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Effect of salinity on growth of the green alga *Caulerpa sertularioides* (Bryopsidales, Chlorophyta) under laboratory conditionsEfecto de la salinidad sobre el crecimiento del alga verde *Caulerpa sertularioides* (Bryopsidales, Chlorophyta) en condiciones de laboratorioZuleyma Mosquera-Murillo¹ and Enrique Javier Peña-Salamanca²¹Universidad Tecnológica del Chocó, Facultad de Ciencias Básicas. Carrera 22 No.18 B-10, Quibdó, A. A. 292. Colombia²Universidad del Valle, Departamento de Biología. Calle 13 No.100-00, Cali, A.A. 25360. Colombia

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ABSTRACT

Background. Salinity, temperature, nutrients, and light are considered essential parameters to explain growth and distribution of macroalgal assemblages in coastal zones. **Goals.** In order to evaluate the effect of salinity on the growth properties of *Caulerpa sertularioides*, we conducted this study under laboratory conditions to find out how salinity affects the distribution of this species in coastal tropical environments. **Methods.** Five ranges of salinity were used for the experiments (15, 20, 25, 30, and 35 ppt), simulating *in situ* salinity conditions on the south Pacific Coast of Colombia. The culture was grown in an environmental chamber with controlled temperature and illumination, and a 12:12 photoperiod. The following growth variables were measured weekly: wet biomass, stolon length (cm), number of new fronds and rhizomes. In the experimental cultures, growth (increase in wet biomass and stolon length) was calculated as the relative growth rate (RGR), expressed as a percentage of daily growth. **Results.** Significant differences ($p < 0.001$) were found between 15 ppt and the remaining treatments (20, 25, 30, and 35 ppt). The highest growth rates were recorded at 25 ppt (4.82 % d⁻¹), while at 15 ppt, whitening and progressive deterioration of *C. sertularioides* fragments were observed. These results are consistent with the natural habitat of *C. sertularioides* in Tumaco Bay (Nariño, Colombia), where it is found in areas with average salinities of 24 ppt during its annual cycle. **Conclusions.** The physiological responses obtained in this study will be valuable in exploring possible effects of environmental conditions on the growth and distribution of *Caulerpa* in tropical estuaries.

Key words: Algal growth, *Caulerpa sertularioides*, distribution, indoor culture, salinity.

RESUMEN

Antecedentes. Salinidad, temperatura, nutrientes y luz se consideran parámetros esenciales para explicar el crecimiento y la distribución de las comunidades de macroalgas en la zona costera. **Objetivos.** El objetivo de este estudio fue evaluar el efecto de la salinidad sobre las propiedades de crecimiento de *Caulerpa sertularioides*. **Métodos.** Cinco rangos de salinidad fueron utilizados para los experimentos (15, 20, 25, 30 y 35 ppt), simulando las condiciones de salinidad encontradas en la costa del Pacífico sur de Colombia. El cultivo se realizó en una cámara ambiental con temperatura e iluminación controladas, manteniendo un fotoperiodo 12:12. Se midieron semanalmente variables tales como, biomasa húmeda, longitud estolón (cm), número de hojas nuevas y rizomas. En el laboratorio, el crecimiento se calculó como la tasa de crecimiento relativo (RGR), expresada como porcentaje de crecimiento diario. **Resultados.** Se encontraron diferencias significativas ($p < 0.001$) entre el tratamiento de 15 ppt y los restantes (20, 25, 30 y 35 ppt). Las tasas de crecimiento más altas se registraron a 25 ppt (4.82% d⁻¹), mientras que a 15 ppt se observó blanqueamiento y el deterioro progresivo de los fragmentos de *C. sertularioides*. Estos resultados son consistentes con el hábitat natural de *C. sertularioides* en la Bahía de Tumaco (Nariño, Colombia), donde la especie se encuentra en áreas con salinidades promedio de 24 ppt en su ciclo anual. **Conclusiones.** Las respuestas fisiológicas obtenidas en este estudio son valiosas para explorar posibles efectos de las condiciones ambientales sobre el crecimiento y la distribución de *Caulerpa* en estuarios tropicales.

Palabras clave: *Caulerpa sertularioides*, crecimiento de algas, cultivos in vitro, distribución, salinidad.

