Indian Journal of Geo Marine Sciences
Vol. 46 (07), July 2017, pp. 1320-1326

Phosphorus accumulation in mudflats in bottom-sowing culture for Manila clam *Ruditapes philippinarum* zone

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Received 30 July 2013 ; revised 25 February 2014

To evaluate impact of bottom-sowing culture for Manila clam *Ruditapes philippinarum* on sediments, 12-cm-long sediment cores were sampled in Daguhe Estuary, Jiaozhou Bay, China. The seeding density was 1.1-1.5 kg/m^2 as juvenile clam was 0.3-0.5 g/individual and the stock density of clam that could be harvested was 450-1600 individual/m^2. Whereas the bottom-sowing culture in the estuary trended to increase TP (total phosphorus) and IP (inorganic phosphorus) content in sediments, it trended to reduce Ex-P (exchangeable phosphorus) content. The bottom-sowing culture in the estuary had no significant effects on IP accumulation and it could reduce TP or Ex-P accumulation significantly.

[**Keywords:** phosphorous content; accumulation flux; bottom-sowing culture]

Introduction

Whereas aquaculture continues to bring benefits to society, it may have negative impacts on environments[1]. Sediments in suspended shellfish culture zones are affected by filter-feeding, bio-deposition, and excretion of shellfish[2-6]. Besides these effects, benthic shellfish influences sediments by bio-turbation[7] and sediments are affected by the harvest of benthic shellfish directly[8]. Benthic shellfish is cultured globally, e.g., only the annual production of Manila clam *Ruditapes philippinarum* in China reached 1.8 million tons in 2003[9].Self-pollution of benthic shellfish culture worsens as increased scale [10, 11]. Whereas phosphorus is necessary to phytoplankton that influences carrying capacity of shellfish farm from the point of food supply[12, 13], there will be ecological stress to aquatic organims if phosphorous is excessive[14]. Moreover, the ecological effect of phosphorus depends on its forms[15]. Organic phosphorus (OP) can be used by algae only after its mineralization to inorganic phosphorus (IP). Exchangeable phosphorus (Ex-P) is exchangeable in ions and can be used by phytoplankton directly[16].

As the scale of benthic shellfish culture expands in recent years, the growth rate of shellfish decreases and its mortality rate increases correspondingly. There is an argumentation that self-pollution of shellfish culture and food lack to shellfish may be the inducements, which has relation to phosphorus content and accumulation in sediments. What is impact of benthic shellfish culture on accumulation of phosphorus? What can be used to assess impact of benthic shellfish culture on sediments? There are some disputes about these issues[2, 17]. The present work aims at phosphorous accumulation in bottom-sowing culture zones and its implications to benthic shellfish culture.

Materials and Methods

Sediment cores were sampled in Daguhe Estuary, Jiaozhou Bay, China. In the northwest of

Jiaozhou Bay, Daguhe River had about 80 km^2 of mudflats in its estuary and the water residence time in the estuary was 80 days[18]. About 40% of the mudflat was used for bottom-sowing culture for Manila clam and the production was 3.4 kg/(m^2.year). Deepest water depth of the estuary was 4 m in high tide and there was no macro-algal cover. Juvenile clams with weight 0.3-0.5 g/individual were sowed at density 1.1-1.5 kg/m^2.The clams were harvested by harrows from May to November and the stock density of clam that could be harvested (with weight 8 g/individual) from May to August was 1600, 1100, 633 and 512 individual/m^2 and it was 450 individual/m^2 in other months[19]. Furthermore, phosphate was relative limitation to phytoplankton in the estuary[20]. In February, May, August and November of 2009, 12-cm-long sediment scores were sampled by box sampler at five sites designed at randomly (Fig.1). Mudflat used for bottom-sowing culture (the cultured mudflat), the mudflat without shellfish culture (the uncultured mudflat), the river way was represented by sampling sites E1 and E2, sampling site W1, sampling sites M1 and M2, respectively. Particulate size of sediments (p) in the uncultured mudflat, the river way and the cultured mudflat was 0.04, 0.02 and 0.01mm[21], respectively.

