Co-culture of dulse *Palmaria mollis* and red abalone *Haliotis rufescens* under limited flow conditions

Ford Evans *, Chris J. Langdon

Hatfield Marine Science Center and Department of Fisheries and Wildlife, Oregon State University, Newport, OR 97365, USA

Accepted 21 October 1999

Abstract

A series of experiments was conducted to determine factors limiting the stocking density and growth rates of red abalone in a co-culture system with the macroalgae dulse (*Palmaria mollis*). Co-culture conditions were altered by varying the degree of artificial illumination (0 h, 12 h at night, and 24 h d⁻¹ in addition to ambient sunlight) used to supplement ambient sunlight and water volume exchange rate (1, 6, or 35 d⁻¹). Rates of dulse production, dulse consumption by abalone, ammonia uptake by dulse and ammonia excretion by abalone were measured seasonally over 1 year. Abalone growth rates under co-culture conditions were measured. Maximum abalone stocking densities within the co-culture system were first limited by the amount of algae available for abalone consumption, and then by the capacity of the algae to absorb ammonia excreted by abalone. Degree of supplemental illumination, water volume exchange rate, and abalone body weight all affected maximum stocking densities within the co-culture system. The growth rates of abalone fed dulse grown under all co-culture conditions range: 112±132 μm shell length d⁻¹ compared favorably with that of abalone fed on other algal and artificial diets. Both duration of supplemental illumination and water volume exchange rate affected abalone growth. Overall, the co-culture of dulse and abalone provides the farmer with a reliable supply of nutritious abalone food while ensuring high water quality through uptake of excreted ammonia by the dulse. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Haliotis*; Dulse; Co-culture; Polyculture; Growth; Ammonia
1. Introduction

The abalone aquaculture industry along the eastern Pacific is located in areas that support an abundance of wild kelp (*Macrocystis* spp. or *Nereocystis luetkeana*). These brown macroalgae are easily harvested feed for land-based and offshore abalone farms (Hahn, 1989; McBride, 1998). Kelp supports relatively slow abalone growth rates, typically 30–60 μm shell length (SL) d⁻¹ (Ebert and Houk, 1984; Trevelyan et al., 1998), and limits the geographic expansion of the abalone industry to sites where kelp can be harvested in large quantities (Ebert, 1992). Further, governmental regulation (Mercer et al., 1993; McBride, 1998) and natural events, such as El Niño (Ebert, 1992; McBride, 1998), may reduce availability of harvested kelp.

As an alternative to the collection of wild algae, we investigated the potential of cultivating the nutritious macroalgae dulse (*Palmaria mollis*) with the red abalone in a land-based co-culture system. Dulse was determined to be an ideal candidate for land-based production due to ease of culture, rapid growth rate and capacity to absorb...
dissolved nutrients (Levin, 1991). In addition, Buchal et al. (1998) found dulse to be highly nutritious, supporting abalone growth rates of up to 3.8 mm SL month\(^{-1}\).

Ideally, in a self-sustaining co-culture system, abalone would consume dulse and release both ammonia and carbon dioxide as waste. Dissolved abalone waste products would then be absorbed by the dulse, with inorganic carbon and nitrogen being assimilated into growing dulse tissue (Fig. 1). Traditional land-based abalone farms maintain water quality via rapid water exchange rates, which flush metabolic and other waste products from the culture system. By contrast, dulse serves not only as a food source in co-culture, but also as an in situ biofilter. Therefore, dulse maintains water quality (i.e., absorbs ammonia) within the co-culture system, allowing water flushing rates to be minimized and operating costs reduced. Macroalgae have previously been reported to effectively reduce nutrients in aquaculture effluents (e.g., Cohen and Neori, 1991; Shpigel et al., 1993; Krom et al., 1995; Neori et al., 1996).

Maximum abalone stocking density within such a co-culture system could be limited by (1) the amount of algae available for abalone to consume, and (2) the capacity of the algae to absorb ammonia excreted by the abalone. Both limitations can be addressed by increased algal production rates which would supply more algae as fodder and increase dissolved nutrient uptake rates (Neori et al., 1991; Magnusson et al., 1994; Braud and Amat, 1996). Locations that lack year-round abundant natural sunlight, such as the Pacific Northwest, may require supplemental artificial illumination to enhance algal production and therefore abalone yield.

This paper describes a series of experiments conducted at the Hatfield Marine Science Center (HMSC), Newport, Oregon, USA, which were designed to determine limiting factors affecting the stocking density of red abalone in a co-culture system with dulse. Experiments were carried out during Fall (August–October, 1996), Winter (November 1996–January 1997), Spring (March–April 1997), and Summer (May–July, 1997) because season-dependent factors such as water temperature and solar radiation are major parameters affecting the co-culture system. Finally, the growth rates of abalone within the co-culture system were measured.

2. Material and methods

2.1. Dulse production

Dulse was collected from Fidalgo Bay, Washington, USA, and maintained at HMSC until used in experiments. Dulse rosettes were cultured in 110-l cylindrical polyethylene tanks filled with UV-filtered seawater and kept in suspension via vigorous aeration. All culture tanks were partially immersed in seawater baths to reduce daily temperature fluctuations and to minimize temperature differences among treatments caused by different seawater exchange rates. All experiments were conducted under ambient seawater conditions at HMSC (temperature range 8–14°C; salinity range 25–35 g l\(^{-1}\)).

