

1 **Tolerance response of *Lessonia flavicans* from the sub-Antarctic ecoregion of**
2 **Magallanes under controlled environmental conditions**

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25 **Abstract**

26 Environmental heterogeneity plays a key role in spatio-temporal distribution of organisms,
27 their ecology and their evolutionary biology, with their physiological response, or tolerance
28 to the environment defining their distributional range. The macroalgae of the sub-Antarctic
29 ecoregion of Magallanes are subject to a wide range of environments, resulting from
30 geomorphological processes (glacial erosion in the Quaternary), oceanographic gradients,
31 and drastic seasonal variations of photoperiod and irradiance (winter <8 hours of light,
32 summer >17 h). We examined the tolerance response of *Lessonia flavicans* to contrasting
33 environments (three salinities, two temperatures and two photoperiods) under controlled
34 laboratory conditions. Our results suggest that *L. flavicans* has limited salinity tolerance
35 that is affected by temperature and photoperiod. Summer temperature ($9^{\circ}\text{C} \pm 0.02$) and
36 photoperiod (18:6 h L:D) and salinity 32 seem optimal conditions for *L. flavicans*
37 sporophyte development. Results of the present study provide key information for culturing
38 a species of high economic and biological value, and could aid in predicting the species
39 potential tolerance response to environmental fluctuations in the wake of global changes.

40

41 **Keywords:** Environmental heterogeneity, photoperiod, salinity, temperature, Sub-Antarctic
42 macroalgae

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44 **Introduction**

45 Understanding the factors that limit geographical distribution of species has been essential
46 for both ecology and evolutionary biology (Sexton et al. 2009). Biotic and abiotic factors
47 might interact synergistically, and physiological response can restrict the range of
48 distribution and abundance of organisms (Bozinovic et al. 2011). There is a need for a

49 better understanding of how environmental factors over time and space may affect the
50 fitness, growth, survival and reproduction of species (Sexton et al. 2009; Kearny and Porter
51 2009).

52 In high-latitude ecosystems abiotic parameters such as temperature, salinity, and
53 photoperiod are considered key variables affecting the survival, reproduction, and
54 development of macroalgae species (Mansilla et al. 2004; Rambov et al. 2012) and
55 structuring their geographical distribution (Wiencke and Dieck 1990). Among these
56 variables, temperature is one of the most important factors that condition geographical
57 distribution of macroalgae (Kirst and Wiencke 1995). Temperature affects growth rates,
58 reproductive periods, and survival rates (van de Hoek 1982; Breeman 1988). Photoperiod
59 can also limit the development of macroalgae, as light and dark cycles are responsible for
60 the onset of the different phases of the life cycles (Hay 1981; Gaines and Lubchenco 1982;
61 Dhargalkar 2004) and metabolic processes (Gómez et al. 1998) of many species.

62 The macroalgae present in the channels and fjords of the southernmost waters of
63 Chile (from 41°S to 56°S) are subject to diverse and often harsh environmental conditions
64 that result in differences in composition, richness and community structure as compared to
65 other temperate coasts in the Americas (Ojeda 2013). Glacial erosion due to the back and
66 forth movement of ice during the Quaternary drove the geomorphology of these fjords that
67 were proglacial lakes during the Last Glacial Maximum (Kilian et al. 2007). Large
68 differences of current velocity, temperature, salinity and wave exposure within fjords and
69 channels build strong oceanographic gradients (Silva and Calvete 2002) in a region with an
70 abrupt coastal geomorphology and high local variability of fresh water inputs and
71 substratum types (Valdenegro and Silva 2003). This wide environmental heterogeneity
72 probably plays a fundamental role in the spatial distribution of macroalgae. Another

73 important peculiarity of this ecoregion is the drastic seasonal variations of photoperiod and
74 irradiance. In winter, photoperiod reaches a minimum of 6 hours of light and irradiance of
75 23.2 Wm^{-2} , but in the summer the photoperiod reaches a maximum of 18 hours of light and
76 an irradiance of 164.7 Wm^{-2} (Santana et al. 2010; Ojeda 2013).