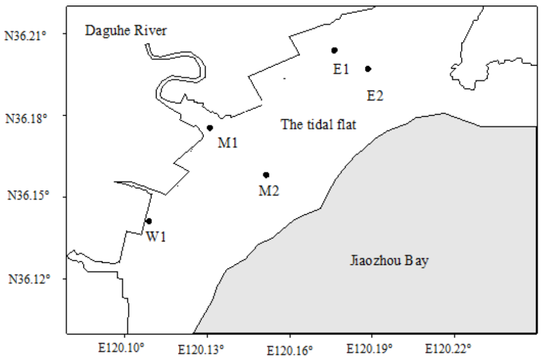

Fig.1—Daguhe Estuary and sampling sites

Sediment cores were segmented at interval of 2cm. The weighted wet sediment was dried to constant weight at 60℃ to determine water content. Moreover, density of wet sediment was measured by the Archimedes method. Dried sediments were ground by mortar and pestle, during which its natural particulate size was kept[22]. The form of phosphorous that was exchanged by hydrochloric acid (HCl) was inorganic phosphorous (IP) and the kind of phosphorus that was exchanged by magnesium chloride ($MgCl_2$) was exchangeable phosphorous (Ex-P). Different forms of phosphorous were measured by the following methods (Table 1) and phosphorous content (μg/g) was calculated by dry weight of sediment.

Water content, φ (%), was calculated by $(w_{wet} - w_{60}) / w_{wet}$, where w_{wet} and w_{60} was wet weight of sediment (g) and weight of sample dried to constant weight at 60℃(g), respectively.

Accumulation flux of phosphorous, R (g/(m^2.year)), was calculated by $C_i S \rho_d 10^{-2}$, where C_i, S and ρ_d was phosphorus content (μg/g), sedimentation rate of the estuary (cm/year) and dry bulk density of sediment (g/cm^3), respectively.

Dry bulk density of sediment was calculated by $(1-\varphi)/[(1-\varphi)/\rho_{wet} + \varphi/\rho_{water}]$, where ρ_{water} was density of seawater that was regarded as 1.027g/cm^3.

If the homogeneity of variances was satisfied, the temporal and spatial differences of water content, contents and accumulation fluxes of phosphorus from February to November were analyzed by the Two-Way ANOVA. Otherwise, the distribution characteristics were analyzed by the Dunnett test, one kind of multiple comparisons based on observed values. Moreover, the regional classification for study areas was conducted by PCA (principal component analysis). Density of wet sediment, particulate size and water content of sediment, and content of phosphorus were selected as independent parameters. Furthermore, correlations among these parameters were analyzed by the CORRCOEF function in Matlab7.0 and the significant level was 5%.

Table 1—Forms of phosphorus in sediment and extraction method

Phosphorus Form	Extraction method	Measurement
Exchangeable phosphorus (Ex-P)	Dried 1.0g SPM is added into 25mL $MgCl_2$ (1mol/L). After 2-h oscillation, the mixture is centrifuged (4000r/min, 10mins). The supernatant is collected to determine Ex-P content.	
Inorganic phosphorus(IP)	Dried 0.1g SPM was added into 25mL HCl (1mol/L). After 2-h oscillation, the mixture is centrifuged (4000r/min, 10mins). The supernatant is collected to determine IP content.	Phosphomolybdate blue spectrophotometry
Total phosphorus(TP)	Dried 0.1g SPM is added into 25mL oxygenant which consisted of $K_2S_2O_8$ (0.15mol/L) and NaOH (0.15mol/L). After 1-h nitration at 120℃ and 0.12MPa, the mixture is centrifuged (4000r/min, 10mins). The supernatant is collected to determine TP content.	

