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Silence of the clams: health status of *Mya arenaria* clams in the Saguenay–St. Lawrence Marine Park

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Abstract

The cumulative effects of pollution of intertidal clam populations should be investigated to ensure the sustainable perennity of our resources. The purpose of this study was to examine the health status of intertidal clams and tissue levels of essential and non-essential elements at sites under anthropogenic stress. Clams were collected at two anthropized sites, a St. Lawrence Estuary (SLE) beluga high-residency area and reference site in the Saguenay–St. Lawrence Marine Park (Québec, Canada). Clam health status was determined by the condition factor (CF: wet weight/shell length ratio), growth index (GI: shell length/age), air survival time and weight loss index (WLI). Elemental analysis was also performed in soft tissues. The data revealed that clams from at least one of the harbor/marina sites had reduced CF, GI and WLI. Air survival time was not affected at the anthropized sites but was significantly higher at the St. Lawrence Estuary beluga high-residency area. The clams were contaminated by Ag, Al, Cd, Cu, Hg and V, with a decrease in essential cations (K, Ca, Mg) suggesting altered osmoregulation. Although the individual metals in tissues were not found at harmful concentrations based on reported data, the combined effects of non-essential elements could not be excluded. More research will be needed to better understand the cumulative effects of various stressors, such as low salinity, algal toxins and elemental composition, on clam health status.

Keywords: *Mya arenaria, condition factor, growth, air survival, elemental contamination, osmoregulation*

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1. Introduction

Bivalves are marine invertebrates which are ecologically valuable for coastal ecosystems and contributing to the fixation of atmospheric CO_2 for shell formation and growth [1, 2]. The soft-shell clam *Mya arenaria* is an important member of the benthic community, forming an interphase between sediments and water column in marine environments [3]. The burrowing activity and their predation (bird, fish, worms and marine mammals) contribute to sediment oxygenation and other nutrient exchanges, ensuring the functioning of the benthic community in intertidal mud flats. Indeed, clams are found in both subtidal and intertidal surfaces in the eastern/northern coast of North America, representing one of the most prevalent species in this area [4]. In addition, *Mya arenaria* clam harvesting has been a highly prized traditional food source for coastal communities for over two centuries and constitutes a potential pathway of food contamination for the human population. In general, management of clam stocks includes monitoring the renewal and occurrence of potential human pathogens such as microorganism (coliform) and toxic phytoplankton (red tide) outbreaks [5], but these assessments are not provided for all feral clam sites. Clams, like other endobenthic mollusks, reproduce by external fertilization during early summer in the months of June–July [6]. Spawning usually follows increases in water temperature (12–20 °C). Other factors include food availability and quality, which can be compromised by the occurrence of pollutants and toxins. A valuable parameter for stock assessment for fishery conservation is the maintenance of clam populations where at least 50% of the population attains sexual maturity [7, 8]. This usually occurs when individuals attain shell lengths of >2.5 cm for this species. These assessments involve non-destructive methods such as the condition factor (clam total weight/shell length) and age evaluation (major grooves on shells), which are sometimes followed (but requires the sacrifice of animals) by the gonado-somatic index to determine the relative gonad size before spawning. However, annual variations in the gonado-somatic index in *M. arenaria* from the lower SLE in relation to the gonad development (gametogenesis) showed some uncertainties [9]. For example, endocrine disruption in vitellogenesis could occur, leading to a higher gonado-somatic index with a high lipid accumulation but with fewer eggs [10].

Following two decades of ecotoxicology research with *Mya arenaria* clams in the lower SLE and Saguenay Fjord area, three simple biomarkers were identified to assess water quality status [11]. These were based on the condition factor described above, the growth index (GI: shell length/age) and air survival time. The air survival time determines the capacity of bivalves to withstand air emersion, which could be compromised when multiple stressors, such as pollution, food quality/availability, pathogens and abiotic factors, are at play (temperature, salinity, pH, anoxia, etc.) [12]. The so-called stress on stress (SOS) response is a biomarker that

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measures the capacity of organisms to survive in air emersion and considers the initial physiological state (stress) of the organisms. These three simple biomarkers were associated with various molecular biomarkers of toxicity, such as oxidative stress/lipid peroxidation, DNA strand breaks, metal uptake and induction of heavy metal detoxication proteins or metallothioneins [11]. This suggests that pollution and other stressors nominally influence apical endpoints such as clam size, growth and reduced resilience to air emersion.

The purpose of this study was to examine the relationships between essential and non-essential elements/metals and the health status of intertidal clams in the Saguenay–St. Lawrence Marine Park. Health status was examined by following changes in CF, GI and air survival as proposed by Blaise et al. [11]. Elemental tissue concentrations were also determined for essential (Ca, Co, Cu, Fe, K, Mg, Mo, Ni and Zn) and non-essential elements/metals as proxies of anthropogenic pollution. Changes in element/metal tissue loadings were studied in relation to the clams' health status. Finally, considering that contaminants have been identified as a threat to the recovery of the St. Lawrence Estuary beluga whale population, an endangered marine mammal listed in Canada's Species at Risk Act, information on the presence of contaminants in its prey and habitat is warranted.

