

Habitat characteristics and seasonal changes of *Laurencia brongniartii* (Ceramiales, Rhodophyta) in Kagoshima, Southern Japan

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SUMMARY

The seasonal change in size of *Laurencia brongniartii* was observed for the year 2002 at Banshobana Park, Satsuma Peninsula and Tsuchihama Beach, Amami Oshima, both in Kagoshima Prefecture, Japan. Additionally, the vertical distribution of this species was also studied at Tsuchihama Beach (second site), where the largest populations were found. Maximum lengths were observed in October – November with a size of 100 ± 6 mm at Banshobana Park and 111 ± 5 mm at Tsuchihama Beach. Line-transects conducted at Tsuchihama Beach, were analyzed to determine the vertical distribution of *L. brongniartii* due to depth and the slope of the substrate. Alga was found growing in areas restricted to low levels of light.

Key words: habitat, *Laurencia brongniartii*, Rhodophyta, seasonality.

INTRODUCTION

Laurencia brongniartii has been reported from the Atlantic, Indian and Pacific Oceans (Papenfuss 1943; Taylor 1960; Hackett 1974; Saito and Womersley 1974; Cribb 1983; McDermid 1988; Cabioch *et al.* 1990; Silva *et al.* 1996; Bula-Meyer and Norris 2001; Haroun *et al.* 2002). Within Japan, *L. brongniartii*, has been previously reported from the southern islands of the Ryukyu Archipelago, Southern Kyushu Island, and the Pacific coast of Shikoku and Southern Honshu Island (Okamura 1936; Saito and Takata 1974).

In the early 1990s, a number of surveys were conducted on indigenous macroalgae of Japan which found hundreds of species of algae that produce bioactive compounds capable of controlling antibiotic-resistant micro-organisms or exhibiting antitumor activity (Kamei *et al.* 1995; Miyazaki *et al.* 1995; Harada *et al.* 1996a, 1996b, 1997; Horikawa *et al.* 1995, 1996, 1999). In particular, *L. brongniartii*, collected from Amami Oshima Island, was discovered to produce compounds that are effective in controlling *Staphylococcus aureus*, *Aspergillus fumigatus*, *Trichophyton*

rubrum, *Streptomyces acidiscabies*, and *Streptomyces scabies* (Miyazaki *et al.* 1995; Horikawa *et al.* 1996). This has resulted in interest to commercialize this species, which would provide a new fisheries industry for Amami Oshima Island. However literature on *L. brongniartii* in Japan, are limited to taxonomical and morphological studies (Saito 1967; Saito and Takata 1974; Abe *et al.* 1998). Therefore additional information on the habitat characteristics, abundance and seasonality of *L. brongniartii* are needed.

There are many factors that can affect the abundance and seasonal pattern of algae. For example, physical variables such as light, temperature, salinity and wave action affect the distribution and abundance of algae (Lobban and Harrison 1994). Ecological variables such as herbivory stress and competition may also have some impact (Little and Kitching 1996). Preliminary field observations in Amami Oshima Island suggest that *L. brongniartii* preferred low-light areas of the coral reef and was under no stress from herbivores. We also noted that maximum size appeared to occur in the autumn season. Compared to two studies on Australian *L. brongniartii*, similar low-light conditions were also observed (Saito and Womersley 1974; May and Larkum 1981).

However in order to clarify the habitat characteristics of *L. brongniartii*, the effects of the coral reef on the light gradient must be considered. Light attenuation in a marine environment increases as depth increases (Lobban and Harrison 1994). Additionally, light gradients can also be affected by terrain (Anthony and Hoegh-Guldberg 2003). The topography of a coral reef is complex and can cause low-light conditions to occur in relatively shallow areas. Specifically, the numerous fissures and crevices, as well as the sloping walls of the reef can cause isolated areas of low-light (Brakel 1979; Baynes 1999; Anthony and Hoegh-Guldberg 2003).

