

RESEARCH ARTICLE

A Study on the Distribution and Level of Cadmium in Scallop *Bractechlamys vexillum* (Reeve 1853) from the Visayan Sea, Philippines

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ABSTRACT

Bivalves such as scallop *Bractechlamys vexillum* are considered an essential resource for livelihood and revenues in the Visayan Sea, central Philippines. To date, there are several reports that the local marine ecosystems are contaminated with heavy metals like cadmium; hence, these species are also susceptible to bioaccumulation of cadmium because they feed mainly by filtering particles from contaminated water. In recent studies, scallops are suggested to be a potential bioindicator for cadmium contamination due to their ability to accumulate and tolerate the metal. This research aims to examine the anatomical distribution of cadmium in *B. vexillum* and measure the relationship between shell weight and cadmium concentration. The sites for this study include Carles, Iloilo, Madridejos, Cebu, and Cawayan, Masbate. Five organs were analyzed: adductor muscle, digestive gland, gonad, gill, and mantle. The analysis for quantification of cadmium in the different scallop parts was carried out by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES Model ICPE-9820, Shimadzu, Kyoto, Japan). Results showed that cadmium preferentially accumulates in the digestive gland, accounting for 76.39% of the total metal concentration. Moreover, a significant positive correlation ($r = 0.798$, $p < 0.01$) between the cadmium content in the whole digestive gland and shell weight were observed. Thus, this tissue tends to have a potential marker of metal contamination in the environment.

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Received: April 29, 2020
Accepted: November 17, 2020

Keywords: *cadmium, anatomical distribution, scallop, Visayan Sea, contamination*

1. INTRODUCTION

Heavy metal contamination has been widely studied for decades now and reported as a potential hazard posing threats to local marine ecosystems and marine diversity (Khanh et al. 2013). Harmful metals like cadmium (Cd) can accumulate in aquatic organisms and in humans consuming these organisms. Bivalves such as scallop *Bractechlamys vexillum* (Reeve 1853) are considered an essential resource for livelihood and profit in the Visayan Sea, central Philippines. It is also one of the most commonly captured fishery products because they generally inhabit shallow water (Acabado et al. 2018). Scallops accumulate cadmium as they feed by filtering particles from water, including metals from sediment and harmful substances caused by pollution (Julshamn et al. 2008). In the study of Khanh et al.

(2013), scallops can be a potential bioindicator for cadmium contamination because of their ability to accumulate and tolerate the metal, capacity to withstand changes in environmental conditions, sedentary and long-living life, easy identification and sampling, high abundance, and availability. Hence, the bioaccumulation of cadmium in this organism could be a basis for evaluating its capacity to reflect the level of contamination in the Visayan Sea.

In the Philippines, bioaccumulation of heavy metals in aquatic organisms has been an interest for researchers. Some studies suggest that the determination of the anatomical distribution of cadmium in scallops will provide necessary information on the species' economic value by eliminating the tissues with a high-level accumulation of cadmium. Therefore, this research aims to (1) examine the anatomical distribution of cadmium in

B. vexillum and (2) measure the relationship between the shell weight and cadmium concentration.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

All the reagents used in this study were analytical reagent grade. Nitric acid (65%), hydrogen peroxide (30%), and cadmium standard (1000 mg/L Cd in nitric acid) were purchased from Merck (Darmstadt, Germany). Ultrapure water (18.2 MΩcm resistivity) produced by a Milli-Q water system (Millipore, Massachusetts, USA) was used for all dilutions.

2.2 Study site

The Visayan Sea (located between 11° to 12° North latitude and 123° to 124° East longitude), having an estimated area of 10,000 km², is bounded by the islands of Masbate to the north, Iloilo to the west, Leyte to the east and Cebu to the south. *B. vexillum* was collected from validated sampling sites in Carles, Iloilo (CI), Madridejos, Cebu (MC), and Cawayan, Masbate, CM (Figure 1).

