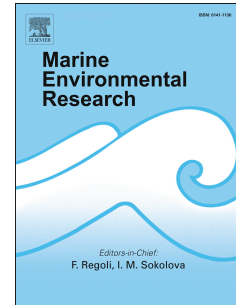


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1 **Effects of kelp phenolic compounds on the feeding-associated mobility of the**
2 **herbivore snail *Tegula tridentata***

3
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15
16 **Abstract**

17 *Tegula tridentata*, is a common herbivore gastropod inhabiting the subtidal *Lessonia*
18 *trabeculata* kelp forest, which tends to show higher densities after kelp harvesting. We
19 investigated if harvested kelp beds may harbor higher densities of herbivore
20 invertebrates, and the underlying mechanisms. Thus, we evaluated if the exudates of *L.*
21 *trabeculata* change the seawater levels of soluble phenols, known to have a deterrent
22 effect against the feeding behavior of some herbivore invertebrates. Finally we
23 investigated whether the increase in *T. tridentata* densities in harvested kelp grounds
24 could be related to a decrease in the seawater levels of soluble phenols. Our results
25 showed that the density of invertebrate herbivores increased up to 32% in harvested
26 kelp grounds. We provide the first estimate of the rate of phenolic exudation by *L.*
27 *trabeculata*, and we demonstrate that *T. tridentata* changes its food dependent
28 movement in the presence of exudates with synthetic phloroglucinol. We suggest that
29 the recovery of harvested kelp ecosystems can be jeopardized by increased herbivory
30 triggered by water-borne changes in the levels of herbivore deterrent compounds.

31 **Key-words:** Kelp harvesting, *Lessonia trabeculata*, Herbivory, Community
32 composition, Ecosystem disturbance, Effects-community

33

34

35 1. Introduction

36

37 Understanding the dynamics of kelp forest ecosystems implies unraveling the
38 complexity of trophic and non-trophic interactions (Estes et al., 2004; Kefi et al., 2015),
39 particularly those including the kelp itself (Bertness et al., 2001; Dayton, 1985).
40 Herbivory is the main interaction affecting the abundance and distribution of kelp
41 species (Vásquez & Buschmann, 1997). Actually, inverse relationships between kelp
42 densities and herbivore densities have been found in several kelp ecosystems
43 worldwide, from Chile (Fariña et al., 2005; Vásquez & Santelices, 1990) to South
44 Africa (Fricke, 1979; Levitt et al., 2002) or Norway (Sjøtun et al., 2006).

45 Chile is the most important producer of kelp from natural populations in the world, as
46 much as 9% of the worldwide sources for alginic acid come from landings of *Lessonia*
47 species in this country (Vásquez, 2008; Vásquez et al., 2012). Commercial kelp
48 extraction has the power to disrupt both top-down and bottom-up processes of kelp
49 dynamics regulation (Steneck et al., 2002) risking kelp ecosystem's ability to persist.
50 Harvesting is likely to affect not only the productivity of kelp populations but also the
51 diversity of associated fauna, which tends to respond to harvesting impacts with a shift
52 on the composition of the community towards an increase in the number of grazers
53 (Fariña et al., 2005; Perreault et al., 2014; Steneck et al., 2002; Vásquez & Santelices,
54 1990). The traditional harvesting of the subtidal kelp *L. trabeculata* entails reductions in
55 kelp densities that may range from 3 to 0.2 ind.m⁻², and has been associated to
56 significant increases in the density of the herbivore snail *Tegula tridentata* (Vásquez &
57 Santelices, 1990). We point out two possible mechanisms for this herbivory response to
58 kelp harvesting. The first one assumes that the removal of whole sporophytes may
59 prompt increased inter-algae distances (Vásquez & Santelices, 1990) which, above a
60 certain threshold, may promote a reduction of the whiplash effect of blades and stipes,
61 favoring the access of snails to the algae and substrata (Vásquez & Buschmann, 1997).
62 This mechanism does not consider the display of directional movement towards the
63 harvested kelp. However, the high densities of the herbivore snail *Tegula tridentata*
64 found in kelp stands with low kelp density (Perreault et al., 2014; Subida & Pérez-
65 Matus, pers. obs.) suggest a directional displacement of herbivores. Hence, we suggest a
66 second mechanism related to herbivores' responses to water-borne cues. We
67 hypothesize that the removal of whole sporophytes may reduce the amount of kelp
68 individuals releasing water-soluble herbivory deterrent compounds, and so herbivores'
69 move towards food (kelp) sources located in areas with lower seawater concentration of
70 these compounds.

71 In brown algae, polyphenolic metabolites are the characteristic and the most extensively
72 studied group of secondary compounds (Amsler, 2008). Polyphenolic compounds, like
73 phlorotannins and bromophenols, play several important ecological roles, specifically as
74 herbivore deterrents (Amsler & Fairhead, 2006; Shibata et al., 2006, 2014). While
75 phlorotannins are stored inside vegetative cells of the outer cortical layer (Amsler, 2008;
76 Shibata et al., 2006), bromophenols are released to the medium through extracellular
77 secretions (Shibata et al., 2006, 2014). So, brown algae may release phenolic
78 compounds into the surrounding water either directly via exudation (Jennings &
79 Steinberg, 1994; Shibata et al., 2006; 2014) or indirectly via tissue erosion or grazing
80 (Koivikko et al., 2005). These compounds are typically large, polar and water soluble,
81 and also usually difficult to measure (Stern et al., 1996).

82 The existing information allows us to predict that kelp harvesting provokes a reduction
83 in the concentration of phenolic compounds in the seawater, triggering an increase in

84 the mobility of the herbivore *Tegula tridentata* towards the harvested kelp bed. Using
85 the productive and heavily exploited kelp ecosystem of central Chile, we set three
86 objectives to shed light on the cascading effects of harvesting. First, we assessed if
87 abundance of grazers increases in harvested areas. Second, we monitored the chemical
88 composition of seawater to determine if *Lessonia trabeculata* exudes significant
89 amounts of phenolic compounds, and to provide the first laboratory estimate of
90 exudation rates for this species. Finally, we experimentally studied the influence of
91 varying concentrations of synthetic phloroglucinol (the basic monomer of polymeric
92 phlorotannins) on the food-dependent movement of the herbivore gastropod *Tegula*
93 *tridentata*.

