

## RESEARCH ARTICLE

# Coastal pollution from the industrial park Quintero bay of central Chile: Effects on abundance, morphology, and development of the kelp *Lessonia spicata* (Phaeophyceae)

Carolina Oyarzo-Miranda<sup>1,2,3</sup>, Nicolás Latorre<sup>1,2,3,4</sup>, Andrés Meynard<sup>1,2,3</sup>, Jorge Rivas<sup>1,2</sup>, Cristian Bulboa<sup>1,2</sup>, Loretto Contreras-Porcía<sup>1,2,3\*</sup>

**1** Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile, **2** Centro de Investigación Marina Quintay (CIMARQ), Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile, **3** Center of Applied Ecology and Sustainability (CAPES), Santiago, Chile, **4** Programa de Doctorado Medicina de la Conservación, Facultad de Ciencias de la Vida, Universidad Andres Bello, Santiago, Chile

\* [lorettocontreras@unab.cl](mailto:lorettocontreras@unab.cl)



## OPEN ACCESS

**Citation:** Oyarzo-Miranda C, Latorre N, Meynard A, Rivas J, Bulboa C, Contreras-Porcía L (2020) Coastal pollution from the industrial park Quintero bay of central Chile: Effects on abundance, morphology, and development of the kelp *Lessonia spicata* (Phaeophyceae). PLoS ONE 15(10): e0240581. <https://doi.org/10.1371/journal.pone.0240581>

**Editor:** Hans-Uwe Dahms, Kaohsiung Medical University, TAIWAN

**Received:** June 17, 2020

**Accepted:** September 30, 2020

**Published:** October 15, 2020

**Copyright:** © 2020 Oyarzo-Miranda et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the manuscript and its Supporting Information files.

**Funding:** This work was supported ANID FONDECYT N° 1170881 to LC-P as PI and ANID PIA/BASAL FB0002.

**Competing interests:** The authors have declared that no competing interests exist.

## Abstract

The industrial park of Quintero Bay (QB) in the central coast of Chile was established in the 1960s, presents high levels of pollution due to the industrial activity, and it is known as one of the five Chilean “sacrifice zones”. *Lessonia spicata* is the most important habitat-forming kelp species in the intertidal along the central and south shores of Chile, and currently there are no morphometric and population studies of *L. spicata* (or other seaweed species) nor studies about the effects of pollution on its development in QB and neighbouring sites. In this context, the aims of this study were (i) to register the abundance and morphological features of *L. spicata* populations from Ventanas, Horcón and Cachagua (sites with different pollution histories and located only up to 40 km from the QB); ii) to determine the heavy metals (HMs) concentration in seawater and marine sediments; and (iii) to evaluate *in vitro* the effects of exposure to seawater from the three sampling sites on spore release and early developmental stages, up to the juvenile sporophyte. Results showed that the chronically exposed Ventanas kelp population had the smallest adult individuals in comparison with the other sites. Ventanas and Horcón registered high HMs concentration in the seawater and marine sediments exceeding the international permissible limits (e.g in seawater Cu 20–859  $\mu\text{g L}^{-1}$ ; sediments Cu > 50,000  $\mu\text{g kg}^{-1}$ ). Unexpectedly in Cachagua, a site often considered unpolluted, high concentrations of Cu and As were also registered in the seawater (859 and 1,484  $\mu\text{g L}^{-1}$ , respectively) and of As in marine sediments (20,895  $\mu\text{g kg}^{-1}$ ). Exposure of gametophytes to the seawater from Ventanas resulted in a developmental delay compared to the other treatments; however, low sporophyte production was determined in all treatments. Our results indicate that QB, more notably Ventanas, induce highly negative effects on individual development, and consequently on seaweed populations, which suggest a long-term negative impact on the community structure of these marine zones. Furthermore, the high concentrations of HMs reported here at Cachagua suggest a recent expansion of

**Abbreviations:** Ca<sub>T</sub>, Treatment using seawater from Cachagua; CCME, Canadian Council of Ministers of the Environment; EPA, Environmental Protection Agency; FG, Female gametophyte; G, Germinated spore; HMs, Heavy metals; Ho<sub>T</sub>, Treatment using seawater from Horcón; MG, Male gametophyte; PAHs, Polycyclic aromatic hydrocarbons; PEL, Probable effect levels; QB, Quintero Bay; S, Spore; TEL, Threshold effect levels; U, Undifferentiated gametophyte; Ve<sub>T</sub>, Treatment using seawater from Ventanas.

pollution along the central coast of Chile, evidencing effects on the marine ecosystem health even on sites far from the pollution source.

## 1. Introduction

Heavy metals (HMs) are inorganic pollutants naturally present in the environment, which have increased significantly due to anthropogenic action [1]. These metals generate alterations in the normal metabolism, development, and fitness of animals, plants and seaweeds, depending on their concentration, distribution and speciation [2–4]. Several reports have evidenced the direct impact of heavy metal pollution on loss of biodiversity in coastal ecosystems [5–8].

Seaweeds are bioindicators and biomonitors of heavy metal enrichment [9, 10], especially brown seaweeds (Phaeophyceae), which are capable of adsorbing more heavy metals than other species because of the alginate and fucoidan matrix present in their inner cell membrane [11, 12]. Species of Phaeophyceae, such as *Scytosiphon lomentaria*, *Macrocystis pyrifera* and *Ectocarpus siliculosus*, are highly tolerant to Cu excess [13–15]. In contrast, species of the genus *Undaria* and *Lessonia*, are sensitive to high concentrations of HMs, principally Cu, which trigger oxidative stress, cell damage and negative effects on spore release, germination and gametophyte development [4, 16–20]. Recently, Contreras-Porcía et al. 2020 [17] demonstrated that binary and tertiary mixtures of Cu, Cd, and polycyclic aromatic hydrocarbons (PAHs) have negative synergistic effects on spore settlement of the kelps *Lessonia spicata* and *M. pyrifera*.

The life cycle of kelps (order Laminariales) consists of two phases, a microscopic haploid gametophyte ( $n$ ), alternating with a macroscopic diploid sporophyte ( $2n$ ), which respond differently to environmental variables [21]. Specifically, the spores and gametophytes are more sensitive to chemical pollution exposure than the adult sporophytes [17, 22]. Moreover, microscopic stages play a critical role in the persistence and sustainability of kelp populations, and therefore, harmful effects of pollution may reduce or cause local extinction [23, 24]. Indeed, the adult sporophyte populations are an indicator of the pollution levels because as soon as recruitment occurs, they are immediately subjected to run-off and other pollutants present in the seawater and marine sediments [25]. For example, sporophytes of *Laminaria japonica* exposed to chronic pollution (East Japanese Sea) show anatomic alterations, inhibition of the generative tissue development, and a delay in maturation of the sorus [26]. The negative effects on any of the life stages, produce direct and indirect economic, biological, and ecological effects, which have repercussions on higher levels of biological organization, such as population, community, and ecosystem [7, 27, 28].

Along the coast of Chile are located several industrial parks that concentrate energy production companies, fuel refineries and others, which have a long history of pollution problems, affecting terrestrial and coastal zones around the industrial area [29–31]. One of them is the industrial area of Puchuncaví-Ventanas (32° 44' S 71° 30' W), located in Quintero Bay (QB) at the central coast of Chile. This industrial park began operation in 1961, and it is characterized by high pollution levels due to historical discharges of petroleum, gaseous pollutants and atmospheric particulates, as well as the deposition of heavy metals from diverse industrial facilities, including coal-fired power plants, a copper refinery and smelter, natural gas terminals, and cement companies, among others [30–35]. Since the industrial activities in the zone were largely unrestrained and their impacts were poorly regulated during several decades, the QB is known nowadays as one of the five Chilean sacrifice zones [31]. Previous studies about marine

pollution in this zone reported historical and current concentrations of HMs such as Al, Mo, Fe, Cr, Cu, and Zn, and PAHs that exceed the permissible limits of the US Environmental Protection Agency (US EPA) quality guidelines for seawater, and were higher than quality standards allowed in molluscs, crustaceans and kelps for human consumption [30, 36].

It is worth highlighting that at present there is no information about the status of kelps forest in the Quintero Bay, such as *L. spicata*, despite their key economic and ecological role. *L. spicata* is an important ecosystem engineer in the low intertidal zone along the central and south coasts of Chile [37]. This kelp facilitates the recruitment of algae and invertebrates and provides habitat and shelter for several associated species in its holdfast and blades, modulating local biodiversity and community structure [38]. In addition, intertidal beds of *L. spicata* are an important commercial resource exploited by seaweed-based industries for alginate, and animal consumption [39, 40]. Based on this previous knowledge, we formulated two hypotheses: (i) *L. spicata* populations chronically exposed to seawater from polluted sites that are closer to QB would be more affected in terms of abundance and morphometric features than more distant kelps populations, and (ii) the *in vitro* exposure of *L. spicata* to seawater from polluted sites closer to QB would have a higher negative effects on spore release and early developmental stages than the exposure to seawater from more distant sites. In this context, the aims of this study were (i) to register the abundance and morphological features of *L. spicata* populations from Ventanas, Horcón and Cachagua (sites with different pollution history and located only up to 40 km from the QB), ii) to determine the heavy metals (HMs) concentration in seawater and marine sediments, and (iii) to evaluate *in vitro* the effects of exposure to seawater from the three sampling sites on spore release and early developmental stages of this kelp.