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Communicating editor: J. H. Kim

Received 15 April 2003; accepted 14 August 2003.

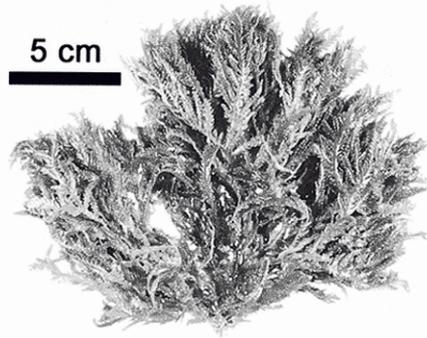


Fig. 1. *Laurencia brongniartii* from Amami Oshima Island taken during October 2002.

The objective of this study was to characterize the habitat and determine the abundance and distribution of *L. brongniartii* in a coral reef and to observe its seasonality. It was hypothesized that: (i) increasing depth would positively increase abundance; (ii) steep terrain would positively affect abundance in shallow water; and (iii) maximum size of *L. brongniartii* would occur during the autumn season.

MATERIALS AND METHODS

Description of the study sites

The study was conducted in two areas of Kagoshima Prefecture, Japan from January to December 2002. Monthly size of *L. brongniartii* (Fig. 1) and basic water quality assessments were conducted at Banshobana Park (31°15'N, 130°26'E), Ei Town, Satsuma Peninsula and Tsuchihama Beach (29°24'N 129°40'E), Kasari Town, Amami Oshima Island (Fig. 2).

Banshobana Park is located on the southern coast of the Satsuma Peninsula on the island of Kyushu. The area is a small consolidated rocky platform shore. The shorelines on either side of this shore are composed largely of sand. The base rock is of volcanic origin and at low tides expansive bedrock and large boulders are exposed. During high tides, the boulders and rock platform are entirely submerged. Heavy surf is common during the autumn and winter months. *L. brongniartii* is found in a confined area within a miniature cove. The cove is approximately 125 m wide by 200 m long, from land to sea. Boulders are found haphazardly throughout the perimeter of the cove. The central area is comprised of largely sand with occasional boulders. The cove faces south, and the algae are only found along the subtidal, eastern edge.

Tsuchihama Beach is a wide coral reef located on the north-eastern side of Amami Oshima Island. The

coral reef is approximately 120 m from shore to offshore, ending in a steep drop-off. Patch reefs are located for another 100 m. This reef extends for a few kilometers on either side of the study location. Throughout the reef, there are crevices and fissures. Strong currents are not unusual. The beach faces east and is exposed to strong surf during the summer and autumn months.

Seasonality and water quality

In a preliminary survey, conducted during 2000, the average monthly lengths of *L. brongniartii* were found to decrease from January to March in Banshobana Park and Tsuchihama Beach and increase thereafter. Therefore, *L. brongniartii* were observed during the spring tides of every month at Banshobana Park and likewise at Tsuchihama Beach with a few exceptions (February through March, July and December 2002) by skin-diving. The length of each specimen was measured *in situ* with a stainless steel ruler, marked in millimeters. Thirty algae were randomly selected at each site for measurement. The length of each specimen was measured by setting one end of the ruler next to the holdfast on the substrate, and recording the length of the main axis. All measurements were taken to the nearest millimeter and recorded on site.

The water temperature and salinity of each site were determined electronically (YSI Model 85, Yellow Springs Institute Inc., Ohio, USA) and the concentrations of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorous (DIP) were determined by chemical analysis (DR/2000 Portable Spectrophotometer, Hach Co., Colorado, USA). There were some difficulties in analyzing the values for nitrite, nitrate and ammonia in a timely manner, therefore the results were combined as DIN.

Line transect survey of Tsuchihama Beach

Tsuchihama Beach was selected as the site to determine how the light gradient affects the abundance and distribution of *L. brongniartii* in a coral reef. Depth and substrate slope were the factors used to describe the light gradients in the reef. Depth was used to account for the variability of light due to attenuation. Substrate slope was used to account for the influences of reef topography.

