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Microbiological quality of undulated surf clam (*Paphia undulata*) in selected areas in Samar, Philippines

KRISANTO L. BACNUTAN, KIRBY ULYSSES M. MOMO, RYAN JAMES A. PAGTABUNAN AND JERSON C. SORIO*
College of Fisheries and Marine Sciences, Samar State University
Catbalogan City, Samar Philippines 6700
*Email: jerson.sorio@ssu.edu.ph

Abstract. Bivalves are filter feeders that ingest particles from the surrounding water, including pathogens, which could cause illness in consumers. In Samar, Philippines no data on the microbial quality of undulated surf clam (*Paphia undulata*) have previously been reported. Thus, this study was conducted to determine the microbial quality of the species in selected areas in Samar, Philippines (Pinabacdao, Zumarraga and Villareal). The total plate count (TPC) and total *Vibrio* count were beyond the standard limits set by the Food and Drug Administration (FDA), although *Salmonella* was not detected in any samples. The TPC of undulated surf clam was 5.9-6.3 log CFU·g^{-1} in Pinabacdao, 4.9-6.0 log CFU·g^{-1} in Zumarraga, and 6.3-7.2 log CFU·g^{-1} in Villareal. The total *Vibrio* count was 5.1-5.7 log CFU·g^{-1} in Pinabacdao, 3.9-4.6 log CFU·g^{-1} in Zumarraga, and 6.1 log CFU·g^{-1} in Villareal. Bivalves in these areas should undergo purification process, such as relaying or depuration.
Keywords: Bivalve, Undulated surf clam, Food safety, Microbiological quality

Introduction

The undulated surf clam *Paphia undulata*, locally known as "mayahini" in Samar, Philippines, is a popular bivalve shellfish harvested mainly for food. They grow in all tropical seas and are considered to be a valuable food item by people residing in coastal areas. It is a commercially important bivalve mollusk in the Philippines (Villarta and del Norte-Campos 2010). For the species globally, a total harvest of 17,763 metric tons was recorded, with a value of approximately 7 million USD and export value of 20 million USD for the processed product in the year 2009 (Chanrachkij 2013).

The microbiological quality of shellfish varies depending on environmental conditions and the bacterial load of the water in which the shellfish are grown (Simental and Martinez-Urtaza 2008). Infections and illnesses brought about by the consumption of contaminated shellfish is a recognized problem worldwide. Pathogenic bacteria contaminate mollusks through wastewater and by those that are naturally occurring in the aquatic environment (Rippey 1994). Contamination mainly occurs due to the fact that they are filter feeders (Burkhardt and Calci 2000). Moreover, some species of naturally-occurring *Vibrio* contribute to the contamination of bivalves, leading to the outbreak of illnesses with symptoms such as diarrhea, abdominal pain, vomiting and in some cases, death (Yilmaz and Bilgin 2005).

In general, information on the microbiological quality of shellfish is important since these commodities are known to be filter feeders that accumulate small particles including pathogenic

bacteria. They are usually eaten as raw or slightly cooked. Raw or undercooked seafood have been identified as major vehicles of *Vibrio parahaemolyticus* infection to humans (Venkitanarayanan and Doyle 2001) and considered as one of the major causes of food-borne illness in the world (Wong *et al.,* 2000) and in Asia (Ma *et al.* 2014). In the United States of America, about 70% of deaths due to foodborne diseases main caused by *Salmonella* (CDC 2000). Presently, there are no existing scientific data on the microbiological quality of undulated surf clams in Samar, Philippines. It is important to have this baseline data since surf clams are being sold and marketed in Samar as well as other places in the country. Hence, this study aims to evaluate the microbiological quality of undulated surf clams and the water in which they are harvested from selected sampling sites.

Materials and Methods

Sample collection: Approximately 50 bivalve samples were collected for microbial analysis in every sampling station i.e. Pinabacdao (11°37'00.8"N, 124°59'22.4"E), Zumarraga (11°39'37.5"N, 124°53'33.1"E), and Villareal (11°36'20.8"N, 124°53'50.1"E) in the province of Samar, Philippines (Fig. 1), and 100 samples were collected for morphometric data. Water parameters, such as dissolved oxygen, temperature, salinity, pH and conductivity were measured using a portable multi-meter. Sampling was done in the months of March, June and September 2018.

