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Effects on Growth of Juvenile Abalones *Haliotis tuberculata* Under Chronic Exposition to Metals Released from the Dissolution of an Aluminium-Based Galvanic Anode

Laureen Nivelais² · Alexandre Levallois¹ · Olivier Basuyaux² · Katherine Costil¹ · Jean-Marc Lebel¹ · Solveig Larsonneur² · Gwendoline Guichard² · Antoine Serpentine¹ · Christelle Caplat¹

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Abstract

In the marine environment, the galvanic anode cathodic protection system (GACP) undergoes oxidation and releases metals in the forms of ions or oxy-hydroxides into the environment. The objective of the present study was to investigate the toxicity of a cocktail of metals released from the dissolution of an aluminium-based galvanic anode (~95% Al, ~5% Zn) on the abalone *Haliotis tuberculata*. Juveniles were exposed for 16 weeks (i.e. 12 weeks of exposure and 4 weeks of decontamination phase) and their growth, intake rate, conversion rate and metallic concentrations were monitored. A total of 6 conditions were tested: a control, 4 concentrations based on Al and a trophic control. Results showed that the mortality reached 57% for individuals exposed to 1125 µg L⁻¹ of Al, and the abalone growth significantly decreased for an Al concentration greater than 495 µg L⁻¹. At the highest exposure concentration, intake rate measurements revealed that the appetite of abalones was affected, supported by the large increase in the conversion rate which was indicative of a poor feed efficiency. The monitoring of metallic concentrations showed that *H. tuberculata* strongly bioconcentrated Al relative to zinc. The diet did not appear to be the primary pathway for metal entry. Concentrations that significantly impacted abalone growth and survival during the experiment were higher than those found in natural environment, but the bioconcentration of Al into the tissues of a primary consumer such as abalone may be a potential pathway for Al to enter food webs.

Marine ecosystems, and particularly coastal and estuarine environments, are under pressure due to human activities. Since the beginning of the industrial period, the coastline anthropisation has dramatically accelerated, generating inputs of diverse substances such as metals, directly or indirectly into environment. The seawater is a highly corrosive environment and it is common to apply corrosion protection systems on immersed metal structures such as cathodic protection by impressed current (ICCP) or by galvanic anodes

(GACP). These latest are preferred for several reasons including an easy installation and less maintenance (Roy 1968; Roche 2015). The GACP consists of controlling the corrosion of a metal surface by means of an anode, of which electrochemical potential is lower than that of the structure to be protected (Guibert 2009). The anodes are metallic alloys composed in majority by one metal, aluminium (Al) or zinc (Zn) and trace concentrations of other elements. Al is preferred over Zn, due to its high current capacity, low specific weight as well as a high thermal and electrical conductivity and the use of aluminium-based galvanic anodes for cathodic protection of metal structures has been practiced since the early 1960s (Roy 1968; Shibli et al. 2007). Reactions of oxidation occur with the dissolution of the anodes and induce the release in seawaters of the metals that compose them. Throughout their lifetime, the anodes thus contribute to the release of Al and Zn but also toxicologically important trace elements such as cadmium and lead (Reese et al. 2020). If anodes are usually installed on all types of immersed metal structures (boat hulls and in harbours), their contribution to an enrichment of metals in the marine

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environment was extended to the growing settlements of offshore wind farms. If we consider a wind farm offshore of about 75 wind turbines, aluminium-based galvanic anodes can represent a mass of about 15 tons per foundation, which corresponds to an estimated transfer of metals into water of 105 kg day^{-1} or around 45 tons of Al and 2 tons of Zn per year (Kirchgeorg et al. 2018).

Overall, the contribution of aluminium-based galvanic anodes to metal concentrations in marine waters has been reported to be limited to confined areas presenting low hydrodynamic conditions and low depths, where aggregation of corrosion products were observed in surface of sediments located only at proximity of the anodes (Deborde et al. 2015; Caplat et al. 2020). In these particular conditions, it has been shown that the sediments were more enriched by Zn than Al (Caplat et al. 2020). This result contrasts with the abundance of Al in the anode composition and may suggest a potentially toxic effect on benthic organisms in relation to the fate of Zn released from the aluminium-based galvanic anodes.

If some studies related the Al effect on some marine organisms (Harford et al. 2011; Golding et al. 2015; Gillmore et al. 2016), authors focused on the acute toxicity of Al with bioassays performed from Al salt solutions (often Al chloride). Recently, Bell et al. (2020) investigated the effect of galvanic anodes during acute exposures on an amphipod species, *Corophium volutator*, a diatom, *Phaeodactylum tricorutum*, and a bacterium, *Aliivibrio fischeri*, but without considering the dissolution of the anode from the electrochemical reactions involved in real conditions. Further research is thus needed to assess the chronic impact of galvanic anodes on marine organisms.

The main objective of our study was to examine in conditions closed to in situ, the chronic toxicity of metals released from an aluminium-based galvanic anode on juvenile abalone, *Haliotis tuberculata* (Linnaeus, 1758). The abalone is a grazing gastropod that feeds on various macroalgae and preferentially on the red alga *Palmaria palmata*. Besides their ecological importance as primary consumers, abalones are economically important since they are widely consumed in Asian countries, and among the most studied models as marine gastropods in ecotoxicological studies (Bryan et al. 1977; Huang et al. 2010; Mottin et al. 2010; Liu et al. 2011; Minguez et al. 2014). Several studies have already investigated the bioaccumulation of metals by abalone which appears to be a sensitive organism (Guo et al. 1998; Skinner et al. 2004; Huang et al. 2010; Torres et al. 2020), but none has ever considered the bioaccumulation of aluminium, leading to the choice of *Haliotis tuberculata* for this experiment. Juvenile abalones (around 1 year for $20.2 \pm 0.1 \text{ mm}$) were exposed for 12 weeks to 6 experimental conditions using an electrochemical device reproducing the dissolution of an aluminium-based galvanic anode in a marine environment. The 6 conditions tested were: a control, a trophic

control (non-contaminated water + contaminated algae), and 4 increasing concentrations of aluminium-based galvanic anode degradation products. After 12 weeks of exposure, all the surviving individuals were exposed for a further 4 weeks in natural seawater for a decontamination phase. Every 4 weeks, ingestion rate is calculated, and the abalone were measured and weighed in order to calculate several indices such as growth rate and conversion rate. Finally, the bioaccumulation of the different metals constituting the galvanic anode was measured in the whole tissues of abalones but also in algae used to feed the abalones in order to study the involvement of feed in the bioaccumulation processes.

