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Nutrient cycling in ecological aquaculture wastewater treatment systems: vertical distribution of benthic phosphorus fractions due to bioturbation activity by *Tegillarca granosa*

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ABSTRACT: Fractionation of sediment phosphorus (P) was conducted to examine the distribution and bioavailability of P fractions in a blood clam *Tegillarca granosa*-based ecological aquaculture wastewater treatment system. In a laboratory experiment, either a low or high density of clams, or no clams (control), were added to a polypropylene chamber containing aquaculture wastewater and an 8 cm sediment layer. Distribution and bioavailability of inorganic P (Inorg-P) — which included exchangeable P (Ex-P), metal oxide-bound P (NaOH-P), calcium-bound P (HCl-P), redox-sensitive P (BD-P), and detrital P (De-P) — and organic P (Org-P) in the sediment were investigated after 30 d using a sequential extraction procedure (SEDEX). Results showed that *T. granosa* bioturbation activities markedly changed the contents of P fractions, especially Ex-P, NaOH-P, BD-P, and Org-P ($p < 0.05$). Total Inorg-P varied from 1.83 to 6.75 µmol g^{-1}, with an average of 4.29 ± 1.37 µmol g^{-1} among the 3 groups. Org-P ranged from 5.01 to 9.76 µmol g^{-1} and accounted for 22 to 41 % of total P (TP). Despite some variation in contents at different depths, the average proportions of P fractions to TP were, in ascending order, Ex-P, BD-P, NaOH-P, HCl-P, De-P, and Org-P. Inorg-P had the highest proportions (73.83 %) of TP. Bioavailable P (Ex-P, NaOH-P, and BD-P) proportions were 61, 58.23, and 44.44 %, respectively, relative to the control. The variant contents of the P fractions were most probably due to enhanced organic matter consumption and mineralization, metal oxide dissolutions, and the increased bioavailable P in the bioturbated sediment. This study suggests that *T. granosa* can modify the concentrations, distribution, and bioavailability of sedimentary P, as well as reduce Org-P. Our study further advances the knowledge on P cycling and provides theoretical assistance for monitoring P in aquatic environments.

KEY WORDS: Phosphorus fractions · Sediments · *Tegillarca granosa* · Aquaculture wastewater · Bioavailable phosphorus

1. INTRODUCTION

Aquaculture is the agroindustrial activity with the highest growth rate worldwide. The production of aquaculture has an annual growth rate of 8% and contributes 44.1% of the world fish supply of 167.2 million metric tonnes (Mt) (FAO 2016). Hall et al. (2011) estimated that aquaculture production will grow from 60 Mt in 2010 to 100 Mt by 2030 to meet the ever-increasing demand for fish food production. In aquaculture, similar to any other food production industry, inputs (e.g. chemicals, water, feeds, antibiotics) are necessary to generate the intended products. Because of the incomplete transformation of these in-

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puts to final products, and poor management, aquaculture wastes are increasingly discharged into the surrounding environment (Crab et al. 2007, Cubillo et al. 2016). Wastes associated with high levels of nitrogen (N) and phosphorus (P) can lead to critical environmental damage and irreversible ecosystem degradation. Previous research has found that about 67% of P applied to aquaculture facilities (e.g. ponds) as feed accumulates in sediments (Masuda & Boyd 1994).

P, while a common pollutant in aquaculture effluent waters, is one of the most essential and critically limited nutrients governing the growth of animals and plants (Shilla et al. 2009). It can be classified in various forms such as organic P (Org-P), exchangeable or loosely sorbed P (Ex-P), metal oxide-bound P (NaOH-P), calcium-bound P (HCl-P), redox-sensitive P (BD-P), and detrital P (De-P) (Wang et al. 2019). Variations in P fractions in the sediment dictate the P exchange between the sediment and overlying water, the water quality, and P bioavailability in the system (Zheng et al. 2004). The amount of P released from the sediment to the bottom water depends on the different chemical-physical conditions and the biological environment as well as the P fractions (distribution and quantity) within the sediment (Zheng et al. 2004, Jin et al. 2006, Łukawska-Matuszewska & Bolałek 2008, Shilla et al. 2009). Benthic P can best be estimated by determining total P (TP) and its individual P fractions separately, owing to its different chemical and biological properties in sediment (Ruttenberg 1992).

In most enclosed aquaculture ecosystems P is generated in part from the sediment (internal loading) and from anthropogenic activities (Kaiserli et al. 2002). The content of P released and trapped by surface sediments greatly depends on the amount of organic particles available in the overlying water and the discharging systems. P produced from sediment degradation activities is carried in porewater and eventually released to the water column where it is easily accessible to plankton species (Zhang et al. 2008). Excess P in the environment can affect primary productivity, resulting in eutrophication (e.g. Roy-Poirier et al. 2010) as well as changing the biogeochemical cycling of nutrients in the ecosystem (Meng et al. 2015, Wang et al. 2015, Bastami et al. 2018). In the long run, the P content in aquatic systems becomes buried/accumulated in the sediments.

Sediments are actively involved in aquatic nutrient uptake and regeneration processes under different biological conditions (Reddy et al. 2005, Zhu et al. 2008) and contribute a paramount role in the cycling of P (Wang et al. 2015). Sediment's ability to retain P depends on its physiochemical characteristics and the oxidation-reduction conditions at the sediment-water interface (SWI) (Bostic & White 2007). Reservoirs, estuaries, and earthen aquaculture pond bottoms can play an important role as a source or sink of sedimentary P (Wang et al. 2017). Deposition of sediment P can occur through assimilation in calcium carbonate minerals and/or organic matter (OM) mineralization (Jin et al. 2006, Ribeiro et al. 2008, Küster-Heins et al. 2010), which can be affected by environmental conditions (Huo et al. 2011) and bioturbating organisms such as Venus clam and blood clam *Tegillarca granosa*.

