

# Effect of Water Depth on Seasonal Variation in the Chemical Composition of Akamoku, *Sargassum horneri* (Turner) C. Agardh

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## Abstract

The brown alga, Akamoku, also known as *Sargassum horneri*, was harvested at depths of 1 - 2 and 5 m in the oceanic areas of the Chikuzen Sea off the coast of Fukuoka, Japan, and its chemical composition (moisture, protein, lipid, ash, carbohydrate and total dietary fiber content) was investigated. The aim of this study was to elucidate the relationship between the chemical components and the growth of *S. horneri* as recyclable resource for a sustainable future. *S. horneri* at an ocean depth of 5 m grew rapidly. The biomass was abundant from January until April. After maturation and gamete release at the middle of April, plant senescence began occurring, and only short plants remained at the sea bottom. On the other hand, *S. horneri* at an ocean depth of 1 - 2 m grew slowly and could be harvested until early June. The protein, lipid and total dietary fiber content of *S. horneri* at an ocean depth of 5 m showed apparent gender-based differences.

## Keywords

Seaweed, Chemical Composition, Total Dietary Fiber, Seasonal Variation, *Sargassum horneri*

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## 1. Introduction

Seaweed contains mucilage polysaccharides such as agar, alginate and carrageenan. These phycocolloids have

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been used as gelling agents and emulsifiers in the food and pharmaceutical industries [1]. Brown alga polysaccharides exhibit antitumor and antiviral activity [2] [3]. Additional brown alga components have also been reported to possess additional physiological activities, including prevention of blood coagulation; leukotriene-mediated chloride absorption; regulation of bone metabolism; and effects on ingestion and excretion of cholesterol [4]-[8].

Thus, the demand for seaweed as a source of foods and biochemicals is expected to increase in the future. However, a limited number of seaweed species are available for practical use. One of the remaining unexplored seaweed species is *Sargassum horneri*, a brown alga growing in the coastal sea.

We reported on the seasonal changes in the chemical composition of moisture, protein, lipid, ash, carbohydrate, total dietary fiber (TDF), calcium, magnesium and zinc contents of *S. horneri* [9]. We have also reported on the changes in the general components after the parboiling process of *S. horneri* harvested from the Chikuzen Sea. We revealed that the chemical composition of *S. horneri* varied with its growth and maturity. An increase in TDF was observed after the appearance of receptacles, which appeared to correspond to the highest maturity, suggested by an increasing amount of fucoidan content and viscous exudates from the tissues of *S. horneri*. *S. horneri* is rich in dietary fiber and minerals, and can be developed for human consumption. The optimum season to harvest alga for human consumption may be early spring, when its growth and maturity are complete, and its dietary fiber and mineral contents have reached their maximum values.

On the other hand, *S. horneri* is a component of *Sargassum* beds, which are an important nursery ground for living marine organisms, such as fishes, crabs, sea urchins, abalones and so on. *Sargassum* beds perform the role of water quality purification and the prevention of red tide buildup [10] [11]. Seaweed species show great variation in their nutrient content in relation to various environmental factors, and most environmental parameters display seasonal variation [12] [13].

For its practical use as an edible resource, information on the influence of growing depth on the nutritional components and the seasonal variations of *S. horneri* in relation to its growth and maturity is important. In particular, *S. horneri* is a component of *Sargassum* beds, which also play important roles in resource enhancement of the coastal fisheries. In order to maintain the balance of the marine biomass and for its use as an edible resource, it is useful to understand the growth and maturity of *S. horneri*, and the relation to its nutritional value.

In addition, seaweed has recently been identified as a potential alternative source of renewable biofuel production [14]-[16]. *S. horneri*, which grows rapidly for a short period of time, may be a suitable material for biofuel. It is important to understand the relationship between the algal life cycle and chemical constitution such as carbohydrate content in *S. horneri*.

In the present study, seasonal changes in the chemical compositions with regard to moisture, protein, lipid, ash, carbohydrate and TDF content of *S. horneri* harvested in Chikuzen Sea at ocean depths of 1 - 2 and 5 m were investigated to elucidate the influence of growth at a certain ocean depth on the chemical components of *S. horneri* in relation to its growth and maturity.

