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Effects of Deep Seawater on the Growth of a Green Alga, *Ulva* sp. (Ulvophyceae, Chlorophyta)

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In order to examine the effects of deep seawater (mesopelagic water in the broad sense) on the growth of macroalgae, the growth and nutrient uptake (nitrate and phosphate) of *Ulva* sp. (Ulvophyceae, Chlorophyta) were investigated by cultivation in deep seawater (taken from 687 m depth at Yaizu, central Japan, in August 2001), surface seawater (taken from 24 m depth), and a combination of the two. Culture experiments were carried out in a continuous water supply system and an intermittent water supply system, in which aerated 500-mL flasks with 4 discs of *Ulva* sp. (cut sections of ca. 2 cm^2) were cultured at 20°C water temperature, 100 μmol photons $m^{-2} \cdot s^{-1}$ light intensity, and a 14:10 light:dark cycle. Nutrient uptake by *Ulva* sp. was high in all seawater media in both culture systems. The frond area, dry weight, chlorophyll *a* content, dry weight per unit area, and chlorophyll *a* content per unit area of *Ulva* sp. at the end of the experimental period were the highest in deep seawater and the lowest in surface seawater in both culture systems. These values, except for dry weight per unit area and chlorophyll *a* content per unit area, for each seawater media in the intermittent water supply system were higher than those in the continuous water supply system. We conclude that not only deep seawater as the culture medium but also the seawater supply system is important for effective cultivation of macroalgae.

Key Words: deep seawater, frond area, growth, nitrate, nutrient uptake, phosphate, *Ulva* sp.

INTRODUCTION

Recently, seawater taken from the zone deeper than the euphotic zone, which is often referred to as "deep seawater," has been used for the culture of fish, shellfish, and other animals as well as phytoplankton in Japan. This is because deep seawater is rich in nutrients and minerals, contains only a small number of bacteria, and is stable at low temperature in comparison with surface seawater (Nakashima 2002).

It is known that the primary productivity of phytoplankton is high in oceanic areas where deep seawater naturally upwells into the euphotic zone (Ryther 1969; Takahashi *et al.* 1981). It is believed that this is mostly due to the abundance of inorganic nutrients in deep seawater. However, it was reported that the biomass and the rate of photosynthesis of phytoplankton is lower in newly upwelled than upwelled aging seawater that contain organic chelators (Barber and Ryther 1969; Barber *et al.* 1971; Kanda *et al.* 1985; Nakashima 1988). This suggests that not only the supply of light and inorganic nutrients but also other factors, such as the presence of certain trace elements and chelators, are needed for phytoplankton to proliferate (Terry and Caperon 1982; Nakashima 1988, 1992). Nakashima *et al.* (1991) showed that some phytoplankton could not efficiently utilize nutrients in deep seawater without the presence of chelators and that if certain trace elements were present, the uptake rates were affected by the concentrations of other trace elements. Macroalgae culture experiments using deep seawater have rarely been performed (Ohno *et al.* 2000). Furthermore, there have been very few studies of nutrient uptake by macroalgae using deep seawater.

In order to examine the potential use of deep seawater to enhance macroalgal growth, the nutrient uptake (nitrate and phosphate) and growth of *Ulva* sp. (Ulvales, Chlorophyta) were investigated by cultivation in deep seawater, surface seawater, and a 1:1 combination of the two.

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MATERIALS AND METHODS

The deep seawater and surface seawater used in the present experiment were pumped from a depth of 687 m and 24 m, respectively, in August 2001, at the Suruga Bay Deep Seawater Plant (Yaizu, Shizuoka Prefecture, central Japan). The pumped seawater from both depths was immediately filtered using Whatman GF/C glass fiber filters, poured into 20-L polyethylene tanks, transported to the Japan Marine Science and Technology Center (JAMSTEC), and stored in a dark room (5°C).