T. granosa is a shallow-burrowing benthic filter-feeding marine bivalve which naturally lives and is usually cultivated on mud in the intertidal zone with a water salinity of around 10–32 ppt (Kuang & Sun 1995) and optimum temperature ranging from 20 to 30°C. This species is mostly found distributed throughout the Indo-Pacific region, including South Africa, Australia, Polynesia, Japan, and China (Nguyen et al. 2017). It has a high economic value as food, and is cultivated aquaculturally. Globally, the production of *T. granosa* increased from 31298 to 66 195 metric tons (t) from 2007 to 2016 (FAO 2016). For China, Taiwan, and South Korea, the FAO Yearbook of Fishery Statistics reports that yearly capture production of this species rose from around 1415 t in 1995 to 6503 t in 1999. The coast of Zhejiang Province, China, is among the largest producers of blood clam, occupying ~100 km^2 of mudflats production area. The Guangdong and Fujian coastal regions are popular for field farming, with each production area covering up to 50–60 ha (Mao et al. 2019).

The feeding habit of *T. granosa* is related to the bottom feeds where it lives, and includes organic detritus (98%), phytoplankton, and unicellular algae (Phuc 1997). *T. granosa* has 2 equal shells held together with a ligament and opened and closed with muscles, and strong, burrowing feet that it uses to bury itself in the sand. It can burrow between 1 and 4 cm down the sediment (Nicholaus et al. 2019). Like other clams, *T. granosa* draws in water and filters out organic detritus/phytoplankton for feeding (Phuc 1997, Ramli & Abu Hasan 2013). During sediment reworking in a process known as bioturbation, *T. granosa* can potentially influence the cycling and distribution of benthic biogeochemical nutrients and remediate aquaculture wastewater (Nicholaus et al. 2019). Bioturbation by invertebrate species affects the internal loading

and cycling process of P in an aquatic ecosystem (Kaiser et al. 2002). Accordingly, bioturbation activities can directly or indirectly affect a number of processes, such as redox potential, acid-base property, dissolved oxygen (DO), OM, microbial community, and iron-aluminum oxides/hydroxides that are thought to primarily control the distribution of P fractions (Li et al. 2016).

Various studies on the distribution of sedimentary P fractions in both marine and freshwater sediments have been performed (e.g. Huo et al. 2011, Meng et al. 2014, Yang et al. 2016, 2018, Cao et al. 2019); however, understanding the vertical distribution of benthic P fractions in aquaculture wastewater treatment systems is important for improving the nutrient cycling and optimization of the system. This study aimed to quantify the contents of the major P fractions in clam-bioturbated pond sediment and to investigate the influence of *T. granosa*'s ecological activity on the distribution and bioavailability of P fractions in an ecological aquaculture wastewater treatment system. Studying bivalve bioturbation impacts on the distribution and speciation of P fractions may further improve the understanding of the mechanisms of aquaculture wastewater treatment systems.

2. MATERIALS AND METHODS

2.1. Bivalve, aquaculture wastewater, and sediment collection

Aquaculture wastewater and sediment were collected from the Ningbo Shrimp Production Center in Ningbo, Zhejiang Province, China. For consistency, reliability, and homogeneity, the experimental sediments were thoroughly homogenized by stirring and sieving to remove large particles and macrofauna before being distributed into the respective experimental chambers (Wang et al. 2015, Hou et al. 2018). The sediment was passed through a 1 mm mesh diameter sieve to remove large particles/macrobenthos. The freshly collected sediment had a porosity of 51.8% and OM content of 7.9%, and it was comprised of 31% sand and 66% silt and clay. Locally farmed blood clams *Tegillarca granosa* (average biovolume ± SD, 4.92 ± 0.43 g ml^{-1}) were collected from a local commercial farm. The clams were acclimatized for 15 d prior to the experiment. Throughout the study, the clams fed on the naturally existing organic detritus/phytoplankton/algae from the aquaculture wastewater.

2.2. Experimental design

A laboratory experiment was carried out for 30 d in an artificially constructed mesocosm system designed with a plastic bucket (0.55 m^3) filled with approximately 480 l of aquaculture wastewater and 12 small polypropylene (PP) chambers (0.125 × 0.125 × 0.25 m = 0.00391 m^3). An 8 cm thick layer of well-sieved sediment was spread on the bottom of each PP chamber, and all chambers were filled with aquaculture wastewater. The experiment consisted of 3 treatments: control (no clams), low density (1 clam $chamber^{-1}$), and high density (4 clams $chamber^{-1}$). The treatment densities were set according to the optimal stocking densities equivalent to 63–252 clams m^{-2}. Each treatment group consisted of 4 replicates. With the lids removed, the chambers (with sediment + clams + aquaculture wastewater) were carefully lowered down into the mesocosm. Aeration was provided every other day, during which approximately 50% of the water in the mesocosm was replaced with fresh aquaculture wastewater. Water temperature was maintained at 23 ± 0.5°C, and salinity ranged from 26 to 29 PSU.

2.3. Post-treatment sampling

Sampling of sediment and water for measurements of P fractions and overlying-water P (OWP) occurred at the end of the experiment (Day 30). All experimental chambers were carefully taken out and ~20 ml of overlying water (for phosphate analysis) was collected by gently siphoning with rubber tubing 1 cm above the sediment surface. Then the entire remaining overlying water was gently siphoned out of each chamber. The sediment core in each chamber was sliced into 3 sections at different intervals to create 3 sampling areas: (1) surface (0–2 cm), (2) middle (2–4 cm), and (3) bottom (4–8 cm), for sediment P fractions, OM, and total N (TN) analysis. Sediment samples were collected using a 30 cm long Plexiglas, and all subsamples were immediately placed in sterile and sealable plastic bags with air excluded before storage at −20°C for later analyses.

