Performance of a new artificial abalone hatchery culture system in terms of settlement of larvae and growth and survival of postlarval *Haliotis discus discus* (Reeve)

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**ABSTRACT:** A new abalone postlarval culture system, Stott’s abalone postlarval production system (SAPPS), is being developed as an alternative method for settling larvae and on-growing postlarval abalone. SAPPS was tested in trial 1 using two commercially available artificial diets, Adam and Amos (Adam; supplied by Adam and Amos, Mt Barker, SA, Australia) and Cosmo (Cos; supplied by Cosmo, Matsuyama, Japan), against the diatom biofilm method (Diatom). In trial 2, SAPPS was tested against the previous production system (PPS, used by Stott et al. 2002). Larvae were induced to metamorphose and the resulting postlarvae were on-grown in 10-L flow-through tanks for 28 days in their respective treatments. The final survival of postlarvae was significantly higher (*P* < 0.05) in the SAPPS-Cos group (56.7 ± 11.15%) than in the Diatom (9.4 ± 2.7%) and SAPPS-Adam groups (8.5 ± 1.1%). The final length of postlarvae in SAPPS-Cos (1065 ± 73 μm) was significantly higher (*P* < 0.05) than those in the other two treatments (average of 841–883 μm). In trial 2 the metamorphosis rate was improved by using *Spirulina platensis*. The final length of postlarvae in SAPPS (1449–1471 μm) was significantly higher (*P* < 0.05) than PPS (1065–1075 μm) and Diatom (993 μm). There is potential for SAPPS to be used as an alternative to the current diatom method in the culture of postlarval abalone.

**KEY WORDS:** alternative culture system, postlarval abalone, settlement induction, *Spirulina* powder, Stott’s abalone postlarval production system.

**INTRODUCTION**

Many problems have been identified with the current method of raising abalone on diatom biofilms. Abalone hatcheries often suffer 90–100% mortality by 2 months post-settlement.¹ Although *Haliotis discus hannai* hatcheries in Northern Japan have relatively high postlarval survival, those in other regions of Japan, where species such as *Haliotis discus discus* are produced, still have low survival (A Stott, pers. comm., 2004). Management of diatom biofilms in abalone hatcheries is often challenging, and Hahn² and Seki³ suggest that control of the initial food supply is still one of the most critical problems in hatchery seed production. It is a difficult task to maintain a supply of readily ingestible and digestible food (diatoms) for growing postlarval abalone.⁴

Artificial microparticle diets may offer a more controlled and efficient source of nutrition for postlarval abalone. In Hahn’s study, an artificial microdiet is used at Argenton Experimental Station, France, to rear postlarval abalone in the critical 1–3-month post-settlement age period.² However, farmers feed the diet directly to the water column and wait for it to settle. Leaching is well documented in artificial microdiets,⁵,⁶ and this problem is compounded by the fact that abalone feed slowly.⁷,⁸ Bissett et al. observed that bacteria proliferated on an artificial diet after immersion in seawater for 2 days.⁹ Additionally, applying a diet directly to the water column results in the diet falling to the bottom of the tanks and being inaccessible to young postlarvae attached to plastic plates or the sides of tanks.

Ogino and Ohta coated glass plates with artificial diets using alginate as a fixer.¹⁰ The diet-coated glass plates were used to feed juvenile *H. discus discus*. Sagara and Sakai also used the same method for rearing trials of juvenile *H. discus dis-
**MATERIALS AND METHODS**

**Short explanation of trial 1**

Trial 1 was conducted over a period of 4 weeks to test the SAPPS method (using two commercially available abalone diets) against the Diatom method. Two plates from each treatment—SAPPS-Cos (diet supplied by Cosmo, Matsuyama, Japan), SAPPS-Adam (diet supplied by Adam and Amos, Mt Barker, SA, Australia) and Diatom—were placed into nine 10-L flow-through tanks (three treatments replicated three times each). Broodstock at Banda Marine Laboratory, Tokyo University of Fisheries, were induced to spawn using UV light and the resulting larvae were used in the trial when they were deemed ready to settle. Once the larvae had metamorphosed into postlarvae (on the experimental plates), the trial was commenced to compare SAPPS (using the two commercial diets) against Diatom in terms of postlarval growth and survival. Food was resprayed onto SAPPS plates every second day and measurements for postlarval growth were taken weekly, with postlarval survival being calculated every second day.