95 2. Methodology

96 The present study was divided in three parts addressing each of the proposed objectives:
97 1) Effect of kelp harvesting on kelp-associated invertebrates and epifauna (field survey),
98 2) Measurement of kelp exudation of phlorotannins in seawater (laboratory experiment),
99 3) Effect of changes in the phlorotannin concentration in seawater on the food-
100 dependent movement of an herbivore snail (laboratory experiment). All experiments
101 were conducted at the Estación Costera de Investigaciones Marinas from the Pontificia
102 Universidad Católica de Chile (Las Cruces, central Chile).

103 We focused our work on *Lessonia trabeculata*, one of the most conspicuous species of
104 brown algae in Chile (Camus & Ojeda, 1992; Santelices, 1991). *L. trabeculata* is fertile
105 all year and, in the absence of grazers, can recruit and colonize free grounds (Tala et al.,
106 2004; Vásquez, 1989 in Vásquez & Santelices, 1990). This brown alga is the main
107 primary producer in northern and central Chile, and an important foundation species,
108 since it serves as habitat for several invertebrate and vertebrate consumers that feed
109 directly on it or on its associated assemblages (Dayton, 1985; Tala & Edding, 2007). On
110 the central coast, these consumers are mainly composed by invertebrate herbivores and
111 omnivores; in terms of species richness mollusks and crustaceans are the most diverse
112 groups, while mollusks and echinoderms are the most representative in terms of
113 biomass (Fariña et al., 2005). The herbivore sea-urchin *Tetrapygus niger* and the
114 herbivore snail *Tegula tridentata*, are commonly dominant inhabitants of this bottom
115 kelp species (Fariña et al., 2005; Vásquez & Buschmann, 1997; Villegas et al., 2008). *T.*
116 *tridentata*, for instance, is an important grazer in kelp habitats of Chile (Graham et al.,
117 2007; Vásquez & Buschmann, 1997; Villegas et al., 2008).

118 As several of its congeners, *T. tridentata* may be considered as a generalist herbivore
119 (Steinberg, 1985; Watanabe, 1984) with a clear feeding and habitat preference for
120 *Macrocystis* rather than *Lessonia* species (Graham, 2007; Watanabe, 1984). This
121 preference might be associated to an avoidance of plant chemical defenses, as suggested
122 by Steinberg (1985), since *M. integrifolia* show lower content in phenolic herbivore
123 deterrent compounds in vegetative cells than *Lessonia spicata* (Pansch et al., 2008).
124 However, in monospecific *L. trabeculata* stands from central Chile, *T. tridentata* can be
125 the dominant herbivore species (Perreault et al., 2014) and at some sites its massive
126 colonization may even resemble that of feeding fronts (Lauzon-Guay & Scheibling,
127 2009; Pérez-Matus & Subida, per. obs.). *Tegula* shows a rhipidoglossan radula, which,
128 in an experiment with the intertidal *T. funebris* feeding on *Laminaria* blades, was
129 shown to provoke superficial (to release cell liquid contents without damaging cells) as
130 well as deep incisions (with algal tissue removal) in the blade tissue (Hickman &
131 Morris, 1985).

132

133 Objective 1. In order to assess the effect of kelp harvesting on kelp-associated non-
134 epiphyte and epiphyte invertebrates we sampled the subtidal kelp forest of *Lessonia*
135 *trabeculata* at Los Molles (central Chile), in April 2013. Within this site, three kelp
136 grounds were selected according to the time elapsed from harvesting (treatments): a
137 non-harvested site used as control (NH: 32° 12.920'S; 71° 31.580'W), a site harvested
138 two months before the sampling (H2: 32° 12.578'S, 71° 31.653'W) and a site harvested
139 12 months before the sampling (H12: 32° 13.453'S; 71° 31.523'W). Kelp grounds of
140 contrasting recovery time since harvest were spaced between 600 and 1500 m apart.

141 Kelp-associated non-colonial invertebrates (except epiphyte ones) were surveyed *in situ*
142 via SCUBA diving. *In situ* identification was performed to the lowest possible
143 taxonomic level. At each kelp ground (NH, H2, H12), four band-transects (50 x 2 m)
144 were placed parallel to the shoreline and approximately 100 m apart in order to capture
145 heterogeneity in local shore topography. Depths varied within each transect, ranging
146 from 5 to 20 m. Density estimates were obtained by separate counts within the entire
147 band transect (100 m²). The abundance of epiphyte invertebrates living on *L.*
148 *trabeculata* sporophytes was estimated by collecting two kelp sporophytes of
149 approximately the same size (total length and holdfast diameter), at two non-contiguous
150 transects. Kelp extraction was performed by covering kelps with a mesh bag ($\phi=0, 5$
151 mm), in order to retain the epiphyte fauna, and then removing the entire kelp using a
152 crowbar. Kelp sporophytes and their associated invertebrates were preserved with
153 formaldehyde 4% and stored in plastic bags until samples were processed in the
154 laboratory. During this procedure, one of the sporophytes collected at the NH site was
155 lost. In the laboratory, each sporophyte was gently washed with tap water through a 500
156 μm sieve and the collected invertebrates (not larger than 3 cm) were preserved with
157 70% ethanol and stored for further sorting, identification and counting. Organisms were
158 counted and identified under a binocular microscope to the lowest possible taxonomic
159 level.