The floating type of *Ulva* sp. (Ulvophyceae, Chlorophyta), which is considered to be difficult to maturity (Hiraoka *et al.* 1998), was collected at Nojima, Yokohama, central Japan, in November 2001 (Fig. 1). The collected alga was transported to JAMSTEC within 1 h and its surface wiped with cotton saturated with sterilized seawater. Then the alga was stocked in sterilized artificial seawater (Sealife, Marine-tec, Tokyo, Japan), aerated at 20°C water temperature under 100 μmol photons $m^{-2} \cdot s^{-1}$ light intensity and a 14 h:10 h light:dark (LD) cycle for 5 months.

The surface of the stored *Ulva* sp. was wiped again with cotton saturated with sterilized seawater, and then 48 discs (ca. 2 cm^2) were cut from the central part of the frond using a cork-borer. They were precultured for 2 days at 20°C, 100 μmol photons $m^{-2} \cdot s^{-1}$, and 14 h:10 h LD cycle. Twenty-four discs without flaws were then chosen and cultured in two systems. One was a continuous water supply system (fresh culture media in a refrigerator are pumped into a 500-mL flask through a fixed-quantity pump, and the culture media from the 500-mL flask are discarded through a fixed-quantity pump, and the culture medium exchange rate is 500 mL day^{-1}) (Fig. 2). The other was an intermittent water supply system (exchange rate and frequency 500 mL once daily). Four discs were cultured in each 500-mL flask for 10 days under the previously stated conditions (20°C, 100 μmol photons $m^{-2} \cdot s^{-1}$, and 14 h:10 h LD cycle). Three culture media (deep seawater, surface seawater, and a 1:1 combination of the two) were examined in the present study. Disc areas were measured on the day of culture initiation and 2, 4, 6, 8, and 10 days thereafter. Each disc was photographed using a digital camera, and the disc area was measured against NIH images (free computer software of the National Institutes of Health, Bethesda, MD, USA).

For analysis of nitrate and phosphate contents, water was sampled from each medium every 2 days. The samples were filtered using Millipore filters (0.45 μm HA) and stored at –20°C until analysis. Concentrations of nitrate and phosphate were determined by colorimetric methods (Strickland and Parsons 1972) using an autoanalyzer (Traccs 2000, Bran+Luebbe, Tokyo, Japan).

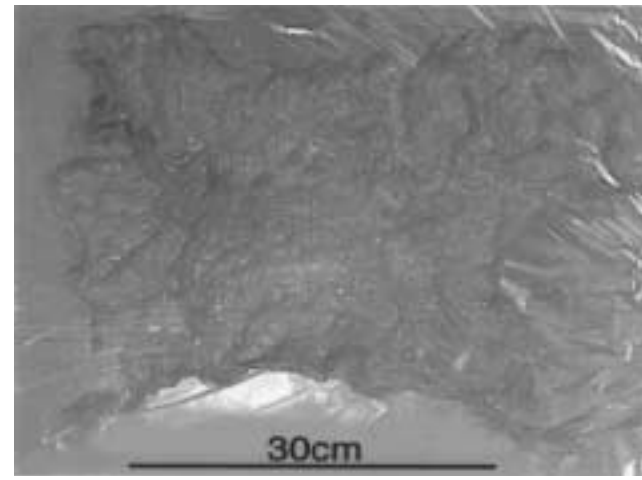

Fig. 1. Photograph of *Ulva* sp. used in the present experiments.

Chlorophyll *a* contents and dry weight were measured after the culture experiment. To obtain the extract of chlorophyll *a*, a section (ca. 40 mm^2) was cut from the central part of each cultured disc and placed in 1 mL of N, N-dimethyl-formamide solution at –20°C for 48 h. Absorbance of the extract was measured at 646.8 and 663.8 nm with a spectrophotometer (Ultrospec 3000, Pharmacia Biotech, Uppsala, Sweden), and the chlorophyll *a* content was calculated using the formula of Porra *et al.* (1989):

$$\text{Chlorophyll } a \ (\mu g \ mL^{-1}) = 12.00 \ A_{663.8} - 3.11 \ A_{646.8},$$

where $A_{663.8}$ and $A_{646.8}$ are absorbances at 663.8 and 646.8 nm, respectively. The remaining part of the cultured frond disc was dried at 80°C for 48 h and the dry weight measured.

