



Ingestion of contaminated kelps by the herbivore *Tetrapygyus niger*: Negative effects on food intake, growth, fertility, and early development

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ABSTRACT

Macrocystis pyrifera reaches distant areas after detachment, accumulate heavy metals, and serve as trophic subsidy. In this context, effects on both adults and larvae of *Tetrapygyus niger* fed with polluted kelps were determined by assessing growth, fertility, and early larval development. Results revealed that sea urchins fed with polluted kelps from highly impacted zone (HIZ) showed a lower growth (3.6% gained weight) and gamete release (358 cells mL⁻¹) than those fed with non-impacted kelps (NIZ) (19.3% and 945 cells mL⁻¹). The HIZ treatment showed a developmental delay in comparison to NIZ, accounted mainly by the abundance of malformed 2-arm pluteus larvae (10–15%) during most of the culture. Malformed 4-arm pluteus larvae showed a constant increase, reaching 37% at the end of the culture. Thus, the pollutants ingested by sea urchins can be transferred to their offspring and cause negative effects in their early development, categorizing *M. pyrifera* as a pollutant carrier.

1. Introduction

In the marine environment, industrial or domestic wastewater are the main sources of pollutants worldwide (approximately 44%) (Walker et al., 2012). Due to the dynamics of natural processes on earth and human activities, pollutants can be transported long distances, via air or sea currents, and generate an anthropogenic impact even in places where human population density is low or zero (Bedient et al., 1994). In this context, the introduction of pollutants in the environment may have negative effects both in the ecosystems and in human health (Curtis et al., 2006; Lohmann et al., 2007).

Seaweeds, especially kelps, are able to accumulate a great diversity of pollutants, such as metals, phenolic compounds, nutrients surplus, among others (Contreras et al., 2005; Evans and Edwards, 2011; Yu et al., 2016). This occurs principally due to the characteristics of the cell wall, which contains, for instance, alginates that could chelate pollutants, such as metals (Evans and Edwards, 2011). In addition, some kelps such as *Macrocystis pyrifera* (Linnaeus) C. Agardh (distributed along the Chilean coast) can float for long periods of time after being detached by

both natural and anthropogenic factors (Tenger et al., 1995; Hobday, 2000). Drifting kelps maintain their metabolism and can maintain or even generate new reproductive structures, depending of the biotic and abiotic environmental factors (Macaya et al., 2005; Rothäusler et al., 2018). As previously demonstrated, when transported by the currents, kelps can eventually reach the coast again and be used as food by marine and terrestrial organisms, providing a significant trophic subsidy to these ecosystems (Bustamante et al., 1995). We argue here that if these kelps come from or pass through a polluted zone, while drifting, they could bioaccumulate pollutants and exert negative effects through trophic cascades when they are consumed by herbivores such as sea urchins (Vanderklift and Wernberg, 2008).

Among the pollutants, organic compounds and heavy metals cause several negative biological effects. Particularly, heavy metals at high concentrations can cause negative effects on reproductive success, survival rates, and generate lacerations and malformations (Reijnders, 1980; Tanabe, 2002; Golovanova, 2008; Anselmo et al., 2011). For example, Anselmo et al. (2011) evidenced an abnormal development in the sea urchin *Psammechinus miliaris* (Müller) which presented

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malformations in the body and arm bars when exposed to polychlorinated biphenyls (PCBs). Similar effects and a developmental delay in sea urchin larvae were also evidenced when exposed to high levels of zinc and other heavy metals (Kobayashi and Okamura, 2004, 2005). Moreover, these pollutants are bioaccumulated and biomagnified along the trophic network; therefore, as the trophic level increases, the pollutants also increase their concentration (Jamieson et al., 2017; Romero-Romero et al., 2017; Hao et al., 2019).

In Chile, one of the most polluting industrial parks of the country (founded in 1961) is located at Quintero Bay (Valparaíso Region), containing nowadays 15 companies and an operating port. These companies carry out several poorly regulated industrial processes such as metal and oil refinery, coal-fired thermoelectric generation and cement production, among others (Comisión de Recursos Naturales, Bienes Nacionales y Medio Ambiente, 2011; Salmanighabeshi et al., 2015). Indeed, the marine ecosystem in this area is highly polluted with polycyclic aromatic hydrocarbons (PAHs) and heavy metals, principally Al, As, Cd, Cu, Fe and Zn (Parra et al., 2015; FIC-ALGA 2016; Oyarzo-Miranda et al., 2020). This kind of pollution not only affects the health of the resident human population but several types of organisms and probably the entire marine ecosystem of Quintero Bay.