160 In order to assess the significance of the all-group differences in univariate assemblage
161 descriptors between the three kelp grounds, non-parametric Kruskal-Wallis rank-sum
162 tests were performed. A modified robust Brown-Forsythe Levene-type test based on the
163 absolute deviations from the median, was previously applied in order to ensure that data
164 met the homoscedasticity assumption. Multivariate data on invertebrates' composition
165 and structure in the three kelp grounds (NH, H2, H12) were analyzed separately for
166 kelp-associated and kelp epiphyte invertebrates, with the Primer 6 software package
167 (PRIMER-E, Plymouth). Non-metric dimensional scaling (nMDS) and hierarchical
168 agglomerative clustering, using the UPGMA algorithm, were performed on the Bray-
169 Curtis similarity matrix of the fourth-root transformed abundance data, in order to
170 analyze the occurrence of sample groupings, according to the *a priori* defined groups (=
171 kelp grounds NH, H2, H12). For epiphyte invertebrates, it was not possible to assess the
172 significance of all-group differences due to the low number of kelp replicates available
173 per kelp ground. Thus, to identify the most characteristic species at each ground, a one-
174 way similarity percentage analysis (SIMPER) was performed (Clarke & Gorley, 2006).
175 For the non-epiphyte invertebrate assemblages, one-way analysis of similarities
176 (ANOSIM) was used to assess the significance of kelp ground groupings (Legendre,
177 1998). For both kelp-associated and epiphyte invertebrates, each taxon was assigned a
178 trophic level based on existing literature.

179

180 Objective 2. In order to assess if the kelp *Lessonia trabeculata* exudates phenolic
181 compounds to the seawater and to get the first estimate of its exudation rate, kelps were

182 collected at Punta Tralca (33° 25.470'S; 33° 25.470'S), a sheltered bay located near the
 183 marine laboratory of Las Cruces where the experimental work was conducted. The
 184 experiment, with two trials, was carried out in tanks filled with filtered seawater (FSW),
 185 where kelp sporophytes were allowed to exudate. Three kelp sporophytes were collected
 186 in May 2014 (used in the first trial), and another three in July 2014 (used in the second
 187 trial), by SCUBA at depths ranging between 2.5-2.7 m and 4-4.7 m, respectively. All
 188 sporophytes were weighted (wet weight) in the field and transported to the laboratory.
 189 For each sporophyte, the entire holdfast and a portion of the blades showing few signs
 190 of herbivory were cleaned to remove epiphytes and weighted (wet weight, Table 1).
 191 Each kelp part (holdfast and blades) was placed in a separate tank with a known volume
 192 (14 and 17 L, in trials 1 and 2, respectively) of FSW, maintaining the amputated
 193 extremity out of the water to avoid the release of phenolic compounds through the
 194 damaged tissue. One tank containing only FSW was used as control. Water samples of
 195 50 ml were collected from each tank 6 hours after the beginning of the experiment, and
 196 stored in dark conditions at -18° C, until their analysis. The concentration of soluble
 197 phenols in each water sample was measured by the Folin-Ciocalteu technique adapted
 198 by Koivikko et al. (2005). Water samples were freeze-dried to concentrate the phenols
 199 and then resuspended in a known amount of distilled water. An aliquot (250 µL) was
 200 mixed to 1250 µL of dH₂O, 500 µL of 1 N Folin–Ciocalteu reagent, and 1 mL of 20 %
 201 NaCO₃, incubated for 45 min at room temperature in darkness and centrifuged at 5000
 202 rpm for 3 min. In a preliminary scanning of the samples, absorbance was read at a range
 203 of 220-400 nm; in experimental samples two absorbance peaks were found near 230 and
 204 270 nm, which was never observed in the control sample. Then, the absorbance was
 205 read at 760 nm, and the total content of phenols was expressed as mg L⁻¹, based on a
 206 standard curve with purified phloroglucinol (79330 Aldrich). Exudation rates of soluble
 207 phenols were calculated as µg of phloroglucinol per g of wet weight of *L. trabeculata*
 208 per hour. Owing to the lack of knowledge about the phenolic composition of *Lessonia*
 209 *trabeculata*, we used phloroglucinol as standard, since phloroglucinol polymers, namely
 210 phlorotannins, are amongst the most common phenols of brown algae. Furthermore
 211 phloroglucinol has been used as a standard in a wide number of ecophysiological
 212 studies (e.g. Abdala-Díaz et al., 2006; 2014; Amsler & Fairhead 2006; Jennings &
 213 Steinberg 1994, Koivikko et al. 2005, Gómez & Huovinen 2010).
 214

Table 1. Wet weight (g) of *Lessonia trabeculata* used in the experiment for the measurement of kelp exudation of phenols in seawater. Kelps 1-3 were used in the first trial (May 2014) and kelps 4-6 were used in the second trial (July 2014).

	Kelp 1	Kelp 2	Kelp 3	Kelp 4	Kelp 5	Kelp 6
Holdfast	295	730	470	950	1,310	1,900
Blade	755	785	1,020	1,000	1,200	1,120
Entire plant	1,675	3,660	3,500	6,180	6,075	10,640

215
 216 In order to test if *Lessonia trabeculata* exudates can change the concentration of
 217 phenolic compounds in the surrounding seawater, phenols concentration (in mg of
 218 phenols per total volume of FSW in the tank) was measured and compared between the
 219 control and the remaining experimental tanks. Since brown macroalgae show seasonal
 220 variation in phenolic content (Abdala-Díaz et al., 2006; Tala et al., 2013) the time of
 221 each trial (May and July) could have affected experimental results. Thus, a block design
 222 was used, with factor trial as block. Since data did not meet the error normality

223 assumption of ANOVA, a GLM with quasipoisson error distributions was used instead
224 (residual deviance = 13 on 11 degrees of freedom). Since the exudation differences
225 between holdfast and blades were not consistent across all sporophytes used in the
226 experiments (GLM with negative binomial distribution $p < 0.05$, res.dev. = 9.5 on 5 df),
227 we were not able to assess which part of *L. trabeculata* showed higher exudation rates.
228 The limit for statistical significance was set at 0.05. All statistical analyses were
229 performed with the R statistical language (R Core Team, 2014).

