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Heavy Metal Levels and Length-Weight Dynamics of *Anodontia philippiana* (Reeve, 1850) from Barobo and Hinatuan, Surigao del Sur, Philippines

*Ritchen R. Ecleo, Joycelyn C. Jumawan, Exur M. Buenaflor, & Jashin J. Rosal**

Department of Biology, College of Mathematics and Natural Sciences, Caraga State University, Butuan City, Agusan del Norte, Philippines

*Corresponding Author
*Email:jjrosal@carsu.edu.ph
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A B S T R A C T

Anodontia philippiana is among the commercially valuable edible clams found in Brgy. Wakat, Municipality of Barobo, and Brgy. Loyola, Municipality of Hinatuan, Surigao del Sur, Philippines. However, anthropogenic activities may pose a threat to the abundance and quality of this species. This study aimed to determine the levels of lead (Pb), nickel (Ni), chromium (Cr), and mercury (tHg) in both the sediments and flesh of *A. philippiana* using Atomic Absorption Spectrophotometry (AAS). Additionally, the length-weight relationship (LWR) of *A. philippiana* was assessed to determine the size structure of the species' population. Among the heavy metals tested, only the concentration of Cr (59±0.38 ppm) in Hinatuan exceeded the prescribed tolerable limit of ≥50 ppm (FAO, 1993). Conversely, the heavy metal levels in the flesh of *A. philippiana* consistently remained below tolerable limits. Furthermore, *A. philippiana* from Barobo exhibited a positive allometry (B>3), while those from Hinatuan showed a negative allometry (B<3), suggesting the influence of environmental stressors on growth patterns. The LWR in *A. philippiana* exhibited significant differences (P<0.001) between the two stations. Despite heavy metal concentrations in *A. philippiana* flesh and sediments were mostly below standard limits, the observed negative allometric growth pattern in Hinatuan indicates the presence of ecological stressors in the sediments. This study underscores the potential impact of heavy metals on the growth patterns of *A. philippiana.* It is recommended that regular monitoring of heavy metal concentrations be conducted in these areas. Further studies can provide better insights into morphometric patterns in *A. philippiana* and other commercially significant bivalves.

Keywords: *allometry, bioaccumulation, environmental stressors, growth patterns, LWR*

1 Introduction

Heavy metals are metallic elements or metalloids that pose a significant risk to the environment and human health due to their toxic properties. These substances are present in varying concentrations in the Earth's crust and can enter soil and water systems through natural processes and human-related activities (Tchounwou et al. 2012). Heavy metal pollution occurs when the concentration of these elements exceeds the required or desired levels in living organisms, leading to various dysfunctions in cellular components. The pollution of heavy metals has become a pressing environmental issue due to anthropogenic activities and industrialization. Human-related activities such as mining, smelting, fossil fuel usage, waste disposal, and manufacturing processes are the primary sources of heavy metal pollution (Asati et al. 2016).

Once these elements enter the food chain through soil and water, they can accumulate in the tissues of living organisms, affecting their health and survival. While some heavy metals are essential for daily nutrition, excessive amounts of others, such as lead, nickel, chromium, and mercury, can harm organisms and cause issues with growth, reproduction, and survival. The environment plays a crucial role in transporting and distributing heavy metals, and various environmental factors, such as soil and water properties, can influence their dispersion. The bioavailability of heavy metals can be affected by the physical and chemical conditions of the environment, making some areas more prone to contamination than others. Heavy metal pollution in the environment can lead to biodiversity loss and ecosystem changes (Jaishankar et al. 2014).