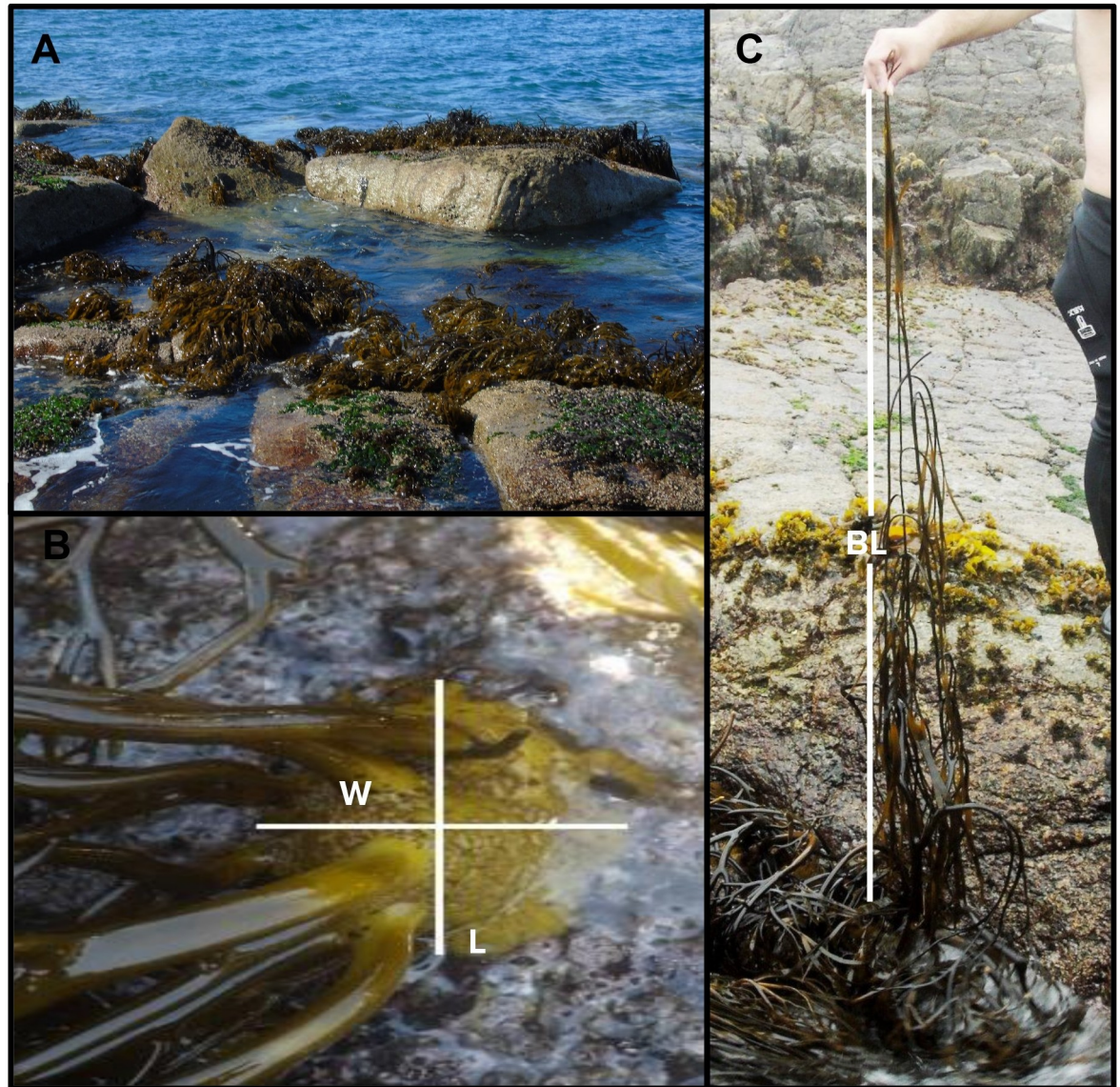
## 2. Materials and methods

### 2.1. *In situ* analysis

**2.1.1. Sampling sites.** We defined the industrial park located in Quintero Bay (32° 44' 31" S, 71° 29' 33" W) as a pollution focus. Then, we selected three sampling sites, due to their order of proximity to the focus, from south to north: Ventanas, Horcón and Cachagua (S1 Fig). Ventanas (32° 44' 31" S, 71° 29' 33" W) and Horcón (32° 42' 33" S, 71° 29' 19" W) were closer to QB and had serious environmental precedents [30, 41, 42], while Cachagua (32° 35' 04" S, 71° 27' 22" W) was the farthest site from QB. This site was declared as a natural sanctuary in 1979 and does not have a history of direct anthropogenic impact [13, 43, 44]. The specific sampling sites were outside of the delimited protected area, and only part of the reproductive tissue (1–2 cm<sup>2</sup>) was collected, where extraction permits are not required [45].

**2.1.2. Material treatment.** For the analysis of total HMs concentration, all glass and plastic materials (flasks, plastic bottles, Petri dishes, glass pipettes, among others) were treated according to the protocol established by the US EPA [46]. The material was incubated on the non-ionic detergent Extran 7% (Merck, Germany) for 12 h. Then, the material was rinsed three times with distilled water, incubated on nitric acid 5% (Merck, Germany) for 12 h. After incubation, the material was washed with distilled water and MilliQ water (18,2 MΩ cm) three times. Finally, it was dried on a Class II laminar flow chamber (Cientec, Model JSCB-1200SB), avoiding any contact with metal.

**2.1.3. Abundance and morphometric features.** The study was carried out in July (2018), because it corresponds to the season with the highest fertility of *L. spicata*, as reported previously by Araujo and Faugeron [47]. For the three sites, we randomly established four areas of 2.25 m<sup>2</sup> at the low intertidal zone where populations of *L. spicata* occur. In each area, we quantified the number of holdfasts, the length of the longest blade and the diameter of the width and length of each holdfast (Fig 1). Based on Vega et al. [27], the diameter of the holdfast was



**Fig 1. Morphological features recording.** A) *Lessonia spicata* intertidal populations examined at Cachagua sampling site, B) view of a holdfast of *L. spicata* showing its length (L) and width (W), and C) illustration of the measurement of the longest blade (BL) in an individual of *L. spicata*.

<https://doi.org/10.1371/journal.pone.0240581.g001>

used to classify the size structure of *L. spicata* population: recruits and juvenile (< 10 cm), young (10–19 cm) and adult sporophytes (> 20 cm). Additionally, the holdfast area was calculated using the following equation:

$$\text{Holdfast area} = \pi * \text{diameter}(\text{width} * \text{length})$$

**2.1.4. Collection and analysis of total concentration of heavy metals in seawater and marine sediments.** Seawater and marine sediments samples from Ventanas, Horcón and Cachagua were collected manually in an area close to the *L. spicata* population (3 m perimeter), in triplicate for each matrix. For seawater and sediment samplings, the material was

rinsed with seawater from each site three times. Samples of seawater were taken using 500 mL acid-washed low-density polyethylene bottles, which were placed in plastic bags. In the case of marine sediment, around 50 cm of surface sediment was collected using 50 mL Falcon tubes. Both types of samples were placed in a cooler (treated for HMs) with ice packs and kept at 8–10°C for transporting. Once at the laboratory of Quintay Marine Research Center (CIMARQ) (33° 11' 34.567" S, 71° 42' 8.842" W), sediment samples were placed on plastic Petri dishes and oven-dried for 12 h at 45°–50°C. All samples were sent to the Laboratory of Elemental Analysis (LAE) at the Universidad Andrés Bello, Concepción. We selected Cu, As, Cd, Ag, and Pb because of their toxicity in marine organisms and high levels in QB according to previous reports [34, 43, 48, 49]. The analyses of the total concentration of HMs were carried out through inductively coupled plasma-mass spectrometry (ICP-MS Aurora M90 model Bruker, USA).

EPA3051/3050 method [50] was used for sample treatments.

Because Chile lacks quality guidelines for seawater and marine sediments, the results of the HMs concentrations were contrasted with international criteria. In the case of seawater samples, they were compared with the values established by the US EPA standards in the "National Recommended Water Quality Criteria" [51]. These standards compare two criteria according to the exposure time (acute and/or chronic), where exceeding these values indicates possible damage to marine organisms. On the other hand, sediments samples were compared with the values established by the "Canadian Sediment Quality Guidelines for the Protection of Aquatic Life" [52, 53], which applies two values: threshold effect level (TEL) indicates a criterion that damage to marine organisms may occur if this value is exceeded; and possible effect level (PEL) corresponds to the lowest value associated with adverse effects in marine organisms.

## 2.2. *In vitro* experiments

**2.2.1. Treatments.** We collected 10 L of seawater from each sampling site, and they were stored in high-density pre-treated polystyrene containers. Then, seawater was filtered through 0.22 µm pore membrane (Sartorius, Goettingen, Germany) and stored in new glass bottles in darkness without any enriched culture medium at 15°C during all the experimental time (two months).

**2.2.2. Kelp sampling for *in vitro* experiments.** The biological material was collected from Cachagua due to its low polluted environmental history. Blades with sori, were collected randomly in an area of 10 m<sup>2</sup>. Then, the sori were cleaned to eliminate epibionts using the following sequence: washing with iodine at 9% dissolved in 0.22 µm filtered-seawater, rinsed with distilled water, cleaned superficially with ethanol at 5% using a paint brush, rinsed with 0.22 µm filtered seawater plus GeO<sub>2</sub> at 4% m/v, and finally rinsed with 0.22 µm filtered-seawater. Then, they were dried with absorbent paper, and transported in a cooler at 10°C to the Laboratory of Ecology and Molecular Biology in Algae (LEBMA, [www.lebma.cl](http://www.lebma.cl)) in Santiago. We used the study of Contreras et al. [18] as a reference to classify the developmental stages: Spore (S), Spore with germ tube (G), Undifferentiated gametophyte (U), Female gametophyte (FG) and Male gametophyte (MG) (S2 Fig). The summary of the *in vitro* experiments was schematized in S3 Fig.

**2.2.3. Effect of treatment on spore release.** Individual discs of 1 cm in diameter were cut from randomly selected sori. Each disc was blot-dried with absorbent paper, air dried on trays and exposed to light for 1 h to stimulate the spore release. Then, groups of four discs were placed in pre-treated amber glass vials of 5 mL containing 4 mL of treatment. The glass vials were kept under continuous agitation at 120 rpm on an orbital shaker (SEA STAR™), under controlled conditions (15°C and 20–30 µmol photons m<sup>-2</sup> s<sup>-1</sup>) during the entire assay. The

number of spores was recorded after 1, 2, 3, 5 and 7 hours, by counting three samples in a haemocytometer (Neubauer 0.100 mm, 0.0025 mm<sup>2</sup>) using a light microscope (Leica ICC50 HD, Germany).

**2.2.4. Effect of treatment on spore settlement.** After three hours of spore release, 58 µL of the spore solution (with an average density of  $5 \times 10^3$  cells mL<sup>-1</sup>) were inoculated in glass Petri dishes containing 7 mL of treatment (n = 15). The count of settled spores was performed using an inverted microscope (Eclipse Ts2, Nikon, Japan) in ten areas of 1 mm<sup>2</sup> per Petri dish at 48 h, and after the first medium change at 120 h. The culture conditions for all experiments were: photoperiod of 12:12, 10–15°C, and 20–30 µmol photons m<sup>-2</sup> s<sup>-1</sup>.

**2.2.5. Effect of treatment on gametophyte development.** The same cultures used for the spore settlement experiment (2.8) were maintained and monitored for the development of gametophytes. The development of gametophyte was recorded by counting the number of cells per stage (S, G and U) 6 times during 40 days of exposure. A total of 10 areas of 1 mm<sup>2</sup> were evaluated per replicate (n = 15). The 7 mL of treatment were changed every seven days.

**2.2.6 Effect of treatment on sexual differentiation, sporophyte production and fertility.** New cultures were conducted for sexual differentiation, sporophyte production and fertility experiments. For that, we added 65 µL of spore solution ( $5 \times 10^3$  cell mL<sup>-1</sup>) in each Petri dish containing 7 mL of 0.22 µm filtered-seawater obtained from CIMARQ. After 25 days, the seawater was changed for the different treatments, data was recorded 3 times during 25 days of exposure, which was changed every seven days. Petri dishes were maintained at the same culture conditions mentioned on point 2.9. We evaluated a total of 7 areas of 1 mm<sup>2</sup> per Petri dish (n = 13).

Cell numbers per stage (U, MG and FG) for sexual differentiation, and sporophyte numbers for sporophyte production were quantified, and then fertility was determined. Percentage of fertility was calculated according to Oppliger et al. [54], using the following equation:

$$\% \text{ Fertility} = \frac{\text{Number of Sporophytes}}{\text{Number of female gametophyte} + \text{Number of Sporophytes}} (x100)$$

## 2.3. Data analyses

**2.3.1. Abundance and morphometric features.** R software was used to perform all statistical analyses. We first checked for normality and homoscedasticity of the data by performing a Shapiro Wilk test and a Bartlett test, respectively. Based on these results, we performed a Kruskal-Wallis test, followed by a post-hoc Mann-Whitney U test to compare the sampling sites on each measured variable (i.e. abundance, holdfast area and blade length).

**2.3.2. PCA for morphological features and heavy metal concentrations.** To examine data variability, a principal component analysis (PCA) was carried out [55] using abundance data, morphological features, and HMs concentrations among the sampling sites.

**2.3.3. Effect of seawater on spore release, settlement, gametophyte development, sexual differentiation, sporophyte production and fertility.** Due to the distribution of the data, for all the *in vitro* experiments we performed a Kruskal-Wallis test (K-W), followed by a posteriori Mann-Whitney pairwise test to compare the treatments. For spore release, treatments were compared in terms of the accumulated number of spores released after 1, 2, 3, 5 and 7 h, each hour being analysed independently. For spore settlement, treatments were compared at 48 and 120 h after the beginning of the cultures. For gametophyte development, we performed two statistical analyses, (i) independently, a K-W on the percentage per day of each development stage (S%, G%, U%) and (ii) a two-way ANCOVA on all the recorded times per stage between the treatments. For sexual differentiation we compared the percentage of U, FG and MG after

4, 10 and 25 days of exposure between the treatments, also Dunn's test was performed to compare the percentage per stage per treatment during the different exposure time. We compared the sporophyte production (%) and fertility (%) at 4, 10 and 25 days of exposure to the treatments. Finally, to evidence variation along the experimental time in these two descriptors, we performed another K-W test between the recorded times on each treatment.

