Training Center, Fremantle, Western Australia. The commercial scale trial was conducted at Great Southern Marine Hatcheries, Albany, Western Australia. The commercial trial was run for 24 wk with the final diatom counts done at 23 wk.

Diatom Stock Culture and Cell Volume

Stock cultures for all three diatom species, *Cocconeis sublittoralis*, *Achnanthes longipes* and *Navicula* cf. *jeffreyi* were grown in sterile 50-mL culture flasks with 25 mL of *f*/2 media (Guillard & Ryther 1962). Diatom cell volume was determined by measuring the length and width of 6 cells per species using an inverted microscope. The cell volume was then calculated using the following formula:

$$Cell\ volume = \frac{4}{3}\pi ab^2$$

where a = longest radius and b = shortest radius.

Laboratory Experiments

Diatom Culture

Stock cultures of *C. sublittoralis* and *N.* cf. *jeffreyi* were held in a constant temperature room at 18 ± 1°C and *A. longipes* at 24 ± 1°C. Stock cultures were used to inoculate (1 mL) experimental cultures.

Cocconeis sublittoralis

Cocconeis sublittoralis was isolated from local ocean waters in Fremantle, Western Australia. This species was cultured under two light intensities, shaded (830 ± 80 lux) and unshaded (1217 ± 135 lux), and two temperature regimes, low (18°C ± 0.5) and high (25°C ± 0.5). Each treatment combination was replicated four times (total of 16 cultures). For each temperature a flow through water bath was set up to maintain constant temperatures. The light source was a 36W fluorescent cool white light placed underneath the water baths.

Achnanthes longipes

Achnanthes longipes was isolated from local ocean waters in Albany, Western Australia. Part A: two light intensities, shaded (256 ± 14 lux) and unshaded (1412 ± 21 lux) and 2 temperatures, high (25°C) and low (18°C).

Part B: two light intensities, shaded (891 ± 34 lux) and unshaded (4404 ± 78 lux), and two temperature regimes, low (18 ± 0.07°C) and high (25 ± 0.03°C).

For each temperature a flow through water bath was set up to maintain constant temperatures. Culture flasks were placed on weighted racks within the water baths. One of the water baths received heated water from a sump containing a heater and a pump. The other received water at room temperature. The room was chilled by an air conditioner. Light was provided by two fluorescent cool white globes hanging above the water baths, with a photoperiod of 12 L: 12 D. Each of the treatment combinations was replicated four times (total of 16 cultures).

Coculture of *Cocconeis sublittoralis* and *Navicula* cf. *jeffreyi*

Three treatment combinations, single cultures of *C. sublittoralis* and *N.* cf. *jeffreyi* and combined cultures of both species were replicated four times. *Navicula* cf. *jeffreyi* was isolated from local ocean waters at Port Fairy, Victoria. Each was inoculated with 1.5 mL of stock culture. All cultures were held in a controlled temperature room at 18 ± 1°C at 1400 ± 20 lux. After one week the *f*/2 media (Guillard & Ryther 1962) was replaced every 2–3 days (after every cell count) to prevent any nutrient deficiencies during the growing period.

Laboratory Scale Measurements

Cell density was estimated by counting cells directly under an inverted microscope (*C. sublittoralis* at ×200 magnification in 20 fields of view; *A. longipes* at ×400 magnification in 10 fields of view; Coculture of *C. sublittoralis* and *N.* cf. *jeffreyi* was determined from 10 fields of view at ×400 magnification). The number of diatom cells cm^{-2} was then calculated. Specific growth rates were calculated using the following formula:

$$Specific\ growth\ rate\ (\%\, day^{-1}) = 100 \times (\ln(final\ cell\ count) - (\ln(initial\ cell\ count))/number\ of\ days$$

Biochemical Analysis of Diatoms

Eight tissue culture flasks per diatom species were prepared for biochemical analysis. The flasks contained 25 mL of f/2 medium and were inoculated with 1 mL of diatom stock solution. After inoculation the flasks were stored within a constant temperature growth cabinet at 18 ± 1°C, on a 12 h L: D cycle at 796 ± 19.39 lux for 12 days. Total protein, lipid, carbohydrate, and ash contents in the three diatom species were determined at each cultures stationary phase. Each sample was filtered through 25-mm Whatman GF/C glass microfibre filters. To rupture diatom cells, all samples were homogenized with a mortar and pestle prior to assays.

Protein Determination

Total soluble protein was measured using a modified Lowry method based on Dorsey et al. (1978) and adapted by Mercz (1994) and Buttery (2000). Bovine serum albumin (BSA) was

used as the protein standard. Soluble protein was extracted by heating the sample at 100°C in a Biuret solution for 60 min. After incubation 0.5 mL of Folin phenol reagent was added. The absorbance of the supernatant was read at 660 nm with a Cary 50 Probe UV-visible spectrophotometer (Varian Inc.).

Carbohydrate Determination

Total soluble carbohydrate was determined using the phenol-sulfuric acid method of Kochert (1978) and Ben-Amotz et al. (1985) incorporating the modifications of Mercz (1994) and Buttery (2000). The samples were homogenized in 1 M H_2SO_4 and after heating at 100°C for 60 min, 0.3 mL of the supernatant was transferred into a fresh vial and made up to 2 mL with deionized water. Sets of glucose standards were prepared and 1 mL of 5% (w/v) phenol solution was added to all samples, after which, 5 mL of concentrated H_2SO_4 was added. Absorbance was read at 485 nm.

Lipid Determination

The total lipid determination was based on the method of Bligh and Dyer (1959) as modified by Kates and Volcani (1966) and adapted by Mercz (1994) and Buttery (2000) using a methanol:chloroform:deionised water (2:1:0.8, v/v/v) solution. The vials were dried under a stream of ultra pure N_2 gas and placed in a vacuum desiccator over silica gel overnight and then weighed.