There have been studies on heavy metal accumulation in bivalves in Cagayan Valley (Raju 2021), Davao Oriental (Bersaldo et al. 2022), and Manila Bay (Montojo et al. 2021). These studies indicate that bivalve mollusks, such as clams, are known to have the ability to accumulate heavy metals and are widely used as bioindicators for monitoring heavy metal pollution in aquatic environments. Due to their efficient filter-feeding mechanism, these animals directly ingest metal-enriched particles, demonstrating their bioaccumulation capability in their habitat. Heavy metal accumulation in bivalves and the environment has become more frequent with increased human activity. Prolonged exposure to these pollutants can adversely affect the growth and morphology of bivalves, particularly in terms of length and weight (Raju 2021). Moreover, high concentrations of these heavy metals can also pose risks to humans, depending on the mode of exposure and the nutritional state of the exposed marine organism (Tchounwou et al. 2012).

Anodontia philippiana (Reeve, 1850) is commonly found in mangrove mudflats and intertidal sandy beaches. These clams are significant in abundance, biomass, and density. They play a crucial role in the food chain and food webs of mangrove forests, serving as primary mollusks (Myers et al. 2011). *A. philippiana* is widely distributed in the Indo-West Pacific region. In the Philippines, it is considered a seafood delicacy known as "Imbao" in Surigao del Sur. Due to their large size, high protein content, and ample flesh, they are highly valued in the region (Bersaldo et al. 2022). These clams provide a vital source of accessible protein for higher trophic levels, contributing to the shift from primary forest production to higher trophic levels in animal production (Carter 2014).

Surigao del Sur is a province characterized by an extensive network of streams and oceans, where small-scale gold mining activities are prevalent (Quisil et al. 2014). The Sorex River, also known as the Tambis River System, flows through the municipality of Barobo and neighboring areas, which are well-known for small-scale mining activities (Fajardo et al. 2015). This river serves as the primary source of water that empties into the Hinatuan coast, where fishing and artisanal fishery are the primary livelihoods of local communities. Despite the popularity of commercially sold *A. philippiana* as a food source in the Municipalities of Barobo and Hinatuan, limited information is available regarding the levels of metal contamination, including lead (Pb), nickel (Ni), chromium (Cr), and mercury (tHg), as well as the allometry, length-weight relationships, and the physical and chemical variables of the *A. philippiana* habitat. This study aims to determine the physicochemical properties of water in the coastal areas of Barobo and Hinatuan, Surigao del Sur, establish the length-weight relationship (LWR) of *A. philippiana*, and quantify the concentration of Pb, Ni, Cr, and tHg in both *A. philippiana* flesh and sediments collected from the sampling stations.

2 Materials and Methods

Study Area

The study was conducted in two municipalities of Surigao del Sur— Brgy. Wakat, Municipality of Barobo and Brgy. Loyola, Municipality of Hinatuan, Surigao del Sur, Philippines (Figure 1). Both are mangrove areas and local collection sites for the commercially sold *A. philippiana*. The sampling site in the Municipality of Barobo (8°34'9.876" N, 126°6'50.652" E) is located relatively far from residential areas and mining sites. On the other hand, Brgy. Loyola in Hinatuan (8°21'12.1824" N, 126°19'7.1004" E) serves as the primary exit point of the Tambis River system. This river system is a slow-moving water channel, approximately 15-20 m in width, stretching up to 20 km, and known for the highest number of small-scale mining activities in the Province of Surigao