230

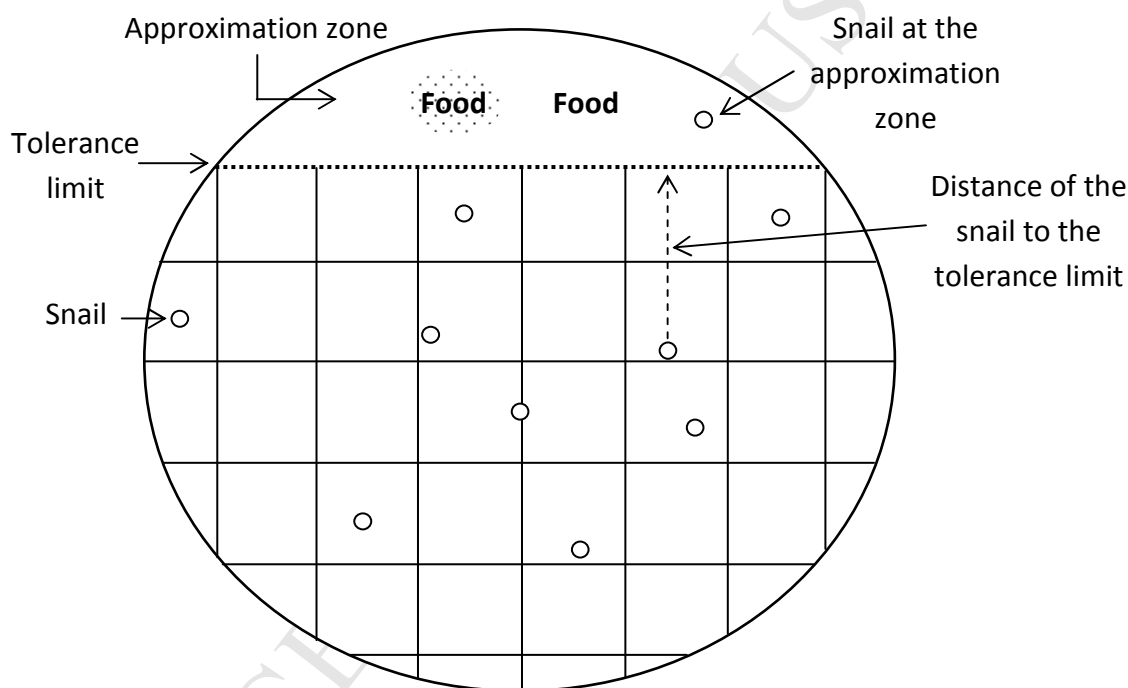
231 Objective 3. In order to assess the effect of phloroglucinol dissolved in seawater on the
232 food-dependent movement of the herbivore snail *Tegula tridentata*, a three-trial
233 laboratory experiment was conducted. The experiment consisted in placing starving *T.*
234 *tridentata* individuals in tanks with different food treatments (each treatment
235 corresponding to a different level of water-releasable phenols added to the food), and
236 monitoring snails' food-dependent movement for 6 h. Thus, experimental treatments
237 were the four phloroglucinol concentrations used to prepare each food treatment,
238 defined after Steinberg (1988): 0 mg/mL (control C0), 8 mg/mL (treatment C1), 16
239 mg/mL (treatment C2) and 22 mg/mL (treatment C3). Synthetic phloroglucinol (Sigma
240 P-3502) was used to manipulate the concentration of the total phenols in each tank. We
241 assumed phloroglucinol to effectively simulate the effect of kelp phenols, for the
242 reasons already explained in the previous objective.

243 Four cylindrical tanks filled with 200 L of FSW and maintained under natural
244 photoperiod, were used in each trial. The bottom of each tank ($\emptyset = 92$ cm) was marked
245 with a grid of 10 x 10 cm cells, and with a line defining a semi-circle with a radius of
246 about 22 cm from the tank wall. The line of the semi-circle corresponded to the
247 'tolerance limit' to the 'approximation zone' - the area where snail's food was placed
248 during the experiment (Fig. 1). Each tank was set with different food treatments.

249 Snails' food consisted in 40 ml agar disks prepared by heating an aliquot of kelp extract
250 with agar and letting cool to 50 °C. Kelp extract was obtained from homogenization of a
251 constant amount of chopped kelp blades (in order to ensure a constant input of natural
252 kelp phenols) in seawater, in a ratio of 1/3, at room temperature and in light conditions.
253 Phloroglucinol, at different concentrations depending on the food treatment considered,
254 was dissolved in an aliquot of kelp extract at room temperature, added to the (cooled)
255 aliquot containing agar, and shaken vigorously. The mixture was immediately poured
256 into Petri dishes and allowed to harden. Polyphenols bind to agar in solution preventing
257 hardening, so the concentration of agar in the different disks varied (2.5%, 2.9% and
258 3.3% of the final mixture) to ensure that disks containing different levels of polyphenols
259 were similar in texture (Steinberg, 1988).

260 *Tegula tridentata* snails were collected on May 2014, at Punta de Tralca, where
261 previous studies showed the occurrence of strong herbivory pressure of *T. tridentata*
262 over *L. trabeculata* (Pérez-Matus & Subida, per. obs.). In the laboratory, snails were
263 measured, marked with nail polish to allow its identification during the experiment, and
264 maintained in tanks with running seawater under starvation for 2 to 3 days. Five hours
265 prior to the experiment, 10 marked snails were transferred to each experimental tank for
266 acclimation. Afterwards, snails were carefully placed at the center of each tank and 2
267 agar-food disks were placed at the wall side of the approximation zone (Fig. 1). Since
268 phloroglucinol gradually leaches out from agar disks (Steinberg, 1988), snails at
269 different treatments were gradually exposed to different concentrations of
270 phloroglucinol dissolved in the FSW of the tank. At each treatment, the position of each
271 snail in the tank grid was recorded at 7 different times: half an hour after the beginning
272 of the experiment and every following hour, for a period of 6 h (Steinberg, 1988).