### 3. Results

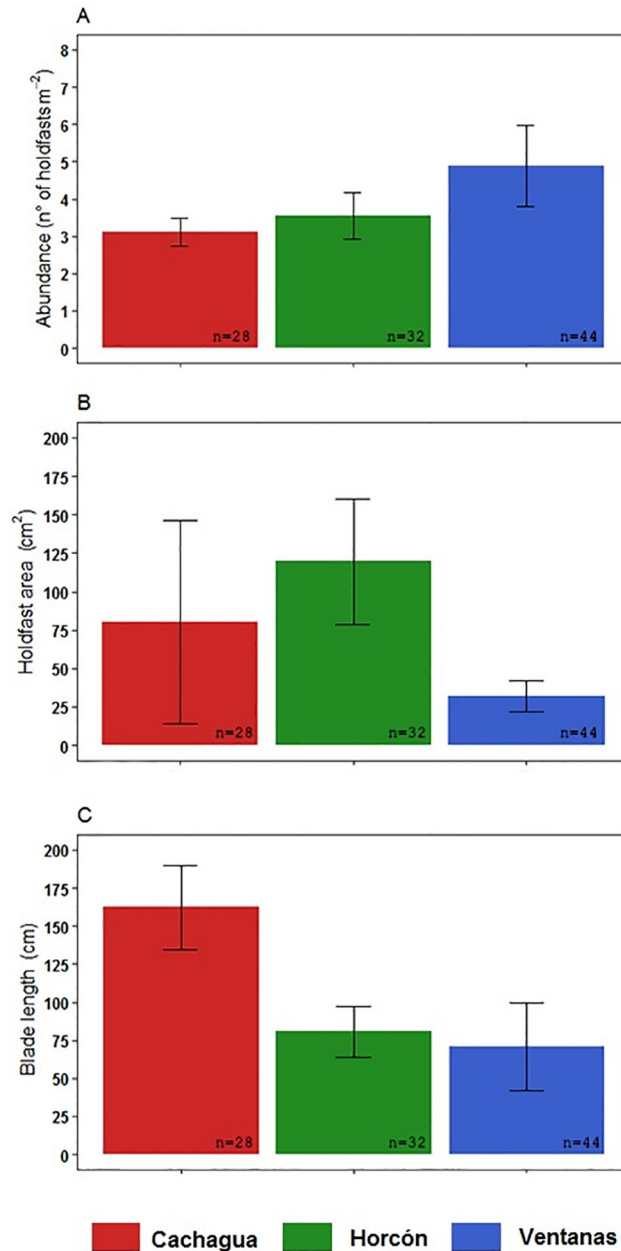
#### 3.1. *In situ* analysis

**3.1.1. Abundance and morphometric features.** The K-W analysis revealed significant differences in abundance and morphometric traits between *L. spicata* populations from the different sampling sites. Abundances were significantly different among Ventanas and Cachagua (K-W = 0.028). Ventanas had the highest abundance ( $4.8 \pm 2.4$  holdfast  $m^{-2}$ ) in comparison with Horcón ( $3.5 \pm 1.4$  holdfast  $m^{-2}$ ) and Cachagua ( $3.1 \pm 0.8$  holdfast  $m^{-2}$ ) (Fig 2A). The holdfast area of *L. spicata* from Ventanas was also significantly different to Cachagua (K-W = 0.029), where Ventanas showed the smallest holdfast area ( $32 \pm 9.9$   $cm^2$ ) in comparison with this site ( $80 \pm 26.1$   $cm^2$ ) and Horcón ( $120 \pm 40.5$   $cm^2$ ) (Fig 2B). Similarly, the length of blades was significantly different between Ventanas and Cachagua (K-W = 0.029), and between Horcón and Cachagua (K-W = 0.029) (Fig 2C). Ventanas displayed the shortest blades ( $71 \pm 28.7$  cm), in comparison to the intermediate blades from Horcón ( $81 \pm 16.4$  cm), and the longest blades from Cachagua ( $163 \pm 27.6$  cm). In relation to the size structure of *L. spicata* population, Cachagua showed 39.3% of recruits and juvenile plants, 42.9% of young and 17.9% of adult sporophytes. Horcón registered 9.4%, 59.4% and 31.3%, respectively (Fig 3). Finally, Ventanas presented only 63.6% of recruits and juvenile, 36.4% of young sporophytes and 0% of adult sporophytes (Fig 3).

**3.1.2. Heavy metal concentration in seawater and marine sediments.** Regarding seawater from the three sampling sites, Cu and As occurred at maximal concentrations exceeding international normative for aquatic life by several orders of magnitude (Table 1). The Cu maximum values registered at each site, ordered from highest to lowest, were as follows: Cachagua ( $859 \mu g L^{-1}$ ), Ventanas ( $741 \mu g L^{-1}$ ) and Horcón ( $46 \mu g L^{-1}$ ). These values exceeded the EPA criteria for acute exposure ( $4.9 \mu g L^{-1}$ ) by 175 times in Cachagua, 151 times in Ventanas and 9.4 times in Horcón. In the case of As, the highest value was registered in Cachagua ( $1,484 \mu g L^{-1}$ ), followed by Horcón ( $449 \mu g L^{-1}$ ) and Ventanas ( $348 \mu g L^{-1}$ ). All three exceeded the EPA standard value for acute exposure ( $69 \mu g L^{-1}$ ) by 22, 7 and 5 times, respectively. On the other hand, Cd, Ag and Pb presented lower values than those recommended by international standards (Table 1).

In marine sediments, Cu and As concentrations exceeded international standards by several orders of magnitude (Table 2). Specifically, the maximum Cu concentration was registered in Horcón ( $82,203 \mu g kg^{-1}$ ), followed by Ventanas ( $32,128 \mu g kg^{-1}$ ), both exceeding the CCME value for TEL ( $18,700 \mu g kg^{-1}$ ) by 4 and 2 times, respectively. In the case of As, the concentrations exceeded the CCME value for TEL ( $7,240 \mu g kg^{-1}$ ) by 3 times in Cachagua ( $20,895 \mu g L^{-1}$ ), 2 times in Horcón ( $14,008 \mu g L^{-1}$ ) and 1.8 times in Ventanas ( $13,743 \mu g L^{-1}$ ). On the contrary, Cd, Ag and Pb in the sediments showed lower values than those recommended by international criteria (Table 2).

**3.1.3. Relationship between morphometric features and heavy metal concentrations.** Results of the PCA based on morphological characters and HMs concentrations are shown in Fig 4. The cumulative proportion of the first two principal components (Dim1 = 57.1% and Dim2 = 25.2%) explained more than 80% of the total variation in the data. This analysis evidenced three discrete kelp population clusters in relation to morphology, abundance, and

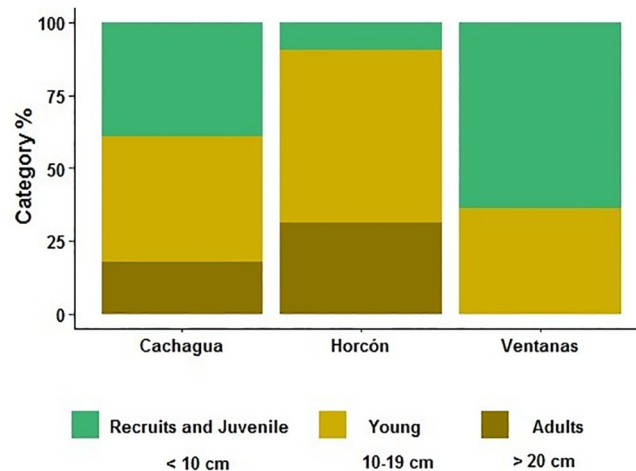


**Fig 2. Comparison of morphological features.** Average of A) abundance, B) holdfast area, and C) length of the longest blade of *Lessonia spicata* population from Cachagua, Horcón and Ventanas.

<https://doi.org/10.1371/journal.pone.0240581.g002>

concentrations of HMs in seawater and sediments (Fig 4). The most important morphological trait that allowed Cachagua data to differentiate was blade length, followed by kelp abundance (Fig 4). Furthermore, the most important environmental factors that allowed to separate Cachagua's kelps population from the others in Dim1 were As and Cu concentrations in seawater and As in marine sediment (Tables 1 and 2).





**Fig 3. Comparison of size structure based on holdfast diameter.** Percentual presence of three categories: recruit and juvenile, young, and adult sporophyte of *Lessonia spicata* populations. Each bar corresponds to one of the locations in this study: Cachagua, Horcón and Ventanas.

<https://doi.org/10.1371/journal.pone.0240581.g003>

## 3.2. In vitro experiments

**3.2.1. Effect of treatments on spore release.** After three hours of exposure, spore release was higher using seawater from Horcón and Cachagua than with seawater from Ventanas (Fig 5). This tendency persisted until the end of the experiments (7 h) (spore release in Cachagua  $841 \pm 229 \times 10^3$ , Horcón  $760 \pm 212 \times 10^3$  and Ventanas  $583 \pm 156 \times 10^3$  spore  $\text{mL}^{-1}$ ). Significant differences were registered in the concentration of spores released between Cachagua and Ventanas (K-W = 0.042).

**3.2.2. Effect of treatments on spore settlement.** From 48 h to 120 h of exposure to the treatments, the number of settled spores in Horcón and Ventanas decreased 25%, while Cachagua showed an increase of 53% (Fig 6). Significant differences were registered between Cachagua and Horcón at 120 h of exposure (K-W = 0.014).

**3.2.3. Effect of treatments on gametophyte development.** The K-W test showed significant differences between treatments, and post hoc analyses showed that these differences were mainly between Ventanas and Horcón in comparison to Cachagua (S1 Table). The treatments Cachagua and Horcón had a higher percentage of undifferentiated gametophytes at day-16 (17% and 12%, respectively) in comparison with Ventanas (9%) (Fig 7), but K-W test did not show significant differences. Also, on day-25-30 of exposure, Horcón and Ventanas increased significantly (K-W = 0.003) their percentage of undifferentiated gametophytes (29% and 28%)

**Table 1. Range of concentrations of heavy metals in seawater from Cachagua, Horcón and Ventanas (2018).**

Site	Range of concentration ( $\mu\text{g L}^{-1}$ )				
	Cu	As	Ag	Cd	Pb
Cachagua	19–859	64–1,484	0.111–0.143	0.162–0.196	0.233–0.234
Horcón	20–46	6–449	0.057–0.207	0.065–0.335	0.363–2.261
Ventanas	28–741	9–348	0.010–1.191	0.091–0.243	0.093–3.425
acute	4.9	69	33	1.9	210
chronic	3.1	36	79	2	8.1

<https://doi.org/10.1371/journal.pone.0240581.t001>

**Table 2. Range of concentrations of heavy metals in marine sediments from Cachagua, Horcón and Ventanas (2018).**

Site	Range of concentration ( $\mu\text{g Kg}^{-1}$ )				
	Cu	As	Ag	Cd	Pb
Cachagua	8,256–9,135	<b>20,271–20,895</b>	19–29	25–31	2,502–3,651
Horcón	<b>45,070–82,203</b>	994–14,008	28–66	20–80	2,115–3,109
Ventanas	5,285– <b>32,128</b>	776–13,743	10–70	17–49	1,310–3,709
TEL	18,700	7,240	700	-	30,200
PEL	108,000	41,600	4,200	-	112,000

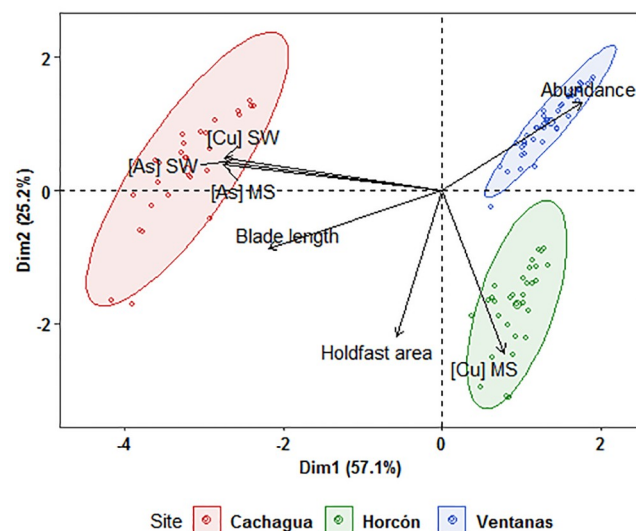
These values correspond to the heavy metal concentrations in seawater ( $\mu\text{g L}^{-1}$ ) determined by ICP-MS. In bold are indicated values which exceeds the international norm [51] for acute (less than 24 h) and chronic exposure (more than 24 h).