2.3 Sample collection

The sample collection was carried out last March–June 2019 (dry season) and August–October 2019 (wet season) to assess the organ-specific accumulation of cadmium in *B. vexillum*. The scallop samples were authenticated by the Invertebrate Section of the National Fisheries Research and Development Institute (NFRDI), according to Carpenter and Niem (1998). Ten scallops were collected and pooled from each sampling site. The shell's length and height were measured using a Vernier caliper from the hinge to the maximum parallel dimension to the nearest 0.1 mm. The soft tissues extracted from scallops were carefully dissected to obtain the mantle, gills, digestive gland, gonad, and adductor muscle (Figure 2). Since the kidney was of small size and fragile, this organ was included in the digestive gland. The fresh weight of each organ was also measured and stored at -20°C prior to homogenization and analysis. The temperature was monitored by placing a digital thermometer with a probe in the freezer.

2.4 Determination of cadmium concentration

Cadmium concentrations were quantified according to AOAC Official Method 999.10

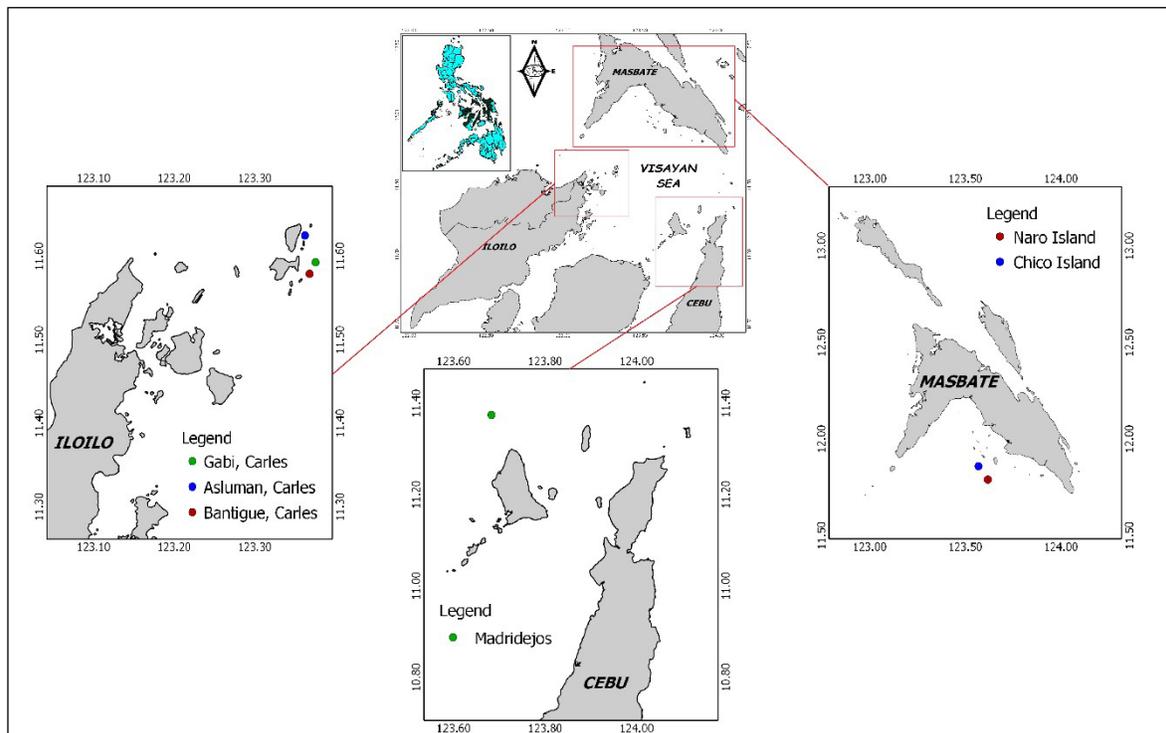


Figure 1. Sampling sites of *B. vexillum* in the Visayan Sea.

(AOAC 2010) with slight modification. Aliquot of samples (0.5 ± 0.1 g) were digested with 7 mL HNO_3 (65% by volume) and 1 mL H_2O_2 (30% by volume) using a microwave digestion system (Ethos ONE, Italy). The digestion was programmed into two steps: ramping temperature to 200°C for 10 minutes and holding for another 20 minutes at 200°C . After the digestion process, each sample was diluted to 25 mL with 0.1 N HNO_3 .

