Vietnam Journal of Agricultural Sciences

p-ISSN 2588-1299
e-ISSN 3030-4520

VJAS 2025; 8(4): 2723-2732
https://doi.org/10.31817/vjas.2025.8.4.05

Changes in Microplastic Particles and the Biochemical Composition of Blood Cockles, *Anadara granosa*, During Five-day Depuration

Nguyen Thi Thao Tran[1], Nguyen Thanh Tam[1], Vo Van Tuan[1], Nguyen Ngoc Ha[2,3], Nguyen Phuc Thuong[1], Dang Thi Thanh Hoa[1], Nguyen Thi Bach Mai[1] & Nguyen Phuc Cam Tu[1*]

[1]Faculty of Fisheries, Nong Lam University, Ho Chi Minh City 71300, Vietnam

[2]Research Institute for Biotechnology and Environment, Nong Lam University, Ho Chi Minh City 71300, Vietnam

[3]Faculty of Biological Sciences, Nong Lam University, Ho Chi Minh City 71300, Vietnam

Abstract

Depuration is a widely used post-harvest technique to reduce contaminants in shellfish. However, its effects on the nutritional quality of blood cockles (*Anadara granosa*) in the removal of microplastics (MPs) are not well understood. This study aimed to assess the changes in the biochemical composition of cockle meat during a five-day MP depuration process. Cockles were subjected to a controlled depuration protocol, with samples collected at day zero, three, and five for analyses of MP abundance and biochemical composition. Standard methods were used to determine the MPs, proximate composition (including ash, proteins, lipids, and carbohydrates), and mineral content (Ca, Mg, P, and Fe). The results showed that the five-day depuration period effectively reduced the mean MP load by 71.6%. The process removed larger MPs and films, resulting in a higher relative abundance of smaller MPs (<500µm) and fibers. However, this process resulted in a statistically significant decrease in the biochemical composition of the cockles. By day five, the contents of biochemical composition had declined significantly. These findings demonstrate that while depuration is an effective process for reducing MP contamination in *A. granosa*, it decreases the nutritional quality.

Received: July 11, 2025
Accepted: November 10, 2025

Correspondence to
Nguyen Phuc Cam Tu
npctu@hcmuaf.edu.vn

ORCID
Nguyen Phuc Cam Tu
https://orcid.org/0000- 0001-6073-8320

Keywords

Anadara granosa, blood cockle, depuration, microplastic, proximate composition

Introduction

Plastic contamination is a critical and pervasive global issue, with approximately eight million metric tons of mismanaged plastic

debris entering marine environments annually (Birnstiel *et al.*, 2019; Ribeiro *et al.*, 2022). Due to their durability, these plastics accumulate and degrade into smaller particles known as microplastics (MPs), typically defined as synthetic polymer fragments less than 5mm in size (Birnstiel *et al.*, 2019; Covernton *et al.*, 2022; Ribeiro *et al.*, 2022; Liu *et al.*, 2023). These MPs originate either indirectly, from the fragmentation of larger macroplastics, or directly, from sources like hygiene products, synthetic textile fibers, and urban runoff (Birnstiel *et al.*, 2019).

Bivalves, such as oysters and blood cockles, are highly susceptible to MP contamination due to their extensive filter-feeding habits (Wootton *et al.*, 2022). Their feeding mechanisms, which efficiently capture particles between 5-300µm, make them prone to ingesting MPs along with other particulate matter (Xu *et al.*, 2024). As a non-selective filter organism, blood cockles (*Anadara granosa*) – a widely farmed species in the Indo-Pacific – readily accumulate MPs, raising significant environmental and health concerns (Mohan *et al.*, 2024; Rahmatin *et al.*, 2024). Contamination of MPs in *A. granosa* is documented across Southeast Asia, such as Thailand (Goh *et al.*, 2021) and Indonesia (Saleh *et al.*, 2023). In Vietnam, MPs have been identified in various bivalve samples, including *A. granosa* (Dao *et al.*, 2023; Tu *et al.*, 2025). This ingestion of MPs raises concerns for food safety, as MPs, along with the pollutants they adsorb, can potentially transfer to human consumers through the food chain (Liu *et al.*, 2023).

To mitigate risks, depuration is a common post-harvest practice in shellfish aquaculture, traditionally reducing microbiological contaminants (e.g., *Escherichia coli*) and biotoxins in bivalves (Lee *et al.*, 2008). Recently, studies have investigated its effectiveness in reducing the MP content. Depuration significantly reduces MPs in bivalves; efficacy increases with time but rarely reaches 100%. For example, a 93-hour period reduced MPs in mussels by 30-47% (Birnstiel *et al.*, 2019), while a five-day period lowered the concentration in oysters by 73% (Covernton *et al.*, 2022). Extending the duration to seven days achieved an 80% reduction in other mussel species (Pizzurro *et al.*, 2024). The process is more effective for larger MPs (>1,000µm) and granules than for smaller fragments and fibers (Pizzurro *et al.*, 2024). This incomplete removal occurs because some MPs are quickly expelled from the gills, while others are retained longer in deeper tissues, such as the digestive tract (Birnstiel *et al.*, 2019; Covernton *et al.*, 2022; Pizzurro *et al.*, 2024). The efficacy of depuration is influenced by the characteristics of the MP (type, size, and surface properties) and the duration of depuration (Paul *et al.*, 2023). Based on these findings, Covernton *et al.* (2022) recommended a five-day depuration period for industrial applications to effectively lower MP abundance in bivalves for human consumption.