Nitrogen concentration of incoming seawater at HMSC ranges seasonally from 0.4 to 1.4 mg NO\(_3\)–N l\(^{-1}\), while dissolved phosphate is generally undetectable (Dionex DX 500 chromatograph; Anne C. Sigleo, EPA, Newport, Oregon, personal communication). In addition to nutrients present in ambient seawater, dulse cultures were fertilized by the
addition of sodium nitrate (NaNO₃) and monosodium phosphate (NaH₂PO₄). Fertilizers were delivered in batch applications during Fall 1996, and on a continuous basis during Winter 1996, Spring 1997 and Summer 1997. For batch fertilization, water flow was turned off and nutrients were added resulting in a final concentration of 4.76 mg N l⁻¹ and 4.32 mg P l⁻¹. Water flow was resumed after 8 h. Continuous fertilization allowed uninterrupted water flow, with nutrients being injected via peristaltic pump into the source water. Continuous fertilization rate was adjusted such that there was always a detectable quantity of N and P in the effluent of each culture tank as measured with Hach water quality kits (Hach, CO, USA). Fertilizer concentrations of the inflowing seawater reached a maximum of 196 mg NaNO₃ l⁻¹ and 50 mg NaH₂PO₄ l⁻¹ in Summer 1997.

A 3 × 3 factorial experiment was used to determine dulse production under three levels of supplemental illumination and three water volume exchange rates. Supplemental illumination was supplied for 0 h d⁻¹ (0 h), 12 h d⁻¹ at night (12 h) and 24 h d⁻¹ (24 h). Water volume exchange rates were 1 d⁻¹ (1×), 6 d⁻¹ (6×), and 35 d⁻¹ (35×). All treatments were replicated in triplicate.

Supplemental illumination was provided by 1000-W metal halide lamps (Sylvania, NH, USA) installed over the dulse tanks, delivering 11–24 mol photon m⁻² d⁻¹ of photosynthetically active radiation (PAR) at tank water level. In addition, dulse cultures received PAR from ambient sunlight, which ranged seasonally from 2.6 mol photon m⁻² d⁻¹ in Winter to 30.7 mol photon m⁻² d⁻¹ in Fall. Ambient light intensity was recorded every 15 min using a calibrated, fixed LI-COR quantum sensor (model Li-185B), while light intensity under the 1000-W lamps was measured with a calibrated hand-held LI-COR quantum sensor. Due to complications in data retrieval, portions of the Spring 1997 ambient light readings were derived from data collected by the Tillamook People’s Utility District, Tillamook, Oregon.

Water temperature was recorded with either mercury maximum/minimum thermometers or every 2 h with temperature loggers (Optic Stowaways, Onset, MA, USA), placed in 110-l culture tanks under 24 h/1× and 24 h/35× treatments. Due to equipment limitations, temperature loggers were only placed in culture containers that demonstrated the highest and lowest average daily water temperatures.

Dulse growth rates were determined by measuring the weekly increase in wet weight of dulse after a minimum acclimation of 1 week under the lighting and flow conditions described above. Dulse was spin-dried in a domestic washing machine for 3 min to minimize error in weight measurements due to seawater retained on the surface of the algae. Dulse production per unit area (P, g wet wt m⁻² d⁻¹) was calculated as:

\[
P = \frac{(W_f - W_i)}{(SA \times d)}
\]

where \(W_f\) was the final wet weight (g) and \(W_i\) was the initial wet weight (g) for each weekly growth period, \(SA\) was the surface area of the 110-l experimental container exposed to light (0.155 m²), and \(d\) was the length of the growth period in days. After each weekly weighing, dulse stocking densities were adjusted to 9 g wet wt l⁻¹ (1 kg tank⁻¹).

The effects of supplemental illumination and water volume exchange rate on dulse production were analyzed using a two-factor ANOVA. In addition, regression analysis
was used to quantify the relationship between dulse production (g wet wt m$^{-2}$ d$^{-1}$) and total daily PAR (mol photon m$^{-2}$ d$^{-1}$).

2.2. Dulse consumption by abalone

Weight-specific abalone consumption rates were determined for abalone of approximately 10, 20, 40 and 80 mm SL. Animals were held in perforated 0.5-l containers submerged in 110-l co-culture tanks and exposed to one of two treatments, 0 h/35$^\circ$C or 24 h/35$^\circ$C. Experimental containers were stocked with 8, 4, 2, or 1 abalone from each of the 10, 20, 40 and 80 mm SL size groups, respectively. Controls were set up with dulse alone to correct for non-grazing causes of dulse weight change. All treatments were replicated in triplicate. Temperatures were recorded with data loggers every 2 h (Optic Stowaways).

Abalone were acclimated for 3 weeks under experimental conditions, then offered a known weight of pat-dried dulse grown in cultures identical to assigned treatments (i.e., 0 h/35$^\circ$C or 24 h/35$^\circ$C treatments as per Section 2.1). Uneaten dulse was collected approximately 7 days later, pat-dried, and weighed. Abalone were removed from the container, gently pat-dried to remove water retained in the mantle cavity, and weighed. Total weight of dulse consumed was calculated as:

$$TC = W_o - W_r + \left[ Gc \times \left( W_o + W_r \right) / 2 \right]$$

(2)

where TC was the total dulse consumption per container (g), $W_o$ was the weight of dulse offered (g), $W_r$ was the weight of dulse recovered (g), and Gc was the weight change of control dulse (%). Due to the varying amounts of dulse offered to the different abalone size-classes, it was necessary to apply the dulse control growth rate to the average dulse weight present in the container over the measured feeding period. Rate of dulse consumption ($C$, g dulse abalone$^{-1}$ d$^{-1}$) was then calculated as:

$$C = TC / (n \times d)$$

(3)

where $n$ was the number of abalone per container, and $d$ was the duration of the feeding period in days.