The cadmium concentration was determined by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES Model ICPE-9820, Shimadzu, Kyoto, Japan). The instrumental conditions and settings are given in Table 1. Internal quality control measures were conducted, such as blanks, mid-standard, and spiking or fortifying the samples with known cadmium concentration. All samples were measured in triplicate. Method validation was also

carried out to ensure the accuracy and reliability of analytical results. The calculated method detection limit was $0.05 \mu\text{g Cd/g}$, and the variability of reproducibility of the measurements was $<5\%$.

2.5. Statistical analysis

Data analysis was carried out using the statistical software package SPSS version 21. The One-Way Analysis of Variance (ANOVA) was used to assess significant differences in cadmium levels in different scallop tissues from various sampling sites. Statistically, different areas were determined using Duncan's Multiple Range Test. The Pearson correlation test was used to check for significant relationships between the levels of cadmium and shell weight of *B. vexillum*. The significance was set at a 95% confidence level ($p < 0.05$)

Table 1. Instrumental settings for Shimadzu ICP-OES 9820

ICP-OES settings	
Carrier gas flow (L/min)	0.7
Plasma gas flow (L/min)	10
Auxillary gas flow (L/min)	0.6
Torch	Mini
Wavelength (nm)	214.438
Nebulizer type	Concentric glass
Spray chamber type	Glass cyclonic spray chamber
Plasma viewing mode	Radial

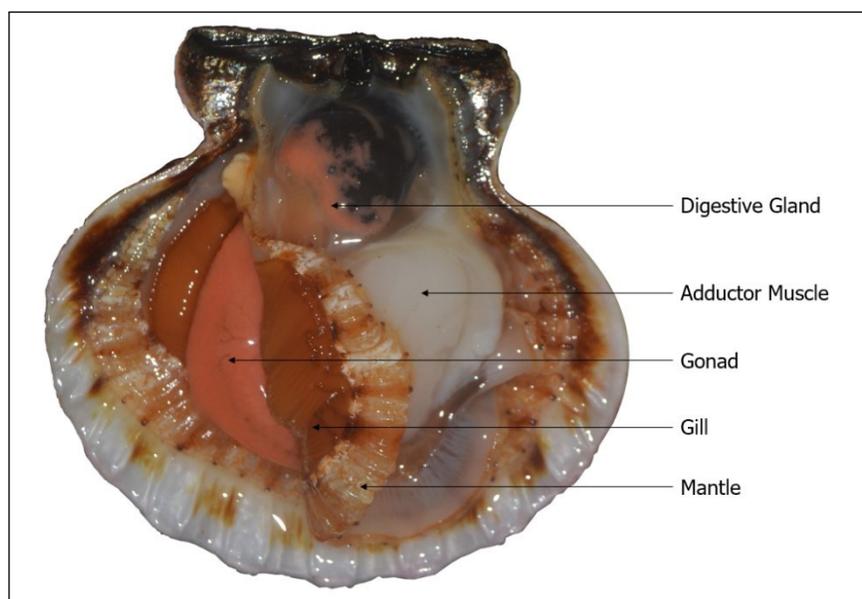


Figure 2. Anatomical fraction of *B. vexillum* used for cadmium analysis

3. RESULTS

3.1. Levels of cadmium in tissues of scallop

Cadmium concentrations in muscles and tissues of *B. vexillum* collected from different sampling sites around the Visayan Sea are given in Table 2. Scallops of approximately 60 mm shell height were used. It can be observed that the cadmium concentrations in all edible portions, such as adductor muscles and gonads of scallops, exceeded the maximum concentration set at 1.0 mg/kg wet weight by the European Food Safety Authority (2017) and the Philippines (BFAR 2001). In contrast, mantle collected from Gigantes Is., Carles, Iloilo, Cawayan, Masbate and Madridejos, Bantayan Is., Cebu during wet season passed the regulatory limit of cadmium. It should be noticed that the level of cadmium decreases during the wet season if compared with seasonal variation.