Materials and Methods

Biological Materials

The Abalone, *Haliotis tuberculata*

The experiment was performed on abalones from the France Haliotis farm located in Plouguerneau (Brittany, France). They averaged $20.2 \pm 0.1 \text{ mm}$ in shell length (around 1 year). After an acclimatization period of 7 weeks, the 630 abalones were separated into groups of 35 individuals homogeneous in size (ANOVA, $p > 0.05$), each group placed in 20 L tanks for 16 weeks (12 weeks of exposure and 4 weeks of decontamination phase). In order to carry out growth measurements, abalones were individually marked with varnish on the shell (circular mark) after being dried. The animals were fed once a week with the red alga *Palmaria palmata*. Five ingestion control tanks containing water and contaminated algae at different concentrations were also used to determine the amount of naturally degraded algae in an abalone-free environment.

The Red Algae, *Palmaria Palmata*

The algae for the abalone feed were harvested along the west coast of Cotentin (Normandy, France) every month. The algae were conditioned for one week in separate tanks of 250 L with the same metallic concentrations as the abalone exposure tanks before being distributed to animals (70 g/abalone tank). Abalones of the trophic control, which were in uncontaminated water, were fed with algae exposed to condition C2 (described below). A quantity of algae ($28 \pm 8 \text{ g}$) was collected every month to monitor metallic concentrations in *Palmaria palmata*.

Experimental Device

All experiments were realized at Blainville-sur-mer (west coast of Normandy, France) in the experimental centre

« Synergie mer et littoral » (SMEL), in a thermoregulated room (16 °C) with a supply of natural sea water passed through a sand filter.

A total of 6 seawater conditions based on total Al concentration determination were tested: control (C), trophic control (TC), $74.7 \pm 5.7 \mu\text{g L}^{-1}$ (C1), $165.5 \pm 11.3 \mu\text{g L}^{-1}$ (C2), $494.9 \pm 51.3 \mu\text{g L}^{-1}$ (C3) and $1124.5 \pm 205.0 \mu\text{g L}^{-1}$ (C4) each in triplicate, leading to a total of 18 tanks. We targeted these concentrations because the lowest was near from concentrations measured in polluted sites and the others allowed the study of effects on organisms. All tanks were equipped with a bubbling system and the water was renewed 3 times a week by siphoning. Physicochemical parameters were daily controlled and remained stable between the different conditions throughout the experiment: temperature of 16.4 ± 0.05 °C, salinity of 34.0 ± 0.1 ‰, and pH of 8.00 ± 0.02 .

Electrochemical Device

An electrochemical experimental device with natural seawater was settled to simulate the dissolution of an aluminium-based galvanic anode (GACP) in order to obtain a transfer of metals with the same chemical speciation as in the marine environment. The device was equipped with a saturated calomel electrode (SCE), a platinum-titanium alloy counter electrode (CE) and an Al anode (Al Bera15, Bac Corrosion® Control) immersed in a single tank. The assembly was electrically connected to a galvanostat (Amel, 2049 model) as already described in other publications (Caplat et al. 2010). The water was agitated by an air diffuser. The galvanic anode was composed of seven elements (Al, Zn, Fe, Si, In, Mn and Cu) with the mass percentages of each metal given by the supplier: 93.2% to 97.1% for Al, 2.5% to 6.5% for Zn, 0.13% max for Fe, 0.1% max for Si, 0.01% to 0.03% for In, 0.01% max and 0.006% max for Mn and Cu, respectively. In order to accelerate the dissolution of the anode, it was submitted to a controlled electric current of 200 mA over a period of 22 h. The operating time of the galvanostat was controlled by a programmer.

Each day, every 20 L tanks were emptied, rinsed and filled with natural seawater contaminated by the stock solution. After 22 h, the seawater solution was collected at a high concentration of anode degradation products ($[\text{Al}] = 7624 \pm 656 \mu\text{g L}^{-1}$, $[\text{Zn}] = 357 \pm 36 \mu\text{g L}^{-1}$). Dilutions were made with seawater 3 times a week in the different exposure tanks to reach the target concentrations.

Measures of Metallic Concentrations in Water and in Biota

Every week, seawater samples were collected to monitor the total concentrations of Al and Zn by atomic absorption

spectrometry (AAS). Al was analysed by a Zeeman Atomic Absorption Spectrometer Z-AAS (AA240Z – Varian) equipped with a graphite tube atomizer (FTA 120, Varian) and a programmable sample dispenser (PSD 120, Varian). Analyses were performed with a matrix modifier solution (2 g L^{-1} of $\text{Mg}(\text{NO}_3)_2$) and a temperature program developed for the seawater matrix. Each digested sample was analysed 4 times, and the mean of values was used for evaluation. Zn was analysed by atomic absorption spectrometry in an air–acetylene flame (F-AAS, Varian AA240F). Non-specific absorptions were corrected with a deuterium lamp. Each sample was analysed in triplicate, and mean values were used for evaluation. The limit of detection (DL) was defined as three times standard deviation (SD) of blank analyses. A DL of $3.1 \mu\text{g L}^{-1}$ ($n = 30$) and of $6.6 \mu\text{g L}^{-1}$ ($n = 45$) were given for Zn and Al, respectively.

Five abalones were collected in each tank (3×5 individuals par condition of concentration) to monitor metallic concentrations in all tissues at T0, 4 weeks, 8 weeks, 12 weeks and 16 weeks. The total visceral mass was weighed and then placed in an oven at 80 °C to dehydrate the samples. Algae were also analysed to monitor metallic concentrations. Once removed from the contaminated water, the algae were rinsed with natural uncontaminated seawater before being placed in the incubator at 80 °C for dehydration. Samples were then crushed with Teflon balls and mineralized using a microwave digestion technique. Around 0.2000 ± 0.0010 g of tissues were exactly weighed and transferred to Teflon Microwave bomb containing 10 mL of nitric acid (69% VWR—AnalR NORMAPUR) and placed in a microwave oven (Berghof®, MWS-2) under controlled temperature and power (200 °C, 1000 W). The digested solution was transferred to labelled bottles adding ultrapure water to 50 mL final volume and stored at 4 °C up to metal analyses. Total concentrations of several elements (Al, Ag, As, Ca, Cd, Cu, Fe, In, K, Mg, Mn, Ni, Pb and Zn) were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Vista MPX, Agilent Technologies Santa Clara, USA). Results were expressed in $\mu\text{g L}^{-1}$ for seawaters and in $\mu\text{g g}^{-1}$ dry weight (dw) for abalones and algae. To assess the quality of the biota treatment procedure, a standard reference material SRM NIST-1566b oyster's tissue (National Institute of Standards and Technology, Gaithersburg, MD, USA) was analysed at the same time as samples. The relative errors were less than 10% for all metal concentrations recorded in both standard reference materials, excepted for Pb and Al concentrations which were ranged between 10 and 40%.

Bioconcentration factors (BCFs) were used to assess the capacity of organisms to accumulate Al and Zn taking into consideration all pathways as contaminated water and food (Adams et al. 2000). BCFs were calculated as the following ratio:

$$\text{BCF} = \frac{\text{metal concentration in the organism (mg kg}^{-1} \text{ fw)}}{\text{metal concentration in water (mg L}^{-1})}$$

The metal concentration in abalones in fresh weight (fw) was calculated from the average water content of the abalones. For the algae, BCFs have been calculated from the average metal concentration of all algae used during the experimentation.