Frond area, dry weight, chlorophyll *a* contents, dry weight per unit area, and chlorophyll *a* content per unit area were statistically compared between the water supply systems in each culture medium and among the culture media in both water supply systems (ANOVA and Fisher's PLSD posthoc test).

RESULTS

At the beginning of the experiment, the concentrations of nitrate and phosphate were 14.9 μM and 2.1 μM,

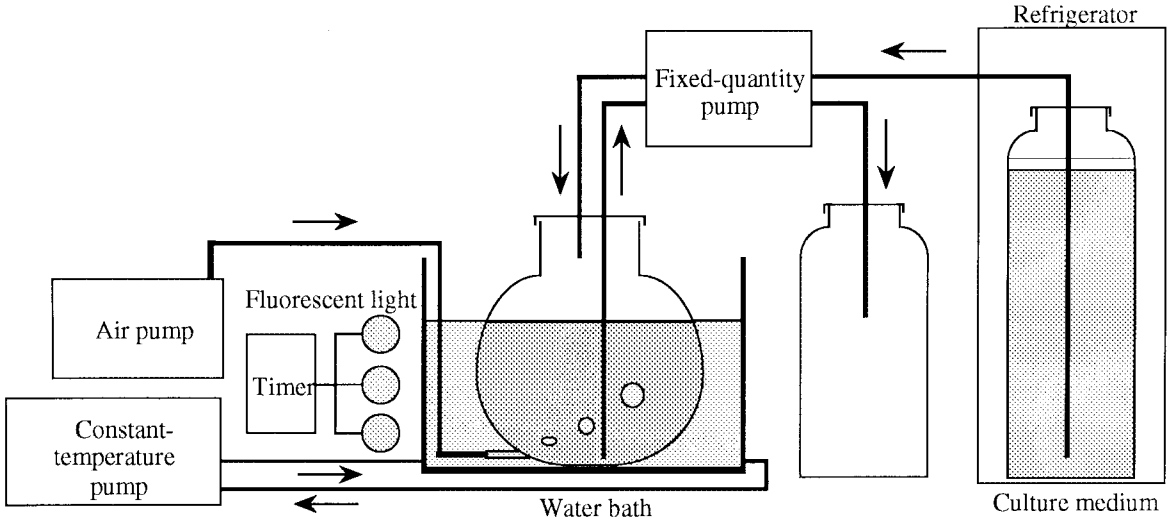

Fig. 2. Illustration of the continuous water supply system and culture apparatus.

Table 1. Concentrations of nitrate and phosphate during the period 10-day incubation period in three culture media using two water supply systems

		Continuous water supply system			Intermittent water supply system		
Day of culture		Deep water	Mixed	Surface water	Deep water	Mixed	Surface water
Nitrate	0	14.9	8.0	2.6	14.9	8.0	2.6
(μM)	2	2.7	0.4	0.2	0.2	0.0	0.1
	4	0.0	0.1	0.1	0.1	0.5	0.1
	6	0.6	0.1	0.2	0.1	0.0	0.1
	8	0.1	0.2	0.2	0.1	0.1	0.1
	10	0.4	0.3	0.2	0.0	0.0	0.1
Phosphate	0	2.1	1.1	0.6	2.1	1.1	0.6
(μM)	2	1.2	0.3	0.1	0.8	0.3	0.1
	4	0.6	0.2	0.1	0.6	0.3	0.1
	6	0.9	0.3	0.2	0.6	0.1	0.1
	8	0.3	0.2	0.1	0.7	0.2	0.1
	10	1.2	0.5	0.1	1.2	0.2	0.1

respectively, in the deep seawater, 8.0 μM and 1.1 μM, respectively, in the mixed seawater, and 2.6 μM and 0.6 μM, respectively, in the surface seawater in both culture systems (Table 1). In the continuous water supply system, concentrations of nitrate and phosphate in the culture media decreased to 2.7 μM and 1.2 μM or less, respectively, from day 2 and remained at a low level (less than 0.6 μM and 1.2 μM, respectively) until the end of the experiment (Table 1). On the other hand, in the intermittent water supply system, concentrations of nitrate and phosphate in the culture media were low (less than 0.2 μM and 1.2 μM, respectively) from day 2 to day 10 (Table 1).