Cadmium was highly concentrated in the digestive gland ranging from 20.33 to 72.65 mg/kg wet weight, approximately having 13-fold higher than that observed in the gonad, ranged from 1.70 to 5.05 mg/kg wet weight, which had the second-highest concentration. The highest concentration in adductor muscle, being the usual edible portion of scallops in the Philippines, was observed during the dry season in Chico Is., Cawayan, Masbate, with a mean value of 6.31 ± 0.42 mg/kg wet weight. Scallops from Madridejos, Bantayan Is., Cebu exhibits the lowest mean concentration of 1.12 ± 0.05 mg/kg wet weight. Nonetheless, cadmium concentrations distributed in each organ still exceeded the maximum allowable limit of 1.0 mg/kg wet weight instated by the European Union (EU) and the Philippines. Levels were noticeably dispersed, resulting in a significant difference ($p < 0.05$) in each tissue's cadmium concentration among different sampling sites.

Table 2. Cadmium concentration (mean \pm SD, $\mu\text{g}\cdot\text{g}^{-1}$ wet weight basis, n = 10) in muscles and organs of *B. vexillum* from different sampling sites around the Visayan Sea

Sampling Sites	DRY SEASON				
	Adductor Muscle	Digestive Gland	Gonad	Gill	Mantle
Chico Is.(CM)	6.31 ± 0.42^d	42.20 ± 0.57^b	5.05 ± 0.50^c	7.73 ± 0.74^e	2.40 ± 0.23^a
Naro Is. (CM)	3.94 ± 0.40^b	51.40 ± 1.14^d	3.69 ± 0.24^b	3.67 ± 0.06^a	2.16 ± 0.07^a
Asluman (CI)	3.11 ± 0.01^c	39.33 ± 1.95^{ab}	3.94 ± 0.05^b	5.60 ± 0.09^d	2.40 ± 0.03^a
Bantigue (CI)	2.09 ± 0.19^a	35.97 ± 1.90^a	2.67 ± 0.08^a	2.83 ± 0.12^c	1.57 ± 0.10^b
Gabi (CI)	1.65 ± 0.19^a	30.20 ± 1.87^c	3.10 ± 0.06^a	4.31 ± 0.08^b	2.12 ± 0.07^a
Madridejos (MC)	3.78 ± 0.39^b	72.65 ± 2.62^e	3.97 ± 0.16^b	3.97 ± 0.15^{ab}	3.00 ± 0.32^c
WET SEASON					
Chico Is.(CM)	2.05 ± 0.04^a	20.33 ± 0.71^a	2.88 ± 0.21^b	2.22 ± 0.24^b	1.75 ± 0.15^b
Naro Is. (CM)	2.69 ± 0.26^b	22.13 ± 1.00^a	5.47 ± 0.71^d	2.44 ± 0.06^b	1.65 ± 0.04^b
Asluman (CI)	1.81 ± 0.18^a	29.50 ± 7.21^b	1.70 ± 0.01^a	1.52 ± 0.08^a	0.79 ± 0.11^a
Bantigue (CI)	3.37 ± 0.11^c	31.80 ± 1.75^b	1.76 ± 0.05^a	2.42 ± 0.17^b	1.59 ± 0.15^b
Gabi (CI)	3.11 ± 0.44^{bc}	50.43 ± 2.97^c	3.45 ± 0.16^{bc}	3.49 ± 0.18^c	1.75 ± 0.07^b
Madridejos (MC)	1.12 ± 0.05^d	45.20 ± 2.83^c	3.53 ± 0.24^c	1.42 ± 0.07^a	0.75 ± 0.05^a

Values in the same column with different superscript letters are significantly different ($p < 0.05$).