These values correspond to the heavy metal concentrations in surface marine sediments ( $\mu\text{g Kg}^{-1}$ ) determined by ICP-MS. In bold are indicated values which exceeds the international norm [52, 53] for threshold effect levels (TEL) and Probable effect levels (PEL).

<https://doi.org/10.1371/journal.pone.0240581.t002>

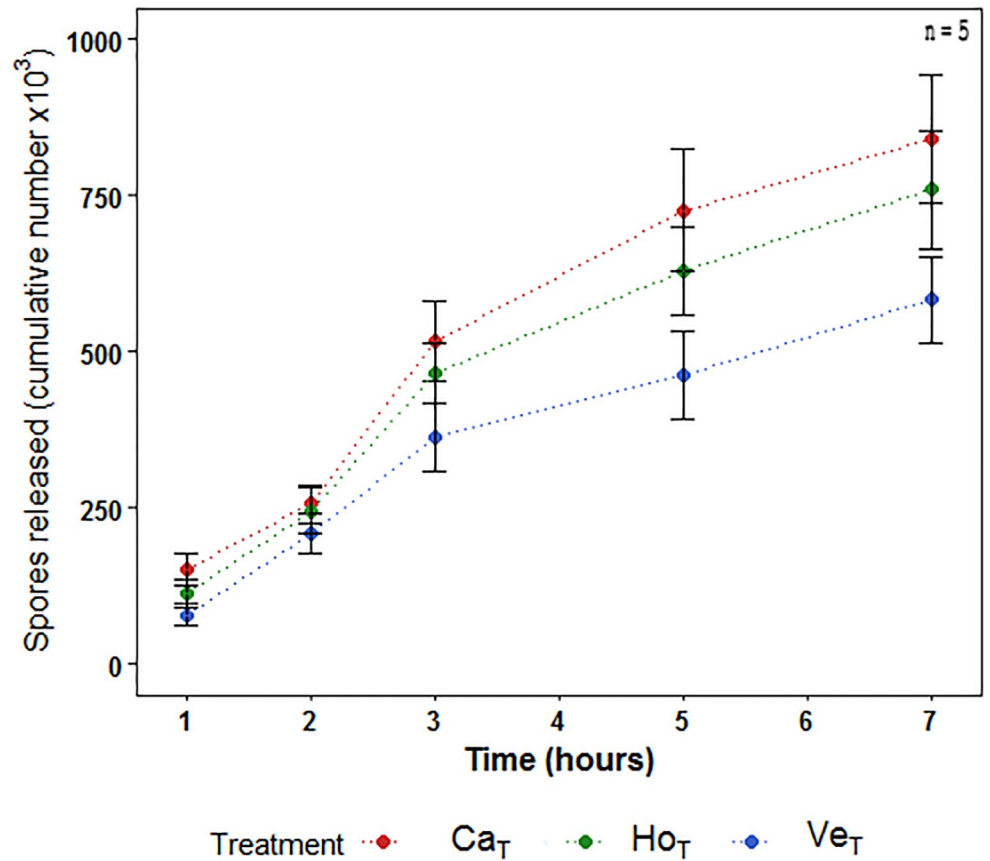
in comparison to Cachagua (11%) (Fig 7). However, to compare if there were significant differences between the treatments over time, in relation to the percentage of each stage, a two ways ANCOVA was performed independently by stage. These results, evidenced that, the percentage of germinated spores ( $F = 0.002$ ) and undifferentiated gametophytes ( $F = 0.0003$ ) differed significantly between the treatments, where Ventanas had a higher percentage of germinated spores during a longer period compared with Cachagua. It is worth mentioning that the three treatments evidenced a high percentage of spores (35 ~ 46%) that did not develop during the entire exposure period (Fig 7).

**3.2.4. Effect of treatments on sexual differentiation.** At the beginning of the exposure, Cachagua and Horcón showed a small percentage of Female gametophyte (FG) (3%), which increased significantly to 50% and 74%, respectively ( $K-W = \text{Cachagua} = 0.027$ ,  $\text{Horcón} < 0.001$ ) over time (Fig 8). In Ventanas treatment, FG just increased from 2% to 39% ( $K-W = 0.024$ ) (S2 Table). Also, undifferentiated gametophytes (U) remained high at



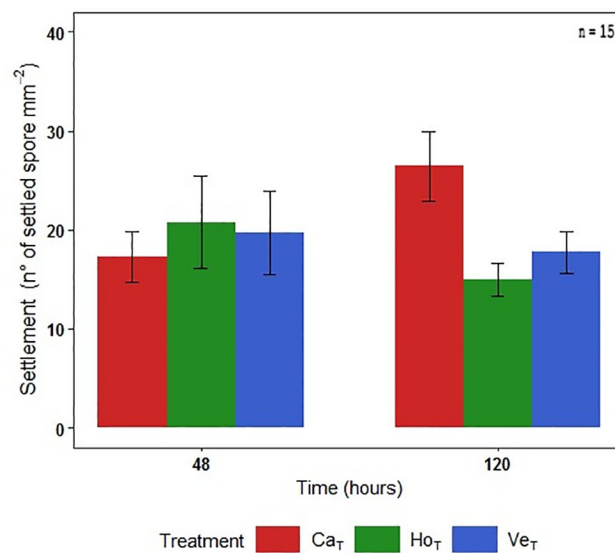
**Fig 4. Principal components analysis of morphological features and heavy metals concentration.** PCA includes abundance, holdfast area and blade length (Fig 2), plus the concentration of [As] and [Cu] in seawater (SW) and marine sediments (MS) (Tables 1 and 2).

<https://doi.org/10.1371/journal.pone.0240581.g004>



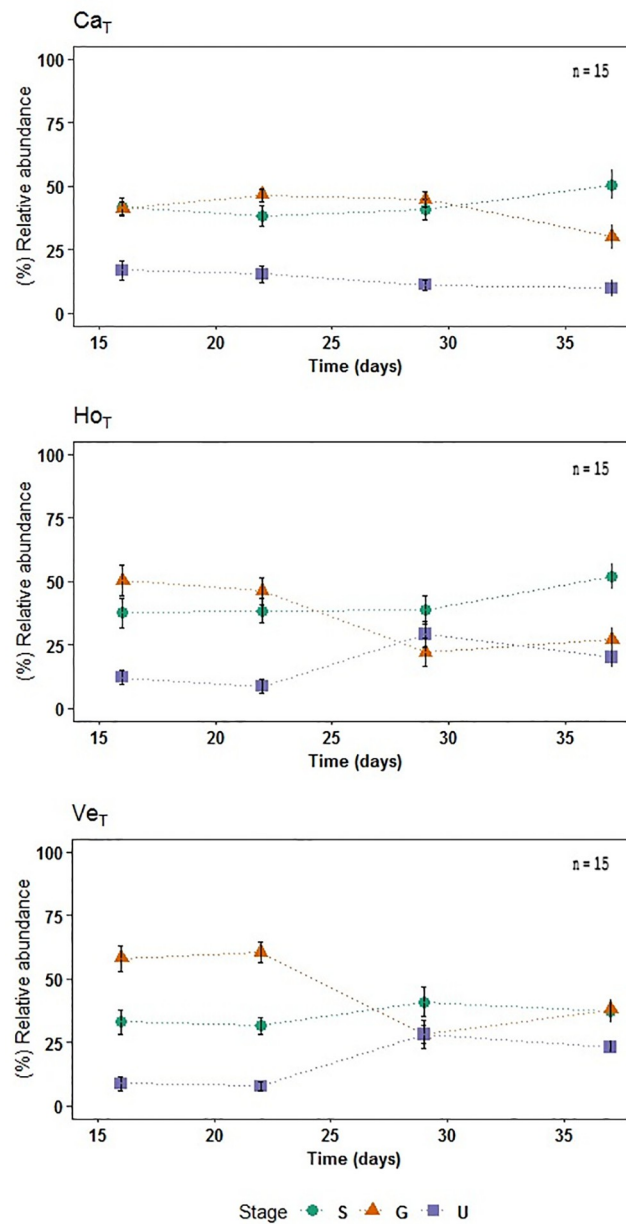
**Fig 5. Spore released.** Accumulated spores released by sori of *Lessonia spicata* (number of spores released mL<sup>-1</sup>, average ± S.D (n = 5). Ca; Cachagua, Ho; Horcón, Ve; Ventanas. T; treatment.

<https://doi.org/10.1371/journal.pone.0240581.g005>



**Fig 6. Settlement.** Number of settled spores of *Lessonia spicata* per treatment showing SD (n = 15), at 48 and 120 h of exposure.

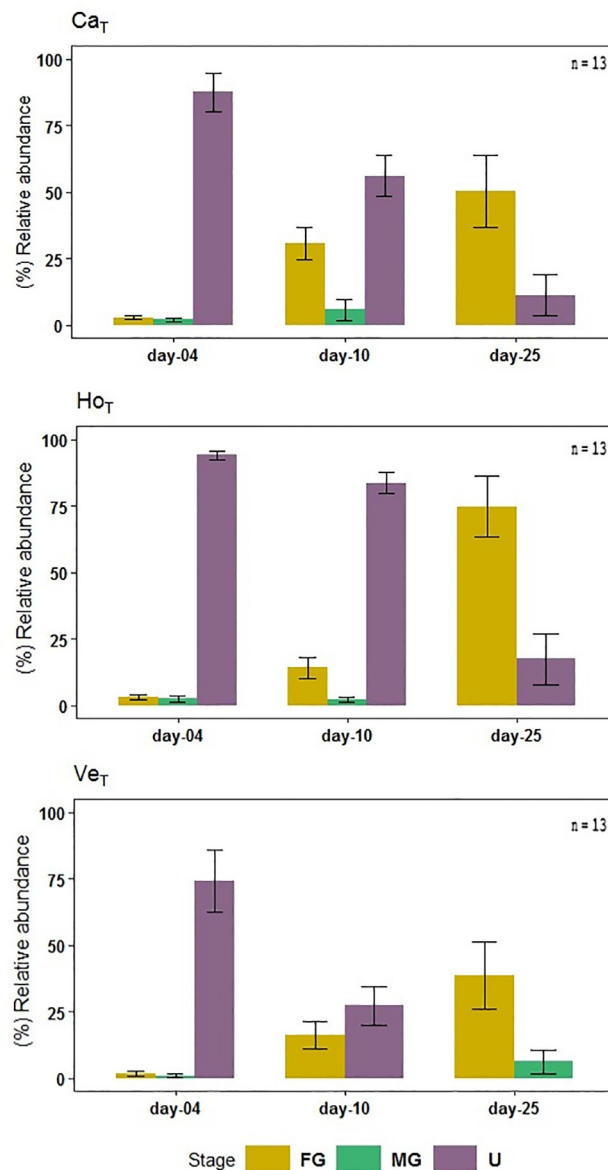
<https://doi.org/10.1371/journal.pone.0240581.g006>



**Fig 7. Gametophyte development.** Development from settled spores to undifferentiated gametophyte during 37 days of exposure to the treatments, showing SD (n = 15). The legend indicates the following life cycle stages: Spores (S), Spores with germ tube (G) and Undifferentiated gametophyte (U) (Fig 3A–3C). Ca: Cachagua, Ho; Horcón, Ve; Ventanas. T; treatment.