## 2. Materials and Methods

### 2.1. Sampling of the Seaweed

Samples of the seaweed *S. horneri* were collected once per month from November 2005 to June 2006 in the Chikuzen Sea, off the coast of the Fukuoka prefecture, Japan. Sampling was carried out at a depth of 1 - 2 and 5 m in ocean areas near Nagasakibana, Oshima, Munakata City. The seaweed harvested from December 2005 was used for chemical component analysis. In addition, respective branches of male and female plants were analyzed from January, when their gender differences were most discernible. The harvested plants were cleaned and washed with distilled water to remove epiphytes and encrusting materials; their lengths and weights were subsequently measured after blotting out any excess water. The upper two-thirds of the full length of the plants were used as the edible portions, and the bottom one-third rigid portions were discarded. Seasonal changes in the chemical composition of the edible portions were determined. After 0.5 - 2 kg of edible portions from a number of seaweed plant were lyophilized, the dried samples were pulverized in a coffee mill and pooled. The homogenized samples were stored in polyethylene bottles at  $-30^{\circ}\text{C}$  until further analysis.

### 2.2. Maturity Index

Judgment of concerning gender differences was made on the basis of the receptacles of the alga, and the maturi-

ty index was expressed as the ratio of the number of plants with discernible gender differences distinction to the total number of plants.

## 2.3. Analytical Methods

Moisture, protein, lipid, ash, carbohydrate and TDF contents were determined using standard AOAC methods [17]–[22]. The homogenized samples consisted of the edible portion of *S. horneri* (0.5 - 2 kg of *S. horneri*; 4 - 12 plants). Moisture, protein, lipid, ash and TDF were measured using methods described by Murakami *et al.* [9].

Carbohydrate contents were calculated as weight differences between the total weight and the sum of the amounts of moisture, protein, lipid and ash contents.

## 2.4. Statistical Analysis

Results are expressed either as mean values or as mean values  $\pm$  standard deviation. The Student's *t*-test was used to determine statistical differences in the lengths and weights measured for each depth group. The Wilcoxon-Mann-Whitney test was used to compare the chemical composition of dry-weight samples of two depth groups. Differences with *p* values  $<0.05$  were considered statistically significant. Statistical analyses were performed using Kaleida Graph ver. 3.6 (Synergy Software; HULINKS, Tokyo, Japan) on Windows 7 (Microsoft, Tokyo, Japan).

## 3. Results

### 3.1. Seasonal Changes of Biomass

Data on the biomass are shown in **Table 1** and **Figure 1**. In the middle of November, the mean length of the plants was  $14.8 \pm 3.2$  cm at 1 - 2 m ocean depth, and  $71.1 \pm 31.1$  cm at 5 m ocean depth. The mean length of the plants increased to a maximum value of  $8.97 \pm 1.88$  m by the middle of January at 5 m ocean depth, and  $1.81 \pm 0.08$  m by the middle of February at a 1 - 2 m ocean depth (**Figure 1**). The maximum plant lengths were 12.7 m at 5 m ocean depth, and 6.5 m at 1 - 2 m ocean depth (**Table 1**). The mean weight of the plants increased to a maximum value of  $16.6 \pm 0.82$  kg by the middle of March at 5 m ocean depth, and to  $3.96 \pm 1.65$  kg by the middle of February at 1 - 2 m ocean depth (**Figure 1**). The maximum plants weight was 28.7 kg at 5 m ocean depth and 0.63 kg at 1 - 2 m ocean depth (**Table 1**). During the study period, the mean length and weight of plants harvested at 5 m ocean depth were significantly greater than those of plants harvested at 1 - 2 m ocean depth ( $p < 0.0001$ ; **Table 1**). Both mean length and weight of the plants increase earlier and to a greater length at 5 m than 1 - 2 m ocean depth (**Figure 1**). As *S. horneri* age, they are torn to pieces or separated from rocks in the sea, resulting in their drift depositions into the sea. *S. horneri* flowed out at 5 m ocean depth from the middle April. On the other hand, *S. horneri* were harvested until the middle of June at 1 - 2 m in ocean depth.

The gender differences became discernible after the middle of January (5 m ocean depth, 80%; 1 - 2 m ocean depth, 5%). Male and female receptacles were clearly discernible by the middle of March (5 m ocean depth) and April (1 - 2 m ocean depth; **Figure 1**).