Along the temperate South Pacific coast, from northern Perú to the Strait of Magallanes in Southern Chile, the herbivore sea urchin *Tetrapygus niger* (Molina 1782) is known to feed mainly on kelps, both attached and drifted ones from other localities (Roa, 1990; Rodríguez, 2003). The grazing behavior of sea-urchins can play a key role in driving the switch between two alternative states of temperate rocky reefs, erect algal forest and coralline barrens (Rodríguez, 2003; Perreault et al., 2014). In this context and considering that marine ecosystems are highly impacted by industrial pollution in Quintero Bay, the following working hypothesis emerged: the fitness of sea urchins fed kelps from sites closer to the contamination focus would be more affected in terms of their feeding behavior, growth, fertility and early development, than those fed kelps from more distant sites. In this context, the objective was to demonstrate negative effects on the feeding behavior, growth, fertility, and early developmental stages of *Tetrapygus niger* being fed by *Macrocystis pyrifera* from sites highly impacted by industrial pollution.

2. Material and methods

The algae used for the feeding experiments were manually collected in mid-spring 2019 from a non-impacted zone (NIZ), specifically Algarrobo Bay ($33^{\circ}21'22''S$; $71^{\circ}39'36''W$) located 76 km south of Quintero Bay (Fig. 1). These algae were separated into two groups: the first group corresponding to 15 *Macrocystis pyrifera* adult individuals (approximately 4–5 m) which was transplanted during 60 days to the highly impacted zone (HIZ) Caleta Horcón ($32^{\circ}42'24''S$; $71^{\circ}29'34''W$), close to the also highly impacted zone of Quintero Bay (Fig. 1a). The transplant was made in a long-line culture system previously installed in Caleta Horcón by our research group (FIC-ALGAS, 2016) and following Westmeier et al. (2013) without fragmenting the holdfasts (Fig. 1b). The individuals were hung to the line with zip ties attached to the holdfast at 4 m deep. The second group of 15 *M. pyrifera* individuals was also collected from Algarrobo and considered as NIZ treatment. Both groups of algae were transported to Quintay's Marine Investigation Center (CIMARQ), University Andrés Bello, where they were transferred into two different covered 5000 L tanks and maintained with a constant seawater flux (filtered at $1\ \mu\text{m}$) and aeration for 48 h (acclimation period) until the beginning of the feeding essays.

2.1. Sampling and acclimation of *Tetrapygus niger*

24 adults of *T. niger* individuals (85–100 mm of test diameter) were manually collected from Quintay ($33^{\circ}11'36''S$; $71^{\circ}41'59''W$) in mid-summer 2019, after obtaining local fishermen's authorization and guidance. It is known that in this species both sexes present mature

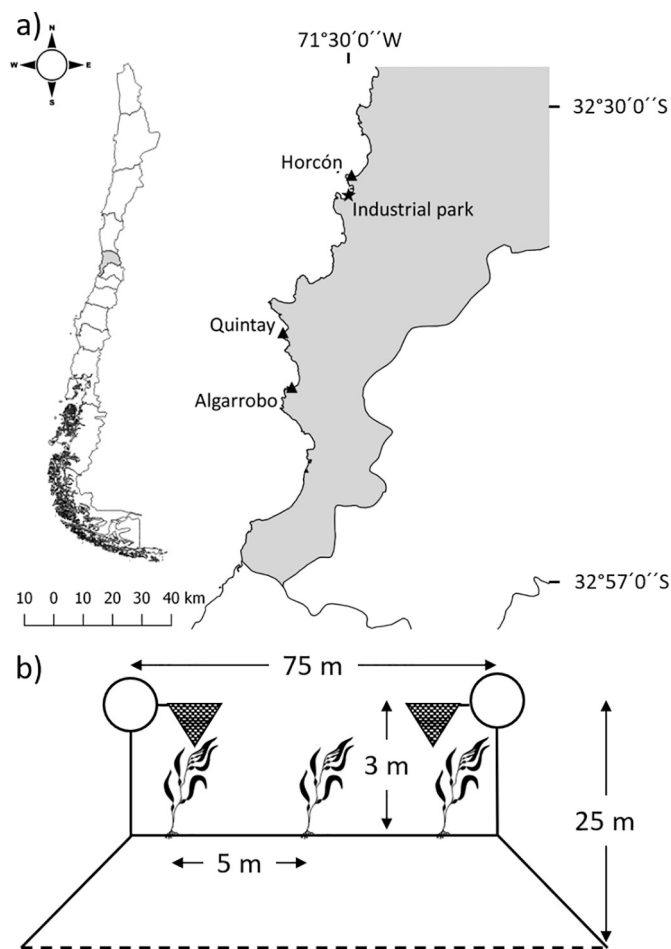


Fig. 1. Georeferencing of the locations in this study (a). Long-line culture system previously installed (b), with 20 mm diameter nylon ropes (solid lines) suspended by buoys (circles) 3 m deep at 25 m from the seabed (dashed line), triangles represent the sea surface with tide variation.