Biomagnification factors (BMFs) were used to assess the transfer of Al and Zn from algae to abalones (Gray 2002). BMFs were calculated as the following ratio:

$$\text{BMF} = \frac{\text{metal concentration in abalones (mg kg}^{-1} \text{ fw)}}{\text{metal concentration in algae (mg kg}^{-1} \text{ fw)}}$$

The metal concentration in abalones and algae in fresh weight (fw) was calculated from the average water content of the abalones and algae.

Mortality and Growth Assessment

The health status of abalones was daily checked to remove dead individuals and calculate mortality rates. TL_{50} , time at which mortality reaches 50%, have been calculated from cumulated mortality according time. When cumulated mortality reached 50%, the number of weeks was recorded to determine the TL_{50} .

At each study date, all the individuals were dried with paper towel and then weighed (to the nearest 0.01 g) and measured (to the nearest 0.01 mm). These data were used to calculate the growth of abalones through the weight growth rate (WGR in % d^{-1}) and the linear growth of the shell (LG in mm d^{-1}) determined as follows:

$$\text{WGR} = \left(\left(\frac{\text{fW}}{\text{iW}} \right)^{\frac{1}{t}} - 1 \right) \times 100$$

$$\text{LG} = \frac{\text{fL} - \text{iL}}{t}$$

With iW = initial weight (g), fW = final weight (g), iL = initial length (mm), fL = final length (mm), t = duration of experimentation (days).

Intake Rate and Conversion Rate Assessment

Every week, the remaining algae from each tank was collected in a sieve, drained with a salad spinner and weighed. The intake rate (IR in g of feed. $\text{ind}^{-1} \text{ day}^{-1}$), and the conversion rate (CR in g of feed intake. g of fresh weight increase $^{-1}$) were calculated after the 12 weeks of experimentation as follows:

$$\text{IR} = \frac{(\text{iW} - \text{fW}) - \text{Td}}{n \times t}$$

$$\text{CR} = \frac{(\text{iW} - \text{fW}) - \text{Td}}{g}$$

With iW = the total weight of initial algae supplied (in g), fW = the final weight of algae remaining in the tanks (in g), Td = the total weight of algae degraded in the ingestion controls (in g), n = the number of individuals considered over the 12 weeks, t = the duration of the experiment (in days) and g = The average weight increase in abalone between T0 and the 12th week (in g).

Statistical Analyses

Statistical analyses were carried out with R studio software version 3.6.3. The normality of the data was tested with the Shapiro–Wilk test and the homogeneity of variances with a Bartlett test. In the case where the data verified these hypotheses, an analysis of variance (ANOVA) followed by a Tukey post-hoc test was performed to compare the parameters. If the data did not follow a normal distribution and/or homoscedasticity, a non-parametric Kruskal–Wallis (KW) test followed by Dunn's test was used. Differences were considered significant at p -value < 0.05. All results were presented as mean \pm SEM (Standard Error of the Mean). In order to calculate various EC_x values, we realised nonlinear regressions (using the Hill equation) through the Excel® macro REG-TOX (Vindimian 2012). For each dose response curve, the EC_{10} , EC_{20} and EC_{50} of weight growth rate and linear growth were calculated using the Al concentrations measured in the water during the exposure and Al concentrations measured in abalones' tissues after 12 weeks of exposure.

Results

Metallic Concentrations in Waters and Abalones and Bioconcentration Factors

The mean of Al and Zn concentrations effectively measured in seawaters exposed to Al-based anode and prepared in the 5 conditions of tanks are presented in Table 1.

Before the start of exposure, the basal value of Al content in abalone was $33.7 \pm 4.1 \text{ mg kg}^{-1} \text{ dw}$ and that of Zn about $195.3 \pm 29.8 \text{ mg kg}^{-1} \text{ dw}$ (Fig. 1). As soon as 4 weeks, the results showed a clear increase in the average amount of Al recorded accumulated in abalone tissues in relation to the different concentrations (Fig. 1A). Indeed, it was 48 times higher in condition C4 ($2390.4 \pm 211.2 \text{ mg kg}^{-1}$) than in the control ($49.9 \pm 13.3 \text{ mg kg}^{-1}$) after 4 weeks of exposure

Table 1 Aluminium and zinc concentrations measured in water ($\mu\text{g L}^{-1} \pm \text{SEM}$) during the exposure period for the different conditions

Conditions ($\mu\text{g L}^{-1}$)	Control	C1	C2	C3	C4
Aluminium	10.1 ± 3.0	74.7 ± 5.7	165.5 ± 11.3	494.9 ± 51.3	1124.5 ± 205.0
Zinc	11.2 ± 0.6	14.2 ± 0.6	16.4 ± 0.8	26.5 ± 2.5	46.9 ± 8.8

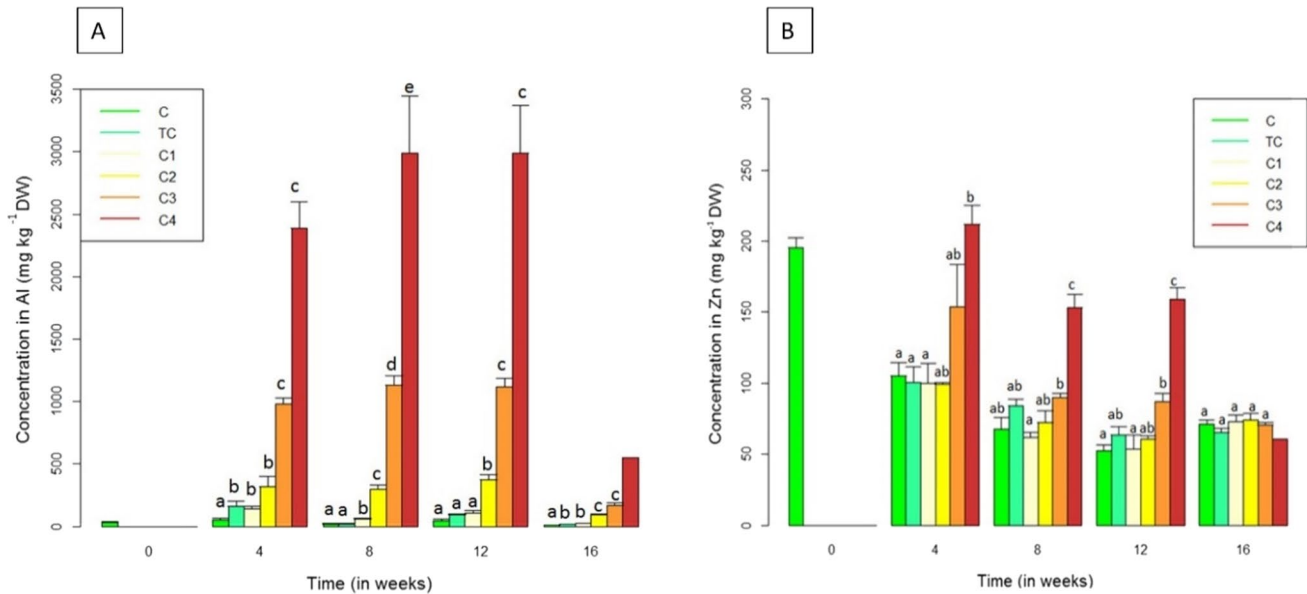


Fig. 1 Concentrations of aluminium (A) and zinc (B) in abalones for the 12 weeks of exposure and 4 weeks of decontamination under the 6 experimental conditions. Metals levels are expressed as mg kg^{-1} of flesh (mean values \pm SEM, $n=3$). The significance of data is pre-