INTRODUCTION

Macroalgae of the *Caulerpa* genus (Chlorophyta: Bryopsidales) are of interest in the marine environment for several reasons. The high growth rates that some species exhibit, and their ability to propagate from asexual fragments have generated serious negative impacts on natural communities (Biber & Irlandi, 2006; Glardon *et al.*, 2008; Pérez-Ruzafa *et al.*, 2012). Three species, *C. taxifolia* (M. Vahl) C. Agardh, *C. brachypus* Harvey and *C. racemosa* (Forsskål) J. Agardh, originally found in warm tropical waters grow rapidly and are therefore classified as invasive plants (Klüser *et al.*, 2004; Lapointe & Bedford, 2010). The factors that determine growth have been evaluated for several *Caulerpa* species (Khou *et al.*, 2007; Theil *et al.*, 2007; Burfeind & Udy, 2009; Guo *et al.*, 2015). Salinity and temperature, together with nutrients and light, are considered essential parameters for the further cultivation of seaweed on a large scale (Scrosati, 2001; Lapointe & Bedford, 2010). Furthermore, physiological responses of *Caulerpa* to those abiotic parameters are used to discuss the implications for the management of this green alga for possible invasions (West & West, 2007; Lirman *et al.*, 2014). Little is known, however, about the impact of salinity on the growth of *C. sertularioides* in tropical environments, such as the Colombian Pacific, where oceanographical conditions are constantly changing due to extensive river discharges and high precipitation patterns along the coast (Tejada *et al.*, 2003). Particularly, on the Pacific coast of Colombia, *C. sertularioides* inhabits intertidal and shallow subtidal areas (Peña, 1998; 2008). No seasonality of natural populations was registered; however, great biomass was observed during low rainfall season within creeks along the Bay of Tumaco (Marín & Peña, 2014). Unlike other species within this genus, such as *C. taxifolia* and *C. racemosa*, there are several studies that address their tolerance to different environmental conditions and their conditions as invasive plants (Piazzi *et al.*, 2001; Lirman *et al.*, 2014). Osmotic acclimatization in response to changes in salinity is a fundamental tolerance mechanism that conserves the stability of the intracellular medium and is therefore essential to maintain an efficient functional state in the cells (Kirst, 1990; Peña *et al.*, 1999; Ospina *et al.*, 2006). It has been suggested that algae can regulate their cell volume by modifying the internal water potential in response to changes in salinity (Goulard *et al.*, 2001; Eggert *et al.*, 2007). Although most marine algae can tolerate fluctuations in salinity over the short term, large variations of this parameter can significantly affect some biochemical processes involved in photosynthesis and growth, altering the biomass, distribution, and productivity of a great number of species (Sousa *et al.*, 2007; Choi *et al.*, 2006; Theil *et al.*, 2007). Those results demonstrated the plasticity and adaptation of *Caulerpa* species to different salinity gradients compared to other siphonous algae and, therefore, their capacity to spread out in shallow coastal environments.

The aim of this study is to evaluate growth conditions under different salinity conditions of the green alga *C. sertularioides* and its effect on the distribution and colonization of the species in the estuary.

MATERIALS AND METHODS

Culture conditions and experimental design. Fragments of *C. sertularioides* were brought from Tumaco Bay, Pacific coast of Colombia (1° 45' - 2° 00' N; 78° 30' - 78° 45' W). The bay comprises a 350 km² area with depths varying between 0 and 50 m (Tejada, 2002). Algal fragments were collected from the intertidal zone, during low tide and stored in paper towels moistened with seawater, packed in polyethylene

bags and stored in a polystyrene icebox until transportation to the laboratory, according to West and Calumpong (1988). Fragments were cleaned of other benthic materials (sand, shell fragments, etc.) and kept in an environmental chamber with average temperatures of 27 ± 0.37 °C and controlled illumination ($40\text{--}50 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). A 12:12 cycle of light/darkness was maintained during two (2) weeks for their acclimation. Material for subsequent experiments was then selected from these fragments. Fragments of *C. sertularioides* consisted of a basal portion (stolon), rhizome and 3-4 erect axes (fronds); fresh weight was between 0.2 and 2.0 g and was calculated at the beginning of the experiments (West & West, 2007). Five ranges of salinity were used for the experiments (15, 20, 25, 30, and 35 ppt). These salinities were chosen after examination of historical data of salinity recorded for Tumaco Bay by the oceanographical and Hydrological Research Center of the Colombian Army at the Pacific coast, located in the Port of Tumaco (Tejada *et al.*, 2003). Six replicates of each salinity range were set up and the experiment was repeated twice, with a culture period of 4 (four) weeks each time, and measurements taken every 8 days. Mini-aquaria of 0.5 L capacity were filled with artificially filtered seawater enriched with Provasoli, stirred with aerators (modified by West & McBride, 1999) (10 ml L^{-1}), and changed weekly.