3.2. Distribution of cadmium in tissues of scallop

The body distributions of cadmium in muscles and tissues of *B. vexillum* are presented in Figure 3. The digestive gland clearly showed the highest proportion targeted by cadmium accumulation, which accounts for 76.39% of the total cadmium distribution. The next largest amount was present in the gonad, which contained approximately 7.17% of the total cadmium in *B. vexillum*. The gill and adductor muscle accumulated 6.88 and 5.83%, respectively. Furthermore, the mantle had the lowest fraction of cadmium, accounting for 3.73% of the total distribution.

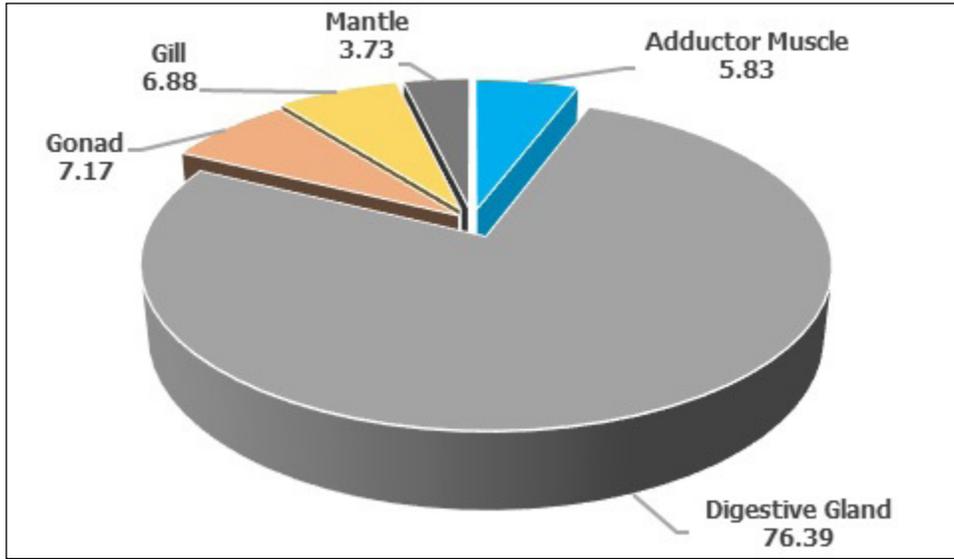


Figure 3. Percentage distribution of cadmium in different tissues of *B. vexillum*

3.3. Correlation between shell weight and Cd concentration

Correlation analysis between cadmium concentration in different organ and shell weight of *B. vexillum* are illustrated in Figure 4. The digestive gland and mantle showed a significant correlation between shell weight and cadmium concentrations, having a correlation coefficient of 0.80 ($p < 0.01$) and 0.67 ($p < 0.05$), respectively (Table 3). On the other hand, adductor muscle, gonad, and gill showed no significant difference between shell weight and cadmium concentration ($p > 0.05$). Out of the five tissues, cadmium accumulated in the digestive gland and mantle were positively associated with their body weight. The significant coefficients revealed that accumulative cadmium content in the scallop's tissue was elevated with increased body weight. The cadmium concentration in the edible portions of scallops, such as adductor muscle and gonad, showed little or no change as the weight of scallop increases ($r < 0.30$).

