

Bacterial and parasitic contamination in blood cockles (*Anadara antiquata* Linnaeus, 1758), and effect of depuration on bacterial load using simple bio-filter in closed circulatory system

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Abstract

Blood cockle (*Anadara antiquata* Linnaeus, 1758) serves as a vital food and protein source for many coastal communities in Fiji. However, no studies have been conducted on bacterial and parasitic contamination in blood cockles in Fiji, leaving a significant knowledge gap. The present work thus aimed to determine the presence, types, and prevalence of parasites in blood cockles across four sites in Fiji, as well as to evaluate the effectiveness of depuration in reducing bacterial loads using a closed circulatory system with a simple biofilter. Blood cockles were collected from Nasese Coast, Viwa Island, Bau Landing, and Tailevu in the Central Division of Viti Levu, Fiji. Ectoparasites were observed on the shell surface, while endoparasites were identified in the digestive tract, gills, and muscles. The depuration system's effect on bacterial loads, including total plate count (TPC), coliforms, and *Vibrio* spp., was monitored over 48 h. Both endoparasites and ectoparasites were detected, with a mean prevalence of 29.3% at Nasese Coast, 0% at Viwa Island, 4% at Bau Landing, and 6.7% at Nasilai Coast. In tanks maintained at room temperature, bacterial contamination in blood cockles decreased to undetectable levels within 48 h of depuration. However, in tanks with elevated temperatures, TPC, coliforms, and *Vibrio* spp. increased by 48 h, resulting in 100% mortality of the blood cockles. The present work demonstrated that room-temperature depuration with a simple biofilter could be an effective and economical method for improving food safety and reducing health risks associated with consuming blood cockles.

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Introduction

Bivalves are filter-feeding organisms that play a vital role in maintaining ecosystem health by keeping waters clean through filtration, and providing various ecosystem services. The blood cockle (*Anadara antiquata* Linnaeus, 1758), a bivalve in the Arcidae family, feeds on organic detritus and microalgae from tidal flats and seawater. This filtration process clears seawater, promotes primary production, and converts plant material into consumable forms that support the food web (Tawake *et al.*, 2001; Buhadi *et al.*, 2013; Kawai *et al.*, 2014). In Southeast Asia, blood cockles primarily inhabit mudflats (Khalil *et al.*, 2017; Yulinda *et al.*, 2020), but they are also found in coastal areas between mangroves and shallow coral reefs.

In Fiji, blood cockles serve as a significant food and protein source for many coastal communities (Richards *et al.*, 1994). They are harvested year-round for household consumption, and sold in municipal markets and roadside stalls, supporting the socioeconomic livelihoods of individuals and communities (Tawake, 2003; Kawai *et al.*, 2014). Blood cockles are typically sold directly to consumers without prior processing or value addition.

The consumption of contaminated seafood is a leading cause of human infections, hospitalisations, and fatalities, particularly in developing countries with limited resources (Ali *et al.*, 2020). Despite their importance in Fiji, the parasitology and bacteriology of *A. antiquata* remain poorly understood and undocumented. Parasites infect seafood organisms,

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including bivalves, as ectoparasites or endoparasites, using the host as a source of nutrition (Abowei *et al.*, 2011; Wood and Lafferty, 2015). Studies on *Anadara* species in Malaysia have revealed the presence of ectoparasites and endoparasites such as *Balanus* sp., copepods, *Nematopsis* sp., metacercaria, and cestode larvae (Joey and Hassan, 2020). Other parasites, including *Nematopsis* sp., turbellarians, trematodes, *Perkinsus* sp., and *Spiroxys* sp. have been reported in *A. granosa* (Uddin *et al.*, 2011; Putra *et al.*, 2021).

In addition to parasites, the filter-feeding habits of blood cockles increase their susceptibility to accumulating pathogenic bacteria from their environment (Hossen *et al.*, 2014). Coastal areas exposed to pollution and anthropogenic waste further elevates this risk. Pathogens such as *Vibrio* spp., *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp., and *Clostridium botulinum* are commonly associated with seafood, and have been linked to food poisoning and infections (Iwamoto *et al.*, 2010; Noorlis *et al.*, 2011; Martinez-Urtaza *et al.*, 2016; Ramachandran and Raymond, 2019; Khasanah *et al.*, 2021). Faecal coliforms and *E. coli* have been detected in blood cockles from seafood markets in Bangkok, Thailand, along with a high prevalence of *Vibrio* spp., which are major contributors to foodborne gastrointestinal illnesses (Atwill and Jamsripong, 2021).