2. Methods

2.1. Study sites and clam collection

Adult *Mya arenaria* clams (>4 cm longitudinal shell length with a mean age of 8 ± 0.12 years from N = 80 individuals) were sampled in the Saguenay–St. Lawrence Marine Park sites (**Figure 1**). The reference site was Baie du Moulin Baude (BAU), sampled in areas located 3–5 km upstream the outfall of Rivière du Moulin à Baude, known to contain inputs of municipal effluent and agriculture runoffs of the municipality of Sacré Coeur [13]. Based on previous surveys from the past 20 years, this site proved a suitable reference site with no signs of direct inputs of contamination [11] (Blaise et al., 2016). Two anthropized sites were selected at the villages of Tadoussac (TAD) and Baie-Sainte-Catherine (BSC), close to harbors supporting both recreational and commercial boating activities (e.g., whale-watching excursions). The last site, Bay Sainte-Marguerite (BSM), located in the Fjord-du-Saguenay National Park and upstream of the Saguenay River, is an area characterized by regular visits of beluga whales during summer. Indeed, the BSM site has been identified as a beluga residency area and is frequented by females and young feeding on various food sources such as worms (*Hediste diversicolor*), crustaceans, *Mya arenaria* clams and small fish [14, 15]. Although this site does not support any harbors or marina, the site faces the Saguenay Fjord commercial seaway and the Sainte-Marguerite River, a suspected source of unintended industrial and domestic waste products [16, 17]. Between 40 and 60 clams were collected at each of the 4 sites during low tide, in the morning, in the last week of September 2020. The clams were transported back to a local facility for processing. Clam wet weight and longitudinal length were measured, and their age was determined by the number of major grooves. A subset of 15 clams were randomly sampled for air survival time evaluation and the remainder were stored at −85 °C. Surface water samples (1 L) were collected to determine the basic physico-chemical analysis following standard methods for pH, conductivity, salinity and dissolved oxygen [18].

2.2. Elemental analysis

Elemental analysis of clam tissues was performed as previously described [18]. Briefly, soft tissues from randomly picked individuals (N = 6) were prepared and weighted for ion coupled plasma mass spectrometry [19]. Soft tissues were added to a Quartz digestion vessel in 4 mL 8 M of nitric acid and heated at 240 °C for 15 min using a microwave oven. After cooling, the samples were completed to 50 mL of 1% hydrochloric acid (HCl) solution. Elemental analysis included the elements using the main isotope, and the data were expressed as μg/g wet weight.

2.3. Clam health status evaluation

CF (whole mass/shell length), GI (shell length/age) and air survival time (lethal time in days) were determined as previously described [20]. Clam age was estimated by the number of major grooves on shells (annual growth rings). Air survival time was determined by placing 15 live clams at each site in plastic plates and incubating at 15 °C under 80% humidity. The weight was determined on each day and measured each day until mortality as determined by the absence of any shell reaction after handling (shells remain open following death). The air survival data were expressed as lethal time (days). The weight loss index (WLI) at the time of death was also determined by measuring clam weight on each day until death. The decrease in weight is thought to originate from dehydration/loss of water. The WLI was calculated as follows: $\text{WLI}\ (\%) = 100 \times (\text{weight}_{\text{to}} - \text{weight}_{\text{tdeath}})/\text{weight}_{\text{to}}$.

2.4. Data analysis

Clams (15 or 6 for biomarker and elemental analysis, respectively) were collected at three polluted sites (TAD, BSC, BSM) and one reference site (BAU) in the St. Lawrence Estuary and Saguenay Fjord. Following normality and variance homogeneity confirmation (Bartlett test), the data were subjected to an analysis of variance (ANOVA) followed by Tukey's test, using the BAU site as the reference site. In the case of any deviance from normality of heterogenous variance, the data were log-transformed before analysis. Correlation analysis of biomarker data (encompassing all treatments) was also performed using the Pearson-moment procedure. Principal component analysis was also performed to highlight the most important exposure and effect biomarkers. Significance was set at $p < 0.05$ and the SYSTAT software package was used (version 13, SanJose, CA, USA).

3. Results

The surface water properties were determined at the clam collection sites during low tide (**Table 1**). Water pH and conductivity were relatively stable, at pH 8.1–8.3 and 26,204–28,363 μS cm^{-1}, respectively, across the sites. Salinity closely followed conductivity and ranged between 23.68 and 28.96 PSU. Dissolved oxygen in water was very stable at the sites, with a mean concentration of 10.4 ± 0.15 mg/l.

Health status was determined by following changes in weight/length ratio (condition factor, CF), the growth index (GI), air survival and the weight loss index (WLI) (**Figure 2**). The CF was significantly lower at the anthropized sites BSC and TAD (**Figure 2A**). The GI was significantly lower at BSC (**Figure 2B**).