Regression analysis was used to create predictive models of abalone feed consumption ($C$) as a function of live body weight (g).

2.3. Ammonia uptake by dulse

Two 110-l culture tanks in Winter and four 110-l culture tanks in Summer were stocked with dulse at 9 g l$^{-1}$, exposed to 0 h/35$^\circ$C or 24 h/35$^\circ$C treatments (see Section 2.1), and, in Summer, provided with or without supplemental fertilization. All other dulse culture conditions were the same as those described in Section 2.1. The concentration of ammonia in the inflowing seawater was adjusted by the addition of ammonium sulfate ([NH$_4$]$_2$SO$_4$) to 21 $\mu$mol TAN l$^{-1}$. Total ammonia nitrogen
(TAN, NH$_4^+$-N + NH$_3$-N) concentrations in the inflowing and outflowing seawater were measured every 6 h over a 24-h period using the Solorzano method reported by Parsons et al. (1984). Ammonia uptake rates by dulse for each sample period of the 24-h experiment were determined as:

$$U = (f \times [N_f - N_o]) / W$$

where $U$ was the ammonia uptake rate (µmol TAN kg$^{-1}$ h$^{-1}$), $f$ was the flow rate (l h$^{-1}$), $N_f$ and $N_o$ represented ammonia concentrations (µmol TAN l$^{-1}$) in the inflow and outflow, respectively, and $W$ was the weight of dulse per tank (kg). Water temperature, pH and PAR were measured concurrently over the 24-h sample period. Due to time and labor constraints, measurements were taken from only one tank per treatment.

2.4. Ammonia excretion by abalone

In Winter 1996 and Summer 1997, groups of abalone (10, 20, 40 and 80 mm SL) were acclimated for 3 weeks in triplicate abalone/dulse co-cultures exposed to 0 h/35 h and 24 h/35 h treatments (see Section 2.1). After acclimation, groups of five 10-mm, five 20-mm, two 40-mm, and one 80-mm abalone were removed and each size group was added to separate 1-l beakers partly filled with UV-sterilized seawater. Initial ammonia concentration in each beaker was determined using the Solorzano method reported by Parsons et al. (1984). The beakers were then covered with “Parafilm”. After approximately 5 h of incubation in darkness, ammonia concentration in each beaker was remeasured. Abalone were removed from the beakers, gently pat-dried to remove water retained in the mantle cavity and weighed. Ammonia excretion rate was calculated as:

$$E_{ab} = V \times (C_f - C_i) / (n \times h)$$

where $E_{ab}$ was the ammonia excreted per individual abalone per hour (µmol TAN abalone$^{-1}$ h$^{-1}$), $V$ was the volume of water in the beaker (l), $C_f$ and $C_i$ were the final and the initial ammonia concentrations in the beaker (µmol TAN l$^{-1}$), respectively, $n$ was the number of abalone in the beaker, and $h$ was the length of incubation in hours. Ammonia excretion per gram (whole wet weight) abalone was calculated as:

$$E_g = V \times (C_f - C_i) / (W \times h)$$

where $E_g$ was the weight-specific ammonia excretion rate (µmol TAN g abalone$^{-1}$ h$^{-1}$), and $W$ was the average abalone weight (g) per beaker.

Regression analysis was used to quantify the relationship between ammonia excretion rate per abalone (log[µmol TAN abalone$^{-1}$ h$^{-1}$]) and abalone body weight (log[g]). In addition, a three-way ANOVA was used to determine the effect of season, body weight and light treatment on weight-specific ammonia excretion rates (µmol TAN g abalone$^{-1}$ h$^{-1}$).
2.5. Estimating maximum stocking density

The above data allowed co-culture systems (three light treatments × three water volume exchange rates) to be balanced in two ways. First, animals could be stocked such that they consume exactly as much dulse as is produced within the co-culture unit (i.e., balance based on dulse production/consumption). Second, animals could be stocked such that they excrete exactly as much ammonia as could be absorbed by the dulse (i.e., balance based on ammonia absorption/excretion). In addition, models were run to compare maximum abalone stocking densities for two abalone size classes (10 and 80 mm) and two seasons (Winter and Summer). The dilution of abalone-excreted ammonia due to water volume exchange was also considered in determining maximum abalone stocking densities.

Dulse consumption by abalone and ammonia uptake by dulse were assumed to be independent of water volume exchange rate. Further, dulse consumption by abalone in the co-culture system under 12 h of supplemental illumination was assumed to be similar to dulse consumption by abalone under 24 h of supplemental illumination.