Bacterial infections from blood cockles often result from improper cooking or raw consumption. Depuration, a method of allowing shellfish to purge contaminants, is commonly used to eliminate pathogens. In Fiji, household-level depuration typically involves immersing shellfish in clean water for at least a day before consumption or cooking (Ledua *et al.*, 1996). A study by Singh *et al.* (2021) highlighted the use of biofilters in closed water circulatory systems as a simple and cost-effective depuration method for bivalves. Research by Nakamura and Shinotsuka (2007) and Srisunont *et al.* (2020) demonstrated that elevated water temperatures could enhance the physiological activity of blood cockles. A previous work on a coastal marine bivalve showed maximised physiological activity at 35°C causing increased filtration rate and faecal matter production (Masilamoni *et al.*, 2002). This suggests the potential to expedite the depuration process under controlled conditions.

Despite the widespread use and consumption of blood cockles, only three published studies have

focused on this species in Fiji (Squires *et al.*, 1973; Butler, 1983; Richards *et al.*, 1994). Additionally, no research has examined the parasitology of blood cockles in Fiji, representing a significant knowledge gap. The present work thus aimed to identify the presence, types, and prevalence of parasites in blood cockles at four sites in Fiji, and to monitor bacterial contamination levels during depuration in a closed water circulatory system. Parasitology was only assessed prior to depuration. Such research would be crucial for developing improved food safety practices, and ensuring the sustainable use of economically important species.

Materials and methods

Parasite sampling

Blood cockles (*A. antiquata*), ranging in size from 3 to 5 cm, were collected from four sites in the southeast region of Viti Levu, Fiji, for a parasitology study. The sampling sites included Nasese Coast, Viwa Island, Bau Landing, and Tailevu in the Central Division of Viti Levu, Fiji's main and largest island (Figure 1). Fifteen individual blood cockles were collected from each location, with sampling conducted three times a week over a period of four weeks.

To ensure freshness, the collected samples were placed in polystyrene boxes equipped with aerators. Each box contained water, sediment (sand and gravel), and bivalves from their respective sites (Duobinis-Gray *et al.*, 1991). The samples were transported to the laboratory on the same day as collection for analysis.

Parasite examination

The length and weight of each bivalve, with and without the shell, were measured using a Vernier calliper and an electronic balance. The adductor muscles were severed with a scalpel to open the shells. Targeted organs were then extracted for examination under a dissecting and light microscope, following the method described by Danford and Joy (1984).

Endoparasite observations were focused on the digestive tract, gills, and muscle tissue. The wet mount method was employed for the digestive tract, while the smear method was used for the gills. Muscle specimens were squashed between glass slides with a drop of seawater for microscopic observation. All