2.4. Laboratory analysis

Sequential extraction and measurement of P fractions. To extract the P fractions, a sequential extraction was performed in triplicate with 0.5 g freeze-dried sediment samples in 50 ml centrifuge tubes

using a sequential extraction method (SEDEX) modified from Ruttenberg (1992) and Yang et al. (2016) (Fig. 1). This method analyzes the most commonly known sedimental P fractions: Ex-P, NaOH-P, HCl-P, BD-P, De-P, and Org-P. During the extraction process, modifications were made following Yang et al. (2016) to reduce interference from the citrate-dithionite-bicarbonate (CDB) reagent with the SEDEX process. Fig. 1 shows the detailed extraction process of all the extracted P species. The concentration of Org-P was measured as the difference between 1 M HCl extractable P (24 h) before and after high-temperature combustion (550°C, 2 h) of the sediment. Inorganic P (Inorg-P) was the sum of the 5 P forms Ex-P, BD-P, NaOH-P, HCl-P, and De-P after extraction by the SEDEX method. TP was calculated as the sum of Inorg-P and Org-P (Slomp et al. 1996). To avoid underestimation of Org-P concentration (Ruttenberg 1992), a separate extraction procedure was applied (Yang et al. 2016). In addition, other vital conditions for the extracts were maintained (Zhang et al. 2010, Lin et al. 2013), and all samples were analyzed in triplicate after filtration (GF/F, Whatman, 0.45 µm). The OWP and all P fraction extracts (supernatants) were analyzed by the ascorbic acid-phosphomolybdate blue method (Wang et al. 2005) using a WESTCO SmartChem discrete analyzer.

Sediment total organic matter (OM). The OM contents of the sediment samples were determined by igniting a dry 0.2 g sediment sample for 4 h at 550°C (mass loss on ignition, LOI; White & Reddy 1999).

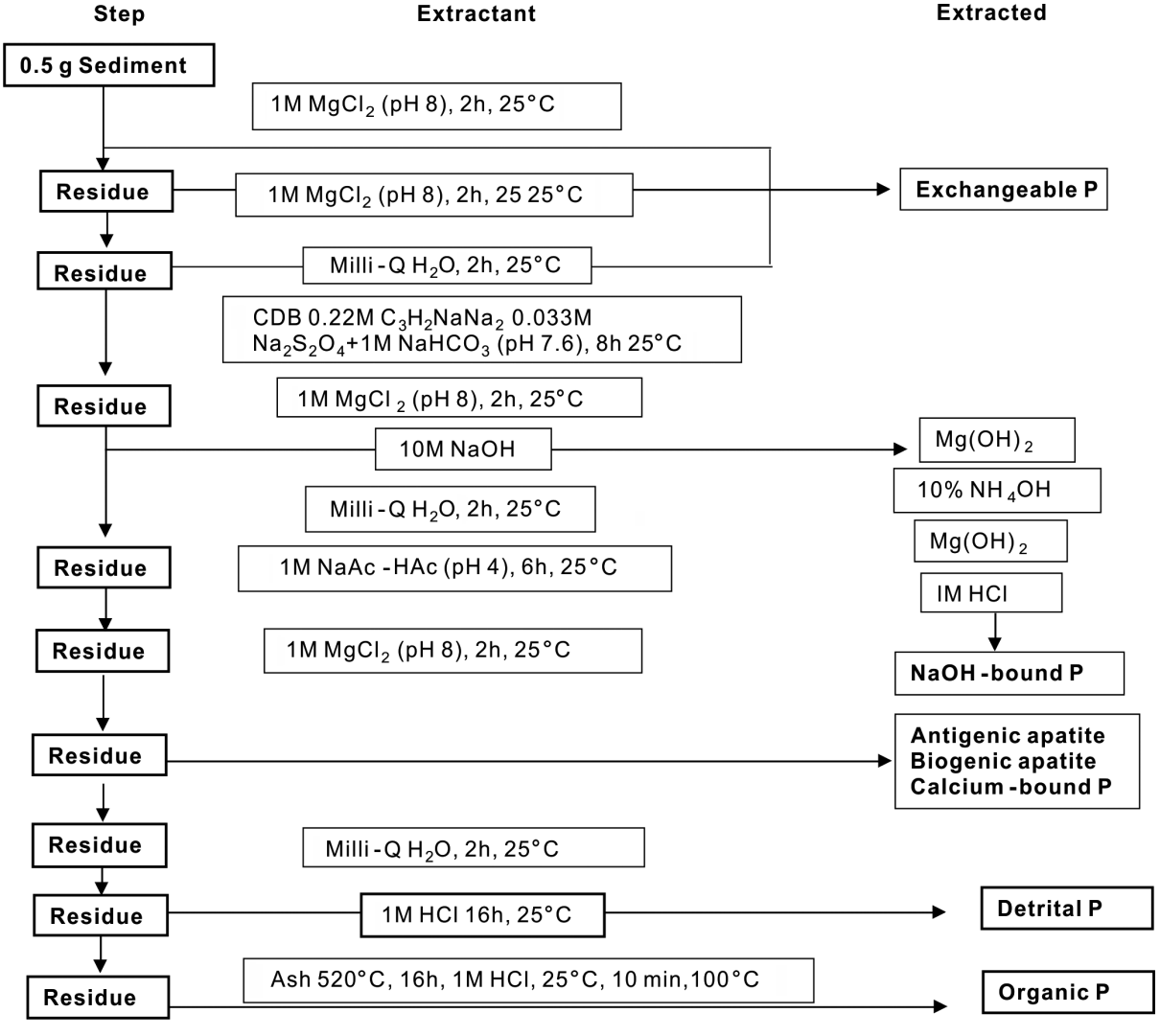

Fig. 1. Phosphorus (P) fractions extraction procedure (SEDEX) for aquatic surface sediments, modified from Ruttenberg (1992) and Yang et al. (2016)

2.5. Statistical data analysis

P fraction concentrations were analyzed using a multivariate analysis of variance (MANOVA) and a 1-way ANOVA in SPSS 16.0, with density, time, and depth as independent factors. Tukey's post hoc test was conducted to identify statistically significant data groups. The normality and homoscedasticity of data were tested prior to each statistical analysis. Pearson correlation analysis was used to identify relationships between P fractions and other diverse physicochemical parameters. For all statistical tests, results were considered significant at $p < 0.05$. Figures were drawn with the Origin Pro 8.0 scientific graphing and data analysis software.