In the intermittent water supply system, nutrient uptake quantity generally decreased with the growth of the frond area of *Ulva* sp. On the second day, when the nutrient uptake quantity was the greatest, nitrate and phosphate uptake quantity per unit area was 27.9 $\mu g \cdot cm^{-2} \cdot day^{-1}$ and 3.8 $\mu g \cdot cm^{-2} \cdot day^{-1}$, respectively, in the deep seawater, 18.6 $\mu g \cdot cm^{-2} \cdot day^{-1}$ and 2.9 $\mu g \cdot cm^{-2} \cdot day^{-1}$, respectively, in the mixed seawater, and 5.9 $\mu g \cdot cm^{-2} \cdot day^{-1}$ and 1.8 $\mu g \cdot cm^{-2} \cdot day^{-1}$, respectively, in the surface seawater. The utilization rates ($Ca/Ci \times 100$, where Ca is the concentration of the nutrient after 24 h in the culture medium and Ci is the initial concentration of the nutrient in the culture medium) were higher

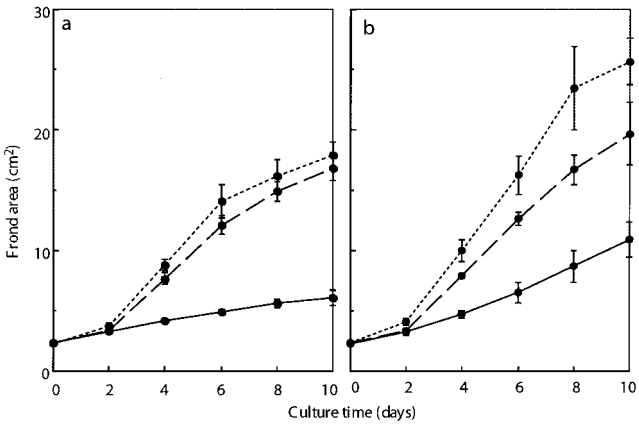

Fig. 3. Growth of the frond area of *Ulva* sp. cultured in the continuous water supply system (a) and intermittent water supply system (b). Solid lines, cultured in surface seawater; broken lines, cultured in mixed seawater; dotted lines, cultured in deep seawater. The values are expressed as mean ± SD of 4 discs.

for nitrate than for phosphate. These values were in excess of 94% for nitrate and 43-90% in phosphate.

Growth of the frond areas in *Ulva* sp. cultured in the deep seawater, mixed seawater, and surface seawater in both culture systems are shown in Fig. 3. The frond area was consistently largest in the deep seawater and smallest in the surface seawater after day 4 in both culture systems ($P < 0.05$; not significant for the value in deep seawater vs mixed seawater on day 4 in the intermittent culture system). In the intermittent water supply system, frond areas cultured in surface seawater, mixed seawater, and deep seawater were significantly larger than those in the continuous water supply system at the end of the experiment ($P < 0.05$). In the continuous water supply system, the differences between the frond areas cultured in deep seawater and mixed seawater were smaller than those in the intermittent water supply system.

Photographs of all cultured frond discs on the final experimental day are shown in Fig. 4. The color of discs cultured in deep seawater was darker than that of those cultured in the other two media. The color of discs cultured in the continuous water supply system was darker than that of those cultured in the intermittent water supply system.

The frond area, dry weight, chlorophyll *a* content, dry weight per unit area, and chlorophyll *a* content per unit area of *Ulva* sp. cultured in each medium on the final day of the experiment are shown in Fig. 5. Frond area, dry weight, and chlorophyll *a* content of *Ulva* sp. were