Ash Determination

Total ash weights were determined by following the methods of Mercz (1994) and Buttery (2000). The glass microfiber filter papers were placed within crucibles before being combusted at 475°C for 24h. After combustion, the samples were weighed to 5 decimal places and total ash was calculated by subtracting the initial filter paper weight.

Commercial Scale Nursery Trial

Diatom Culture

Monospecific diatom strains of the species *N*. cf. *jeffreyi*, *C. sublittoralis* and *A. longipes* were scaled up from 50 mL culture flasks to 200 mL culture bags and then transferred to 1.5 L culture bags; 1.5 L culture bags were then used to inoculate 60 L culture bags. All cultures were laid horizontally on benches in a constant temperature room at 18 ± 1°C. Large bags were then used to inoculate round shallow tanks (120 L), these tanks were held outdoors under clear perspex roofing to provide natural light. Seawater for larger cultures was sterilized by chlorination/dechlorination. Nutrients for 50-mL cultures and 200-mL culture bags were supplied as *f*/2 media (Guillard & Ryther 1962) and Microalgae food (MAF, Manutech, Port Lincoln, Australia) was added to the larger cultures at a concentration of 30 g 1000 L^{-1}. Nursery tanks were initially inoculated every week with 15 L of diatom culture (per species), and supplied with nutrients. Tanks remained static with very low aeration for 24 hrs after each inoculation. After the first 6 wk tanks were inoculated fortnightly because of problems with maintaining dense diatom cultures outdoors as the temperature and light levels decreased. Additionally settlement plates were flipped every fortnight to ensure more consistent coverage of diatoms over the plates.

Commercial Scale Tank System

Experimental nursery tanks (390 L) equipped with 3 baskets holding 20 plates (600 × 300 mm) each were used (Fig. 1). Removable platelets (120 × 170 mm) were fastened to the center of 6 settlement plates (per tank) and used to determine diatom density. Filtered seawater (1-μm) was supplied by a spray bar at a constant rate of 6 L per minute. Each tank held three airlines running parallel to the plates. Tanks were positioned in a semi-enclosed area with clear perspex roofing and walls providing natural light to the tanks. Four treatments were tested, three

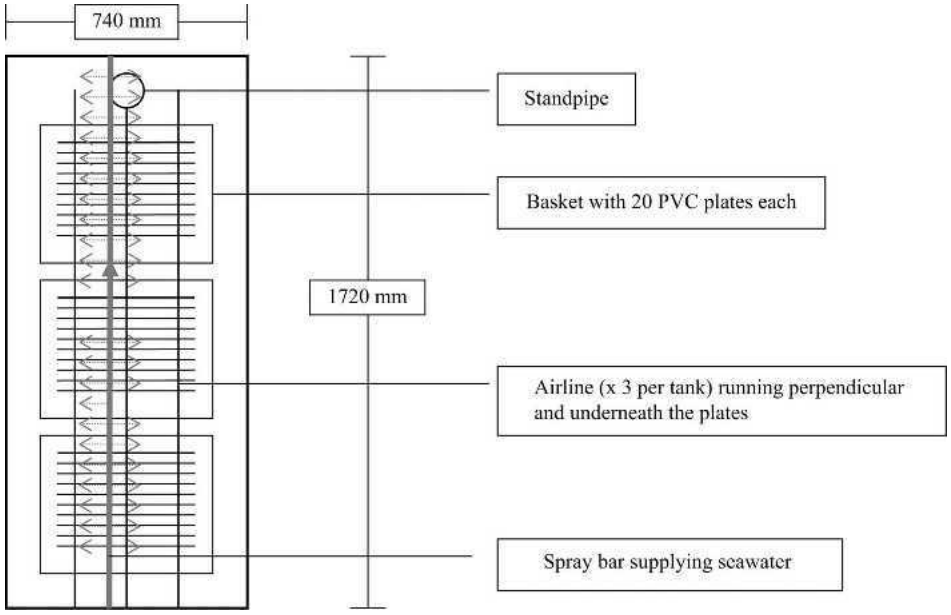

Figure 1. Design of experimental nursery tanks for commerical scale nursery trial.

single species diets (*N.* cf. *jeffreyi, C. sublittoralis* and *A. longipes*) as well as a mixed diet combining all three species. Treatments were randomly assigned to three tanks each. Tanks were stocked with 2,400 juvenile abalone (*Haliotis laevigata*) (40 juveniles per plate) (4.07 ± 0.08 mm shell length). The juveniles were 4 months old and obtained from a commercial abalone farm (Great Southern Marine Hatcheries) in Albany, Western Australia.

Measurements

Platelets were removed regularly (prior to inoculations) and diatom density determined using an inverted microscope at ×400 in 10 random fields of view. The number of diatom cells cm^{-2} was calculated. At both the beginning and end of the trial the shell length and weight of 200 juveniles per tank was recorded, animals were weighed in subsets of 50 animals. A further 2,200 juveniles were added to each tank by weight. Each fortnight 50 randomly selected animals from each of the 12 tanks were measured (shell length). Specific growth rates were calculated using the following formula:

$$\textit{Specific growth rate } (\%\, day^{-1}) = 100 \times (\ln(\textit{final shell length}) - (\ln(\textit{initial shell length}))/\textit{number of days}$$

Temperature and Light

The light intensity was measured using a Lutron LX-103 light meter. Light was measured in units of lux. All measurements were multiplied by a correction factor (1.20) for fluorescent lighting. Temperature was measured using submersible temperature loggers (eTemperature Version 2.04).

