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Master's Thesis

COMPARATIVE STUDY OF ECOLOGICAL AND PHYSIOLOGICAL PROPERTIES
BETWEEN TWO EURYHALINE RED ALGAE

March 2024

Graduate School of Marine Science and Technology
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Master's Course of Marine Resources and Environment

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Abstract:

Euryhaline red algae such as *Bostrychia* and *Caloglossa* are widely distributed in marine, brackish, and freshwater habitats from tropical to temperate regions, and some species shows particular vertical and horizontal distribution patterns in estuaries, where salinity, photon flux densities (PFD) and desiccation time vary depending on depth and distance from the sea. It is known that two *Caloglossa* species indicate clear distribution pattern in temperate estuaries; *C. ogasawaraensis* is more abundant in downstream than *C. continua* and the vertical zone of *C. ogasawaraensis* is below than that of *C. continua*. However, mixed or reverse zonation patterns of these species have occasionally been observed or either species has been absent in some habitats. Therefore, this study aimed to investigate primary environmental factors that regulate the distribution pattern of the two *Caloglossa* species. Three sampling sites were selected at the estuary of Tama River; both salinity and PFD were higher in the order of site 1, site 2 and site 3. The thallus coverage, which was measured using 10 x 10 cm quadrat, was 57 % (site 1), 54% (site 2) and 85 % (site 3) in *C. ogasawaraensis*, and 0 % (site 1), 5 % (site 2) and 39 % (site 3) in *C. continua*. After 2 hour desiccation treatment, *C. continua* showed higher relative water content of thallus (58 %) than *C. ogasawaraensis* (10 %) however, there was no significant difference. When thalli were cultured for four days after 8 hour desiccation treatment, the proportion of alive thallus area was higher in *C. continua* (95.7 %) than *C. ogasawaraensis* (48.9 %). In culture experiment under various salinity (8, 32 and changing condition between 8 and 32) and PFD (5 and 60 $\mu\text{mol}/\text{m}^2/\text{s}$), the relative growth rate was not significantly different among the six conditions in both species. These results suggest that desiccation tolerance is more responsible for distribution pattern of the two species than salinity and PFD. Higher algal biomass of both species at site 3, which is more shaded than other sites, is probably due to low

desiccation stress. The vertical distribution of *C. continua* above *C. ogasawaraensis* can be partly explained by the difference in desiccation tolerance. The outcome of this study might help to understand the ecology, physiology and distribution patterns of euryhaline species worldwide.

1. Introduction

Estuarine habitats support a high level of biodiversity, serving as nurseries and breeding grounds for a diverse range of flora and fauna, including fish, crustaceans, mollusks, bird species and different groups of macroalgae (Schwartz 2009). Macroalgae commonly grow on the surface of pneumatophores, trunks, and prop roots of mangrove trees, which are an important source of primary production through photosynthesis, providing food and habitat for various marine organisms in brackish or estuarine environments (Burkholder and Almodóvar 1973, Lugo and Snedaker 1974, Rodriguez and Stoner 1990). Estuarine stressors such as salinity, light, temperature, nutrients, water movements (Kirst 1989), sedimentation, substratum (Kautsky & Kautsky 1989), and ice scouring (Wetthey 1985) are the main factors influencing the growth and distribution of these intertidal algae. The primary regulating factor in estuaries is salinity, which is mainly determined by freshwater runoff and the prevailing ebb and flow of the tides. Seasonal variations in freshwater discharge, tide variations resulting from the solar and lunar cycles, storms and winds, and local fluctuations in currents are all factors that contribute to salinity variations (Mangelsdorf 1967). Compared to freshwater environments, estuary habitats have a relatively low biodiversity (Hartog, 1967) because most freshwater and marine algae cannot withstand a large salinity fluctuation. However, some macroalgal species are tolerant against such salinity fluctuations and show high biomass in brackish regions such as estuaries and salt marshes (Biebl, 1962). Significantly, euryhaline algae *Bostrychia* and *Caloglossa* can grow more than double salinity than seawater, demonstrating the maximum tolerance to salinity (Almodovar and Biebl 1962).

As the tides periodically rise and fall, intertidal algae go through phases of immersion and emersion, followed by desiccation (Bell 1993). They are exposed to varying frequencies and

durations of emergence during these periodic episodes. Water loss at emersion times may have significantly impact on their physiological processes, including photosynthesis and dark respiration. Once drought progresses above a certain point, intertidal seaweed photosynthetic rates decrease (Bidwell and Craigie 1963, Brown and Johnson 1964, Imada et al. 1970, Brinkhuis et al. 1976, Hodgson 1981, Dring and Brown 1982, Bell 1993, Lipkin et al. 1993, Delgado et al. 1995). This suggests that the photosynthetic activity of seaweed is negatively impacted by drought. However, some species exhibit an initial increase in photosynthetic rate while they are just starting to dry out (Brinkhuis et al. 1976, Dring and Brown 1982, Gao and Aruga 1987, Bell 1993). For example, compared to while fully submerged, the net photosynthesis of the tropical intertidal seaweed *Ahnfeltiopsis concinna* was increased with 20% water loss (Beach and Smith 1997). Similarly, Johnson et al. (1974) reported the photosynthetic rates of temperate middle and upper intertidal algae, even after an appreciable amount of drying in air, to be 1.6 to 6.6 times higher than when submerged.

The euryhaline red algal genus *Caloglossa* (Harvey) G. Martens belong to the family Delesseriaceae, are broadly distributed in the eulittoral and uppermost sublittoral zones of estuaries and salt marshes from tropical to temperate regions, adhering to substrata such as pneumatophores or trunks of mangrove trees, stems of reeds and other solid objects (King and Puttock 1994). They commonly occur with other red algal genera *Bostrychia* and *Catenella* to form mangrove associations, the so-called “*Bostrychietum*” group (Post 1936). Some *Caloglossa* species are also recorded in freshwater streams or true marine habitats (Kumano 2002, West et al. 2015), showing a remarkable euryhaline nature. *Caloglossa* species are taxonomically distinguished based on vegetative traits, such as rhizoidal arrangements, angle of divergence at the thallus nodes, presence or absence of adventitious branches, number of cell rows cut off from

the axial cells around the node, and position of cystocarp on the blades (Krayesky et al. 2011). At present, 22 species of *Caloglossa* are taxonomically accepted (Guiry and Guiry 2024). These algae experience higher in salinity, temperature, nutrient levels and desiccation fluctuations due to repeated emersion-immersion cycles (West et al. 1993). The degree of tolerance to desiccation and the extent of recovery during re-submergence following desiccation seem to determine the upper limits of the vertical distribution of intertidal macroalgae (Schoenbeck and Norton 1979, Hodgson 1981, Dring and Brown 1982, Druehl and Green 1982, Smith and Berry 1986, Madsen and Maberly 1990, Lipkin et al. 1993).

The algae grow in the upper region exposed to high irradiance fluctuations. Due to tidally driven emersion-immersion cycles, these algae are exposed to desiccation daily during the semi-diurnal tidal events. During exposure, intertidal macroalgae experience various potentially stressful environmental conditions, including nutrient limitation, high light, desiccation, and osmotic stress (Davison and Pearson 1996). The physiological adaptations to emersion-related stress in macroalgae have been previously examined (Johnson et al. 1974, Dromgoole 1980, Dring and Brown 1982, Oates and Murray 1983, Oates 1985, 1988, Matta and Chapman 1991, Henley et al. 1992, Bell 1993, Beach and Smith 1997). Notably, the physiological tolerance to desiccation of mangrove red algae were documented by Biebl (1962) and Mann and Steinke (1988). Although several other studies on the zonation pattern and species composition of epiphytic algae have been reported from Puerto Rico (Almodo´var and Biebl 1962, Kolehmainen and Hildner 1975), Brazil (De Oliveira 1984), Japan (Tanaka and Chihara 1987), Kenya (Coppejans and Gallin 1989), and South Africa (Phillips et al. 1996), which environmental stressors regulate the zonation pattern of these epiphytic algae are not fully understood. In addition, structure, biomass and re-colonization of the Australian *Bostrychietum* group were documented by Davey and

Woelkerling (1985). Most of the patterns coincide in that it is possible to recognize distinct vertical algal zones along the roots and pneumatophores. Tidal inundation, wetting frequency, desiccation tolerance, and salinity and photon flux density (PFD) have been cited as possible significant factors influencing the vertical distribution of mangrove algal epiphytes (Phillips et al. 1996).