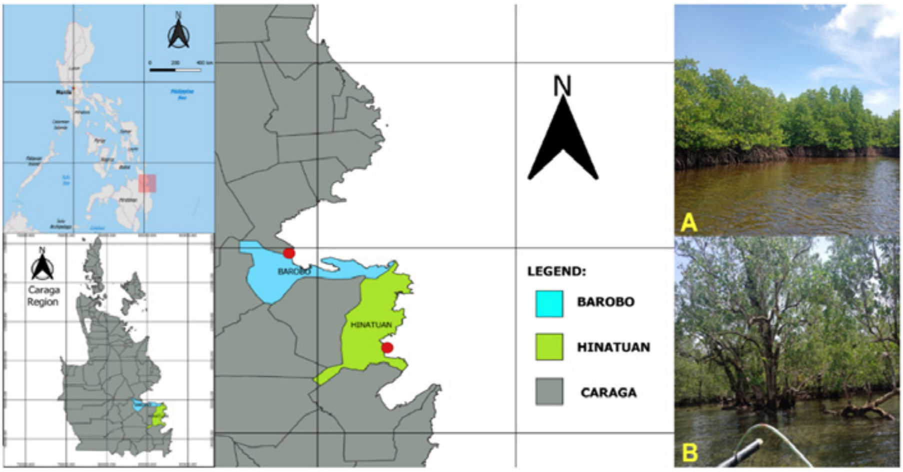

Figure 1. Map showing the study sites in Brgy. Wakat, Municipality of Barobo (blue; A) and Brgy. Loyola, Municipality of Hinatuan (green; B), Surigao del Sur, Philippines

del Sur (Fajardo et al. 2015). Sampling was done during the low tides of August 2022 to ensure efficient collection of *A. philippiana* and sediments.

Sample collection and preparation

Determination of physicochemical parameters (pH, dissolved oxygen, electroconductivity, temperature, total suspended solids, and salinity) was done using the Hanna Multiparameter Portable Meter (Hanna Instruments, Ltd.). The probe was submerged in the surface water until measurements were displayed and data were logged.

The sediment samples were collected using the protocol of the Association of Analytical Communities (AOAC) Official Method 999.11 for analyzing samples, with slight modifications based on the availability of *A. philippiana* in the sediment stations, which were subjectively selected. Pooled wet sediment samples were collected using an improvised non-metal grab sampler. Non-sediment components were removed by hand. The *A. philippiana* samples in these areas were hand-picked for Length-Weight Relationship analysis. The *A. philippiana* adults were measured for their shell length (cm) using a vernier caliper and an analytical scale for the weight (g). The mature individuals (at least 3.5 cm; n=15) were utilized for heavy metal analysis. The flesh was removed from their shells and pooled. The collected samples were subsequently placed in a resealable bag, properly labeled, stored in an ice-filled chiller, and transferred to the laboratory in less than 24 hours for digestion and analysis.

Heavy metal analysis of sediment

Careful homogenization of the material and equipment was carried out in the laboratory to prevent cross-contamination. Methods for the acid digestion of sediments of the United States Environmental Protection Agency (U.S. EPA) were used (U.S. EPA 1996). Personnel trained in acid-related stages performed the digestion operation by transferring a sample weighing 1-2 g (wet weight) or 1 g (dry weight) to a digestion vessel. An acid vapor scrubber system was recommended for the fume hood during the operation for safety purposes. The sample underwent heating and cooling and concentrated HNO_3 until the reaction was complete. After the evaporated solution, water and 30% H_2O_2 were added, and the sample was subjected to another round of heating and cooling. The resulting digest was filtered, centrifuged, or settled before analysis by Flame Atomic Absorption Spectrometry (AAS) for Pb, Ni, and Cr, while Cold Vapor AAS for tHg.

Heavy metal analysis of A. philippiana flesh

HM metal in flesh was done following the protocol of the Association of Official Analytical Chemists (AOAC) International (2019). The pre-treatment involved drying a 10-20 g test portion of the samples using a drying oven, water bath, or hot plate at 100°C. The ashing process involved gradually raising the temperature to 450°C at a maximum rate of 50°C/h, allowing it to sit for at least eight hours or overnight, and adding 1-3 mL of water to wet the ash. The ash dissolved in 0.1M HNO_3, and the analytes using flame and graphite techniques were identified. After digestion, the residue was dissolved in 10.0-30.0 mL of 0.1M HNO_3, and the calibration curves were constructed using at least three standards with background corrections. The analytes of interest, namely Pb, Ni, and Cr, were analyzed using Flame AAS, while Cold Vapor AAS was used to determine the concentration of tHG.