gametes throughout the year, and consequently, potentially they have the ability to spawn multiple times during this time (Zamora and Stotz, 1993). Before feeding trials, these individuals were maintained in starvation for two days inside eight 15 L glass tanks with constant seawater flux (filtered at $1\ \mu\text{m}$) and aeration, at $15\text{--}17\ ^{\circ}\text{C}$ and natural light with a 13:11 photoperiod. Posteriorly, these 24 sea urchins were assigned into two treatment groups of 4 tanks each, being fed kelps from: i) highly impacted zone (HIZ) or ii) non-impacted zone (NIZ) (according to the origin of the *M. pyrifera* individuals) (see below). A third group, consisting of twelve additional *T. niger* individuals collected from Quintay was maintained in starvation for two days and used as control group for fertility experiments. For fertility experiments, we sampled 36 additional *T. niger* individuals from Algarrobo, Horcón and Quintay (12 per site), which were used to compare NIZ and HIZ against non-treated natural population. In every case, initial and final sea urchin weight was established after capture and at the end of the feeding experiment.

2.2. Feeding experiments: *T. niger* consumption and growth

After the starvation period, two groups of twelve *T. niger* individuals each were fed either HIZ or NIZ *M. pyrifera* individuals for three weeks. Specifically, 200 g of fresh kelp tissue was given to sea urchins per week and per tank. Additionally, 200 g of HIZ or NIZ kelp tissue per tank without *T. niger* individuals were cultivated in eight additional tanks for three weeks, in order to assess kelp tissue loss associated with in vitro culture conditions.

Once per week, kelp tissue samples were replaced with new ones, and their mass was determined and compared with the initial values. The percentage of kelp mass consumed per tank was estimated as follows:

$$C\% = \frac{\{Ki - [Kf * (1 - L)]\}}{Ki} * 100$$

where: C% = percentage of kelp consumption; Ki = initial kelp weight; Kf = final kelp weight; L = proportion kelp weight loss due in vitro culture conditions.

Regarding the *T. niger* growth assessment, as previously mentioned, sea urchin weight was measured after capture and at the end of the feeding experiments. More precisely, to calculate the percentage of weight gain (or loss), as a growth measure of *T. niger*, the change in weight was divided by the initial weight and multiplied by 100 [weight growth (%)].

In all the cases, the tanks were cleaned with 1 µm filtered seawater twice a week, and dead *T. niger* individuals were removed from the tanks.

2.3. Gamete release and larval development experiments

After the feeding experiments, sea urchins were injected 1 mL of 0.5 M KCl intracoelomically to induce gamete release, according to Gago and Luís (2011). Male and female gametes, from the two feeding treatments, were used to establish independent groups of embryos. For that, the sperm obtained from 6 males per treatment was collected in different Petri dishes without addition of seawater. The eggs from 4 females per treatment were placed in independent glass flasks filled with 250 mL of 0.22 µm filtered seawater. Additionally, 1.5 mL eggs were collected and fixed with a drop of Lugol's iodine solution and observed under an optical microscope (Leica Dm500, Switzerland), to count the number of eggs released per mL. Then, fertility was established for the NIZ and HIZ feeding treatments, and also for the three sea urchins groups collected from the field in Algarrobo (NIZ), Horcón (HIZ) and Quintay (control site). Fertility was standardized by female weight and calculated as the number of egg cells mL⁻¹ per female gram (cells mL⁻¹ g⁻¹).

For fertilization, the sperm from the 6 males per treatment was mixed to make a sperm pool, and 1 mL of this mix was added to the eggs from the female individuals of the same treatment. The three