Results

From February to November, TP content in the cultured mudflat was significantly higher than that in the river way (p<5%) (Fig.2). TP content of the estuary including the mudflats and the river way in February, May, August and November was 238.10±48.73, 181.94±34.33, 128.26±43.44 and 118.20±51.18 μg/g, respectively. Except August and November, there was significant difference of TP content between any two sampling months (p<5%). IP was the principal form of phosphorous in the estuary (Fig.3). IP content in the river way was significantly lower than that in the cultured mudflat (p<5%). IP content of the estuary in February, May, August and November was 160.97±38.96, 132.43±30.05, 91.56±27.49 and 73.17±34.40 μg/g, respectively. Except August and November, there was significant difference of IP content between any two months (p<5%). The distribution characteristic of Ex-P content was dissimilar to that of TP or IP content (Fig.4). There was significant difference of Ex-P content between the uncultured mudflat and the river way (p<5%). Ex-P content of the estuary in February, May, August and November was 7.33±5.05, 1.38±0.83, 3.42±1.31 and 3.33±2.45μg/g, respectively. Except August and November, there was significant difference of Ex-P content between any two other months (p<5%).

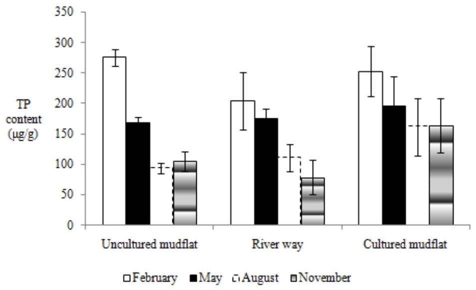

Fig.2—TP content in sediments in Daguhe Estuary

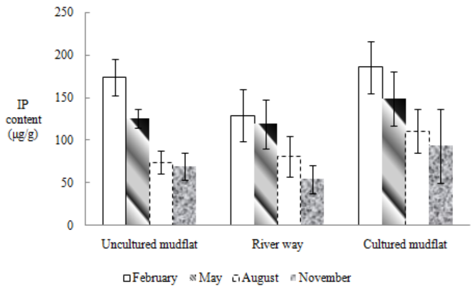

Fig.3—IP content in sediments in Daguhe Estuary

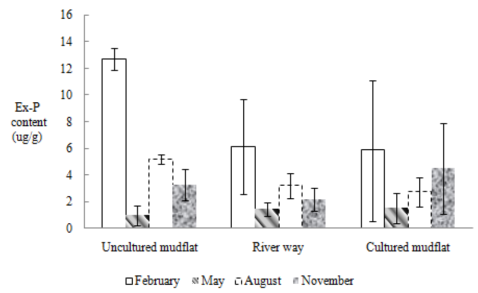

Fig.4—Ex-P content in sediments in Daguhe Estuary

The first principal component of these parameters was expressed as $-0.42\,p -0.47\,\rho_{wet} +0.46\,\varphi +0.41TP-0.40IP-0.26Ex\text{-}P$ and the second one was $0.37\,p -0.18\,\rho_{wet} -0.22\,\varphi +0.42TP + 0.43 IP + 0.65Ex\text{-}P$. Two principal components represented the whole information of those parameters used for PCA, during which the first one accounted for 71.71%. Moreover, the first principal component represented physical characteristics of sediments such as density of wet sediment, particulate size and water content of sediment and the second one reflected chemical characteristics of sediments. According to the scores of principal components, values of original data expressed in the coordinate determined by principal components, the study areas were classified (Fig.5). If the first principal component was used as classification standard, the uncultured mudflat and the river way belonged to the same kind of regional area. If the second principal component was used as the evaluating index, the cultured mudflat and the uncultured mudflat belonged to the same kind of area.