Data analyses

The length-weight relationship was determined by the equation $W = aL^b$, where 'W' is the total body weight, 'a' is the regression intercept, 'L' is the average length, and 'b' is the regression coefficient (Urban and Campos 1994; Urban 2000). The regression coefficients 'a', 'b,' and the coefficient of determination (R^2) were assessed by least squares regression inquiry of the logarithm transformed LWR illustration Log W = b log L + log a. The value of 'b' was used to determine the growth pattern: b=3, isometric growth; b>3, positively allometric growth; and b<3, negatively allometric growth. The strength of the relationship between the variables X and Y was indicated by the regression analysis's coefficient of determination 'R^2' values (Vasconcelos et al. 2018).

Significant differences between the groups of heavy metals were determined using the Mann-Whitney U test. Any correlations between variables of the length-weight were determined using Spearman rank order correlation and point biserial correlation. Differences with $P<0.05$ were considered statistically significant.

3 Results and Discussion

Physico-chemical analysis of surface water

The physical and chemical characteristics of seawater were analyzed at two coastal stations in Surigao del Sur (Table 1). The results indicated that, out of the six parameters tested in the Municipality of Barobo, the electroconductivity (EC) and temperature met the required minimum water quality standards according to DAO (2016) and USEPA (1976) guidelines. Similarly, in Hinatuan, the pH, temperature, and total suspended solids were within the required minimum levels. However, the dissolved oxygen (DO) and salinity levels at both stations, along with the pH and total suspended solids (TSS) in Barobo and the electrical conductivity (EC) in Hinatuan, did not meet the required standards. Further analysis revealed no significant difference in the physicochemical parameters between the two stations ($P>0.05$).

The physicochemical parameters are indicators of water quality in the area (Awoyemi et al. 2014). The impacts of human activities on water quality are extensive and diverse, to the extreme point that they destroy the natural flow and limit water use that may affect the marine organism and their habitat (Bhadja and Kundu 2012). Notably, the dissolved oxygen (DO) for both areas did not reach the minimum levels, with 2.74±0.31 ppm and 2.08±0.38 ppm in Barobo and Hinatuan, respectively. DO is the concentration of oxygen gas dissolved in water. Sufficient DO is critical for bivalves' respiration, which can significantly affect their growth and reproduction. Low DO levels can cause hypoxia, which may adversely affect marine organisms (Ning et al. 2011).

Heavy metal analysis of sediments

Concentrations of heavy metals in the sediments from the Municipalities of Barobo and Hinatuan are summarized in Table 2. The order of the average concentrations of heavy metals in the sediment in Barobo is Pb>Cr>Ni>tHg, while samples in Hinatuan are Cr>Pb>Ni>tHg. Only Cr

Table 1. Physicochemical characteristics of seawater in the Municipalities of Barobo and Hinatuan stations in comparison to the recommended levels of local and international standards

Parameters	Mean±SD		P-value	DAO (2016)	USEPA (1976)
	Barobo	Hinatuan		Range/ Min. levels	
pH	6.65±0.51*	7.12±0.10	0.10	7.0–8.5	6.5–8.5
Dissolved Oxygen (ppm)	2.74±0.31*	2.08±0.38*	0.20	5	5
Electroconductivity (µs/cm)	48.36±0.96	58.21±19.16*	1.00	30–55	–
Temperature (ºC)	30.22±0.18	30.98±0.06	0.10	26–30	29–32
Total Suspended Solids (mg/L)	24.51±0.09*	35.33±12.21	0.70	25	25
Salinity (ppm)	31.78±0.50*	59.33±4.00*	0.10	100	75

**Mean values with an asterisk indicate levels not within the standard limits*

(59±0.38 ppm) exceeded the tolerable limit set by FAO (1993). The concentration of tHg, on the other hand, is less than the reported limit of GFAA and ICP-MS with a value of 0.002 ppm. There are no significant differences in heavy metal concentration from both sites (P>0.05).