Environmental circumstances are changing due to continuous global climate change, which is detrimental to the distribution and abundance of some seaweeds and promotes the prevalence of others (Muth et al. 2019, Umanzor et al. 2023). These effects might be seen in shallow and intertidal coastal habitats, where seaweeds already have to adapt quickly and dramatically to abiotic changes at tiny temporal and geographical scales (cm to m) (Valdivia et al. 2011, Umanzor et al. 2019). Seaweeds living in these conditions are resilient to sporadic stressors and continue to grow. However, seaweed physiological tolerance thresholds may eventually be exceeded when stress grows in intensity, frequency, or duration and involves the interaction of many stressors. Seaweeds are subject to constant stress; those living in harsh environments or on the boundaries of their typical ranges are particularly susceptible to escalating stress levels (Umanzor et al. 2023). Seaweeds may be exposed to extremely cold or extremely hot temperatures, and periods of darkness that can last for more than 10 hours in a 24-hour period, in the intertidal habitats of temperate estuaries (Umanzor et al. 2023). Additionally, seaweeds in this area are periodically and seasonally exposed to extreme desiccation (Lindstrom 2009). Depending on the season, rainfall, and air temperature, sediment inputs from nearby freshwater discharges affect the purity of the water (Hood and Berner 2009). A significant and increasing number of studies demonstrate that variations in freshwater discharge and watersheds impact downstream water qualities, affecting estuarine dynamics across various trophic levels (Arimitsu

et al. 2016). Because of their phenotypic plasticity to a wide range of conditions and their ability to adapt to new environmental conditions or mitigate through ecosystem engineering processes, these changes may create favorable or unfavorable conditions for intertidal seaweeds like *Caloglossa* and *Bostrychia* (Bertness et al. 1999, Weslawski et al. 2010, McCabe and Konar 2021).

In Japan, two *Caloglossa* species, *C. continua* and *C. ogasawaraensis*, are widely distributed in estuaries and brackish lakes from Tohoku to Okinawa (Guiry and Guiry 2024). Morphologically, the blades of *C. continua* are wider and weakly constricted at the nodes, endogenous branches are produced by an axial cell above the nodes, and absence of adventitious branches, whereas *C. ogasawaraensis* is characterized by narrower and more linear blades with strongly constricted at the nodes, no endogenous branches, and adventitious branches formed from the lateral pericentral initials (King and Puttock 1994). Yoshizaki et al. (1985) reported a clear horizontal and vertical zonation pattern of these species in the estuarine habitats of Kido River, Chiba Prefecture; *C. ogasawaraensis* was usually more abundant in downstream than *C. continua* and the zone of *C. ogasawaraensis* is below that of *C. continua*. However, *C. continua* was absent in some of the parts of the Kido River. In my preliminary examination, the distribution pattern mentioned above was confirmed in Sakuta River, adjacent to Kido River, but mixed or reverse zonation patterns were occasionally observed in the upstream region (personal-observation). However, *C. ogasawaraensis* were abundant in the downstream region, while a very few amount of *C. continua* were present in that areas.

The aim of this study is to investigate the primary environmental factors that regulate the zonation pattern of these two species. In particular, *C. continua* and *C. ogasawaraensis* were compared by (a) examining coverage in three different environmental points,

(b) measuring water content of thalli during desiccation to examine the water retention abilities, and (c) monitoring the growth rate under various culture conditions to evaluate the stress tolerance against desiccation, salinity change and high photon flux densities (PFD).

2. Materials and Methods:

2.1. Sample collection and coverage examination: Due to the abundance of *C. continua* and/or *C. ogasawaraensis*, three sites (35°32'40.3"N 139°44'41.2"E; 35°32'41.9"N 139°44'30.7"E; 35°32'42.2"N 139°44'27.7"E) around Daishibashi Bridge in Tama River, Kawasaki Kanagawa Prefecture, were selected in this study (Fig. 1). At site 1, *C. ogasawaraensis* was only found attaching to large plastic bags (> 2 m in diameter) filled with gravels. At site 2, both species were attached to rectangular wooden sheet (Approximately 100.0 × 70.0 cm: length × wide). At Site 3, both species were attached to artificial wooden sticks (approximately 30.0 cm diameter and 1.0 m long) under the bridge (Fig. 2). All the objects were previously present in the sampling areas by the municipality for protecting the River dikes from erosion. In August 2023, salinity were measured using a salinity meter (Es-51, Horiba, Ltd. Kyoto, Japan) in each site during high tide when water submerged these targeted species. PFD was also measured using Quantum PAR meter (APM092, Ast Optoelectronics Co., Ltd, Jiangsu, China) during morning (10:30 AM), noon (1:30 PM) and evening (5:00 PM) among sites.

To examine the density of each species, the coverage of each species was examined using a 10 cm x 10 cm quadrat at haphazardly selected 27 points for *C. ogasawaraensis* and 12 points for *C. continua*.

In June 2021, during the primary survey of Sakuta River, Chiba Prefecture, thalli of both *Caloglossa* species were collected to establish unialgal cultures. Collected samples were

immediately brought back to the laboratory and cleaned by using tap water to remove sands and epiphytes. The apical parts of healthy thalli (around 3mm) cut off using forceps were put into clean 60 ml plastic cup containing 10 mL of Provasoli's enriched seawater modification (PES2) medium (Provasoli L.1966) with a salinity 8. Both species were cultivated at temperature 20°C, 5 $\mu\text{mol}/\text{m}^2/\text{s}^1$ PFD and a photoperiod of 12:12 h L:D in a Cool Incubator (MIR-554-PJ, PHCbi, Tokyo, Japan). The culture media were changed every two weeks (Karsten et al. 1993).

2.2. Measurement of relative water content: Due to the presence of varying habitats (open and shaded) and also varying desiccation periods, the water retention abilities of both species were compared among sites because it may be related to the desiccation tolerance. On 17th June, 2023 collected samples of both taxa were immediately brought back to the laboratory and cleaned by using tap water to remove sands and epiphytes. Then, surface water of the samples were blotted using tissue paper. The water loss of thalli were monitored under a desiccated condition. 10-15 thalli were weighed, then maintained for two hours at 26°C (A3001, Ikuta sangyou Co., Ltd, Japan), 70-80 $\mu\text{mol}/\text{m}^2/\text{s}^1$ of PFD and 77- 80% of relative humidity. PFD and humidity were measured using by Light meter (LI-250, LI-COR, USA) and Thermo Recorder (TR-72nw, T&D Corporation, Nagano, Japan), respectively. Thallus weight was measured at 30 min intervals, and after the experiment, the samples were oven dried for 48 hrs at 50°C in a Dry oven (DRA430DA, Toyo Seisakusho Kaisha, Ltd., Japan). The relative water content (RWC) was calculated using the following formula (Ji et al. 2002);

$$\text{RWC (\%)} = (\text{desiccated Weight} - \text{dry Weight}) / (\text{fresh weight} - \text{dry weight}) * 100$$

2.3. Desiccation experiment: The desiccation tolerance of the two species was evaluated by monitoring the growth rate after periodic desiccation. Healthy unialgal strains cultured for more than 18 months were used for this experiment. Firstly, the 2-3 mm long apical part of the thalli

was cut off using a razor blade (Feather –S, Razor Co., Ltd, Japan) and maintained under 20°C temperature, 100 $\mu\text{mol}/\text{m}^2/\text{s}^1$ of PFD, 16:8 L: D, 80 – 85% of relative humidity. A 2 mL of PES media was added to each well of a 24 well culture plate. Four thalli were exposed to each of the three desiccation conditions, 0 hr (no desiccation), 4 hr (10:00-14:00) or 8 hr (10:00-18:00), for two days, and then these thalli were cultured in PES medium for two days. Digital pictures of the apical fragments before and after the experiments were taken using a camera attached to an inverted microscope (XD30-PHM, Kenis, Osaka, Japan), and then the initial and final areas were calculated using ImageJ (<https://imagej.nih.gov/ij/index.html>). Finally, the recovery rate of each species was calculated using the following formula: final area / initial area.