**General explanation of trial 2**

Trial 2 was conducted over a period of 4 weeks to test the SAPPS method (using the Cos diet ground down to two different particle sizes) against the PPS method (the Cos diet at two different particle sizes) and the Diatom method. Two plates from each treatment—PPS-small particle size (PPS-S), PPS-large particle size (PPS-L), SAPPS-small particle size (SAPPS-S), SAPPS-large particle size (SAPPS-L) and Diatom—were placed into 15 10-L flow-through tanks (five treatments replicated three times each). Larvae for the experiment were obtained as for trial 1. Once the larvae had metamorphosed into postlarvae (on the experimental plates), the trial was commenced to compare SAPPS (S and L) against PPS and Diatom in terms of postlarval growth and survival. Food was reapplied onto the SAPPS and PPS plates every second day and measurements for postlarval growth were taken weekly, with postlarval survival being calculated every second day.

**Approximate analysis and particle size determination of the diets**

An experimental version of the commercially available artificial microdiet ‘Plate Powder’ (Adam) was supplied by Adam and Amos (Mt Barker, SA, Australia). A sieve was used to select particles that were smaller than 53 μm. The Cos diet is a commercially available diet for juvenile abalone, which was supplied by Cosmo (Matsuyama, Japan). The diet was processed into powdered form using a Millser 700G (Iwatana, Tokyo, Japan). The particle size was then taken down to less than 53 μm with a sieve. In trial 2, the Cos diet was milled to create two different particle sizes (S and L). The small particle size was achieved by using an I-mill 5 series Jet Miller (Japan Pneumatic Engineering, Tokyo, Japan). The large particle size was achieved with the same methodology as used for the Cos diet in trial 1.

Diatom were scraped from five blocks (10 plates per block) of plastic corrugated culture plates (45 cm x 45 cm) that were placed out at the same time and for the same duration as the plates used.
in trial 1. Diatom samples were condensed and then centrifuged five times (at 8000 r.p.m. for 5 min) using a Tomy Centrifuge SRX-201 (Tomy Digital Biology, Tokyo, Japan) to remove salts and other dissolved matter from the water. Water was removed from the diatoms with an EYELA Freeze-dryer (EYELA, Tokyo, Japan).

The proximate analysis of the diets for moisture, crude protein, crude lipid and crude ash was carried out as outlined in Hernandez et al.18

The dry particle size of the artificial diets was determined by randomly taking 40 particles and measuring the size under a compound microscope fitted with an ocular eyepiece.

Identification of diatoms

Attached diatoms were completely removed from plastic plates that were identical in size (22.5 cm × 11.5 cm) to those used in trials 1 and 2. The plates were prepared in exactly the same way as described below and three plates were sampled separately for each trial. The samples were preserved in 5% formalin and then transported to Tokyo University of Fisheries, Tokyo, Japan. The common diatoms in the samples were identified and their density enumerated (twice for each sample) using a counting chamber.

Methodology for Stott’s abalone postlarval production system as used in trial 1

A solution of agar was prepared by dissolving 10 mg/mL of agar powder (Kokusan Chemicals, Tokyo, Japan) in boiling deionized water. Once the temperature of the solution had fallen to 50°C, 100 mg/mL of microparticle diet was added and the solution was mixed well. The resulting solution was then transferred to a MK-7 fine mist sprayer (Calmar, New York, USA) and the temperature was maintained at approximately 48°C. Each plastic plate was sprayed (individually) after being removed from the tank and allowed to drain for approximately 10 s. After spraying the plate, it was quickly placed back into the tank. Plastic plates were sprayed by holding them approximately 30 cm away from the sprayer and spraying the entire surface. Each side of the plastic plates was sprayed twice (approximately 0.4 mL of solution per side).

Preparation of diatom biofilm-covered plastic plates

The plastic plates (22.5 cm × 11.5 cm) for the Diatom treatment were prepared by placing them in 3-ton flow-through tanks outside, approximately 4 weeks prior to the experiment, and encouraging naturally occurring diatoms to attach. The tanks were covered with 90% UV block shade cloth, were supplied with vigorous aeration and received unfiltered seawater at a rate of 6 L/min. The diatom plates were rinsed lightly with seawater before being used in the experiment. The same method for diatom plate preparation was also used in trial 2.

Spawning and larval metamorphosis (trial 1)

Broodstock H. discus discus, at a ratio of two females to one male, were induced to spawn at Banda Marine Laboratory, Tokyo University of Fisheries, using UV light. Once hatched, the larvae were reared at three larvae/mL in a 100-L flow-through system with a flow rate of 400 mL/min and a temperature of 17°C. Larvae were deemed competent for settlement when the third tubule on the cephalic tentacle appeared.

Seven days before introducing the plastic plates into the experimental tanks, plastic plates sprayed with the artificial diet were preconditioned with abalone trail mucus (two juveniles/plastic plate). This was done by placing the plastic plates horizontally in tanks that were stocked with juvenile H. discus discus (length 20–35 mm). Food was resupplied every 2 days (in SAPPS-Adam and SAPPS-Cos) using the above stated methods. After the 7-day abalone mucus conditioning period, the plastic plates (excluding the diatom biofilm-covered plastic plates) were placed in a freshwater bath for 30 min. This process was carried out in an attempt to rid the plastic plates of attached living organisms, such as microalgae, bacteria etc.