77 One of the most characteristic macroalgae groups of the sub-Antarctic ecoregion of
78 Magallanes, Chile (48°S to 56°S ; Rozzi et al. 2006) are those of the family Lessoniaceae
79 (Searles 1978). The genus *Lessonia* Bory 1825 (based on *L. flavicans*) is native to the
80 Falkland Islands or Islas Malvinas (Searles 1978) and includes a group of large tree-like
81 brown algae that live in the lower intertidal and shallow subtidal zones (up to 20 m deep) of
82 rocky shores (Martin and Zuccarello 2012). *Lessonia* spp have major ecological roles in the
83 structuring of benthic marine communities (Villouta and Santelices 1984; Vasquez and
84 Santelices 1984) and are commercially exploited for the extraction of alginic acid (Steneck
85 et al. 2002).

86 The ecophysiology of *Lessonia* species in the sub-Antarctic ecoregion of
87 Magallanes is to a large extent unknown. The available information about this genus in the
88 Pacific coast of South America is based on studies of *L. nigrescens* Bory de Saint-Vincent
89 and *L. trabeculata* Villouta and Santelices in northern Chile (Hoffmann et al. 1984;
90 Santelices and Ojeda 1984; Ávila et al. 1985). Research on phycocolloids of *Lessonia*
91 species from the sub-Antarctic region of Chile (Chandía et al. 2005; Chandía and Matsuhira
92 2008) show that some species of this brown macroalgae have important carbohydrate
93 compounds with specific properties that make them highly interesting for biotechnology
94 applications, in terms of productivity and nutritional value (Mansilla et al. 2012a; Astorga
95 and Mansilla 2013).

96 Given the ecological importance and economical potential value that *Lessonia* spp
97 might have in the near future, the study of their biology and ecophysiology is essential to
98 guide cultivation efforts for these species under controlled laboratory and hatchery
99 conditions. Additionally, the region's vulnerability to environmental changes (Mansilla et
100 al. 2012b) is pressing for the study of the tolerance of *Lessonia* spp to them. The objective
101 of this study was to evaluate the tolerance of sub-Antarctic *L. flavicans* to extreme salinity,
102 temperature and photoperiod conditions characteristic of the sub-Antarctic Magellanic eco-
103 region.

104

105 **Materials and methods**

106 Fertile *L. flavicans* foliage was collected from the rocky subtidal areas of the Magallanes's
107 sub-Antarctic ecoregion in June 2012. The foliage was transported in polyethylene bags
108 with sea water to the University of Magallanes's Laboratorio de Macroalgas Antárticas y
109 Subantárticas (LMAS). The blades with sori were treated beforehand using cold freshwater
110 to eliminate epiphytes according to Ávila et al. (2010) recommendations for the cultivation
111 of brown algae. The fertile sections of foliage were carefully rinsed two times with
112 pasteurized (2 hours at 70 °C, see Little et al. 1980) sea water, cut off with razor blades and
113 placed in glass slides inside Petri dishes with pasteurized, 0.45 µm-filtered sea water, and
114 kept in semi-darkness ($5 \mu\text{E m}^{-2} \text{s}^{-1}$ and at 8°C) for 24 hours in order to release the spores
115 (Ávila et al. 1985). Once spores were released, the water with foliage fragments was
116 disposed of. The microscopic phase of *L. flavicans* was kept in a plant growth chamber at 9
117 $\pm 1^\circ\text{C}$, photoperiod 12:12 h (Light:Darkness, L:D; Ávila et al. 1985), irradiance of $10 \mu\text{E m}^{-2}$
118 s^{-1} and Provasoli as growth medium (Ávila et al. 2010) that was renewed every 7 days.
119 The growth of the microscopic phase of *L. flavicans* was measured weekly in photographic

120 records acquired with a digital camera Moticam 2000 attached to an OLYMPUS CX31
121 microscope.