3. RESULTS

3.1. Vertical distribution of benthic P fractions

The concentrations of P fractions in the freshly sieved sediments were: 2.6, 2.9, 5, 6.7, 11.03, 13.94, and 36.81 µmol g^{-1} for BD-P, Ex-P, NaOH-P, HCl-P, De-P, Org-P, and TP, respectively. The concentrations and percentage contributions for the different P fractions in the treatments are shown in Fig. 2 and Table 1. TP in the bioturbated sediment (i.e. the low-density and high-density treatments) ranged from 13.75 ± 5.78 to 31.72 ± 7.46 µmol g^{-1}, with an average of 22.73 ± 6.62 µmol g^{-1} (Fig. 2), suggesting a decrease of the sedimentary TP. Ex-P in bioturbated sediments displayed significantly higher concentrations between layers in each treatment, particularly in the surface (ANOVA, $F_{2,18} = 1.841$, $p = 0.043$) and the middle layer ($F_{2,18} = 1.328$, $p = 0.019$) (Fig. 2A). The mean concentration of Ex-P in the entire 8 cm sediment profile of the control, low-density, and high-density treatments was 2.06 ± 0.4, 2.82 ± 0.1, and 4.2 ± 1.7 µmol g^{-1}, respectively (Fig. 2A). NaOH-P contents (average ± SD) in the clam-bioturbated chambers varied between 1.51 ± 0.4 µmol g^{-1} (low-density) and 4.85 ± 0.3 µmol g^{-1}

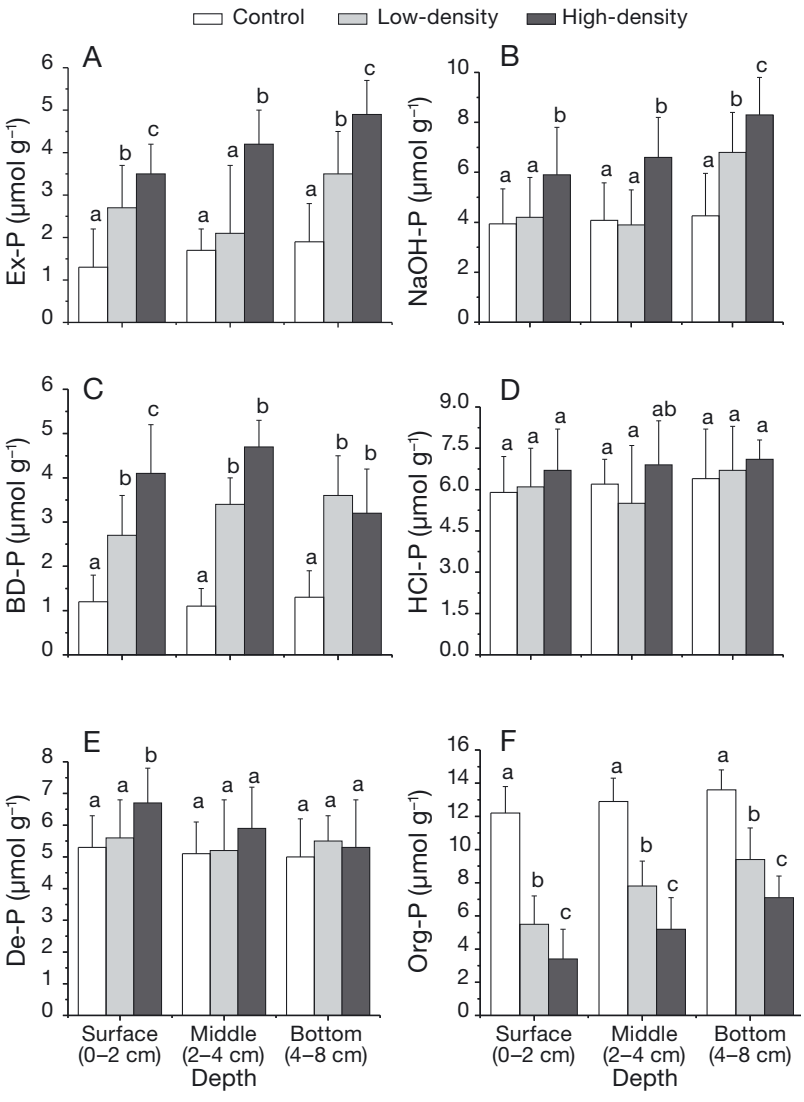

Fig. 2. Phosphorus (P) concentrations (mean ± SD; n = 4) of the vertical distributions of different P fractions: (A) exchangeable or loosely sorbed P (Ex-P), (B) metal oxide-bound P (NaOH-P), (C) redox-sensitive P (BD-P), (D) calcium-bound P (HCl-P), (E) detrital P (De-P), and (F) organic P (Org-P), measured in the 0–8 cm sediment profile in the *Tegillarca granosa* treatments. Letters denote significant differences ($p < 0.05$) within the same treatment

Table 1. Contribution of each phosphorus (P) fraction to sediment total P in the *Tegillarca granosa* treatments. BD-P: redox-sensitive P; De-P: detrital P; Ex-P: exchangeable or loosely sorbed P; HCl-P: calcium-bound P; NaOH-P: metal oxide-bound P; Org-P: organic P

Treatment	Contribution (%)					
	Ex-P	BD-P	NaOH-P	HCl-P	De-P	Org-P
Control	8.6	10	7.1	17	13	44.3
Low-density	12.3	13	15	14	8.6	37.1
High-density	15.9	18	17	17	11	26.1

(high-density) (Fig. 2B). The amount of NaOH-P was significantly increased among the treatments, by 17.1% (high-density) and 15% (low-density) compared to the control.

Both low- and high-density treatments showed significantly increased amounts of sedimentary BD-P than the control (Tukey's HSD, $p < 0.05$; Fig. 2C). BD-P concentrations were 1.1 to 1.3 µmol g^{-1} (1.25 ± 0.5 µmol g^{-1}), 2.7 to 3.6 µmol g^{-1} (3.15 ± 0.65 µmol g^{-1}), and 3.2 to 4.1 µmol g^{-1} (3.65 ± 0.7 µmol g^{-1}) for the control, low-density, and high-density treatments, respectively. The concentration of Org-P was decreased greatly between the bioturbated and the control sediment at all depths (ANOVA, Tukey's HSD, $p < 0.05$; Fig. 2F); Org-P was decreased by 19% (low-density) and 37% (high-density) compared to the control. The concentration of TP decreased sharply in the high-density clam treatment group, especially in the 0–2 cm sediment layer (ANOVA, $F_{2,18} = 13.017$, $p = 0.0038$) compared to the deeper (4–8 cm) sediments (ANOVA, $F_{2,18} = 7.695$, $p = 0.044$; Fig. 3).