At the commencement of the experiment, two prepared plastic plates were hung vertically in each 10-L experimental tank and approximately 2000 larvae were introduced. Larvae were allowed to settle for 48 h, and then the plastic plates were removed and the number of larvae that had completed metamorphosis was determined. Any larvae remaining in the tanks were removed and the tanks were cleaned, ready for the postlarval grow-out phase.

Postlarval grow-out for trial 1

The trial consisted of three treatments—SAPPS-Adam, SAPPS-Cos and Diatom—each replicated three times (total of nine experimental units) in a completely randomized design. Each experimental unit was assigned a 10-L flow-through circular tank
with a flow-rate of 250 mL/min. All experimental units were subject to natural lighting in the laboratory and there was no manipulation of the photoperiod.

Food was supplied using the above methods, on day 1 (after the 48-h settlement period) and every second day thereafter. Plastic plates covered with diatom biofilm were not changed because the bloom persisted for the duration of the trial. Tanks were siphoned every second day, the siphoned water samples were examined and the number of dead postlarvae was tallied to calculate percentage survival.

Postlarvae were sampled every 7 days by randomly removing 10 individuals (20 individuals from each tank were measured at the end of the trial) with a soft brush from plastic plates in each tank. Observations were made of the gut and also to determine that the postlarvae were alive. The size (maximum shell length) of postlarvae was taken using a compound microscope fitted with an ocular eyepiece.

Water temperature was measured daily with a standard thermometer and averaged 16.5 ± 0.9°C. The trial was terminated after 28 days.

Methodology for previous production system

The Cos diet (batch 2) was applied to the hard plastic corrugated plates (22.5 cm x 11.5 cm) by a similar method to that used by Ogino and Ohta. Approximately 120 mg/mL of the Adam diet and 15 mg/mL of sodium alginate was mixed in deionized water. The resulting paste was thinly applied to the clean plastic plate with a soft brush, and the plastic plates were dipped into a solution of calcium chloride. The sodium alginate was thus converted to insoluble calcium alginate gel. Recoating was conducted every second day by removing postlarvae from the plastic plates, cleaning and drying the plastic plate, and then recoating them as explained above. The new plates were placed in the tank when the old plates were removed and postlarvae were removed with a brush so as to give them every change of landing on the new plates. The Cos diet was supplied in excess.

Methodology for Stott's abalone postlarval production system as used in trial 2

Several modifications to SAPPS were made in an attempt to improve larval settlement and postlarval growth and survival in trial 2. New settlement technology (as described in Stott et al.) was adopted where Spirulina platensis powder was applied to plastic plates during the conditioning period. Additionally, the concentration of the artificial diet was increased to 120 mg/mL and the concentration of the agar was decreased to 7 mg/mL. Unless otherwise stated, the methodology was the same as that used in trial 1.

Spawning and larval settlement (trial 2)

Spawning and larvae settlement was conducted as for trial 1. The only difference was that the larvae were raised at a temperature of 20°C. Larvae were deemed competent for settlement when the third tubule on the cephalic tentacle appeared.

A 7-mg/mL solution of agar that contained 80 mg/mL of S. platensis powder was sprayed onto plates in the SAPPS treatments. PPS plates were coated (by brush) with a 15-mg/mL solution of sodium alginate that contained 80 mg/mL of S. platensis powder. Five days before introducing the plastic plates into the experimental tanks, all plates (including diatom biofilm plates, which were prepared as for trial 1) were conditioned with abalone trail mucus by putting two juvenile/plastic plates into the tanks. The plastic plates were placed vertically in tanks that were stocked with juvenile H. discus discus (length 30–40 mm). The S. platensis powder was resupplied every 2 days (in SAPPS-Adam and SAPPS-Cos) using the above-stated methods. Food was not reapplied for PPS treatments. After conditioning, plastic plates coated with the artificial diet were soaked in freshwater for 30 min. Diatom biofilm plates were sprayed with freshwater.

At the commencement of the experiment, two prepared plastic plates were hung vertically in each 10-L experimental tank and approximately 1200 larvae were introduced. The remaining (free swimming) larvae were removed from the tanks after 18 h because of heavier then expected settlement on plates. The number of larvae that completed metamorphosis on the plastic plates was enumerated after 48 h. At this time, because of very high larval attachment and metamorphosis, the number of postlarvae on the plates in each tank was adjusted to 250 postlarvae. The tanks were subsequently cleaned, ready for the postlarval grow-out phase.
unit was assigned a 10-L flow-through rectangular tank with a flow-rate of 300 mL/min. All experimental units were subject to natural lighting in a laboratory and there was no manipulation of the photoperiod.