2.4. PFD and salinity experiment: To establish a relationship between stress tolerance and distribution pattern, relative growth rate under different PFD and salinity was compared between the two species. Approximately, 2 – 3 mm long apical thalli of *C. continua* and *C. ogasawaraensis* strains were incubated at 20°C, 16:8 L: D and the relative growth rate (RGR) was compared at the two PFD (5 or 60 $\mu\text{mol}/\text{m}^2/\text{s}$), and three salinity conditions (8, 32 and 8 for 12 hrs and 32 for 12 hours). After four days of incubation, the initial and final areas of each thallus were calculated using ImageJ software (<https://imagej.nih.gov/ij/index.html>), and finally, the RGR was calculated using the following formula: $[\text{RGR} = (\ln L_t - \ln L_i) t^{-1}]$ where, L_i = initial and L_t = final area after t days (Karsten et al. 1993).

2.5. Statistical analysis: Data were analyzed using Microsoft Office Excel (<https://office.microsoft.com/excel>). Biomass data were analyzed using one way ANOVA to determine the influence of environmental parameters on the density of each species among sites. Also, pair wise t-test was used to evaluate the variation of biomass of *C. continua* and *C. ogasawaraensis* at site 3. The water retention ability of *C. ogasawaraensis* among sites was

analyzed using one way ANOVA to determine the physiological variation. Two way ANOVA was conducted to analyze the effect of salinity and PFD on the growth of both species. A significant level is $P < 0.05$.

3. Results

3.1. Field observation: PFD at sites 1 and 2, highly fluctuated during morning, noon and evening however, at site 3, it was constantly low throughout the day. During low tide, salinity differences among sites indicated that both site 1 and 2 had higher salinity than at site 3 (Figs. 3, 4). The coverage of *C. ogasawaraensis* was higher at site 3 (85 %) than at sites 1 (57 %) and 2 (54 %), respectively, but there was no significant difference ($P = 0.1219$). The coverage of *C. continua* at site 3 (39 %) was significantly higher than that of *C. continua* (5 %) at site 2 ($P = 0.0029$) (Fig. 5).

3.2. Water content: Water content of thallus dropped with an increase in desiccation time (Figs. 6 and 7). After 0.5 hours of desiccation, *C. ogasawaraensis* thalli at site 2 retained a higher amount of water (52%) than those at site 1 (25%) and site 3 (20%), and this difference became small at more extended desiccation conditions (Fig. 6). Although the water retention ability of these species largely varied among sites, a non-significant ($P > 0.05$) relationship was found between the water retaining ability and habitat during each desiccation period.

Following 0.5 hours of desiccation, *C. continua* thalli at site 1 and 2 retained water content of 85 % and 87 %, respectively, and this difference became small at longer desiccation conditions (Fig. 7). There was no significant difference in water retention ability between the sites in both species.

3.3. Effects of Desiccation on growth: The recovery rate of *C. continua* was higher than *C. ogasawaraensis* after 4 and 8 hrs of desiccation. After four hours of desiccation, *C. continua* grew usually and the alive part of the thallus became darkish around the mid-rib. In addition, the basal cutting part of the thallus including the surrounding edges became dried, while the apical region remained alive after 8 hrs of desiccation periods (Fig. 8). However, during 4 hrs of desiccation, the *C. ogasawaraensis* thallus color became faded and part of the thallus died. Subsequently, after 8 hrs of desiccation treatment, the thallus completely died and looked whitish-pale (Fig. 8). After 4 hrs of desiccation treatment, the recovery percentages (%) of *C. continua* and *C. ogasawaraensis* were 1.05 and .61, respectively (Table 1). The recovery percentages (%) of *C. continua* and *C. ogasawaraensis* were .95 and .48, respectively following an 8-hour desiccation.

3.4. Effect of salinity and PFD on the growth of these two species: In *C. ogasawaraensis* cultured under 5 $\mu\text{mol}/\text{m}^2/\text{s}$ PFD, the RGR at 32 psu was lower than that at 8 psu and the changing condition (Fig. 9). This tendency was the same at 60 $\mu\text{mol}/\text{m}^2/\text{s}$ PFD. No significant variation were found among salinity conditions ($P = 0.161$). The RGR was higher at 60 $\mu\text{mol}/\text{m}^2/\text{s}$ PFD than at 5 $\mu\text{mol}/\text{m}^2/\text{s}$ PFD regardless of salinity condition. No significant difference was found for PFD ($P = 0.185$) and interaction between salinity and PFD ($P = 0.923$). Likewise, *C. continua* cultured under 5 $\mu\text{mol}/\text{m}^2/\text{s}$ PFD, the RGR at 32 salinity was lower than that at 8 salinity and the changing condition (Fig. 10). This tendency was the same at 60 $\mu\text{mol}/\text{m}^2/\text{s}$ PFD. No significant variation were found among salinity conditions ($P = 0.1414$). The RGR was higher at 60 $\mu\text{mol}/\text{m}^2/\text{s}$ PFD than at 5 $\mu\text{mol}/\text{m}^2/\text{s}$ PFD regardless of salinity condition. No significant difference was found for PFD ($P = 0.1314$) and interaction between salinity and PFD ($P = 0.6224$).

4. Discussion

In our study, the highest biomass was estimated in site 3 followed by site 2 for both species (Fig. 5). The lowest photon flux density was recorded at site 3 because of shade under Daishibashi Bridge. These conditions may reduce emersion stress and increase algal biomass (Yokoya et al. 1999, Zhang et al. 2014). The biomass fluctuations among the sites might happen due to various environmental factors, such as waves, sediment in suspension and nutrient availability which are known to cause variations on a small scale (Seangkaew et al. 2016). Furthermore, macroalgal biomass in the estuarine habitats might vary due to the pollution status of a water body (Billah et al. 2016). Similarly, the more significant biomass variability among the *Bostrychia*, *Caloglossa* and *Catenella* species were reported in a subtropical estuary in southern Brazil (Mendonça and Lana 2021). The biomass hierarchy of dominant macroalgal taxa followed as, *B. kelanensis* > *C. stipitata* > *C. leprieurii* > *C. ogasawaraensis* > *C. adhaerens* in Miri estuary, Sarawak, Malaysia (Billah et al. 2016), but the biomass of *C. leprieurii*, *C. stipitata* and *C. ogasawaraensis* significantly fluctuated by temporal variations in the Miri estuary (Billah et al. 2016). Likewise, Middelboe et al. (1998) studied macroalgal environmental variables in 202 sites of 26 estuaries in Denmark and mentioned that algal species richness is highly regulated by salinity, availability of hard substratum, higher mean depth like vertical and horizontal extension and nutrient availability. In addition, the specific growth rates of mangrove macroalgae such as *B. moritziana* and *C. leprieurii* were reported to be decreased due to limiting nitrate and phosphate concentrations under laboratory experiments (Ryder et al. 1999). Besides, massive siltation load associated with meteorological disturbances (e.g., floods and cyclones) have influenced the mangrove macroalgal biomass in the Saint Lucia estuary, South Africa (Steinke & Naidoo, 1990). Moreover, the grazing intensity has been examined to influence algae biomass, especially

during the high tide when the substratum is submerged (Wada and Wowor 1989, Underwood and Barret 1990, Laegdsgaard and Johnson 1995, Crowe 1996). Besides, estuarine algae covered by muds have been examined to reduce the photosynthetic activity resulting in lower algal biomass production. However, after one year of removing the mud from algae, the biomass production significantly increased (Steinke and Naidoo 1990). Martins et al. (2001), reported that freshwater intake is one of the primary sources of N, P, and silt in estuaries. In this study, site 3 had higher biomass which was located at the upstream. Site 3 was located under Daishibashi Bride with shaded conditions, whereas site 2 and site 3 were exposed to the sunlight. Shaded habitat probably went through lower desiccation stress than the open habitats, which resulted higher biomass in site 3.