122

123 *Tolerance Experiment:* We performed a set of four consecutive experiments with young
124 sporophytes (diameter, 10–16 mm; fresh weight, 10–20 mg) to evaluate the effect of
125 salinity, temperature, and photoperiod on the growth of *L. flavicans* plantings. We used
126 young sporophytes to avoid the artifacts involved in the fragmentation of larger thalli and
127 the consequent disruption of physiological processes. Further, sporophytes might be also
128 the plant units to use in future culturing of the species. The sporophytes were grown in
129 Provasoli growth medium inside an incubation chamber (BioRef 19L) with temperature and
130 photoperiod control. Considering the salinity variations present in the ecoregion's natural
131 environment (Valdenegro and Silva 2003; Kilian et al. 2007; Ríos et al. 2007), three
132 salinity levels (14, 23 and 32) were evaluated in each of the four experiments. The salinity
133 levels were obtained following Yokoya et al. (1999) protocol, and confirmed with an
134 ATAGO S/Mill refractometer. The water was filtered through 0.45 μm and then pasteurized
135 to reduce the number of micro-organisms. Five replicated 500-ml glass jars containing two
136 young *L. flavicans* sporophytes were set at each of the three salinity levels. A different
137 combination of temperature and photoperiod levels was used in each of the four
138 experiments. These levels corresponded to maximum and minimum temperature and
139 photoperiod values registered in shallow benthic ecosystems of the sub-Antarctic ecoregion
140 of Magallanes (Ríos et al. 2007; Ojeda 2013): 9°C and 5°C, and 18:6 h L:D and 6:18 h L:D,
141 respectively. All the experiments were performed using an irradiance of $70 \pm 2 \mu\text{E m}^{-2} \text{s}^{-1}$,
142 an illumination level considered optimum for cultivation of sporophytes of *L. nigrescens*
143 (Ávila et al. 1985).

144 The cultures were kept for 25 days and every 4 days the growth medium was
145 changed and the sporophyte biomass (fresh weight) inside each jar was measured. The
146 biomasses of the two sporophytes inside each jar were summed thus obtaining five
147 independent replicas for each salinity level. Prior to weighing the excess of water from the
148 sporophytes was removed using absorbent paper, and the fresh weight was assessed using
149 an analytical RADWAG AS 220/C/2 scale

150

151 *Data analysis:* Repeated-measures ANOVA was performed to evaluate the effect of salinity
152 on the biomass of *L. flavicans* sporophytes during the cultivation period in each of the four
153 combinations of temperature and phoperiod tested. Biomass (fresh weight) of sporophytes
154 inside each culture jar and date of measurement was standardized by the initial biomass.
155 The sphericity assumption was checked using the Mauchley test and, if violated, the
156 Greenhouse-Geisser and Huynh-Feldt adjusted probabilities are provided. All data analysis
157 was performed with the STATISTICA 7.1 software, using a 95% confidence rate ($p < 0.05$).

158

159 **Results**

160 *Development of gametophytes in laboratory:* Spores of *L. flavicans* settled to their growth
161 substratum, and during the first 48 hours, formation of the germination tube was observed
162 (Fig. 1a, b). The differentiation of male and female gametophytes started on day 11 of
163 cultivation (Fig. 1c) and gametophytes with an average diameter of $45 \pm 10 \mu\text{m}$ were
164 observed on day 15 (Fig. 1d). At approximately 18 days of culture, fertilization of the
165 female gametophytes was observed (Fig. 1e). Rhizoids were observed and the longitudinal
166 and periclinal divisions commenced on day 24 of culture. A polystromatic layer with a

167 prolonged morphology was differentiated, which corresponds to a young *L. flavicans*
168 sporophyte (Fig. 1f).

169 *Effects of salinity on sporophyte biomass:* Salinity of 14 led to a reduction of the biomass
170 of *L. flavicans* sporophytes in the four combinations of temperature and photoperiod tested
171 (Fig. 2, Table 1). Sporophyte biomass was reduced to zero after 17 days (at 5 °C) and 13
172 days (at 9 °C) of culture in the 6:18 h L:D photoperiod, and after 25 days of culture at 9 °C
173 in the 18:6 h L:D photoperiod. However, the reduction of sporophyte biomass was minimal
174 at 5 °C and 18:6 h L:D photoperiod.

175 The biomass of *L. flavicans* sporophytes cultured at 5 °C and photoperiod of 6:18 h
176 L:D was reduced to half of the initial value at salinity of 23 but did not show a net change
177 at a salinity of 32 (Fig. 2a). When temperature was set to 9 °C, the reduction of sporophyte
178 biomass in this photoperiod was higher because it was zero after 13 days of culture at
179 salinity of 23 and 40% lower than initial values at salinity of 32 (Fig. 2b).