A one-way ANOVA (factor = salinity) was done to test for significant differences (5%) in total new growth. Data were tested for homogeneity of variances using Cochran's test. Where significant differences were found, Tukey's HSD test was used for means comparison. The R statistical package version 2.12.0 and SPSS 17.0 were used for the analyses.

Analysis of algal growth. The following growth variables were measured weekly: wet biomass, stolon length (cm), and number of new fronds and rhizomes. In the experimental cultures, growth (increase in wet biomass and stolon length) was calculated as the relative growth rate (RGR), expressed as percent daily growth, applying the following equation used by different authors (Arecas, 1995; Anderson *et al.*, 1997; Marinho-Soriano *et al.*, 2002): $\text{RGR} = [(W_f/W_i)^{1/t} - 1] \times 100$, where RGR = relative growth rate, W_f = final wet weight, final length, W_i = initial wet weight, initial length; t = time interval elapsed between the two observations.

RESULTS

Salinity had a significant effect on the growth of *C. sertularioides* ($p < 0.001$; Table 1; Figs 1a- b) during the culture period. The highest growth rates in terms of wet biomass during the culture period occurred for salinities of 25 and 30 ppt, with mean values of $2.262 \pm 0.242 \text{ \% d}^{-1}$ and $1.408 \pm 0.215 \text{ \% d}^{-1}$, respectively (mean \pm SD, $n = 18$), that remained constant during the experiment. The lowest growth rates were recorded for salinities of 15 and 35 ppt, which had growth rates of around 1 \% d^{-1} and lower. At a salinity of 15 ppt, growth rates were negative as a result of the progressive deterioration of the material at this salinity concentration (Fig. 1a).

The fragments of *C. sertularioides* cultivated at different salinity concentrations produced growth of the existing stolons and new stolons, except at 15 ppt salinity (Fig. 1b). The highest stolon growth rates were observed at salinity concentrations of 25 and 30 ppt, with average values of $2.638 \pm 0.712 \text{ \% d}^{-1}$ and $3.177 \pm 1.305 \text{ \% d}^{-1}$, respectively (mean \pm SD, $n = 18$). The lowest growth rates were observed at 15 ppt salinity ($1.616 \pm 0.760 \text{ \% d}^{-1}$).

Table 1. ANOVA results of differences in RGR (% d⁻¹) of *C. sertularioides* fragments under different salinity ranges under laboratory conditions.

	Biomass				
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Salinity	4	176.8	44.20	15.54	1.89x10 ⁻⁹
Time	1	0.03	0.03	0.011	0.9141
Salinity/Time	4	2.37	0.59	0.209	0.9327
Residuals	80	227.4	2.84		
	Stolon length				
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Salinity	4	136.1	34.04	6.34	0.000175***
Time	1	0.31	0.30	0.05	0.8114
Salinity/Time	4	22.3	5.58	1.04	0.3917
Residuals	80	429.5	5.36		

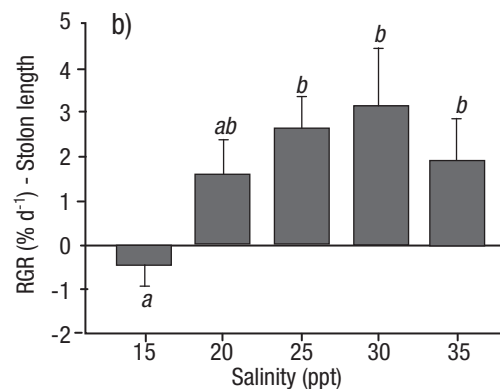
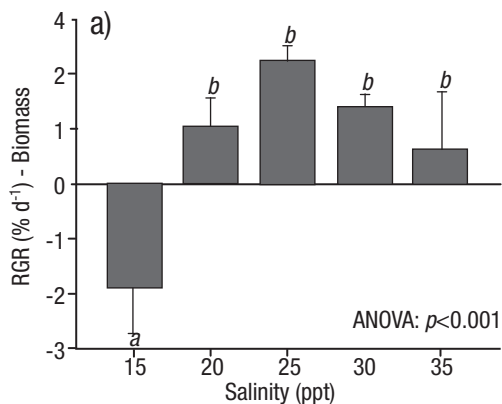
Significant codes (***), (**) = 0.001, (*) = 0.01, (.) = 0.05. Significant difference = 0.01.