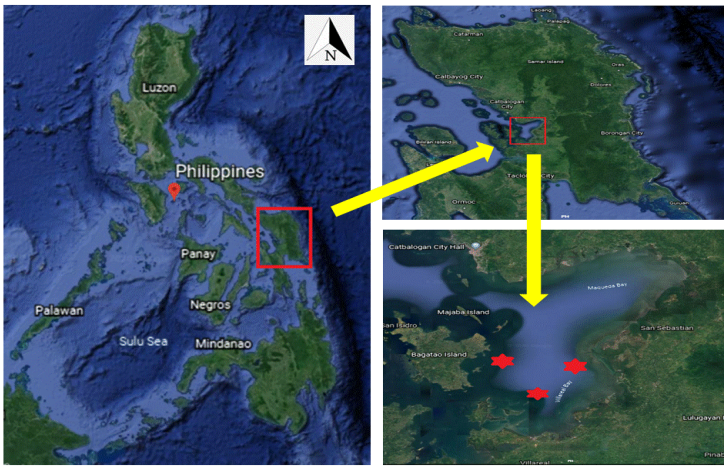

Fig. 1. Sampling stations in Samar, Philippines.

Collection of bivalve and water samples: The bivalve samples were cleaned on site by rubbing and scrubbing with clean seawater to remove mud and sediment. They were drained and placed in a sealed plastic bag, and chilled (below 5°C) in styrofoam boxes with ice. Water samples were collected together with the bivalves. Sterile bottles (200 ml) were used to collect water samples near the growing area and were held at temperatures below 5°C (iced) until analysis. All bivalve and water samples for microbiological analysis were brought to the microbiology laboratory in Samar State University's Mercedes Campus, in Samar, Philippines within 6 h.

***Microbiological analysis of water and shellfish*:** The bivalve and water samples were subjected to the following analyses: Total plate count (APHA 1970), total *Vibrio* count, and detection of *Salmonella* (Food and Drug Administration 2004). All analyses were done in triplicate.

Sample preparation: Fifty fresh clam samples were shucked using a sterile knife to remove the meat. The meat was collected aseptically in a sterile Erlenmeyer flask and homogenized using a hand-held blender. The homogenized meat was then dispensed and used for all microbial analysis.

***Total plate count (TPC)*:** Ten grams of homogenized meat sample was weighed and homogenized in 90 ml peptone water. For water samples, 10 ml of sample was added to 90 ml peptone water. Serial dilution (up to 10^6) was conducted for both sets of samples followed by spread plating onto nutrient agar plates with 2% NaCl. After incubation at 37 ºC for 24 h, colonies were counted using a colony counter and recorded as $CFU \cdot g^{-1}$ or $CFU \cdot ml^{-1}$.

Total Vibrio count: Ten grams of homogenized meat sample was homogenized in 90 ml alkaline peptone water and serially diluted up to 10^6; 10 mL of sampled water was processed in the same way. Spread plating was then done onto TCBS agar (Titan Biotech Ltd.) plates, which were incubated at 35 ºC for 24 h. After incubation, colonies were counted and recorded as $CFU \cdot g^{-1}$ or $CFU \cdot ml^{-1}$.

Detection of Salmonella: Twenty-five grams of homogenized meat sample was homogenized in 225 ml of lactose broth and incubated at 35 °C for 24 h. After incubation, 1 ml of the pre-enrichment broth was inoculated into tetrathionate broth (TTB) and was incubated for 24 h at 35 °C. It was then streaked on xylose lysine deoxycholate (XLD) agar (Titan Biotech Ltd.) plates and incubated for 24 h at 35 °C. Typical *Salmonella* sp. colonies were examined.

***Statistical analysis*:** Data on the hydrobiological and microbiological parameters were subjected to descriptive statistics, one-way ANOVA and a post-hoc analysis, the Holm-Sidak test, to determine significant difference. All statistical analyses were performed using the software Sigma Plot 11.0. The level of significance was set at a level of $p < 0.05$.

Results and Discussion

Hydrobiological parameters: The temperature of the seawater varied between 25.0˚C and 29.5˚C. Meanwhile, the dissolved oxygen (DO) of the water ranged between 12.03 and 14.36 $mg \cdot L^{-1}$. The pH of the water ranged from 7.00 to 7.98, and the salinity ranged from 24.36 to 29.97 ‰ (Table I).

Measurement of microbiological quality: Assessment of the microbiological quality of surf clams in the selected municipal waters of Samar, Philippines was conducted. This was done to determine whether the bivalve samples collected in the site conform to the microbial standard limit set by the Food and Drug Administration (2013).