In regard to De-P, significant differences were observed at the surface sediment (0–2 cm) of both clam treatments (Fig. 2E), whereas none were recorded in the deeper layers (2–8 cm depth). HCl-P was the least affected fraction, with non-significant variation (ANOVA, $F_{2,18} = 3.091$, $p > 0.05$) among all treatment groups. However, HCl-P displayed minor variation with an increasing trend from the control, low-density, to high-density treatments across the 0–8 cm depths (Fig. 2D). Generally, the bioavailable P (Ex-P, NaOH-P, HCl-P, and BD-P) increased among the bioturbated sediments (Fig. 4), with order rank: Ex-P > NaOH-P > BD-P > HCl-P, suggesting that the distributions and availability of the P fractions were related to the clam bioturbation activities.

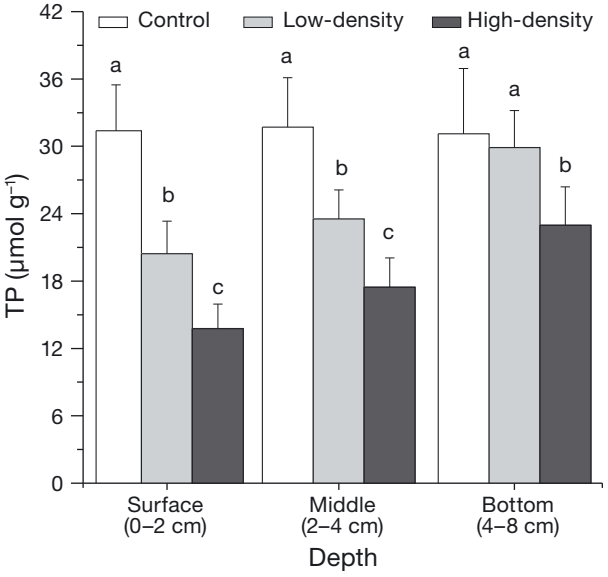

Fig. 3. Concentration (mean ± SD; n = 4) of vertical total phosphorus (TP) in the 3 sediment layers for each *Tegillarca granosa* treatment group. Letters denote significant differences ($p < 0.05$) within the same treatment

The overall descending order of P species contribution to sediment TP was: Org-P, De-P, HCl-P, NaOH-P, BD-P, and Ex-P for control; Org-P, Ex-P, NaOH-P, De-P, BD-P, and HCl-P for low-density; and Org-P, Ex-P, NaOH-P, De-P, BD-P, and HCl-P for high-density treatments (Figs. 2 & 4, Tables 1–3).

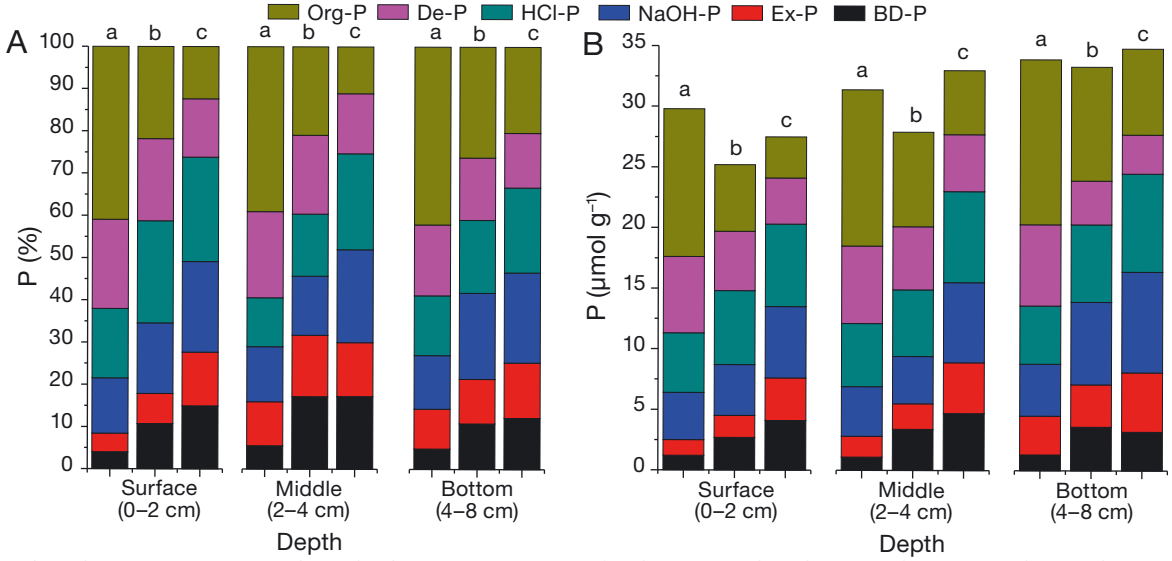

Fig. 4. (A) Relative proportions and (B) absolute concentrations of sedimentary phosphorus (P) fractions in the 3 sediment layers in the experimental groups: (a) control, (b) low density, and (c) high density of *Tegillarca granosa*. BD-P: redox-sensitive P; De-P: detrital P; Ex-P: exchangeable or loosely sorbed P; HCl-P: calcium-bound P; NaOH-P: metal oxide-bound P; Org-P: organic P

3.2. P fraction contributions to TP

The sediments from the blood clam treatments showed lower contributions of the Org-P fraction to sediment TP, and slightly higher for BD-P, NaOH-P, and De-P, compared to the non-bioturbated sediments (control), suggesting a decrease of organic wastes and increased level of bioavailable P. The contribution of each treatment group's NaOH-P concentration to each group's TP showed a stronger direct relationship with increasing bioturbation activities on the sediments; the higher the density of the clams, the higher the NaOH-P concentration in the sediment (Table 3). In the control sediment, Ex-P contributed the least (8.6%) to TP, whereas in the low- and high-clam density treatments, Ex-P contributed about 12.3 and 15.9% to TP respectively (Fig. 4, Table 1). Sediment from the high-density group had the highest NaOH-P concentration, which contributed about 17.1% to sediment TP. Inorg-P in the bioturbated sediments dominated the Org-P, constituting between 62.9 and 73.9% (68.4% ± 11.28%) of the TP (Fig. 4, Table 1).