sented for each date compared to the control. For each time exposure, a letter common to two conditions indicates that there is no significant difference between them

(ANOVA, $p < 0.05$). Moreover, it can also be noticed that abalone from the TC condition ($164.2 \pm 39.1 \text{ mg kg}^{-1}$) incorporated significantly more Al into their tissues compared to those from the control condition (ANOVA, $p < 0.05$). During the decontamination phase, the Al level significantly decreased in tissues of abalones exposed to all conditions; this decrease reached 85% for the C3 concentration. In the same time, the Zn concentrations (Fig. 1B) varied considerably less than the Al concentration throughout the exposure. Before the depuration period (12 weeks), only the abalones exposed to condition C3 and C4 showed a significantly higher Zn concentration compared to the control (ANOVA, $p < 0.05$).

Figure 2 shows the evolution of BCF values related to Al and Zn over time under the different experimental conditions. At the start of the exposure, the Al BCF was 902 ± 109 . At 4 weeks, Al concentration in water was below the limit of detection for the abalones under control and TC conditions, as the result BCF could not be calculated. Few differences were found between the different conditions, except during the decontamination period when BCF increased significantly from 164 ± 41 for the

control to 2462 ± 230 for C3 condition. Between the 8th and 12th week, there was a significant increase for BCF value of TC condition (ANOVA, $p < 0.05$), other values remained stable over time (ANOVA, $p > 0.05$). For Zn, at the start of the exposure, the bioconcentration factor was 5223 ± 18 . During the 4th and 8th week, BCF values of animals under C and TC conditions were significantly higher than values recorded for C4 condition. Throughout the exposure, BCFs values significantly decreased by 50% for the control and by 45% for C1. During the decontamination period, there was no significant difference between conditions (ANOVA, $p > 0.05$). Supplemental Data 1 summarises the specific concentrations and BCFs of Al and Zn in abalones during exposure.

Except for Zn and Al, the concentrations of the other elements analysed in abalones (i.e. Ag, As, Ca, Cd, Cu, Fe, K, Mg, Mn, Ni, Pb) did not show significant variations (ANOVA, $p > 0.05$).

The biomagnification factor (BMF) of Al and Zn in the trophic control condition was also calculated in abalones using the metal content in the algae. After 8 weeks of exposure, BMF for Al was 0.17 ± 0.04 for the control, 0.13 ± 0.01

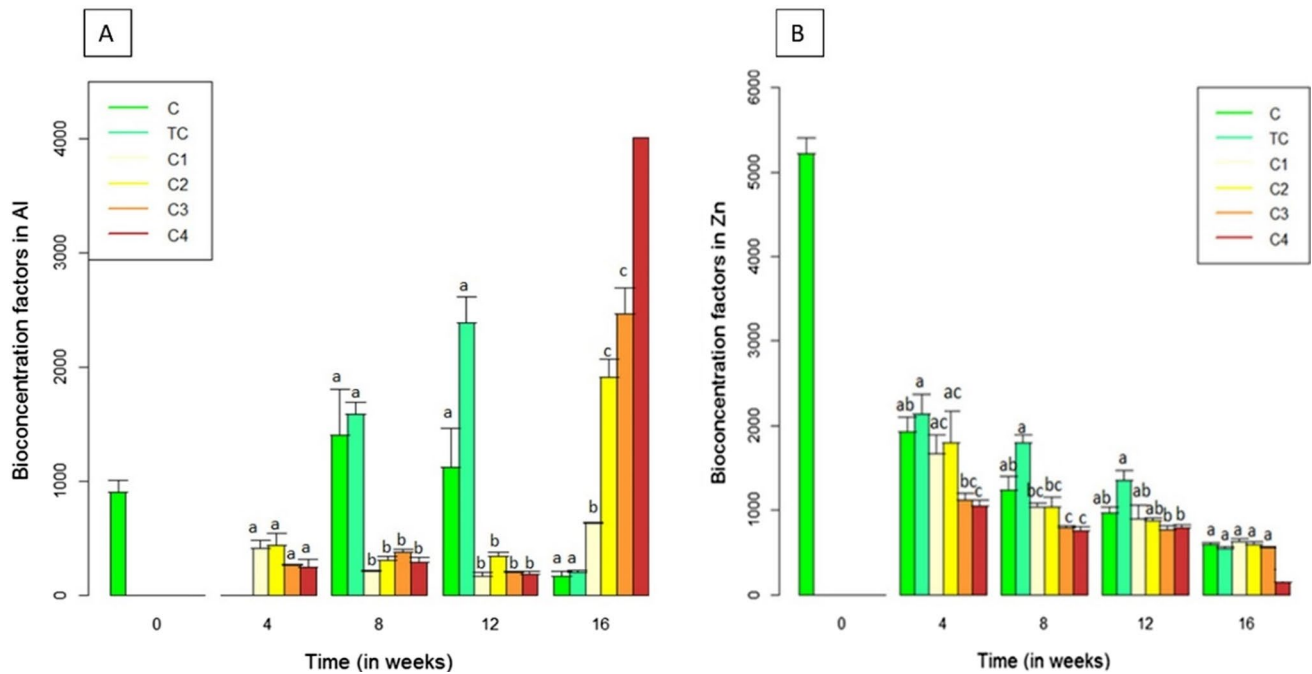


Fig. 2 Bioconcentration factors of aluminium (A) and zinc (B) in abalones for the 16 weeks of exposure (12 weeks of exposure and 4 weeks of decontamination) under the 6 experimental conditions (mean values \pm SEM, $n=3$). The significance of data is presented for

each date compared to the control. For each time exposure, a letter common to two conditions indicates that there is no significant difference between them

for the trophic control and 2.17 ± 0.18 for the C4 condition (KW, $p < 0.05$). Concerning Zn, after 8 weeks of exposure, BMF did not show significant differences (KW, $p > 0.05$).