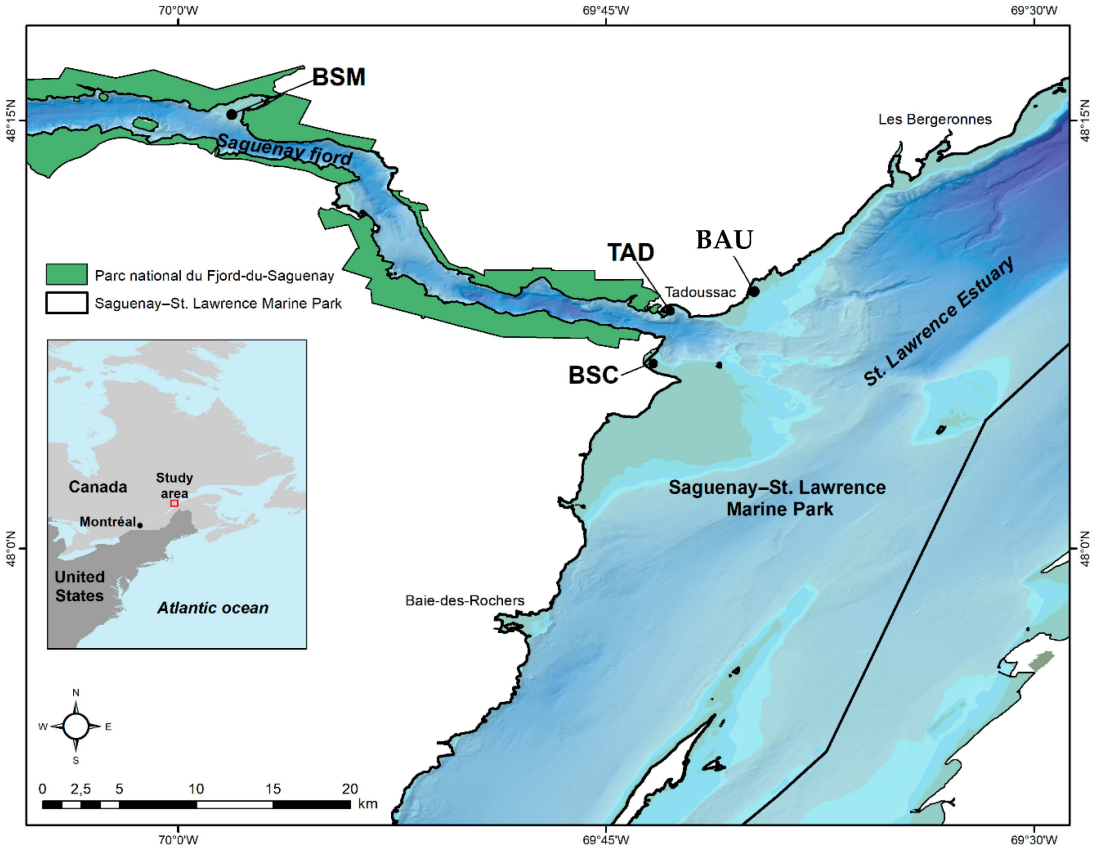

Figure 1 • Study area. The locations of the four *Mya arenaria* intertidal sampling sites: TAD (Baie de Tadoussac), a site subject to wharf, marina and wastewater discharge pollution; BAU (Baie du Moulin Baude), a reference site with no known or apparent source of pollution; BSC (Baie Sainte-Catherine), a site also subject to wastewater discharge and wharf pollution; BSM (Baie Sainte-Marguerite), a St. Lawrence Estuary beluga whale high-residency area.

Table 1 • Surface water physico-chemical characteristics.

Parameters	BAU	TAD	BSC	BSM
pH	8.29 ± 0.1	8.1 ± 0.05	8.15 ± 0.1	8.23 ± 0.1
Conductivity (μScm^{-1})	28,362 ± 280	26,204 ± 240	27,510 ± 220	26,738 ± 250
Salinity (psu [1])	28.96 ± 1	23.68 ± 1	26.18 ± 3	25.12 ± 2
O_2 (mg/l)	10.1 ± 0.1	10.9 ± 0.1	10.3 ± 0.2	10.7 ± 0.2

[1] Practical salinity units: g/l; micro Siemens/cm: μScm^{-1}.