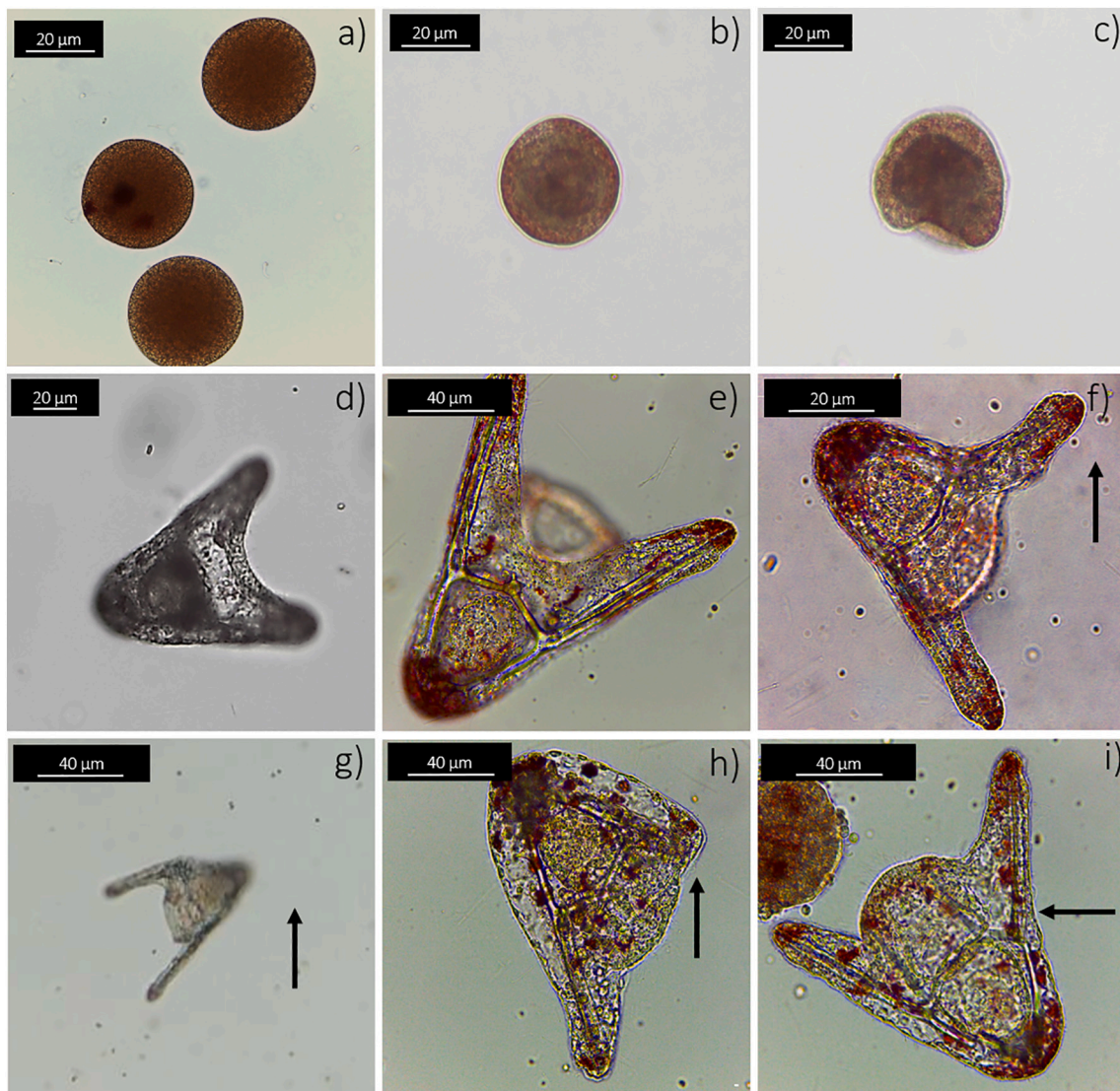


Fig. 2. Larval development stages of *T. niger*. Normal development stages of *T. niger* are shown in (a-e) and correspond to egg, gastrula, prism, 2-arm-pluteus and 4-arm-pluteus, respectively. Malformations of larval development of *T. niger* induced by consumption of polluted kelps are shown in (f-i). Arrows indicate the following malformations and abnormalities in the larval development: arm bar deviation (f and i), crossed tip (g) and deleted arm bar (h).

independent treatment populations of embryos (NIZ and HIZ, and control of the feeding experiments) were reared to the 24 h gastrula larvae stage (Fig. 2a-b); then, larvae were collected and transferred to 500 mL glass flasks (4 flasks per treatment) filled with 0.22 μm filtered seawater with constant aeration and dim light. The larvae were fed with *Chaetoceros* spp. (cultured in CIMARQ) in a density of 1500 to 2000 cells mL^{-1} , and seawater was replaced every 2 days. Before each seawater change, 200 μL of culture medium was collected (after gentle homogenization) and observed under an optical microscope (Leica Dm500, Switzerland). Between 30 and 50 larvae were observed, which were used to assess their developmental stage and register morphological malformations according to Anselmo et al. (2011) and Carballeira et al. (2012). More precisely, the relative percentual abundance of each larval stage (gastrula, 2-arms-pluteus and 4-arms-pluteus; see Fig. 2), and the relative percentual abundance of malformed larvae (with morphological malformations) and normal larvae for each larval stage were calculated for 12 days.

2.4. Data analyses

For consumption, growth and fertility experiments, the mean obtained for each tank (3 individuals per tank) was considered as a replicate (4 replicates per treatment). A one-way ANOVA or a Kruskal-Wallis was carried out (after a normality and homoscedasticity analyses) to determinate significant differences between treatments, followed by post-hoc Tukey's or Dunn's tests to individualize specific treatment differences. In order to identify statistical differences between treatments in the early developmental stages of *T. niger*, a one-way ANOVA was applied for each sample day comparing the relative percentual abundance of each larval stage. Moreover, an ANOVA was done to compare the relative percentual abundance of normal and abnormal larvae for each stage of the HIZ treatment. All the statistical analyses were carried out in R: The R project for Statistical Computing (R Core Team, 2020).