Metallic Concentrations in *Palmaria Palmata*

The concentration for Al and Zn and BCFs in the algae *Palmaria palmata* under the 6 experimental conditions after two weeks of exposure are presented in supplementary data 2. Al concentrations in algae significantly increased from $310.6 \pm 54.7 \text{ mg kg}^{-1}$ for the control to $2281 \pm 372 \text{ mg kg}^{-1}$ for C3 condition (KW, $p < 0.05$). In the same time, Zn concentrations in the algae significantly increased from C3 ($156.8 \pm 25.6 \text{ mg kg}^{-1}$) compared to the control ($39.6 \pm 1.6 \text{ mg kg}^{-1}$).

Mortality Rates

After 12 weeks of exposure, mortality rate for the C4 condition was significantly different from other conditions and reached 57%. The values allow to establish a TL_{50} of 11.5 weeks. For other conditions, mortality rate averaged 3%.

Growth, Intake Rate and Conversion Rate

The concentration of the anode degradation products significantly influenced the weight growth (WGR) and the

linear growth (LG) (ANOVA, $p < 0.05$) after 12 weeks of the experiment (Fig. 3A and B). In both cases, the values decreased when concentrations increased. The WGR decreased by 33% between the control and C3, from $1.26 \pm 0.03 \text{ j}^{-1}$ to $0.85 \pm 0.06 \text{ j}^{-1}$ whereas for condition C4, the weight growth rate decreased by 80%, from $1.26 \pm 0.03 \text{ j}^{-1}$ to $0.26 \pm 0.14 \text{ j}^{-1}$. Regarding the LG, effects were also significant from concentration C3 onwards with a 30% decrease observed compared to the control, and a 70% drop for concentration C4. For the trophic control, we did not observe a significant difference in abalones' growth (ANOVA, $p > 0.05$). EC_{50} of WGR and LG were evaluated to $1690.7 \mu\text{g L}^{-1}$ and $2007.6 \mu\text{g L}^{-1}$, respectively, based on Al concentrations in water (Table 2). On the other hand, the EC_{50} were, respectively, about 702.6 mg kg^{-1} and 762.7 mg kg^{-1} based on Al concentrations in abalones' tissues.

The intake rate (IR) and conversion rate (CR) significantly varied according to the concentrations tested (Fig. 3C and D). The IR decreased with increasing concentrations; it was about $0.16 \pm 0.01 \text{ g of feed. ind}^{-1} \text{ day}^{-1}$ for the control, $0.12 \pm 0.01 \text{ g of feed. ind}^{-1} \text{ day}^{-1}$ for the C3 condition and $0.08 \pm 0.001 \text{ g of feed. ind}^{-1} \text{ day}^{-1}$ for the C4 condition. On the other hand, the IR for the trophic control was significantly higher, about $0.16 \pm 0.01 \text{ g of feed. ind}^{-1} \text{ day}^{-1}$. In contrast, the CR increased and reached a value of $11.1 \pm 1.1 \text{ g of feed intake. g of fresh weight increase}^{-1}$ for

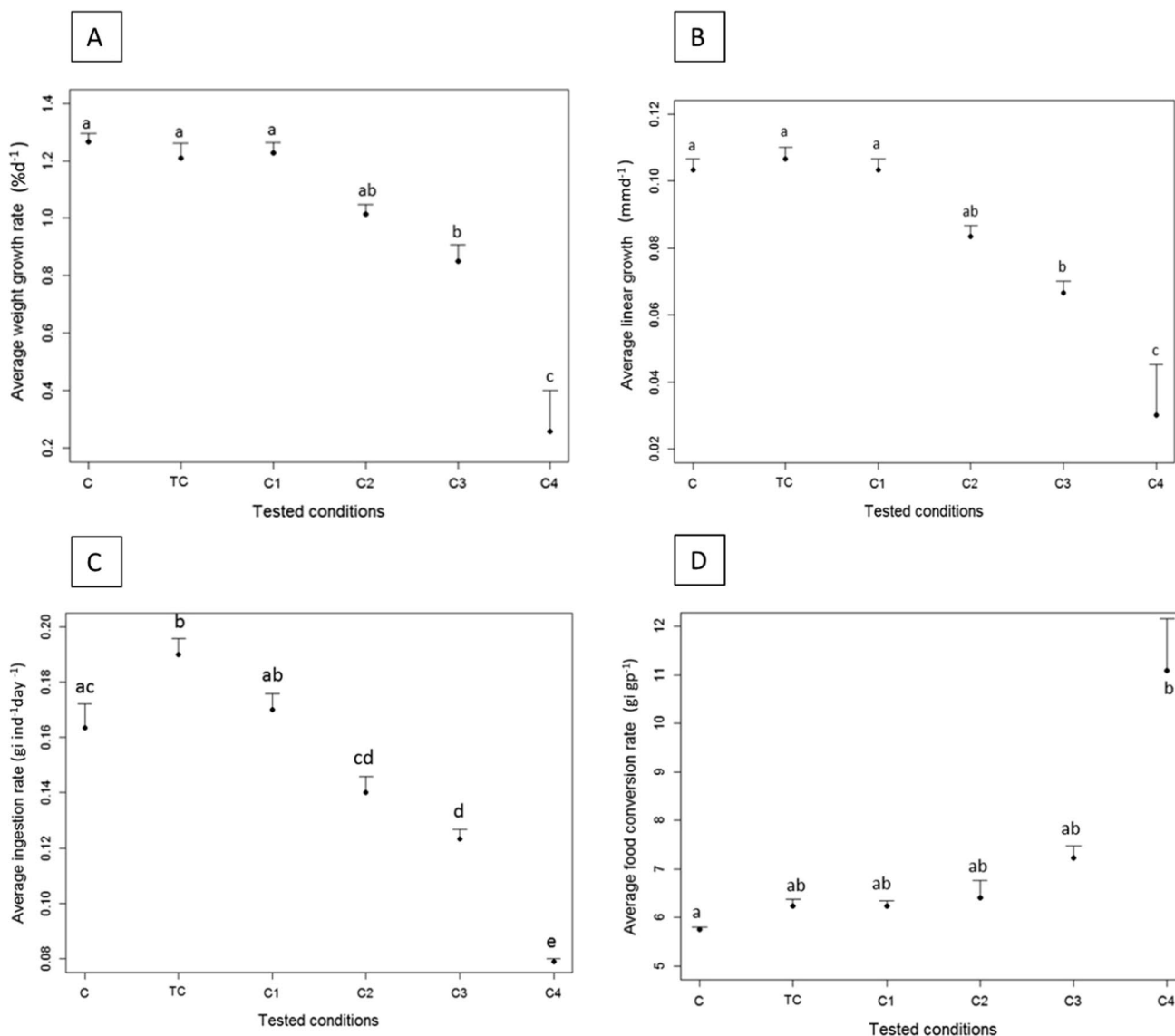


Fig. 3 Evolution of the average weight growth rate (in % d⁻¹ ± SEM) (A), the average linear growth (in mm d⁻¹ ± SEM) (B), the average intake rate (IR in g feed intake individual⁻¹ day⁻¹) (C), and the average conversion rate (CR in g feed intake⁻¹ g of fresh weight gain⁻¹)