Table 2. Concentrations of sediment total inorganic phosphorus (Total Inorg-P) and total organic phosphorus (Total Org-P) in the 3 sediment layers and of overlying-water phosphate (OWP) in the *Tegillarca granosa* treatments

Phosphorus	Depth (cm)	Control	Low-density	High-density
Total Inorg-P (μmol g^{-1})	0–2	3.23	5.94	8.82
	2–4	3.78	5.41	7.53
	4–8	3.56	4.78	5.92
Total Org-P (μmol g^{-1})	0–2	11.30	5.42	3.58
	2–4	12.42	7.16	5.35
	4–8	10.02	9.34	7.01
OWP (μmol l^{-1})	1[a]	11.7	19.02	26.84

[a]For OWP, a water sample was collected 1 cm above the sediment surface

Table 3. Pearson correlation coefficients between the concentrations of different phosphorus (P) fractions, overlying-water phosphate (OWP), organic matter (OM), and total nitrogen (TN). BD-P: redox-sensitive P; De-P: detrital P; Ex-P: exchangeable or loosely sorbed P; HCl-P: calcium-bound P; NaOH-P: metal oxide-bound P; Org-P: organic P; TP: total P. $^{*}p < 0.05$, $^{**}p < 0.01$

	Ex-P	BD-P	NaOH-P	HCl-P	Org-P	De-P	TP
Ex-P							
BD-P	0.414						
NaOH-P	0.137	0.131					
HCl-P	−0.196	0.024	−0.345				
Org-P	0.952**	0.795*	0.169	−0.847*			
De-P	0.262	0.371	0.058	−0.284	0.503		
TP	0.779**	−0.346	0.875**	0.439	0.983**	0.187	
OWP	0.796**	0.585*	0.898*	0.035	0.894**	0.192	0.952**
OM	0.881**	0.763*	0.817**	0.367	0.899*	0.521	0.938**
TN	0.567**	0.135	0.774**	−0.308	0.412*	0.687*	0.592**

3.3. Pearson correlations

Generally, the sediments exposed to bioturbation by blood clams showed increased amounts of total Inorg-P and reduced contents of total Org-P than the control sediments (Tables 1 & 2). High-density chambers had significantly higher concentrations of dissolved OWP compared to both control and low-density chambers (ANOVA, $p < 0.05$; Table 2). TP was significantly positively correlated with some of the P fractions (Ex-P, $r = 0.779$, $p < 0.01$; NaOH-P, $r = 0.875$, $p < 0.01$), as was Org-P with Ex-P ($r = 0.952$, $p < 0.01$) (Table 3). In addition, NaOH-P, Ex-P, Org-P, and TP were positively correlated with OWP ($r = 0.857$, $p < 0.01$; Table 2), which indicates that the amount of OWP was greatly dependent on the bioavailable P in the sediment.

4. DISCUSSION

Bivalve mollusks (e.g. clams, oysters, mussels, scallops) can play a role in engineering aquatic environmental stewardship and sustainability by promoting a healthy sedimental aquatic environment. For instance, bioturbating clams (e.g. blood clam *Tegillarca granosa*, Venus clam, razor clam) can remove N and P accumulated in the sediment and water as they rework the sediment through feeding and burrowing activities (Newell 2004, Lukwambe et al. 2018, Nicholaus et al. 2019, Zhao et al. 2019).

In this study, *T. granosa* significantly influenced the contents, distribution, and bioavailable P fractions within the sediments and the amounts of P in the overlying water (Figs. 2 & 4, Table 2). This may imply that variations in the contents of P fractions in the surface sediment (0–8 cm depth, treatment groups) are attributable to the different amount of OM mineralized and decomposed by the clam's bioturbation and its synergic chemical activi-

ties such as redox reactions (Hou et al. 2018). Sedimental deposited P can be altered and returned to the water column through various biological and physical activities such as OM, temperature, pH, redox fluctuations, and bioturbations (Jin et al. 2006, Chen et al. 2011). Thus, in the sediment, the extent of P adsorption by OM is dependent on the quantity and quality of the OM present (Wang et al. 2006). The decomposition of OM releases phosphate ions, which adsorb to the surface of Fe oxides/hydroxides and clay soils, which are the major carriers of Ex-P in sediments (Andrieux-Loyer & Aminot 2001, Yang et al. 2016).

Ex-P is the loosely absorbed, labile, and exchangeable P that comprises porewater P (Kaiserli et al. 2002). Various factors including water dynamics and redox potential can promote the Ex-P (Chen et al. 2011) in the sedimentary aquatic ecosystem. Ex-P was one of the most significantly increased P fractions (clam-treated chambers) and accounted for 15.9% (high-density treatment) and 12.3% (low-density treatment) of the TP compared to the control (8.6%) (Table 1). This can be related to the bioturbation behavior of the clam to enhance transportation of oxygen, water irrigation, and OM mineralization in the sediment (Kaiserli et al. 2002, Søndergaard et al. 2003, Biswas et al. 2009). The clam's capacity to enhance sediment oxygen consumption (SOC) can also be supported by our recent study that investigated SOC specifically by *T. granosa* (Table 4 in the present study; Nicholaus et al. 2019). The enhanced redox conditions due to increased O_2 supply within the sediment can largely affect the distribution of Ex-P (Łukawska-Matuszewska & Bolałek 2008). Similarly, reduced OM particle size is among the influential factors controlling the contents and availability of Ex-P in sediments (Yang et al. 2016) due to an increased surface area and more binding sites of adsorbed P (Meng et al. 2015, Yang et al. 2019). The bioturbation of clams in the PP chamber might have increased the surface area and resuspension, and reduced the grain size of sediment, resulting in a higher adsorption rate of Ex-P. However, the mineralization process by the clam during OM decomposition can increase the escape of sedimentary Ex-P into the water column, increasing the phosphate flux across the SWI (Nicholaus & Zheng 2014), leading to effluent reduction.