While extensive research addresses the quantification and elimination of MPs in bivalves through depuration, the specific impact of this process on the biochemical composition of bivalves remains underexplored. Depuration without feeding, coupled with environmental stressors (e.g., handling, transport, air exposure, or fluctuating temperatures), induces significant physiological stress in bivalves. Under such conditions, bivalves mobilize stored energy reserves, resulting in measurable changes to their proximate composition (Patterson *et al.*, 1999). For A. granosa, the key unanswered question is not whether biochemical composition will change, but the rate and extent of this change relative to MP elimination efficacy. Although one study confirmed nutritional depletion in A. granosa during a 14-day heavy metal depuration (Tu *et al.*, 2020), the optimal conditions and duration required for effective MP depuration, and the corresponding nutritional consequences, are currently unknown and may differ significantly.

Therefore, this study aimed to evaluate the practical trade-off between enhancing food safety (reducing MP load) and the concurrent, inevitable loss of commercial and nutritional value (degradation of biochemical composition). By simultaneously quantifying these competing factors, this research will provide the first direct data to determine whether MP depuration is a viable, efficient, and economically sound post-

harvest strategy for the *A. granosa* industry. This information is essential for developing evidence-based guidelines that balance between product safety and quality.

Materials and Methods

Blood cockles

The sampling site, Thanh An commune, Ho Chi Minh City, was selected based on unpublished data indicating relatively high concentrations of MP contamination. Market-sized *A. granosa* (50-60 individuals kg^{-1}) were collected from an extensive bivalve farming area. *A. granosa* individuals were thoroughly scrubbed to remove mud and biofouling using onsite water. They were transported dry in an icebox to the Thu Duc Aquaculture Research and Production Facility, located at the Research Institute for Aquaculture No. II.

Depuration experiment

Seawater used for depuration was collected from the sea, filtered through a 5µm filter (salinity 24-26‰), and contained a background MP density of 1.0-1.4 MPs L^{-1}. Upon arrival at the laboratory, the cockles were thoroughly washed with filtered seawater. A total of 220 cockles (average body weight 21.2 ± 1.1 g individual (ind.) $^{-1}$ were selected. Forty cockles were immediately frozen -18 ± 2°C to determine initial MP abundance; the remaining 180 were used in the purification trial. A. granosa were randomly stocked in three replicate glass tanks 65cm × 45cm × 45cm) for five days, with 60 cockles per tank. Tanks were securely covered to prevent MP contamination. During depuration, the seawater was continuously aerated, siphoned to remove fecal matter, and partially replaced (30-50%) daily to maintain optimal quality. Water parameters were monitored daily using handheld meters and maintained within optimal ranges (temperature 26.9-27.5°C, salinity 24-26‰, DO ≥ 80% saturation, and pH 7.5-8.0). Crucially, no food was supplied throughout the purification to facilitate gut content clearance.

Sample collection, processing, and analysis

Sample collection

Cockles were sampled at three time points: zero (before stocking), three, and five days after stocking. At each time point, approximately 15-20 cockles from each tank were collected and combined into a pooled sample. The cockles were cleaned, and their total body weight was measured using an analytical balance (accuracy 0.01-g). The fresh flesh of each cockle was dissected from the shell and weighed. Nutritional and MP analyses were performed only on the soft tissues. Cockle tissue samples were homogenized and frozen at -18 ± 2°C before analysis.

Microplastic extraction and quantification

MP extraction from cockle tissue followed a modified procedure by My *et al.* (2023). Fresh tissue was initially homogenized. For digestion, a 5-g aliquot was weighed into a 250-mL glass beaker, and 50mL of 10% potassium hydroxide (KOH) was added (1:10 sample-to-reagent ratio, g mL^{-1}). The sealed beaker was incubated at 60°C for 48 hours, followed by 24 hours at room temperature to ensure complete tissue digestion.

Following digestion, density separation was performed by adding sodium chloride (NaCl) to the digestate (6g per 20mL solution). The mixture was homogenized using a magnetic stirrer for 15-30 minutes and then allowed to stand until the solid residue had settled. The resulting supernatant was passed through a Whatman GF/B membrane filter (47mm diameter, 1µm pore size). To maximize recovery, the remaining residue was washed twice with saturated NaCl solution, and the washings were passed through the same filter. The filter containing the extracted material was stored in a clean, covered Petri dish for subsequent analysis.

For MP analysis, the filter was examined under an Olympus SZ51 stereomicroscope at 40× magnification. Following established protocols (Masura *et al.*, 2015; Beaman *et al.*, 2016; My *et al.*, 2023), MPs were counted, measured, and characterized: the shape was classified as fragments, fibers, pellets, and films. The largest dimension was measured and categorized into four classes: <100µm, 100-500µm, 500-1,000µm, or >1,000µm. The MP colors were divided into red, yellow, green/blue, brown, white, and black.

Quality assurance/quality control (QA/QC) for microplastic analysis

Strict anti-contamination protocols were implemented across all stages of sample processing and analysis. To prevent the external MP introduction, all work surfaces and equipment were thoroughly cleaned, and contact with plastic materials was strictly avoided. All prepared solutions (distilled water, KOH, NaCl) were filtered through glass fiber filters before use. Furthermore, samples were kept securely covered between steps to minimize airborne contamination. The efficacy of these measures was validated using procedural blanks, which underwent the entire analytical process alongside the cockle samples. Any MPs detected in the blanks were subtracted from sample counts to correct for background contamination.

