Addition of carbon dioxide, followed by irradiance increase, as optimization strategy for the cultivation of the red seaweed Kappaphycus alvarezii

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Abstract
This work aimed to verify the effects of adding CO₂ and irradiance to Kappaphycus alvarezii cultivation. Thalli were cultured in vitro for 2 weeks under low irradiance (50 μmol photons m⁻² s⁻¹) with the addition of CO₂ according to the following concentrations: 142 × 10⁻⁵ ppm (1CO₂, 0.1 L min⁻¹), 285 × 10⁻⁵ ppm (2CO₂, 0.2 L min⁻¹) and 428 × 10⁻⁵ ppm (3CO₂, 0.3 L min⁻¹), followed by culture under higher irradiance (200 μmol photons m⁻² s⁻¹) for 2 more weeks. Control had no CO₂ addition. Afterwards, growth rate, chlorophyll a and carotenoid content were quantified. Samples were submitted to light microscopy and transmission electron microscopy. Growth rates of different treatments or control between experimental periods showed no significant differences, except for 2CO₂ treatment at the end of each period. Kappaphycus alvarezii cultivated with CO₂ addition showed cell wall thickening and increasing quantity of starch granules, chlorophyll a and carotenoids compared with control. Exposed to high irradiance, control samples showed signs of stress, such as changes in chloroplast, starch granules quantity decrease and total chlorophyll a and carotenoids increase. Samples cultivated with CO₂ showed an increase in the quantity and size of the starch granules and an increase in the number of organelles (mitochondria) related to energy generation and cell construction (Golgi complex). Such alterations suggest cellular response after CO₂ addition, such as the formation of starch reserves and cell wall thickening, which could make plants more tolerant to environmental stress during transport from indoor condition to sea farms.

Keywords Aquaculture · Carotenoids · Floridean starch · Inorganic carbon · Transmission electron microscopy

Introduction
Kappaphycus alvarezii (Doty) Doty ex Silva (Rhodophyta, Gigartiniales) and Eucheuma spp. represented the highest production among seaweeds in 2016, reaching 12.2 million t (FAO 2018). Both species are the primary source for carrageenan extraction, a colloid obtained from red seaweed, which is used as a thickener and stabilizer in many industrial activities. However, the world market shows a preference for K. alvarezii over Eucheuma denticulatum for its stronger gel (kappa carrageenan) (Hayashi et al. 2010).

Kappaphycus alvarezii was introduced in Brazil as an alternative to native carrageenan species, which have low farming potential. In 2008, the cultivation of the species started on the coast of Santa Catarina State on an experimental scale. After 3 years, the results showed low temperature during winter to be a limiting factor for seaweed survival and growth (Nunes 2010). However, favourable growth rates were observed during summer and autumn (Hayashi et al. 2011a), highlighting the need to keep seaweeds sheltered during winter so that they would be ready for placement in farms in early spring. Experimental farm units for commercial assignment should be running in Santa Catarina’s coast in the next years, depending on the biomass grown in tanks and indoor conditions. Tank cultivation is an alternative to maintain and increase biomass during the cold months; in such conditions, it is possible to control the physicochemical parameters of the