Statistical Analysis

All data analyses were carried out using Statistica software (version 6.0. StatSoft, Inc. 2002). Normality of all data was checked graphically using histograms and with the Kolmogorov-Smirnov test. Homogeneity of variances was tested using the Levene test.

Laboratory Experiments

Comparisons of *C. sublittoralis* cell density over time were done using repeated measures analysis of variance (ANOVA) followed by Tukey *post hoc* comparisons. Specific growth rate comparisons were analyzed using one-way ANOVA and Tukey *post hoc* comparisons. Between days 4 and 6 the low temperature shaded data was not included in the analysis as all data were negative and therefore there was no growth.

Growth data of *A. longipes* did not conform to assumptions of normality or homogeneity of variances. To determine whether the initial density of diatoms was significantly different within Part A and Part B a Kruskal-Wallis test was performed. Where appropriate, one-way ANOVA or Kruskal-Wallis test was used to determine significant differences in growth between the treatments and followed by Tukey *post hoc* comparisons. For part A and B, comparisons of specific growth rate over time were carried out using repeated measures ANOVA and Tukey *post hoc* comparisons. Comparisons of specific growth rate over the whole period were tested using one-way ANOVA with Tukey *post hoc* comparisons. Cell density over time and specific growth rate in the coculture experiment were compared using repeated measures analysis of variance (ANOVA) followed by Tukey *post hoc* comparisons. Cell density data was square root transformed to meet the assumptions of normality and homogeneity of variances. Analysis of biomass data was done using repeated measures ANOVA and one-way ANOVA where appropriate.

Biochemical Analysis

Lipid data were log transformed. One-way ANOVA was used to determine significant differences in levels of protein, carbohydrate, and lipid, followed by Tukey *post hoc* comparisons.

Commercial Scale Nursery Trial

Where appropriate, one-way ANOVA was used to determine significant differences in growth between the diet treatments, followed by Tukey *post hoc* comparisons.

RESULTS

Laboratory Experiments

The cell length and width of the diatoms used in this study were different between the species of diatoms (Table 1) resulting in different cell volumes. *Navicula* cf. *jeffreyi* has a much smaller cell volume than *Cocconeis sublittoralis* or *Achnanthes longipes*.

Cocconeis sublittoralis

There was a significant difference in cell density between the growing conditions (df = 3, F = 5.77, $P = 0.01$) and the measurements over time (df = 2, F = 40.21, $P < 0.001$). The cell density of *C. sublittoralis* increased faster in the lower temperature (18°C) and shaded treatment (830 lux) but then decreased between 4 and 6 days (Fig. 2). Two and four days after inoculation the cell density was highest in the lower temperature and shaded treatment combination compared with both

TABLE 1.

Comparison of cell length, width, and volume for *Cocconeis sublittoralis*, *Achnanthes longipes* and *Navicula* cf. *jeffreyi* ($n = 6$). (mean ± SE).

Diatom Species	Cell Length (μm)	Cell Width (μm)	Cell Volume (μm^3/cell)
Cocconeis sublittoralis	48.66 ± 2.98	27.30 ± 1.72	20,183.52 ± 3,281.19
Achnanthes longipes	73.64 ± 4.66	46.90 ± 5.58	96,874.21 ± 26,414.17
Navicula cf. *jeffreyi*	11.18 ± 0.82	7.56 ± 0.60	366.71 ± 85.50

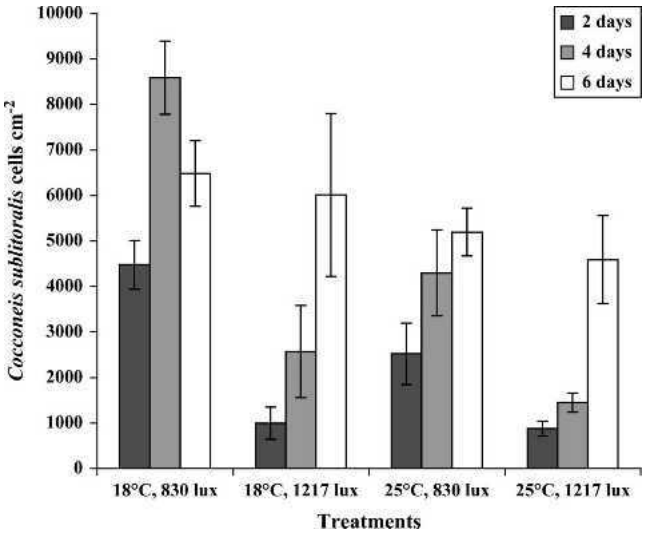

Figure 2. Cell density of *Cocconeis sublittoralis* (cells $cm^{-2} \pm$ S.E.) at 2, 4 and 6 days after inoculation when growing at four treatment combinations (2 temperatures × 2 light intensities) (n = 4).

unshaded treatments (posthoc $P = 0.04$, $P = 0.01$ for lower and higher temperature respectively). At day 6 there was no significant difference in cell density between the treatments (df = 3, F = 0.51, $P = 0.68$).

Specific growth rates were highest in the low temperature unshaded treatment between 2 and 4 days and in high temperature unshaded treatment between 4 and 6 days (Table 2).

Specific growth rates between days 2 and 4 were not significantly different ($F_{3,12} = 1.08$, $P = 0.39$), however between days 4 and 6 both unshaded treatments (high and low temperature) were significantly higher than the high temperature shaded treatment ($F_{2,9} = 7.51$, $P = 0.012$). Specific growth rates were highly variable between replicates in both the shaded treatments after 4 days (Table 2).

Achnanthes longipes

Throughout both trials *A. longipes* maintained a higher mean cell density in unshaded (1,412 and 4,404 lux) than in shaded (256 and 891 lux) culture flasks (Fig. 3A & B, respectively). In the first experiment (Part A) the initial starting density (measured at day 4) was not significantly different between any of the treatments (χ^2 (3) = 6.25, $P = 0.1002$). In

TABLE 2.