D of abalones after 12 weeks of exposure according to the experimental conditions. A letter common to two conditions indicates that there is no significant difference between them

Table 2 Ecotoxicological parameters of the weight growth rate and the linear growth calculated in juveniles *H. tuberculata* using the AI concentrations measured in the water during the exposure and AI concentrations measured in abalones' tissues after 12 weeks of exposure. EC_X=effective concentration (in µg L⁻¹ or in mg kg⁻¹) which induced an effect of X% on the population (10- 50%)

	AI concentration in water (µg L ⁻¹)		AI concentration in abalone (mg kg ⁻¹)	
	Weight growth rate	Linear growth	Weight growth rate	Linear growth
EC ₁₀	309.8	217.7	167.3	103.7
EC ₂₀	442.8	494.2	284.1	216.6
EC ₅₀	1690.7	2007.6	702.6	762.7

the C4 condition. There was no significant difference (KW, $p > 0.05$) between the values of the other conditions and the control, which range from 5.76 ± 0.03 to 7.23 ± 0.25 g of feed intake. g of fresh weight increase⁻¹.

Depigmentation Phenomenon

We also observed depigmentation at the foot of some abalone in concentrations C3 and C4. This phenomenon appeared at the end of the contamination phase when some individuals had the middle and the border of the foot completely depigmented (Fig. 4).

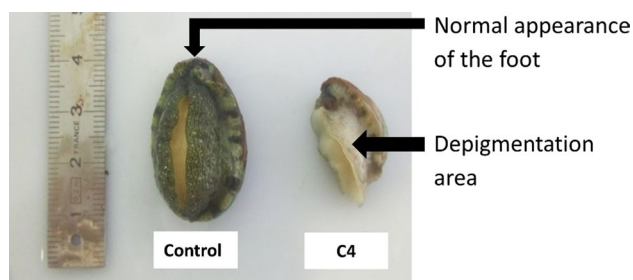


Fig. 4 Depigmentation phenomenon observed in abalones for the C4 condition after 12 weeks of exposure compared to an abalone from the control group

Discussion

As shown in previous studies, the toxicity of aluminium salts cannot be extended to the toxicity of aluminium-based galvanic anodes because of the cocktail effect of the metals and the difference in bioavailability of the chemical species to organisms (Caplat et al. 2010). To our knowledge, the present study is the first one to focus on the chronic impact of an aluminium-based galvanic anode on a gastropod, the abalone *H. tuberculata*.

Results of metallic concentration monitoring confirmed that abalones differently assimilated the principal constituents (Al and Zn) of the galvanic anode. A previous study showed that Zn concentrations in the tissues of *H. tuberculata* collected in the English Channel were less variable than those of other metals, particularly in muscles (Bryan et al. 1977). Zn concentration in the foot, viscera and digestive gland varied from 28 to 656 mg kg⁻¹ depending on the sampling site. Analyses of 12 trace metals in different organs of *Haliotis rubra* from two sites in Victoria, Australia revealed that Al was below the detection limits of the instrumental techniques used which were 1 mg kg⁻¹ (Skinner et al. 2004). Zn concentrations for wild populations were higher than those measured for cultivated abalones, and ranged from 8 to 13 mg kg⁻¹ depending on the site. These authors found that copper and Zn concentrations decreased with increasing abalone length. In the present study, metals basal values were higher but that confirmed the Zn/Al ratio in abalone tissues. After only 4 weeks of exposure, our results showed a clear increase in the average concentration of Al in abalone tissues compared to the control individuals, 20 times higher for C3 concentration and 48 times higher for C4 concentration. From 8 weeks of exposure, Al levels remained almost unchanged, probably suggesting an equilibrium between metal entry and detoxification mechanisms (Gupta and Singh 2011). Zn concentrations showed a different profile, after 8 weeks of exposure, a decrease in levels of approximately 30% was observed for all conditions suggesting that the abalones had already reached the maximum of

Zn concentrations at the beginning of the exposure, and were therefore in the process of detoxification. At the end of the decontamination phase, only Al concentrations in abalone drastically dropped for all exposure conditions, suggesting that abalones were able to depure this metal from their tissues once in an uncontaminated water. Only the C4 concentration, induced a significant mortality rate (57%), indicating that abalones were largely affected by this high concentration after 12 weeks of exposure whereas for the concentrations less than or equal to 495 µg L⁻¹ of Al, concentrations significantly higher than those found in the environment, the mortality rate did not exceed 3%. Indeed, in coastal waters, total Al concentrations commonly vary from 0.1 to 40 µg L⁻¹ but in heavily polluted ports much higher concentrations have already been reported as on the Egyptian coast (106 µg L⁻¹) (Abdel Ghani 2015), on the northern coast of Chile (132 µg L⁻¹) (Valdés et al. 2011), or in Port Botany (151 µg L⁻¹) (Jahan and Strezov 2017) which is close to our C2 and C1 conditions. Considering that EC₁₀ and EC₂₀ as equivalent to No Observed Effect Concentration (NOEC), and Lowest Observed Effect Concentration (LOEC) (OECD 201, 2006), the most polluted sites would not cause any direct effect on the growth of abalones (EC₁₀ = 309.8 µg L⁻¹ for WGR, EC₁₀ = 217.7 µg L⁻¹ for LG). Nevertheless, it is significant to mention that an extreme concentration of 1,170 µg L⁻¹ has already been reported in the port of Yamba in Australia, where the authors found an average concentration as high as 224 µg L⁻¹ (Jahan and Strezov 2017). For such concentration, we can expect significant effects in abalone that are close to what we observed for conditions C3 and C4 in our experimental conditions.

It is important to keep in mind that the degradation of galvanic anodes is a cocktail of metals and it is difficult to determine the origin of the toxicity, which may be attributed to a synergic effect between aluminium and zinc. From this, we can deduce that the tolerance limit of a young abalone to the metals released from the anode is between C3 ([Al] = 495 µg L⁻¹, [Zn] = 26.5 µg L⁻¹) and C4 ([Al] = 1124 µg L⁻¹, [Zn] = 46.9 µg L⁻¹) in our experimental conditions.