Overall, after 2 hrs of desiccation, the RWC of *C. continua* (58%) was higher than that of *C. ogasawaraensis* (10%) (Figs. 6, 7). Since *C. continua* is distributed in more upper zone than *C. ogasawaraensis*, higher RWC may contribute to survival during long desiccation in low tide. Schonbeck and Norton (1979) investigated the possibility that upper intertidal seaweeds may possess morphological mechanisms for avoiding desiccation, but there was no evidence that upper littoral species had a higher water retaining ability. In addition, Beer and Eshel (1983) investigated different populations of *Ulva* species growing at different intertidal levels and found no apparent difference in water retaining ability. The evidence obtained in our study showed that upper intertidal species are more resistant to water loss than their lower intertidal counterparts. It seems that water retention ability is not a necessary primary strategy for upper intertidal species to adapt to their harsh habitat. Lipkin et al. (1993) suggested that, when evaluating the mechanisms enabling high intertidal algae to survive the harsh conditions of their habitat, the

lowest hydration level that they can survive and the length of exposures that they can withstand at these low RWC should be considered.

In intertidal habitats, the upper portion was subjected to longer desiccation time than the lower portions, so *C. continua*, which appears above *C. ogasawaraensis*, may be more tolerant to desiccation conditions. Davison and Pearson (1996) reported that the ability to withstand emersion is considered the primary determinant of the presence of the species and controls the upper distributional limits in some intertidal species. In our laboratory experiment, the number of living cells and recovery percentage (%) after desiccation treatment of *C. continua* was higher than that of *C. ogasawaraensis* (Table 1). Similarly, *B. calliptera* showed higher tolerance against high light intensities, lower desiccation rates, and higher photosynthetic recovery rate after desiccation treatment than *C. leprieurii*, and this property could explain that *B. calliptera* grows in an upper intertidal position in the prop roots (Pena et al. 1999). Mann and Steinke (1988) reported, after high levels of desiccation, both photosynthetic and respiratory rates of *B. radicans* recovered fully, while *C. leprieurii* only recovered some of its initial activity. This indicates that *B. radicans* is more tolerant against desiccation and grows in an upper vertical position than *C. leprieurii*. In addition, Ogata & Matsui (1968) suggested that the ability to recover metabolically after re-immersion indicates the tolerance of seagrass against desiccation. While *C. continua* has a higher survival and recovery rate than *C. ogasawaraensis*, it typically grows in a higher position in the field.

In the PFD experiments, the growth of both species showed better RGR at 60 $\mu\text{mol}/\text{m}^2/\text{s}$, and the same result for *C. ogasawaraensis* was shown in Karsten and West (1993). However, Pena et al. (1999) reported that the efficiency of low-light utilization could be an important adaptation that helps *Bostrychia* and *Caloglossa* colonize intertidal and subtidal habitats. Also, Cribb (1978)

noted an increase in algal development further inward in mangrove forests in the Moreton Bay region and found *C. lepriurii* growing in very shaded conditions. Because PFD differs depending on the season and time, it is necessary to evaluate the influence of photoperiod and fluctuation of PFD on their growth.

For both species, RGR was lower at 32 than 8 and changing conditions. When *Caloglossa* species are placed into culture, the positive growth results in low salinities, which might indicate that this is the natural optimum salinity range for growth. However, *C. ogasawaraensis* showed the highest growth rate in 32 salinity (Karsten and West 1993), while other factors than salinity might affect the difference in their biomass among the sites.

5. Conclusion and perspective

The comparison of environmental stressors between various habitats covering more expansive areas from both upstream and downstream might provide useful information to specify regulating factors for the vertical zonation patterns of euryhaline algae. Three sampling sites were selected at the estuary of Tama River because the biomass and distribution pattern of *C. continua* and *C. ogasawaraensis* were distinct among the three sites. The highest and lowest salinity and PFD were observed at sites 1 and 3. The coverage of both species was higher at site 3 than at the other sites. *C. continua* showed higher relative water content than *C. ogasawaraensis*, but there was no significant difference. Percentage of survival rate were higher in *C. continua* than in *C. ogasawaraensis* after desiccation treatment. Stress tolerance experiments under various salinity and PFD indicated that the relative growth rate was not significantly varied in both species. These results suggest that desiccation tolerance is more

responsible for the distribution pattern of the two species than salinity and PFD. The higher algal biomass of both species at site 3, which is more shaded than other sites, is probably due to low desiccation stress. Consequently, we can say that desiccation is one of the factors that regulate the vertical zonation patterns of these two species. Considering other abiotic factors like wave actions and turbidity might be helpful for better understanding the zonation pattern of these taxa. Furthermore, examining of the growth and seasonal succession among different habitats and substrata might also provide helpful information on the zonation patterns. The outcome of this study might help to understand the ecology, physiology and distribution patterns of euryhaline red algae including *Caloglossa*, *Catenella* and *Bostrychia* species, which are essential components in mangrove ecosystems.

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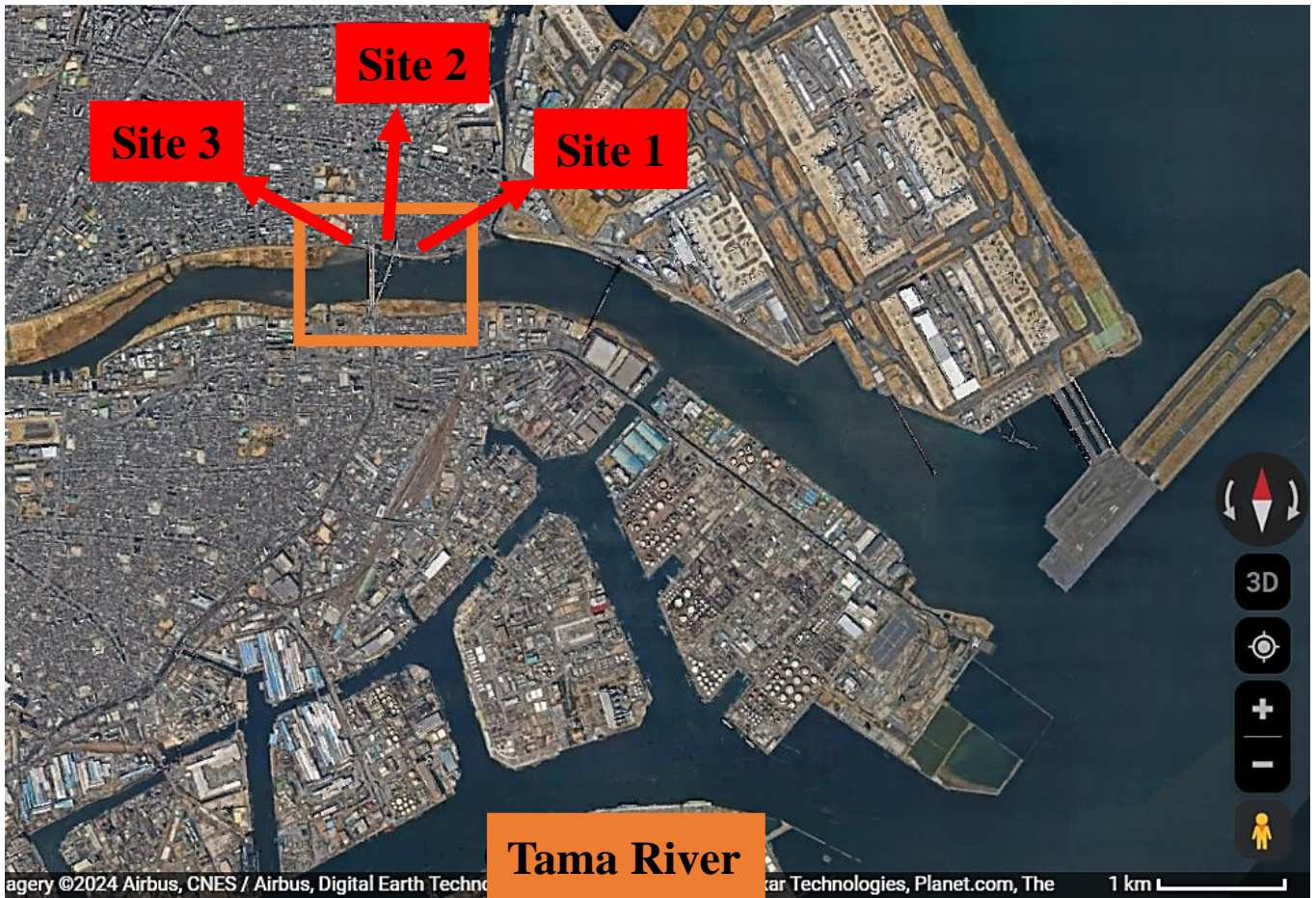