The survey was conducted during October 24 and 25, 2002. Five transects were laid out parallel to each other, in 20 m intervals. Each line started from a North-South baseline, marked by flags along the beach at the high tide mark (24 October, 2002), with all transects laid due East (180°) using a surveyor's compass. A weighted nylon rope, marked every 25 cm, was used to measure depth at appropriate intervals to determine the contour of the bottom terrain. Time was also measured so that all depth measurements could

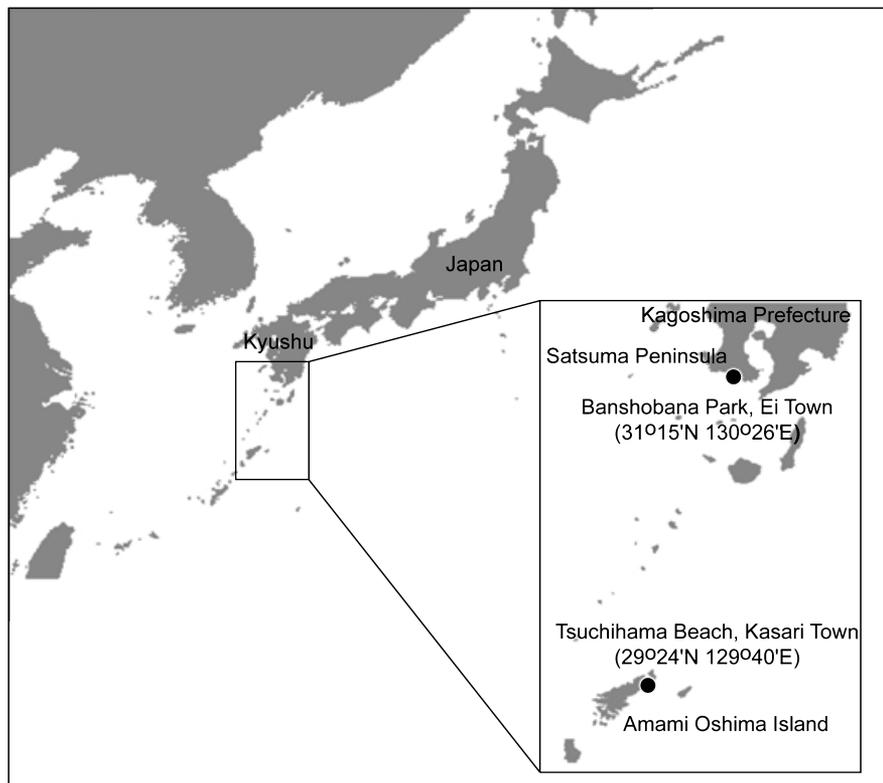


Fig. 2. Map of the study-site, Kagoshima Prefecture, Japan. (●, Amami Oshima Island), Tsuchihama Beach, Kasari Town, 29°24'N 129°40'E; and (●, Satsuma Peninsula), Banshobana Park, Ei Town, 30°15'N 130°26'E.

be standardized to the lowest astronomical tide according to the local tide chart (Hydrographic Department, Japan Coast Guard 2001).

Surveys were designed to determine coverage, population density, substrate slope and location of *L. brongniartii* populations. The average biomass of each population was also recorded, but was not included in the analysis due to the large variation in epiphytic organisms found on the algae among the quadrats. However, they are included as reference. A single population of *L. brongniartii* is defined as a group of algae within 2 m of the line transect and not within 2 m of another group. Five 50 × 50 cm quadrats were randomly placed within each population and the time, depth, population density and coverage were recorded for each quadrat. Slope was measured by an angle meter that was attached to a 1 m length of PVC pipe, and laid parallel to the transect in the middle of each quadrat. Depth at each quadrat was measured similarly to that described for measuring the terrain contour. Sampling was conducted over the entire length of the transect, wherever there was a population of *L. brongniartii*.