3. Results

Kelp consumption by *Tetrapygyus niger* was significantly higher (average 27.5%) when fed kelps from the non-impacted zone (NIZ, Algarrobo), compared to sea urchins fed with kelps from the highly impacted zone (HIZ, Caleta Horcón) (19.3%) (Fig. 3a). It was also observed that in the NIZ treatments, the individuals gained an average of 19.3% of body weight during the three weeks of the feeding experiments, while in the HIZ treatment only a 3.6% (in relation to their initial weight) (Fig. 3b).

Both for feeding experiments and for sea urchins directly collected from the field, fertility was significantly higher in NIZ than HIZ (Fig. 4).

In the feeding experiments average fertility of the NIZ treatment was 945 cells $\text{mL}^{-1} \text{g}^{-1}$, while HIZ and control (sea urchins collected from Quintay after two days of starvation) were 385 and 448 cells $\text{mL}^{-1} \text{g}^{-1}$, respectively (Fig. 4a). The fertility determined from individuals directly collected from NIZ was 1106 cells $\text{mL}^{-1} \text{g}^{-1}$, and only 358 and 686 cells $\text{mL}^{-1} \text{g}^{-1}$ from HIZ and control individuals, respectively (Fig. 4b). No significant differences ($P > 0.05$) were registered within NIZ or HIZ in both laboratory and field experiments.

The development of *T. niger* eggs after fecundation from the NIZ treatment occurred as under the standard culture conditions described by Fuentes and Barros (2000) (Fig. 5a). Gastrula state was reached 24 h after fecundation. The embryos reached the 2-arm-pluteus stage 2 days after fecundation, attaining a 100% relative abundance at day 4 (Fig. 5a). At day 6 of culture, the 4-arm-pluteus stage reached a 90% presence, and 100% at day 8, staying like this until the end of the culture (Fig. 5a). It is worth mentioning that there were no significant differences in the relative percentual abundance for each larval stage between the NIZ and the control treatment. On the other hand, HIZ showed 22.2% lower 4-arm-pluteus relative abundance (63.2%) at day 6 in comparison to NIZ (Fig. 5b), being significantly different to the NIZ treatments at days 6, 8 and 10, but not at day 12. Indeed, on days 6, 8 and 10, the HIZ relative abundance of the 4-arm-pluteus stayed at approximately 68–80% and only reached a 100% on day 12 (Fig. 5b).

Only HIZ treated embryos showed morphological malformations during their larval development, such as arm atrophy, arm bar deviations, deleted or fractured arm bars, or a poorly formed apex with crossed tip (Fig. 2f-i), in comparison with the NIZ or control treatments which did not show any morphological malformations (Fig. 2d-e). The relative abundance of normal versus abnormal larvae in the HIZ treatment showed a different developmental kinetics depending on the larval stage considered (Fig. 6). For the 2-arm pluteus stage, at days 2 and 4 of culture, significant statistical differences were observed between the % abundance of normal and abnormal larvae, whereas at days 6 and 8 no significant differences were detected (Fig. 6). Particularly, from days 6 to 12 post fecundation, the relative abundance of abnormal 2-arm pluteus larvae remained relatively high, namely between 5% and 15% (Fig. 6). Indeed, even as late as day 12, 2-arm pluteus abnormally developed larvae were found in the HIZ treatment.

In relation to the 4-arm-pluteus embryos, the HIZ treatment always showed significant differences between normal and abnormally developed larvae ($P < 0.05$). The relative abundance of the abnormal larvae increased from day 6 to 12 from 14% to 37% (Fig. 6). Normal developed larvae reached its maximum relative abundance of 93% at 6 days of culture, then decreased to 64% (at day 12), which corresponded to the largest number of abnormalities registered for this stage (37%) (Fig. 6).

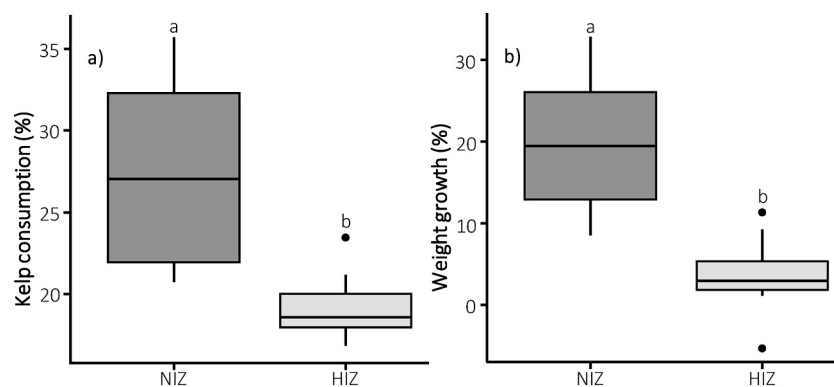


Fig. 3. Kelp consumption (a) and growth (b) of *T. niger* fed with non-impacted zone (NIZ) and highly impacted zone (HIZ) *M. pyrifera* individuals, after 3 weeks of the feeding experiments under culture conditions. Letters above histograms indicate results of Tukey tests; means with the same letter are not significantly different at $P = 0.05$.