180 Sporophytes cultured at a temperature of 5 °C and a 18:6 h L:D photoperiod
181 increased biomass by more than 50 % at salinity 23 and triplicated it at salinity of 32 (Fig.
182 2c). The increase of the culturing temperature to 9 °C led also to an increase of sporophyte
183 biomass at salinity 23 and particularly at salinity of 32 where sporophyte biomass at the end
184 of the experiment was six times more than that at the beginning of it (Fig. 2d).

185

186 **Discussion**

187 Development characteristics of *Lessonia flavicans* microscopic phases meet those described
188 for the order Laminariales (Kain 1979; Lüning 1980; Ávila et al. 1985). Some differences
189 between *L. flavicans* and the congeneric *L. nigrescens* were observed because the first
190 young sporophytes of *L. flavicans* were obtained after 24 days of culture, whereas in *L.*

191 *nigrescens* this occurs on day 18 (Ávila et al. 1985). The presence of species-specific
192 differences highlights the importance of identifying the optimal cultivation conditions for
193 gametophytes and sporophytes of *L. flavicans* and other species if culture protocols for
194 Laminariales of sub-Antarctic regions are to be provided. Additionally, knowledge of the
195 optimal growth conditions allows for a better understanding of adaptive processes, survival
196 and development, reproductive cycles and spatial distribution of the species (Hoffmann and
197 Santelices 1982; Hoffmann et al. 1984; Yokoya et al. 1999). For example, recent studies
198 suggests different tolerance to temperature among two cryptic species of the genus
199 *Lessonia*, where gametophytes of *L. berteriana* Montagne (northern Chile distribution) are
200 more tolerant to higher temperatures than gametophytes of *L. spicata* (Suhr) Santelices
201 (southern Chile distribution; Oppliger et al. 2012).

202 Net increase of *Lessonia flavicans* biomass occurred only in sporophytes cultured in
203 the photoperiod characteristic of summer (18:6 h L:D) whereas biomass gain was none or
204 minimal in sporophytes cultured in the winter photoperiod (6:18 h, L:D). The combination
205 of the photoperiod and temperature characteristic of summer (9 °C) lead to the highest
206 increase of sporophyte biomass but this temperature did not promote biomass increase of
207 sporophytes cultured in the winter photoperiod. The biomass gain of sporophytes cultured
208 at 5 °C and the 18:6 L:D photoperiod was lower than that of sporophytes cultured at 9 °C
209 and same photoperiod. Hence, the length of day seems to be a primary environmental
210 condition to consider when culturing *L. flavicans* sporophytes followed by temperature.
211 This seems not to be the case for the sporophytes of *L. nigrescens* that show maximum
212 growth at 10 °C independently of photoperiod (Ávila et al. 1985; Martínez 1999). The
213 gametophytic and sporophytic phases of *Lessonia* populations at high latitudes have lower

214 tolerance to high temperatures than populations at low latitudes (Martínez 1999; Oppliger
215 et al. 2012).

216 Field experiments with *Macrocystis pyrifera* (Linnaeus) C. Agardh performed in
217 Southern California have shown that temperature and irradiance are key environmental
218 conditions for sporophyte development because at very high temperatures or very low
219 irradiance their growth was inhibited (Dean and Jacobsen 1984). Other species of "kelp"
220 such as *Pterygophora californica* and *Eisenia arborea* present in California have a different
221 behavior compared to *M. pyrifera*, since the sporophytes of those species can tolerate high
222 temperatures (Matson and Edwards 2007). Additionally, while *E. arborea* can produce
223 embryonic sporophytes at high temperatures, *P. californica* cannot (Matson and Edwards
224 2007). Likewise, recent studies show that seasonal changes of temperature and light had an
225 effect on the early survival and growth of *Ecklonia radiata* (C. Agardh) J Agardh.
226 Gametophyte growth rates of this species were positively related to day length, with the
227 fastest growing zoospores released when the days were long. Survival was greatest in the
228 coolest temperature, while optimum growth was achieved in higher temperatures (Mohring
229 et al. 2013).