Food was supplied using the above methods on day 1 (after the 48-h settlement period) and every second day thereafter. Plastic plates covered with diatom biofilm were not changed because the bloom persisted for the duration of the trial. Tanks were siphoned every second day, the siphoned water samples were examined and the number of dead larvae was tallied to calculate percentage survival.

Postlarvae were sampled every 7 days by randomly removing 10 postlarvae (20 postlarvae from each tank were measured at the end of the trial) with a soft brush from plastic plates in each tank. Observations were made of the gut and also to determine that the postlarvae were alive. The size (maximum shell length) of postlarvae was taken using a compound microscope fitted with an ocular eyepiece.

Water temperature was measured daily with a standard thermometer and averaged 19.8 ± 1.4°C. The experiment was terminated after 28 days.

Visual observations were also recorded for any obvious problems with the supply of food etc. using the three different methods.

**Statistical analysis**

Statistical analysis was conducted using the computer package SYSAT (SPSS Inc., Chicago, IL, USA). Normality was determined by normal probability plots and homogeneity of variances was confirmed by way of Levene’s test. One-way ANOVA were then carried out and differences in treatment means were determined using Tukey’s test.

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### RESULTS

#### Proximate analysis of diets and diatom density

The Adam and Cos diets used in trial 1 varied in terms of chemical composition (Table 1). The Adam diet had a high protein and low lipid content compared with the Cos diet. The sizes of wet and dry particles in the Adam diet were slightly larger than in the Cos diet. The diatom biofilm had a very high ash and lipid content and a very low protein content. The Cos diet used in trial 2 was very similar in composition to that used in trial 1. Cos-S had an average particle size of 3.0 ± 2.2 μm, whereas Cos-L had an average particle size of 21.3 ± 11.8 μm.

### Table 1 The size and composition of the different diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Adam†</th>
<th>Cos (trial 1)‡</th>
<th>Cos-S/L (trial 2)§</th>
<th>Diatom¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size (average ± SD; μm)</td>
<td>32.0 ± 10.0</td>
<td>28.0 ± 8.0</td>
<td>3.0 ± 2.2/21.3 ± 11.8</td>
<td>ND</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>7.5</td>
<td>6.8</td>
<td>9.2</td>
<td>–</td>
</tr>
<tr>
<td>On a dry matter basis (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>54.4</td>
<td>34.3</td>
<td>37.6</td>
<td>18.0</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>2.5</td>
<td>4.9</td>
<td>4.8</td>
<td>8.0</td>
</tr>
<tr>
<td>Crude ash</td>
<td>14.1</td>
<td>17.1</td>
<td>16.7</td>
<td>52.8</td>
</tr>
</tbody>
</table>

†Commercially available artificial microdiet ‘Plate Powder’ (Adam) supplied by Adam and Amos (Mt Barker, SA, Australia); ‡commercially available diet (Cos) supplied by Cosmo (Matsuyama, Japan); §Cos-S had an average particle size of 3.0 ± 2.2 μm, whereas Cos-L had an average particle size of 21.3 ± 11.8 μm; ¶analysis of diatom biofilm that was processed into powder. ND, not determined; SD, standard deviation.

More than 50% of the diatom cells in the biofilm for trial 1 were from *Navicula* spp. and *Amphora* spp. (Table 2). Other dominant species included *Cyclophora tenuis*, *Entomoneis* sp. and *Nitzschia* spp. The biofilm had a brownish coloration that persisted for the duration of the trial. The total density of diatom cells was 2.1 ¥ 10^5 cells/cm². The diatom biofilm in trial 2 was conditioned with abalone mucus and the species composition was more diverse than that of trial 1. Although species such as *Navicula* spp. and *Amphora* spp. were still relatively dominant, smaller firmly attached diatoms such as *Cocconeis* spp. also became dominant. The total diatom cell density was 8.4 ¥ 10^4 cells/cm².

#### Results from trial 1

The average number of larvae that completed metamorphosis on the plastic plates in each tank was 381 ± 64, 377 ± 24 and 334 ± 182 for Diatom, SAPPS-Adam and SAPPS-Cos, respectively (Table 3). The percentage metamorphosis rate of larvae for the above treatments was 19.0, 18.8 and 16.7%, respectively.
Final survival of postlarvae was significantly higher ($P < 0.05$) in the SAPPS-Cos treatment (56.7 ± 11.2%) compared with the other treatments, 9.4 ± 2.7 and 8.5 ± 1.1% for Diatom and SAPPS-Adam, respectively (Fig. 1). At the end of the first week, the Diatom treatment had a significantly lower ($P < 0.05$) postlarval survival rate than the SAPPS treatments, and the SAPPS-Cos treatment had a significantly higher ($P < 0.05$) postlarval survival rate than SAPPS-Adam. Approximately 30% of the settled larvae in Diatom failed to complete settlement (attached to plates but failed to complete metamorphosis; no development of peristomal shell). The survival rate of postlarvae in SAPPS-Adam and Diatom dropped sharply in the second week of the experiment. SAPPS-Cos had a slow consistent fall in survival for the duration of the experiment.