Sediments play a vital role in the marine ecosystem, providing a habitat for marine organisms such as the mangrove clams *A. philippiana*. Sediments are also the primary contact point for the toxicity of heavy metals that accumulate in the ecosystem (Zahra et al. 2014). Heavy metals are known to have detrimental effects on the environment even in minute amounts. Heavy metals at low concentrations in seawater can negatively affect marine bivalves, reducing growth rates, decreasing reproduction, and increasing mortality (Chen et al. 2010). However, it is noteworthy that among the sediments and heavy metals tested, only Cr from the Municipality of Hinatuan exceeded the prescribed limit set by international standards. Brgy. Loyola, the collection site for sediments in Hinatuan, is situated at the drainage basin of the Tambis River system, where small-scale gold mining activities are particularly active, especially upstream of this water body (Fajardo et al. 2015). Although mercury and cyanide are commonly used in small-scale gold mining, the presence of Cr is usually associated with gold deposits (Haldar 2016). Hence, the elevated levels of Cr contamination could be due to the extraction of Cr-containing ore upon gold mining. Cr can be harmful when taken in high concentrations over an extended period (Sireli et al. 2006). Another study by Azmat et al. (2018) and Fawad et al. (2017) suggested that heavy metals like Cr can build up in marine organism muscles, particularly in bivalves, and can affect their metabolic and enzymatic processes.

Heavy metal analysis of A. philippiana flesh

Table 3 summarizes the concentration of heavy metals in the flesh of *A. philippiana* collected from the Municipalities of Barobo and Hinatuan. In the Municipality of Barobo, the highest concentration was observed for Pb (0.5±0.02 ppm), followed by Cr (0.25 ppm), Ni (0.25 ppm), and tHg (0.01 ppm). Cr (0.37±0.02 ppm) was the highest in the Municipality of Hinatuan, followed by Pb (0.35±0.01 ppm), Ni (0.25 ppm), and tHg (0.01 ppm). All heavy metals tested are below the tolerable limits set by FAO/WHO (1984) and the European Commission (2006). Similarly, there is no significant difference between the levels of heavy metals among the two sites (P>0.05).

Mangrove clams, being benthic eaters, can acquire food and toxic chemicals from the environment, making them efficient bioindicators of heavy metal contamination (Tamele and Loureiro 2020). The effects of heavy metals on marine organisms are widely studied. The accumulation of heavy metals in the muscles of bivalves can have detrimental effects on their morphology and growth. Studies have demonstrated that even small amounts of heavy metals can induce oxidative stress, DNA damage, and gene expression changes,

Table 2. Mean concentration of heavy metals in sediments collected from Barobo and Hinatuan, Surigao del Sur, Philippines

Heavy Metal	Barobo (in ppm)	Hinatuan (in ppm)	Tolerable limits (in ppm)	References
Lead	5.12±0.22	17.80±0.06	≤100	FAO (1999)
Nickel	1.63±0.06	11.80±0.50	≤25	WHO (2001)
Chromium	5.03±0.22	59.00±0.38**	≤50	FAO (1993)
Mercury	<0.002*	<0.01*	≤0.1	FAO (1999)

*Reporting limit by AAS; **Concentration beyond the tolerable limits*

Table 3. The mean concentration of heavy metals in the flesh of *A. philippiana* collected from the Municipalities of Barobo and Hinatuan, Surigao del Sur, Philippines

Heavy Metal	Barobo (in ppm)	Hinatuan (in ppm)	Tolerable limits (in ppm)	
			FAO/WHO (1984)	EC (2006)
Lead	0.5±0.02	0.35±0.01	≥ 1.50	≥ 0.50
Nickel	<0.25*	<0.25*	≥ 80.0	≥ 1.50
Chromium	<0.25*	0.37±0.02	≥ 13.0	≥ 20.0
Mercury	<0.01*	<0.01*	≥ 13.0	≥ 1.00