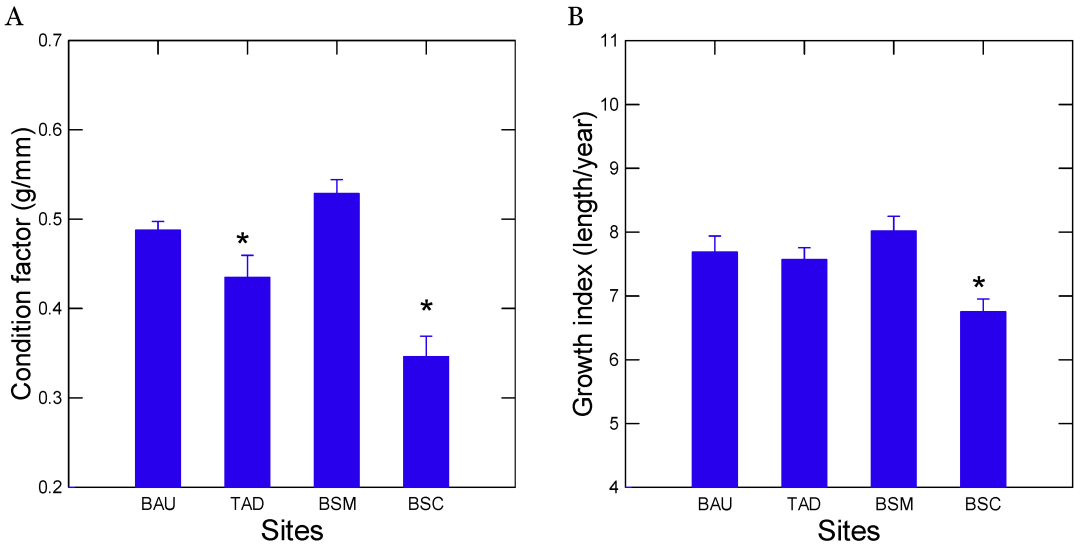

Figure 2 • *Cont.*

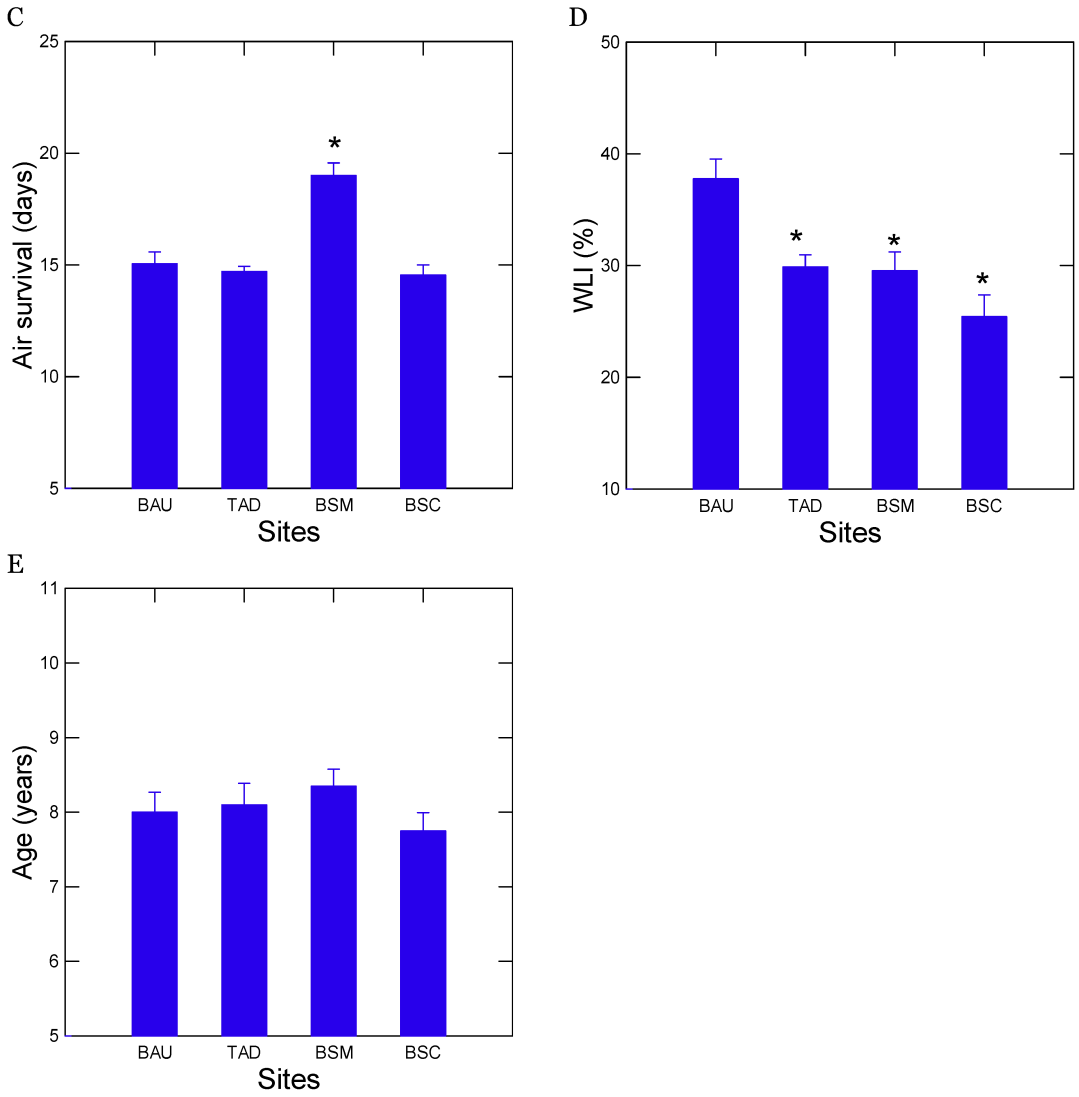

Figure 2 • Health status of collected *Mya arenaria* clams. Health status of feral clams were determined by the condition factor (CF) or clam weight/shell length (**A**), the growth index (GI) (**B**), air survival or lethal time (**C**), the weight loss index (WLI) (**D**) and mean age (**E**). The data represent the mean with the standard error. The star symbol * indicates significance ($p < 0.05$) from the reference BAU site.

The capacity of clams to survive air emersion was not significantly reduced across the sites (**Figure 2C**). A significantly higher air survival time was observed in clams at the BSM site. The WLI of clams at the time of death was significantly lower in clams from the BSC, TAD and BSM sites (**Figure 2D**). There were no significant changes in clam age between sites (**Figure 2E**). Correlation analysis revealed that CF was significantly associated with the GI (r = 0.30) and air survival time (r = 0.40). While the mean age of sampled clams did not significantly vary, it was significantly correlated with CF (r = −0.68), suggesting that CF was lower in aged animals.

The levels of essential trace elements were examined in *Mya arenaria* clams (**Table 2**). Only elements that significantly differed between sites were reported. At the anthropized sites, there was a general tendency of decreased essential elements (Ca, Co, Fe, K, Mg, Mo, P and Ni) indicative of compromised osmoregulation for the hard cations K, Ca and Mg. In other cases, the following elements were increased at either the anthropized sites or the BSM site: Cu, Fe, Ni and Zn. Correlation analyses revealed that the CF was significantly correlated with Fe (r = −0.51), Mg (r = −0.56) and Zn (r = 0.41) (**Table 3**). The GI was correlated with Co (r = −0.46), Mo (r = −0.48) and Ni (r = −0.48). LT was correlated with Mg (r = −0.68) and total P (r = −0.44) tissue levels. The WLI was correlated with K (r = 0.50).

The levels of non-essential trace elements were examined in *Mya arenaria* clams (**Table 4**). The elements Ag, Cd, Cr, Hg, Se, Sn and V were elevated in at least one the three sites (**Table 4**). The elements Ag, Al, As, Ba, Be, Bi, Li, Mn, P and Sr were decreased in at least one site compared to cthe reference site BAU. Correlation analysis revealed that the CF was correlated with Al (r = −0.46), As (r = −0.5), Cd (r = 0.51), Li (r = −0.48), Lu (r = −0.49), Sm (r = −0.44), Sc (r = −0.54), Ag (r = 0.69) and Tb (r = −0.43). The GI was significantly correlated with Al (r = −0.50), Be (r = −0.46), Ce (r = −0.52), Cr (r = −0.47), Gd (r = −0.46), La (r = −0.45), Li (r = −0.47) and Lu (r = −0.50). LT was correlated with Al (r = −0.51), As (r = −0.59), Ba (r = −0.54), Cd (r = 0.63), Pb (r = −0.5), Li (r = −0.55), Hg (r = 0.46), Pr (r = 0.50), Sc (r = −0.46) and Ag (r = 0.73). The weight loss index (WLI) was significantly correlated with Cd (r = −0.63), Pb (r = 0.55) and Ag (r = −0.46). Hence, the following elements were the most significantly associated with the effect biomarkers: Ag, Li, Cd and Al.