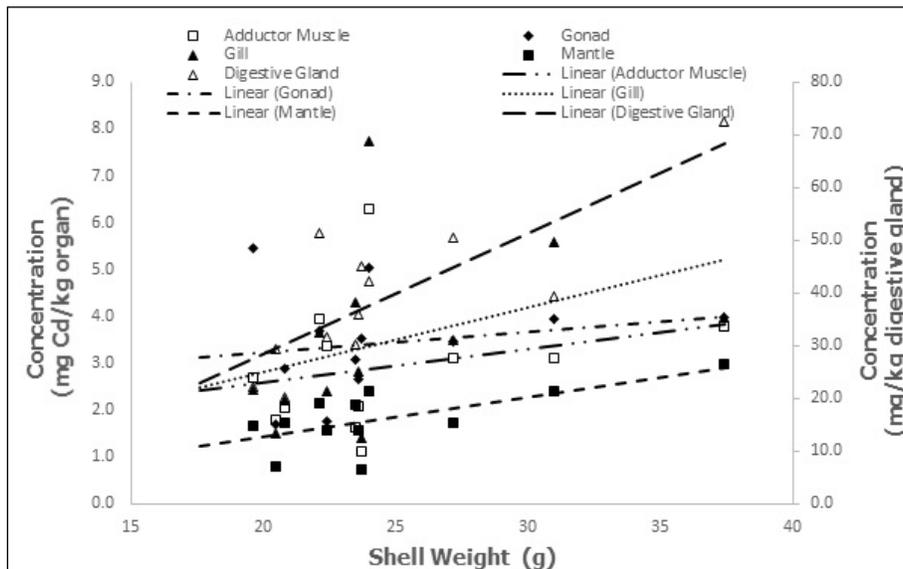


Figure 4. Variation of Cd concentrations with shell weight in the tissues of *B. vexillum*

Table 3. Linear regressions analysis for the relationship between shell weight vs. concentration of Cd in different organs

Regression Analysis	Adductor Muscle	Digestive Gland	Gonad	Gill	Mantle
Slope	0.0709	2.2912	0.0436	0.1374	0.0854
y-intercept	1.1714	-17.2157	2.3589	0.0813	-0.2786
Correlation coefficient	0.2590	0.7981**	0.1949	0.3857	0.6661*

*, ** indicate significance level at 5% and 1%, respectively.

4. DISCUSSION

Cadmium metal does not readily breakdown in the environment, but its chloride and sulfates are easily dissolved. The predominant cadmium speciation are $CdCl^+$, $CdCl_2$, and $CdCl_3^-$. Once discharged to the environment, it constantly disseminates, making it bioavailable to marine organisms. Marine bivalves effectively acclimatize it due to their stationary and filter-feeding behavior (Neff 2002).

Among other marine bivalve species, scallops have shown to accumulate high levels of cadmium in their tissues. Hence, the bioaccumulation of cadmium in scallops can still be considered a bioindicator of contamination, even if cadmium is not detected in the water. This might be due to the possibility that cadmium has been diluted, or it settled down the sediments where scallops inhabit. In fact, many studies reported a high concentration of cadmium in their tissues even without contamination recorded in their environment (Mauri et al. 2000; Julshamn et al. 2008; Metian et al. 2008).

In *B. vexillum*, the digestive gland contains the largest concentrations of cadmium. Most studies concerning the organ distribution of Cd in scallops suggest that it is a general characteristic of the *Pectinidae* family. In a study conducted by Uthe and Chou (1987), more than 90% of the total cadmium burden accumulated in the digestive gland of the Sea scallop, *Placopecten magellanicus*, with less than 1% in the adductor muscle. Similar findings were reported in *Pecten maximus* (Metian et al. 2007; Julshamn et al. 2008; Saavedra et al. 2008); *Chlamys varia* (Bustamante and Miramand 2005; Metian et al. 2007); *Adamussium colbecki* (Mauri et al. 2000); and *Mizuhopecten yessoensis* (Gao et al. 2016). Furthermore, Metian et al. (2008) confirmed that the digestive gland and kidneys contained most of the metal burden in *Camptopallium radula*. Elevated Cd levels in the digestive gland can be attributed to dietary uptake, which serves as Cd's major accumulation pathway in scallops (Metian et al. 2007; Pan and Wang 2009).

Contrary to the above reports, Bach et al. (2014) stated that cadmium was highly concentrated

in the kidney of *Chlamys islandica*, accounting for 92% of the overall body burden of cadmium. It was supported by Wang and Lu (2017). The target organ for Cd accumulation in *Pectinidae* family appears to vary within species depending on several factors such as environmental contamination, dietary pathway, and interspecific variation in the capacity of scallops to concentrated cadmium (Saavedra et al. 2008; Bach et al. 2014). In this study, the kidney was not included in the present tissue specific determination because it was too small to dissect. Instead, it was treated as part of the digestive gland. Hence, cadmium concentration in the digestive gland reflects the metal content of both organs.