273 We measured four response variables to describe the food-dependent movement of
 274 *Tegula tridentata* in the tanks. The average distance to the tolerance limit (Dml, in cm)
 275 was calculated as the average of the distances of each snail position to the tolerance
 276 limit, at each time. The final distance to the tolerance limit (Dfl, in cm) was calculated
 277 as the distance between the position of each snail at the end of the experiment and the
 278 tolerance limit. The escape response (Re) was measured as the number of times each
 279 snail was recorded at the tank wall at a height of 20 cm or more. The approximation
 280 response (Ra) was measured as the number of times each snail was recorded in the
 281 'approximation zone' (Fig. 1). For each response variable, the significance of the
 282 differences between treatments (C0, C1, C2 and C3) was assessed through a one-way
 283 analysis of variance (ANOVA), after graphically assessing that the assumptions of
 284 normality and homoscedasticity were met. Contrasts were used to perform pairwise
 285 post-hoc comparisons to assess the significance of the differences between: i) control
 286 (C0) and all treatments pooled together (C1 + C2 + C3), ii) C1 and C2, iii) C3 and C3.
 287 The limit for statistical significance was set at 0.05. All statistical analyses were
 288 performed with the R statistical language (R Core Team, 2014).



289
 290 Fig. 1. Schematic drawing of the experimental set-up for the laboratory experiment
 291 carried out to assess the effect of phloroglucinol on the food-dependent movement of
 292 *Tegula tridentata*.

293

294 3. Results

295

296 3.1 Effect of kelp harvesting on kelp-associated invertebrates

297

298 *Epiphyte invertebrates*. A total of 54 different epiphyte invertebrate taxa were found
 299 living on the kelp *Lessonia trabeculata* in the studied site. Assemblages at the H2 kelp
 300 ground showed the lowest average number of taxa (13; Table 2). This pattern became
 301 also evident by the low similarity between the assemblages inhabiting *L. trabeculata* at
 302 H2 in comparison with the assemblages from the remaining kelp grounds (< 25%, Fig.

303 2). This dissimilarity was highly determined by the highest abundances at H2 of the
 304 herbivore amphipod *Bircenna* sp. (contribution to dissimilarity with H12 and NH
 305 assemblages of 28 and 12%, respectively) and of the herbivore snail *Tegula tridentata*
 306 (contribution to dissimilarity with H12 and NH assemblages of 9 and 6%, respectively).
 307 It is remarkable that *T. tridentata* was recorded only at the H2 ground, where it
 308 contributed to about 13% of the total abundance. The analysis of trophic groups
 309 revealed an increment of herbivory on the H2 area (88% of herbivores) when compared
 310 to the H12 (56%) and the NH control area (59%). Carnivores and suspension feeders
 311 showed a dramatic reduction in H2 (3% and 7%) when compared to NH (19% and 20%)
 312 and H12 (23% and 17%).
 313

Table 2. Univariate descriptors (average \pm standard error) of invertebrate epifauna and non-epifauna assemblages at the studied *Lessonia trabeculata* grounds. H2: harvested 2 months before sampling, H12: harvested 12 months before sampling, NH: non-harvested, SE: standard error. Abundance (epifauna) represents the average number of individuals per sporophyte; abundance (non-epifauna) represents the number of individuals per m² (= density).

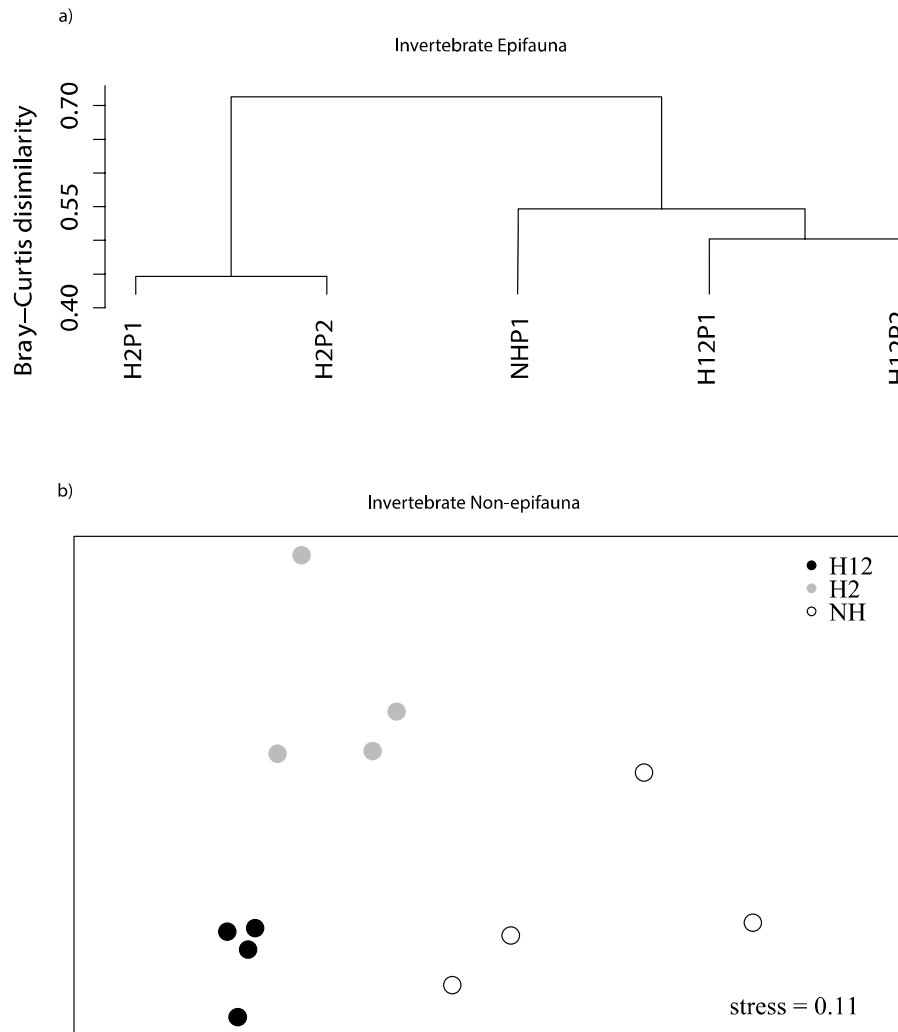
	Invertebrate epifauna			Invertebrate non-epifauna		
	NH*	H2	H12	NH	H2	H12
Total n° of taxa	26	13 \pm 1	22.5 \pm 0.5	6 \pm 5.75	4.1 \pm 2.66	6.2 \pm 5.56
Abundance	130	66 \pm 9	41 \pm 16	176.6 \pm 1.41	94.7 \pm 50.79	81.6 \pm 67.12