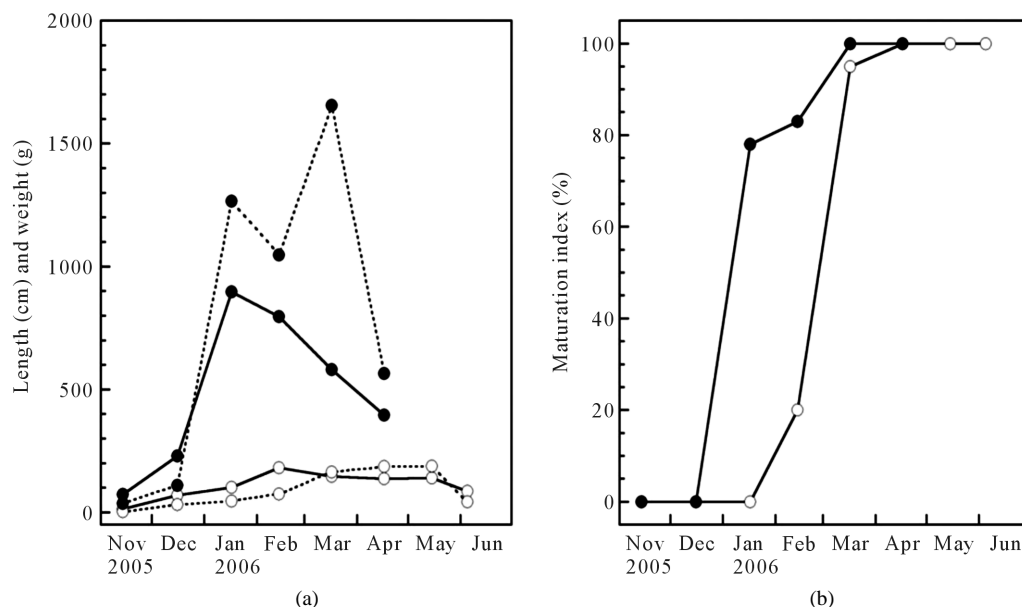
The chemical composition of *S. horneri* was measured using samples collected from December 2005. After the middle of January 2006, the gender differences became clearer, so the chemical compositions of the male and female plants could be separately determined.

### 3.2. Seasonal Changes in Chemical Components

Seasonal changes in chemical components were apparent on a wet- weight basis for evaluation of brown algae as a food resource (**Figure 2**). The dry-weight basis for evaluating *S. horneri* as a biofuel was moisture content, deduced from sample weight. The dry-weight basis is calculated by subtracting the moisture content from the wet-weight basis. The chemical compositions of *S. horneri* harvested at an ocean depth of 1 - 2 m (from December 16, 2005, to June 5, 2006) and 5 m (from December 16, 2005, to April 17, 2006) are shown in **Table 2**.

#### 3.2.1. Moisture Contents

The moisture contents of *S. horneri* are shown in **Figure 2** and **Table 2**. The primary component of *S. horneri* throughout the season is moisture (approximately 90%). According to the wet-weight basis, gender differences



**Figure 1.** Seasonal changes in plant length, weight and maturity index of *Sargassum horneri*. Plants of *S. horneri* were harvested from Chikuzen Sea, Fukuoka prefecture, and their length and wet weight were measured. Nine to 66 plants were harvested from November 14, 2005, to June 5, 2006. The results are presented as mean values. Maturity index was expressed as the ratio of the number of plants with discernible gender distinction to the total number of plant. ○: Ocean depth of 1 - 2 m; ●: Ocean depth of 5 m; —: Length or maturity index, .....: Weight.