Table 2 • Change in essential elements in clam tissues.

Elements	BAU	BSC	BSM	TAD
Ca	961 ± 105	764 ± 18 *,b	598 ± 30 *,b	477 ± 22 *,b
Co	0.66 ± 0.03	0.78 ± 0.08	0.42 ± 0.005 *,b	0.23 ± 0.02 *,b
Cu	2.5 ± 0.7	2.3 ± 0.17	3.9 ± 0.37	5.7 ± 0.4 *,a
Fe	693 ± 30	881 ± 52	472 ± 30 *,b	1122 ± 271 *,a
K	1253 ± 102	990 ± 22 *,b	851 ± 10 *,b	1130 ± 33
Mg	725 ± 30	781 ± 13	408 ± 25 *,b	576 ± 43 *,b
Mo	3.2 ± 0.9	4.7 ± 0.8	1.64 ± 0.2 *,b	0.84 ± 0.02 *,b
P	986 ± 20	991 ± 23	700 ± 35 *,b	774 ± 47 *,b
Ni	16.2 ± 1.4	24 ± 4 *,a	7.6 ± 1.1 *,b	3.9 ± 0.13 *,b
Zn	10.7 ± 0.4	10.3 ± 0.36	13 ± 0.6 *,a	15 ± 0.5 *,a

The data are the mean with standard error expressed as μg/g. The star * symbol represents significance in comparison with the Bau reference site. Increased levels are labeled as [a] and decreased levels are labeled as [b].

Table 3 • Clam health status and elemental loadings in tissues.

	Age	Condition factor	Growth index	Lehtal time	Weight loss	Targets affected
Essential elements						
Ca	−0.18	−0.24	0.01	−0.25	0.37	0
Co	0.20	−0.33	**−0.46**	−0.31	0.13	1
Cu	−0.03	0.13	0.20	−0.01	−0.10	0
Fe	−0.25	**−0.51**	−0.18	−0.34	0.08	1
K	−0.24	−0.15	0.14	−0.31	**0.50**	1
Mg	−0.06	**−0.56**	−0.33	**−0.68**	0.33	2
Ni	−0.10	**−0.42**	−0.23	**−0.52**	0.36	2
P	−0.00	−0.34	−0.23	**−0.44**	0.39	1
Zn	−0.01	**0.41**	0.28	0.33	−0.29	1
Non-essential elements						
Al *	0.15	**−0.46**	**−0.5**	**−0.51**	0.18	3
As	−0.03	**−0.50**	−0.32	**−0.59**	0.21	2
Ba	0.0	−0.38	−0.28	**−0.54**	0.30	1
Be	0.22	−0.31	**−0.46**	**−0.41**	0.11	2
Cd*	0.27	**0.51**	−0.03	**0.63**	**−0.63**	3
Ce	0.16	−0.38	**−0.52**	−0.20	0.03	1
Cr	0.23	−0.35	**−0.47**	−0.34	0.02	1
Gd	0.09	−0.35	**−0.46**	−0.19	0.06	1
Li*	0.14	−0.48	**−0.47**	**−0.55**	0.22	3

Table 3 • *Cont.*

	Age	Condition factor	Growth index	Lehtal time	Weight loss	Targets affected
La	0.31	−0.08	**−0.45**	0.09	−0.19	1
Pb	−0.29	−0.26	0.14	**−0.50**	**0.55**	2
Lu	0.05	**−0.49**	**−0.52**	−0.26	0.05	2
Mn	0.02	−0.27	−0.24	−0.28	0.28	0
Hg	0.35	0.35	−0.14	**0.46**	−0.37	1
Mo	0.23	−0.35	**−0.48**	−0.34	0.02	1
Pr	0.13	0.27	0.1	**0.50**	−0.27	1
Sm	−0.02	**−0.44**	−0.39	−0.27	0.11	1
Sc	−0.18	**−0.54**	−0.28	**−0.46**	0.23	2
Ag*	0.20	**0.70**	0.23	**0.73**	**−0.46**	3
Se	−0.24	**−0.49**	−0.11	**−0.66**	0.15	2
Sr	−0.06	−0.19	−0.10	−0.18	0.23	0
V	−0.23	−0.36	−0.09	−0.15	0.06	0
Sn	**−0.41**	−0.1	0.37	−0.09	0.05	1
Tb	−0.028	**−0.43**	−0.38	−0.28	0.12	1
Y	0.01	−0.37	−0.37	−0.25	0.11	0
# of elements	1	13	11	15	4	

Significant correlations are in bold (r > 0.4). The star symbol * indicates elements that were correlated with at least three biomarkers.

Table 4 • Change in non-essential elements in clam tissues.