2.6. Abalone growth under co-culture conditions

Growth rates of abalone cultured using three water volume exchange rates (1 × , 6 × , and 35 × ) and two light treatments (0 and 24 h) were measured over a 139-day growth study. Fifty 10-mm abalone were stocked into each of 18 cylindrical mesh cages (43 cm × 10 cm, diameter × height) and randomly assigned to a 110-l co-culture tank exposed to one of the above water volume exchange rates and light treatments. It was predicted that at this abalone stocking density, dulse production from each treatment would be able to adequately feed all abalone. Cages were placed at the bottom of each tank with the dulse tumble-cultured above (Fig. 1a). Abalone shell length (longest linear dimension) and pat-dried body weight were recorded on July 31, 1997. Abalone were fed ad libitum on dulse from their respective co-culture tanks once per week. On December 18 and 19, 1998, abalone were harvested. Abalone shell length and pat-dried body weight were again recorded. Abalone soft tissue (foot, viscera, etc.) was weighed on a tarred aluminum dish and dried in a convection oven at 60°C for 24 h to determine moisture content.

Abalone growth rates were determined as follows:

\[
\Delta SL = ( L_t - L_i ) / d
\]

and

\[
SGR = 100 \times \left( \ln W_t - \ln W_i \right) / d
\]

where \( \Delta SL \) was the shell length increase (\( \mu \text{m SL d}^{-1} \)), \( L_t \) and \( L_i \) were the final and the initial abalone shell lengths (\( \mu \text{m} \), respectively, SGR was the specific growth rate (percentage d\(^{-1} \)), \( W_t \) and \( W_i \) were the final and the initial abalone dry meat weights (g), respectively, and \( d \) was the duration of the growth trial in days. Due to size variation in
initial abalone weights, a two-factor ANCOVA was used to test the significance of water volume exchange rate and supplemental illumination on ΔSL and SGR, using initial abalone length or whole wet weight as a covariate.

Ammonia concentrations in the co-culture water were measured 3 months after beginning the experiment. Duplicate water samples were taken from each of the

Fig. 2. Dulse production (g wet wt m⁻² d⁻¹) over four seasons as a function of water volume exchange rate (1, 6, or 35 d⁻¹) and level of supplemental illumination (0, 12, or 24 h d⁻¹). Growth trials were 1-week-long with Fall, Winter, Spring and Summer average water temperatures of 12.6, 12.4, 12.3 and 13.2°C, respectively.

Fertilizers NaNO₃ and NaH₂PO₄ were added to excess. Error bars represent ±1 standard deviation generated by a two-factor ANOVA.
co-culture tanks every 6 h over a 24-h period and mg TAN l⁻¹ determined using the Solorzano method reported by Parsons et al. (1984). Water temperature, salinity, and pH were measured concurrently to estimate concentrations of free-ammonia nitrogen (FAN, mg NH₃-N l⁻¹; Bower, 1978).

Fig. 3. Dulse production (g wet wt m⁻² d⁻¹) with 35 water volume exchanges d⁻¹ as a function of total daily photosynthetically active radiation (PAR, mol photon m⁻² d⁻¹) for Winter-only ($r^2 = 0.96$, $n = 9$), Spring–Summer–Fall ($r^2 = 0.87$, $n = 27$), and all four seasons (dotted line, $r^2 = 0.81$, $n = 36$). See Fig. 2 for average season water temperatures.

Fig. 4. Dulse production (g wet wt m⁻² d⁻¹) as a function of season for three water volume exchange rates (1, 6, or 35 d⁻¹) and three levels of supplemental illumination (0, 12, or 24 h d⁻¹). See Fig. 2 for average season water temperatures.
3. Results

3.1. Dulse production

Water volume exchange rate had a positive effect on algal production (Fig. 2) in Fall, Spring, and Summer (ANOVA, \( P < 0.01 \), but not in Winter (ANOVA, \( P > 0.05 \)). The greatest effect of flow was seen during Summer when productivity increased from 123 g wet wt m^{-2} d^{-1} in the 24 h/1 \times \text{treatment} to 414 g wet wt m^{-2} d^{-1} in the 24 h/35 \times \text{treatment}. Dulse production in Winter, however, only increased from 237 g wet wt m^{-2} d^{-1} in the 24/1 \times \text{treatment} to 320 g wet wt m^{-2} d^{-1} in the 24 h/35 \times \text{treatment}.

Duration of supplemental illumination had a positive effect on dulse production for all seasons (ANOVA, \( P < 0.01 \)). The effect was most dramatic in Winter when dulse production increased from 14 g wet wt m^{-2} d^{-1} in the 0 h/35 \times \text{treatment} to 320 g wet wt m^{-2} d^{-1} in the 24 h/35 \times \text{treatment}. With 35 water volume exchanges d^{-1}, there was a significant linear relationship (Fig. 3) between total daily PAR (natural and supplemental light combined) and dulse production using data collected from all seasons:

\[
P = 12.75 + 7.76 \times \text{PAR} \quad (r^2 = 0.81, n = 36)
\]

where \( P \) was the dulse production (g wet wt m^{-2} d^{-1}) and PAR was the PAR received per day (mol photon m^{-2} d^{-1}). Dulse production between seasons was compared using ANCOVA with average daily PAR as the covariate. Winter production was found to be significantly higher than for all other seasons (\( P < 0.01 \)) at comparable light intensities; therefore, Eq. (9) was split to represent production as a function average daily PAR for Winter only and for Spring, Summer, Fall combined (Fig. 3):

\[
P (\text{Winter}) = -28.90 + 13.94 \times \text{PAR} \quad (r^2 = 0.96, n = 9)
\]

and

\[
P (\text{Spring, Summer, Fall}) = -70.238 + 9.55 \times \text{PAR} \quad (r^2 = 0.87, n = 27).
\]

Most treatments showed a seasonal decline in dulse production from Fall to Winter followed by an increase in dulse production in the Spring and Summer (Fig. 4).