**Table 1.** Effect of depth on plant length and wet-weight of *Sargassum horneri*.

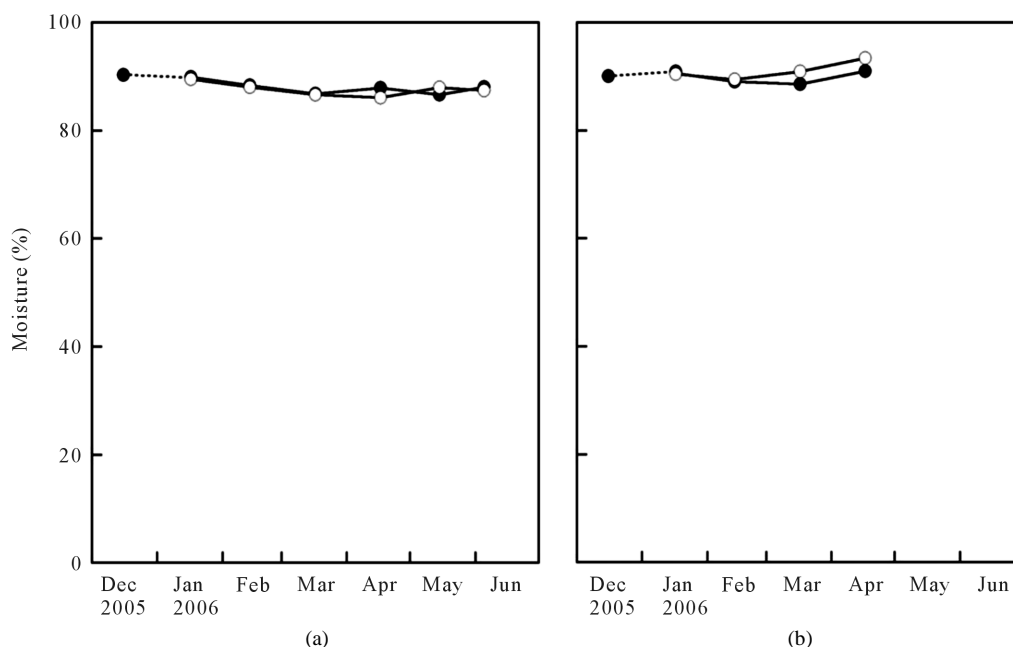
	Length (cm)		Weight (g)	
	Mean ± SD	Range	Mean ± SD	Range
Ocean depth of 1 - 2 m (n = 250)	89.0 ± 90.5	8.0 - 652	75.8 ± 108.5	0.7 - 627
Ocean depth of 5 m (n = 93)	389 ± 318	11.0 - 1270	546 ± 697	2.1 - 2874

Plants of *S. horneri* were harvested at the ocean depth of 1 - 2 m (from November 14, 2005, to June 5, 2006) and 5 m (from November 14, 2005, to April 17, 2006) of Chikuzen Sea, Fukuoka prefecture and their length and wet weight were measured. "n" is the number of sample plants. *t*-test; \* *p* < 0.0001.

**Table 2.** Chemical composition of *Sargassum horneri* on dry-weight basis.

	Ocean depth of 1 - 2 m (n = 13)		Ocean depth of 5 m (n = 11)	
	Mean ± SD	Range	Mean ± SD	Range
Moisture (%)	88.1 ± 1.3*	86.2 - 90.3	90.6 ± 1.3*	88.6 - 93.4
Protein (mg/g dry)	93.5 ± 38.9	52.4 - 195	101 ± 32	59.4 - 150
Lipid (mg/g dry)	4.03 ± 0.51**	2.98 - 4.99	5.64 ± 1.97**	2.64 - 8.59
Ash (mg/g dry)	258 ± 26	222 - 312	277 ± 27	228 - 311
Carbohydrate (mg/g dry)	645 ± 48	533 - 695	617 ± 50	555 - 692
TDF (mg/g dry)	477 ± 63***	390 - 589	409 ± 56***	321 - 495

The dry weight was obtained by dehydration as described in the preceding legend. The chemical composition of *S. horneri* harvested at the ocean depth of 1 - 2 m (from December, 16, 2005, to June 5, 2006) and 5 m (from December, 16 2005, to April 17, 2006) were averaged. TDF: total dietary fiber. Wilcoxon-Mann-Whitney test; \* *p* < 0.001, \*\* *p* < 0.05, \*\*\* *p* < 0.02.



**Figure 2.** Seasonal variations in the moisture contents on the wet weight basis of *Sargassum horneri*. The moisture contents in the samples harvested from December 16, 2005, to June 5, 2006, were determined. Mean values are calculated from 2 specimens from homogenized sample (0.4 - 1.9 kg of edible portions). The gender differences were distinctly observed after the end of January. (a) Ocean depth of 1 - 2 m; (b) Ocean depth of 5 m.  $\cdots\bullet$ : Immature plant,  $\bullet$ : Male plant,  $\circ$ : Female plant.

were not significant (Figure 2). However, the mean moisture contents for plants harvested at 5 m ocean depth ( $90.6\% \pm 1.3\%$ ) was higher than that for plants harvested at 1 - 2 m ocean depth ( $88.1 \pm 1.3\%$ ; Table 2).

### 3.2.2. Protein Contents

The seasonal changes in protein contents of *S. horneri* are shown in Figure 3. The maximum protein content was 19.0 mg/g at an ocean depth of 1 - 2 m, and 14.8 mg/g at an ocean depth of 5 m on wet-weight basis in December, with a tendency to decrease throughout the remainder of the study period. The protein content in the plant at an ocean depth of 1 - 2 m increased in March. On the other hand, gender differences regarding protein content in the plant at an ocean depth of 5 m were observed. The protein content of male plants increased in March (11.7 mg/g), while the protein content of the female plants did not show a tendency to decrease in January and February (11.0 and 10.6 mg/g, respectively).