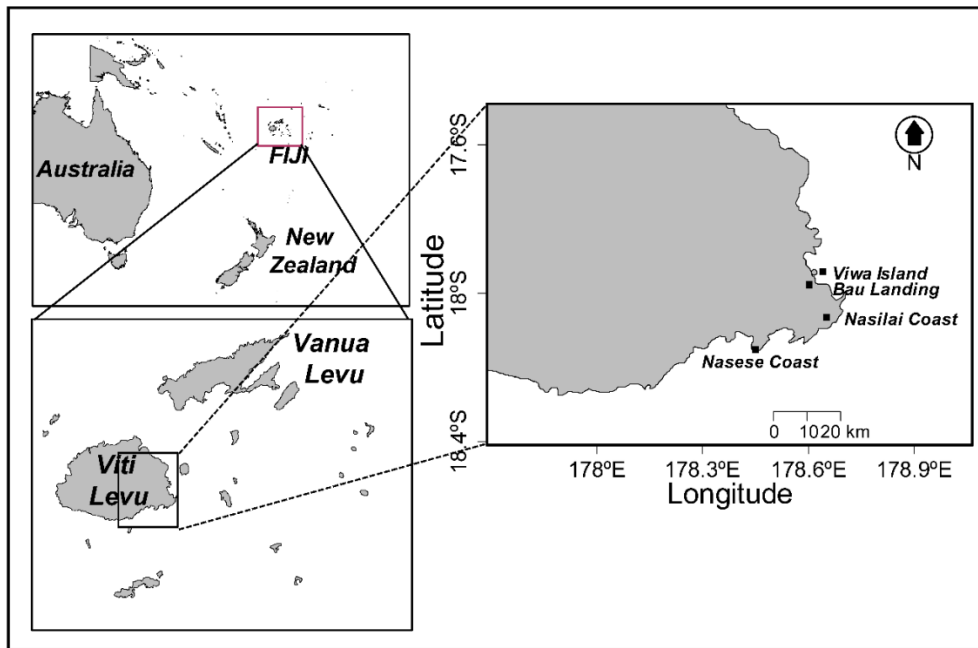


Figure 1. Sample collection sites for parasite and depuration studies on the main island of Viti Levu, Fiji, located in the south-eastern region. The parasite study sampling sites included Viwa Island, Bau Landing, Nasilai Coast, and Nasese Coast. Depuration study sampling was conducted at the Nasese Coast. The inset shows the location of Viti Levu within Fiji, situated in the South Pacific region.

detected parasites were preserved in a 70% ethanol solution, and stored for further analyses. Parasite identification was conducted using a combination of online reference materials, textbooks, and scientific journals on parasitology, parasitic diseases, and invertebrates.

Data analysis

Parasite pervasiveness prevalence and mean intensity were estimated using Eqs. (1) and (2), as given by Fernando (1972) and Margolis *et al.* (1982):

$$P = \frac{K}{n} \times 100 \quad (\text{Eq. 1})$$

$$I = \frac{\sum d}{K} \quad (\text{Eq. 2})$$

where, P = prevalence, representing the proportion of bivalves infected with parasites as a percentage, K = number of infected individuals, n = sample size, and $\sum d$ = sum of all parasites collected.

Depuration sample collection and site

Blood cockles, ranging in length from 3 to 5 cm, were collected from the Nasese Coast in the Suva area (Figure 1). The collection site was a sandy saltwater marine environment. Samples were

gathered by hand from depths ranging from 0.5 to 2 m, and placed in polythene bags.

At the collection site, a water sample was collected using a sterile airtight glass bottle, which was submerged about 1 m below the surface and held facing against the current flow for 1 min. This water sample was analysed for pH, salinity, dissolved oxygen (DO), and bacteriology. Seawater temperature was measured using a digital thermometer at the collection site.

Sediment samples were collected aseptically by scooping from the seafloor, and placing them in sterile polythene bags. Over 200 live blood cockles, ranging from 3 to 5 cm, were gathered by hand from the seabed sediment, and stored in polystyrene boxes with an aerator. Immediately after collection, a sample of four blood cockles was placed in a sterile polyethylene bag, and transported in an ice-filled cooler for total aerobic plate count (TPC) and coliform count analyses. The transportation time from the collection site to the laboratory was approximately 30 min.

Depuration experimental set-up and sampling

In the laboratory, blood cockles that did not respond to tactile stimuli were considered dead and discarded, along with those exhibiting shell damage.

The remaining bivalves were divided into two portions of 100 individuals each. Prior to the trials, the specimens were acclimatised in a tank containing sterilised seawater to confirm their viability. The bivalves (100 individuals per portion) were then transferred to equal-sized silica glass aquarium tanks, each measuring 150 × 70 × 45 cm. Each tank was fitted with an aerator/water regulator system that continuously cycled water through a biofilter made of wool and sterilised sand, returning it to the tank. The first tank was equipped with a heated closed water circulatory system maintained at 35°C and included a biofilter setup. The second tank had a standard closed water circulatory system with only the biofilter setup.

The bivalves were placed on mesh platforms in the tanks, positioned at least 20 cm below the water surface, and 20 cm above the tank's bottom surface. Four bivalves were aseptically removed from each tank at the start of the experiment (referred to as the zero-hour sample), and placed in a sterile homogenising bag. The depuration tanks were filled with sterile seawater, and the temperature of the tank water was measured prior to introducing the bivalves. A water sample was also collected aseptically, with 10 mL taken from below the water surface into sterile vials.

Bivalve and water samples were collected every 4 h for up to 48 h. At each sampling interval, the pH, salinity, temperature, and dissolved oxygen (DO) were measured. All water and bivalve samples were analysed for heterotrophic bacteria, *Vibrio* spp., and coliform load.

