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Effect of salinity on growth, agar content and gel strength for two agaroid seaweed species: Gracilariopsis longissima and Gracilaria gigas

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ABSTRACT

In Indonesia two species of Rhodophyte macroalgae are commonly cultured in brackish coastal ponds in a range of fluctuating salinity conditions. These are *Gracilariopsis longissima* and *Gracilaria gigas*. To evaluate species performance under the relevant range of salinities, we performed replicate laboratory experiments in which both species were exposed to different salinities ranging from 5 ppt to 30ppt under semi-controlled conditions. Highest growth rates were observed for *G. longissima* at a salinity of 10ppt. while for *G. gigas* similar highest growth rates were observed at 10ppt and 15ppt. However, *G. gigas* growth was less sensitive to small salinity differences at the lower salinity range whereas *G. longissima* performed better and more stable than *G. gigas* at the higher salinity range of 15 – 20 ppt. Apart from a few notable exceptions, *G. longissima* had a generally higher agar content than *G. gigas*, whereas *G. gigas* had a generally higher gel strength than, *G. longissima*. Our results show that neither species was superior in growth and quality criteria than the other species along the whole range of brackish water salinity conditions and farmers need to choose between them depending on their pond conditions and market quality criteria.

Introduction

The red algae order of *Gracillariidae* is globally distributed and mainly cultivated for their hydrocolloid content. Hydrocolloids are biochemical compounds with the gelling characteristics useful in cosmetics and biological research (as growth medium for bacterial plates) and as food additives (Buschmann et al., 2001; Hayashi & Reis, 2012; Sousa et al., 2021). The specific hydrocolloid derived from Gracilariales species is agar and is mostly used as a gelling agent in food (Gioele et al., 2017).

Global production of *Gracillariidae* has increased during the past two decades from 55.5 thousand tonnes up to 5.18 million tonnes and ranks 3rd of the seaweed species most cultivated (FAO, 2022). Indonesia is the second largest producer of seaweeds and produced over 9.6 million tonnes of algae in 2020, representing 27.4 % of the global production of all algae (FAO, 2022). This substantial increase during the past two decades is due to the governmental policies in support of seaweed culture and the stated goal of Indonesia to become a world leader in seaweed production (Naufal et al., 2022). In order to reach this, the Indonesian government has developed a seaweed development roadmap (Rimmer et al., 2021).

Cultivation of *Gracillariidae* is done in multiple ways (Mouedden et al., 2024) such as in the open sea, where they are attached to hanging systems (e.g. rafts) or on the sea floor (Alveal, 1986; Santelices & Ugarte, 1987; Buschmann et al., 1995; Buschmann et al., 2001). Other cultivation methods are land-based such as tank or pond production (Santelices & Doty, 1989; Rejeki et al., 2014). In Indonesia the bulk of the seaweed is grown in ponds, so called '*tambaks*', either in monoculture or in polyculture. These ponds are located near the coast with fluctuating salt and brackish water conditions. The further away these ponds are from the sea, the less predictable and stable the salinity levels may be. Water salinities are furthermore affected by factors such as land subsidence, precipitation and seawater intrusion (Rahmawati et al., 2013). This results in exposure to a broad range of environmental conditions which is known to affect growth and composition in different seaweed species (Haroon et al., 2018). Selection of species to match the various salinities is therefore important.

Pond owners in different regions in central Java cultivate *G. longissima* (formerly known as *Gracilaria verrucosa*; Guiry & Guiry, 2024) and this practice is expanding. The seaweed is grown by

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broadcasting into ponds, also known as the “broadcast” method (Fig. 1a) (Rejeki et al., 2014). After a cultivation period of 35–45 days, the seaweeds are harvested by hand and air dried. This can either be done by spreading them out on the ground, or by placing them on so called ‘*para-para's*’: hanging nets on a bamboo structure placed in the sun (Fig. 1b). The advantage of the para-para's is that the water can drip off, enhancing the drying process. In addition, being kept from the ground keeps the seaweeds clean, and allows a higher product value.