Specific growth rate (% cells $day^{-1} \pm$ SE) of *Cocconeis sublittoralis* grown under four treatment combinations (2 temperatures ×2 light intensities) (*n* = 4).

Treatment	Day 2–4	Day 4–6
Low temperature shaded	32.60 ± 20.61[a]	NG
Low temperature unshaded	47.21 ± 52.73[a]	42.51 ± 28.57[a]
High temperature shaded	26.63 ± 16.76[a]	9.51*[b]
High temperature unshaded	25.06 ± 12.63[a]	57.71 ± 76.58[a]

NG, no growth, cell density decreased

* Highly variable between replicates, one replicate with NG.

Means, within a column, with different superscript letters are significantly different ($P < 0.05$).

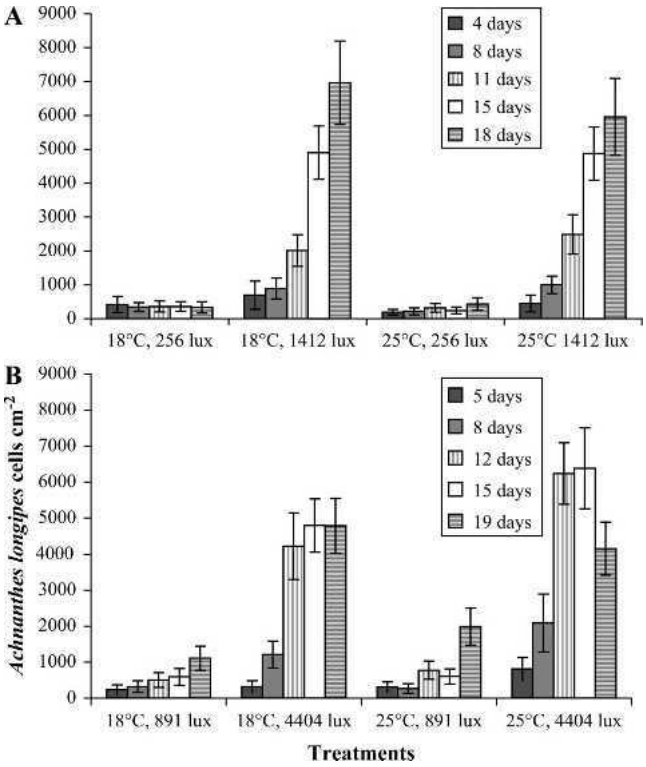

Figure 3 (A and B). Growth of *Achnanthes longipes* (cells $cm^{-2} \pm$ S.E.) over time when growing four treatment combinations (2 temperatures × 2 light intensities) (n = 4) .

both shaded treatments (high and low temperature) the cell density was low throughout the experiment. In contrast, in both unshaded treatments (high and low temperature) the cell density continued to increase as the trial progressed. The unshaded treatments were significantly different from the shaded treatments at day 11 (χ^2 (3) = 12.17, $P = 0.0068$), day 15 (χ^2 (3) = 11.54, $P = 0.0091$), and at day 18 (χ^2 (3) = 11.54, $P = 0.0092$). Specific growth rates (SGR) were significantly different over time (df = 12, F = 3.86, $P < 0.001$). Over the whole experiment, from day 4–18, the SGR was significantly higher in the high temperature unshaded treatment (54.61 ± 9.54% cells day^{-1} ± SE) than in both the shaded treatments (high temperature 12.61 ± 12.10 and low temperature 0.37 ± 8.73% cells $day^{-1} \pm$ SE) (df = 3, F = 8.34, $P = 0.002$). Although cell density increased up to day 18 (Fig. 3A), the SGR was highest between days 8 and 11 (24.93 ± 4.50% cells $day^{-1} \pm$ SE), suggesting that growth is slowing after this time.

In the second part of the experiment (Part B), the number of cells in the high temperature unshaded treatment was significantly higher than in all other treatments (Fig. 3B), ($\chi^2(3) = 8.06$, $P = 0.045$ up to day 15. After 15 and 19 days the density of *A. longipes* was significantly higher in both unshaded treatments compared with the shaded treatments ($\chi^2(3) = 11.53$, $P = 0.009$). The SGR was significantly different between treatments over time (df = 12, F = 4.69, $P < 0.001$). Between days 5 and 8, the SGR in the low temperature unshaded treatment (33.44 ± 2.82% cells day^{-1} ± S.E.) was significantly higher than in the high temperature shaded treatment where the SGR was negative. Between day 8 and 12 and 12 and 15 there was no significant difference in specific growth rates between treatments. The SGR between days 15 and 19 was significantly

higher in the high temperature shaded treatment (20.69 ± 6.58% cells day^{-1} ± SE) than in the high temperature unshaded treatment, where cell density decreased (Fig. 3B).

Overall, at lower temperature (18°C) the cell density was highest at intermediate light intensity (1,412 lux) whereas at higher temperature (25°C) the cell density was similar in intermediate (1,412 lux) and highest light intensity (4,404 lux) but decreased between 15 and 19 days in the highest light treatment.