The digestive gland and kidney play an essential role in metabolism and detoxification. The food is digested and consumed in the digestive gland. It is commonly stated that approximately 80% of the total cadmium concentration is bound to cytosolic proteins such as metallothionein-like proteins (Viarengo et al. 1993; Mauri et al. 2000; Zhukovskaya et al. 2012). The multifaceted functions of metallothionein include involvement in homeostasis, protection against oxidative damage, metabolic regulation, and could be induced by heavy metals otherwise capable of hindering gametogenesis, suppressing embryogenesis, and impeding development (Marie et al. 2006; Mao et al. 2012). Hence, metallothionein is an essential factor influencing the toxicity of metals in aquatic invertebrates. However, according to Evtushenko et al. (1986), cadmium binding by cytoplasmic proteins was observed in various bivalves, but the results obtained were dissimilar and heavily dependent on exposure conditions, contamination levels, and methods of separation of proteins.

Several studies recommended selective evisceration of cadmium-rich organ, leaving only muscle tissue for consumption, as this tissue does not easily accumulate metals. Moreover, different parts of the scallops differ in economic value. The removal of the most toxic part (digestive gland and kidney) could be one of the possible remedies to minimize dietary exposure to cadmium, given they are the ones with the lowest economic value. It may also be a solution

for scallops to export in countries like Europe if evisceration were taken into account by European Union legislation (Gutiérrez et al. 2007; Julshamn et al. 2008; Saavedra et al. 2008; Bach et al. 2014). Nevertheless, the suggested technique is ineffectual for scallops from the Visayan Sea due to high cadmium levels in the adductor muscle.

Changes in bivalve metabolism rates with seasonal variation, as well as metal bioavailability in the surrounding environment, may be responsible for the disparity of cadmium concentration in scallops. Seasonal variations have been reported to be higher in dry than in wet season. Lower metal concentration during the wet season could be attributed to run-offs and hydrodynamics of the Visayan sea. Moreover, biological variables such as size, sex, or changes in tissue composition and reproductive cycle need to be considered. Such seasonal fluctuations were largely correlated with seasonal changes in flesh weight during the development of gonadic tissues (Otchere 2003; Bustamante and Miramand 2005). A similar increase of cadmium levels during the dry season was observed by Góngora-Gómez et al. (2018). Contrary to these results, Bjerregaard and Depledge (1994) concluded that cadmium uptake and bioaccumulation in crustaceans and mollusks are higher at low salinities during the rainy season.

Compared to finfishes and crustaceans, bivalves have a very low degree of enzyme system activity that could metabolize contaminants, making them bioindicators of heavy metal pollution (Otchere 2003). However, Yap et al. (2009) stated that age or size are important factors in determining the concentration of metals in organisms. The significant positive coefficient of cadmium content in the digestive gland and mantle with their weight revealed that the turn-over rate of metals in other soft tissue of scallop is minimal. Whereas cadmium concentration in adductor muscle and gonad were not affected by the size, suggesting a metabolic regulation of this metal in those tissues. Because cadmium has no established physiological role, such constant rates may result from detoxification processes that occur in the scallop tissues rather than from a specific homeostatic mechanism (Bustamante and Miramand 2005). Similar results were observed in oysters *Crassostrea gigas* (Rasmussen et al. 2007) and blue mussel *Mytilus edulis* (Cossa et al. 1980), wherein tissue weight influenced cadmium concentration.

Bioindicators or biomonitoring organisms can be identified as organisms or groups of species used for indicating adverse contamination effects

(Nikinmaa 2014). There are some characteristics to be considered “good” bioindicators. The most important ones are: (a) high levels of contaminants will accumulate without death; (b) it has an adequate abundance and wide distribution for repeated sampling and comparison; (c) its lifespan is long enough for comparison between different ages; (d) it can afford suitable target tissue or cell for further study at microcosmic level; (e) it can be easily sampled and easily raised in the laboratory; and (f) it has a strong dose-effect relationship (Hamza-Chaffai 2014).