Table 1

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Season</th>
<th>Supplemental illumination (h d^{-1})</th>
<th>( n )</th>
<th>Value ( a )</th>
<th>Value ( b )</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.0</td>
<td>Winter</td>
<td>0</td>
<td>12</td>
<td>0.077</td>
<td>0.848</td>
<td>0.99</td>
</tr>
<tr>
<td>11.0</td>
<td>Winter</td>
<td>24</td>
<td>12</td>
<td>0.097</td>
<td>0.886</td>
<td>0.99</td>
</tr>
<tr>
<td>11.7</td>
<td>Spring</td>
<td>0</td>
<td>11</td>
<td>0.085</td>
<td>0.899</td>
<td>0.99</td>
</tr>
<tr>
<td>12.2</td>
<td>Spring</td>
<td>24</td>
<td>12</td>
<td>0.072</td>
<td>0.956</td>
<td>0.97</td>
</tr>
<tr>
<td>13.5</td>
<td>Summer</td>
<td>0</td>
<td>12</td>
<td>0.121</td>
<td>0.740</td>
<td>0.99</td>
</tr>
<tr>
<td>13.9</td>
<td>Summer</td>
<td>24</td>
<td>12</td>
<td>0.106</td>
<td>0.823</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Table 2
Seasonal total ammonia nitrogen (TAN, μmol NH_3−N + NH_4^+−N l\(^{-1}\)) uptake rates by 1 kg of dulse averaged over a 24-h period under either of two levels of supplemental illumination (0 or 24 h d\(^{-1}\)). All treatments received 35 water volume exchanges d\(^{-1}\). Fertilized treatments received 196 mg NaNO\(_3\) l\(^{-1}\) and 50 mg NaH\(_2\)PO\(_4\) l\(^{-1}\).

<table>
<thead>
<tr>
<th>Season</th>
<th>Fertilizer present</th>
<th>Supplemental illumination (h d(^{-1}))</th>
<th>Average daily temperature (°C)</th>
<th>Average daily PAR (μmol m(^{-2}) s(^{-1}))</th>
<th>Seasonal dulse production (g wet wt m(^{-2}) d(^{-1}))</th>
<th>TAN uptake rate (μmol TAN kg(^{-1}) h(^{-1}))</th>
<th>TAN uptake rate/Seasonal dulse production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>No</td>
<td>0</td>
<td>11.0</td>
<td>19.8</td>
<td>14.0</td>
<td>14.0</td>
<td>1.00</td>
</tr>
<tr>
<td>Winter</td>
<td>No</td>
<td>24</td>
<td>11.0</td>
<td>283.2</td>
<td>319.7</td>
<td>676.7</td>
<td>2.12</td>
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<tr>
<td>Summer</td>
<td>No</td>
<td>0</td>
<td>15.1</td>
<td>75.5</td>
<td>216.6</td>
<td>726.6</td>
<td>3.35</td>
</tr>
<tr>
<td>Summer</td>
<td>No</td>
<td>24</td>
<td>15.1</td>
<td>347.5</td>
<td>413.5</td>
<td>1305.9</td>
<td>3.16</td>
</tr>
<tr>
<td>Summer</td>
<td>Yes</td>
<td>0</td>
<td>15.1</td>
<td>81.3</td>
<td>216.6</td>
<td>826.3</td>
<td>3.81</td>
</tr>
<tr>
<td>Summer</td>
<td>Yes</td>
<td>24</td>
<td>15.1</td>
<td>298.1</td>
<td>413.5</td>
<td>1009.3</td>
<td>2.44</td>
</tr>
</tbody>
</table>
exceptions to this trend were dulse cultures in either 12 h/1 × or 24 h/1 × treatments. These dulse cultures showed a continual decline in production throughout the year from Fall to Summer. Epiphytic fouling of dulse in these cultures became increasingly severe during Spring and Summer.

3.2. Dulse consumption by abalone

The relationship between abalone weight and dulse consumption rate for abalone between 0.1 g and 100 g wet wt, was described by the general equation:

\[ C = aW^b \]  

where \( C \) was the dulse consumption rate (g dulse wet wt abalone\(^{-1}\) d\(^{-1}\)), \( W \) was the whole wet abalone body weight (g), and \( a \) and \( b \) were constants derived through regression analysis (Table 1). In addition, ANCOVA indicated feed consumption \( \log g \) was affected by season \( (P < 0.05) \), but not light treatment \( (P > 0.05) \) with average abalone body weight \( (\log g) \) as the covariate.

3.3. Ammonia uptake by dulse

A positive relationship was seen between ammonia uptake by dulse \( (\mu\text{mol TAN h}^{-1} \text{kg}^{-1} \text{dulse}) \) and seasonal dulse growth rate \( (\text{g wet wt m}^{-2} \text{d}^{-1}, \text{Table 2}) \). Seasonal dulse
Table 3
Estimated maximum stocking densities of 10 mm abalone per 110-l co-culture tank based on a balance of dulse production/consumption or total ammonia nitrogen (TAN) absorption/excretion (with and without ammonia dilution due to 35 water volume exchanges d⁻¹), as a function of season and level of supplemental illumination (0 or 24 h d⁻¹).