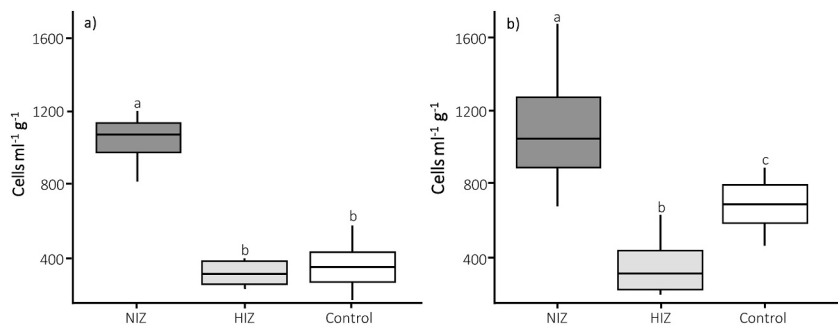


Fig. 4. (a) Fertility of *T. niger* fed kelps from a non-impacted zone (NIZ) and from a highly impacted zone HIZ, determined after three weeks of the feeding experiments under in vitro culture conditions. (b) Fertility of *T. niger* directly determined from field-collected sea urchins from Algarrobo and Caleta Horcón. In both cases control treatment corresponds to individuals from Quintay natural stock. Letters above histograms indicate results of Tukey tests; means with the same letter are not significantly different at $P = 0.05$.

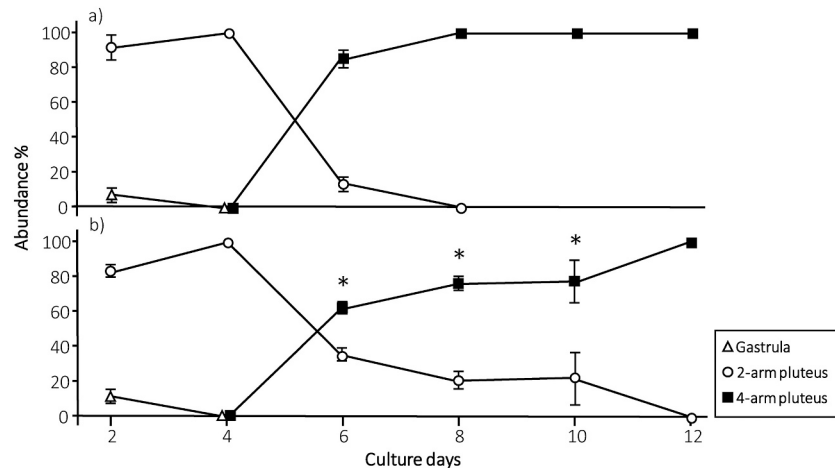


Fig. 5. Larval development of *T. niger* from (a) NIZ and (b) HIZ feed treatments. Gastrula (triangles), 2-arm-pluteus (circles) and 4-arm-pluteus (squares) abundance are represented as a percentage of the total larval number for every 2-culture day. Asterisks represent statistical differences observed in the larval abundance between the NIZ and HIZ treatments, as determined by a one-way ANOVA ($P \leq 0.05$).

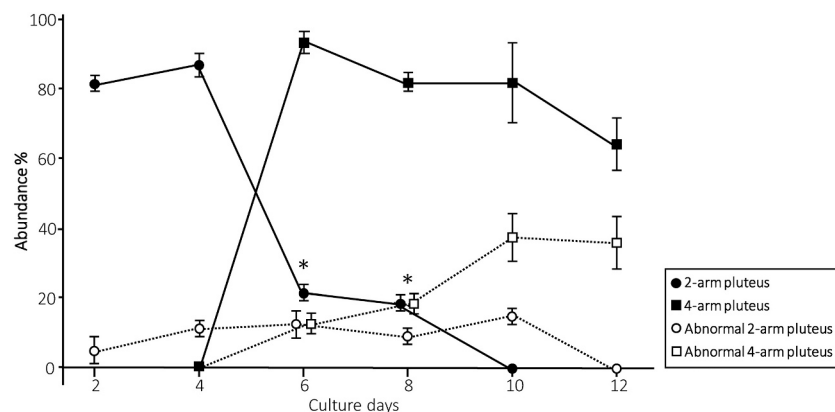


Fig. 6. Normal (filled symbols) and abnormal (open symbols) larval development of *T. niger* from HIZ treatment. Circles correspond to 2-arm-pluteus and squares to 4-arm-pluteus larval stage. Asterisks show lack of statistical differences ($P \leq 0.05$).