* Data correspond to a single observation, due to the lost of one replicate

314

315 *Kelp-associated non-epiphyte invertebrates*. A total of 38 non-epiphyte invertebrates
 316 were found in the *L. trabeculata* kelp grounds. Taxa richness was significantly lower at
 317 H2 (Kruskal-Wallis $\chi^2 = 7.53$, $df = 2$, $p = 0.023$, Table 2). The multivariate structure
 318 and composition of the invertebrate assemblages was significantly different among the
 319 three kelp grounds (Fig. 2; ANOSIM $R = 0.76$, $p < 0.005$). Dominance was highest at
 320 H2, where the herbivore snail *T. tridentata* alone contributed to more than 70% of the
 321 total density of invertebrates. Thus, a 23 and 55% increase in herbivore contribution to
 322 total density was observed at this kelp ground in relation to NH and H12, respectively.

323



324
325

326 Fig. 2. Invertebrates associated to *Lessonia trabeculata* grounds. Dendrogram of
327 epiphyte assemblages (a) and nMDS of non-epiphyte assemblages (b) at each of the
328 studied kelp grounds. H2: harvested 2 months before sampling, H12: harvested 12
329 months before sampling, NH: non-harvested, P1 and P2: sample replicates.

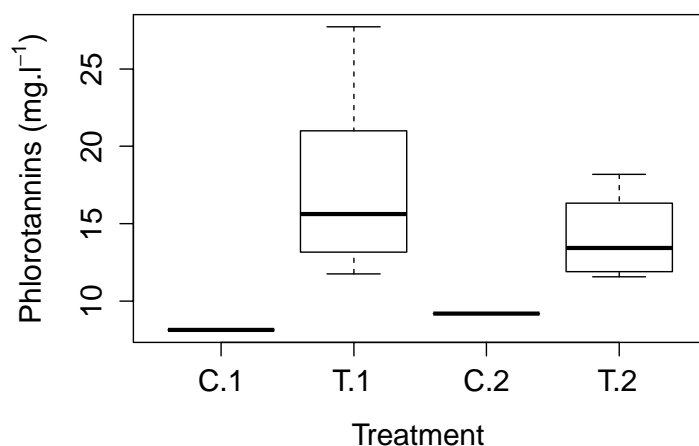
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331 3.2 Measurement of kelp exudation of phenolic compounds in seawater

332

333 We found significant differences in the concentration of phenolic compounds between
334 the seawater of tanks with (T1 and T2) and without (C1 and C2) *Lessonia trabeculata* in
335 both trials (Fig. 3; GLM $F = 5.56$, $p = 0.04$, res. dev. = 13 on 11 df). The rate of
336 exudation of phenols, which was significantly higher in the first experiment (GLM, $F =$
337 22.4 , $p < 0.005$, res.dev. = 16.1 on 10 df), varied between 2.0 ± 0.27 and 4.6 ± 0.85
338 $\mu\text{g}\cdot\text{g}_{(\text{ww})}^{-1}\cdot\text{h}^{-1}$.

339



340 Fig. 3. Concentration of soluble phenolic compounds detected in experimental tanks
 341 with (T) and without (C) *Lessonia trabeculata*, in trials 1 and 2.

342

343 3.3 Effect of changes in seawater concentration of phloroglucinol on the food-dependent 344 movement of the snail *Tegula tridentata*

345

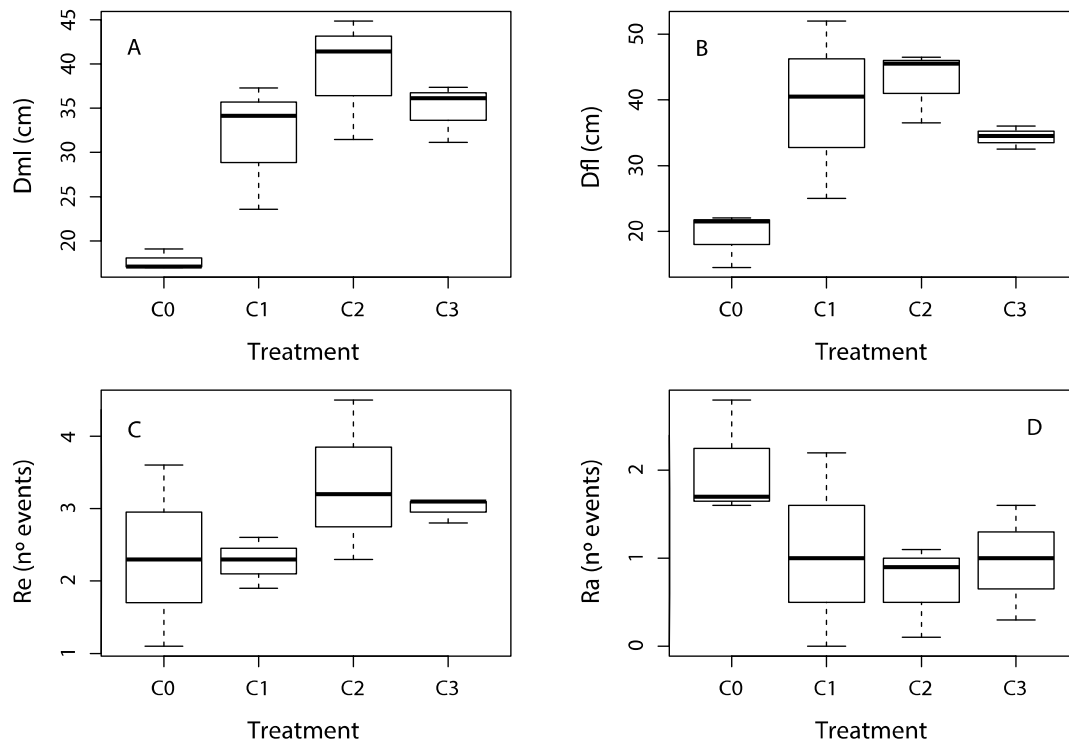
346 Three of the four variables used to characterize the food-dependent movement of *Tegula*
 347 *tridentata* snails showed clear trends between the control and the three treatments
 348 (different levels of phloroglucinol) (Fig. 4). Significant differences among treatments
 349 were found for the average distance (Fig. 4a; $F = 9.26$, $df = 8$, $p = 0.006$) and final
 350 distance (Fig. 4b; $F = 5.46$, $df = 8$, $p = 0.020$) to the tolerance limit. Post-hoc contrast
 351 tests revealed that snails moved, on average, further away from the food and reached a
 352 greater distance to the tolerance limit in treatments with phloroglucinol (Dfl, $p = 0.005$;
 353 Dml, $p = 0.001$). Although snails tended to approach the feeding area (Ra) more often in
 354 the control tank (Fig. 4d), no significant differences were found among records of this
 355 variable. Similarly, no significant differences among treatments were found for snails'
 356 escape response (Re) although snails tended to escape less at the control and at the
 357 lowest phloroglucinol concentration treatment (Fig. 4c).