Accumulation of metal content in scallops is typically correlated with metal content in the environment. In any case, not all species having the same characteristics are appropriate as bioindicators; they are required to reach a significant correlation level. In the present research, the digestive gland of *B. vexillum* appears to have potential biological indicators of trace metals, specifically cadmium. Nevertheless, the utility of these bivalves can be further explored by using some other parameters used in controlled conditions such as temperature, pH, and age. Hopefully, this would give these species a more precise understanding of the process of absorption of metal contaminants (Yusof et al. 2004).

Although the digestive gland is the primary organ for accumulating toxic cadmium metal, the concentration in the adductor muscle of *B. vexillum* collected from the Visayan Sea exceeds the regulatory limit (1.0 mg/kg wet weight) instated by the EU and the Philippines. Consequently, it would be of particular interest to investigate the source of cadmium in the Visayan Sea. It is also recommended to examine the correlation of cadmium content in scallops with water and sediments as additional support that this species can be a potential bioindicator of contamination. In addition, the study of cadmium content in shells of *B. vexillum* and risk assessment should be conducted.

5. CONCLUSION

The distribution of cadmium accumulation in different soft tissues of *B. vexillum* was observed. The digestive gland was the organ that contributed the highest proportion to the overall metal concentration in scallops. In addition, a positive correlation coefficient was found between digestive gland and shell weight. Thus, this tissue seems to have a desirable potential marker of metal contamination in the environment.

6. ACKNOWLEDGMENT

This study was funded by the National Fisheries Research and Development Institute as one of the component projects of the Seafood Quality and Safety Program. The researchers would like to thank the Bureau of Fisheries and Aquatic Resources Region V, VI, and VII and the Local Government Units for their kind assistance in collecting samples used for this study.

AUTHOR CONTRIBUTIONS

Perelonia KBS: Drafting and editing methodology, Validation, Investigation, Formal analysis, Visualization. Banicod RJS: Formal analysis, Investigation, Writing—review and editing, Visualization. Benitez KCD: Methodology, Formal analysis, Investigation, Writing—review and editing. Tadifa GC: Investigation, Writing—review and editing. Tanyag BE: Investigation, Writing—review and editing. Cambia FD: Conceptualization, Writing review and editing, Supervision. Montojo UM: Conceptualization, Writing—review and editing, Project administration, Funding acquisition.

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.

ETHICS STATEMENT

No animal or human studies were carried out by the authors

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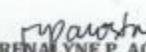
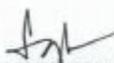
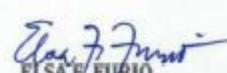
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ANNEX

 <p>NFRDI Integrated Research Laboratory Rm. 511 Floor Corporate 101 Bldg., Mother Ignacia Ave., Quezon City Telefax No. (02) 352-3596 E-mail: nfrdi.integratedlab@email.com</p>	<h3>REPORT OF ANALYSIS</h3>	IRL-Form No. 02 Report of Analysis Date Issued: 01/06/2016 Revision No.: 0
Page 1 of 1		
Lab. No. : 2017-1069	Date Submitted: 12/28/2017	
Sample : Scallop	Date Analyzed : 01/05/2018	
Source of Specimen : Carles, Iloilo	Date Reported : 01/08/2018	
Submitted by : Bryan S. Tanyag		
Company & Address : National Fisheries Research and Development Institute Corporate 101 Bldg. Mother Ignacia Avenue South Triangle Quezon City		
WORK REPORT		
English Name : Distant Scallop		
Family : Pectinidae		
Scientific Name : <i>Bractechlamys vexillum</i> (Reeve, 1853)		
References :		
Carpenter, K.E. Niem, V.H. (eds.). 1998. FAO Species identification guide for fishery purposes. The Living Marine Resources of the Western Central Pacific. Volume 1. Seaweeds, Corals, Bivalves and Gastropods. Rome, FAO.		
Analyzed by:	Verified by :	
 RENALYNE P. ACOSTA Aquaculturist I	 LUZ R. ROMENA Aquaculturist II	
	Noted by:	
	 ELSA F. FURIO OIC, CFRDD	
<hr/> <i>"Ensuring Sustainable Fisheries Through Research and Development"</i>		