Bacteriological analysis

Tank water samples were serially diluted and plated in triplicates on standard plate count agar using the pour plate technique. The plates were then incubated at 37°C for 48 h.

Bivalve shells were thoroughly cleaned using sterile seawater. The shells were opened aseptically by cutting the adductor muscles, and the meat was transferred to sterile homogenising bags. The meat from two bivalves was weighed, diluted 10-fold with sterile seawater, and homogenised in a digester. Serially diluted replicates were plated using the spread plate method to count the total aerobic bacterial load and coliform load. The streak plate method was used for counting *Vibrio* spp. in the bivalves.

Marine agar was used for heterotrophic bacteria: M-endo agar for coliforms, and TCBS

(thiosulfate-citrate-bile salts-sucrose) agar for *Vibrio* spp. Colonies were counted, and the total plate count (TPC) load in bivalve samples was expressed as colony-forming units per gram of sample (CFU/g), and in tank water as colony-forming units per millilitre of sample (CFU/mL). Heterotrophic and coliform loads in parasite bivalve samples were also determined using the same procedure.

Results

Both endoparasites and ectoparasites were detected in the blood cockle samples. Four endoparasites and one ectoparasite were identified in the blood cockles from the four sampling sites in south-eastern Viti Levu. The identified endoparasites included *Mytilicola* sp., a small copepod; *Nematopsis* sp. and *Perkinsus* sp., parasitic protozoans; and *Spiroxys* sp., a nematode commonly found as an aquatic endoparasite. The ectoparasite identified was *Semibalanus balanoides*, an acorn barnacle. Parasites found in each sampling area and different body sections are represented in Table 1.

Parasite-infected samples exhibited some distinct differences compared to non-infected samples. Clinical symptoms included pale gills and an excess of mucus in infected samples. However, not all infected individuals displayed symptoms, and some could not be morphologically distinguished from non-infected individuals. The prevalence and intensity of parasite infections at the four sites are presented in Table 1. The Nasese Coast had the highest number of parasites compared to the other sites, with most of the parasites found attached to the mantle surface of the blood cockles. Samples from Viwa Island did not harbour any parasites, while samples from Bau Island contained only the ectoparasite *S. balanoides*. Nasilai Coast showed a similar prevalence of *Mytilicola* sp. to Nasese Coast. The mean prevalence was 29.3% at Nasese Coast, 0% at Viwa Island, 4% at Bau Landing, and 6.7% at Nasilai Coast.

Water quality parameters, such as pH, conductivity, salinity, dissolved oxygen, total dissolved solids, and temperature were similar across all four sites (Table 2). Coliforms were present at comparable levels at all sites, while the total plate count (TPC) was highest at Nasilai Coast compared to the other sites.

Blood cockle samples from Nasese Coast showed varying bacterial contamination levels across

Table 1. Blood cockles (*Anadara antiquata*) parasite intensity and prevalence of four sampling sites.

Site	Parasite	Σ Parasite	Σ Parasite sample	Σ Sample	Intensity (I)	Prevalence (P) (%)
Nasese Coast	<i>Mytilicola</i> sp.	10	5	15	2	33.3
	<i>Nematopsis</i> sp.	53	13	15	4.1	86.7
	<i>Spiroxys</i> sp.	2	2	15	1	13.3
	<i>Perkinsus</i> sp.	2	2	15	1	13.3
	<i>Semibalanus balanoides</i>	0	0	15	0	0
Viwa Island	<i>Mytilicola</i> sp.	0	0	15	0	0
	<i>Nematopsis</i> sp.	0	0	15	0	0
	<i>Spiroxys</i> sp.	0	0	15	0	0
	<i>Perkinsus</i> sp.	0	0	15	0	0
	<i>Semibalanus balanoides</i>	0	0	15	0	0
Bau Landing	<i>Mytilicola</i> sp.	0	0	15	0	0
	<i>Nematopsis</i> sp.	0	0	15	0	0
	<i>Spiroxys</i> sp.	0	0	15	0	0
	<i>Perkinsus</i> sp.	0	0	15	0	0
	<i>Semibalanus balanoides</i>	4	3	15	1.3	20
Nasilai Coast	<i>Mytilicola</i> sp.	5	4	15	1.3	26.7
	<i>Nematopsis</i> sp.	1	1	15	1	6.7
	<i>Spiroxys</i> sp.	0	0	15	0	0
	<i>Perkinsus</i> sp.	0	0	15	0	0
	<i>Semibalanus balanoides</i>	0	0	15	0	0

Table 2. Water quality parameters of four sampling sites during sample collection.