Inorg-P species such as NaOH-P represent water-soluble and biologically available P which is usually easily available to algae and mostly responsible for promoting eutrophication in water bodies (Zhou et al. 2001, Ribeiro et al. 2008). Proportionally, NaOH-P showed higher concentrations in the sediments bioturbated by the clams. The significant increase of NaOH-P relative to the control can be related to the resultant activities of *T. granosa* bioturbation processes such as improved oxygenation in the sediment (Nicholaus et al. 2019, Zhao et al. 2019; Table 4 in the present study). Due to the strong chemical relationship between O_2, ferrous (FeII) and ferric (FeIII) oxides, Fe(OOH)-P, and goethite and hematite (Zak & Gelbrecht 2002), more P adsorption processes likely took place as a result of a stimulated redox reaction (Bastami et al. 2018).

NaOH-P fractions are controlled by the chemical reaction of Fe and Al occurring at the sediment surface (Huo et al. 2011). High oxygen supply may increase the availability of electron acceptors, hence promoting the formation of Fe(III)-P. However, when conditions turn anoxic, ferric oxides are reduced to ferrous oxides and some P ions can be released to overlying water (Bastami et al. 2018, Hou et al. 2018). Adsorption of P from the clay part and Fe/Al (hydr)oxides is based on ligand exchange, whereby [OH-] substitutes P. This implies that the bioirrigation activities of clams may affect pH and can result in the subsequent increase of the negatively charged absorbing oxides. This suggests that the increased amounts of NaOH-P among the treatments were due to unstable equilibrium between the P sorption (binding) and desorption (release) rate which was probably enhanced by the presence of clam disturbances. Our results of NaOH-P are in congruence with Zhang et al. (2011), who reported that bioturbation by the clam *Corbicula fluminea* increased the NaOH-P content in sediment, as well as with Hou et al. (2018), who reported similar results with the sea cucumber *Apostichopus japonicus*.

HCl-P is considered to be an inert and refractory form (Rydin 2000) and contributes to the long-term burial of P in sediments (Kaiserli et al. 2002, Jin et

Table 4. Evidence from the literature for sediment oxygen consumption by bivalve clams (including *Tegillarca granosa*) in aquatic sediments

Clam species	Consumption rate (mmol O_2 m^{-2} h^{-1})	Reference
T. granosa	1.26–5.87	Nicholaus et al. (2019)
Cyclina sinensis	4–8.5	Nicholaus & Zheng (2014)
Sinonovacula constricta	1.73–3.30	Zhao et al. (2019)
Macoma (*Limecola*) *baltica*	1417	Karlson et al. (2005)
Corbicula fluminea	2500–3000	Zhang et al. (2011)

al. 2006). HCl-P is the product of OM decomposition (Yang et al. 2016, 2018). In the present study, to a large extent, the HCl-P proportions remained unchanged, especially in the bottom sediment layers, compared to the initial contents. This tendency may have been the result of *T. granosa*-induced disturbance which could only span mostly within the surface depth (0–2 cm) and was thus unable to influence the post-depositional transformation of Org-P, as well as the regeneration of HCl-P under anoxic conditions (Cha et al. 2005). Similarly, the detritus of aquatic animals can influence the HCl-P proportions in the sediments (Ruttenberg 1992, Bastami et al. 2018). Moreover, the sedimentary HCl-P concentrations depend on the availability of calcium and manganese (Mn) in the sediment (Kaiserli et al. 2002), and from direct combination between calcite and P, which always exists in relatively stabilized forms, e.g. $Ca_5(PO_4)_3OH$, $Ca_2HPO_4(OH)_2$, and $Ca_3(HCO_3)_3PO_4$ (Cassagne et al. 2000). This can further be supported by the fact that HCl-P is not easily available to phytoplankton (Zheng et al. 2004), thus its resistance to biological influences.

BD-P is a labile redox-sensitive P fraction (Zhou et al. 2001, Kaiserli et al. 2002), and an internal source for P loading (Rydin 2000). The concentration of BD-P in the present study was markedly affected in sediments treated with the clams, with a notable difference between the surface and middle layer (low- and high-density treatments). Küster-Heins et al. (2010) stated that BD-P bound to reducible iron and manganese P fractions are potentially mobile in the sediment, and can be released upon reduction of the reactive oxidized species of iron and manganese from the anoxic zone. Under anoxic conditions, occluded Fe(III) oxides are reduced to soluble Fe(II) oxides, causing the dissolution of the ferric hydroxide and promoting iron-bound phosphate ions to release from the sediment (Łukawska-Matuszewska & Bolałek 2008). Evidence of SOC by different clams (see our Table 4) into the sediment could imply a higher supply of DO within the sediment during this study and enhanced redox conditions that favored an oxic zonation and subsequently, more P sorbed to Fe(III) (Søndergaard et al. 2003). Furthermore, in our previous studies (Nicholaus & Zheng 2014, Zhao et al. 2019), we found that bioturbation by clams increased the oxic layer of the surface sediment. Therefore, we suggest that the adjustment of the BD-P concentration among the treatments is likely due to stimulated oxic zonation owing to the improved DO distribution by the clams. Similarly, physicochemical conditions like redox potential (Huo et al. 2011) and sediment resuspension can greatly affect various P reactions in the sediment. Khalil et al. (2007) stated that during the oxidation of OM in oxidizing sediments, a certain portion of P released in porewater is adsorbed on iron oxyhydroxides, carbonates, and clay minerals. Moreover, a substantial amount of P is retained in the sediment through adsorption to Fe oxides in the oxic surface layer (Jensen et al. 1995).