Table 2  Main species and density of diatoms that occurred in the biofilm for trials 1 and 2

<table>
<thead>
<tr>
<th>Species of diatom</th>
<th>Trial 1 (not conditioned)</th>
<th></th>
<th></th>
<th></th>
<th>Trial 2 (conditioned)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell density (%)</td>
<td>Final density</td>
<td>Cell density (%)</td>
<td>Final density</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(cells/cm²)</td>
<td>(%)</td>
<td>(cells/cm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Achnanthes longipes</strong></td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>1.5 × 10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Achnanthes sp.</strong></td>
<td>–</td>
<td>–</td>
<td>5</td>
<td>4.4 × 10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Amphora spp.</strong></td>
<td>16</td>
<td>3.4 × 10⁴</td>
<td>19</td>
<td>1.6 × 10⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cocconeis sp. 1</strong></td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>8.2 × 10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cocconeis sp. 2</strong></td>
<td>–</td>
<td>–</td>
<td>5</td>
<td>4.1 × 10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cyclodora tenuis</strong></td>
<td>3</td>
<td>6.3 × 10³</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diatom</strong></td>
<td>1</td>
<td>2.1 × 10³</td>
<td>1</td>
<td>7.7 × 10²</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Entomoneis sp.</strong></td>
<td>1</td>
<td>2.1 × 10³</td>
<td>1</td>
<td>7.7 × 10²</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nitzschia spp.</strong></td>
<td>3</td>
<td>6.3 × 10³</td>
<td>4</td>
<td>3.1 × 10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Navicula sp. 1</strong></td>
<td>40</td>
<td>8.4 × 10⁴</td>
<td>17</td>
<td>1.4 × 10⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Navicula sp. 2</strong></td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>1.7 × 10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Melosira nummuloides</strong></td>
<td>2</td>
<td>4.2 × 10³</td>
<td>3</td>
<td>2.7 × 10³</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Psammodictyon panduriforme</strong></td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1.3 × 10²</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rhoicosphenia abbreviata</strong></td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1.3 × 10²</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tabularia investiens</strong></td>
<td>2</td>
<td>4.2 × 10³</td>
<td>1</td>
<td>1.3 × 10²</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>30</td>
<td>6.3 × 10⁴</td>
<td>29</td>
<td>2.5 × 10⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>2.1 ± 0.3 (×10⁵)</td>
<td>100</td>
<td>8.4 ± 3.4 (×10⁴)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3  Results in the three different treatments for postlarval Haliotis discus discus (trial 1)†

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. larvae metamorphosed ± SD (% metamorphosis)</th>
<th>Shell length ± SD (µm)</th>
<th>Daily growth rate (µm)</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAPPS-Adam†</td>
<td>377 ± 24 (18.8)a</td>
<td>841 ± 45b</td>
<td>19</td>
<td>8.5 ± 1.1b</td>
</tr>
<tr>
<td>SAPPS-Cos§</td>
<td>334 ± 182 (16.7)a</td>
<td>1065 ± 73a</td>
<td>27</td>
<td>56.7 ± 11.2a</td>
</tr>
<tr>
<td>Diatom¶</td>
<td>381 ± 64 (19.0)a</td>
<td>883 ± 69c</td>
<td>21</td>
<td>9.4 ± 2.7b</td>
</tr>
</tbody>
</table>

† Different superscripts in the same column denote significant differences in means ($P < 0.05$); † initial size of postlarvae was 306 ± 10 µm (n = 50); ‡ commercially available artificial microdiet ‘Plate Powder’ (Adam) supplied by Adam and Amos (Mt Barker, SA, Australia); § commercially available diet (Cos) supplied by Cosmo (Matsuyama, Japan); ¶ analysis of diatom biofilm that was processed into powder.

SAPPS, Stott’s abalone postlarval production system; SD, standard deviation.
trial and at the end of week 2 still had a survival rate of 83.1 ± 1.5%.

The final average length of postlarvae in SAPPS-Cos (1065 ± 73 mm) was significantly higher (P < 0.05) than those in Diatom (883 ± 69 mm) and SAPPS-Adam (841 ± 45 mm) (Fig. 2). The average daily growth rate of postlarvae ranged from 19 mm/day for SAPPS-Adam to 27 mm/day for SAPPS-Cos.