Proximate composition analysis

The proximate composition of the cockle flesh was determined using standard analytical methods (Association of Official Analytical Chemists (AOAC), 1997). First, the moisture content was quantified gravimetrically by oven-drying about 20g of homogenized tissue at 60°C for 24 hours. The resulting dried tissue was pulverized into a fine powder and stored for all subsequent analyses. The ash content was determined on a 2-g aliquot of the dried powder by incineration in a muffle furnace (550^{o}C, 4h) (AOAC Method 938.08, 1997). The crude protein was analyzed by measuring total nitrogen (N) in a 0.5-g sample using the Kjeldahl method (AOAC Method 955.04, 1997). Digestion involved concentrated H_2SO_4 and a $K_2SO_4/CuSO_4$ catalyst, followed by distillation using a Kjeltec 2100 unit (Foss Tecator, Hoganas, Sweden). Crude protein was calculated using a nitrogen-to-protein conversion factor of 6.25. Total lipids were quantified using a modified Folch *et al.* (1957) protocol. A 0.5-g dried sample was extracted with 10mL of a chloroform:methanol (2:1 v/v) solution, followed by two wash-and-dry cycles with 10.0mL of hexane. The residue was weighed to calculate lipid content. Finally, the total carbohydrate content was colorimetrically determined using the phenol-sulfuric acid method (Dubois *et al.*, 1956) with glucose as the standard, following sample extraction with 80% H_2SO_4 (Myklestad & Haug, 1972). All results were expressed as percentages.

Mineral analysis

The mineral composition of the cockle samples was quantified using established analytical protocols. For the calcium (Ca), magnesium (Mg), and iron (Fe) analyses, an ashed sample was first digested in 6M HCl and brought to a final volume of 50mL. The concentrations of Ca and Mg were quantified by complexometric titration using a 0.01M EDTA solution (Vietnamese National Standards, 2018). Total Ca and Mg were utilized in an aliquot buffered to pH 10.5 and titrated with the Eriochrome black T indicator. For Ca alone, a separate aliquot was adjusted to pH 12-13 with 1M NaOH and titrated using calcein; Mg was then calculated by difference. The Fe content was measured spectrophotometrically at 510nm using the o-phenanthroline method (AOAC, 1997; Method 944.02). This involved treating an aliquot of the digestate with a hydroxylamine hydrochloride, sodium acetate, and o-phenanthroline solution to develop the colored complex. Phosphorus (P) was analyzed separately: the sample was first decomposed with H_2SO_4 and H_2O_2, followed by colorimetric analysis via the ascorbic acid method (AOAC, 1997; Method 995.11).

Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics (Version 22.0). Before analysis, data were assessed for the assumptions of normality and homogeneity of variance using the Kolmogorov-Smirnov and Levene's tests, respectively. A one-way analysis of variance (ANOVA) was employed to detect differences in the proximate composition and MP abundance among the three time points, with Tukey's HSD test used for post-hoc pairwise comparisons. For all analyses, statistical significance was set at a *P*-value of less than 0.05.

Results

Microplastic abundance and characteristics during depuration

Initially, MPs were detected in 100% of the non-depurated control samples (day zero), with a

mean abundance of 9.98 ± 1.54 MPs ind. $^{-1}$ (or 1.60 ± 0.20 MPs g wet wt. $^{-1}$) (**Table 1**). The depuration process proved effective, significantly reducing the mean MP load by 45.3% after three days (to 5.46 ± 2.14 MPs ind. $^{-1}$) and by a total of 71.6% after five days (to 2.83 ± 0.84 MPs ind. $^{-1}$). The mean values for day zero were statistically different from both day three and day five. But the means for day three and day five were not statistically different from each other (**Table 1**).

The MP composition exhibited significant shifts in shape, color, and size distribution throughout the depuration period. Morphologically, the relative loads of MP shapes changed dramatically over five days, as shown in **Figure 1A**. Initially (day zero), the sample was dominated by fiber and film (0.733 MPs g^{-1} each, 42.3% each), followed by fragments (0.200 MPs g^{-1}, 11.5%) and a small presence of pellets (0.067 MPs g^{-1}, 3.8%; **Figure 2**). By day three, the MP concentration was halved, dropping significantly to approximately 0.9 MPs g wet wt $^{-1}$. This intermediate point featured a compositional shift where film and pellet shapes constituted the most significant proportions, followed by fragments and fibers. The depuration remained effective, achieving a further reduction by day five, when MP levels reached a minimum of 0.466 MPs g wet wt^{-1}. Fiber remained the most numerous shape, with fragments and films contributing smaller amounts. Fragments showed the most significant proportional decrease, with a decreasing ratio of 91%. Pellets were virtually absent by day five.

Regarding the color distribution, black MPs were the most abundant (roughly 0.600 MPs g wet wt. $^{-1}$) and represented the largest fraction (34.6%), followed by white (0.466 MPs g wet wt^{-1}, 26.9%) and red (0.333 MPs g wet wt^{-1}, 19.2%). At day three, white became the most numerous color fraction, followed by red and black. On day five, black and white MPs were the largest groups, with minor contributions from brown MPs. The other colors were removed entirely from the sample during the depuration process (**Figure 1B**).