According to farmers, *G. gigas* performs better under lower salinities while *G. longissima* does better under higher salinities, while buyers prefer *G. longissima* based on presumed higher product quality. However, little is known with certainty about the difference between the two species in terms of growth and quality (agar content and gel strength) under differing and variable salinity conditions. The aim of this study was to compare and contrast biomass production and product quality of the two cultured seaweed species (*G. gigas* and *G. longissima*) for a range of salinities as relevant to their pond culture in Indonesia.

Material and methods

Seaweed cuttings (Fig. 2) were collected from Losari, central Java, Indonesia. The seaweed obtained was from starting cultures used by local farmers. At the start of the experiment 100 g of seaweed was placed in separate 36L aquaria at the outdoor facilities of the Laboratory of Marine Sciences at the campus of the University of Diponegoro in Semarang, Indonesia. Artificial seawater with different salinities (5, 10, 15, 20 and 30ppt) was used to fill the aquaria. The different salinities were randomly distributed over the aquaria. The experiment was done in triplicates and ran for 35 days. To ensure the presence of nutrients, F/2 medium was added every second week. The tanks were aerated and a shading cloth was used to protect the seaweeds from excess UV radiation.

Biomass was determined weekly by taking out the seaweed, drying it gently with a paper towel and then weighing it (Resetarits et al., 2024). Temperature (Water Quality Checker YSI® Pro 20; 0.1 °C), pH (EZDO 7200 with ORP sensor) and salinity (digital ATAGO® PAL-06S; 1 ppt accuracy) were measured three times every working day (morning, noon and afternoon). Nitrogen and phosphorus content of the water were measured at the start, after two weeks and at the end of the experiment with the use of a Wavelength Dispersive X-ray Fluorescence (WDXRF) combined with a Rigaku Supermini200 sensor at the Diponegoro University laboratory facilities. After the cultivation period of 35 days, the biomass was sun dried and weighed to determine the dry matter content, agar content and gel strength by the laboratorial facilities of Universitas Diponegoro following Rejeki et al. (2014) and Nauta et al. (2025).

Fig. 2. Habitus picture of the two species. *G. longissima* (**A**), this species is more branched compared to *G. gigas* (**B**), which grows lengthier and has fewer and smaller branches.

Measurements of agar and gel strength are destructive measurements, negatively affecting the course of the experiment. Therefore, only biomass (fresh weight) was measured weekly to follow the growth, while agar and gel strength were solely measured at the end of the experiment to assess the final product which is of relevance to the farmers. The Specific growth rate (SGR) was determined using the formula given below in which d = day, T = number of days of cultivation, Wo = initial wet weight, Wt = wet weight at harvest (Nauta et al., 2025). Statistical analysis was done using GraphPad Prism (V8.2.1). Generic

Fig. 1. Farmer using the broadcast method to stock his pond (**A**). After harvest the seaweeds are placed on a ‘*para-para*’, a structure where nets are placed over a bamboo construction to let them dry (**B**). (Photos: R.W. Nauta).

testing for differences was done using 1-way ANOVA and t-tests were performed to assess the difference between specific treatments and the two tested species.

$$SGR(\% \bullet day^{-1}) = (\ln(Wt) - \ln(Wo))/T \bullet 100$$

Results

Water parameters

The water temperature in the aquaria differed during the day due to the outdoor condition. On average (±SD) it was 29.0 ± 2.4 °C with an observed minimum of 25.1 °C in the morning and a maximum of 39.6 °C in the afternoon. Mean pH levels of the water was 8.82 ± 0.30. Nutrient (nitrate and phosphate) levels were on average respectively 1.68 ± 0.60 mg/L and 0.252 ± 0.098 mg/L (Table 1).

Seaweed growth

In terms of growth, the two species showed both similarities and differences in their response to different salinity levels (Fig. 3). Both species showed a culture preference for water between 5 and 30 ppt, with highest growth rates at salinities intermediate to these extremes. The highest growth was observed for *G. longissima* at a salinity of 10ppt. For *G. gigas* similar highest growth were observed at 10ppt and 15ppt, while at higher salinities, growth rates were notably lower (Fig. 4).