358

359

360

361



362 Fig. 4. Boxplots of the *Tegula tridentata* movement response variables. Dml (average
 363 distance to tolerance limit), Dfl (final distance to tolerance limit), Re (escape response)
 364 and Ra (approximation response). Phloroglucinol concentrations: C0 (0 mg/ml), C1 (8
 365 mg/ml), C2 (16mg/ml), C3 (22 mg/ml).
 366
 367

368 4. Discussion

369 Our results showed that the density of invertebrate herbivores increased in about 30% in
 370 harvested grounds of the kelp *Lessonia trabeculata*. The mechanisms that trigger this
 371 response of herbivores to kelp harvesting are still poorly understood, and we shed light
 372 on our prediction that the decrease in the number of kelp sporophytes exuding
 373 secondary herbivore-deterrent metabolites, like soluble phenolic compounds, can trigger
 374 the movement of herbivores towards harvested areas. First we demonstrated that *L.*
 375 *trabeculata* has the ability to change the concentration of phenolic compounds in the
 376 surrounding seawater, by providing the first estimate of the rate of phenolic exudation
 377 by this kelp. Finally we demonstrated that the herbivore snail *Tegula tridentata* might
 378 change its food-dependent movement, avoiding approaching food in the presence of
 379 phenolic exudates, by recognizing water-borne chemical cues. On that account, this
 380 study sets out a novel perspective on the ecosystem-level effects of this harvesting
 381 activity, by considering not only the direct effect of removing a habitat forming species,
 382 but also the indirect effects mediated by changes in the chemical composition of the
 383 seawater driven by the extraction of kelp individuals.

384 Marine invertebrates are usually negatively affected by kelp harvesting either due to the
 385 direct or indirect loss of food, shelter, spawning and nursery grounds (Fariña et al.,
 386 2005; Kelly, 2005; Vásquez & Santelices, 1990). So, as expected, the harvesting of *L.*
 387 *trabeculata* led to a shift in the structure of the associated invertebrate assemblages,
 388 both in terms of species (decrease in the number of species and subsequent increase in
 389 the dominance) and trophic (increase in the number of herbivores) composition. The

390 herbivory increase in the samples taken two months after harvesting is in agreement
391 with the results presented by other authors, which suggest this pattern to be related with
392 a decrease in the density of adult kelp sporophytes after the harvesting (Vásquez &
393 Santelices, 1990; Villegas et al., 2008). Although some authors attribute this response to
394 a facilitation of herbivores' access to the algae (Vásquez & Buschmann, 1997; Vásquez
395 & Santelices, 1990), herein we explored the potential effects of water-borne substances
396 from macroalgae (Amsler, 2008), namely herbivory deterrent phenolic compounds
397 released by *L. trabeculata*.

398 Little is known about the specific composition of *L. trabeculata*'s herbivore deterrent
399 compounds. The scarce information available for this species refers to general terms
400 such as soluble phenolic content (Chandía et al., 2004), total phenolic content (Guinea
401 et al., 2012) or phlorotannins (Storz et al., 2009), despite the determination procedure
402 being the same in all studies (Folin-Ciocalteu method for determination of total soluble
403 phenolic content). Polyphenolic compounds are, in effect, the most abundant secondary
404 metabolites produced by brown algae (Amsler, 2008). Besides phlorotannins, brown
405 algae from the orders Laminariales (e.g. *Lessonia*) and Fucales, are also rich in
406 halogenated phenols, in particular those of bromine and iodine (La Barre et al., 2010).
407 In this study, however, we were constrained to use the Folin-Ciocalteu method, which
408 does not allow differentiating between classes of phenolic compounds. Nevertheless,
409 according to Shibata et al. (2006) we would expect *L. trabeculata* to present the
410 following classes: i) phlorotannins, stored inside vegetative cells of the outer cortical
411 layer; ii) bromophenols, releasable through extracellular secretion; and eventually iii)
412 other molecules not yet identified by the available analytical techniques. Defensive
413 phenolic compounds can be expressed constitutively (constantly produced to avoid
414 grazing) or can be induced in response to stimulus (Macaya & Thiel, 2008). The
415 constitutive phenolic defenses exhibited by *Lessonia* species (Martinez & Correa, 1993;
416 Rothäusler et al., 2005) are expected to be less variable in response to grazers
417 abundance and environmental conditions than induced defenses (Karban and Baldwin
418 1997). Therefore, kelp harvesting activities and the resulting increase in the number of
419 grazers are not likely to induce the production of these chemical defenses.

420 Adult individuals of *L. trabeculata* maintained in laboratory had the ability of changing
421 the chemical composition of the surrounding seawater. The levels of soluble phenolic
422 compounds exuded by *L. trabeculata* were within the detection limits of the Folin-
423 Ciocalteu technique, with an average of $3.28 \mu\text{g}\cdot\text{g}_{(\text{wet wt})}^{-1}\cdot\text{h}^{-1}$, exuded into the
424 surrounding seawater. This rate is lower than the one recorded *in situ* for the sublittoral
425 kelp *Ecklonia radiata*, that reached up to $10 \mu\text{g}\cdot\text{g}_{(\text{dry wt})}^{-1}\cdot\text{h}^{-1}$ (Jennings & Steinberg,
426 1994). Assuming that all the measured exudates fall into the class of halogenated
427 phenols (Shibata et al., 2006), the values mentioned above are likely to overestimate the
428 real concentration of these compounds exuded to the water column, as none of these
429 two studies took into account the dilution provoked by water movement. These polar
430 metabolites are highly water-soluble and can rapidly dissolve away from the surface of
431 the producing kelp (Le Barre et al., 2010; Steinberg et al., 2001).