The depuration process resulted in an apparent change in the size composition of the MPs retained in the cockles (**Figure 1C**). Initially (day zero), the size fraction 100-500µm was dominant (approximately 1.2 MPs g wet wt. $^{-1}$), constituting the vast majority (69.2%) of the total burden. The 500-1,000µm and >1,000µm fractions made up smaller proportions, while the

Table 1. Microplastic abundance (mean ± standard deviation) in the cockles during the depuration process

Day	MPs individual^{-1}	MPs g wet wt. $^{-1}$
Day 0	9.98^{a} ± 1.54	1.600^{a} ± 0.200
Day 3	5.46^{b} ± 2.14	0.800^{b} ± 0.346
Day 5	2.83^{b} ± 0.84	0.466^{b} ± 0.115

Note: wt.: weight. Mean values in the same column followed by different letters are statistically different (Tukey's test, P <0.05).

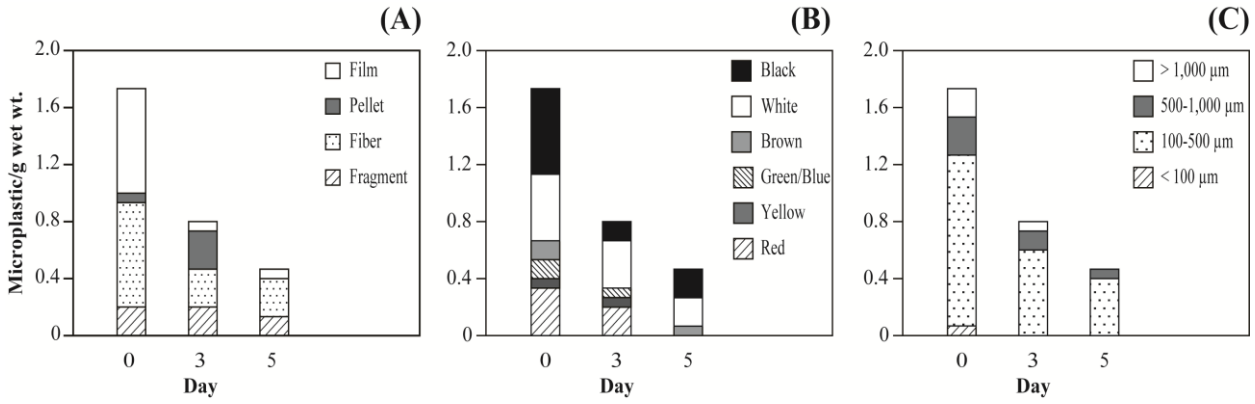

Figure 1. Mean number of microplastics per gram (wet wt.) in cockle flesh during the depuration process (A) by shape; (B) by color; (C) by size

<100µm fraction was the least abundant. By day three, the 100-500µm fraction, although reduced (0.600 MPs/g wet wt.$^{-1}$), remained the most significant contributor, followed by 500-1,000µm; the < 100µm fraction was absent. Day five recorded the lowest overall concentration (0.466 MPs g wet wt.$^{-1}$). The 100-500µm fraction, though significantly reduced from day zero, still comprised the largest single category. The proportion of 500-1,000µm remained relatively stable or only slightly reduced compared to day three, and the > 1,000µm fraction had disappeared.

Changes in the biochemical composition and mineral content during depuration

The changes in the biochemical composition of the cockles during the depuration process are presented in **Table 2**. No statistically significant differences were found for whole-body weight, flesh weight, or the condition index across five days (P >0.05).

Depuration-induced changes in the proximate composition occurred, with most components decreasing by day five. All reductions were statistically significant (P <0.05). The ash content decreased from 2.22 ± 0.11% to 1.84 ± 0.09%, while the protein level decreased from 8.44 ± 0.72% at day zero to 6.73 ± 0.16% at day five. A reduction was also observed in carbohydrates, from 0.670 ± 0.074 to 0.402 ± 0.022 mg 100 g^{-1}, while the lipid content fell from 2.83 ± 0.26% to 2.01 ± 0.13% (**Table 2**).

The depuration process induced a significant change in the cockles' mineral profile, with concentrations of most elements declining over a five-day period (**Table 2**). The most substantial loss was observed in Ca, which decreased by nearly 50% from an initial 59.9 ± 14.0 mg 100 g^{-1} to 31.0 ± 5.2 mg 100 g^{-1} by day five (P <0.05). Statistically significant reductions were also recorded for Mg and P. Notably, the primary decline in Mg occurred within the first three days, after which the concentration stabilized. In

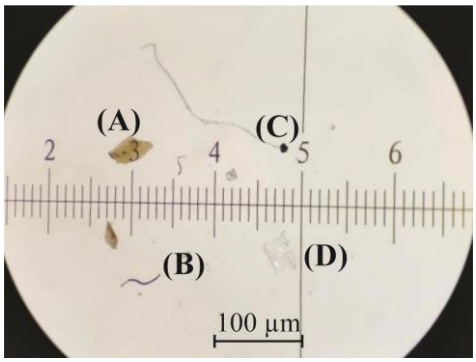

Figure 2. Representative stereomicroscope images of MPs: (A) fragment, (B) fiber, (C) pellet, and (D) film in the cockle samples

Table 2. Variation in the nutritional composition of the cockles during the depuration process (mean ± standard deviation, based on wet wt.)