<https://doi.org/10.1371/journal.pone.0240581.g007>

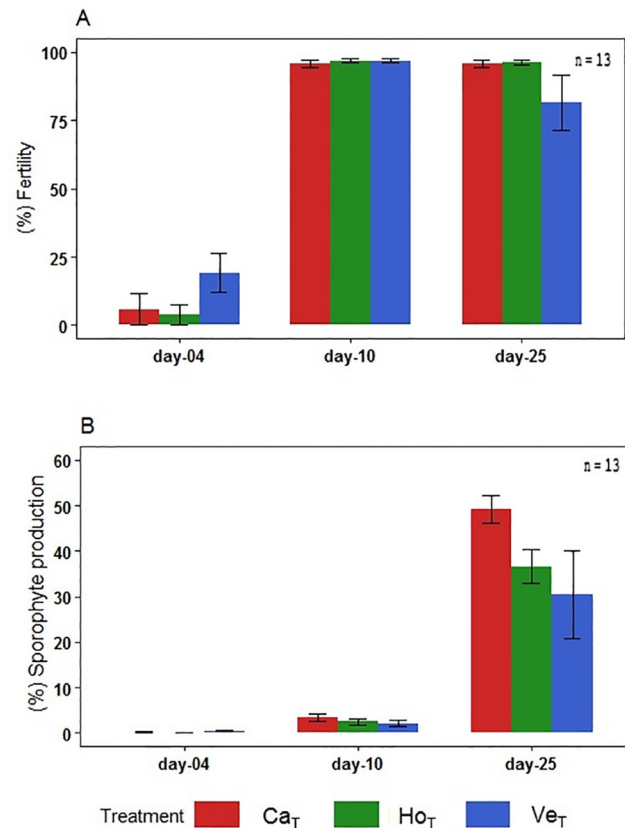
Cachagua and Horcón at days 10 and 25 in comparison to Ventanas (K-W = Cachagua < 0.001, Horcón < 0.001) (Fig 8). A low percentage of Male gametophytes (MG) was registered in all treatments (Fig 8). Indeed, after 10 days of exposure the MG % in all treatment was lower than 10% (Cachagua = 6%, Horcón = 2% and Ventanas = 0%), and at the end of the experiment only Ventanas presented a 6% of MG (Fig 8).



**Fig 8. Sexual differentiation.** Development from undifferentiated gametophyte to sexual gametophytes during 25 days of exposure to treatment, showing SD ( $n = 13$ ). The legend indicates the different stages: Undifferentiated gametophyte (U), Female gametophyte (FG) and Male gametophyte (MG) (S2C–S2E Fig). Ca; Cachagua, Ho; Horcón, Ve; Ventanas. T; treatment.

<https://doi.org/10.1371/journal.pone.0240581.g008>

**3.2.5. Effect of treatments on fertility and sporophyte production.** Between days 4 and 10 of exposure, the three treatments displayed an increase of fertility, corresponding to approximately 96–97% (Fig 9A). Between days 10 and 25, this percentage remained stable in Cachagua and Horcón, but fertility in Ventanas decreased to 81% (Fig 9A). On the other hand, the sporophyte production indicated that Cachagua and Horcón significantly increased their percentage of sporophytes from 0 to 49% and from 0% to 37%, respectively (K-W = Cachagua < 0.001, Horcón < 0.001) (Fig 9B). Ventanas was the treatment with the



**Fig 9. Fertility and sporophyte formation.** A) Trends in the percentage of fertility and sporophyte formation based on the number of female gametophytes, and B) percentage of sporophyte production. Ca; Cachagua, Ho; Horcón, Ve; Ventanas. T; treatment.

<https://doi.org/10.1371/journal.pone.0240581.g009>

lowest percentage of sporophyte production (31%) and showed significant differences in comparison to the other sites (K-W = 0.035).

#### 4. Discussion

There are many factors involved in the fitness and development of seaweeds such as *L. spicata*, especially in terms of pollution impact evaluations. One unexpected result of this work is that the HMs concentration of Cu and As in seawater and sediment samples was above the international normative in all three sampling sites. Additionally, our results supply information that suggests that both adults and early life stages of this kelp are negatively affected once exposed to seawater from sites that are closer to a highly polluted focus, such as the industrial park of Quintero, rather than distant sites. Specifically, in terms of morphological features, all the sampling sites of this study presented average densities of *L. spicata* that were considerably lower ( $< 5$  holdfast  $m^{-2}$ ) than those previously reported in the coast of Central Chile [56, 57]. Additionally, for this species, it is reported that the maximum fertility is observed in July [47]. However, the site that is closest to the pollution source (Ventanas), had the largest percentage of recruits and juveniles (62%) and fully mature adults were absent. Indeed, the adult kelps from Ventanas exhibited the smallest average holdfast area ( $32\text{ cm}^2$ ), the shortest blades (71 cm) and width diameter (8.7 cm), in comparison with the other two sites. On the contrary, *L. spicata*

individuals from the most distant site (Cachagua) showed a higher heterogeneity in terms of morphology and showed the highest average length of blades, implying that this population presented both, young and old individuals. Also, Horcón was slightly separated from Ventanas in the PCA, because it showed the highest concentrations of Cu in marine sediments and the larger kelp average holdfast area, representing a transition zone in terms of morphology. However, the three kelp populations were clearly separated in relation to their morphometry and heavy metal concentrations in seawater and sediments (Fig 4). These results were similar to those reported by Vásquez et al. [58] who found a negative relationship between the size of adult individuals of the kelp *Lessonia trabeculata* and proximity to a mining pollution source. More specifically, these authors observed that populations of *L. trabeculata* were absent in the first 0 to 3 km from the pollution source, whereas from 3 to 5 km kelp individuals were present, and their sizes increased significantly with distance. These results are related with the evidence that heavy metals excess in algae triggers an overproduction of reactive oxygen species (ROS) and leads to an oxidative stress condition, which negatively affects kelps ultrastructure, growth, and physiology [4, 58]. Indeed, a possible explanation for the variation of morphological features among the populations in this study is based on Sáez et al. [16], who demonstrated in *L. trabeculata* that patterns of metal accumulation in kelps parts such as holdfast, stipe and blades are metal specific and each part is impacted differently.

In terms of HMs concentration, all three sites were highly polluted with Cu and As above the international normative; nonetheless, Ag, Cd and Pb concentrations in sediment samples of the three sites were at least one order of magnitude inferior to those previously reported for QB by Parra et al. [41]. The historical environmental information about Ventanas suggests that other pollutants may be present in seawater, such as polycyclic aromatic hydrocarbons (PAHs) resulting from oil refinery activities (in higher concentrations than in Horcón and Cachagua) and could explain the smaller kelp sizes from Ventanas. We did not find specific information about population adaptations to chemicals such as PAHs in seaweeds, but it has been previously demonstrated that photosynthetic organisms under sublethal stresses (e.g. chemicals, temperature) incur a fitness cost, reallocating resources to costly stress responses and maintenance of homeostasis [59]. Accordingly, Ventanas kelp individuals would have been affected in a similar way as terrestrial plants, where physico-chemical stressors result in growth inhibition, decreased biomass and smaller body sizes, plus impacts on other traits that we did not assess such as presence of reproductive tissue or photosynthetic activity [60]. So, based on our results plus the environmental history of Ventanas, we suggest that adult kelps of this site reached shorter sizes than those from less-polluted environments due to excess of different pollutants and to a long-term exposure, i.e. chronic exposure to pollutants.

The analysis of seawater and marine sediments samples showed that our initial premise was wrong because the pollution did not decrease significantly with increasing distance from the industrial focus. In fact, unexpectedly, in relation to Cu and As levels in seawater and sediment samples, all three sites showed levels above the international norm (EPA, [51]) for acute and chronic exposure in seawater, and for TEL and PEL in sediments (according to the CCME, [53]) (Tables 1 and 2). The only exception to this trend was the relatively unpolluted sediments of Cachagua for Cu concentration. Indeed, seawater samples from Ventanas and Horcón exhibited high Cu concentrations similar to those previously reported by Contreras [30] and FIC [36] in QB. Nevertheless, among the three sites, Cachagua showed the highest concentrations of Cu in seawater, and also the highest levels of As in sediments. Regarding Cu levels in sediments, Horcón registered the highest value among the three sites ( $82,203 \mu\text{g kg}^{-1}$ ). Horcón sediments probably showed this high Cu concentration because this sampling site is a fishing cove, where high concentrations of organic matter occur, which facilitates Cu sedimentation and increases the concentration of this metal in marine sediments [61].

In our study, maximum Cu concentrations in sediments were in the same order of magnitude as those reported within QB by De Gregori et al. [62], Sáez et al. [63] (52,000 and 68,000  $\mu\text{g kg}^{-1}$ , respectively) but lower than those of Parra et al. [41]. Specifically, Cachagua and Ventanas displayed maximum Cu concentrations in sediments that were lower compared to the control site reported by Parra et al. [41] (41,000  $\mu\text{g kg}^{-1}$ ); on the other hand, Cu level at Horcón was higher than the control but lower than most of the sampling stations of Parra et al. [41] (range 41,000–1,476,000  $\mu\text{g kg}^{-1}$ ). Moreover, sediment samples from Horcón and Ventanas showed maximum As concentrations that were similar to those reported within QB by Parra et al. [41]; on the contrary, Cachagua's sediment samples exhibited a maximum As concentration that was as high as the most polluted sampling station of Parra et al. [41], which was located just in front of the main copper refinery at QB. The concentration of Cd, Ag and Pb in sediments was also lower than those reported for the sampling station used as control in a previous study of Parra et al. [41], where the concentrations correspond to samples collected closer to the industrial discharge in comparison to our study, i.e. mostly within QB.

In summary, for sediment samples, a mixed result was observed. On the other hand, Cu and As levels in the seawater from all three sites were above the international norm and were higher than those of previous reports for more distant sites such as Cachagua. These results agree with the findings of Celis et al. [64], who reported higher concentrations of HMs (As, Pb and Cu) in Humboldt penguin feces (*Spheniscus humboldti*) from Cachagua Island compared to previous reports. In the past, this site was indeed considered a non-impacted site, with reported Cu concentration values of  $< 1$  to  $5 \mu\text{g L}^{-1}$  in seawater [13, 41]. One possible explanation for the high concentrations of As and Cu found in Cachagua could be the intensification of industrial activity in the Valparaíso region without specific normative for the reduction of pollution in this zone, and the increase and expansion of environmental pollution from QB [65]. Because the water currents along the Humboldt Current System (HCS) flow predominantly northward [66], we can hypothesize that in Cachagua (located north of the industrial focus of QB), the HCS would provide a constant and frequent flux of polluted water masses and/or polluted biomass, carrying high concentrations of HMs. Consequently, this work supplies more evidence to suggest a pollution expansion from Quintero Bay along the Chilean coast.

The results from our *in vitro* experiments showed different responses depending on the developmental process or stage. However, the early life stages of *L. spicata* exposed to Cachagua seawater exhibited a greater performance than when they were exposed to seawaters from the two sites closest to the pollution source of QB. Although the concentration of total Cu and As in the seawater of Cachagua was higher in comparison with the other two sites (see previous section), our results indicate a tendency of higher spore release and spore settlement in the Cachagua treatment than in the Ventanas and Horcón. Spore release of Cachagua was approximately 30% higher than Ventanas, but only 10% higher than Horcón treatment at 7 h of exposure (Fig 5). Similarly, the reduction of spore settlement in both Ventanas and Horcón treatments compared to Cachagua was 25–30% after 120 h of exposure (Fig 6). Because, as previously mentioned, seawater samples collected from all three sites displayed Cu and As levels above the international criteria, it is not likely that these two compounds explain this differential performance. Thus, we hypothesize that additional pollutants (e.g. PAHs) which are present in the seawater from Ventanas and Horcón, and which are probably present at lower concentration in Cachagua's seawater, might significantly enhance the effects of HMs (synergic effects). In fact, previous studies have demonstrated the presence of several PAHs in the seawater of Ventanas and Horcón, such as: acenaphthene, benzo (a) pyrene, phenanthrene, pyrene, among others [36]. Additionally, human activities such as the burning of fossil fuels, coal and biomass, or ship transport and oils spills have been shown to occur frequently in both



sites, all causing a significant rise in PAHs levels [36, 67]. Indeed, Contreras-Porcía et al. [17] recently evidenced synergistic negative effects of binary mixtures of PAHs and HMs (Cu and Cd) on spore release and settlement in *M. pyrifera* and *L. spicata*; probably explaining the lower performance of Ventanas and Horcón treatments compared to Cachagua. On the other hand, Contreras et al. [18] showed that the exposure of *L. berteriana* to Cu concentrations equal or higher than  $8 \mu\text{g L}^{-1}$  cause a decrease (over 50%) on spore release. This would indicate that populations of *L. spicata* that are closer to the QB industrial park in Central Chile have developed a greater tolerance to Cu than *L. berteriana*, and probably other populations of *L. spicata* from less impacted sites.