Reporting limit by AAS

potentially leading to developmental abnormalities (Nordberg et al. 2007; Angeles-Agdeppa et al. 2011). Moreover, chronic exposure to low levels of heavy metals can result in long-term effects on the health and morphology of marine bivalves (Hossen et al. 2015). Other studies have observed changes in bivalve morphology, including reduced shell length and thickness, and alterations in muscle growth and development (Yap et al. 2003; Siringan and Tongson 2011; Navarro et al. 2014; Stewart et al. 2021). The concentrations of heavy metals in bivalves can also have broader ecological consequences. Richir and Gobert (2016) discovered that heavy metal exposure can lead to decreased survival rates of bivalve species, potentially disrupting the food chain and other ecological processes.

Nevertheless, the quantified concentration of metals in the flesh of *A. philippiana* is way below the tolerable limits, some of which are at the lowest possible reporting limit of the AAS (Ni, Cr, and tHg). It is noteworthy also that despite the high concentration of Cr in the sediments of the Municipality of Hinatuan, the accumulated Cr in the flesh of *A. philippiana* was only 0.37±0.02 ppm, which is 35- or 54-fold lower than FAO/WHO (1984) and EU (2006) standards, respectively.

LWR in A. philippiana shells

Collection *A. philippiana* for LWR analysis was done in the Municipalities of Barobo (n=576) and Hinatuan (n=196), Surigao del Sur. The mean length and weight of *A. philippiana* are significantly larger in Hinatuan (5.26±0.57 cm; 35.39±13.62 g) compared to samples from Barobo (3.93±0.84 cm; 20.08±15.40 g) ($P<0.05$) (Table 4). A significant and strong relationship exists between the LWR of *A. philippiana* in Barobo and Hinatuan ($P<0.001$). The *A. philippiana* clams tend to be bigger in size and heavier in Hinatuan than in Barobo. Morphometric analysis showed positive allometric growth in Barobo and negative allometric growth in Hinatuan (Figure 2).

The use of the LWR is significant for the assessment of the growth and production of species. LWR is used for evaluating the weight corresponding to a given length, and condition factors are used for comparing the condition, fatness, or well-being of species based on the assumption that heavier organisms of a given length are in better condition (Froese 2006). Bivalve growth is influenced by biotic and abiotic factors. Among these factors that affect the growth of bivalves are the quality of food sources, water quality, type of sediments, type of bottom, and wave exposure (Babaei et al. 2010).

The negative allometric pattern in the Municipality of Hinatuan indicates that the rate of increase in shell length and weight is not proportional. Although food availability is the main influence on the growth and reproduction of bivalves (Haberle et al. 2020), environmental factors such as the quality of water are also pivotal for the growth of bivalves and lead to negative allometry (Zuo and Cai 2017). Although the concentration of Pb, Ni, Cr, and tHg in the flesh of *A. philippiana* is below the tolerable limits set by international organizations, it was observed that Cr in sediments exceeded the tolerable limit of FAO (1993) and the DO of surface water did not meet the minimum level of DAO 2016-08. The positive allometric growth pattern shown by *A. philippiana* in the Municipality of Barobo indicates that the species is not affected by its biological condition, which can be seen in the lower heavy metal concentrations in both sediments and bivalve muscles. The collection sites are far from the residential area and no known disturbing anthropogenic activities were observed.

Interestingly, the *A. philippiana* from the

Table 4. Mean length and weight of *A. philippiana* collected in Barobo and Hinatuan

Parameters	Mean±SD		P-value	DAO (2016)
	Barobo	Hinatuan		Range/ Min. levels
pH	6.65±0.51*	7.12±0.10	0.10	7.0–8.5
Dissolved Oxygen (ppm)	2.74±0.31*	2.08±0.38*	0.20	5
Electroconductivity (μs/cm)	48.36±0.96	58.21±19.16*	1.00	30–55
Temperature (ºC)	30.22±0.18	30.98±0.06	0.10	26–30
Total Suspended Solids (mg/L)	24.51±0.09*	35.33±12.21	0.70	25
Salinity (ppm)	31.78±0.50*	59.33±4.00*	0.10	100