Day	WB wt. (g ind.$^{-1}$)	Flesh wt. (g ind. $^{-1}$)	CI (%)	Ash (%)	Protein (%)	Lipid (%)
Day 0	21.6^{a} ± 0.5	6.22^{a} ± 0.21	29.0^{a} ± 1.8	2.22^{a} ± 0.11	8.44^{a} ± 0.72	2.83^{a} ± 0.26
Day 3	21.7^{a} ± 0.9	6.96^{a} ± 0.54	32.0^{a} ± 1.6	1.68^{b} ± 0.12	6.84^{ab} ± 0.92	2.05^{b} ± 0.22
Day 5	20.6^{a} ± 0.6	6.04^{a} ± 0.57	29.5^{a} ± 2.0	1.84^{b} ± 0.09	6.73^{b} ± 0.16	2.01^{b} ± 0.13

Day	Carbohydrate (mg 100g^{-1})	Ca (mg 100g^{-1})	Mg (mg 100g^{-1})	P (mg 100g^{-1})	Fe (mg 100g^{-1})
Day 0	0.670^{a} ± 0.074	59.9^{a} ± 14.0	118.0^{a} ± 12.0	84.7^{a} ± 7.6	26.1^{a} ± 4.5
Day 3	0.518^{ab} ± 0.080	36.4^{ab} ± 8.9	97.9^{b} ± 4.4	75.5^{ab} ± 6.5	20.1^{a} ± 1.7
Day 5	0.402^{b} ± 0.022	31.0^{b} ± 5.2	88.4^{b} ± 5.4	69.1^{b} ± 0.6	20.2^{a} ± 1.1

Note: WB: whole body; wt.: weight; ind.: individual; CI: condition index. Mean values in the same column followed by different letters are statistically different (P <0.05).

contrast, the Fe content remained statistically unchanged throughout the trial, indicating it was less susceptible to metabolic depletion during this period.

Discussion

Efficacy of microplastic depuration

The MP content in the cockles in this study (1.60 ± 0.20 MPs g wet wt.$^{-1}$) was comparable to or lower than findings from studies in Vietnam (4.2 ± 3.2 MPs g wet wt.$^{-1}$) (Dao *et al.* (2023), Thailand (4.71 ± 0.06 MPs g wet wt.$^{-1}$) (Goh *et al.* (2021), and parts of Indonesia (< 0.45-3.81 MPs g wet wt.$^{-1}$) (Saleh *et al.* (2023). Besides, the observed MP abundance was markedly lower than that reported for cockles in other Indonesian locations, including Jambi (9.8 ± 2.26 MPs g wet wt.$^{-1}$) (Fitri & Patria (2019) and Banten (567-720 MPs ind.$^{-1}$) (Ukhrowi *et al.* (2021).

The results clearly demonstrate that depuration is an effective method for significantly reducing MP content in *A. granosa*. But our finding that *A. granosa* retained a portion of their MP burden even after five days reflects a well-documented physiological challenge common to many bivalves. Research, such as that on *C. gigas*, shows that residual MP loads often persist despite significant reduction (Covernton *et al.*, 2022). This incomplete clearance is observed across species and environments, with studies on *Mytilus galloprovincialis* and *Perna perna* similarly highlighting the persistence of MPs, particularly fibers, retained within tissues (Birnstiel *et al.*, 2019; Pizzurro *et al.*, 2024). Furthermore, depuration efficacy is species-specific, with documented differences in clearance rates between *Ostrea edulis* and *C. gigas* (Paul *et al.*, 2023). In the present study, MP abundance decreased by approximately 72% (from 9.98 MPs ind.$^{-1}$ to 2.83 MPs ind.$^{-1}$) or 71% (from 1.60 MPs g wet wt.$^{-1}$ to 0.466 MPs g wet wt.$^{-1}$) over the five-day period. Thus, our results for *A. granosa* contribute to a growing consensus: while depuration is a viable mitigation strategy, it is not a panacea, and persistent MP fractions remain a key challenge for bivalve food safety.

This reduction is comparable to, or in some cases, more effective than, clearance rates reported for other bivalve species. For example, a 93-hour depuration significantly reduced the MPs in wild and farmed mussels by 46.79% and 28.95%, respectively (Birnstiel *et al.*, 2019). *C. gigas* showed a 73% reduction in MP concentration after five days of depuration (Covernton *et al.*, 2022). A three-day depuration achieved a 33% reduction in MPs in *M. edulis* and 25% in *C. gigas* (Van Cauwenberghe & Janssen, 2014). More recently, *M. galloprovincialis* exhibited an approximate 80% reduction after seven days (Pizzurro *et al.*, 2024). These comparative results collectively highlight depuration as a potential technique to lessen MP contamination in commercially relevant bivalves.

Despite the significant reduction, the complete elimination of MPs was not achieved after five days. This is common; even extended depuration periods (e.g., seven or ten days) rarely yield 100 removal (Ward *et al.*, 2019; Covernton *et al.*, 2022). This persistent residual load suggests some MPs may have prolonged retention times, likely due to association with tissues beyond the digestive tract, such as the mantle and muscle (Weinstein *et al.*, 2022; Paul *et al.*, 2023).

The retention of low MP levels post-depuration also suggests potential re-ingestion if bivalves were exposed to polluted environments. Furthermore, given the static-renewal system used in this experiment (daily siphoning and partial water changes), we cannot rule out the possibility of re-ingestion of previously expelled MPs from feces and pseudofeces.