Coculture of Cocconeis sublittoralis *and* Navicula *cf.* jeffreyi

The cell density of *N*. cf. *jeffreyi* was significantly higher throughout the experiment when grown separately compared with the combined culture (df = 3, F = 56.63, $P < 0.001$). In contrast, the cell density of *C. sublittoralis* in separate culture was only higher at the start of the experiment (Table 3). After 23 days, the cell density of *C. sublittoralis* increased in the combination treatment, particularly between 28 and 30 days, however the density of *N*. cf. *jeffreyi* decreased after 30 days (Table 3). *Navicula* cf. *jeffreyi* is small with a volume of 366.71 ± 85.50 μm^3 (Table 1). *Cocconeis sublittoralis* is substantially larger with a cell volume of 20,183.52 ± 3281.19 μm^3 (Table 1). In the single culture, the biomass of *C. sublittoralis* consistently remained higher than that of *N*. cf. *jeffreyi* because of cell size (Table 3). After an initial drop in biomass of *C. sublittoralis* in both the monoculture and mixed culture treatments biomass increased and peaked at day 23 (Table 3). At day 23 the biomass of *N*. cf. *jeffreyi* was significantly lower than *C. sublittoralis* in both the single ($F_{6,1} = 8.8052$, $P = 0.03$ One way ANOVA) and mixed cultures ($F_{6,1} = 12.82$, $P = 0.01$ One way ANOVA). There was a significant effect of treatment (df = 3, F = 19.91, $P < 0.001$) and days (df = 10, F = 4.14, $P < 0.001$) for specific growth rates. *Navicula* cf. *jeffreyi* biomass did not differ significantly over time when grown in monoculture or mixed culture with *C. sublittoralis* ($F_{6,1} = 5.47$, $P = 0.32$ One way ANOVA). This was also true for *C. sublittoralis* ($F_{6,1} = 50.24$, $P = 0.11$ One way ANOVA). The SGR in the single *N*. cf. *jeffreyi* culture was highest between days 24–26, whereas the single culture of *C. sublittoralis* was highest between days 26–29 (Table 3). In the combined cultures the SGR of *N*. cf. *jeffreyi* was highest between days 19 and 22 (6.03 ± 3.72% cells day^{-1}), whereas the SGR of *C. sublittoralis* did not peak until between days 29–31 (7.86 ± 9.51% cells day^{-1}) (Table 3).

Biochemical Analysis

Navicula cf. *jeffreyi* had a significantly higher percentage of protein ($F_{2,12} = 8.84$, $P = 0.01$) than the other two species (Table 4). Carbohydrate levels were not significantly different ($F_{2,6} = 0.09$, $P = 0.91$) however lipid was significantly higher in *A. longipes* ($F_{2,6} = 83.02$, $P = 0.00$) (Table 4). Ash content was highest in *N*. cf. *jeffreyi* (25.82% ± 2.75%) however was not statistically analyzed because of having only two replicates.

Commercial Scale Abalone Nursery Trial

Juvenile abalone feeding on *N*. cf. *jeffreyi* and *A. longipes* reached a shell length of only 9.99 ± 3.52 and 9.49 ± 3.21 mm, respectively, in nursery tanks after 24 wk. However, shell length reached 10.71 ± 3.58 and 10.42 ± 3.71 mm in the *C. sublittoralis* and mixed diatom treatments, respectively. Overall specific growth rates (SGR Length as % day^{-1}) were highest in the mixed culture, however they did not differ significantly ($F_{3,8} = 0.45$, $P = 0.73$) (Table 5). There was also no significant difference in daily growth ($F_{3,8} = 0.46$, $P = 0.72$), weight gain ($F_{3,8} = 0.57$, $P = 0.65$) or survival ($F_{3,8} = 0.46$, $P = 0.72$) over the four diatom treatments (Table 5). Mortality was highest during the first months (20 ± 1.38%) but similar in all treatments and dropped to less than 1% after the first month.

The cell volume of *N*. cf. *jeffreyi* is 366 μm^3 whereas *C. sublittoralis* and *A. longipes* are substantially larger with a cell volume of 20,183 μm^3 and 96,874 μm^3, respectively (Table 1). Therefore the cell density of *N*. cf. *jeffreyi* must be around 4–7 times as high to achieve similar biomass than the other two species. In addition *A. longipes* also consist of a mucus thread that can contribute to the food biomass. Throughout the trial, density of the diatom *N. cf. jeffreyi* in both single and mixed culture was highest. All diatom species peaked in density and biomass between weeks 4 and 7 and all diatom treatments declined in density and biomass after week 15 or 18. Overall the density of *N*. cf. *jeffreyi* in the single species culture peaked at 68×10^3 cells cm^{-2} at the beginning of the experiment and density was lowest at week 12 (28×10^3 cells cm^{-2}), biomass (10.43 ± 2.6×10^6 μm^3 cm^{-2}) was also low because of the small cell size (Table 6). *Cocconeis sublittoralis* peaked in cell density at week 4 (30×10^3 cells cm^{-2}) and was lowest at the beginning of the experiment (11×10^3 cells cm^{-2}), whereas biomass was still high (220.7 ± 70.3 × 10^6 μm^3 cm^{-2}) because of the high cell volume. Density of *A. longipes* was low in the first week of the experiment (3×10^3 cells cm^{-2}) and increased to a maximum of only 15×10^3 cells cm^{-2} in week 7, whereas biomass was 1472.5 ± 325.1 × 10^6 μm^3 cm^{-2} because of the large cell volume. In the mix diatom treatment, both *N*. cf. *jeffreyi* and *A. longipes* peaked in cell density at week 7, (68×10^3 and 15×10^3 cells cm^{-2}, respectively) whereas *C. sublittoralis* was highest at week 4 (24×10^3 cells cm^{-2}).

After 4 wk, the biomass of diatoms was highest in the mixed treatment, because of an increase in the density of *N*. cf. *jeffreyi*, *C. sublittoralis* and *A. longipes* (Table 6), reaching 625 ± 333 × 10^6 μm^3 cm^{-2} at week 7. Although cell density of *N*. cf. *jeffreyi* was higher (68×10^3 cells cm^{-2}) than *C. sublittoralis* (16×10^3 cells cm^{-2}) and *A. longipes* (15×10^3 cells cm^{-2}) at this time, the large cell volume of the later two species suggests they were more likely contributing to the large biomass than *N*. cf. *jeffreyi*.