230 Assessing the influence of environmental conditions on the development of kelps
231 seems to be a complex task considering the high variability introduced by species-specific
232 and stage of life cycle effects, the possible seasonality of the responses and the effects of
233 adaptation to local environmental conditions. Our results provide evidence of photoperiod
234 and temperature effects on *L. flavicans* sporophyte growth, although only winter and
235 summer minimum and maximum temperatures were tested. Thus, we consider essential to
236 do further evaluations of the effects of a wider range of photoperiods and temperatures on
237 the development of the gametophytic and sporophytic phases of *L. flavicans*.

238 Salinity seemed also a determining factor of *Lessonia flavicans* sporophyte growth,
239 with salinities of 23 and 14 leading to a reduction of biomass gain, or even resulting fatal to
240 sporophytes. Salinity of 32, typical of Magellanic channels with low freshwater influence,
241 was conducive to the best growth. In this regard, most of the reports about the presence of
242 *L. flavicans* within the sub-Antarctic ecoregion of Magallanes correspond to areas with
243 salinity between 30.7 and 32.2 (Table 2). Hence, salinity might be another crucial variable
244 determining the spatial distribution of the species. In contrast, young sporophytes of the
245 kelp *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl and G.W. Saunders can
246 tolerate very low levels of salinity (6 PSU; Peteiro and Sanchez 2012). The gametophytic
247 and sporophytic phases of *Macrocystis pyrifera* in the sub-Antarctic ecoregion of
248 Magallanes have a wider range of tolerance to salinity than those distributed on the
249 northern Chilean coastline (Buschmann et al. 2004). This wide salinity tolerance allows *M.*
250 *pyrifera* to live in very heterogeneous habitats in the region (Mansilla et al. 2009). Salinity
251 is known to affect the development of several macroalgal species, for example in some
252 species of kelps as *Alaria esculenta* (L.) Greville, the microscopic stages were shown to be
253 more sensitive to salinity changes than the macroscopic stages (Fredersdorf et al. 2009).
254 Likewise, in other species of macroalgae, low salinities inhibited the formation of erect axis
255 and the carposporeling developed only a basal disc in *Hydropuntia caudata* (J. Agardh)
256 Gurgel and Fredericq, (Miranda et al. 2012).

257 In conclusion, our results suggest that *Lessonia flavicans* has a limited tolerance
258 range for salinity, which seems closely linked to salinity of its natural habitats in the sub-
259 Antarctic ecoregion of Magallanes. In addition to salinity, photoperiod and temperature
260 have also an effect on the growth of the sporophytic phase of *L. flavicans*. Summer
261 temperature and photoperiod and a salinity of 32 seem to provide optimal conditions for *L.*

262 *flavicans* sporophyte development. This is key information for culturing of a species of
263 high commercial value. Further, these results could aid in predicting the species potential
264 tolerance response to imminent environmental fluctuations in the wake of global changes.

265

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424 **Figure and Table Legends**

425 Figure 1. Initial development stages (microscopic) of *Lessonia flavicans* under laboratory
426 conditions: i) spores; ii) spores with a developed germination tube; iii) appearance of male
427 and female gametophytes; iv) mature male and female gametophytes; v) mature female
428 sporophyte; vi) young sporophyte.

429 Figure 2. Standardized average fresh biomass (relative to initial value) of *L. flavicans*
430 sporophytes subjected to four combinations of temperature and photoperiod and three
431 salinities during 25 days of culture. Circles represent salinity: 14 PSU, Squares: 23 PSU,
432 Stars: 32 PSU. Error bars represent ± 1 standard error (n = 5).

433 Table 1. Repeated-measures (ANOVA) analysis, evaluating the effect of salinity, on the
434 biomass gain of sporophytes of *L. flavicans* under four combinations of temperature and
435 photoperiod.