Implementing strict QA/QC measures—such as using glass or metal equipment, covering materials with cotton canvas, and running procedural blanks—is essential for minimizing contamination and ensuring the reliability of results. The low MP levels detected in the procedural blanks in this study suggest that laboratory contamination was negligible, supporting that the measured MP levels reflect actual ingestion.

Characteristics of the depurated microplastics

The shift in MP shape and size loads during depuration provides insight into the selective elimination capabilities of *A. granosa*. Initially, film and fiber were the most abundant shapes, but

by day five, fibers predominated, and small particles (100-500µm) became the highest size fraction. This pattern suggests cockles are more effective at eliminating larger MPs and films than smaller MPs and fibers.

Several studies have documented this selective depuration. The flexible morphology of fibers enables them to be more effectively trapped in gills and digestive glands, resulting in longer retention times compared to spherical particles, such as pellets (Woods *et al.*, 2018). In this study, pellets were eliminated most effectively, followed by film, supporting the notion that shape influences depuration efficiency. Furthermore, studies indicate that bivalves can more easily eliminate larger MPs, while smaller MPs may be more difficult to expel and can remain in the digestive glands (Fernández & Albentosa, 2019). Similarly, Ward *et al.* (2019) found that MPs 500 µm have a longer residence time within bivalve digestive tracts.

The prevalence of white and black MPs in our samples, followed by red and green/blue, is generally consistent with common MP types and colors found in marine environments, often reflecting textile shedding and plastic fragmentation (Covernton *et al.*, 2022; Paul *et al.*, 2023). The observed reduction of blue fibers during depuration in *P. perna* suggests that depuration can be more effective for certain fiber types, potentially due to differences in surface properties from additives or pigments (Birnstiel *et al.*, 2019).

Changes in the biochemical composition of blood cockles during depuration

The observed changes in the biochemical composition of *A. granosa* during the five-day depuration period, specifically the reductions in ash, proteins, lipids, carbohydrates, and most mineral contents (**Table 2**), indicate a physiological response to stress induced by starvation conditions. Depuration protocols typically involve periods without feeding to facilitate gut clearance (Lee *et al.*, 2008), which is considered total starvation. Our findings align with other studies on bivalves, which have reported a general decrease in the biochemical composition in bivalve tissue during starvation (Whyte *et al.*, 1990; Liu *et al.*, 2024; 2025). The direct evidence strongly affirms that starvation during depuration drives the observed nutritional shifts.

A bivalve's physiological response to food scarcity is a well-defined, sequential process involving metabolic depression and the catabolism of endogenous energy reserves (Bayne, 1973; Liu *et al.*, 2024; 2025). To conserve energy, the metabolic rate is suppressed from routine to baseline levels. Concurrently, the organism mobilizes its stored nutrients in three distinct phases. The initial, immediate response to starvation is the rapid degradation of glycogen, the primary, most accessible energy store (Whyte *et al.*, 1990; Li *et al.*, 2009; Liu *et al.*, 2024). The significant decrease in carbohydrate content observed here is a classic presentation of this first metabolic phase, consistent with findings in *C. gigas* and other species (Whyte *et al.*, 1990; Li *et al.*, 2009). As glycogen depletes, metabolism transitions to the slower consumption of lipids and proteins. The steady decrease in protein content observed aligns with this secondary metabolic shift. This mobilization of protein—reported in mantle and gills—is critical for survival, as amino acids fuel essential functions via gluconeogenic precursors (Whyte *et al.*, 1990; Patterson *et al.*, 1999; Okumura *et al.*, 2002; Li *et al.*, 2009).

The reduction in ash content, often coupled with increased moisture, is a typical physiological response to bivalve starvation (Whyte *et al.*, 1990). The observed decreases in specific minerals, including Ca, Mg, P, and Fe, in *A. granosa* during depuration are supported by other bivalve studies. For example, in the zebra mussel (*Dreissena polymorpha*), stress from catching and transportation led to significant reductions in body content of Ca and Mg (4 to 4.4 times) over seven days (Martem'yanov, 2000). This mineral loss is considered a "negative" stress response, involving a cation efflux from cells to maintain ion homeostasis by compensating for leakage from the hemolymph (Martem'yanov, 2000).

Conclusions

This study demonstrated that while depuration is an effective method for reducing MP contamination in the blood cockle, *A. granosa*, it concurrently results in a significant

decline in the cockle's nutritional value. The five-day purification process successfully lowered the mean MP abundance by approximately 72%, proving its value as a post-harvest technique to enhance bivalve safety. This practice, however, induced a statistically significant degradation of the cockles' biochemical composition. Further research is needed to develop a method of feeding during depuration that ensures the effective removal of MPs while maintaining nutritional quality.

Acknowledgements

This study was funded by Nong Lam University, Ho Chi Minh City, under grant number CS-CB24-TS-02. We express our gratitude to the Research Institute for Aquaculture No. 2 for providing us with experimental facilities.

References

Association of Official Analytical Chemists (1997). Official Method of Analysis of AOAC International. (1 and 2). Association of Official Analytical Chemists. Gaithersburg, MD, USA. 1061 pages.

Bayne B. (1973). Aspects of the metabolism of *Mytilus edulis* during starvation. Netherlands Journal of Sea Research. 7: 399-410. DOI: 10.1016/0077-7579(73)90061-6.

Beaman J., Bergeron C., Benson R., Cook A.-M., Gallagher K., Ho K., Hoff D. & Laessig S. (2016). State of the Science White Paper: A Summary of Literature on the Chemical Toxicity of Plastics Pollution to Aquatic Life and Aquatic-Dependent Wildlife. United States Environmental Protection Agency.