The appearance of new fronds and rhizomes was observed at all evaluated salinities, excepting 15 ppt salinity. The highest number of new fronds (mean = 2.111 ± 0.787) and rhizomes (mean = 5.111 ± 1.109) was obtained at 25 ppt. The lowest number of new fronds (mean = 1.111 ± 0.192) and rhizomes (mean = 1.167 ± 0.500) was obtained at 35 ppt.

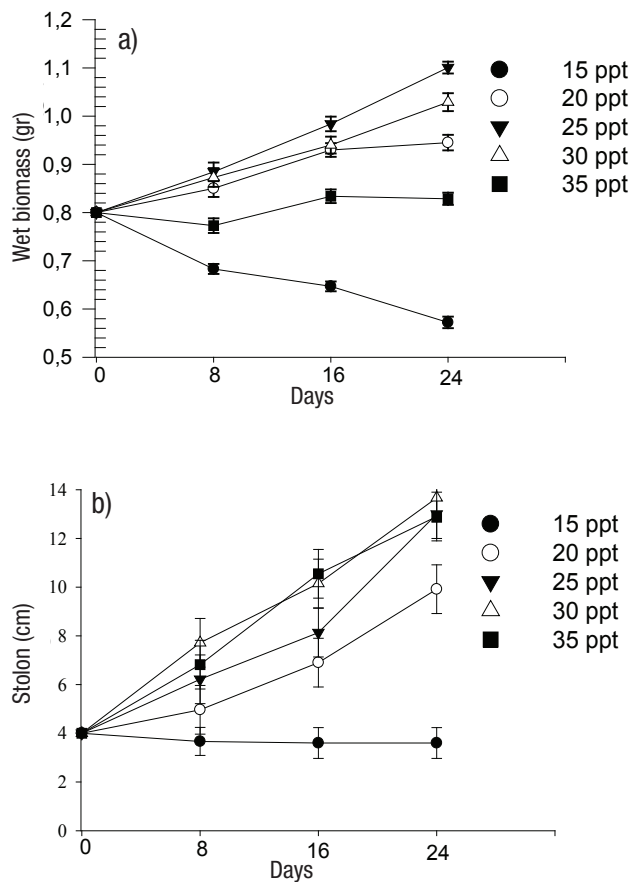
The daily growth rate, determined at three time intervals (Fig. 2), showed that, from the first week of culture, the relative growth rate of *C. sertularioides* varied under the different salinity concentrations evaluated. This behavior was constant during the culture period and was more obvious at salinities of 25 ppt and 30 ppt than at 15 and 35 ppt. During the 24 days of culture, growth rates of 4.82% d⁻¹ and 3.02% d⁻¹ were recorded at salinities of 25 and 30 ppt, respectively. At salinities of 20 and 35 ppt, growth rates of only 2.8% d⁻¹ and 2.6% d⁻¹ were observed.

DISCUSSION

According to these experimental results, *C. sertularioides* growth was significantly affected by changes in salinity. Growth increased with salinities over 20 and up to 30 ppt; the best growth response was obtained at 25 ppt. Variations in salinity can significantly affect the growth, distribution, and productivity of macroalgae (Chesnes & Montague, 2001). These results are consistent with the natural habitat of *C. sertularioides* in Tumaco Bay (Nariño, Colombia), where it is found in areas with average salinities of 24 ppt during its annual cycle (Fig. 3). Indeed, higher biomass of *C. sertularioides* showed a direct relationship with seasonal variation of salinity in the study area (Marín & Peña, 2014). Salinity is clearly one of the key variables influencing abundance and distribution of macroalgal meadows in shallow coastal environments, and it is the factor most easily manipulated through management decisions (Biber & Irlandi, 2006; Pérez-Ruzafa *et al.*, 2012; Lirman *et al.*, 2014).