Fig 1. Study area, Tama River, Kawasaki Prefecture.



Fig 2. Three sites of the Tama River where target species were present.

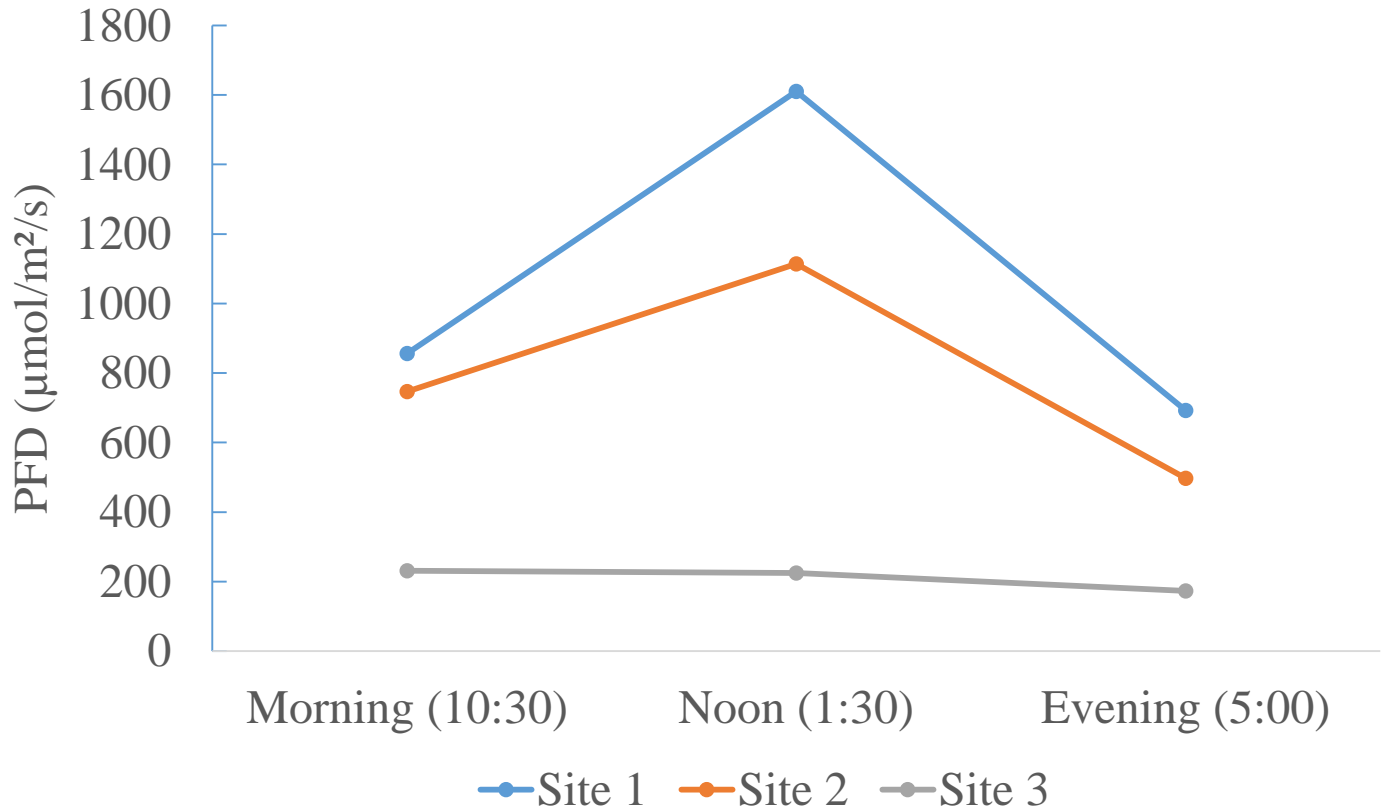


Fig 3. Variation of PFD during morning, noon and evening among sites.

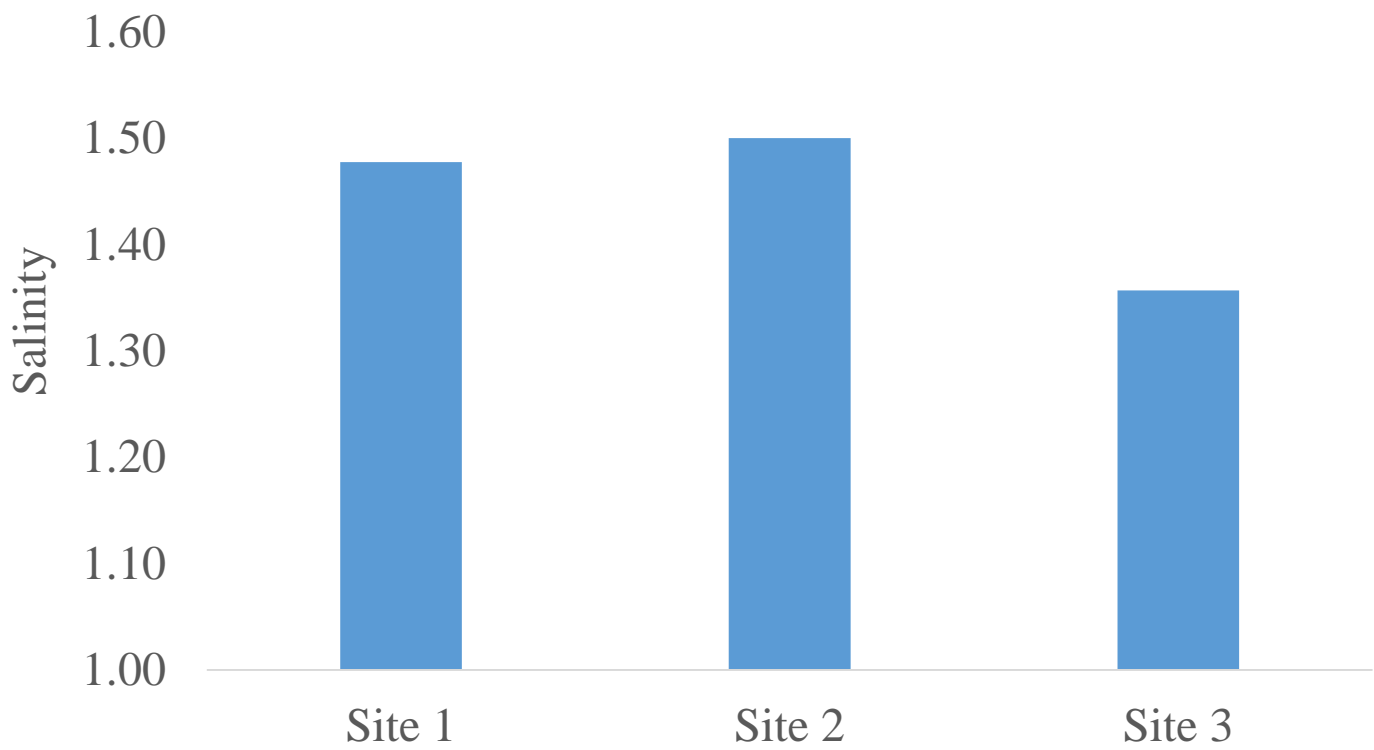


Fig 4. Difference of salinity during high tide among sites.