Birnstiel S., Soares-Gomes A. & da Gama B. A. P. (2019). Depuration reduces microplastic content in wild and farmed mussels. Marine Pollution Bulletin. 140: 241–247. DOI: 10.1016/j.marpolbul.2019.01.044.

Covernton G. A., Dietterle M., Pearce C. M., Gurney-Smith H. J., Dower J. F. & Dudas S. E. (2022). Depuration of anthropogenic particles by Pacific oysters (*Crassostrea gigas*): Feasibility and efficacy. Marine Pollution Bulletin. 181: 113886. DOI: 10.1016/j.marpolbul.2022.113886.

Dao T.-S., Lai D.-M.-T., Nguyen Q.-H., Pham A.-D., La X.-T., Tran A.-T. & Bui X.-T. (2023). Investigation on microplastics in some bivalves at Binh Dien Market in Hochiminh City, Vietnam. IOP Conference Series: Earth and Environmental Science. 1278(1): 012029. DOI: 10.1088/1755-1315/1278/1/012029.

DuBois M., Gilles K. A., Hamilton J. K., Rebers P. A. & Smith F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 28(3): 350-356. DOI: 10.1021/ac60111a017.

Fernández B. & Albentosa M. (2019). Insights into the uptake, elimination and accumulation of microplastics in mussel. Environmental Pollution. 249: 321-329. DOI: 10.1016/j.envpol.2019.03.037.

Fitri S. & Patria M. P. (2019). Microplastic contamination on *Anadara granosa* Linnaeus 1758 in Pangkal Babu mangrove forest area, Tanjung Jabung Barat district, Jambi. Journal of Physics: Conference Series. 1282(1): 012109. DOI: 10.1088/1742-6596/1282/1/012109.

Folch J., Lees M. & Stanley G. H. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry. 226(1): 497-509.

Goh P. B., Pradit S., Towatana P., Khokkiatiwong S., Kongket B. & Moh J. H. Z. (2021). Microplastic abundance in blood cockles and shrimps from fishery market, Songkhla Province, Southern Thailand. Sains Malaysiana. 50(10): 2899-2911. DOI.

Lee R., Lovatelli A. & Ababouch L. (2008). Bivalve depuration: fundamental and practical aspects. *FAO Fisheries and Aquaculture Technical Paper*. Rome.

Li Y., Qin J. G., Li X. & Benkendorff K. (2009). Spawning-dependent stress response to food deprivation in Pacific oyster *Crassostrea gigas*. Aquaculture. 286(3-4): 309-317. DOI: 10.1016/j.aquaculture.2008.09.035.

Liu M., Li T., Zhang H., Ni H., Yuan G., Wang Z., Yang Y., Cong Q. & Dong Z. (2024). Starvation alters lipid and glycogen metabolism in clam *Cyclina sinensis*. Aquaculture Reports. 38: 102353. DOI: 10.1016/j.aqrep.2024.102353.

Liu M., Wei C., Xu W., Tan L., Li L. & Zhang G. (2025). Effect of prolonged starvation on nutrition utilization and transcriptional responses in Pacific oyster (*Crassostrea gigas*). Aquaculture Reports. 40: 102649. DOI: 10.1016/j.aqrep.2025.102649.

Liu Y., Shi H., Chen L., Teng X., Xue C. & Li Z. (2023). An overview of microplastics in oysters: Analysis, hazards, and depuration. Food Chemistry. 422: 136153. DOI: 10.1016/j.foodchem.2023.136153.

Martem'yanov V. I. (2000). The dynamics of the sodium, potassium, calcium, magnesium contents in the fresh water mollusc zebra mussel *Dreissenia polymorpha* during stress. Journal of Evolutionary Biochemistry and Physiology. 36(1): 41-46. DOI: 10.1007/bf02890664.

Masura J., Baker J., Foster G., Arthur C. & Herring C. 2015. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. *NOAA Technical Memorandum NOS-OR&R-48*. National Oceanic and Atmospheric Administration, Marine Debris Division, Silver Spring MD.

Mohan P., Shahul Hamid F., Furumai H. & Nishikawa K. (2024). Beneath the surface: exploring microplastic intricacies in *Anadara granosa*. Marine Environmental Research. 199: 106581. DOI: 10.1016/j.marenvres.2024.106581.

My T. T. A., Dat N. D. & Hung N. Q. (2023). Occurrence and characteristics of microplastics in wild and farmed shrimps collected from Cau Hai lagoon, central Vietnam. Molecules. 28(12): 4634. DOI: 10.3390/molecules28124634.

Myklestad S. & Haug A. (1972). Production of carbohydrates by the marine diatom *Chaetoceros affinis var. willei* (Gran) Hustedt. I. Effect of the concentration of nutrients in the culture medium. Journal of Experimental Marine Biology and Ecology. 9(2): 125-136. DOI: 10.1016/0022-0981(72)90041-X.

Okumura T., Nagasawa T., Hayashi I. & Sato Y. (2002). Effects of starvation on RNA : DNA ratio, glycogen content, and C : N ratio in columellar muscle of the Japanese turban shell *Turbo* (*Batillus*) *cornutus* (Gastropoda). Fisheries Science. 68(2): 306-312. DOI: 10.1046/j.1444-2906.2002.00426.x.