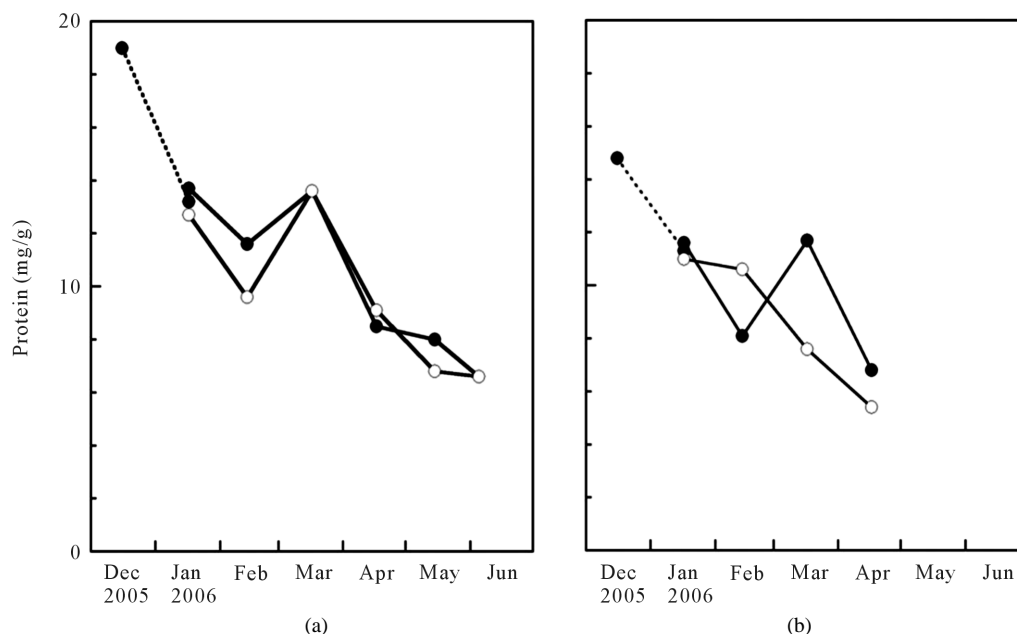
### 3.2.3. Lipid Contents

The seasonal changes in lipid contents of *S. horneri* are shown in Figure 4. The lipid contents (0.3 - 0.8 mg/g) of *S. horneri* showed the lowest value among all chemical constituents. When harvested at an ocean depth of 1 - 2 m, the maximum lipid content in female plants was observed in April (0.61 mg/g); while the maximum lipid content in male plants was observed in May (0.65 mg/g). When harvested at an ocean depth of 5 m, the maximum lipid content in female plants was observed in February (0.57 mg/g), while the maximum lipid content in male plants was observed in March (0.79 mg/g; Figure 4).

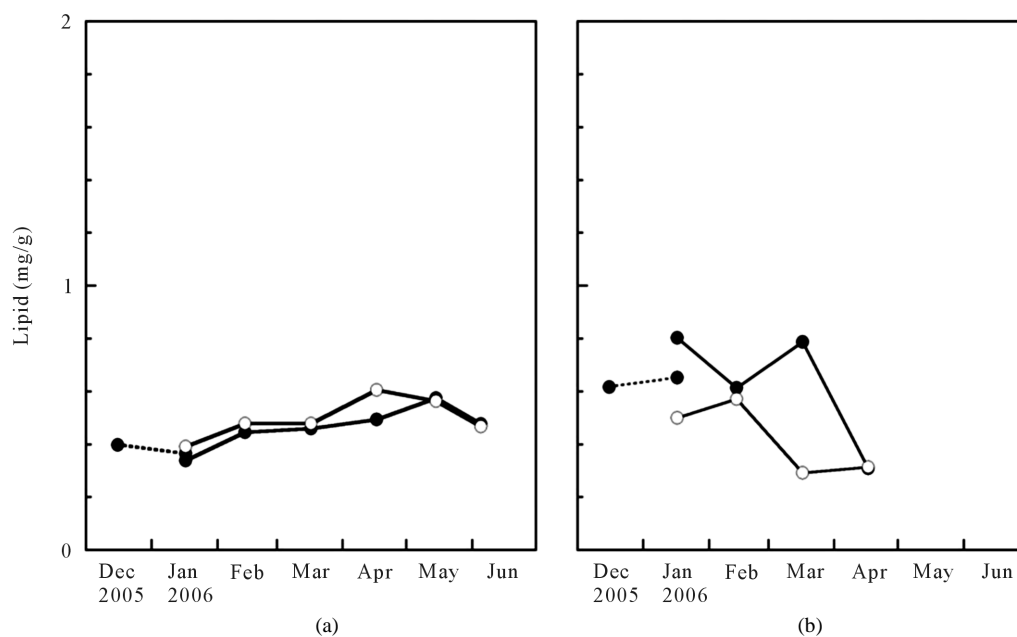
The mean lipid contents on a dry-weight basis were significantly higher for plants harvested at a 5 m ocean depth ( $5.64 \pm 1.97$  mg/g) than for those harvested at 1 - 2 m ocean depth ( $4.03 \pm 0.51$  mg/g;  $p < 0.05$ ; Table 2).