Figures 1a-b. Effect of salinity on Relative Growth Rate (% d⁻¹) of *C. sertularioides*. **a)** Biomass. **b)** Stolon length. Means and standard deviations are shown ($n = 18$). Different letters represent significant differences between salinities as shown by Tukey's test, $p < 0.05$.



Figures 2a-b. Growth of *C. sertularioides* at different salinities (ppt). a) Increase in wet biomass (g). b) Increase in stolon length (cm). Error bars show SD ($n = 18$).

Results of this study showed a progressive deterioration of *C. sertularioides* fragments at 15 ppt of salinity, demonstrating a negative growth rate in terms of biomass and stolon growth (Mosquera-Murillo, 2012). Other species of the *Caulerpa* genus, such as *C. taxifolia* (West & West, 2007) and *C. lentillifera* J. Agardh (Guo *et al.*, 2015), exhibit reduced chlorophyll content and decreased Fv/Fm values, which could be the result of a disorganization of the cellular structure and chloroplasts in turgid cells. The rapid growth of *C. sertularioides* at salinities above 20 ppt observed in this study, as well as its deterioration at lower salinities, has also been reported for other species in this genus, such as *C. taxifolia* (Biber & Irlandi, 2006; West & West, 2007; Theil *et al.*, 2007).

The fragments of *C. sertularioides* grown at higher salinity concentrations (35 ppt) also showed low growth rates ($0.618 \pm 1.047 \% d^{-1}$), but maintained normal coloration. According to Kirst (1990), growth can be reduced near the salinity tolerance level in order to maintain osmotic regulation, which can guarantee survival. The reduction in growth can also be a consequence of the cumulative effect of enzymes and the reduction of turgidity pressure that inhibits cellular division (Lee & Liu, 1999; Liu *et al.*, 2000; Kamer & Fong, 2000). West and West (2007) reported optimal growth rates of *C. taxifolia* at salinities between 22.5 and 30 ppt, with null growth at lower salinities. (Liu & Phang, 2010). Increases and decreases in salinity generate stress in macroalgae, and species that are tolerant to these conditions present different strategies for growth (Liu *et al.* 2000; Ospina *et al.*, 2006; Choi *et al.*, 2010; Guo *et al.*, 2015).

These initial laboratory experiments demonstrated the effect of salinity changes on growth of *C. sertularioides*, and suggest that a range of experiments investigating other environmental factors, such as temperature, light, and nutrient conditions, would be beneficial in understanding the distribution of this species in the region.

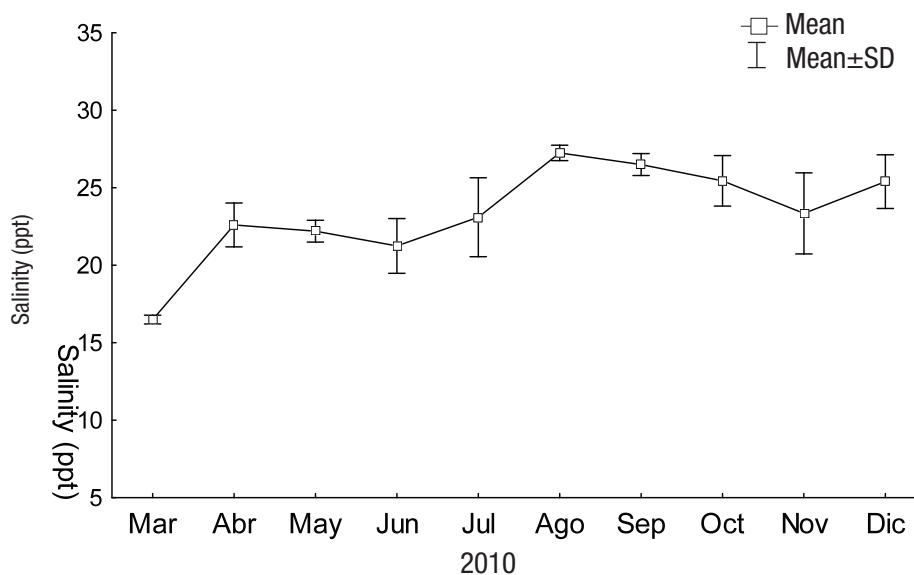


Figure 3. Salinity values recorded at sampling locations in Tumaco Bay (Nariño, Colombia), during 2010 (Marin & Peña, 2014).

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