Patterson M. A., Parker B. C. & Neves R. J. (1999). Glycogen concentration in the mantle tissue of freshwater mussels (Bivalvia : Unionidae) during starvation and controlled feeding. American Malacological Bulletin. 15: 47-50. DOI.

Paul A. T., Hannon C., Švonja M., Connellan I. & Frias J. (2023). Efficacy of microplastic depuration on two commercial oyster species from the west coast of Ireland. Journal of the World Aquaculture Society. 54(5): 1217-1234. DOI: 10.1111/jwas.12989.

Pizzurro F., Nerone E., Ancora M., Di Domenico M., Mincarelli L. F., Cammà C., Salini R., Di Renzo L., Di Giacinto F., Corbau C., Bokan I., Ferri N. & Recchi S. (2024). Exposure of *Mytilus galloprovincialis* to microplastics: accumulation, depuration and evaluation of the expression levels of a selection of molecular biomarkers. Animals. 14(1): 4. DOI: 10.3390/ani14010004.

Rahmatin N. M., Soegianto A., Irawan B., Payus C. M., Indriyasari K. N., Marchellina A., Mukholladun W. & Irnidayanti Y. (2024). The spatial distribution and physico-chemical characteristic of microplastics in the sediment and cockle (*Anadara granosa*) from the coastal waters of East Java, Indonesia, and the health hazards associated with cockle consumption. Marine Pollution Bulletin. 198: 115906. DOI: 10.1016/j.marpolbul.2023.115906.

Ribeiro F., Mitrano D. M., Hacker C., Cherek P., Brigden K., Kaserzon S. L., Thomas K. V. & Galloway T. S. (2022). Short depuration of oysters intended for human consumption is effective at reducing exposure to nanoplastics. Environmental Science & Technology. 56(23): 16716-16725. DOI: 10.1021/acs.est.2c02269.

Saleh R., Daud A., Ishak H., Amqam H., Wahyu A., Stang, Birawida A. B. & Mallongi A. (2023). Spatial distribution of microplastic contamination in blood clams (*Anadara granosa*) on the Jeneponto coast, South Sulawesi. Journal of Namibian Studies. 34: 2154-2179. DOI: 10.59670/jns.v34i.1481.

Tu N. P. C., Ha N. N., Nhan D. T., Dong N. V. & Tri N. N. (2020). Evaluation of heavy metal removal ability in blood cockles by flow-through depuration process. The Third International Conference on Sustainable Agriculture and Environment. Vietnam National University Ho Chi Minh City Publishing House. 411-422.

Tu N. P. C., Oanh P. T. K., Ha N. N. & Tam N. T. (2025). Microplastic distribution in the sediment of the mangrove ecosystem of Can Gio, Ho Chi Minh City, Vietnam, and its reflection in the inhabited cockle. Iranian Journal of Fisheries Sciences (in press).

Ukhrowi H. R., Wardhana W. & Patria M. P. (2021). Microplastic abundance in blood cockle *Anadara granosa* (Linnaeus, 1758) at Lada Bay, Pandeglang, Banten. Journal of Physics: Conference Series. 1725(1): 012053. DOI: 10.1088/1742-6596/1725/1/012053.

Van Cauwenberghe L. & Janssen C. R. (2014). Microplastics in bivalves cultured for human consumption. Environmental Pollution. 193: 65-70. DOI: 10.1016/j.envpol.2014.06.010.

Vietnamese National Standards (2018). TCVN 12598:2018: Fertilizers - Determination of total calcium and magnesium content by volumetric method (in Vietnamese).

Ward J. E., Zhao S., Holohan B. A., Mladinich K. M., Griffin T. W., Wozniak J. & Shumway S. E. (2019). Selective ingestion and egestion of plastic particles by the blue mussel (*Mytilus edulis*) and Eastern oyster (*Crassostrea virginica*): Implications for using bivalves as bioindicators of microplastic pollution. Environmental Science and Technology. 53(15): 8776-8784. DOI: 10.1021/acs.est.9b02073.

Weinstein J. E., Ertel B. M. & Gray A. D. (2022). Accumulation and depuration of microplastic fibers, fragments, and tire particles in the eastern oyster, *Crassostrea virginica*: A toxicokinetic approach. Environmental Pollution. 308: 119681. DOI: 10.1016/j.envpol.2022.119681.

Whyte J. N. C., Englar J. R. & Carswell B. L. (1990). Biochemical composition and energy reserves in *Crassostrea gigas* exposed to different levels of nutrition. Aquaculture. 90(2): 157-172. DOI: 10.1016/0044-8486(90)90338-N.

Woods M. N., Stack M. E., Fields D. M., Shaw S. D. & Matrai P. A. (2018). Microplastic fiber uptake, ingestion, and egestion rates in the blue mussel (*Mytilus edulis*). Marine Pollution Bulletin. 137: 638-645. DOI: 10.1016/j.marpolbul.2018.10.061.

Wootton N., Sarakinis K., Varea R., Reis-Santos P. & Gillanders B. M. (2022). Microplastic in oysters: A review of global trends and comparison to southern Australia. Chemosphere. 307: 136065. DOI: 10.1016/j.chemosphere.2022.136065.

Xu Z., Huang L., Xu P., Lim L., Cheong K.-L., Wang Y. & Tan K. (2024). Microplastic pollution in commercially important edible marine bivalves: A comprehensive review. Food Chemistry: X. 23: 101647. DOI: 10.1016/j.fochx.2024.101647.