The total bacterial count of all samples was beyond the standard limit of < 5 log $CFU \cdot g^{-1}$, except for the Zumarraga site during the 2nd sampling (Table II). This result suggests that the

bivalves harvested in these areas should undergo purification. However, specimens were not collected during the 3rd sampling in Pinabacdao.

Table I. Hydrobiological parameters of *Paphia undulata* growing waters in three sampling stations*

Sampling period	Sampling site	Temperature (˚C)	D.O. (mg·L^{-1})	pH	Salinity (‰)
1st Sampling	Pinabacdao	29.53±0.08^a	12.03±0.08^a	7.68±0.17^a	24.54±0.03^a
	Zumarraga	28.70±0.56^a	12.85±0.04^a	7.00±0.02^a	27.03±0.04^a
	Villareal	28.83±0.38^a	14.36±0.06^a	7.31±0.03^a	28.30±0.03^a
2nd Sampling	Pinabacdao	28.03±0.48^b	11.70±0.09^b	7.98±0.01^b	24.90±0.05^b
	Zumarraga	27.64±0.17^b	12.16±0.06^b	8.03±0.02^b	29.97±0.03^b
	Villareal	28.25±0.23^b	12.89±0.08^b	7.31±0.04^a	29.48±0.18^b
3rd Sampling	Pinabacdao	NT	NT	NT	NT
	Zumarraga	26.48±0.22^c	12.14±0.04^b	7.97±0.09^b	27.07±0.12^a
	Villareal	25.00±0.07^c	12.88±0.04^b	7.97±0.06^b	24.36±0.12^c

*Note: NT - not tested; 1st sampling-March; 2nd sampling-June; 3rd sampling-September. Values with same superscript between sampling periods showed no significant difference.

Table II. Total plate count (log CFU·g^{-1}) of undulated surf clam and water samples from three sampling sites*

Sampling site	1st Sampling		2nd Sampling		3rd Sampling	
	Meat	Water	Meat	Water	Meat	Water
Pinabacdao	6.0±0.21^a	6.3±0.15^a	5.6±0.15^b	5.9±0.30^a	NT	NT
Zumarraga	5.7±0.15^a	6.0±0.15^a	4.9±0.40^b	5.1±0.25^b	5.6±0.15^a	5.2±0.23^b
Villareal	6.1±0.27^a	7.2±0.25^a	5.9±0.21^a	6.3±0.40^b	6.1±0.25^a	5.4±0.25^c

*Note: NT - not tested; 1st sampling-March; 2nd sampling-June; 3rd sampling-September. Values with same superscript between sampling periods showed no significant difference.

Among the three sampling sites, Zumarraga had the lowest mean bacterial count. This may be attributed to the fact that the sampling site was situated in open seawater, far from residential areas and other municipal activities. The bacterial load in shellfish depends on the degree of pollution in the growing area (Adebayo-Tayo *et al.* 2006). As a reflection of this relationship, the total plate counts of the surrounding waters in Pinabacdao and Villareal were higher than the Zumarraga site. It was also observed that the bacterial count during the first sampling was much higher compared to the second and third. The first sampling was conducted in the month of March, which was the beginning of summer, and temperature was notably higher (Table I). The high bacterial count during the first sampling could be attributed to the increased temperature of the surrounding water. It was reported that increasing sea water temperature could increase the bacterial load (Okumus and Stirling 1998). In addition, Colakoglu *et al.* (2010) explained that the increase of bacterial load in clams during summer could be due to the increased human recreational activities, as well as increased industrial and household wastes during this season. Similar results were obtained by Altug *et al.* (2008), in which bacteria levels of clams in northern Marmara Sea were found to increase during summer. Colakoglu *et al.* (2010) also

observed a high value of total aerobic organisms ($2.0x10^4$ CFU·g^{-1}) in striped venus clams (*Chamelea gallina*) during summer. Further, Adjei-Boateng *et al.* (2009) reported that total viable counts in clams during the dry season ($7.0x10^{10}$ CFU·g^{-1}) was higher compared to the wet season.

The standard microbial limit set by the Food and Drug Administration (2013) is <3 log CFU·g^{-1}. Results revealed that the total *Vibrio* count of the bivalve samples exceeded the standard limit. Yellow colonies were more dominant than green-colored colonies. Based on the results, the *Vibrio* count of clams collected in the Zumarraga site was notably lower compared to the other sites (Table III). This result conforms to the findings for total plate count, where the clams collected in this site had lower bacterial load. A third sampling was not conducted due to several problems encountered in the laboratory.