4. Discussion

Sea urchin larvae have long been used as a model organism for bioassays for evaluating water quality for various pollutants (His et al., 1999; Bellas et al., 2005). In previous studies, larvae (most cases) or parental individuals (less frequently) have been directly exposed to pollutants (e.g. Durkina and Evtushenko, 1991). The results obtained in the present investigation showed that a polluted diet has negative effects both on the adult individuals and on their larvae. More importantly, our study revealed that *Tetrapygus niger* fed contaminated *Macrocystis*

pyrifera evidenced a significantly lower kelp consumption than *T. niger* fed non-polluted (or less polluted) algae. Furthermore, we suggest that this low consumption of polluted kelps in addition to the pollutants ingested, probably explain their lower growth, fertility, and higher abundance of malformed larvae during the early life development.

4.1. Effects on adult individuals: kelps consumption, growth and fertility

The lower weight increase of organisms fed with contaminated algae may be directly related to their relatively lower consumption of this

biomass, as described by Heflin et al. (2012), who proved that sea urchins with a lower protein and carbohydrate intake show a lower weight gain. Alternatively, these individuals may have manifested compensatory feeding, i.e. higher intake of polluted algae given a lower nutritional value because they were under environmental stress (Cruz-Rivera and Hay, 2001). Nonetheless, in our case, the feeding experiments revealed that kelp consumption of adult sea urchins in the contaminated HIZ treatment group was lower than in the uncontaminated (or less polluted) NIZ group. Consequently, the lower growth and gamete release of the HIZ compared to NIZ treatment could be related to a lower consumption of algae. In fact, field-collected sea urchin females from the natural population of Horcón, a site highly impacted by the industrial pollution of Quintero Bay, presented a similar response to females of the HIZ treatment, namely a low number of eggs released. It is known that the nutrient content in the diet fulfills an important role in the reproductive performance of herbivores (Mattson, 1980). Indeed, studies in macroalgae have shown that exposure to heavy metals can generate both qualitative and quantitative changes in the lipids profile and lipid metabolism (Jones and Harwood, 1993; Ritter et al., 2008). For example, in the kelp *Laminaria digitata*, it has been shown that copper stress triggers lipoperoxide accumulation and changes in the lipid metabolism, such as induction of the synthesis of oxylipins (oxygenated polyunsaturated fatty acids) among other alterations (e.g. Ritter et al., 2008). An increase in the free fatty acid contents is also induced by copper stress, as demonstrated at the transcriptomic and metabolomic levels in the brown alga *Ectocarpus siliculosus* (Ritter et al., 2014). Alternatively, we suggest that not only the nutritional quality of the algae could be affecting the reproduction performance of the sea urchin *T. niger* but also the presence of pollutants in the diet. In fact, in the sea urchin *Evechinus chloroticus*, reproductively mature females exposed to PAHs stress experienced a 2-fold reduction in fecundity compared to non-exposed females (Lister et al., 2016). This may be due to the generation of lesions in the female gonads and oogenesis inhibition caused by the exposition to PAHs, but the mechanisms are unclear (Schäfer and Köhler, 2009). It has also been shown that heavy metals cause negative effects in sea urchin gonads, displaying necrosis, changes in the morphology of sexual related cells, and a lower gonadosomatic index (Zhadan et al., 1992; Vashchenko et al., 2001). It should be noted that in previous studies, the presence of heavy metals has been demonstrated in HIZ. For example, Oyarzo-Miranda et al. (2020) evidenced high concentrations of heavy metals in the water column of Horcón that exceed the permissible limits of the US Environmental Protection Agency (US EPA), specifically for Cu and As (20–46 $\mu\text{g L}^{-1}$ and 6–449 $\mu\text{g L}^{-1}$, respectively). Other heavy metals reported in this study were Ag, Cd and Pb. Additionally, it is known that in the same area repeated oils spills have occurred during the last decade, introducing PAHs into the water column and sediments (FIC-ALGAS, 2016; Pastene et al., 2019).

4.2. Effects on early larval development

The results of this study demonstrated that the consumption of kelps from highly polluted areas by adult sea urchins generates negative effects on their offspring, suggesting a possible transfer of pollutants. Although the transfer of antioxidants from adult sea urchin females exposed to PAHs to their eggs has been previously shown, which reduced the oxidative damage to lipids and proteins of embryos exposed to PAHs; despite this, a high proportion of these embryos exhibited morphological abnormalities (Lister et al., 2016). Then, it is likely that, in our case, it is the transfer of pollutants from adults to their offspring that explain the occurrence of morphological abnormalities. Coincidentally, another study by Sekiguchi et al. (2018) demonstrated that the polycyclic hydrocarbon benz[a]anthracene (BaA) and its metabolite, 4-monohydroxylated BaA, inhibit indirectly larval skeletogenesis in *H. pulcherrimus*, through inhibiting vascular endothelial growth factor (VEGF) and the VEGF signaling modulator heparan sulfate 6-O endosulfatase (Sulf). Moreover, previous studies proved that direct exposure