Statistical analysis

A mixed model analysis of variance (ANOVA) was used to test if depth and slope and its interaction had a significant effect on the population density and coverage of *L. brongniartii*. Each transect was set up as the block treatment and every transect-depth-slope combination

was nested in each population with quadrats as the replicate. The two-way ANOVA model is:

$$X_{ijklm} = \mu + D_i + S_j + DS_{ij} + T_k + P(DST)_{k(ijk)} + e_{ijklm} \quad (1)$$

where μ is the mean population density or coverage of the algae, D_i is the i th level of the factor depth, S_j is the j th level of the factor slope, DS_{ij} is the i th and j th level of the interaction between depth and slope, T_k is the k th level of the block transect, $P(DST)_{k(ijk)}$ is the l th population nested in the k th transect at depth i and slope j , and e_{ijklm} is the m th quadrat as a residual term. X_{ijklm} is the value of the m th quadrat in population l which is nested in transect k at depth i and slope j .

Homogeneity of variances were tested with Levene's test. The analysis for population density was done by transforming the number of plants per quadrat by the equation:

$$\sqrt{\chi + 0.5} \quad (2)$$

where χ is the number of plants in each quadrat. Likewise, the analysis for coverage was done by transforming percent coverage of each quadrat by the equation:

$$\sin^{-1}(\chi) \quad (3)$$

where χ is the percent coverage of each quadrat.

Slopes and depths were systematically separated into thirds. Depths were categorized so that groups were broken into a <3 m group, a 3–6 m group and a >6 m group. Slopes were likewise separated into groups that were <30°, groups between 30–60° and groups that were on slopes >60°.

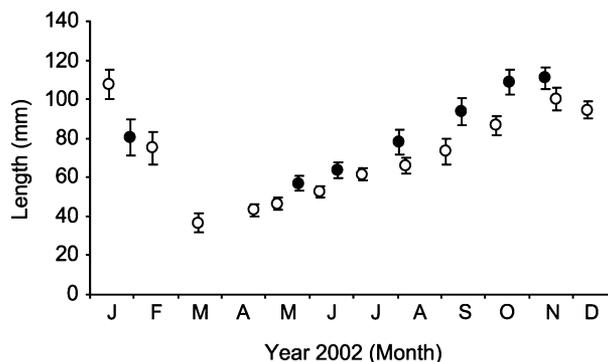


Fig. 3. Average lengths (mm) of 30 specimens of *Laurencia brongniartii* during 2002. (●) Tsuchihama Beach, Kasari Town (Amami Oshima Island). (○) Banshobana Park, Ei Town (Satsuma Peninsula). Error bars indicate the 95% CI.

Differences in the size of *L. brongniartii* from the population in Tsuchihama Beach and Banshobana Park were compared using Student's paired t-test. The test was applied to data when algae were observed to be the largest (November). All statistical analyses were done using SPSS V.11 (SPSS Inc., Chicago, IL, USA).

RESULTS

Seasonality and water quality

L. brongniartii were found on hard substrate in the subtidal zone and were never observed in sand or the intertidal zone. *L. brongniartii* in Banshobana Park were often found growing on the walls of rock or in the crevices between large boulders. Those of Tsuchihama Beach were typically found growing on the walls of reefs in shallower areas and on the flats of reefs in deeper areas. In either location, algae were found as single plants or as a group of plants in a population group. Overall, compared to Banshobana Park, the population density and coverage of *L. brongniartii* is estimated to be at least one order of magnitude larger at Tsuchihama Beach.

The seasonal changes in size of *L. brongniartii* were observed for approximately 1 year, from January to December at Banshobana Park and until November at Tsuchihama Beach (Fig. 3) in 2002. Thirty algae were measured every sampling period. Algae in Banshobana Park decreased in size, from 108 ± 8 mm (average \pm 95% CI) in January to 37 ± 5 mm in March. Algae in Tsuchihama Beach were measured in January, May, June and August through to November the average length in January and June for algae at Tsuchihama Beach was 81 ± 10 mm and 64 ± 4 mm, respectively.