Table III. Total *Vibrio* count (log CFU·g^{-1}) of undulated surf clam and water samples from three sampling sites*

Sampling site	1st Sampling		2nd Sampling		3rd Sampling	
	Meat	Water	Meat	Water	Meat	Water
Pinabacdao	5.7 ± 0.12^a	5.6 ± 0.12^a	5.1 ± 0.30^b	5.3 ± 0.25^a	NT	NT
Zumarraga	4.6 ± 0.25^a	5.2 ± 0.40^a	3.9 ± 0.21^b	4.8 ± 0.15^a	NT	NT
Villareal	6.1 ± 0.25^a	6.1 ± 0.25^a	6.1 ± 0.21^a	5.8 ± 0.35^a	NT	NT

*Note: NT - not tested; 1st sampling-March; 2nd sampling-June; 3rd sampling-September. Values with same superscript between sampling periods showed no significant difference.

The total *Vibrio* count in clams was also observed to be higher during the first sampling than the second. As discussed previously, the first sampling was done in the month of March, when the temperature was warmer (Table I). As reflected in Table III, *Vibrio* were also more abundant in the surrounding water during the first sampling. Several researchers have also noticed higher concentrations of *Vibrio* during warmer seasons because of their proliferation in aquatic environments (Charles-Hernandez *et al.* 2006). Their occurrence was positively correlated with seawater temperature (Marino *et al.* 2005). These natural peaks correspond with an increase in human infections, which are usually highest during summer months (Barbarite 2016). The findings of this study conform to the results of other researchers studying the occurrence of *Vibrio* species in bivalve shellfish. Peralta and Andalecio (2011) detected high levels of *V. parahaemolyticus* in oysters and mussels from Roxas, Capiz ranging from 110-2400 MPN·g^{-1} and the presence of *Vibrio cholera* during the month of March. Colakoglu *et al.* (2010) also detected *V. parahaemolyticus* in striped venus clams (*Chamelea gallina*)twice during summer months.

Results of the present study revealed that all undulated surf clams collected from the sampling stations were negative for *Salmonella* (Table IV) and thus conform to the microbiological standard set by the Food and Drug Administration (2013). Several studies obtained similar findings on the detection of *Salmonella* in bivalves. Sorio and Peralta (2018) detected no *Salmonella sp.* in oysters from Dumangas, Iloilo, Philippines. Colakoglu *et al.* (2010) also obtained negative results for *Salmonella* in striped venus clams (*Chamelea gallina*).

Moreover, Ekawati and Yusmiati (2018) did not detect any *Salmonella* species in blood cockles (*Anadara granosa*).

Table IV. *Salmonella* detection in undulated surf clams from three sampling sites*

Sampling site	1st Sampling	2nd Sampling	3rd Sampling	Standard limit
Pinabacdao	Negative	Negative	NT	Negative (FDA)
Zumarraga	Negative	Negative	Negative	
Villareal	Negative	Negative	Negative	

*Note: NT - not tested; 1st sampling-March; 2nd sampling-June; 3rd sampling-September

In contrast with the findings of this study, *Salmonella* were found in aquatic environments, especially in tropical regions (Much *et al.* 2009). Major sources of *Salmonella* contamination include culture ponds and coastal water used for handling and processing seafood (Upadhyay *et al.* 2010). The presence of *Salmonella* in seawater and seafood is attributed to factors such as climatic conditions (FAO 2010). Several studies observed the presence of *Salmonella* in fish and fishery products such as oysters (DePaola *et al.* 2010) and in freshwater species, i.e. tilapia, rainbow trout, and carp (Nesse *et al.* 2005). Fresh fish, fish meal, oysters and shrimp can carry *Salmonella* if they are caught in contaminated areas or processed in unsanitary conditions, and consumed raw or slightly cooked (Mol *et al.* 2010, Norhana *et al.* 2010).

Conclusions: The total microbial count of undulated surf clams (*P. undulata*) in selected growing areas in Samar, Philippines was beyond the standard limit set by the FDA, although, *Salmonella* was not detected in any samples. It is recommended that bivalves grown and produced in these areas undergo a purification process, such as relaying or depuration before sale and consumption. The microbiological quality of shellfish in a specific area is important to determine, since this commodity is consumed and marketed by many people, especially those who live in coastal communities. Bivalve mollusks are filter feeders that feed non-selectively on particles from their surrounding water, including most pathogenic microorganisms. As such, they could carry bacteria that may cause illnesses to consumers. Further studies may be conducted to identify other pathogenic bacteria that may be present in the bivalves.

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