De-P is diagenetically stable, mostly derived from marine sediments and metamorphic rocks (Ruttenberg 1992, Meng et al. 2014, 2015). In the current study, De-P was among the most abundant form of P, contributing about 21% of the total Inorg-P in the freshly sampled sediment, which is in agreement with previous studies (Meng et al. 2014, Song & Liu 2015). De-P concentrations varied at the 0–2 cm layer ($p < 0.05$), then slightly increased down the sediment depth post clam bioturbation. This probably contributed to more sediment deposition at deeper depths over the 30 d time period. Generally, De-P is rarely influenced by biogenic particles due to its mineralogical properties (Ruttenberg 1992) and is thus normally identified as a permanent sink of P in sediment (Meng et al. 2014).

Org-P can be regarded as an inert P bound to hydrous iron oxides and phytoplankton (Ji et al. 2009). In the present study, Org-P was strongly reduced by the clams in both treatments, suggesting the ability of clams to degrade and minimize organic effluent, which corresponds to previous findings by Zheng et al. (2011) and Nicholaus & Zheng (2014). Further results of the present study suggest that efficient decomposition of OM occurred in the bioturbated sediments, especially in the 0–2 cm layer, based on a notable decrease in concentrations of Org-P. Mechanical activities by the clams, e.g. burrowing, ingestion, and digestion, can reduce the amount of accumulated OM in the sediment (Zhao et al. 2019). In addition, the transformation of Org-P to Inorg-P under enhanced microbial and alkaline phosphatase activities in the sediments can result in a decrease of Org-P fractions (Li et al. 2013, Lukwambe et al. 2018).

The commonly known bioavailable forms of P include Ex-P, NaOH-P, and BD-P (Kaiserli et al. 2002, Wang et al. 2019). In the current study, the bivalve clam *T. granos*a markedly increased the bioavailability level of Ex-P (61%), NaOH (58.23%), and BD-P (44.44%) relative to the control, all together contributing up to 54.56% of the TP. The increase in the bioavailability of the P fraction in the sediment was thought to equally increase the OWP cycling rate and the total P in the overlying water. This hypothesis is supported by our previous study (Nicholaus et al. 2019) which showed a highly increased phosphate

contents in the sediment and total P in the OWP in a pond bioturbated by the blood clam. In the study by Nicholaus et al. (2019), sedimentary P fluxes across the SWI increased significantly among the treated chambers, with concentrations ranging from 8.26 ± 1.23 µmol m^{-2} h^{-1} (control) to 16.02 ± 0.65 µmol m^{-2} h^{-1} (low-density treatment) and 27.97 ± 1.09 µmol m^{-2} h^{-1} (high-density treatment). Thus, the increased bioavailability of different P fraction contents as an outcome of the sediment reworking by *T. granosa* activities during this study can influence the TP released across the SWI. Different biological, mechanical, chemical, and environmental conditions supported by benthic macrofauna such as clams could modify the P within the sediment to be released (Zhou et al. 2001, Wu et al. 2014). The blood clam's bioturbation potential is likely to alter various conditions in the sediment such as sediment resuspension, oxygen-mediated reactions, metal oxide immobilization, and OM degradation, resulting in an increased release rate. P effluxes in sediments inhabited by clams have been reported (Nicholaus & Zheng 2014, Zhao et al. 2019), thus the sediment serves as an important internal source of P for the overlying water.

Furthermore, a sedimental P species like NaOH-P is a redox-sensitive substrate and can be released into the water under unstable redox conditions (Rydin 2000). The availability of the NaOH-P contents can be regulated through redox reaction and subsequently released from sediments across the SWI (Coelho et al. 2004). Qian et al. (2010) suggest that Org-P mineralization, HCl-P solubilization, and NaOH-P separation are important ways in which internal P is released into the water column. On the other hand, microorganisms play a major role in solubilization and mineralization of P in the sediment. Burrow walls created by *T. granosa* and other clams have been found to be rich with microbes responsible for OM mineralization and effluent reduction (Pischedda et al. 2011, Lukwambe et al. 2018, Zhao et al. 2019). Further, Edlund & Carman (2001) suggest that microorganisms play large roles in the abiding and releasing of molecules in the transient SWI, as microorganisms are capable of storing, utilizing, and releasing P compounds. Bioturbation activities by benthic macrofauna such as burrowing, irrigation, resuspension, feeding, excretion, and particle transportation can modify the sediment structure and properties, thereby affecting the diffusive and advective transport of both solutes and OM (Biswas et al. 2009). The positive relationship between the concentration of OWP and the TP in the sediment suggest that the amount of OWP originated from the sediment and its release was probably induced by the activities of the clams.

Generally, significant concentrations of several P fractions mostly at the surface sediment in bioturbated sediments reflect that bioturbation probably produced a stronger effect within the surface layer (0–2 cm). Usually, *T. granosa* can burrow a maximum of 4 cm into the sediment. *T. granosa* is likely to exert 3 major responses in its sedimental environment that are certain to affect the cycling of P: (1) it increases the concentration of O_2 in the burrows, thereby influencing the P-binding capacity of sediment, (2) it facilitates the transport of porewater P from sediment to water, and (3) it can move particulate oxidized compounds down to the reduced zone and reduced compounds up to the oxic zone, thus affecting ferric oxide redox in sediments.

5. CONCLUSIONS

The current study examined the influence of the bivalve *Tegillarca granosa* on the distribution and bioavailability of P fractions in an ecological aquaculture wastewater treatment system. The concentrations of BD-P, Ex-P, NaOH-P, HCl-P, and De-P exhibited an increasing trend, whereas Org-P was reduced among the clam treatments. The chambers containing the clams showed significantly higher bioavailable P (Ex-P, NaOH-P, De-P, BD-P, and HCl-P) fractions relative to the control. Similarly, *T. granosa* significantly increased the total overlying-water P, implying that the concentration of P in the water column can be altered by the amounts of available P fractions. Our results suggest that bioturbation by clams may control the internal loading, distribution, bioavailability, and cycling of sedimental P fractions as well as Org-P reduction in benthic aquatic ecosystems, and hence may be a control measure for benthic organic effluents.

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