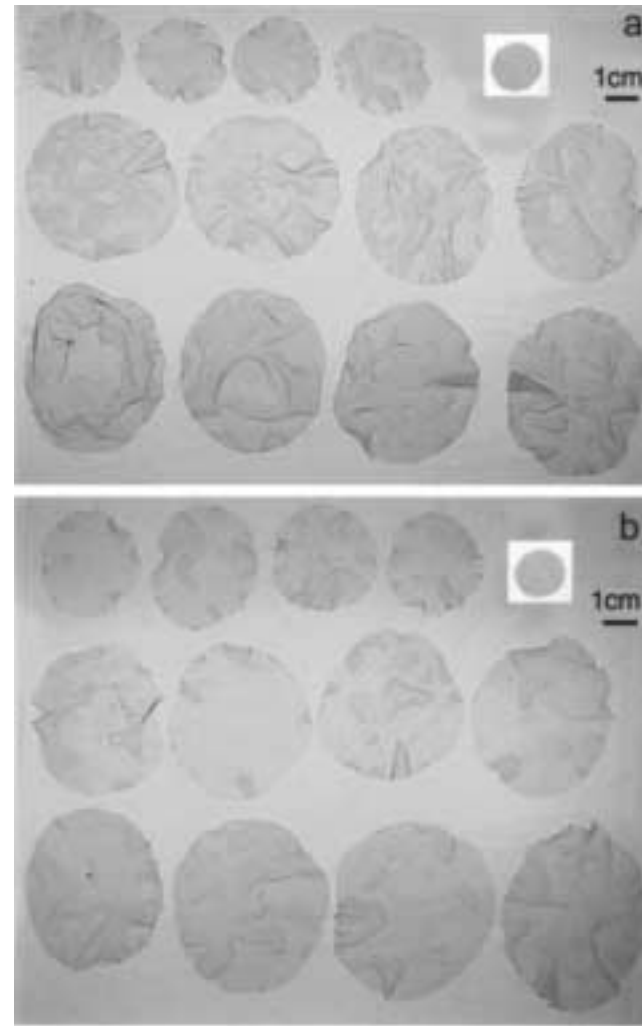

Fig. 4. Photographs of *Ulva* sp. on the initial (enclosed white squares) and final experimental day. Discs were cultured in the continuous water supply system (a) and intermittent water supply system. Upper rows, cultured in surface seawater; middle rows, cultured in mixed seawater; lower rows, cultured in deep seawater.

the greatest in deep seawater and least in surface seawater in both culture systems ($P < 0.05$). In the intermittent water supply system, these values in each culture medium were greater than those in the continuous water supply system ($P < 0.05$; not significant for the value in mixed seawater for dry weight). In each culture system, the chlorophyll *a* content per unit area was the highest in deep seawater ($P < 0.05$), but the difference between that in surface seawater and that in mixed seawater was not significant ($P > 0.05$). Dry weight per unit area did not differ significantly among the culture media in the two culture systems ($P > 0.05$, significant for the value in deep seawater vs mixed seawater in the continuous culture system).