After 15 wk the green alga *Ulvella lens* Crouch started appearing on the plates in all treatments and probably also contributed as a food source. *U. lens* showed a slightly higher cover in the *N*. cf. *jeffreyi* and mixed diatom treatment at 18 wk, 13.72% and 13.06% respectively. *Ulvella lens* was only able to colonize the plates when the diatom density had declined.

Temperature and Light

The average seawater temperature within the nursery tanks declined over the 24 wk trial period (Fig. 4). Light levels were measured throughout June and July and ranged from 16,336 ± 2,812 lux in June to 2,928 ± 426 lux in July (Fig. 4). Light was highly variable over days as well as tanks. Although light was not measured over February through to May it is assumed that this variability would be more pronounced during the sunnier months of February and March.

TABLE 3.

Cell density (cells cm^{-2} ± SE) and biomass (μm^3 cm^{-2} ± SE) of *Navicula* cf. *jeffreyi* and *Cocconeis sublittoralis* over a 37 day growing period when grown separately (monoculture) or together (mixed culture) ($n = 4$).

Treatment		Diatom Species	Days															
			1	4	7	9	11	14	16	18	21	23	25	28	30	32	35	37
Monoculture	Density ($\times 10^4$)	*N.* cf. *jeffreyi*	18.3 ± 2.0	10.0 ± 1.2	10.7 ± 1.1	6.7 ± 1.1	8.3 ± 1.5	9.6 ± 1.8	9.4 ± 1.1	14.4 ± 2.4	14.1 ± 2.6	12.0 ± 2.1	13.2 ± 1.4	7.0 ± 1.2	12.8 ± 1.5	7.8 ± 1.2	16.9 ± 1.9	16.1 ± 2.1
	Biomass ($\times 10^6$)	*N.* cf. *jeffreyi*	67.0 ± 3.9	37.0 ± 2.7	39.5 ± 6.3	24.6 ± 1.2	33.7 ± 4.9	35.1 ± 5.6	34.5 ± 3.3	52.8 ± 3.8	51.9 ± 1.9	43.9 ± 5.9	48.3 ± 5.0	25.5 ± 2.2	46.9 ± 2.7	28.5 ± 1.8	62.0 ± 6.6	59.0 ± 5.4
Monoculture	Density ($\times 10^4$)	*C. sublittoralis*	6.1 ± 1.0	4.5 ± 1.0	4.5 ± 1.0	3.7 ± 1.1	3.7 ± 1.1	3.2 ± 7.7	3.8 ± 1.1	4.1 ± 1.1	4.7 ± 1.1	5.1 ± 0.9	4.5 ± 1.2	3.9 ± 1.1	5.2 ± 1.2	5.1 ± 1.0	4.5 ± 0.7	4.6 ± 0.8
	Biomass ($\times 10^6$)	*C. sublittoralis*	1243.3 ± 67.9	904.2 ± 45.7	904.2 ± 26.4	742.8 ± 57.5	750.8 ± 82.7	653.9 ± 53.4	758.9 ± 119.7	823.5 ± 113.8	952.7 ± 150.6	1033.4 ± 132.5	896.1 ± 108.2	791.2 ± 65.3	1049.5 ± 40.6	1025.3 ± 85.8	896.1 ± 59.5	936.5 ± 34.9
Mixed culture	Density ($\times 10^4$)	*N.* cf. *jeffreyi*	12.8 ± 1.5	6.1 ± 1.3	6.3 ± 1.0	4.6 ± 9.4	5.1 ± 9.0	6.5 ± 1.2	5.2 ± 9.9	6.5 ± 1.3	7.0 ± 1.8	6.3 ± 1.3	4.1 ± 1.0	3.7 ± 9.5	3.7 ± 9.6	3.5 ± 7.8	2.9 ± 8.5	2.5 ± 8.5
	Biomass ($\times 10^6$)	*N.* cf. *jeffreyi*	47.23 ± 4.7	22.4 ± 4.0	23.18 ± 4.1	16.9 ± 0.8	18.6 ± 3.8	23.6 ± 4.8	19.07 ± 1.5	23.6 ± 5.6	25.8 ± 3.1	23.0 ± 4.9	15.1 ± 3.3	13.5 ± 13.6	13.6 ± 2.4	12.8 ± 2.6	10.7 ± 2.8	9.2 ± 1.3
Mixed culture	Density ($\times 10^4$)	*C. sublittoralis*	2.5 ± 0.3	2.2 ± 0.6	2.6 ± 0.8	2.0 ± 0.6	2.2 ± 0.8	2.4 ± 0.8	2.9 ± 0.8	3.8 ± 0.8	4.6 ± 0.8	7.0 ± 0.8	4.3 ± 8.1	4.8 ± 0.9	4.9 ± 0.9	3.4 ± 0.8	3.4 ± 0.6	3.4 ± 0.5
	Biomass ($\times 10^6$)	*C. sublittoralis*	516.7 ± 86.5	444.0 ± 70.2	524.8 ± 65.1	411.7 ± 46.4	436.0 ± 84.9	484.4 ± 91.3	589.4 ± 62.4	775.0 ± 156.0	936.5 ± 104.6	1404.8 ± 363.8	880.0 ± 164.9	976.9 ± 211.1	993.0 ± 167.0	686.2 ± 149.4	694.3 ± 96.4	678.2 ± 96.9

TABLE 4.

Biochemical analysis of three benthic diatom species (% ± SE) (n = 8).