Metals/metalloids	**BAU**	**BSC**	**BSM**	**TAD**
Al	264 ± 6	299 ± 32	172 ± 13 ^{*,b}	156 ± 9 ^{*,b}
As	1.1 ± 0.02	1.1 ± 0.04	0.8 ± 0.03 ^{*,b}	0.91 ± 0.03 ^{*,b}
Ba	1.7 ± 0.03	1.6 ± 0.1	0.9 ± 0.08 ^{*,b}	0.77 ± 0.02 ^{*,b}
Be	0.007 ± 0.001	0.008 ± 0.001	0.005 ± 0.0003 ^{*,b}	0.004 ± 0.001 ^{*,b}
Bi	0.002 ± 0.0004	0.002 ± 0.001	0.001 ± 0.0002 ^{*,b}	0.001 ± 0.0004 ^{*,b}
Cd	0.05 ± 0.002	0.06 ± 0.006	0.08 ± 0.005 ^{*,a}	0.07 ± 0.003 ^{*,a}
Cr	27 ± 2	40 ± 6 ^{*,a}	14 ± 2 ^{*,b}	8.2 ± 0.4 ^{*,b}
Pb	0.26 ± 0.008	0.17 ± 0.011 ^{*,b}	0.11 ± 0.01 ^{*,b}	0.25 ± 0.02 ^{b}
Li	0.3 ± 0.008	0.34 ± 0.03	0.18 ± 0.016 ^{*,b}	0.17 ± 0.004 ^{*,b}
Mn	55 ± 7	44 ± 3 ^{*,b}	23 ± 1 5 ^{*,b}	8 ± 1.2 ^{*,b}
Hg	0.015 ± 0.001	0.015 ± 0.001	0.026 ± 0.002 ^{*,a}	0.004 ± 0.0005 ^{*,b}
Ag	0.067 ± 0.003	0.05 ± 0.004	0.24 ± 0.03 ^{*,a}	0.075 ± 0.003 ^{b}
Se	0.28 ± 0.004	0.29 ± 0.015	0.2 ± 0.008 ^{*,a}	0.28 ± 0.01

Table 4 • *Cont.*

Metals/metalloids	**BAU**	**BSC**	**BSM**	**TAD**
Sr	11.2 ± 0.9	10.2 ± 0.3	8.2 ± 0.4 *,b	6.6 ± 0.32 *,b
V	1.18 ± 0.04	1.48 ± 0.07	1 ± 0.08	2.45 ± 0.7 *,a
Sn	0.09 ± 0.04	0.07 ± 0.02	0.07 ± 0.01	0.24 ± 0.02 *,a
Rare earth elements				
Ce	1.47 ± 0.02	1.9 ± 0.09 *,a	1.21 ± 0.1 *,b	0.81 ± 0.07 *,b
Gd	0.1 ± 0.002	0.12 ± 0.004 *,a	0.09 ± 0.01	0.06 ± 0.008 *,b
La	0.74 ± 0.007	0.97 ± 0.06 *	0.88 ± 0.08 *,a	0.41 ± 0.008 *,b
Lu	0.004 ± 0.001	0.006 ± 0.001	0.004 ± 0.001	0.003 ± 0.001 b
Pr	0.1 ± 0.01	0.1 ± 0.015	0.3 ± 0.07 *,a	0.06 ± 0.007 *,b
Sm	0.12 ± 0.003	0.15 ± 0.004 *	0.11 ± 0.01	0.07 ± 0.009 *,b
Sc	0.07 ± 0.004	0.08 ± 0.002	0.05 ± 0.003 *,b	0.05 0.007 *,b
Y	0.37 ± 0.01	0.45 ± 0.02 *	0.35 ± 0.04	0.24 ± 0.048 b

The data are the mean with standard error expressed as μg/g. The star * symbol represents significance in comparison with the Bau reference site. Increased levels are labeled as [a] and decreased levels are labeled as [b].

In the attempt to gain a more holistic view of the exposure and effect biomarkers in clams, a principal component analysis was performed (**Figure 3**). Based on the factorial scores (**Figure 3A**), they were significantly higher for the anthropized sites, while they were similar for BSM compared to the reference BAU. The analysis revealed that 70% of the total variance was explained by two factors (**Figure 3B**). The effect biomarkers were associated with Ag, Cd, Hg, Se and Zn. While the CF was closely related to toxic metals (Ag, Cd), the GI and the WLI were associated with Zn and Se, respectively.

4. Discussion

Elemental analysis revealed that Al, Cd, Li and Ag were significantly related to the effect biomarkers (CF, LT, GI and WLI) based on correlation analysis. Al levels ranged between 258 and 270 μg/g at the reference site and reached 333 μg/g at one of the anthropized sites (BSC). Al levels were significantly decreased at the BSM and TAD sites, at a concentration range of 150–170 μg/g, in respect to the BAU reference site (260 μg/g). Al levels at the BAU reference site were circa 20 times higher than those in laboratory-reared