<table>
<thead>
<tr>
<th>Season</th>
<th>Supplemental illumination (h d⁻¹)</th>
<th>Maximum stocking density based on dulse consumption/production (abalone tank⁻¹)</th>
<th>Maximum stocking density based on ammonia excretion/absorption Ignoring dilution due to flushing (abalone tank⁻¹)</th>
<th>Including dilution due to flushing (abalone tank⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>0</td>
<td>157</td>
<td>558</td>
<td>19,535</td>
</tr>
<tr>
<td>Winter</td>
<td>24</td>
<td>3007</td>
<td>44,815</td>
<td>1,566,355</td>
</tr>
<tr>
<td>Summer</td>
<td>0</td>
<td>1242</td>
<td>28,272</td>
<td>990,030</td>
</tr>
<tr>
<td>Summer</td>
<td>24</td>
<td>3174</td>
<td>123,198</td>
<td>4,309,445</td>
</tr>
</tbody>
</table>
growth rate appeared to predict ammonia uptake better than instantaneous light intensity, across both season and light treatment.

3.4. Ammonia excretion by abalone

Abalone body weight and light treatment had an effect on weight-specific ammonia excretion rate (μmol TAN g abalone⁻¹ h⁻¹, ANOVA, P < 0.01, Fig. 5) for abalone

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**A. WINTER**

![Graph](Image 1)

**B. SUMMER**

![Graph](Image 2)

Fig. 6. Estimated maximum Winter (A) and Summer (B) stocking densities of 10 mm abalone per 110-l co-culture tank as a function of water volume exchange rate (1, 6, or 35 d⁻¹) and level of supplemental illumination (0, 12, or 24 h d⁻¹). Stocking densities based on dulse production/consumption balance.
Fig. 7. Estimated maximum Winter stocking densities of 10 mm (A) and 80 mm (B) abalone per 110-l co-culture tank as a function of water volume exchange rate (1, 6, or 35 d⁻¹) and level of supplemental illumination (0, 12, or 24 h d⁻¹). Stocking densities based on dulse production/consumption balance.

body weights between 0.1 and 100 g wet wt. Rate of ammonia excretion by individual abalone (μmol TAN abalone⁻¹ h⁻¹) was significantly correlated with abalone weight (g), for each season and light treatment (r² > 0.93). The effect of abalone body weight on weight-specific ammonia excretion rate was primarily the result of the 10-mm abalone size-class showing an increased weight-specific excretion rate compared with 20–80 mm abalone. Excretion rates of 10 mm abalone ranged from 0.04 μmol TAN g
abalone$^{-1}$ h$^{-1}$ with supplemental illumination (Winter and Summer) to 0.1 µmol TAN g abalone$^{-1}$ h$^{-1}$ without supplemental illumination (Winter and Summer). Ammonia excretion rates for 20–80 mm abalone typically ranged between 0.017 to 0.028 µmol TAN g abalone$^{-1}$ h$^{-1}$ with and without illumination, respectively. Season (Winter or Summer) did not significantly affect weight-specific ammonia excretion rate (ANOVA, $P > 0.05$), although average water temperatures varied from 11.0°C in Winter to 12.5°C in Summer.

### 3.5. Estimation of maximum stocking densities for co-culture systems

Estimated maximum Summer stocking densities of 10 mm abalone in the 24 h/35 × treatment ranged from 3174 animals per tank based on dulse production/consumption relationships to 123,198 animals per tank based on ammonia absorption/excretion relationships (Table 3). The latter number (i.e., ammonia absorption/excretion stocking density) is derived when the amount of ammonia excreted by the abalone equals the amount of ammonia absorbed by the dulse, and does not include dilution due to water exchange. This density is raised to over 4,300,000 juvenile abalone per tank when excreted ammonia is diluted with 35 water volume exchanges of fresh seawater d$^{-1}$.

These results suggest that dulse production/consumption limits abalone stocking density to a far greater degree than ammonia absorption/excretion, therefore only the

![Abalone growth](image)

Fig. 8. Abalone shell length increase (A; µm SL d$^{-1}$) and specific growth rate (B; SGR, percentage d$^{-1}$) as a function of water volume exchange rate (1, 6, or 35 d$^{-1}$) and level of supplemental illumination (0 or 24 h d$^{-1}$). Each co-culture tank was stocked with fifty 10-mm abalone. Duration of the experiment was 139 days. Error bars represent ±1 standard deviation generated by ANCOVA (covariate was initial abalone shell length or wet body weight). Average treatment temperatures ranged from 14.6°C to 15.7°C.
Table 4
Measured total ammonia nitrogen (TAN l⁻¹, mg NH₃−N + NH₄⁺−N l⁻¹), pH, water temperature (°C), and free ammonia nitrogen (FAN l⁻¹, mg NH₃−N l⁻¹) in co-cultures stocked with fifty 10-mm abalone and 1 kg dulse exposed to different water volume exchange rates (1, 6, or 35 exchanges d⁻¹) and levels of supplemental illumination (0 or 24 h d⁻¹). All treatments in triplicate. All values averaged over the 24-h sample period.