From early spring to late autumn, the average lengths increased steadily at both locations. The final average length measured for specimens in Banshobana Park was 100 ± 6 mm (December); for those in Tsuchihama Beach the average was 111 ± 5 mm (November).

Table 1. The water quality characteristics of Banshobana Park, Ei Town (Satsuma Peninsula) and Tsuchihama Beach, Kasari Town (Amami Oshima Island) for 2002

| Parameter | Banshobana Park | Tsuchihama Beach |
|--------------------------------|-----------------|------------------|
| Temperature (°C) | | |
| Maximum (August) | 28.2 | 29.0 |
| Minimum (March) | 16.5 | 20.1 |
| Average salinity (psu) | 33.7 | 34.8 |
| DIN ($\mu\text{mol L}^{-1}$) | 3.7 ± 2.2 | 2.2 ± 2.8 |
| DIP ($\mu\text{mol L}^{-1}$) | 0.3 ± 0.2 | 0.2 ± 0.2 |

DIN and DIP values are the average \pm 95% confidence interval.

At both locations, minimum temperatures were recorded during March and maximum temperatures were recorded in August (Table 1). March surface seawater temperatures were 16.5°C and 20.1°C and August surface seawater temperatures were 28.2°C and 29.0°C for Banshobana Park and Tsuchihama Beach, respectively. Salinities were typically 33.7 psu for Banshobana Park and 34.8 psu for Tsuchihama Beach during the year 2002. Dissolved inorganic nitrogen (DIN) averaged 3.7 ± 2.2 $\mu\text{mol L}^{-1}$ and dissolved inorganic phosphorous (DIP) averaged 0.3 ± 0.2 $\mu\text{mol L}^{-1}$ for Banshobana Park (average \pm 95% CI). The DIN and DIP values for Tsuchihama Beach were 2.2 ± 2.8 $\mu\text{mol L}^{-1}$ and 0.2 ± 0.2 $\mu\text{mol L}^{-1}$, respectively.

Line transect survey of Tsuchihama Beach

Five transects were laid out in parallel facing due East at Tsuchihama Beach. Each transect was approximately 280 m in length, with no evidence of *L. brongniartii* on the intertidal portion of the transect. Algae were never found in areas that were uncovered by low tide and were found in low-light areas of the reef.

Figure 4 illustrates a typical cross-section of a transect with Table 2 summarizing the results. This transect is located in the middle of the survey site. Along this transect, 10 populations of *L. brongniartii* were found growing on the reef, no populations were found growing in the upper 3 m. A total of eight populations were found in depths of 3–6 m and the rest were observed in areas deeper than 6 m. Population 6 was found growing in an area of large boulders. Population 4 was found to be the largest patch in the survey site and was found at a depth of 4 m in a narrow deep crevice with 30 plants m^{-2} and coverage of 42%. Population 9 was found growing in fissures on the reef (9 plants m^{-2} and 7% coverage).

Statistical analysis

The average population density of each population is shown in Figure 5. For shallow slopes ($< 30^\circ$), there was a general trend for higher densities at lower depths. A similar trend was observed for slopes that were