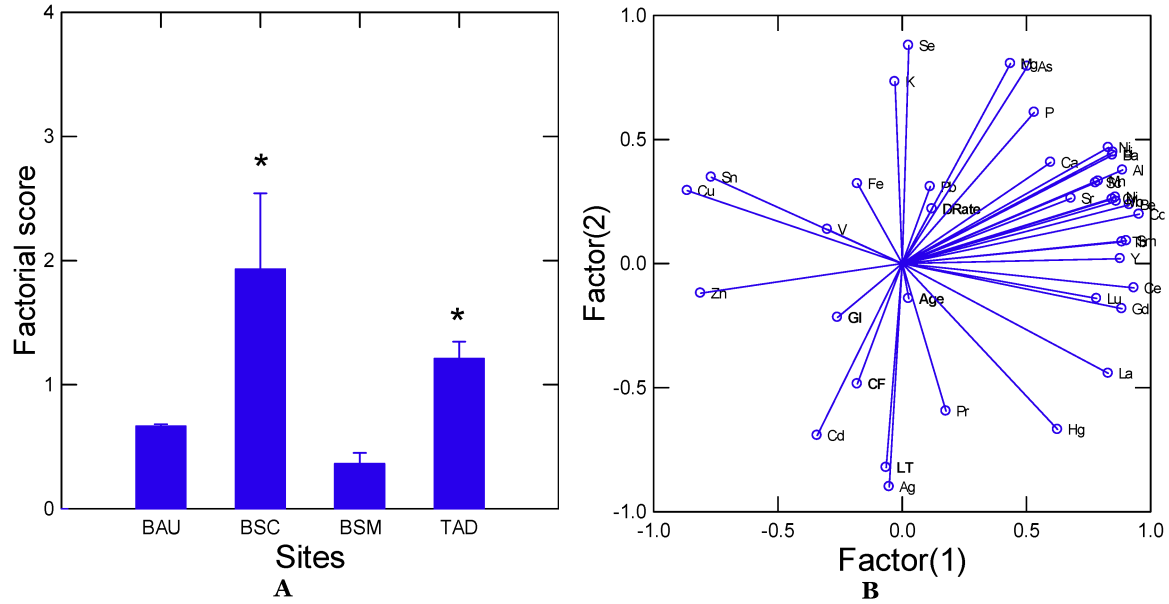

Figure 3 • Principal component analysis of exposure and effect biomarker data. Principal component analysis was performed on the exposure (tissue levels of metals/elements) and effect (age, CF, GI, LT, WLI) biomarker data. The factorial score (**A**) and principal components (**B**) are shown. The total explained variance was 70% by 2 factors (x and y axes) and the major biomarkers are those with factorial weights > 0.75. The star symbol * indicates significant from the reference BAU site.

oysters *Crassostrea gigas*, which is normal for this organism since it is less in contact with sediments [21]. The tissue levels of Al reached 93–113 μg/g in oysters (control oysters were at 10 μg/g) exposed to 300 μg/L Al for 84 days. At this tissue concentration, phagocytosis activity was reduced to 40% of the control, and lipid peroxidation and glycogen contents were significantly reduced. In another study with *Mytilus edulis*, tissue levels of Al reached 309 μg/g dry weight following exposure to 530 μg/L for 13 weeks in contaminated water compared to controls (39 μg/g) [22]. In non-contaminated water, tissue Al concentration was 80 μg/g dry weight compared to controls (39 μg/g). In contaminated water, high Al in the digestive gland was associated with decreased filtering activity from valve closure. This suggests that Al in tissues (>80 μg/g) could inhibit both filtering and feeding activity and is negatively associated with size and growth. A previous study with clams (same size range) in the same area reported baseline values of 75 μg/g at the BAU reference site [20], which was 3.5 times lower than the Al levels in the present study. This suggests that tissue Al levels could vary in time, probably from the release of geochemical Al (increased bauxite mobilization and leachates from rain events). Bauxite is a sedimentary rock rich in alumine and prevails in this area, and strategies are in place to exploit bauxite residues from Al industries in this area [23]. This suggests that Al contamination in this area rose at levels likely to affect health condition and growth. The element V was shown to reach concentrations of 0.1 to 0.4 μg/g in the digestive gland in *Mya arenaria* clams exposed to divalent (reduced) V [24]. At this tissue concentration, increased peroxidase activity, glucose and heme degradation were observed. Tissue concentrations of V were found at a baseline level of 1.1 μg/g and increased to 2.45 μg/g at one anthropized site (TAD) in the present study, albeit V in tissues was not correlated with any of the effect biomarkers. In a previous study with blue mussels *Mytilus* sp., an increase of 0.2 μg/g ww V doubled the levels of metallothioneins, a protective mechanism against toxic metals [25]. In respect to Ag and Cd, their toxicity is much more understood in aquatic organisms. For Cd, the combined tissue levels of Cd (digestive gland and gonad) reached circa 1.25 μg/g ww, leading to the induction of metallothioneins and lipid peroxidation at a threshold tissue concentration of 0.34 μg/g in the burrowing clam *Scrobicularia plana* [26]. In the present study, tissue levels of Cd were circa 4 times below those found in the burrowing clam study, suggesting that the Cd levels found in clams do not pose an immediate danger to the population. In respect to Ag, the combined levels (digestive gland and gonad) reached circa 3.4 μg/g, leading to the induction of metallothioneins and lipid peroxidation at a tissue concentration of 1.025 μg/g Ag. In the present study, tissue Ag levels reached 0.27 μg/g ww, which was also 4 times lower than the tissue concentration able to produce an effect. In another study, exposure to dissolved Ag led to a combined accumulation of 6 ug/g ww [27], which was 22 times higher than the concentration measured in feral clams in the present study. In respect to Li, tissue levels in *Venerupis corrugata* clams reached 8 μg/g following exposure to 800 μg/L for 14 days [28]. At this tissue level, mitochondria electron transport activity and lipid peroxidation were significantly reduced, but protein carbonylation (a marker of protein oxidative damage) was significantly increased. Li levels in clams were in the order of 0.17 to 0.37 μg/g, which is also well below the tissue levels in the former study. These studies suggest that Ag, Cd and Li are unlikely to contribute to the observed changes in CF, GI and LT in clams on their own. Al and V are present at concentrations likely to produce effects in clams, but only tissue Al levels were significantly correlated with the CF, GI and lethal time to air emersion (**Table 2**). Although these studies applied long exposure periods, they do not constitute the complete lifespan of clams (12–15-year lifespan). Another possibility resides in the combined effects of toxic elements such as Cu, Ag and Cd, giving a total loading of 6 μg/g at the most contaminated sites compared to 2.1 μg/g at the reference site, which is sufficient to initiate toxicity. However, this hypothesis should be further confirmed.