**Mean values with an asterisk indicate levels not within the standard limits*

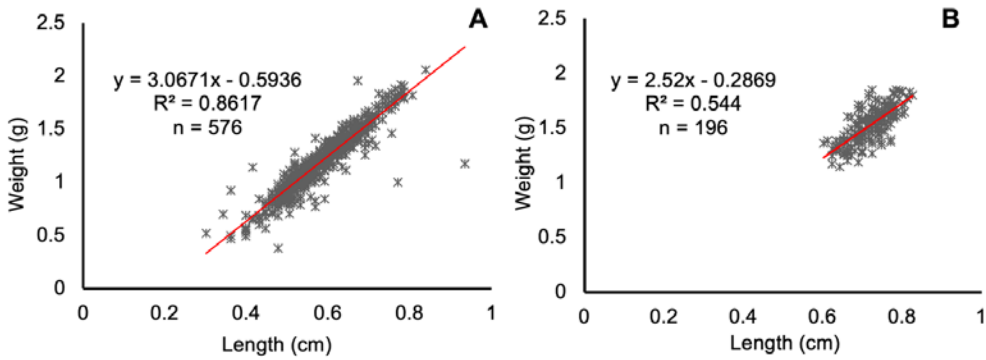

Figure 2. Relationship between the total weight and shell length of *A. philippiana* in the Municipalities of Barobo (A) and Hinatuan (B) with its equations and coefficient of determination R^2 values

Municipality of Hinatuan is larger and heavier than the Barobo samples. Collection sites differ in their soil types—Barobo tends to be sandy, while Hinatuan is characterized as mudflat. In general, coarse-textured soil is the poorest habitat for any marine organism, as it supports less biota than mudflat types. Muddy types of sediments provide more biota than sand because organic matter is more likely to lodge on them, providing food to organisms like the *A. philippiana* (McLean et al. 2021). Moreover, another factor that may contribute to the growth of bivalves is the ease of access to *A. philippiana* in sandy sediments, and the exposure of clam beds during low tide makes them highly visible to harvesters, leading to a decline in the overall size of the population.

4 Conclusion and Recommendations

This study aimed to determine the levels of heavy metals in the sediments and the flesh of *A. philippiana* collected from the Municipalities of Barobo and Hinatuan, Surigao del Sur, alongside the LWR of *A. philippiana* samples. The relative order of the average concentrations of HM in the sediment across two stations is Cr>Pb>Ni>tHg, with Cr from the Municipality of Hinatuan exceeding the tolerable limits. On the other hand, the accumulated heavy metals in the flesh of *A. philippiana* have remained below the tolerable limits. It was also determined that heavy metal concentration in sediments is relatively higher than in the flesh of *A. philippiana.* The LWR of *A. philippiana* showed positive allometry in the Municipality of Barobo and negative allometry in the Municipality of Hinatuan, suggesting environmental stressors affecting growth. It is recommended that heavy metal levels be monitored to capture seasonal variations. Additional research is needed to gain better insights into morphometric patterns concerning HM accumulation in *A. philippiana* and other commercially relevant bivalves. This will help identify direct and indirect anthropogenic stressors, enabling more effective management of these economic resources.

5 Acknowledgement

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Statement of Conflict of Interest

To maintain impartiality in the article's evaluation, Joycelyn Jumawan, who serves as the EIC of JESEG refrained from involvement in the review process of this article.

Author Contribution

RR Ecleo conducted the study, collected data, performed the analysis, wrote the original draft, and JJ Rosal conceptualized the study design, editing, and supervision of the project. RR Ecleo and JJ Rosal wrote the manuscript with input from all authors. JC Jumawan and EM Buenaflor provided technical inputs in the design, implementation, and analyses of the research. All authors approved the final version of the article.

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