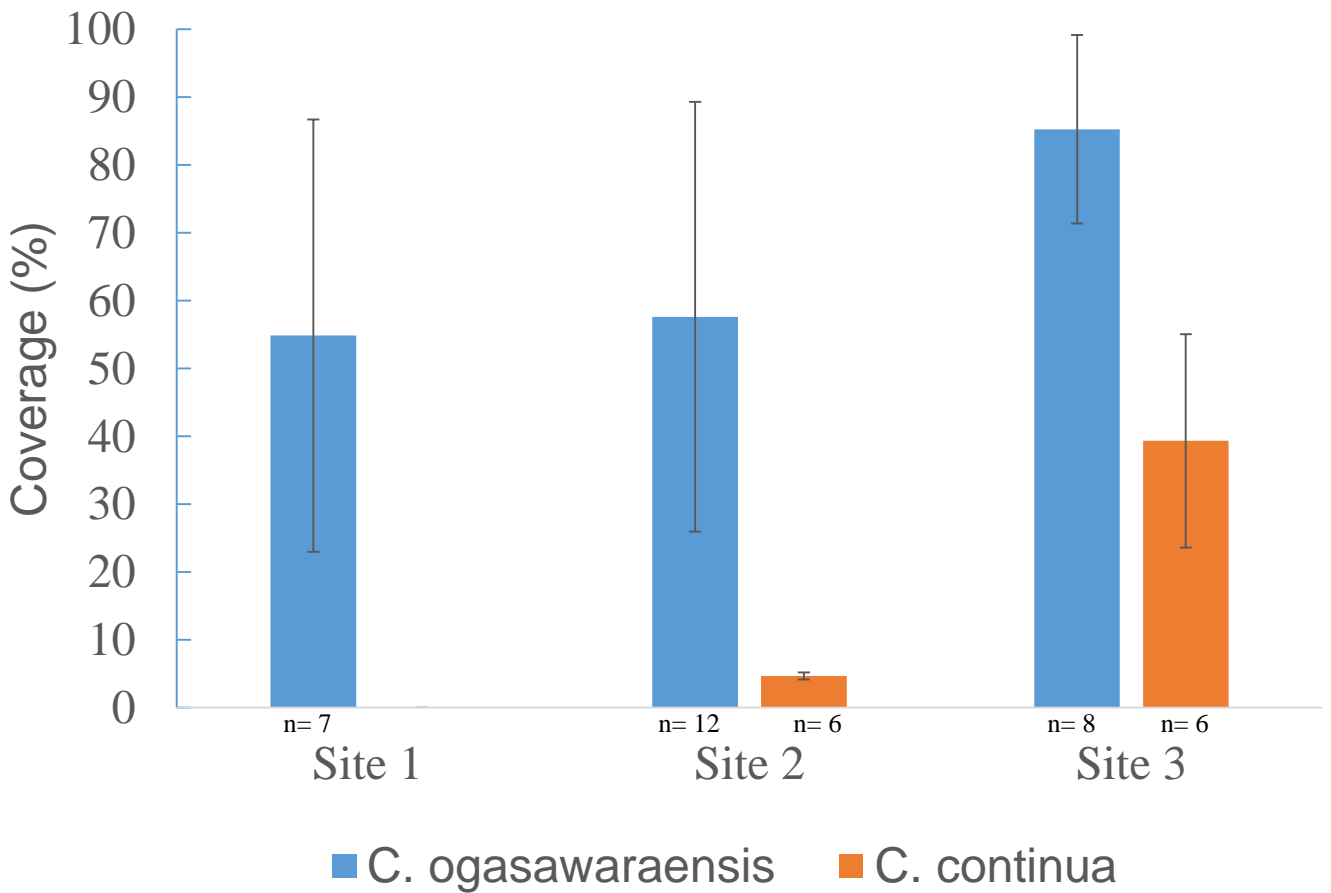


Fig 5. Mean coverage (%) and standard error of *C. ogasawaraensis* and *C. continua* at the three sites.

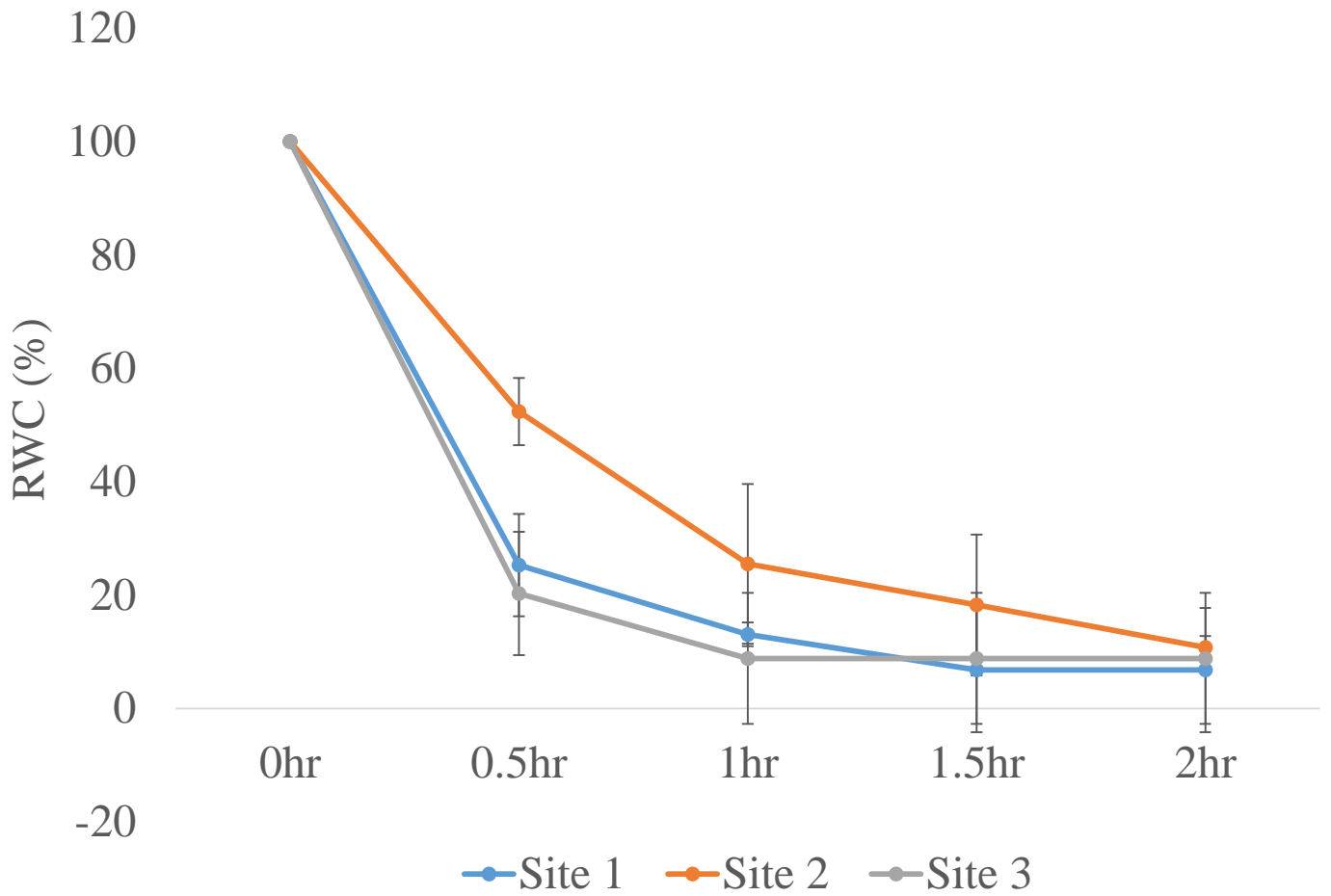


Fig 6. Mean relative water content (%) and standard deviation (n=3) of *C. ogasawaraensis* collected from the three sites.