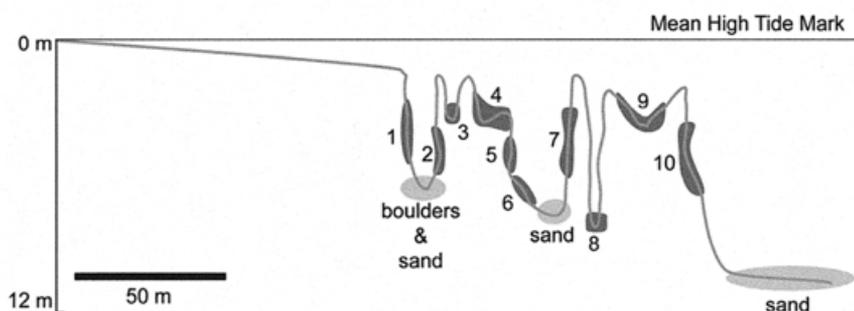


Fig. 4. Cross-sectional view of a typical transect at Tsuchihama Beach, Kasari Town (Amami Oshima Island) in October 2002. Vertical scale indicates depth and the horizontal scale bar in the lower left indicates the horizontal distance. See Table 2 for detailed information on each patch of *Laurencia brongiartii*.

Table 2. The details on each *Laurencia brongiartii* population for Fig. 4. Population density and coverage were measured along the transect to estimate the amount of *Laurencia brongiartii* during October 2002 at Tsuchihama Beach, Kasari Town (Amami Oshima Island)

| Population | Number of plants (m ⁻²) | Coverage per quadrat (% coverage) | Estimated biomass (wet g m ⁻²) |
|------------|-------------------------------------|-----------------------------------|--|
| 1 | 26 | 30 | 382 |
| 2 | 10 | 14 | 44 |
| 3 | 10 | 13 | 40 |
| 4 | 30 | 42 | 1602 |
| 5 | 1 | 21 | 112 |
| 6 | 18 | 25 | 187 |
| 7 | 18 | 26 | 207 |
| 8 | 18 | 20 | 101 |
| 9 | 9 | 7 | 20 |
| 10 | 4 | 4 | 13 |

Estimates are based on five randomly placed quadrats per population.

between 30° and 60°. Extreme slopes, those that were >60°, had the highest population densities in the middle depths.

Table 3 shows the ANOVA result for the root-transformed population density data. Levene's test was used to test for homogeneity of variance and was found to be highly significant ($F_{36,148} = 2.719$; $P < 0.001$). There was no significance between transects ($F_{4,24} = 0.904$; $P = 0.477$), the effects of slope was not significant ($F_{2,24} = 2.861$; $P = 0.077$) and the effects of depth was not significant ($F_{2,24} = 0.870$; $P = 0.432$). There was no significant interaction between slope and depth ($F_{4,24} = 2.215$; $P < 0.098$). However, there was significant variation between replicate quadrats of each population in each transect-depth-slope combination ($F_{24,148} = 7.461$; $P < 0.001$).

The result of the ANOVA for percentage coverage is presented in Table 4. The average percentage coverage per population is shown in Figure 6. The transect and the interaction between slope and depth was not significant ($F_{4,24} = 0.876$; $P = 0.493$ and $F_{4,24} = 1.409$; $P = 0.261$, respectively). The depth was not significant ($F_{2,24} = 0.551$; $P = 0.584$). There was significant vari-

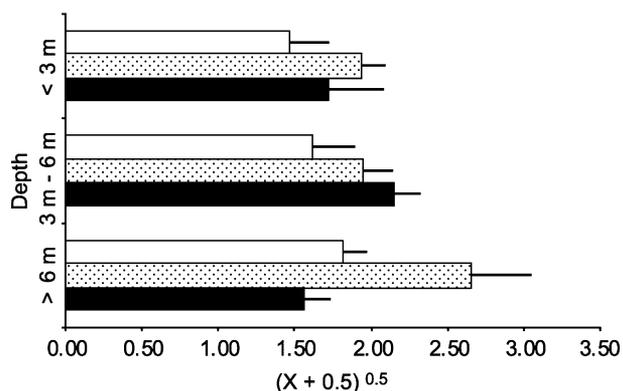


Fig. 5. Root-transformed population density of *Laurencia brongiartii* per 50 × 50 cm quadrat with respect to depth and substrate slope for the October 2002 line-transect survey of Tsuchihama Beach, Kasari Town (Amami Oshima Island). (□) slopes <30°; (▨) slopes 30°–60°; (■) slopes >60°. Error bars indicate the 95% CI.