Site	pH	Conductivity (mS/cm)	Salinity (PSU.ppt)	DO (%)	TDS (ppt)	Temperature (°C)	TPC (CFU/mL)	Coliform (CFU/mL)
Nasese Coast	8.43	45.76	29.66	24.9	22.89	24.1	9600 ± 80	296 ± 42
Viwa Island	8.37	36.39	22.90	30.5	18.19	25.4	10700 ± 56	349 ± 32
Bau Landing	8.85	38.48	24.43	23.7	19.24	23.2	12300 ± 78	302 ± 150
Nasilai Coast	8.35	33.09	20.73	20.7	16.54	26.2	23100 ± 54	264 ± 93

three trials. TPC, coliforms, and *Vibrio* spp. were monitored during the 48-h depuration process. The depuration patterns varied depending on the initial bacterial load. However, all contamination loads in blood cockles reached undetectable levels within 48 h in tanks at normal room temperature (Figure 2). TPC levels decreased in less than 27 h, coliforms in less than 31 h, and *Vibrio* spp. in less than 31 h. In tanks at raised temperatures (35°C), the results were markedly different (Figures 2 and 3). Initially, TPC, coliforms, and *Vibrio* spp. levels decreased in both blood cockles and tank water, but these then increased beyond initial levels by 48 h. This increase in the warmer tanks (35°C) might have been caused by the

mortality of blood cockles, which showed signs of death within 12 h. The tank water became turbid as a result.

In normal tanks, at the end of depuration, there was approximately 0% mortality and a > 99% reduction in TPC, coliform, and *Vibrio* spp. loads in blood cockles across all three trials. The physicochemical parameters did not show significant variation during depuration in normal tanks. However, dissolved oxygen levels sharply decreased after the 12-h mark, and remained low until the end of depuration in the warm tanks. While microbial contaminants decreased during depuration, the ectoparasite and endoparasite counts in blood cockles

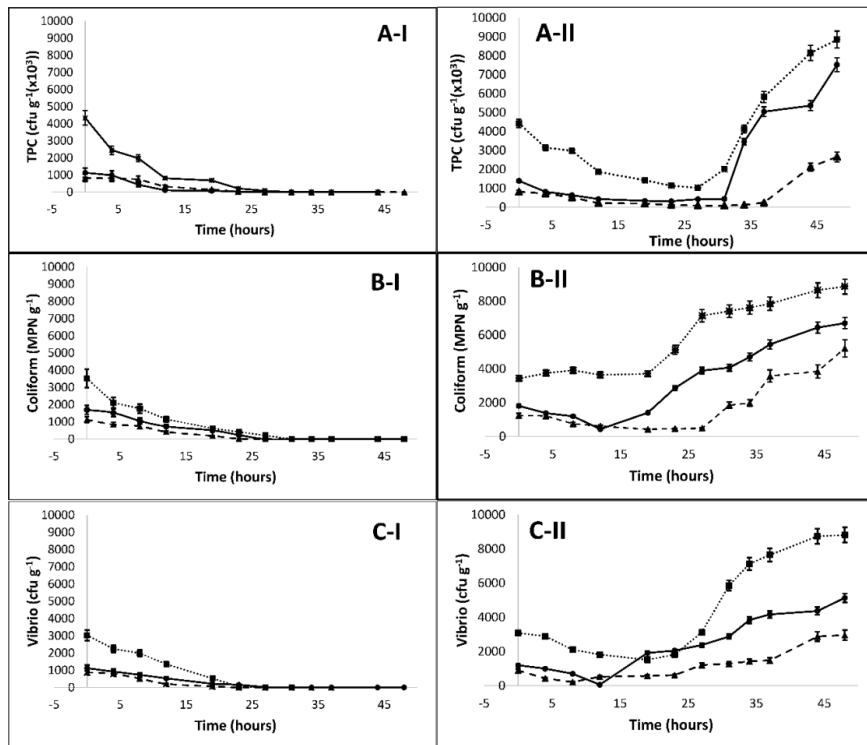


Figure 2. Time series of bacterial load in blood cockles (*Anadara antiquata*) during 48-h depuration period. (A) Total Plate Count Bacteria (CFU/g); (B) Coliform (MPN/mL); (C) *Vibrio* sp.; (I) Closed circulatory system at room temperature; and (II) Closed circulatory system at elevated temperature (35°C). Data points are mean \pm SE of five replicates across three trials.