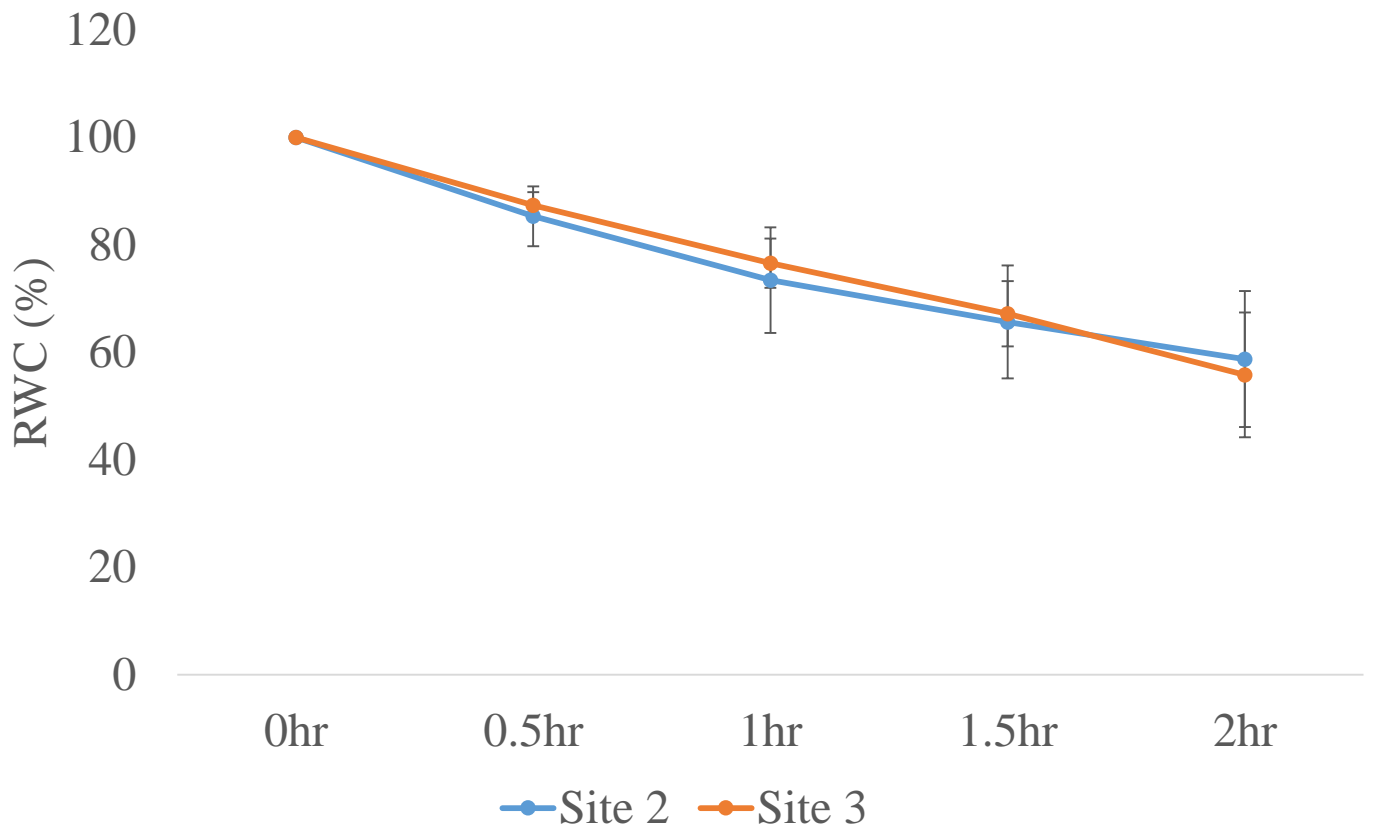
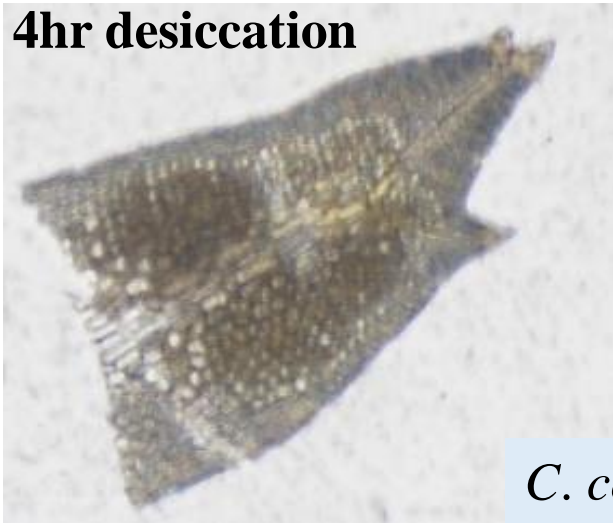


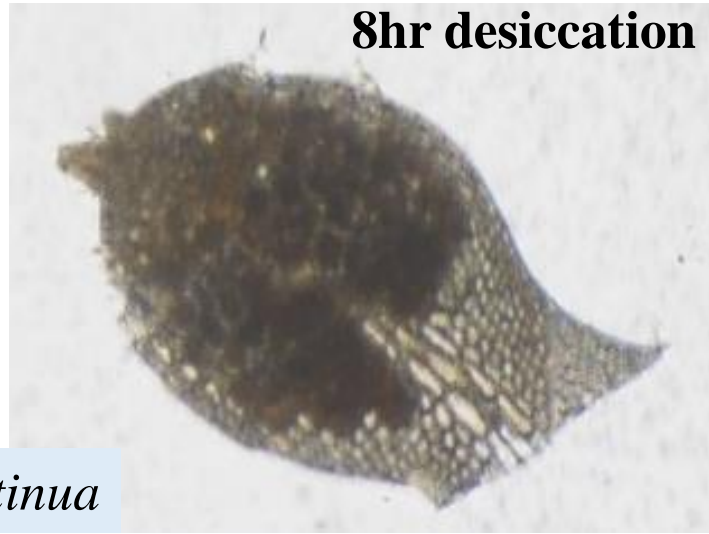
Fig 7. Mean relative water content (%) and standard deviation (n=3) of *C. continua* collected from two sites.

Scale bar: 200µm

4hr desiccation



8hr desiccation



C. continua

The alive part of the thallus remained darkish around the mid-rib

Edges dried and apical region kept alive



Death cell

Alive cell

4hr desiccation



Whitish
Death cell

8hr desiccation

C. ogasawaraensis

Fig 8. Thallus conditions after desiccation treatment

Table 1. Recovery rate of *C. ogasawaraensis* and *C. continua* after 4days desiccation treatment.