Bioavailability of Al and therefore its toxicity essentially depends on its chemical speciation (Gagnon and Turcotte 2007). Al speciation and solubility are dependent on a large range of environmental parameters including pH, temperature, dissolved organic carbon content and the presence of many ligands (Gensemer and Playle 1999). In the marine environment (pH ~ 8), Al speciation is dominated by the soluble aluminate ion $Al(OH)_4^-$ and by the neutral insoluble Al hydroxide $Al(OH)_3$ (Tria et al. 2007). Golding et al. (Derivation of a water quality guideline for aluminium in marine waters, 2015), and Angel et al. (Geochemical controls on aluminium concentrations in coastal waters, 2015) have reported that the solubility of Al depends of its concentration

level with a solubility limit of $500 \mu\text{g L}^{-1}$ and a solution which should be dominated by the soluble form $\text{Al}(\text{OH})_4^-$. Between $500 \mu\text{g L}^{-1}$ and $1000 \mu\text{g L}^{-1}$ the solution would be divided in dissolved and particulate Al hydroxide ($\text{Al}(\text{OH})_3$) and above $1000 \mu\text{g L}^{-1}$, particulate Al would become dominant. Zn could also be found in different forms: divalent ionic (Zn^{2+}), hydrated ions ($\text{Zn}(\text{H}_2\text{O})^{2+}$), Zn complexed by organic ligands (fulvic and humic acids). In the marine environment, approximately 64% of the total release of Zn is recovered as the free ion Zn^{2+} , 14% is complexed with chloride (ZnCl_2), 10% with carbonate (ZnCO_3), 6% with hydroxide ($\text{Zn}(\text{OH})_2$), 5% with sulphate (ZnSO_4) and 1% with bicarbonate (ZnHCO_3^+) (Stanley Jr and Byrne 1990). For a pH of 8.1, the dissolved limit for Zn has been evaluated at $160 \mu\text{g L}^{-1}$ which is slightly higher than the value for the Al (Rousseau et al. 2009).

All organisms naturally accumulate metals without suffering any adverse effects up to a defined level; some elements are essential for biological functions (such as Zn) and are therefore subject to homeostatic regulation (Adams et al. 2000). However, for non-essential metals such as Al, there is not necessarily a regulatory mechanism (McGeer et al. 2003). Zn is an essential trace element for the functioning of many cellular processes, but in excess it is toxic and generates ROS that damage the organism (Boyden et al. 1975; Sies 1986; Mottin et al. 2012). For a marine organism, a metal can become available by two major ways: dissolved uptake, which requires no energy investment; and the ingestion of particulates associated with food which become available to abalones. Absorption of Al by dissolution in various forms can be effected by different routes including paracellular transport, transcellular transport, active transport, ion channels and adsorptive or receptor-mediated endocytosis (Exley and Mold 2015). BCF measures the ability of an organism to accumulate an element in its tissues regarding the concentration of that element in the water (McGeer et al. 2003). It is important to take into consideration that in natural aquatic environments, contaminants adsorb more onto suspended organic matter, but low amount of dissolved or suspended organic matter occurring in controlled conditions (in relation to decantation and sand filtration processes), can increase the levels of contaminants to which the organisms are exposed. *H. tuberculata* lives in the low levels of the intertidal zone, in rocky environments where it hides in crevices. Unlike bivalves that feed by filtering and absorbing large volumes of water, the abalone is a macroalgal grazer. This difference in feeding mode may potentially change the mechanisms involved in contaminant entry processes in abalone. It was this initial hypothesis that led this study to add the trophic control (TC) condition by feeding abalone with algae that had assimilated the metals released from aluminium-based galvanic anode. Results were of particular interest as it was found that after 4 weeks of exposure, abalone from the TC

condition incorporated significantly more Al than those from the control condition. For Zn, levels of the TC condition were not significantly higher than the control but concentrations (63.6 mg kg^{-1}) were of the same order as in condition C2 (60.4 mg kg^{-1}). These results confirm that Al and Zn are equally incorporated by the trophic pathway. A study in a close species, *Haliotis diversicolor*, showed that under natural conditions, Ag, Cd and Hg (non-essential metals) accumulated more through the diet during an exposure of 3 h (Huang et al. 2008). Biokinetic measurements of these toxic metals indicated that they were highly assimilated by abalones from ingested macroalgae. These metals also had a higher potential for biomagnification due to their high dietary assimilation. Dietary uptake is the dominant route for these elements but in our study, the dominant contamination pathway for Al appears to be the exposure medium as illustrated by measuring the BMF after 4 weeks of exposure for the trophic control (0.12 ± 0.03) which showed no significant difference from the control (0.24 ± 0.07). In another study, exposure to Zn showed that *Haliotis discus hannai* had a low Zn accumulation potential (Guo et al. 2013). The incoming Zn was mainly deposited in the viscera. Furthermore, the authors did not find any impact on growth at a concentration of $300 \mu\text{g L}^{-1}$ (ZnCl_2) but the significant metallothionein induction indicated that this species could efficiently detoxify the bioaccumulated metal (Guo et al. 2013). As a trace essential element, Zn appears to be more incorporated by the diet in the present study as shown by the increase in the BMF after 8 weeks of exposure for the trophic control (BMF of 3.8 ± 0.1) compared to the control (BMF of 3.0 ± 0.2); despite this increase there was a non-significant increase in Zn concentrations in abalones for the trophic condition compared to the control.

Results of this study show different responses between abalone and the Pacific oyster *Crassostrea gigas* exposed to the same anode in a previous study (Levallois et al. 2022). First, basal Al concentrations in oysters (20 mg kg^{-1}) were lower than in abalones (33.7 mg kg^{-1}) while basal Zn levels in oysters were 3 times higher (672 mg kg^{-1}) than in abalones (195 mg kg^{-1}). In the present study, the Al accumulation rate for the C3 concentration was 33 compared to T0 whereas for a similar exposure concentration to C3, it was only about 5.25 for oysters. Regarding Zn, the inverse trend was observed as Zn concentrations were much higher and more gradual in the oyster. The Zn accumulation rate after 3 months of exposure was 4.09 compared to 0.81 in abalones showing that the young gastropod does not bioaccumulate Zn in its tissues at all. Abalones regulated this metal in their tissues, resulting in a Zn concentration decrease over the exposure period for all conditions. On the other hand, it is only during the decontamination phase that the abalone eliminate the Al previously accumulated. However, Al is not completely removed, because the accumulation rate for the

C4 concentration at 16 weeks of exposure compared to T0 is still 16.35, which may explain why there is an increase in Al BCF at the end of exposure. This was not the case for oysters, which regulated Al during exposure, as concentrations decreased in the tissues during the entire exposure period without a decontamination phase. These first results of metals accumulation on two mollusc models show different inputs processes related to the metal, the Pacific oyster preferentially bioconcentrate Zn while abalone preferentially bioconcentrate Al.