432 Although in some species of brown algae bromophenols play a more efficient role as
433 herbivore deterrents than the phlorotannins (Shibata et al., 2014), our results showed
434 that the marine herbivore gastropod *T. tridentata* changed its movement behavior, and
435 hence its spatial distribution, in response to the presence of soluble synthetic
436 phloroglucinol, the monomeric base of phlorotannin polymers. So, supposing that *L.*
437 *trabeculata* does not exudate phlorotannins, but only halogenated phenols like
438 bromophenols (Shibata et al., 2006), one would expect a even stronger herbivore
439 deterrence response of natural exudates in the field than the one observed in our

laboratory experiment. In this experiment, snails tended to move away from areas where soluble phenolic compounds were present (deterrence by exudates) regardless of the lack of physical contact (by direct feeding, for instance) between the snails and the source of the exudates. As far as we know this study represents the first attempt to relate herbivore food-dependent movement with levels of kelp defense metabolites in the seawater (water-borne cues). Most studies focus, instead, on the response of macroalgae to water-borne cues of grazers' presence (Amsler, 2008 and references herein) or on the palatability of food sources (Borell et al., 2004; Macaya et al., 2005; Macaya & Thiel, 2008; Shibata et al., 2014; Van Alstyne, 1988).

Palatability studies yielded contrasting results since grazers reacted differently to the gradients of concentration of phenolic compounds used. The feeding activity of the gastropods *Tegula funebris* and *T. brunnea*, for example, was deterred by different concentrations of polyphenols, with highest concentrations showing a stronger deterrent effect (Steinberg, 1985; 1988). Another study examining the deterrence effect of natural phloroglucinol and its oligomers and also bromophenols on the feeding activity of the gastropod *Turbo cornutus*, evidenced varying, or even opposite, effects at varying phenolic compounds' concentration (Shibata et al., 2014). Namely, the authors found that phloroglucinol and phlorotannins at concentrations occurring in algal tissues had feeding deterrent activity against *T. cornutus*, but that the highest deterrence effects were found for bromophenols that are only present on algal exudates. Furthermore they suggest that at low concentrations some of these phenolic compounds can even stimulate the feeding behavior of the studied gastropod.

Although our results show a clear effect of the water-borne presence of synthetic phloroglucinol on the food-dependent movement of the snail *T. tridentata* under laboratory conditions, distinct herbivores can perceive differently the presence of phenolic compounds in the seawater (Jennings & Steinberg, 1997). Furthermore, in nature the water movement can strongly affect this perception (Macaya & Thiel, 2008; Swanson & Druehl, 2002), since the content of phlorotannins can be influenced by temporal variation on water movement (Dubois & Iken, 2012). Therefore, in order to test if our findings apply to other herbivores and to the natural environment, future studies should in the first place, focus on the *in situ* measurement of the seawater concentration of polyphenolic compounds in harvested and non-harvested *Lessonia trabeculata* beds, with similar hydrodynamic conditions. This would allow verifying the assumption that the reduction in the number of adult kelp sporophytes, due to harvesting, can reduce the concentration of dissolved herbivore-deterrent polyphenolic compounds within the kelp bed. The major limitation for such a study is, for the moment, methodological. It would be necessary to sample and further concentrate high amounts of seawater in order to obtain a concentration of polyphenolic compounds falling within the detection limits of the Folin-Ciocalteu method (Tala, pers. comm.). It is possible that the use of different methods for the extraction of kelp phenols, such as HPLC (Shibata et al., 2006; 2014), or the use of different standards in the Folin-Ciocalteu method, would allow improving the phenol extraction procedure. In addition, *in situ* studies should be conducted in order to evaluate the response of *T. tridentata* or other important herbivores (e.g. fishes) to the levels of exuded phenolic compounds into the surrounding seawater.

5. Conclusion

Kelps are key organisms in marine ecosystems as they are major conduits of energy between autotrophs and the rest of the food web. The intensity of *Lessonia* harvesting

490 along the Chilean coast has been rapidly increasing in the last few years, as this species
491 is used by several growing industries. This study provides a deeper insight into the
492 ecosystem-level effects of the commercial harvesting of *L. trabeculata* in Chile. We
493 demonstrated that harvesting activities may have a profound direct and indirect
494 influence on the dynamics of kelp-associated organisms for this kelp species. Moreover,
495 our experimental studies suggest that harvesting might significantly alter the chemical
496 composition of the seawater in a way that influences the spatial distribution of some
497 abundant herbivores like the marine snail *Tegula tridentata*. Due to the removal of
498 whole sporophytes, harvesting of *L. trabeculata* may lead to a decrease in the number of
499 adult sporophytes exuding polyphenolic compounds. By perceiving this reduction in the
500 seawater concentration of herbivore deterrent molecules, *T. tridentata* may select areas
501 where the levels of kelps defenses are lower, moving towards harvested kelp beds. If
502 this proves to be a more generalized response pattern of herbivores, kelp harvesting
503 above certain thresholds may produce a shift on the structure of the kelp associated-
504 communities, which can compromise the growth and development of new kelp
505 sporophytes due to the grazing activities.

506

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508

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517

518 7. References

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Highlights:

Density of invertebrate herbivores increased in harvested grounds of *L. trabeculata*.

L. trabeculata exudates soluble phenolic compounds to the surrounding seawater.

T. tridentata avoided the presence soluble phloroglucinol in the seawater.

Kelp harvesting may lead to reduction in levels of herbivore deterrents in seawater.

Kelp harvesting may indirectly trigger kelp herbivory via water-borne chemical cues.