Spore germination and gametophyte development of *L. spicata* was achieved successfully, with the presence of undifferentiated gametophytes in all three seawater treatments after 13 days of culture, which is within the usual time frame previously reported for this species under similar culture conditions. Although the number of undifferentiated gametophytes was higher in Cachagua's treatment than in the others, and some delay in gametophyte development could be suggested in Ventanas and Horcón treatments compared to Cachagua (see Results), no major developmental differences were evident from data analyses. Nonetheless, all three treatments showed a large proportion of spores that did not germinate, and of germinated spores that did not develop into gametophytes, during the 37 days of the culture period (Fig 7). A previous study on the kinetics of early life stage development of *L. spicata* from central Chile showed that, under no addition of toxic (control condition), one third of spores did not germinate during forty days of culture. Nonetheless, for the rest of more advanced early life stages, this last study showed that each successive life stage (germinated spores, undifferentiated and differentiated gametophytes, and sporophytes) followed a phase of growth and then a sharp decrease in their numbers until its development (with some time delay) to pass to the next life stage. Contreras et al. [18] also demonstrated that when treated with increasing amounts of Cu, as in our case, a large proportion of spores of *L. spicata* do not germinate, and a large proportion of germinated spores do not develop into undifferentiated gametophytes; moreover, very high concentrations of Cu resulted in a complete inhibition of post-germination stages, which was not the case for our three seawater treatments (see Fig 6 of Contreras et al. [18]). This supports the fact that, probably, although the seawater from all three sites considered in the present study had high levels of pollutants, early life stages of *L. spicata* from central Chile were able to cope with these high levels of pollution, even though they undergo sub-lethal effects (such as a delayed development). Undifferentiated gametophytes were observed during the 40 days of exposure in all treatments, lasting longer than the documented normal period of development under standard culture conditions [68]. This is in accordance with the findings by Bond et al. [69] who demonstrated a delay in the development of germlings of *Fucus spiralis* when exposed to an excess of Cu. Similarly, our results agree with Chung & Brinkhuis [70], who found that Cu concentrations greater than or equal to  $50 \mu\text{g L}^{-1}$  caused a delay in gametophyte development of *Laminaria saccharina*. On the other hand, Tala et al. [71] reported the recovery capacity of early life stages of *Lessonia* species after stress, evidencing that surviving spores retained their viability.

Our results showed that under exposure to the three seawater treatments, sexual differentiation of gametophytes was attained during the culture period (at day 25) despite the high HMs concentration used. Although percentages of differentiated gametophytes were lower for Ventanas than Cachagua and Horcón (Fig 8), the proportion of male (5%) to female gametophytes (95%) was extremely female-biased (sex ratio = 0.05) in all treatments. Previous studies have demonstrated that in natural populations of *L. spicata*, male and female gametophytes generally occur in equal proportions (sex ratio = 0.5) [72]. However, this ratio could change under  $T^\circ$ , pH or other environmental fluctuations [20, 72, 73]. For example, Leal et al. [20] evidenced

the interactive effects of pH, T° and Cu on the sex ratio of *M. pyrifera* and *Undaria pinnatifida*. A decrease in the proportion of male to female gametophytes has also been previously reported for the Chilean coast. For example, Oppliger et al. [74] determined that in *L. spicata* populations from cooler southern sampling sites (El Quisco, Las Cruces and Valdivia), the proportion of male gametophytes decreased significantly with temperature, compared to the warmer northern sites. Likewise, Nelson [75] reported that cultivation of *Lessonia variegata* under the combined conditions of 15°C and a photoperiod of 15:9 results in a female-biased sex ratio, suggesting that males are less tolerant to stressful conditions than females. In this context, and according to our results and previous literature, we postulate that male gametophytes of *L. spicata* are more sensitive to polluted seawater than female gametophytes. Moreover, since all three treatments considered in this study exhibited an extremely female-biased sex ratio, this would indicate that *L. spicata* populations from the three sites are probably subjected to sublethal toxic effects resulting from high levels of pollution. All three treatment cultures showed a considerable increase in fertility after 4 days of culture (80–90%) (Fig 9), although the number of male gametophytes formed was extremely low. One possible explanation of this sex ratio bias could be parthenogenesis. Parthenogenesis, frequently involving the development of a female gamete without male fertilization, has been reported in terrestrial and marine organisms, and is a form of asexual reproduction which is prevalent in stressful environmental conditions, in marginal populations [74]. Parthenogenesis can modify the sex ratio, and it has already been reported to occur in *Lessonia* species in nature, as well as under controlled culture conditions [54, 74]. Additionally, we determined that Ventanas was the treatment with the lowest percentage of sporophyte production (31%) which showed significant differences compared to the other two treatments. However, the sporophytes production (25–50%) in all the treatments did not increase significantly over exposure time, indicating sub-lethal toxic effect of pollution on *L. spicata* sporophyte production. A disruption of sporophyte development was also previously demonstrated in *Laminaria hyperborea* chronically exposed to Cu excess, which would affect population's stability in HMs impacted sites [76]. Therefore, our results suggest that chemical pollution has a significant impact on sporophyte production in *L. spicata* populations from sites that are highly impacted by anthropogenic pollution. Moreover, parthenogenesis, which would result from a low viability of male gametophytes under chemical stress conditions, could be operating in these polluted environments, probably explaining the extremely female-biased sex ratio.

Some previous studies demonstrated [77, 78] that there is an interactive effect of *in situ* environmental conditions (such as temperature, day length, light intensity, and ultraviolet radiation) on gametophyte and sporophyte growth and survival. This would explain the temporal (stationary) variation in the gametophyte and sporophyte performance in *Ecklonia radiata* and *Laminaria solidungula*, respectively. Our *in vitro* experiments were performed using sori collected from a single site belonging to the same *L. spicata* population and were additionally subjected to the same laboratory conditions. Therefore, we discard that different environmental conditions would significantly explain the differences in performance between early life stages of *L. spicata* treated with different seawater treatments. On the contrary, it is more likely that the differences observed in gametophyte development (and other early life stages) in our *in vitro* experiments would be related to the different pollutants and concentrations, presents in the three types of seawater used in toxic treatments.

It is important for future studies to highlight that the high concentrations of HMs in all three sampling sites, both near the industrial park of Quintero Bay and 40 km away, suggest an expansion of industrial pollution from QB since the year 2000. In fact, in all three *L. spicata* populations surveyed in this study, the density of adult individuals was significantly lower than previously reported in central Chile. The small sizes of kelps would have negative bottom-up

effects on higher trophic levels [79]. In fact, kelps provide a three-dimensional habitat for biodiversity, which depends on their sizes and morphology. Specifically, Villegas et al. [80] showed that in the case of the kelp *L. trabeculata*, the maximum holdfast diameter is strongly and positively correlated with the size (and the weight) of other morphological traits; and that these morphological variables drive the invertebrate biodiversity. Although a tendency of higher performance of early life stages was observed in the treatment using seawater from Cachagua compared to Horcón and Ventanas, sublethal effects of pollution on early life stages (spore release, settlement, gametophyte and sporophyte production) were observed in all three treatments. This confirms that all three sites have differential but high levels of pollution, which contradicted our initial premise that Cachagua would be less polluted than the other two sites. Remarkably, relatively low sporophyte production and fertility, and an extremely female-biased sex ratio (0.05) were observed under seawater exposure from all three sites. This suggests that parthenogenesis could be operating under these chemically polluted and stressful conditions. In conclusion, this work supports that pollution expansion has already occurred along the central coast from Quintero Bay, negatively affecting the quality of seawater, sediments, kelp populations, and probably the entire marine ecosystem. All the previous results imply that there is an urgent need to improve the environmental normative determining the maximum levels of pollutants allowed, and the environmental pollution control programs and policies of Chile, especially considering foundation kelp species.

## Supporting information

**S1 Fig. Map of sampling sites.** Georeferencing of the sampling sites, the framed area indicates the Industrial Park “Las Ventanas” located in Quintero Bay, Valparaíso Region. From north to south; Cachagua, Horcón and Ventanas.

(TIF)

**S2 Fig. Early developmental stages of *Lessonia spicata*.** Inverted microscope photos of: A. Spore (S), B. Germinated spore (G), C. Undifferentiated gametophyte (U), D. Male gametophyte (MG) and E. Female gametophyte with sporophytes and an egg cell (arrow) (FG).

(TIF)

**S3 Fig. Scheme of *in vitro* experiments.** Scheme about time of developmental stages and each data recording. The colour bars indicate the time of respective stage recording. In the case of spore release, settlement and germination and gametophyte development, the darker colour indicates the data time that was considered for the statistical analysis.

(TIF)

**S1 Table. Statistical analysis of gametophyte development.** Results of Kruskal-Wallis and Mann-Whitney pairwise comparisons between treatments per day per stage during gametophyte development; Spore (S), Germinated spore (G) and Undifferentiated gametophyte (U). Different letters indicate statistical differences between the treatments.

(DOCX)

**S2 Table. Statistical analysis of sexual differentiation.** Results of Kruskal—Wallis and Dunn’s test for each developmental stage percentage (Undifferentiated gametophyte (U), Female gametophyte (FG) and Male gametophyte (MG)) between 4, 10 and 25 days of exposure per treatment.

(DOCX)

## Acknowledgments

The authors acknowledge language support provided by ARGA Intercultural ([www.arga.com.ar](http://www.arga.com.ar)).

## Author Contributions

**Conceptualization:** Carolina Oyarzo-Miranda, Cristian Bulboa, Loretto Contreras-Porcía.

**Data curation:** Carolina Oyarzo-Miranda, Nicolás Latorre, Andrés Meynard.

**Formal analysis:** Carolina Oyarzo-Miranda, Nicolás Latorre, Andrés Meynard, Loretto Contreras-Porcía.

**Funding acquisition:** Cristian Bulboa, Loretto Contreras-Porcía.

**Investigation:** Carolina Oyarzo-Miranda, Cristian Bulboa, Loretto Contreras-Porcía.

**Methodology:** Carolina Oyarzo-Miranda, Cristian Bulboa, Loretto Contreras-Porcía.

**Project administration:** Carolina Oyarzo-Miranda, Loretto Contreras-Porcía.

**Resources:** Loretto Contreras-Porcía.

**Supervision:** Cristian Bulboa, Loretto Contreras-Porcía.

**Validation:** Cristian Bulboa, Loretto Contreras-Porcía.

**Visualization:** Loretto Contreras-Porcía.

**Writing – original draft:** Carolina Oyarzo-Miranda, Andrés Meynard, Jorge Rivas, Cristian Bulboa, Loretto Contreras-Porcía.

**Writing – review & editing:** Carolina Oyarzo-Miranda, Andrés Meynard, Jorge Rivas, Cristian Bulboa, Loretto Contreras-Porcía.