Desiccation period	Alive and recovery percentage (%) of both species	
	<i>C. ogasawaraensis</i>	<i>C. continua</i>
4hr	0.613	1.0504
8hr	0.4893	0.957

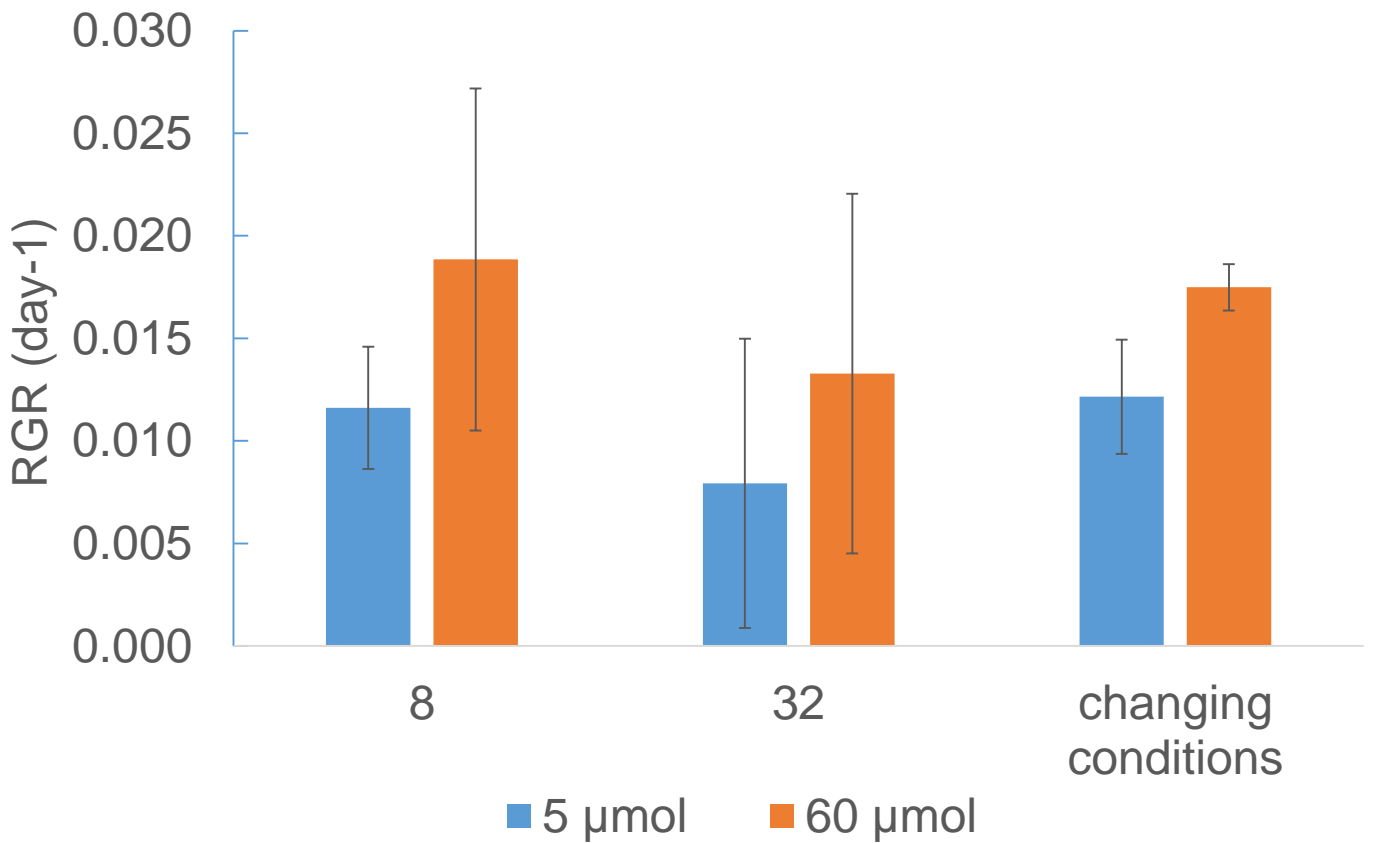


Fig 9. Mean relative growth rate and standard deviation (n=4) of *C. ogasawaraensis* strain.

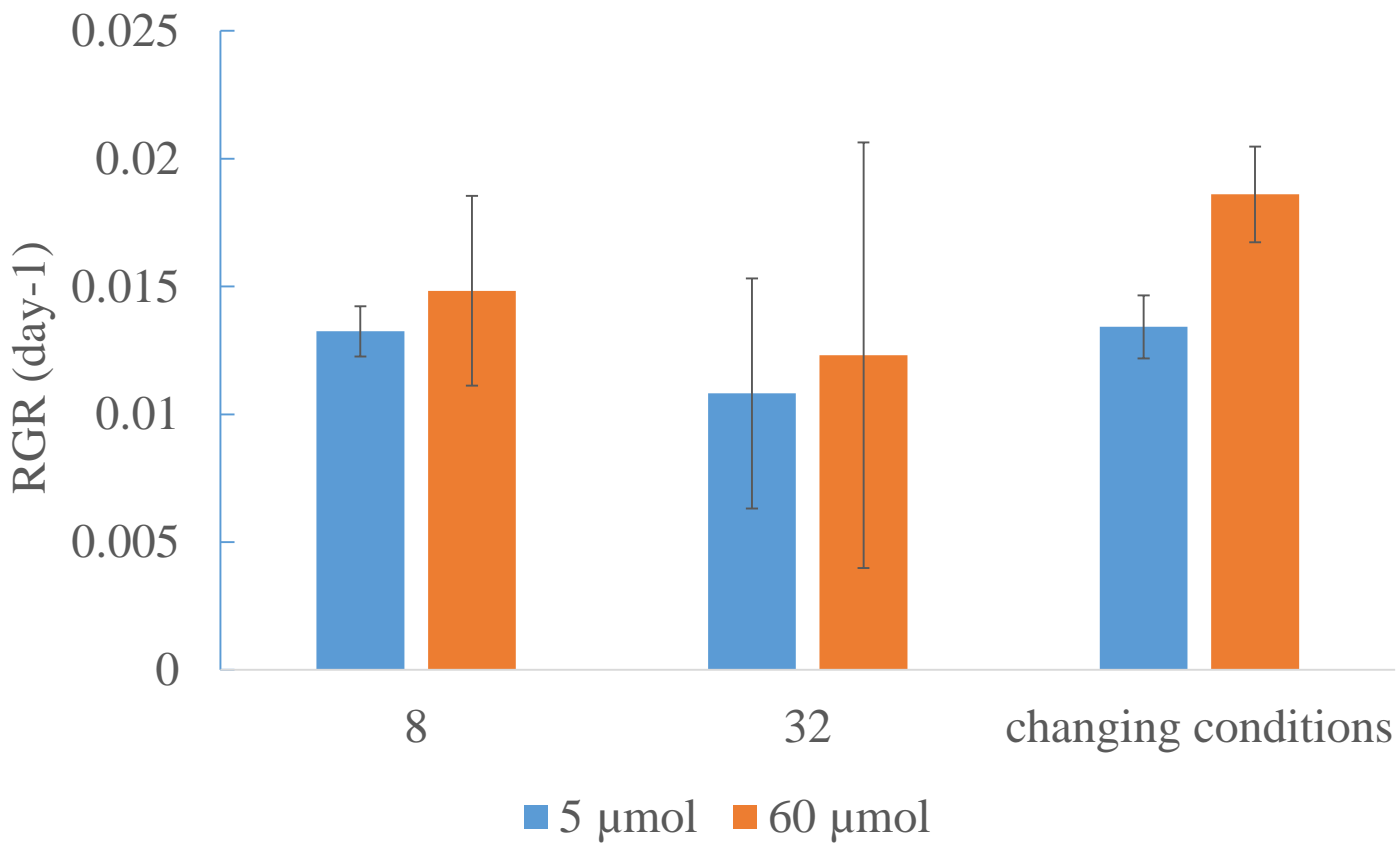


Fig 10. Mean relative growth rate and standard deviation (n=4) of *C. continua* strain.