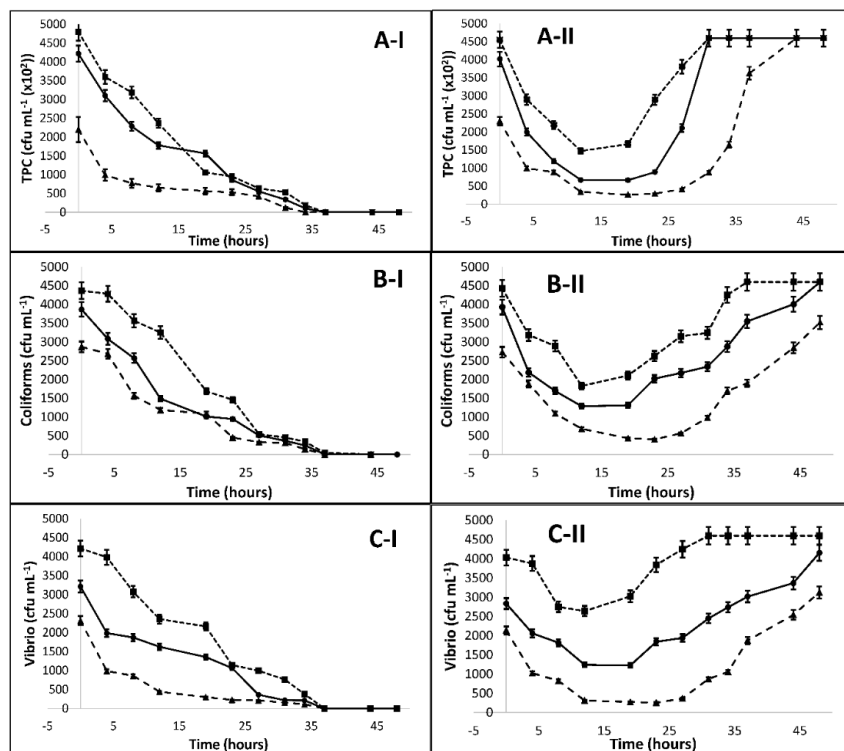


Figure 3. Time series of bacterial load in tank water during 48-h depuration of blood cockles (*Anadara antiquata*). (A) Total Plate Count Bacteria (CFU/mL); (B) Coliform (MPN/mL); (C) *Vibrio* sp.; (I) Closed circulatory system at room temperature; and (II) Closed circulatory system at elevated temperature (35°C). Data points are mean \pm SE of five replicates across three trials. In panels (A-II), (B-II), and (C-II), the trend line flattens above 4,500 (CFU/mL) due to bacterial load being too numerous to count.

from Nasese Coast after depuration did not show a significant difference from pre-depuration levels, as shown in Table 1 ($p < 0.05$).

Discussion

Blood cockles are an important food commodity for coastal communities in Fiji. They are primarily harvested for household consumption, and commercially sold in local municipal markets and roadside stalls (Lewis, 1988; Fay-Sauni *et al.*, 2008). Consumption methods range from uncooked to lightly or fully cooked (Quinn and Davis, 1997). However, the biological contaminant levels in blood cockles are not well documented locally. Therefore, the present work aimed to assess parasitic and bacterial contaminant levels in blood cockles, and evaluate the effectiveness of an economical depuration setup with a biofilter for bacterial contamination reduction.

The present work found that parasite presence, type, and prevalence varied significantly across the four sampling sites. These differences could be attributed to local ecological processes and anthropogenic influences. While clinical symptoms can help differentiate infected from non-infected individuals (Putra *et al.*, 2021), some infected blood cockles do not exhibit any visible symptoms. In these cases, morphological characteristics can be used to identify parasitic infections. Symptoms in infected blood cockles include pale gills (Nainggolan, 2016), increased mucus presence, and irregular shell shapes (Karnisa and Widowati, 2019). However, some parasites do not manifest external symptoms (Sarjito *et al.*, 2013).