## References

1. Walker CH, Colin H, Sibly RM, Hopkin SP, Peakall DB. Principles of ecotoxicology. 4th ed, Taylor & Francis Group; 2012.
2. Chen CW, Kao CM, Chen CF, Dong CD. Distribution and accumulation of heavy metals in the sediments of Kaohsiung Harbor, Taiwan. *Chemosphere* 2007; 66(8):1431–40. <https://doi.org/10.1016/j.chemosphere.2006.09.030> PMID: 17113128
3. Nagajyoti PC, Lee KD, Sreekanth TVM. Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem Lett.* 2010; 8:199–216. <https://doi.org/10.1007/s10311-010-0297-8>
4. Contreras-Porcía L, Meynard A, López-Cristoffanini C, Latorre N, Kumar M. Marine metal pollution and effects on seaweed species. In *Systems Biology of Marine Ecosystems*, Springer International Publishing AG; 2017; 35–48. [https://doi.org/10.1007/978-3-319-62094-7\\_3](https://doi.org/10.1007/978-3-319-62094-7_3)
5. Castilla JC. Environmental impacts in sandy beaches of copper mine tailing at Chañaral, Chile. *Mar Pollut Bull.* 1983; 14:459–64. [https://doi.org/10.1016/0025-326X\(83\)90046-2](https://doi.org/10.1016/0025-326X(83)90046-2)
6. Wong MH. Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere* 2003; 50(6):775–80. [https://doi.org/10.1016/s0045-6535\(02\)00232-1](https://doi.org/10.1016/s0045-6535(02)00232-1) PMID: 12688490
7. Medina M, Andrade S, Faugeron S, Lagos N, Mella D, Correa JA. Biodiversity of rocky intertidal benthic communities associated with copper mine tailing discharges in northern Chile. *Mar Pollut Bull.* 2005; 50(4):396–409. <https://doi.org/10.1016/j.marpolbul.2004.11.022> PMID: 15823301
8. Stuart-Smith RD, Edgar GJ, Stuart-Smith JF, Barrett NS, Fowles AE, Hill NA, et al. Loss of native rocky reef biodiversity in Australian metropolitan embayment. *Mar Pollut Bull.* 2015; 95(1):324–32. <https://doi.org/10.1016/j.marpolbul.2015.03.023> PMID: 25882229
9. Zhou Q, Zhang J, Fu J, Shi J, Jiang G. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. *Anal Chim Acta.* 2008; 606(2):135–50. <https://doi.org/10.1016/j.aca.2007.11.018> PMID: 18082645

10. Farias DR, Hurd CL, Eriksen RS, Macleod CK. Macrophytes as bioindicators of heavy metal pollution in estuarine and coastal environments. *Mar Pollut Bull.* 2018; 128:175–84. <https://doi.org/10.1016/j.marpolbul.2018.01.023> PMID: 29571361
11. Davis TA, Volesky B, Mucci A. A review of the biochemistry of heavy metal biosorption by brown algae. *Water Res.* 2003; 37(18):4311–30. [https://doi.org/10.1016/S0043-1354\(03\)00293-8](https://doi.org/10.1016/S0043-1354(03)00293-8) PMID: 14511701
12. Fertah M, Belfkira A, Dahmane E, Taourirte M, Brouillette F. Extraction and characterization of sodium alginate from Moroccan *Laminaria digitata* brown seaweed. *Arab J Chem.* 2017; 10:S3707–14. <https://doi.org/10.1016/j.arabjc.2014.05.003>
13. Contreras L, Moenne A, Correa JA. Antioxidant responses in *Scytosiphon lomentaria* (Phaeophyceae) inhabiting copper enriched coastal environments. *J Phycol.* 2005; 41(6):1184–95. <https://doi.org/10.1111/j.1529-8817.2005.00151.x>
14. Ritter A, Ubertini M, Romac S, Gaillard F, Delage L, Mann A, et al. Copper stress proteomics highlights local adaptation of two strains of the model brown alga *Ectocarpus siliculosus*. *Proteomics* 2010; 10(11):2074–88. <https://doi.org/10.1002/pmic.200900004> PMID: 20373519
15. Evan LK, Edwards MS. Bioaccumulation of copper and zinc by the giant kelp *Macrocystis pyrifera*. *Algae* 2011; 26(3):265–75. <https://doi.org/10.4490/algae.2011.26.3.265>
16. Sáez CA, Lobos MG, Macaya EC, Oliva D, Quiroz W, Brown MT. Variation in patterns of metal accumulation in thallus parts of *Lessonia trabeculata* (Laminariales; Phaeophyceae): implications for biomonitoring. *PLoS ONE* 2012; 7(11):e50170. <https://doi.org/10.1371/journal.pone.0050170> PMID: 23166836
17. Contreras-Porcía L. Integral impact assessment of heavy metals and organic toxic compounds: effects on the development, growth, and ecosystem function of marine algae. ANID FONDECYT 1170881, 2017–2020
18. Contreras L, Medina MH, Andrade S, Oppliger V, Correa J A. Effects of copper on early developmental stages of *Lessonia nigrescens* Bory (Phaeophyceae). *Environ Poll.* 2007; 145(1):75–83. <https://doi.org/10.1016/j.envpol.2006.03.051> PMID: 16720066
19. Lovazzano C, Serrano C, Correa JA, Contreras-Porcía L. Comparative analysis of peroxiredoxin activation in the brown macroalgae *Scytosiphon gracilis* and *Lessonia nigrescens* (Phaeophyceae) under copper stress. *Physiol Plantarum.* 2013; 149(3):378–88. <https://doi.org/10.1111/pp1.12047> PMID: 23489129
20. Leal PP, Hurd CL, Sander SG, Kortner B, Roleda MY. Exposure to chronic and high dissolved copper concentrations impedes meiospore development of the kelps *Macrocystis pyrifera* and *Undaria pinnatifida* (Ochrophyta). *Phycologia* 2016; 55(1):12–20. <https://doi.org/10.2216/15-87.1>
21. Mabin CJ, Johnson CR, Wright JT. Family level variation in early life cycle traits of kelp. *J Phycol.* 2019; 55(2):380–92. <https://doi.org/10.1111/jpy.12820> PMID: 30506918
22. Coelho SM, Rijstenbil JW, Brown MT. Impacts of anthropogenic stresses on the early development stages of seaweeds. *J Aquat Ecosyst Stress Recovery.* 2000; 7(4):317–33. <https://doi.org/10.1023/A:1009916129009>
23. Flórez-Leiva L, Rangel-Campo A, Díaz-Ruiz M, Venera-Pontón DE, Díaz-Pulido G. Efecto de la sedimentación en el reclutamiento de las macroalgas *Dictyota* spp. y *Lobophora variegata*: un estudio experimental en el Parque Nacional Natural Tayrona, Caribe colombiano. *Bol Invest Mar Cost.* 2010; 39(1):41–56.
24. Thompson SA, Knoll H, Blanchette CA, Nielsen KJ. Population consequences of biomass loss due to commercial collection of the wild seaweed *Postelsia palmaeformis*. *Mar Ecol Prog Ser.* 2010; 413:17–31
25. Fink LA, Manley SL. The use of kelp sieve tube sap metal composition to characterize urban runoff in southern California coastal waters. *Mar Pollut Bull.* 2011; 62:2619–32. <https://doi.org/10.1016/j.marpolbul.2011.09.035> PMID: 22030107
26. Aminina N, Shaposhnikova T. Peculiarities of growth and metabolism in Japanese kelp in habitats exposed to chronic contamination. *Res Plant Biol.* 2012; 2(1):32–40. Available from: <https://updatepublishing.com/journal/index.php/ripb/article/view/2563>
27. Vega JA, Broitman BR, Vásquez JA. Monitoring the sustainability of *Lessonia nigrescens* (Laminariales, Phaeophyceae) in northern Chile under strong harvest pressure. *J Appl Phycol.* 2014; 26(2):791–801 <https://doi.org/10.1007/s10811-013-0167-4>
28. Mineur F, Arenas F, Assis J, Dacies AJ, Engelen AH, Fernandes F, et al. European seaweeds under pressure: consequences for communities and ecosystem functioning. *J Sea Res.* 2015; 98: 91–108. <https://doi.org/10.1016/j.seares.2014.11.004>
29. Fariña JM, Castilla JC. Temporal variation in the diversity and cover of sessile species in rocky intertidal communities affected by Cu mine tailings in northern Chile. *Mar Pollut Bull.* 2001; 42 (7):554–68. [https://doi.org/10.1016/s0025-326x\(00\)00201-0](https://doi.org/10.1016/s0025-326x(00)00201-0) PMID: 11488236

30. Contreras M. Análisis de Riesgo Ecológico por sustancias potencialmente contaminantes en el aire, suelo y agua, en las comunas de Concón, Quintero y Puchuncaví. Quinta Región de Valparaíso. Final Report. Environmental Ministry. 2013. Spanish [http://www.munipuchuncavi.cl/estudios/articulos-55902\\_InformeFinal\\_CEA](http://www.munipuchuncavi.cl/estudios/articulos-55902_InformeFinal_CEA)
31. Larsson N. The brutal reality of life inside one of the world's most polluted cities, Wired Magazine, 2020 [cited: February 24th, 2020]. <https://www.wired.co.uk/article/chile-quintero-pollution>
32. Informe de la comisión de recursos naturales, bienes nacionales y medio ambiente recaído en el mandato otorgado por la sala a fin de analizar, indagar, investigar y determinar la participación de la empresa estatal CODELCO y empresas asociadas, en la contaminación ambiental en la zona de Puchuncaví y Quintero, Final Report. Comisión de Recursos Naturales, Bienes Nacionales y Medio Ambiente. 2011. <https://www.camara.cl/verDoc.aspx?prmTipo=SIAL&prmID=45601&formato=pdf>
33. Bernalte E, Salmanighabeshi S, Rueda-Holgado F, Palomo-Marín MR, Marín-Sánchez C, Cereceda-Balic F, et al. Mercury pollution assessment in soils affected by industrial emissions using miniaturized ultrasonic probe extraction and ICP-MS. *Int J. Environ Sci Technol*. 2015; 12:817–26. <https://doi.org/10.1007/s13762-013-0461-3>
34. Rueda-Holgado F, Calvo-Blázquez L, Cereceda-Balic F, Pinilla-Gil E. Temporal and spatial variation of trace elements in atmospheric deposition around the industrial area of Puchuncaví-Ventanas (Chile) and its influence on exceedances of lead and cadmium critical loads in soils. *Chemosphere* 2016; 144:1788–96. <https://doi.org/10.1016/j.chemosphere.2015.10.079> PMID: 26524148
35. Salmani-Ghabeshi S, Palomo-Marín MR, Bernalte E, Rueda-Holgado F, Miró-Rodríguez C, Cereceda-Balic F, et al. Spatial gradient of human health risk from exposure to trace elements and radioactive pollutants in soils at the Puchuncaví-Ventanas industrial complex, Chile. *Environ Pollut*. 2016; 218:322–30. <https://doi.org/10.1016/j.envpol.2016.07.007>
36. FIC-ALGAS. Cultivo de alga parda *Macrocystis pyrifera* en la zona de Quintero y Puchuncaví: evaluación de la productividad y potencial uso para biorremediación de metales pesados y compuestos orgánicos. N° 30397482–0, 2016.
37. Vásquez JA, Santelices B. Comunidades de macroinvertebrados en discos adhesivos de *Lessonia nigrescens* Bory (Phaeophyta) en Chile central. *Rev Chil Hist Nat*. 1984; 57:131–54. Spanish
38. Álvarez-Campos P, Verdes A. Syllids inhabiting holdfasts of *Lessonia spicata* in Central Chile: diversity, systematics, and description of three new species. *Syst Biodivers*. 2017; 15(6):520–531. <https://doi.org/10.1080/14772000.2017.1285364>
39. Vásquez JA, Piaget N, Vega JMA. The *Lessonia nigrescens* fishery in northern Chile: “how you harvest is more important than how much you harvest”. *J Appl Phycol*. 2012; 24(3):417–26. <https://doi.org/10.1007/s10811-012-9794-4>
40. Ortega KJ, Sáez CA, Macaya EC. Changes in invertebrate assemblages inhabiting *Lessonia spicata* (Phaeophyceae) holdfasts after the 2010 earthquake-mediated coastal uplift in Chile. *Rev Biol Mar Oceanog* 2014; 49(1):129–34. <https://doi.org/10.4067/S0718-19572014000100014>
41. Parra S, Bravo MA, Quiroz W, Querol X, Paipa C. Distribution and pollution assessment of trace elements in marine sediments in the Quintero Bay (Chile). *Mar Pollut Bull* 2015; 99(1–2):256–63. <https://doi.org/10.1016/j.marpolbul.2015.07.066> PMID: 26254027
42. Valenzuela Pérez LF. Coppered lives: The Chilean sacrifice zone of Quintero Bay. 2016 PhD Thesis, University of Sydney. 2016 <http://hdl.handle.net/2123/15914>
43. Ratkevicius N, Correa JA, Moenne A. Copper accumulation, synthesis of ascorbate and activation of ascorbate peroxidase in *Enteromorpha compressa* (L.) Grev. (Chlorophyta) from heavy metal-enriched environments in northern Chile. *Plant Cell Environ* 2003; 26(10):1599–608. <https://doi.org/10.1046/j.1365-3040.2003.01073.x>
44. Zaldívar JR. Homologación de categorías de manejo de áreas protegidas UICN a los Santuarios de la Naturaleza de Chile. Final Report. United Nations Development Programme, Global environmental facility, Environmental Ministry. 2015. Spanish. [http://bdmap.mma.gob.cl/recursos/privados/Recursos/CNAP/GEF-SNAP/Rivera\\_2015.pdf](http://bdmap.mma.gob.cl/recursos/privados/Recursos/CNAP/GEF-SNAP/Rivera_2015.pdf)
45. Requirements that the research fishery requests must fulfil No 461. 1995 Ministry of economy, development and reconstruction, Spanish [http://www.subpesca.cl/portal/618/articles-5959\\_documento.pdf](http://www.subpesca.cl/portal/618/articles-5959_documento.pdf)
46. US EPA (Environmental Protection Agency) Methods for Chemical Analysis of Water and Wastes, Office of Research and Development. US. E.P.A., Cincinnati, Ohio, 1974. [https://www.wbdg.org/FFC/EPA/EPACRIT/epa600\\_4\\_79\\_020.pdf](https://www.wbdg.org/FFC/EPA/EPACRIT/epa600_4_79_020.pdf)
47. Araujo Casares FA, Faugeton S. Higher reproductive success for chimeras than solitary individuals in the kelp *Lessonia spicata* but no benefit for individual genotypes. *Evol Ecol* 2016; 30(5):953–72. <https://doi.org/10.1007/s10682-016-9849-0>
48. Moreno-Garrido I, Pérez S, Blasco J. Toxicity of silver and gold nanoparticles on marine microalgae. *Mar Environ Res* 2015; 111:60–73. <https://doi.org/10.1016/j.marenvres.2015.05.008> PMID: 26002248