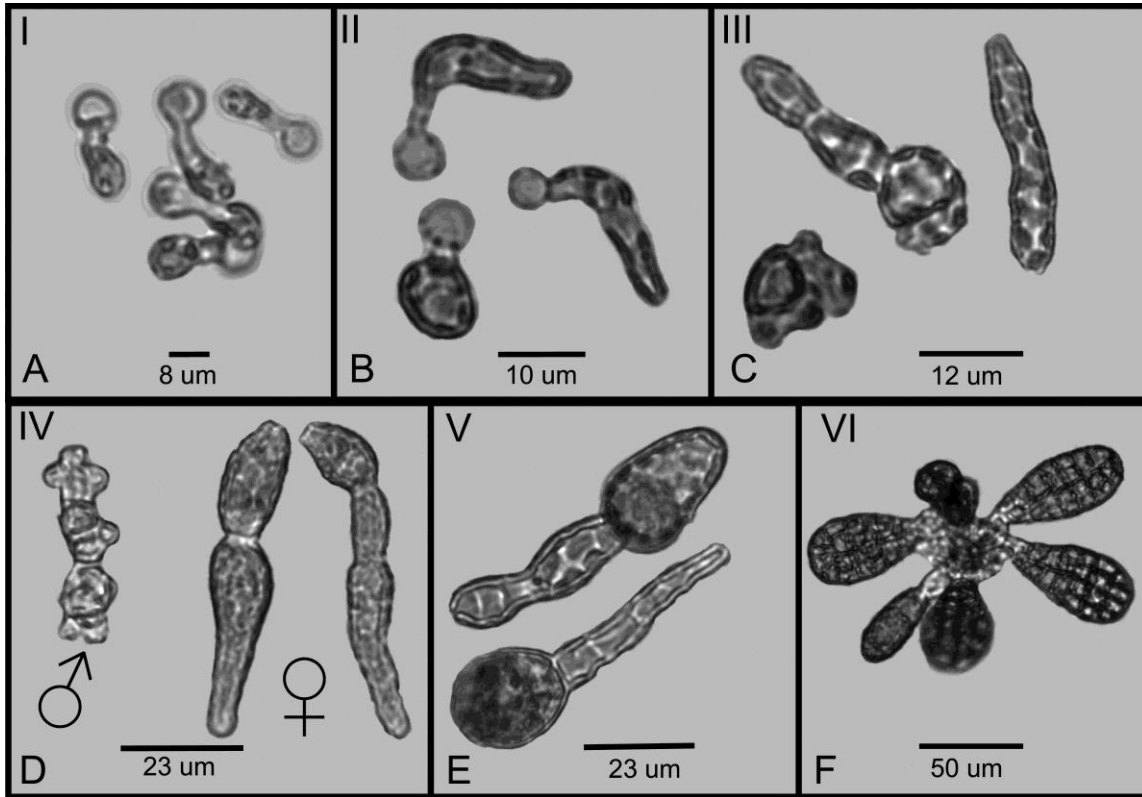
436 Table 2. Reports of *Lessonia flavicans* for the sub-Antarctic Magellanic ecoregion. Salinity
437 values for each site were obtained from Silva and Calvete (2002) and Valdenegro and Silva
438 (2003).