of adult individuals to pollutants lead to alterations of enzymatic activities in their larvae and abnormal development (Durkina and Evtushenko, 1991). The response of the HIZ treatment larvae was similar to those seen in previous studies, where larvae were directly exposed to heavy metals, organic pollutants, or both (Bielmyer et al., 2005; Anselmo et al., 2011; Carballeira et al., 2012). The larval abnormalities observed in this study consisted of arm bar deviations, deleted or fractured arm bars, or a poorly formed apex with crossed tip; the first one being the most common. These morphological malformations can be caused by various mechanisms. Although the participation of hormones, such as estrogen, in larval development in invertebrates is still uncertain, studies in other sea urchins have shown that larvae are sensible to endocrine disruptors. Some organic pollutants such as bisphenol A that have a similar structure to sex steroids, affect the early development and juvenile growth of *Hemicentrotus pulcherrimus* and *Strongylocentrotus nudus* via endocrine disruption (Kiyomoto et al., 2006), possibly by attaching itself to estrogen like receptors (Rubin, 2011). Another study revealed that larvae of *Psammechinus miliaris* are sensible to persistent organic pollutants such as triclosan, hexabromocyclododecane, tetrabromobisphenol and others, presenting abnormalities in the early development such as malformations, development arrest and a lower egg hatch rate (Anselmo et al., 2011).

Heavy metals as pollutants also can generate an abnormal development in sea urchin larvae. For example, lithium chloride, cobaltous chloride, and mercury acetate produce negative effects in the gastrula stage. Meanwhile, manganese, lead, cadmium and zinc generate malformations after the gastrula stage; specially Zn that in low concentration would be toxic to marine biota (Kobayashi and Okamura, 2004). It is worth mentioning that the pluteus arms are essential to swim and feed, and the body bars, as the apex ones, give structural support (Soars et al., 2009). The negative effects on pluteus arms by metals may be related to an alteration of gene expression associated with morphological development (Agca et al., 2009) probably caused by a rise of reactive oxygen species (ROS) (Hardin et al., 1992; Coffman and Davidson, 2001). Since heavy metals accumulate mainly in the soft tissues, including the gonads (Al Najjar et al., 2018), reproductive function would be particularly affected by heavy metals and other organic pollutants such as PAHs, that also bioaccumulate in soft tissues.

According to Carballeira et al. (2012), any morphological abnormalities will lead to a delay in the sea urchin early development. In our study, a considerable percentage of the HIZ larvae remained longer in the 2-arm pluteus stage, and most of them were individuals presenting morphological malformations. However, depending on the severity of the abnormality, the larvae could recover and continue a normal development to adulthood (e.g. Morrioni et al., 2018). We were able to demonstrate the indirect effects of pollutants presents in the diet of adults on their offspring; because the larvae were not constantly exposed to the pollutants, but rather these would be transferred from the parents. In addition, in malformed larvae, with a delayed development due to pollutants, some can recover from their malformations, and a longer time in the plankton could be beneficial, being able to find a less polluted habitat or it could also increase the chances of being predated (Pechenik, 1999). Thus, it is the likely that a reduced viability of malformed *T. niger* larvae would result from predation, or because they would not be able to settle and mature into healthy fit adults. This is important because it can have serious consequences on the persistence of marine herbivore populations under scenarios of increasing environmental pollution.

5. Conclusions

The results obtained in this study demonstrated that organisms fed with algae from impacted zones have negative effects on several biological functions both on themselves and on their offspring. The morphological malformations observed herein in the early developmental stages were similar to those observed when the larvae of sea

urchins are directly exposed to the pollutants. On the other hand, this study could create new research opportunities, related to kelps capacity to bioaccumulate and disperse pollutants to areas with low anthropogenic or industrial impact and exert negative effects through trophic cascades. Future studies on the effects of polluted diet in sea urchins would have to determine more precisely whether pollution impacting biological functions through affecting feeding behavior (reduced kelp consumption) and the total energy available to concurrent activities, or whether the main effects of pollutants on growth, reproduction and behavior are independent between each other and have different organismal causes. More generally, chemical contaminants can result in direct effects not only on biological functions such as growth or reproduction, but also can alter general behavior such as a lessened activity level of organisms; or disrupt more specific behaviors, such as reproductive behaviors, predator-avoidance, or the capacities of herbivores and predators to forage or capture preys. These alterations of biological functions and behaviors resulting from chemical pollution can lead, concurrently or subsequently, to indirect cascading effects at all trophic levels within a community (Saaristo et al., 2018). Under this context, and the drifting capability of kelps such *Macrocystis pyrifera*, non-industrial impacted zones herbivores may be indirectly affected by pollutants dispersed by these mechanisms. In this sense, understanding the relative impacts of pollution on different biological factors of particular species, and linking them to the negative effects observed at higher levels of biological organization (e.g. populations, community, ecosystems) would make possible a more accurate risk assessment and protection of marine environments.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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CRedit authorship contribution statement

L-CP and NL designed the study, participated in the practical work and wrote the manuscript. NL and AM performed the statistical analysis. AM and FO contributed to writing the manuscript. FO designed the graphical abstract.

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