### 3.2.4. Ash Contents

The seasonal changes in ash contents of *S. horneri* are shown in Figure 5. The ash contents were in the range of 26.0 - 37.5 mg/g on a wet-weight basis, were not much less varied throughout the season, although some gender differences were observed between February and April. When harvested at an ocean depth of 5 m, the ash content of the male plants (30.7 mg/g) was higher than that of the female plants (22.6 mg/g) in April.



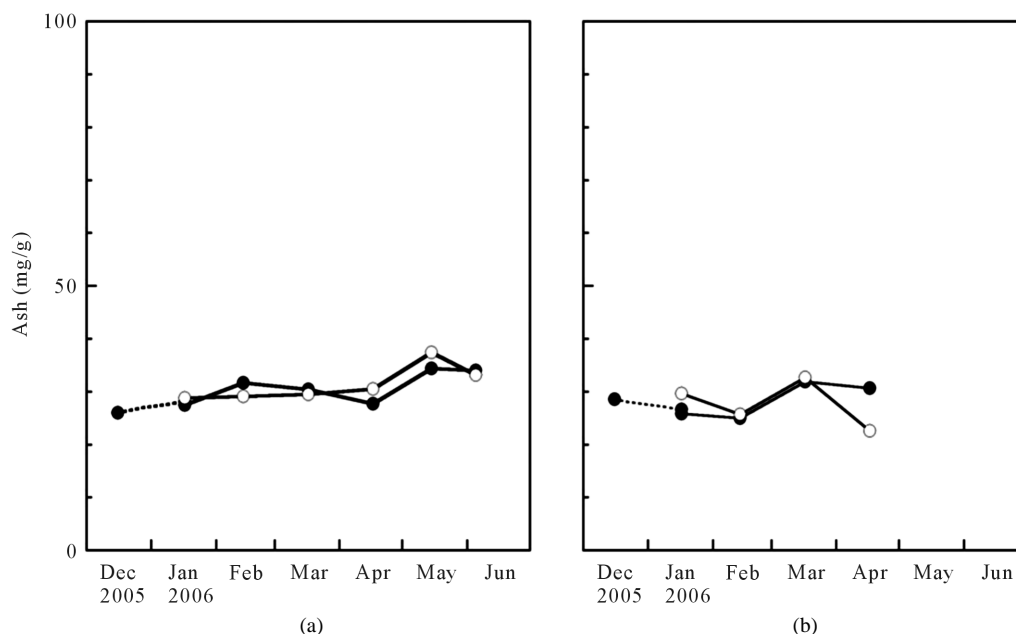
**Figure 3.** Seasonal variations in the protein contents on the wet weight basis of *Sargassum horneri*. The same homogenized samples as used for Figure 2.