Food on plates in the SAPPS-Adam, SAPPS-Cos and Diatom treatments was visible throughout the entire trial. After new food was sprayed onto the plates in the SAPPS treatments and the plates were returned to the tanks, some food loss was apparent. This detached food typically floated on the top of the water and exited the tank via the outlet.

Some pink colonies of bacteria were apparent on plates in SAPPS-Adam and SAPPS-Cos after the end of the second week of the experiment. However, the appearance of these colonies did not coincide with mass or excessive mortality.

Results from trial 2

Three days into the 5-day period for establishing mucus trails on the plates, food was not visible on plates in the PPS-Adam treatment; however, it was renewed at the start of the postlarval grow-out period. Fewer juvenile abalone were observed to be grazing plates in that treatment at night compared with the Diatom and SAPPS plates.

The average number of larvae that completed metamorphosis on the plastic plates in each tank was 727 ± 35, 583 ± 137, 774 ± 68, 888 ± 50 and 862 ± 44 for PPS-S, PPS-L, SAPPS-S, SAPPS-L and Diatom, respectively (Table 4). The percentage metamorphosis rate of larvae for the above treatments was 60.6, 48.6, 64.5, 74.0 and 71.8%, respectively.

Final survival of postlarvae was significantly higher (P < 0.05) in the SAPPS-S (18.8 ± 5.2%) and SAPPS-L treatments (21.0 ± 3.2%) compared with the PPS-S (9.9 ± 2.2%), PPS-L (10.1 ± 1.6%) and Diatom treatments (4.5 ± 1.6%). Mass mortality occurred in all treatments from day 4–7 and dead postlarvae were typically 420–490 mm in length. After the first week, mortality was relatively low in all treatments.

The final average length of postlarvae in the SAPPS-S (1449 ± 138 mm) and SAPPS-L treatments (1471 ± 129 mm) was significantly higher (P < 0.05) than in the PPS-S (1065 ± 71 mm), PPS-L (1075 ± 69 mm) and Diatom treatments (993 ± 42 mm) (Fig. 4). The average daily growth rate of postlarvae ranged from 24 mm/day for Diatom to 41 mm/day for the SAPPS treatments.

DISCUSSION

Stott’s abalone postlarval production system, when used with the Cos diet, was superior to the other treatments in terms of growth (P < 0.05) and sur-
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Survival (P < 0.05) of postlarval H. discus discus. The survival of postlarvae in the SAPPs/Cos diet treatment was four–fivefold higher than that in the Diatom treatment. Additionally, the survival rate in the SAPPs treatments was double that in the PPS treatments. In terms of growth, postlarvae in SAPPs-Cos treatments grew 20% faster in trial 1 and more than 35% faster in trial 2 compared with those in the other treatments. In these trials, SAPPs/Cos was most likely superior to the other treatments because of the fact that a highly nutritious food source was supplied directly to grazing postlarvae on a continuous basis.

The PPS treatment had slightly lower (although not significant) metamorphosis of larvae on the plastic plates when compared with the SAPPs and Diatom treatments. SAPPs utilized the temperature-setting properties of agar to allow food microparticles to be sprayed directly onto the plastic plates. Once a plastic plate has been raised from a tank, most of the water runs off within seconds, so the plate can be quickly sprayed and placed back into the tank. SAPPs, unlike PPS, allows food to be replenished onto the original plastic plates that have abalone attached. PPS requires a clean, dry plate for the alginate to be fixed. Thus, during the abalone mucus conditioning stage, the plastic plates in PPS could not be replenished with food and juvenile abalone soon consumed all the food. As a result, less abalone mucus would have been deposited onto those plates and the attachment and metamorphosis stimulus was lower.

Although the Diatom in trial 1 induced a slightly higher number of larvae to settle on the plastic plates compared with the other treatments, many of the settled larvae died before depositing a peristomal shell. Most authors studying the metamorphosis of larval abalone define metamorphosis as a process where larvae attach to a surface, drop their swimming organs and deposit a peristomal shell. Thus, a high percentage of postlarval mortality in the first week in Diatom might have been because of unsuccessful completion of metamorphosis.

The metamorphosis rate in trial 2 increased more than threefold compared with that in trial 1, by using new technology developed by Stott et al.19 SAPPs and PPS plates were coated with Spirulina powder, which acts as a potent attachment and metamorphosis inducer when combined with abalone mucus. The conditioned diatom biofilm was also a much more effective settlement cue than a non-conditioned diatom biofilm. Although the settlement rate in SAPPs and Diatom was improved from approximately 20% to approximately 70%, a direct comparison cannot be made between the two trials because the culture conditions were slightly different (temperature, tank etc.) and the batches of larvae were different.