The essential cations K, Ca and Mg are tightly regulated in cells and are in equilibrium with Na for osmoregulation and the maintenance of membrane potential and functions. K ions are maintained in tissues by Na/K-ATPases by excluding Na outside cells and maintaining K levels in cells. Clams from anthropized sites showed reduced tissue levels of K, Ca and Mg, suggesting altered osmoregulation. This is consistent with the reduced WLI in the contaminated sites, where clams lose less water (and K) at the time of death compared to the reference site clams. These cations were previously reduced in clams exposed to low salinity stress at 10 PSU [29], well below the measured salinity at the sites investigated. Clams were collected at sites with no direct input of incoming rivers, except for the BSM site, where discharges from the Sainte-Marguerite River diffusively enter the bay, but no changes in water pH, conductivity, salinity and dissolved oxygen were found (**Table 1**). Hypoxia could have lowered K levels, since it was reported that low oxygen levels (0.5–2 mg/L) decreased Na/K-ATPase gene expression in *Ruditapes philippinarum* clams [30]. Decreased activity would have resulted in lower tissue K levels. However, these values are well below those measured at the study sites. Lower pH (from 8.2 to 7.7) than that in the ocean, however, was shown to increase Na/K-ATPase activity and would have increased K levels in tissues. Measured water pH values were between 8.1 and 8.29 at the study sites. The average pH of the ocean surface has decreased from 8.1 to approximately 8.0, with projections indicating a further decline of 0.3–0.5 units by the end of this century [31] . This was also confirmed in *Mercenaria mercenaria* clams treated with low pH (7.8), which had increased Na/K-ATPase activity, which would increase K levels in tissues [32]. This upregulation was most important in biomineralizing cells, suggesting impacts on shell growth and density. In another study, long-term pH acidification to 7.7 led to transgenerational increases in Na/K-ATPase activity in *Ruditapes philippinarum* clam populations [33]. However, no effects was observed for Ca/Mg-ATPase. Hence, based on the above studies, acidification would result in increased K levels in clams, which was not observed in the present study. The essential metal Cu was shown to reduce the number of Na/K-ATPase sites (which would decrease K levels in tissues) at tissue concentrations of 50 μg/g [34], which are circa 8 times higher than the measured levels of Cu in tissues from clams at the anthropized TAD site. In fish, gill and hepatic Na/K ATPase activity was inhibited by both Pb and Zn [35]. However, tissue levels of Zn and Pb were not measured. In another study, Na/K-ATPase activity in carp gills was significantly inhibited at tissue concentrations of 12 μg/g and 8 μg/g for Cu and Cd, respectively [36]. The Cu concentration was in the same range of Cu in tissues in clams collected at the anthropized site TAD, but Cd levels were not significantly different between the sites in the present study. In a previous study with clams at the same sampling sites, Cd levels reached values of 0.14 μg/g [20], which was circa 57 times lower than the carp study. Interestingly, nanoplastics were shown to reduce Na/K-ATPase activity in the gills of the Mediterranean mussel *Mytilus galloprovincialis* [37].

A recent study with clams in the present study area found plastic nanoparticles in soft tissues [38]. Principal component analysis revealed that As, Ca, Cu, Sn and V were closely related to polystyrene-like nanoparticles in tissues, suggesting that plastics could interfere with metal bioavailability and perhaps osmoregulation.

Another potential culprit for altered osmoregulation could be the occurrence of paralytic shellfish toxins from red tides which are known to occur in the study area [5, 39]. In zebrafish gills, reduced Na/K-ATPase activity was observed following exposure to aphantoxin, a toxin known to disrupt respiration and reduce Na/K-ATPase and carbonic anhydrase activities [39]. However, studies on the effects of algal toxins on osmoregulation in clams are lacking at the present time. A previous study revealed the occurrence of 320 toxin-producing algae/L in surface water on the north and south shore of the St. Lawrence Estuary and Saguenay Fjord, in the vicinity of the BSM site [40]. The study revealed that toxin-producing phytoplankton was positively correlated with human population density (probably increasing nutrient levels) and negatively correlated with total phytoplankton counts in water (suggesting a decreased abundance of non-toxic algae). The toxin-producing algae identified were diatoms (*Pseudo nitzschia delicatissima* and *seriatta*) and dinoflagellates (*Dinophysis* sp. and *Prorocentrum cordatum*) in the digestive gland, albeit toxin measurements were not provided. An increase in acetylcholinesterase activity in clams was also significantly related to toxic phytoplankton counts in water, suggesting neurological impacts consistent with paralytic shellfish toxins (saxitoxins). Future research should examine the cumulative effects of algal toxins and pollutants (metals) on osmoregulation in tissues, especially in the nervous tissues of clams. It is noteworthy that organic contamination could also be at play in this area. For example, butyltins and trace metals (Cd, Co and Fe) were found at higher levels in the water column, sediments and clams in this area [41, 42]. Concentrations up to 793 ng Sn g^{-1} d.w were found in zooplankton at the mouth of the Saguenay Fjord and estuary. A poly- and perfluoroalkylated substance (PFAS) contamination problem in the sediment and surface waters in the Baie des Ah!Ah!, upstream the Saguenay Fjord, was recently reported [43]. The PFAS contamination arose from the long-term activities of fire control from military activities/training in Bagotville. However, the extent of contamination, if any, of benthic organisms has yet to be established.

In conclusion, clam health status was determined by following changes at the morphological level showing that the CF, GI and the WLI were reduced in at least one anthropized site (BSC). Air survival time was not significantly affected at the anthropized sites but was increased at the BSM site, although the WLI was significantly reduced. These clams were generally larger in size but were not devoid of contaminants, as evidenced by increased levels of Hg, Ag and Se. For Cd, its sources could be of geological origin in this sector [5, 39]. The following essential elements were reduced at the anthropized sites and the BSM site: Ca, Co, Fe, K, Mg, Mo and Ni. Cu, Fe, Ni and Zn levels were increased at the TAD site, with the exception of Ni, which was higher at the BSC site. The decreases in K, Ca and Mg suggest changes in osmoregulation, which could be explained by hypoxia, low salinity and toxin-producing algae, but not by acidification. However, no evidence of hypoxic and low-salinity conditions was observed in intertidal clams based on the measured dissolved oxygen and salinity levels. However, the combined/cumulative effects of these metals could not be excluded as a potential cause for the observed effects. Based on elemental analysis, more research will be needed to determine the combined effects of toxic elements such as Al, V, Cd, Cu and Ag in feral clam populations.

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Conflict of interest

The authors declare no conflict of interest.

Data availability statement

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