Species	Protein	Carbohydrate	Lipid	Ash
N. cf. *jeffreyi*	24.69 ± 3.0[a]	25.64 ± 4.04[a]	23.50 ± 0.30[a]	25.82 ± 2.75
C. sublittoralis	15.76 ± 2.16[b]	28.02 ± 7.81[a]	21.97 ± 0.58[a]	19.59 ± 0.14
A. longipes	11.60 ± 1.50[b]	29.00 ± 3.64[a]	30.99 ± 0.59[b]	8.80 ± 5.90

* Means, within a column, with different superscript letters are significantly different ($P < 0.05$).

DISCUSSION

Laboratory Experiments

Cocconeis sublittoralis

Results indicate that *Cocconeis sublittoralis* can grow well under both low (830 lux) and high light (1217 lux) conditions and varied temperature levels (18 or 25°C), which suggests this species is well suited to the changing light conditions in nursery tanks on vertical plates. Mean cell density increased faster and was highest in the low temperature (18°C) and shaded (830 lux) treatment after which specific growth rate declined. There was no growth in the low light treatments at the end of the experiment, and cells also became dislodged because of high cell density particularly in the later treatment.

Achnanthes longipes

The final cell density was highest at intermediate light intensity at 1412 lux (Part A) both at low and high temperatures (18°C and 25°C) suggesting that the growth of *Achnanthes longipes* was more influenced by light than by the temperature levels.

Because light intensity controls the light reaction of photosynthesis (Valiela 1984), the light intensity may have limited the rate of photosynthesis resulting in reduced growth rates. In Part B, the density of *A. longipes* in the high temperature unshaded treatment decreased after 15 days (Fig. 3) suggesting that *A. longipes* may have been light saturated or nutrient limited. Lewis et al. (2002) found growth of *A. longipes* to be light saturated at 60 μmol photons $m^{-2}s^{-1}$. This is equivalent to 4,440 lux, using the conversion factor provided in Langhans and Tibbitts (1997). This same author reported an optimal temperature for maximum growth as 26°C, at higher temperatures the growth rate of *A. longipes* dropped considerably. The effect of higher temperature (above 24°C) on growth of the *A. longipes* strain used in this study requires further investigation but is unlikely to be important for temperate abalone nurseries that record maximum temperatures of around 22°C to 24°C.

Co-culture of Cocconeis sublittoralis *and* Navicula *cf.* jeffreyi

Medium changes and thus nutrient replenishments were carried out three times a week post cell density estimates, which accounted for the fluctuations in cell density. Medium changes also resulted in some loss of cells that were not as tightly attached (e.g., older cultures release small biofilm patches into the water column), because of discarding old medium and refilling with new medium. However medium changes ensured that growth did not become limited by the availability of nutrients and was supposed to mimic nutrient addition in nursery tanks. *Navicula* cf. *jeffreyi* responded very quickly to nutrient addition whereas growth of *C. sublittoralis* stayed fairly constant. *Cocconeis sublittoralis* can grow well under lower light condition (see above) and will eventually out compete an early colonizer like *N.* cf. *Jeffreyi*, which was present at much higher cell density at the start of the experiment. These results indicate that *C. sublittoralis* may be a suitable diatom species for commercial abalone nurseries where shading is often necessary to manipulate the species composition and density of microalgae in the tanks and to provide a better environment for the photophobic juveniles.

Biochemical Analysis

Although significant differences were found in biochemical composition between the three diatom species there was no significant difference in growth, which suggests food biomass may be more important. However, juveniles reached a shell length of 10.42 ± 3.71 mm in the mixed diatom treatment and overall the specific growth rate was highest in the mixed culture. Mixed algal diets may provide a better balance of nutrients, if

TABLE 5.

Specific growth rates (SGR), daily growth, weight gain and survival of juvenile abalone feeding on different diatom diets over 24 wk (n = 3, ± SE).

Diet	SGR (% day^{-1})	Daily Growth (μm day^{-1})	Weight Gain (g)	Survival (%)
N. cf. *jeffreyi*	0.51 ± 0.06	34.21 ± 4.51	0.14 ± 0.02	65.80 ± 4.51
C. sublittoralis	0.55 ± 0.03	38.90 ± 2.50	0.14 ± 0.02	61.10 ± 2.50
A. longipes	0.54 ± 0.05	33.94 ± 5.09	0.11 ± 0.03	66.06 ± 5.09
Mixed	0.58 ± 0.05	38.61 ± 3.29	0.15 ± 0.01	61.40 ± 3.30

TABLE 6.

Biomass ($\times 10^6$ μm^3 cm^{-2} ± SE) of diatom feed treatments throughout the experimental period (0–23 wk).

Species	Weeks							
	0	2	4	7	12	15	18	23
N. cf. *jeffreyi* (μm^3 $cm^{-2} \times 10^6$)	25.0 ± 6.1	11.47 ± 3.2	11.87 ± 2.4	14.5 ± 2.5	10.43 ± 2.6	21.7 ± 3.3	12.5 ± 2.8	11.3 ± 2.5
C. sublittoralis (μm^3 $cm^{-2} \times 10^6$)	220.7 ± 70.3	326.5 ± 108.6	604.6 ± 110.3	595.6 ± 134.3	303.2 ± 47.9	292.4 ± 44.2	342.8 ± 73.4	318.6 ± 71.6
A. longipes (μm^3 $cm^{-2} \times 10^6$)	258.3 ± 148.0	895.5 ± 177.0	1196.9 ± 197.6	1472.5 ± 325.1	602.8 ± 114.6	852.5 ± 176.6	465.0 ± 76.8	516.7 ± 83.6
Mixed (μm^3 $cm^{-2} \times 10^6$)	84.7 ± 59.3	197.7 ± 147.9	886.2 ± 486.5	625.0 ± 333.0	350.3 ± 177.3	317.7 ± 159.4	373.8 ± 176.1	281.6 ± 138.4