Table 3. The mixed model ANOVA result for the square-root transformed number of *Laurencia brongiartii* per 50 × 50 cm quadrat data

| Source of variation | MS | df | F | P |
|------------------------|-------|-----|-------|-------|
| Transect | 0.939 | 4 | 0.904 | 0.477 |
| Depth | 0.904 | 2 | 0.870 | 0.432 |
| Slope | 2.973 | 2 | 2.861 | 0.077 |
| Interaction | 2.301 | 4 | 2.215 | 0.098 |
| Population (T × D × S) | 1.039 | 24 | 7.461 | 0.000 |
| Error | 0.139 | 148 | | |

ation between the quadrats of each population of each transect-depth-slope combination ($F_{24,148} = 8.341$; $P < 0.001$). Levene's test was used to test the error variances of the data, resulting in a highly significant result ($F_{36,148} = 3.455$ and $P < 0.001$).

Results of Student's paired *t*-test to test if there was a difference in populations from Tsuchihama Beach and Banshobana Park were highly significant ($t_{29} = 9.280$; $P < 0.001$) with a mean difference of 11.1 mm (Tsuchihama Beach > Banshobana Park).

Table 4. The mixed model ANOVA result for the arcsine transformed percent coverage of *Laurencia brongniartii* per 50 × 50 cm quadrat data

| Source of variation | MS | df | F | P |
|---------------------------|--------|-----|-------|-------|
| Transect | 0.0354 | 4 | 0.876 | 0.493 |
| Depth | 0.0222 | 2 | 0.551 | 0.584 |
| Slope | 0.0945 | 2 | 2.343 | 0.118 |
| Interaction | 0.0569 | 4 | 1.409 | 0.261 |
| Population (T × D × S) | 0.0403 | 24 | 8.341 | 0.000 |
| Error | 0.005 | 148 | | |

DISCUSSION

Laurencia brongniartii was found restricted to areas where the light attenuation effects of depth and the shading effects of the coral reef provided refuge from prolonged exposure to high levels of light. This follows in agreement with observations made by Shepherd and Womersley (1970, 1971) upon the distribution of sub-littoral algae in Australia. Their observations suggest that algae that are typically found in deep water can exist in shallow water when terrain provides refuge from direct irradiance. Prior accounts by Saito and Womersley (1974) indicate that Australian *L. brongniartii* were generally found in waters 4–11 m deep and in areas that were shaded. In a later study, May and Larkum (1981) also noted that Australian *L. brongniartii* was found in deep and shady environments. All of this suggests that *L. brongniartii* is confined to a habitat that is low in light.

A cursory look at Figure 4 also suggests that the population size is relatively small. Out of 10 populations of *L. brongniartii*, eight had population densities <25 plants m⁻² and eight populations had percentage coverage of <30%. This is low compared to other commercially-harvested species, such as *Gracilaria sordida*, which averaged 20–40 plants m⁻² during peak periods (Nelson 1989) and *Mazzaella cornucopiae* which had percent coverage >50% (Scrosati 1999).

Our results show an overall trend for higher population densities and coverage in deeper areas and steeper slopes promoted a similar trend in shallower water (Fig. 4). However, the lack of significance in the statistical analysis is unfortunate. The failure to detect significant results at the 0.05 level, in population density and percentage coverage, is attributed to the significant variation among replicated quadrats in each population that was nested in all possible combinations of transect-depth-slope. This significant variation is probably due to heterogeneity in the size of the sampled population and suggests patchy distribution of *L. brongniartii* within the sampled population. Therefore, random placement of quadrats in the population had chances of enclosing no plants or many plants, which can cause significant variation in the measured variable.