However, while both species showed lowest biomass production at the highest salinity of 30 ppt., for *G. gigas* growth at 20 ppt was equally low as at 30 ppt whereas for *G. longissima* growth at 20 ppt was already better (Fig. 4). While both species showed optimal growth at 10 ppt, *G. gigas* also had optimal (or close to optimal) growth up to 15 ppt, whereafter its growth dropped more rapidly than in the case of *G. longissima*. This meant that *G. gigas* growth was less sensitive to small salinity differences at the lower salinity range whereas *G. longissima* performed better and more stable than *G. gigas* at the higher salinity range of 15 – 20 ppt (even though its optimal growth was also clearly at around 10 ppt) (Fig. 4).

Salinity affected the specific growth rate (SGR) of *G. longissima* negatively with a slope of −0.072 ($R^2 = 0.44$). The SGR of *G. longissima* was the lowest at a salinity 30ppt salinity (Fig. 5). No significant differences were found between the treatments of 5 to 20ppt (one way ANOVA, $F(4,10) = 0.58$, $p > 0.05$). However, the comparison between 10ppt and 30ppt were found to be significant (*t*-test, df = 4, $p < 0.05$).

The effect of salinity on the SGR of *G. gigas* was also negatively correlated, with a slope of −0.096 ($R^2 = 0.376$). For *G. gigas* the SGR was significantly higher at 10 and 15 ppt when compared to the other salinities (*t*-test, df = 4, $p < 0.05$). However, between 5, 20 and 30ppt the SGR of *G. gigas* did not differ significantly.

Comparison of the SGR between the species per salinity (*t*-test) showed that only at 5ppt (df = 4, $p = 0.012$) and 20ppt (df = 4, $p = 0.048$) *G. longissima* had a significant higher SGR.

Agar production

In general, the agar content of *G. longissima* was higher than in *G. gigas*, except at 20ppt (Fig. 6). Agar content at 5ppt only differed significantly with 10ppt for *G. longissima* (*t*-test, df = 4, $p < 0.001$) but

Table 1
Average water conditions in the aquaria during the experiment.

Parameter	Mean ± SD	Min	Max
Temperature (°C)	29.0 ± 2.4	25.1	39.6
pH	8.82 ± 0.30	8.12	9.68
Nitrate (mg/L)	1.68 ± 0.60	0.426	2.762
Phosphate (mg/L)	0.252 ± 0.098	0.070	0.562

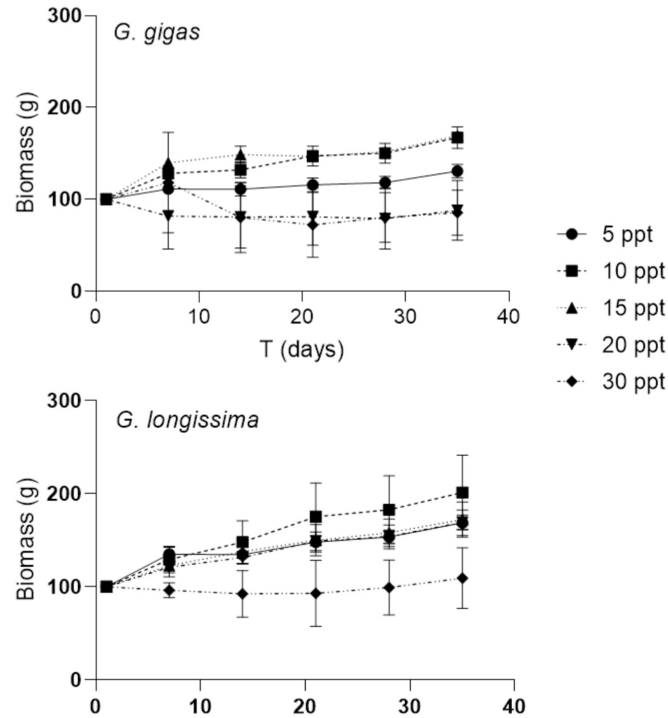

Fig. 3. Growth (± SD) over time of both species per salinity.