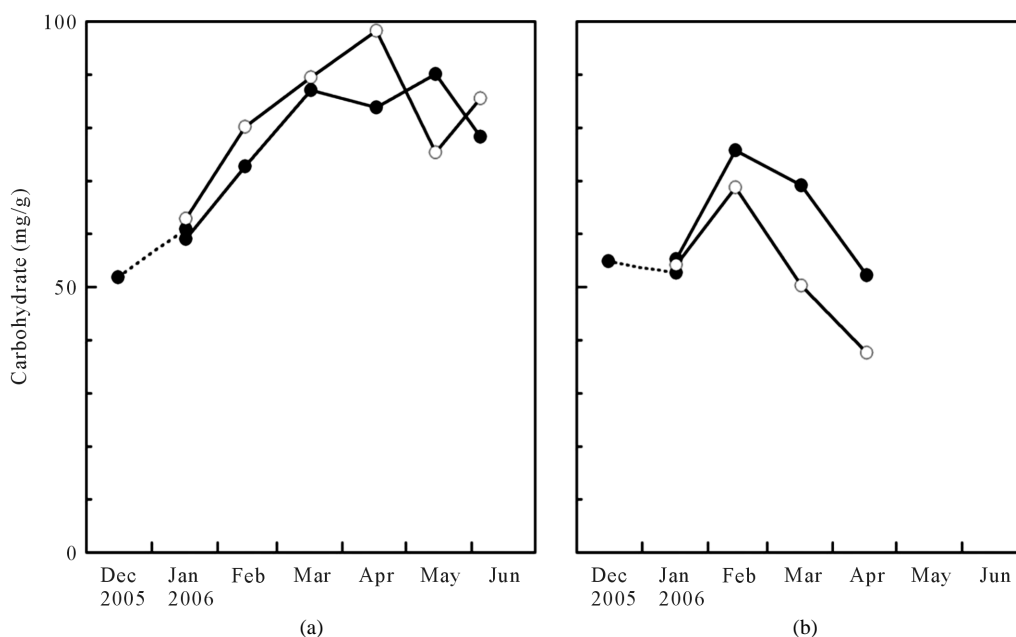
**Figure 4.** Seasonal variations in the lipid contents on the wet weight basis of *Sargassum horneri*. The same homogenized samples as used for Figure 2.

### 3.2.5. Carbohydrate Contents

The seasonal changes in carbohydrate contents of *S. horneri* are shown in Figure 6. The carbohydrate contents were calculated as the differences between the total weight and the sum total of the moisture, protein, lipid and ash contents. The carbohydrate contents of plants at an ocean depth of 1 m increased gradually until April (female, 98.3 mg/g) or May (male, 90.2 mg/g). When harvested at an ocean depth of 5 m, the maximum carbohydrate contents of the plants were observed in February (68.8 mg/g and 75.8 mg/g for female and male, respectively).



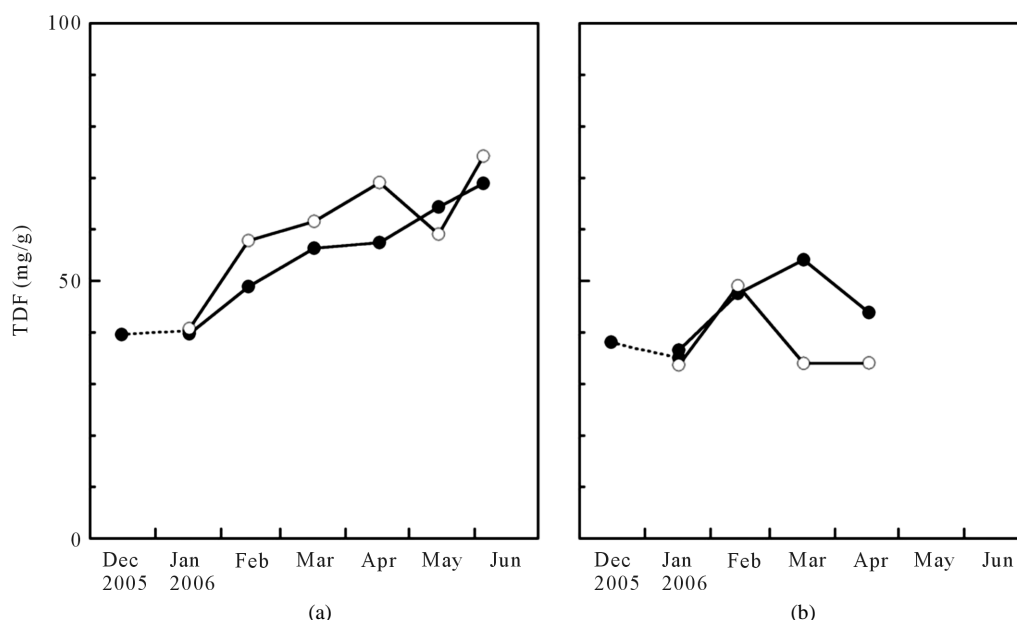
**Figure 5.** Seasonal variations in the ash contents on the wet weight basis of *Sargassum horneri*. The same homogenized samples as used for Figure 2.



**Figure 6.** Seasonal variations in the carbohydrate contents on the wet weight basis of *Sargassum horneri*. The same homogenized samples as used for Figure 2. Carbohydrate contents were calculated as differences between the total weight and the sum of the moisture, protein, lipid and ash contents.