Survival of post larvae in trial 1 dropped sharply during week 2 in the SAPPs-Adam and Diatom treatments. Survival dropped between 47.9 and 64.2% in the aforementioned groups compared with only 11.9% in the SAPPs-Cos treatment. Shilling et al. reported that there is a definite point where postlarval abalone exhaust their residual yolk reserves,20 and Roberts et al. observed this occurring in the second week after larval settlement in Haliotis iris.21 Alternatively, Ohashi suggested that the yolk reserves of H. discus discus postlarvae are exhausted as soon as 6 days post-settlement.22 In the current study, it appears that mass postlarval mortality occurred after the residual yolk reserves of postlarvae were exhausted (between day 8 and 14) in treatments fed the Adams diet and Diatom. Postlarvae might have had problems digesting these two food sources, or the
food sources themselves might not have been nutritionally adequate.

In trial 2, mortality was very high in all treatments in week 1. This mortality coincided with a rapid increase in water temperature from 20°C to more than 23°C. This unseasonably high water temperature, combined with the initial high density of postlarvae, could have resulted in high stress levels. Alternatively, egg quality could have been low, resulting in high mortality of postlarvae at the stage where they change from an endogenous food source to an exogenous food source.23 Searcy-Bernal suggested that postlarval survival is often compromised by boundary layers that cover diatom biofilm plates.24 However, in the current study, compromised by boundary layers that cover dia- plates are coated too thickly with food, the food would cool rapidly when flying through the air. These microdroplets have a large surface area to volume ratio, which means they would cool rapidly when flying through the air. This mist spray system also facilitates a fine film coating of the plastic plates. Recently settled postlarvae do not require large amounts of food and if plates are coated too thickly with food, the food will peel off.17

The average shell length of postlarvae at the end of the first week in SAPPS-Cos was significantly higher (P < 0.05) than in the other treatments. In trail 2 the average length of postlarvae was significantly higher (P < 0.05) in SAPPS treatments compared with PPS and Diatom. Postlarvae supplied with superior nutrition might be more equipped to tolerate stressful culture conditions.

The survival of abalone in SAPPS-Cos in trial 1 was 95% after 1 week, which would suggest that the SAPPS application method did not adversely affect the survival of recently settled postlarval abalone. Although food would have been sprayed onto plates (where postlarvae were attached) three times by that stage, there was no evidence of larvae being drowned in the agar gel solution or solution temperature causing mortality. Mist sprayers produce microdroplets of solution that are projected through the air. These microdroplets have a large surface area to volume ratio, which means they would cool rapidly when flying through the air. This mist spray system also facilitates a fine film coating of the plastic plates. Recently settled postlarvae do not require large amounts of food and if plates are coated too thickly with food, the food will peel off.

Postlarvae feeding on the diatom film could not obtain sufficient nutrition from bacteria, diatoms or their extracellular products. In trail 1, it is likely that some of the dominant species of diatoms, such as *Navicula* spp. and *Amphora bigibba*, could not be utilized effectively as a nutrition source by the early postlarvae. Furthermore, suitable diatoms might have been limiting because of selective feeding by the postlarvae or because of competition with other diatom species. In trial 2 the diatom biofilm plates were conditioned with abalone mucus, which becomes a food source for early postlarvae.25–28 Grazing by juveniles during the conditioning period also results in strongly adhesive solitary forms of diatoms, such as *Cocconeis* spp., becoming dominant.28–30 However, although growth was higher in trial 2 compared with trial 1 for postlarvae feeding on diatom biofilm as a result of higher temperatures, survival was lower. There seemed to be no advantage in conditioning the diatom biofilm in these studies. Thus, overall, the major disadvantage of the natural diatom biofilm method is that the actual diet being offered to the postlarvae cannot be controlled. It is extremely difficult to manage diatom species composition and density when using the natural diatom biofilm method.

In trial 1, the growth and survival of postlarval abalone was highest in Cos, followed by Diatom and Adam. However, as no information was provided on the diets by the manufacturers of both the Cos and Adam diets, no speculation can be made on the performance of the diet as a result of protein source, binders, manufacturing process used etc. Fleming et al. reviewed artificial diets for abalone and found that most artificial diets produced for abalone had an average of approximately 30% crude protein and 4% crude lipid.31 The protein and lipid levels in the Cos diet were closer to these levels than the Adam diet, and this might suggest that the former diet was not nutritionally balanced. However, it must be noted that the Adam diet (as used in the present trial) was an experimental form of ‘Plate Powder’ and its chemical composition is different from the product sold commercially as ‘Plate Powder’. Furthermore, postlarval abalone might require a higher content of lipid than adult abalone require. Diatom biofilm, the natural food of abalone, contains 8.1% crude lipids. Considering that more than 50% of the sample was ash (silicate contained in the diatom cell wall), the crude lipid contents of the digestible intracellular contents of the diatoms could have been as high as 16%. It is likely that the Adam diet was deficient in lipids and this caused low growth and survival rates of postlarval abalone in the PPS-Adam and SAPPS-Adam treatments.
In trial 2, there was no significant difference in terms of the growth and survival of postlarval abalone (P > 0.05) between the Cos-S diet and the Cos-L diet. Grinding the diet to 3 μm is very expensive and would most likely result in higher leaching because of the higher surface area of food directly exposed to water. Grinding the food to less than 53 μm is appropriate for even recently settled postlarvae and would result in reduced costs. It might even be possible to increase the particle size to more than 53 μm, but considering the small size of the mouth of postlarvae and the fact that the nozzle of the sprayer will be more susceptible to blocking, this should be done with caution.