<table>
<thead>
<tr>
<th>Water volume exchange (d⁻¹)</th>
<th>Supplemental illumination (h d⁻¹)</th>
<th>Total ammonia nitrogen (mg NH₃−N + NH₄⁺−N l⁻¹)</th>
<th>pH</th>
<th>Water temperature (°C)</th>
<th>Free ammonia nitrogen (mg NH₃−N l⁻¹)</th>
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</thead>
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<tr>
<td>1</td>
<td>0</td>
<td>0.168</td>
<td>8.1</td>
<td>13.1</td>
<td>0.0037</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0.166</td>
<td>8.1</td>
<td>12.8</td>
<td>0.0035</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>0.157</td>
<td>8.0</td>
<td>12.6</td>
<td>0.0032</td>
</tr>
<tr>
<td>1</td>
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<td>0.188</td>
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<td>0.0056</td>
</tr>
<tr>
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<td>0.171</td>
<td>8.2</td>
<td>13.3</td>
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</tr>
<tr>
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<td>24</td>
<td>0.174</td>
<td>8.1</td>
<td>12.8</td>
<td>0.0042</td>
</tr>
</tbody>
</table>
former will be addressed below. Maximum abalone stocking density in the co-culture tank was affected by water volume exchange rate, light treatment, season and abalone body weight (Figs. 6 and 7). The effect of water volume exchange rate was most notable in Summer when maximum stocking densities of 10 mm abalone range from 946 individuals per tank in the 24 h/1 × treatment to 3174 individuals per tank in the 24 h/35 × treatment. The effect of supplemental illumination was greatest in Winter when 10 mm abalone could be stocked at densities from 157 per tank in the 0 h/35 × treatment to 3007 per tank in the 24 h/35 × treatment. The effect of season was most apparent in the 0 h/35 × treatment where Winter cultures were capable of supporting 157 10-mm abalone per tank, while 1242 10-mm animals could be supported per tank under the same culture treatment in Summer. Abalone body weight also dramatically affected stocking density. In Winter, the 24 h/35 × treatment co-culture system could support 3007 seed-size abalone (10 mm), but only 11 market-size abalone (80 mm; Fig. 7).

### 3.6. Abalone growth rate

A positive effect of both duration of supplemental illumination and water volume exchange rate was seen on abalone shell length increase (μm SL d⁻¹, ANCOVA, \( P < 0.05 \), Fig. 8). Linear growth rates ranged from a low of 111.2 μm SL d⁻¹ in the 0 h/1 × treatment to 131.6 μm SL d⁻¹ in the 24 h/35 × treatment. Under both light regimes, the greatest change in abalone growth rate occurred as water exchange rate increased from 1 to 6 volumes d⁻¹. Growth rate was consistently faster under 24 h versus 0 h supplemental illumination d⁻¹ for all water volume exchange rates. Abalone dry weight gain (SGR, percentage d⁻¹) was also affected by degree of supplemental illumination and water volume exchange rate (ANCOVA, \( P < 0.05 \), Fig. 8). Again, abalone grew slowest in the 0 h/1 × treatment. Average SGR was higher in cultures receiving six water volume exchanges d⁻¹, although abalone SGR in the three different water exchange treatments within each light regime did not differ (ANCOVA, Tukey–Kramer, \( P > 0.05 \)).

Average temperature over the growth trial ranged from a low of 14.6°C in the 0 h/35 × treatment to a high of 15.7°C in the 24 h/1 × treatment. FAN levels were never high enough to affect juvenile abalone growth rate (Harris et al., 1998). Maximum measured ammonia levels remained below 0.006 mg FAN l⁻¹ in abalone culture tanks during the sampling period 3 months after the start of the growth trial (Table 4).

### 4. Discussion

The biomass of abalone that can be sustained by the present co-culture system is dependent on rate of dulse production, and therefore dependent on total daily PAR and water volume exchange rate. *P. mollis* in the present study, cultured with 35 water volume exchanges d⁻¹, showed a positive linear relationship between productivity (g wet wt m⁻² d⁻¹) and total daily PAR. Similarly, Lignell et al. (1987) observed the rhodophyte *Gracilaria secundata*, tumbled in culture via vigorous aeration, grew in a linear relationship with increased light intensity up to 1450 μmol photon m⁻² s⁻¹.
These results suggest that at 35 water volume exchanges d\(^{-1}\), tumble-cultured dulse production was typically seasonally light-limited, not nutrient-limited.

Under high light conditions, dulse cultures were probably nutrient-limited at low water volume exchange rates. A plateau in algal production was typical between 6 and 35 water volume exchanges d\(^{-1}\), suggesting that six exchanges per day supplied dulse with adequate nutrients (DeBoer et al., 1978; Morgan and Simpson, 1981a; Morgan et al., 1980), including inorganic carbon (DeBusk and Ryther, 1984; Neori et al., 1991; Magnusson et al., 1994; Braud and Amat, 1996), under light treatments examined in this study. The lack of a flow effect in Winter 1997 was probably due to cultures being light-limited.