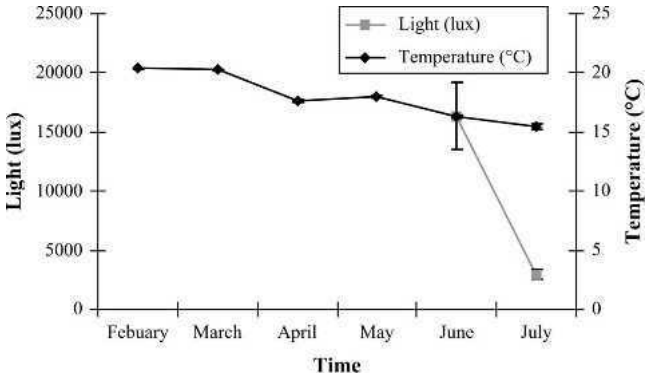

Figure 4. Seawater temperature and light intensity over the experimental period (Mean ± S.E.).

one or more diatom species are lacking in key nutrients needed for growth (Brown et al. 1997). *Navicula* cf. *jeffreyi* contained a significantly higher percentage of protein (24.69 ± 3.0%) than the other two diatom species. The optimal level of protein in an abalone diet depends on the species, however optimal levels have been reported to range from 20% to 35% (Uki & Watanabe 1992, Mai et al. 1995, Britz & Hecht 1997, Coote et al. 2000). The levels of lipid found in the three diatom species are much higher than the reported optimum (3% to 5%) (Mai et al. 1995). Additionally high levels of lipids (≥5%) are thought to be detrimental to growth of abalone (Thongrod et al. 2003), as in other marine herbivores. The previous studies were conducted with larger juveniles (10 mm), whereas the juveniles used in this study were smaller (4.07 ± 0.08 mm) and thus are likely to have different nutritional requirements. However, high levels of carbohydrates (5% to 30%) are thought to enhance growth of abalone (Mercer et al. 1993, Renaud et al. 1999). The time at which diatoms are harvested as well as their culture conditions can affect the biochemical composition (Brown et al. 1993), in the case of this experiment diatoms were harvested during the stationary phase and all three diatom species were cultured under the same conditions (light, temperature, and nutrients).

Commercial Scale Trial

Overall specific growth rates and weight gain were highest in the mixed treatment and diatom biomass was highest in this treatment from week four onwards. Although *N.* cf. *jeffreyi* displayed good growth and high cell density counts, the biomass was not high because of the small cell volume of this species. Therefore using solely density estimates to compare diatom species as a food source for juvenile abalone can be misleading especially so when the diatoms have greatly different cell sizes. Although cell size of diatoms and thus volume may change over time, the estimates of cell volume in this trial were run simultaneously with the commercial trial so it is assumed that differences in cell sizes within the same species would be minimal.

Diatom biomass decreased after week 18 (Table 6) at which time temperature and light intensity (Fig. 4) also declined, most likely influencing the decline in juvenile growth.

The first drop in diatom biomass occurred at week 12, which most likely resulted in a delayed growth response in the juvenile abalone at week 15. Therefore in cooler months when temperature and light are at their lowest, it may be difficult to maintain

juveniles on diatom feeds only and weaning onto formulated feed or supplementing with other algal feeds may be required. Initially tanks were inoculated every week (first 6 wk) however as the light levels declined, maintaining dense outdoor cultures (last stage of scale up culture method) became limiting.

From the laboratory scale studies, it seems that growth of *A. longipes* is more influenced by light than by temperature and thus as the experiment progressed the declining light levels may have limited growth. *Cocconeis sublittoralis* was growing well under low light conditions, which may account for this species maintaining a relatively high biomass between weeks 15 and 23 in the commercial scale experiment, when natural light was declining, whereas the biomass of the other treatments declined (Table 5). Similarly, Watson et al. (2004) found growth of *Cocconeis* sp. was not inhibited at a low light intensity (80 lux). As well, *Cocconeis* spp. are considered to be dominant in subtidal regions, rather than well lit intertidal regions (Round 1971). Recently Takami et al. (2003) reported very promising growth rates of up to 100 μm/day for juvenile *Haliotis discus hannai* (2.9 mm in shell length) feeding on *A. longipes*, however this trial was only conducted for 10 days. The strain of *A. longipes* used in this study was a large strain (73.64 ± 4.66-μm length, 46.90 ± 5.58-μm width), which suggests that this strain may not be suitable for smaller post larval abalone. On the other hand, because a large size range of animals is usually present on plates at the same time, requiring different feed sizes, the use of different species or different strains of the one species at the same time may be more beneficial than monoculture in the nursery.

In the commercial scale trial, cell density and biomass of *N.* cf. *jeffreyi* peaked at the start of the experiment and then reduced within the first 2 wk, possibly because of grazing pressure. In the mixed treatment, biomass of *N.* cf. *jeffreyi* dropped after a peak in biomass of *C. sublittoralis* between weeks 7 and 12, a similar trend was found in the small scale coculture experiment although there was no grazing pressure (no abalone), indicating that this trend is not just because of selective grazing pressure and instead *N.* cf. *jeffreyi* appears to be an early colonizer, whereas *C. sublittoralis* takes a few weeks to establish itself, but will eventually out compete *N.* cf. *jeffreyi*.

Overall, juveniles up to 8 mm in shell length can be maintained on a diatom diet, with a mixed species diet, where feed is provided in a range of cell sizes, after which weaning onto formulated feed may provide a growth advantage. Juveniles feeding on the mixed diet had the highest specific growth rate and weight gain over the experiment, compared with the other treatments, although it was not significant. In low temperature and low light conditions, it may prove difficult to maintain sufficient biomass of diatoms on plates. However, *C. sublittoralis* appears to be more suited to these conditions although supplementing juveniles with other algal feeds may be necessary.

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