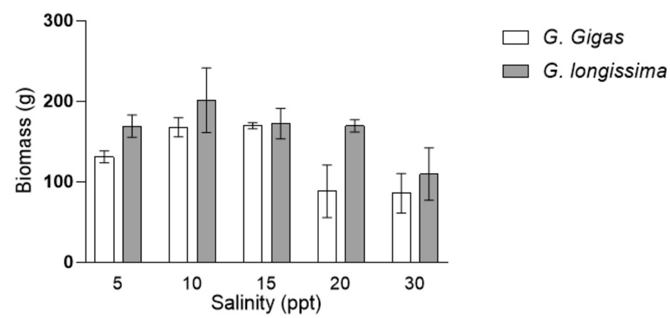

Fig. 4. Mean fresh biomass (± SD) of the two cultivated *Gracillariidae* species over a 35-day period.

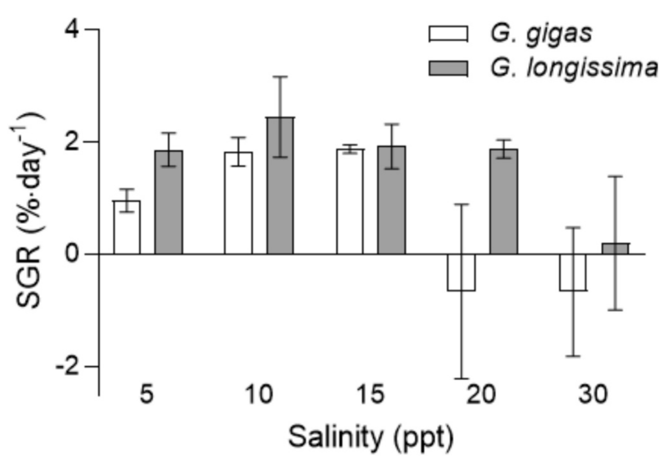

Fig. 5. Specific Growth rates (± SD) of the two seaweed species per salinity.

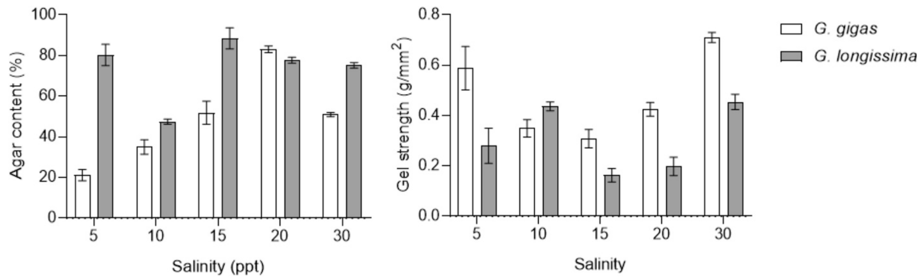

Fig. 6. Agar content and gel strength (quality) of the two tested seaweed species per salinity treatment. Mean values are given ± SD.

not with the other salinities. At 15ppt the highest agar content was measured, significantly higher compared to all other treatments except 5ppt (t-test, df = 4, $p < 0.05$). For *G. gigas* the highest agar content was found at 20ppt, which was significantly higher than all other treatments (t-test, df = 4, $p < 0.001$). Per treatment the two species differed significantly for all salinities (t-test, df = 4, $p < 0.05$). A linear regression analysis for the SGR affecting the agar content showed a weak correlation for *G. longissima* ($R^2 = 0.27$) and a moderate correlation for *G. gigas*, ($R^2 = 0.34$).

Gel strength

Overall, *G. longissima* showed a lower gel strength, except at 10ppt. Comparison between the species per salinity differed for all salinities significantly (t-test, $p < 0.05$). Highest gel strength was found in *G. gigas* at widely divergent salinities 5ppt and 30ppt (Fig. 5), but did not differ significantly from each other (t-test, $p > 0.05$). Lowest values were observed at 10 and 15ppt, both differing significantly with all other salinities for this species.