Both endoparasites and ectoparasites were identified in the blood cockles. Endoparasites included *Mytilicola* sp., *Nematopsis* sp., *Perkinsus* sp., and *Spiroxys* sp. The only ectoparasite found was *Semibalanus balanoides*. A recent study in Aceh Besar, Indonesia, reported the presence of *Perkinsus* sp. and *Spiroxys* sp. in blood cockles (Putra *et al.*, 2021). Similarly, *Perkinsus* sp. and *Nematopsis* sp. were found in blood cockles from Central Java, Indonesia (Karnisa and Widowati, 2019). In the present work, the depuration process did not reduce the ectoparasite or endoparasite load in the blood cockles. Further research is recommended to explore effective means of parasite removal without negatively affecting host survival.

Bivalves, including blood cockles, are known to harbour *Vibrio* spp. more commonly than other seafood (Woodring *et al.*, 2012), posing potential health risks. Ekawati and Yusmiati (2018) reported the presence of *Vibrio* spp. in blood cockles, and Zarkasi *et al.* (2019) identified *Vibrio* spp., *E. coli*, and *Bacillus* spp. in similar species. The present work found that spoilage bacteria (TPC) and common foodborne pathogens such as coliforms and *Vibrio* spp. could be effectively depurated from blood cockles using a simple biofilter system under room temperature conditions. The depuration period was dependent on the initial bacterial load, with lower loads requiring less time for reduction. In the present work, 31 h of depuration was sufficient to reduce all bacterial loads to undetectable levels, regardless of the initial bacterial loads.

Depuration at a raised temperature (35°C) caused the mortality of all blood cockle specimens, and resulted in an increase in bacterial load both in the cockles and the tank water. Mortality at raised temperatures might have been due to temperature stress, as reported by Srisunont *et al.* (2020), where cockles died in large numbers at 32.4°C. The blood cockles required a shorter depuration time to reduce TPC (< 27 h) compared to coliforms, which took slightly longer (< 31 h). *Vibrio* spp. were reduced to undetectable levels in less than 31 h at room temperature. Barile *et al.* (2009) conducted depuration on bivalves such as *Chamelea gallina* and *Mytilus galloprovincialis*, and reported the reduction of *V. parahaemolyticus* to undetectable levels within 36 to 48 h. Based on these results, coliforms would be a better microbiological indicator for assessing the health and safety of blood cockles for human consumption, as their reduction corresponds to the elimination of TPC and *Vibrio* spp.

The depuration system at normal temperatures was effective in reducing all three bacterial types—TPC, coliforms, and *Vibrio* spp. While the depuration setup was effective for eliminating microbial contaminants, it was not suitable for removing ecto- and endoparasites from blood cockles. Additional purification steps are recommended for blood cockles from Nasese before consumption. Blood cockles from Bau Landing were parasite-free, but still contained TPC, coliforms, and *Vibrio* sp. Therefore, depuration of bivalves before consumption or sale is highly recommended.

Visual assessment alone may not be sufficient to distinguish infected blood cockles from uninfected ones during routine harvesting. Thorough cooking is recommended to eliminate bacterial and parasitic loads, ensuring consumer health and safety. The room temperature depuration method with a simple biofilter setup presented here is a cost-effective solution that can be easily integrated between harvest and consumption, improving food safety and reducing health risks. Similarly, Singh *et al.* (2021) reported that closed water circulatory systems can also reduce bacterial load in bivalves such as *Batissa violacea*.

Conclusion

The results presented herein, particularly from the normal temperature tanks, were primarily dependent on the specific depuration setup and characteristics used, such as the closed circulatory system with a sand biofilter in a controlled environment. The present work demonstrated that increasing depuration temperature did not support the purification process. Industrial-scale depuration setups, as well as subsistence-level sales, may involve higher densities of bivalves than in the present work, potentially presenting different challenges not encountered herein.

The present work provided valuable insights into common parasites found in blood cockles, and evaluated the effectiveness of depuration in reducing microbial contamination, aiming to address food safety concerns for consumers who rely on blood cockles as a source of protein. Further studies are needed to examine additional factors such as heavy metals, chemical toxins, toxic bioaccumulation, and pollutants to facilitate more informed decision-making regarding the safety of consuming blood cockles.

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