### 3.2.6. Total Dietary Fiber Contents

Seasonal changes in TDF contents of *S. horneri* are shown in Figure 7. The TDF content of plants at an ocean depth of 1 m increased gradually until June (74.2 mg/g and 69.0 mg/g for female and male, respectively). When harvested at an ocean depth of 5 m, the maximum carbohydrate content of the plants was observed in February (43.9 mg/g and 54.2 mg/g for female and male, respectively). It was apparent that an increase in TDF content from February corresponded to plant maturity (Figure 1).



**Figure 7.** Seasonal variations in the total dietary fiber contents on the wet weight basis of *Sargassum horneri*. The same homogenized samples as used for Figure 2. TDF: total dietary fiber.

The mean TDF content on a dry-weight basis was higher for plants harvested at a 5 m ocean-depth ( $477 \pm 63$  mg/g) than for those harvested at a 1 - 2 m ocean-depth ( $409 \pm 56$  mg/g;  $p < 0.02$ ; Table 2).

#### 4. Discussion

The results of this study show that the chemical compositions of *S. horneri* harvested from the Chikuzen Sea changed according to the growth and maturity of the plant. To our knowledge, this study is first to demonstrate the effect of seasonal variation on the chemical composition of *S. horneri* according to differences in water depth and gender. In order for the use of *S. horneri* as an edible resource to proceed practically, it is important to retrieve basic information on the relationship and the seasonal variation of the growth and maturity of the plant, as well as its nutritional content. In particular, *S. horneri* is a component of *Sargassum* beds, which play important roles in resource enhancement of coastal fisheries. To maintain the balance of the marine biomass [10], it is important to understand the process of growth and maturity of *S. horneri*, as well as its relationship with nutrition, for the use of this plant as an edible resource. In addition, these data can assist in determining at which stage of life the plant contains the highest levels of carbohydrates, and thereby assist in the application of *S. horneri* as a resource of biofuels.

*S. horneri* grows more rapidly in deep water (*i.e.*, an ocean depth of 5 m) than in shallow water (*i.e.*, an ocean depth of 1 - 2 m). *S. horneri* in shallow water may be harvested even after the seaweed in the deep water has flowed out. Moreover, *S. horneri* harvested at an ocean depth of 5 m from February to March, or at an ocean depth of 1 - 2 m from April to June was the most suitable as food nutritionally. At these times, *S. horneri* achieves its maximum length and weight, and the carbohydrate and TDF contents reach their respective peak values. Moreover, *S. horneri* forms receptacles during these periods.

The protein content was observed to increase, depending on gender and ocean depth, which appears to be related to the plant's maturity (Figure 3): at a depth of 1 - 2 m in March (both genders), and at a depth of 5 m in February (female) or March (male). These observations were supported by the results of a recent study of *S. horneri* that was harvested at an ocean depth of 3 m (Murakami *et al.*, 2011). We surmise that protein synthesis may be required for plant maturity.

The lipid content of female plants achieved its maximum levels earlier than that of male plant, both at ocean depths of 1 - 2 m and 5 m. Furthermore, the protein and TDF contents of female plant harvested at an ocean depth of 5 m, achieved their maximum value earlier than those of male plants harvested at the same depth did (February and March, respectively).



The carbohydrate and TDF contents also increased, and correlated with the growth curve and the maturity of plants. These changes appear to be related to the amount of viscous exudate of *S. horneri* (data not shown).

*S. horneri* shreds and flows out subsequently, when going to aging. The seaweed that drifts ashore eventually decomposes causing several aesthetic and environmental problems, including an unsightly appearance and foul odor from decomposition by microorganisms, respectively. It often causes unsightly appearance and foul odor from decomposition by microorganisms. To alleviate these problems, we are investigating the possibility of utilizing *S. horneri* as a recyclable resource for sustainable edible and biofuel consumption by using the information that we generate throughout the plant's lifecycle. Specifically, *S. horneri* may be used as a biofuel before flowing out or after drifting ashore. Therefore, it is more advisable to harvest the upper half or third of the plant than to harvest the whole plant from the point of the *Sargassum* bed retention.

*S. horneri* can be used as an edible resource for extended periods, with growth and maturity during harvesting that depend on the ocean depth in the growth area. *S. horneri* can be utilized as a recyclable resource for a sustainable future both nutritionally and energetically; the upper part of the plant harvested at peak maturity for food, and entire aging plant harvested for biofuel before flowing out or after they have drifted ashore.

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