Ammonia is one of the most toxic waste products that accumulates in intensive aquaculture systems, with the unionized form (NH\(_3\)) being more toxic than the ionized form (NH\(_4^+\)) for most organisms (Kinne, 1976; Spotte, 1979). Harris et al. (1998) showed free ammonia nitrogen (FAN) levels as low as 0.025 mg l\(^{-1}\) can negatively affect abalone growth rates. The use of dulse as an in situ biofilter can help reduce FAN within the co-culture system. Average daily ammonia uptake by dulse appeared to be positively affected by algae growth rate across seasons (Table 2), consistent with results reported by Magnusson et al. (1994) and Cohen and Neori (1991). Ammonia uptake by dulse was affected only minimally by the presence of nitrate fertilizer. This is consistent with published results that macroalgae take up ammonia in preference to, or independently of, nitrate–nitrite (D’Elia and DeBoer, 1978; Morgan and Simpson, 1981b; Wallentinus, 1984; Neori et al., 1991; Krom et al., 1995).

Only a few researchers have reported ammonia excretion rates for abalone (Barkai and Griffith, 1987; Kismohandaka et al., 1995). The effect of abalone size-class on weight-specific ammonia excretion rate found in the present study was due to 10 mm abalone excreting a disproportionate amount of ammonia. No size-class effect on weight-specific ammonia excretion was found when 10 mm (0.1 g live weight) abalone were excluded from the analysis. The absence of a size-class effect on weight-specific ammonia excretion rate for the larger animals (> 1 g live weight) was unexpected (Eckert et al., 1988), and may be an artifact due to experimental error. Interestingly, these results agree with those of Kismohandaka et al. (1995), who found no significant effect of body size on weight-specific ammonia excretion rate of _Haliotis cracherodii_ with meat weights over 50 g. Excretion rates for all abalone size-classes were consistently greater for abalone acclimated in tanks without supplemental illumination versus those acclimated in tanks receiving 24 h supplemental illumination d\(^{-1}\). It was possible that dulse cultured in the absence of supplemental illumination had a higher protein content (Morgan and Simpson, 1981a; Rosen et al., in press), and therefore resulted in elevated nitrogenous waste production (Lovell, 1989). Further, it is possible that animals acclimated to treatments receiving no supplemental illumination were more active because of the nocturnal behavior of abalone (Hayashi, 1988). Such behavior modification may have resulted in increased metabolic waste production.

Stocking densities of abalone in the co-culture system were, in all cases, limited by the capacity of dulse to supply food not it’s capacity to remove ammonia. Therefore, maximum abalone stocking densities closely follow dulse production rates. The positive effect of daily PAR and water volume exchange rate on dulse production increased.
maximum abalone stocking densities. Poor Winter dulse production in Oregon and other temperate regions will probably necessitate the use of supplemental illumination to allow abalone to be cultured at economically viable densities. Further, development of management strategies will be required to rapidly grow dulse with water volume exchange rates less than 6 d⁻¹ (i.e., pH control, nutrient addition, etc.) which would allow farms to minimize released effluent, reduce dependence on a constant supply of high salinity seawater, and reduce seawater pumping costs.

The exponential relationship between abalone body weight and dulse consumption rate as seen in this and other studies (e.g., Barkai and Griffith, 1987) indicates that the co-culture system is best suited for production of seed abalone (10–20 mm SL). Greatly expanded dulse culture would be required to produce enough dulse to support large numbers of market-sized abalone (80 mm). The use of artificial diets or wild harvested algae in combination with dulse may prove economically beneficial for commercial production of market-sized abalone.

The growth rates of abalone fed dulse grown under all co-culture conditions (range: 112–132 μm SL d⁻¹) compare favorably with those of abalone fed on other algal and artificial diets. Fleming et al. (1996) reviewed growth rates of abalone when fed a variety of artificial diets, ranging from 30 μm SL d⁻¹ in Australia (3–18 mm SL) to 160 μm SL d⁻¹ in Japan (NNKKK diet, 7–20 mm SL). Abalone growth rates on natural diets are reported to range from 0.8 μm SL d⁻¹ for H. iris (20 mm SL) fed Ulva lactuca (Stuart and Brown, 1994) to 139 μm SL d⁻¹ for H. discus hannai (24–34 mm SL) fed Eisenia bicyclis (Uki et al., 1986). More common to the Eastern Pacific is the alga Macrocystis pyrifera. Trevelyan et al. (1998) reported juvenile H. rufescens fed M. pyrifera grew 33 μm SL d⁻¹. Similarly, in New Zealand, Stuart and Brown (1994) found juvenile H. iris fed M. pyrifera grew 34 μm SL d⁻¹. Although dulse production was poor, abalone growth rates in 24 h/1 × treatments were high (averaging 124 μm SL d⁻¹), which suggested water quality in low-flow cultures was maintained at adequate levels and dulse nutritional quality remained high.

This study demonstrated the effectiveness of co-culture as a viable method of abalone production. Dulse was effective as both an in situ biofilter and as a food source capable of supporting rapid abalone growth. Abalone stocking density within the co-culture system was limited by the availability of dulse for abalone consumption, and therefore on the amount of PAR available and water volume exchange rate. Overall, the co-culture of dulse and abalone provides the farmer with a reliable supply of nutritious abalone food while ensuring high water quality through uptake of excreted ammonia by the dulse.

Acknowledgements

The authors would like to thank Gunther Rosen, Carl Demetropoulos, and Yu Shinmyo for help in collecting water samples, culturing dulse and measuring abalone. Ambient light data were provided by John Chapman, EPA, Newport, Oregon. Thanks to Dr. Susan C. McBride for review of the manuscript. Juvenile abalone were purchased from The Cultured Abalone, Goleta, California. This research was supported by grant
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