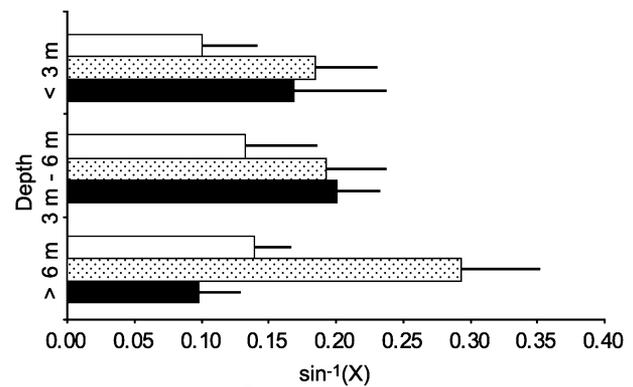


Fig. 6. Arcsine-transformed percentage coverage of *Laurencia brongniartii* per 50 × 50 cm quadrat with respect to depth and substrate slope for the October 2002 line-transect survey of Tsuchihama Beach, Kasari Town (Amami Oshima Island). (□) slopes <30°; (▨) 30°–60°; (■) slopes >60°. Error bars indicate the 95% CI.

An additional factor that may have affected the analysis was the existence of populations in shallow, flat areas. For example, in Figure 4, population 4 and 9 are illustrated as an example of two such populations. However, this is an artifact of the figure and does not truly reflect the population's terrain. In fact, there exists three other populations under similar conditions in other transects. However, what is not shown is the microhabitat of the population. As is typical of most coral reefs, there are numerous fissures and crevices in the reef that can provide protection from high levels of light (Brakel 1979; Baynes 1999; Anthony and Hoegh-Guldberg 2003). In the case of population four and nine of Figure 4, the sampled populations were found in such crevices; however, they were systematically categorized according to our sampling program.

Regarding the seasonality of *L. brongniartii*, an increase in size occurred from March through November at either location and after December and through February, the fronds bleached and decayed which is depicted as a decrease in size (Fig. 3). Analysis of herbarium specimens collected from Amami Oshima Island and Banshobana Park during 2000 and 2001 (deposited in the Faculty of Fisheries, Kagoshima University) show that mature tetrasporophytes and gametophytes were found during the months of August through December. Observations of herbarium and *L. brongniartii* *in situ* suggest that there was no impact of herbivores and the locations of populations indicate no possibility of dessication. Effects of wave and surges were assumed to be equivalent over both locations in our study, and therefore were not considered in the model; however, both were observed to be similar at either location.

However, environmental data gathered at both locations suggests that the differences between minimum and maximum water temperatures may have been a factor in the determining maximum size, because there was a significant difference in size between locations.

March recorded the lowest surface water temperatures and August had the highest surface water temperatures for both locations. The temperature range between these periods were 16.5°C to 28.2°C for Banshobana Park and 20.1°C to 29.0°C for Tsuchihama Beach. We suggest that plants in Tsuchihama Beach are able to attain larger sizes because they are in waters that are closer to their optimum growth temperature longer than that of plants in Banshobana Park. Experiments to confirm this are currently in progress.

Other factors such as DIN and DIP were not considered to be a significant factor in the differences in growth and size; these waters are heavily influenced by the Japan Current, which is characterized by its low nutrient conditions (Coastal Oceanography Research Committee 1985). Salinity was also deemed to be typical of coastal waters.

In conclusion, the size of *L. brongniartii* was largest during November at either location and there was a trend toward higher population densities and coverage in deeper areas or in steep slopes when areas were shallow. With respect to the hopeful commercialized harvesting of this species, our results suggest that the relatively small populations of *L. brongniartii* might complicate sustainable harvesting. Maximum yield would occur in the autumn months, but this also coincides with the maturation period, which should be considered in any harvesting program. We suggest additional studies to evaluate the recovery rates, harvesting intensity and harvesting period of *L. brongniartii* to maintain a sustainable resource.

ACKNOWLEDGMENTS

This research was made possible by the support of the Office of Amami Oshima, Kagoshima Prefecture, Japan.

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