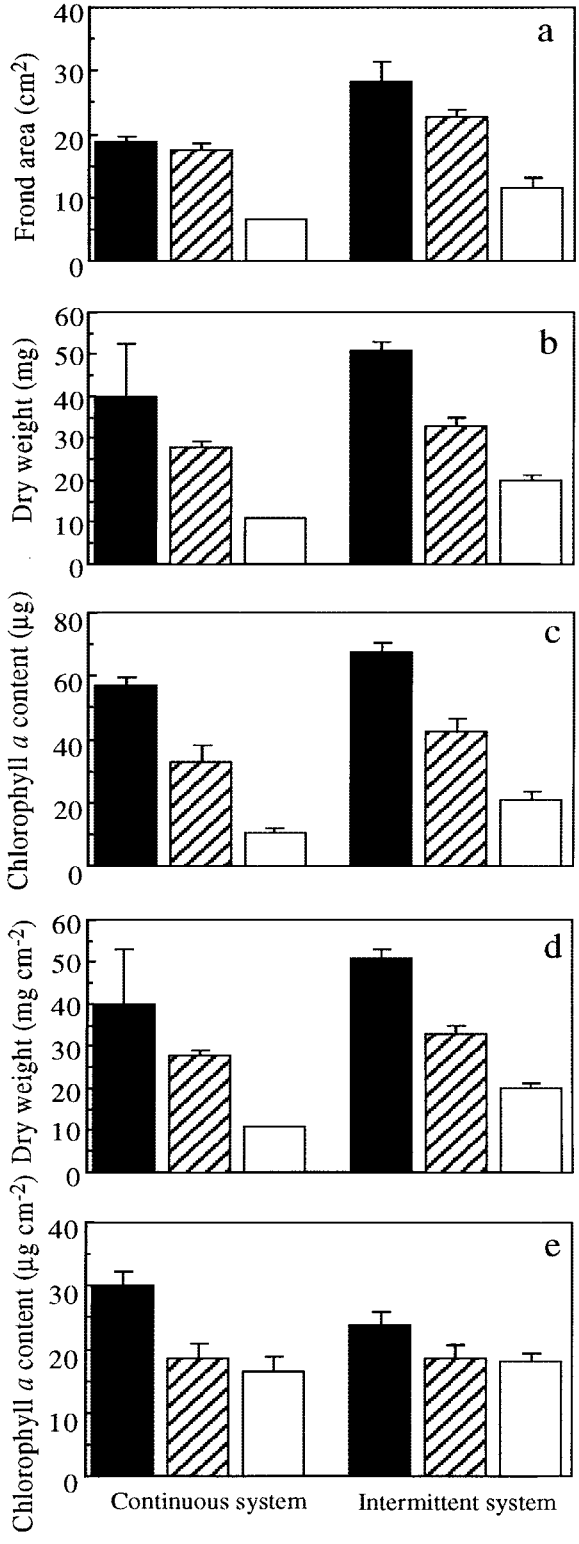

Fig. 5. Frond area (a), dry weight (b), chlorophyll *a* content (c), dry weight per unit area (d), and chlorophyll *a* content per unit area (e) of *Ulva* sp. on the final experimental day in the continuous water supply system and intermittent water supply system. Solid bars, cultured in deep seawater; hatched bars, cultured in mixed seawater; open bars, cultured in surface seawater. Values are expressed as mean ± SD of 4 discs.

DISCUSSION

It was believed that some phytoplankton cannot take in sufficient nutrients from deep seawater owing to the lack of certain trace elements and / or chelates (Terry and Caperon 1982; Nakashima 1988, 1992). In the present study, *Ulva* sp. efficiently utilized nitrate and phosphate from deep seawater to 94-100% and 43-90%, respectively. This indicates the possibility that nutrient uptake by macroalgae from deep seawater is more efficient than that by phytoplankton.

It is known that the productivity and biomass of phytoplankton are high in upwelling areas (Ryther 1969; Takahashi *et al.* 1981). In macroalgae, Nakashima (2002) reported that the biomass of *Gelidium* spp. (Rhodophyta) (an ingredient in agar) was higher in an area affected by local upwelling than in another area of Miyake Island, Japan. Recently, Ohno *et al.* (2000) succeeded in the off-season culture of *Undaria pinnatifida* (Harvey) Suringar (Phaeophyta) using a mixture of deep seawater and surface seawater and reported that the kelp grew well and matured. In the present study, we showed that the frond area and dry weight of *Ulva* sp. were greater when cultured in deep seawater and mixed deep when cultured in surface seawater (Fig. 5). Therefore, macroalgae grow better in deep seawater than in surface seawater. These results indicate that other macroalgae growing in the near-shore area would show higher growth and productivity when a local upwelling approache the shores. Thus, deep seawater can be regarded as a natural circulating culture medium.

It is generally believed that marine plankton take in nitrogen and phosphorus at a fixed ratio. In the seawater used in the present study, the nitrate utilization rates by *Ulva* sp. were higher (greater than 94%) than those of phosphate (43-90%). This result indicates the possibility that in *Ulva* sp., nitrogen is the limiting factor compared to phosphorus in the seawater used in the present study.

We found that the growth and yield of *Ulva* sp. were different in our two culture systems. The growth and yield of *Ulva* sp. were higher in the intermittent water supply system than in the continuous water supply system (Fig. 5). The intermittent water supply system may be an efficient culture method for *Ulva* sp. using a restricted quantity of deep seawater.

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