Although it is still not clear if the type of binder used in the application method affected postlarval abalone growth and survival, SAPPS incorporated 50% less binder (in terms of weight) than PPS. McShane et al. found that increasing the amount of agar in an artificial diet from 2 to 10% resulted in lower ingestion of the diet by Haliotis rubra. In trial 2, compared with trial 1, the amount of agar used in the solution was decreased by 30%. However, because of the different culture conditions between trials 1 and 2, it is unclear whether agar concentration affected postlarval growth and survival.

Stott’s abalone postlarval production system was superior to PPS for raising postlarval abalone for a number of reasons. First, SAPPS resulted in little mortality because of direct handling of postlarvae, and postlarvae were supplied directly with artificial food. PPS, in contrast, requires the postlarvae to be removed from the plastic plates before food can be renewed. Stott et al. observed that postlarvae, once they are dislodged from the plastic plates, do not return unless they are approximately 1200 μm or greater in length. Thus, the postlarvae in PPS would have only been feeding on the food that had fallen from the plastic plates or that had been dislodged from the plastic plates when the postlarvae were removed. Handling stress would also be high in PPS because the postlarvae had to be removed from the plastic plates (if they landed back on the plates during a plate change) with a soft brush every second day, to allow the plastic plates to be changed. Cleaning the tanks in PPS was also difficult because most of the postlarvae were attached to the bottom of the tank. This was in contrast to SAPPS, where the postlarvae remained attached to the original plastic plates for the duration of the trial.

Second, SAPPS provided a simple and effective method of applying a food mixture extremely thinly and evenly onto the plastic plates. Ogino and Ohta used a similar method to PPS in a feeding trial for juvenile abalone and found that they could not use flow-through water because of the food falling off the plates, decomposing and compromising the water quality. They most likely experienced problems because they applied the diet too thickly to the plates. This problem was overcome in the present study in PPS by painting the diets very thinly onto the plastic plates. That said, it must be acknowledged that painting plates would not be economically viable on a large scale. However, spraying the diet, as in SAPPS, could be economically viable on a large scale if an appropriate spray device and techniques were developed.

Stott’s abalone postlarval production system also had many advantages over the diatom method. In SAPPS, artificial diets were used so no lighting was required to culture and sustain favorable food. Additional tanks are also required when using the diatom method to encourage growth of diatom biofilm on plastic plates. In winter this process can take more than 4 weeks, adding to costs related to pumping, tank cleaning etc. SAPPS only required tanks for conditioning the plastic plates with abalone mucus (5–7 days) and for growing-out the postlarvae.

A noticeable disadvantage of SAPPS is that food, if not consumed by postlarvae or leached from the plastic plates, will become a medium for bacteria proliferation. Pink colonies of bacteria were noticeable after the second week on some plastic plates in the SAPPS-Adam and SAPPS-Cos treatments. Plastic plates with a low density of postlarval abalone were especially susceptible. Although the bacteria didn’t adversely affect postlarval growth or survival, it seems that it is important to maintain a relatively high density of postlarvae on plates when using SAPPS. Another problem with SAPPS is that the nozzle of the spray device will block if the temperature of the mixture containing the food and agar falls below approximately 40°C and the mixture hardens. Thus, it is important to maintain the temperature of this mixture at approximately 50°C. Additionally, food that was sprayed onto areas of the plastic plates where water had not sufficiently drained was lost when the plastic plates were returned to the tanks. However, the amount of food loss was minimal.

Stott’s abalone postlarval production system has the potential to be used as an alternative to the diatom method for the culture of postlarval abalone. Further trials will also be conducted on a commercial scale to develop spraying techniques and machinery to cut down on labor costs when spraying the plates.
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REFERENCES

19. Stott AE, Takeuchi T, Koike Y. Testing various substances that have been bound to plastic plates with agar to induce larval settlement and metamorphosis of abalone Haliotis discus discus (Reeve). Aquaculture 2004; 231: 547–557.
30. Suzuki H, Ioriya T, Seki T, Arura Y. Changes of algal community on the plastic plates used for rearing the abalone.