Our results of the different parameters reflecting the growth and nutrition of young abalones under control conditions are in agreement with values found in a previous study focusing on the importance of *Palmaria palmata* in the diet of *H. tuberculata* (Basuyaux et al. 2018). As shown in the results, all parameters were significantly affected from 494.9 $\mu\text{g L}^{-1}$ of Al. The drop of the IR from concentration C3 suggests that the appetite of abalones was affected. The slight increase in the IR for the trophic control condition may be the result of a slight hormetic effect. It is common during exposures that some physiological functions are stimulated at low concentrations before dropping drastically at higher concentrations (Calabrese and Baldwin 2001). This could be related to overcompensation in response to disturbances as have already been shown in several bivalve species during Zn exposure (Sauvé et al. 2002). For healthy young abalones, a CR of 6 g g^{-1} is considered to be the standard (Basuyaux 1997), which is in agreement with our results for the control condition (5.76 g g^{-1}). A large increase in the CR at the C4 condition (11.1 g g^{-1}) is indicative of poor feed efficiency. Exposure to the degradation products of galvanic anodes greatly affected the physiology of abalones from C3 concentration. The abalone organism may be under excessive stress due to contamination, involving energy in detoxification processes to the detriment of growth. Decreased appetite and bioavailability of nutrients may also be the origin; once contaminated at high concentrations, algae may be less attractive to abalone. Depigmentation phenomena observed on the foot of some abalone exposed to the highest concentrations, attesting of a strong effect on the physiology of gastropods. Similar phenomena have already been reported in literature, notably in juveniles of *Haliotis rufescens* exposed to supersaturated oxygen, which showed depigmentation, lethargy and swelling of the tissues (Elston 1983). Depigmentation phenomena have also already been observed in several Indian carp species in response to cadmium and Zn (40 $\mu\text{g L}^{-1}$) alone and in combinations (Rani et al. 2017). As the foot of abalones is in constant contact with the tank walls, metals may be adsorbed in quantity by the molluscs. In the environment, such depigmentation can affect the camouflage of abalone, resulting in increased predation pressure as an affected individual would be more visible to predators.

Even if Al has no biological function, it has already been shown that it can substitute for essential metals in some biochemical processes, as in the case of its complex with ATP and perhaps other nucleotides where Al^{3+} can replace Mg^{2+} ions; it is also an effective and occasional competitor to Ca^{2+} and $\text{Fe}^{2+}/\text{Fe}^{3+}$ (Exley and House 2011). To our knowledge, very few studies have investigated the biological effects of aluminium-based galvanic anode on marine organisms. No acute toxicity of Al from galvanic anodes was reported on the bacterium *A. fischeri*, low effects on algal growth on *P. tricornutum* and significant mortalities on the amphipod *C. volutator* were found but at a concentration of 100 mg L^{-1} which is not environmentally relevant (Bell et al. 2020). The global conclusions of the authors indicated no direct environmental impact of galvanic anodes on the tested organisms. Considering Al in all its forms, various CL_{50} values have been reported in the bibliography: 3 000 $\mu\text{g L}^{-1}$ for the coral *Acropora tenuis* (Negri et al. 2011); 10 mg L^{-1} for the copepod *Nitocra spinipes* (Bengtsson 1978) and 28 mg L^{-1} in the sea urchin *Heliocidaris tuberculata* (Golding et al. 2015). Nevertheless, it is important to consider that the different chemical species of Al influence its bioavailability and therefore its toxicity for marine organisms, as demonstrated by Caplat et al (2010) who compared the biological effects of Al and Zn released from galvanic anodes to their conventional sulphate salt. The study involved exposed embryos and sperms of the sea urchin, *Paracentrotus lividus*, with developmental abnormalities, fertilization rate and mitotic abnormalities as biomarkers. The results showed that Al and Zn from the dissolution of the galvanic anode were less toxic than sulphate salts. The use of Al salts is consequently not the right solution to assess the ecotoxicological effects of galvanic anodes on marine organisms which is why it is necessary to study the effect of GACP more specifically.

In contrast to Al, Zn is an essential trace element for the regulation of many cellular processes (Watanabe et al. 1997). One of the few studies on zinc-based galvanic anodes indicated that oysters *C. gigas* were sensitive to acute Zn toxicity (10.2 \pm 1.2 mg L^{-1}), 81.8% of the oysters died at the end of the short-term assay, but the bivalves were only moderately affected by chronic Zn exposure (0.53 \pm 0.04 mg L^{-1}) (Mottin et al. 2012). In vitro exposure of abalone *H. tuberculata* haemocytes to ZnCl_2 demonstrated a decrease in cell viability as well as morphological and physiological changes in cells at a concentration of 6 538 $\mu\text{g L}^{-1}$ (Mottin et al. 2010). A study on young abalones of the species *Haliotis discus hannai* concluded that the minimum requirement for dietary Zn was 16–18 mg kg^{-1} from ZnMet against 35 mg kg^{-1} from ZnSO_4 because its bioavailability was lower for juvenile abalone (Tan and Mai 2001). These concentrations were higher compared to the environmental concentration of Zn. In the present study, the Zn concentration in water reached a maximum of 47 $\mu\text{g L}^{-1}$. Referring to the recommendations of

Tan and Mail (2001) on a closely related species, the Zn concentrations in the algae fed to abalone in the control condition (39 mg kg^{-1}) are sufficient for optimal growth. These Zn concentrations increased to 193 mg kg^{-1} for the algae in condition C4 indicating that just by feeding, the amount of Zn provided to abalones is much higher than their requirements. Bioconcentration results obtained suggest that Al is more important as a cause of the observed biological effects but the metal cocktail effects should be taken into consideration in further study.

Conclusion

The uptake by abalone of Al and Zn from the degradation of an aluminium-based galvanic anode is very different. Abalone regulated Zn in their tissues leading to a decrease in the concentrations in their tissues during the exposure period while a clear increase in Al concentrations in abalone tissues was recorded in relation to the different exposure concentrations. Only the highest concentration tested resulted in significant mortality and we did not observe any significant difference between the other conditions more closed to the in situ Al concentrations. Results showed a significant decrease in ingestion rate and growth as well as depigmentation phenomena in the abalones from an Al concentration of $495 \mu\text{g L}^{-1}$ and the EC_{50} for weight and linear growth were estimated at $1690.7 \mu\text{g L}^{-1}$ and $2007.6 \mu\text{g L}^{-1}$, respectively. In view of the environmental concentrations, the degradation products of aluminium-based galvanic anodes do not appear to present a direct risk to *H. tuberculata*. In order to estimate the global environmental risk of galvanic anodes, it is necessary to consider other species and other stages of development. In a future study, it could be also interesting to focus on older abalones which may be more resistant but some physiological processes (i.e. reproduction) may be affected. Moreover, the literature generally shows that the larval stages of molluscs are more sensitive than juveniles. Growth restriction caused by a contaminant leads to a prolongation of the pelagic life of the larvae and therefore an increase in the risk of predation, disease and dispersal, resulting in reduced population recruitment. In addition, galvanic anodes will be used more and more in the next few years, particularly on offshore wind farms and in port structures. This could modify basal level of metal concentrations (such as Al and Zn) in waters, especially in ports or in closed areas with low water circulation.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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