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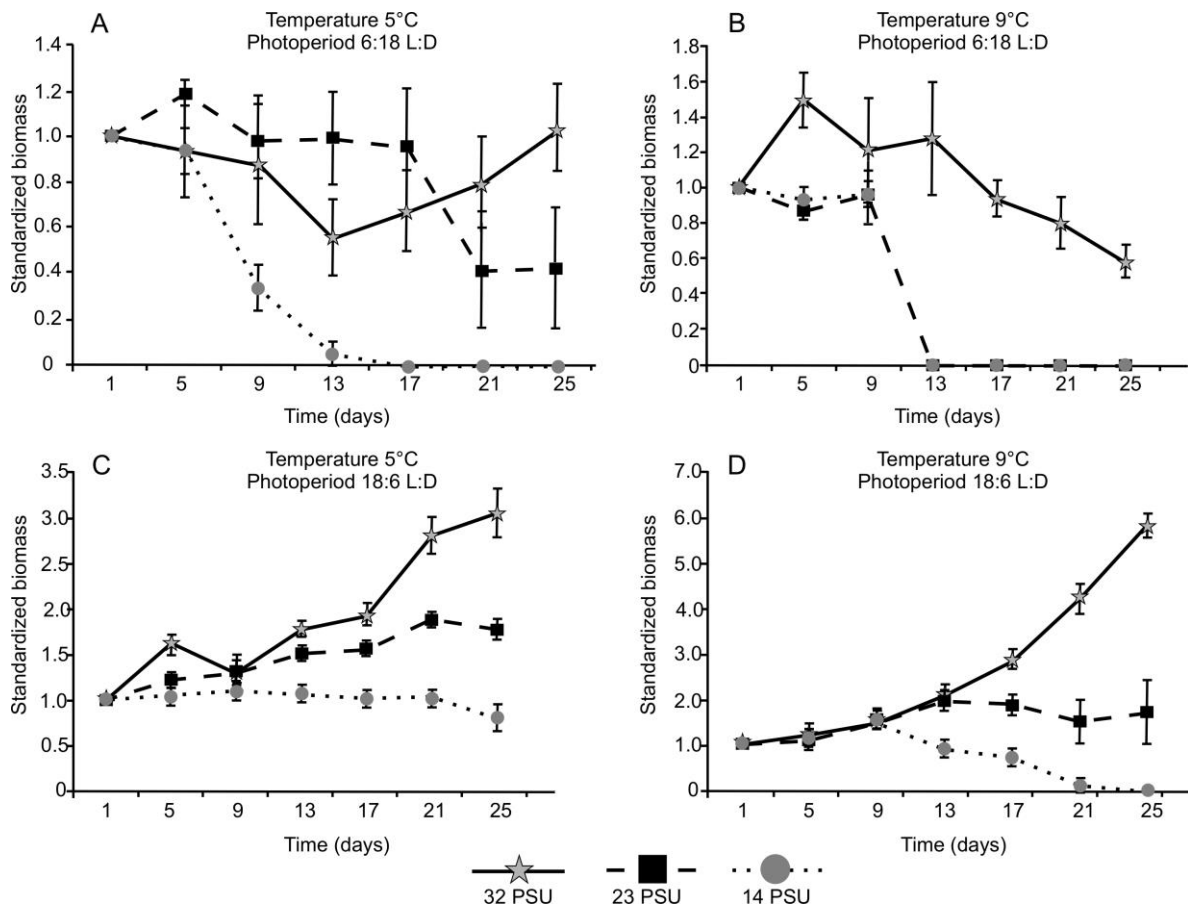


443

444 Figure 1

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448 Figure 2

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451 Table 1

Temperature 5°C	Photoperiod 6:18 L:D					Temperature 9°C	Photoperiod 6:18 L:D				
	Degr. Of Freedom	MS	F	p			Degr. Of Freedom	MS	F	P	
Intercept	1	34.75	50.56	0.000		Intercept	1	27.93	87.93	0.000	
Salinity	2	3.56	5.19	0.023		Salinity	2	5.53	17.42	0.000	
Error	12	0.68				Error	12	0.31			
Time	5	0.75	9.64	0.000		Time	5	2.46	75.77	0.000	
Time*Salinity	10	0.31	4.04	0.004		Time*Salinity	10	0.20	6.30	0.001	
Error	60	0.07				Error	60	0.03			

Temperature 5°C	Photoperiod 18:6 L:D					Temperature 9°C	Photoperiod 18:6 L:D				
	Degr. Of Freedom	MS	F	p			Degr. Of Freedom	MS	F	P	
Intercept	1	216.84	1379.97	0.000		Intercept	1	281.41	474.82	0.000	
Salinity	2	7.62	48.49	0.000		Salinity	2	37.12	62.64	0.000	
Error	12	0.15				Error	12	0.59			
Time	5	1.20	26.99	0.000		Time	5	3.07	13.66	0.000	
Time*Salinity	10	0.96	21.50	0.000		Time*Salinity	10	7.31	32.58	0.000	
Error	60	0.04				Error	60	0.22			

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454 Table 2

Localities	Lattitue S	Longitude W	Salinity	Reference
Trinidad Channel	49° 53.2'	75° 13.0'	30.69	Searles (1978)
Punta Santa Ana	53° 38'	70° 55'	31	Chandia et al. (2005); Martin and Zuccarello (2012)
Ocasion Channel	54° 33.4'	71° 59.7'	31.15	Searles (1978)
Beagle Channel	55° 0'	69° 02.2'	32.17	Searles (1978)

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