Discussion

Our results show that *G. gigas* grows best at lower salinities whereas *G. longissima* grew best over a wider range of salinities. Especially when comparing the two species, *G. longissima* outcompeted *G. gigas* at the higher salinities. Production for both species is optimal in relatively moderate to low salinity, but at salinities higher than about 20 ppt, *G. gigas* performs quite poorly in contrast to *G. longissima.* Raikar et al. (2002) found for *Gracilaria* species from Japan, Malaysia and India that they attained their optimum growth at salinities that resembled normal seawater salinity conditions (35ppt). So the species of preference for farmers will differ depending on salinity in ponds and the ability of the farmer to regulate salinity in the ponds. The latter depends on the position of the specific ponds with respect to tides, sea level, rainfall, and flooding (Jarecki & Walkey, 2006).

When agar content is assessed, it is *G. longissima* which appears to give much better overall results than *G. gigas* across the range of salinities. Interestingly, other literature (Daugherty & Bird, 1988) showed lower agar production at lower salinity (17ppt) compared to 25 and 33 ppt for *G. longissima* (there named by the old name *G. verrucosa*), however, Bird (1988) did find the contrary with higher agar content at 17 ‰ compared to 33 ‰. In addition, Marinho-Soriano and Bourret (2003) found a positive correlation of agar yield with salinity.

For gel strength the opposite is observed: in general, *G. gigas* has the highest gel strength except at the joint optimum growth conditions of 10ppt. Examining Fig. 6, it appears that at 10 ppt both species' agar content and gel quality may be inversely related. When one species has a peak in agar content compared to the other species, then the other species has a peak in gel strength and vice versa. Optimizing both agar content and gel strength as inversely-related quality descriptors by selecting between species and culture conditions does not seem possible. However, while gel strength is an indicator for the quality of the seaweed, the (pre)processing of the agar strongly affects the quality of the produced agar (Yarnpakdee et al., 2015). Further (chemical) analysis is therefore advised to assess the quality of the agar in more detail.

As this research was performed under semi-controlled condition, some factors potentially affecting production could not be assessed. With *G. longissima* being a cosmopolitan, it could be that local variation within the species could explain the differences observed, as local varieties can adapt to conditions to which they are exposed to. This could be enhanced by the propagation methodology used by farmers as they use vegetative reproduction as is the case for other hydrocolloid producing seaweeds (Charrier et al., 2017). Therefore, the individuals that thrive best under local conditions (low salinity) will have the advantage and can outcompete individuals which grow less. Another factor that was not assessed for, was the fluctuation of salinity. In the ponds where aquaculture is done in Indonesia, there are often large and unpredictable variations in water quality (Fakhri et al., 2015). Nejrup and Pedersen (2012) showed that temporal variability in salinity levels negatively affected the growth of *Gracilaria vermiculophylla*. Our results do not assess this factor, and it could well be that *G. gigas* could deal with this better, resulting in a higher production in the field.

Conclusion

Where farmers are mainly interested in the production of marketable weight, processors value the quality of the seaweed based on the agar content and the quality of the agar which is commonly expressed as gel strength. This was the initial cause for this research, as there was no information which of the two species (*G. gigas* and *G. longissima*) does indeed perform better on which aspect. Farmers were favourable to *G. gigas* due to the assumed higher growth rate, whilst buyers preferred *G. longissima* because of its assumed higher agar production and quality. Based on our study we can state that *G. gigas* does not over-perform *G. longissima* in growth or agar content and thus the assumptions of the farmers are likely incorrect. In contrast, we do see higher agar content in *G. longissima*, supporting the notion of the buyers. Nonetheless, if gel strength were the parameter to base prices upon, it would be best to cultivate *G. gigas.* However, fluctuations in environmental parameters other than salinity were not extensively assessed and could affect the growth of the two species differently.

CRediT authorship contribution statement

R.W. Nauta: Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **R.W. Ariyati:** Writing – review & editing, Project administration, Methodology, Data curation. **L. L. Widowati:** Writing – review & editing, Methodology, Investigation, Data curation. **A.O. Debrot:** Writing – review & editing, Validation, Data curation. **S. Rejeki:** Writing – review & editing, Supervision, Project administration.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical clearance

No ethical clearance was needed for this experiment as no animals were used.

Competing interests

All authors declare to have no competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

Code availability

Not applicable.

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