49. Sfakianakis DG, Renieri E, Kentouri M, Tsatsakis AM. Effect of heavy metals on fish larvae deformities: a review. *Environ Res* 2015; 137:246–55. <https://doi.org/10.1016/j.envres.2014.12.014> PMID: 25594493
50. U.S. EPA. "Method 3050B: Acid Digestion of Sediments, Sludges, and Soils," Revision 2. Washington, DC; 1996. <https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf>
51. US EPA National Recommended Water Quality Criteria—Aquatic Life Criteria Table; 1999. [cited: 2019, Jan 15] <https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table#table>
52. Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME). Protocol for the Derivation of Canadian Sediment Quality Guidelines for the Protection of Aquatic Life 1999. [cited: 2018, Dec 13] <http://ceqg-rcqe.ccm.ca/download/en/226>
53. Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME). Summary Table 1999. [cited: 2018, Dec 13] [https://www.elaw.org/system/files/sediment\\_summary\\_table.pdf](https://www.elaw.org/system/files/sediment_summary_table.pdf)
54. Oppliger LV, Correa JA, Peters AF. Parthenogenesis in the brown alga *Lessonia nigrescens* (Laminariales, Phaeophyceae) from central Chile. *J Phycol* 2007; 43:1295–301. <https://doi.org/10.1111/j.1529-8817.2007.00408.x>
55. Reid MK, Spencer KL. Use of principal components analysis (PCA) on estuarine sediment datasets: the effect of data pre-treatment. *Environ Poll* 2009; 157(8–9):2275–81. <https://doi.org/10.1016/j.envpol.2009.03.033> PMID: 19410344
56. Tala F, Edding M. Growth and loss of distal tissue in blades of *Lessonia nigrescens* and *Lessonia trabeculata* (Laminariales). *Aquat Bot* 2005; 82(1):39–54. <https://doi.org/10.1016/j.aquabot.2005.02.009>
57. Tala F, Edding M. First estimates of productivity in *Lessonia trabeculata* and *Lessonia nigrescens* (Phaeophyceae, Laminariales) from the southeast Pacific *Phycol Res*. 2007; 55(1):66–79. <https://doi.org/10.1111/j.1440-1835.2006.00447.x>
58. Vásquez JA, Matsuhiro B, Vega MA, Pardo LM, Véliz D. The effects of mining pollution on subtidal habitats of northern Chile. *Int J Environ Pollut*. 2000; 13:2–25. <https://doi.org/10.1504/IJEP.2000.002330>
59. Whitehead A, Clark BW, Reid NM, Hahn ME, Nacci D. When evolution is the solution to pollution: Key principles, and lessons from rapid repeated adaptation of killifish (*Fundulus heteroclitus*) populations. *Evol Appl*. 2017; 10(8):762–83. <https://doi.org/10.1111/eva.12470> PMID: 29151869
60. Sulmon C, van Baaren J, Cabello-Hurtado F, Gouesbet G, Hennion F, Mony C et al. Abiotic stressors and stress responses: What commonalities appear between species across biological organization levels?. *Environ Pollut*. 2015; 202:66–77. <https://doi.org/10.1016/j.envpol.2015.03.013> PMID: 25813422
61. Chandía C, Salamanca M. Long-term monitoring of heavy metals in Chilean coastal sediments in the eastern South Pacific Ocean. *Mar Poll Bull* 2012; 64:2254–2260. <https://doi.org/10.1016/j.marpolbul.2012.06.030> PMID: 22841495
62. De Gregori I, Pinochet H, Gras N, Muñoz L. Variability of cadmium, copper and zinc levels in molluscs and associated sediments from Chile. *Environ Poll* 1996; 92(3):359–68. [https://doi.org/10.1016/0269-7491\(95\)00077-1](https://doi.org/10.1016/0269-7491(95)00077-1)
63. Sáez CA, Roncarati F, Moenne A, Moody AJ, Brown MT. Copper-induced intra-specific oxidative damage and antioxidant responses in strains of the brown alga *Ectocarpus siliculosus* with different pollution histories. *Aquat. Toxicol* 2015; 159:81–9. <https://doi.org/10.1016/j.aquatox.2014.11.019> PMID: 25521566
64. Celis JE, Espejo W, González-Acuña D, Jara S, Barra R. Assessment of trace metals and porphyrins in excreta of Humboldt penguins (*Spheniscus humboldti*) in different locations of the northern coast of Chile. *Environ Monit Assess* 2014; 186(3):1815–24. <https://doi.org/10.1007/s10661-013-3495-6> PMID: 24464330
65. Muñoz AA, Klock-Barría K, Sheppard PR, Aguilera-Betti I, Toledo-Guerrero I, Christie DA, et al. Multi-decadal environmental pollution in a mega-industrial area in central Chile registered by tree rings. *Sci Total Environ* 2019; 696:133915. <https://doi.org/10.1016/j.scitotenv.2019.133915> PMID: 31461694
66. Thiel M, Macaya EC, Acuña E, Arntz WE, Bastias H, Brokordt K et al. The Humboldt current system of northern and central Chile. *Oceanogr. Mar. Biol. Annu. Rev.* 45: 195–344. <http://www.bedim.cl/publications/thieletalOMBAR2007.pdf>
67. Pastene M, Quiroga E, Hurtado CF. Stable isotopes and geochemical indicators in marine sediments as proxies for anthropogenic impact: A baseline for coastal environments of central Chile (33° S). *Mar. Poll. Bull* 2019; 142:76–84. <https://doi.org/10.1016/j.marpolbul.2019.03.005> PMID: 31232351
68. Oppliger LV, Correa JA, Engelen AH, Tellier F, Vieira V, Faugeton S. et al. Temperature effects on gametophyte life-history traits and geographic distribution of two cryptic kelp species. *PLoS ONE* 2012; 7(6): e39289. <https://doi.org/10.1371/journal.pone.0039289> PMID: 22723987

69. Bond PR, Brown M, Moate R, Gledhill M, Hill SJ, Nimmo M. Arrested development in *Fucus spiralis* (Phaeophyceae) germlings exposed to copper. *Eur J Phycol.* 1999; 34(5):513–521. <https://doi.org/10.1080/09541449910001718871>
70. Chung IK, Brinkhuis BH. Copper effects in early stages of the kelp, *Laminaria saccharina*. *Mar Pollut Bull.* 1986; 17(5):213–18. [https://doi.org/10.1016/0025-326X\(86\)90603-X](https://doi.org/10.1016/0025-326X(86)90603-X)
71. Tala F, Véliz K, Gómez I, Edding M. Early life stages of the South Pacific kelps *Lessonia nigrescens* and *Lessonia trabeculata* (Laminariales, Phaeophyceae) show recovery capacity following exposure to UV radiation. *Phycologia* 2007; 46(4):467–70.
72. Oppliger LV, Correa JA, Faugeron S, Beltran J, Tellier F, Valero M, et al. Sex ratio variation in the *Lessonia nigrescens* complex (Laminariales, Phaeophyceae): Effect of latitude, temperature, and marginality. *J Phycol.* 2011; 47(1): 5–12. <https://doi.org/10.1111/j.1529-8817.2010.00930.x> PMID: 27021705
73. Leal PP, Hurd CL, Sander SG, Armstrong E, Fernández PA, Suhrhoff TJ, et al. Copper pollution exacerbates the effects of ocean acidification and warming on kelp microscopic early life stages. *Sci. Rep* 2018; 8(1):14763. <https://doi.org/10.1038/s41598-018-32899-w> PMID: 30283041
74. Oppliger LV. Reproduction at the range limits of Laminariales at the Chilean and European coasts. PhD Thesis Pontifical Catholic University of Chile. 2010. <https://hal.archives-ouvertes.fr/tel-01146450>
75. Nelson WA. Life history and growth in culture of the endemic New Zealand kelp *Lessonia variegata* J. Agardh in response to differing regimes of temperature, photoperiod and light. *J Appl Phycol.* 2005; 17(1):23–28. <https://doi.org/10.1007/s10811-005-5521-8>
76. Hopkin R, Kain JM. The effects of some pollutants on the survival, growth and respiration of *Laminaria hyperborea*. *Estuar Coast Mar Sci.* 1978; 7(6):531–553. [https://doi.org/10.1016/0302-3524\(78\)90063-4](https://doi.org/10.1016/0302-3524(78)90063-4)
77. Mohring MB, Kendrick GA, Wernberg T, Rule MJ, Vanderklift MA. Environmental Influences on Kelp Performance across the Reproductive Period: An Ecological Trade-Off between Gametophyte Survival and Growth?. *PLoS ONE.* 2013; 8(6): e65310. <https://doi.org/10.1371/journal.pone.0065310> PMID: 23755217
78. Roleda MY. Stress physiology and reproductive phenology of Arctic endemic kelp *Laminaria solidungula* J. Agardh. *Polar Biol.* 2016; 39:1967–1977 <https://doi.org/10.1007/s00300-015-1813-x>
79. Schiel DR, Foster MS. The population biology of large brown seaweeds: ecological consequences of multiphase life histories in dynamic coastal environments. *Annu Rev Ecol Evol Syst.* 2006; 37:343–72. <https://doi.org/10.1146/annurev.ecolsys.37.091305.110251>
80. Villegas MJ, Laudien J, Sielfeld W, Arntz WE. *Macrocystis integrifolia* and *Lessonia trabeculata* (Laminariales; Phaeophyceae) kelp habitat structures and associated macrobenthic community off northern Chile. *Helgol Mar Res.* 2008; 62(1): 33–43 <https://doi.org/10.1007/s10152-007-0096-1>