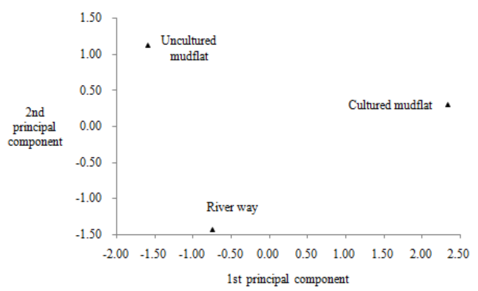

Fig.5—Regional classification for study areas

Based on water content (Fig.6), sedimentation rate of Daguhe Estuary[23,24], dry bulk density of sediment (Fig.7) and phosphorus content, accumulation flux of phosphorus was calculated (Fig.8). There was similar accumulation flux of TP in the river way and the cultured mudflat, which were significantly less than that in the uncultured mudflat ($p<5\%$). Moreover, the accumulation flux of Ex-P in the cultured mudflat was significantly less than that in the uncultured mudflat ($p<5\%$).

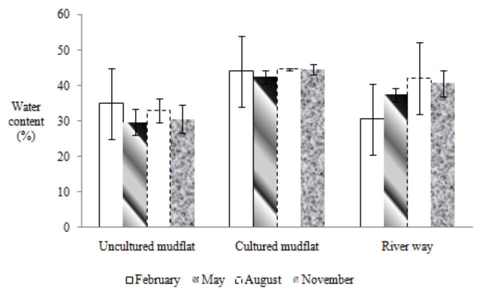

Fig.6—Water content of sediments in Daguhe Estuary

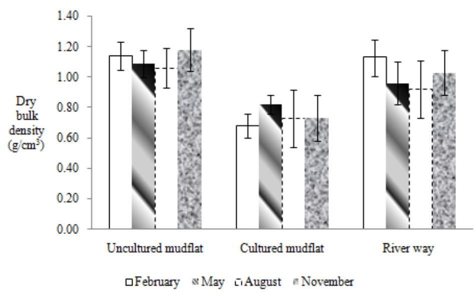

Fig.7—Dry bulk density of sediments in Daguhe Estuary

Discussion

There was significant correlation between TP and IP content ($R=0.84$, $p<5\%$) or Ex-P content ($R=0.40$, $p<5\%$), which meant that the distribution characteristics of TP content were similar to those of IP or Ex-P content. If particulate size of sediment is smaller than 67μm, there is positive significant correlation between particulate size and TP content[25]. Moreover, phosphorus excretion rate increased with increased water temperature[26] and the bio-deposition rate of Manila clam in Jiaozhou Bay was 49.5-1527.4 mg/(individual.d)[27].

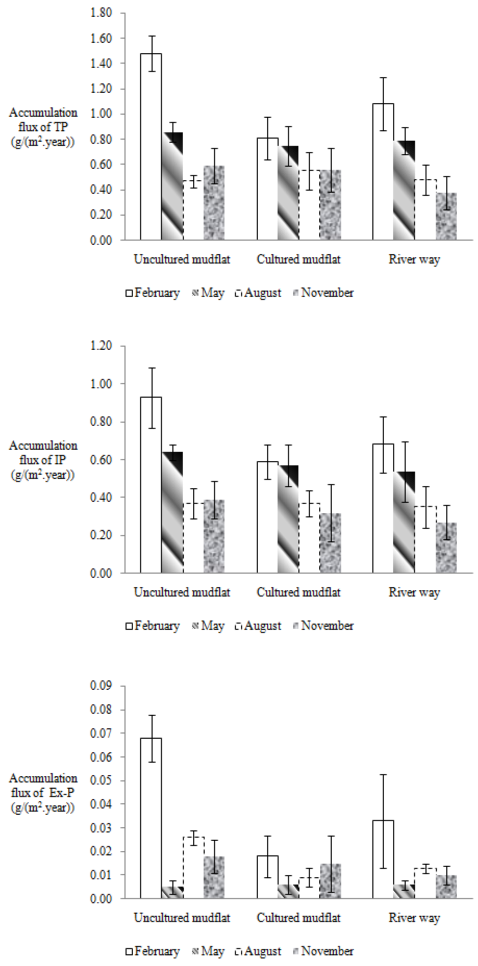

Fig.8—Accumulation flux of phosphorus in sediments

Organic matters content in the cultured mudflat in Daguhe Estuary was high[21] and there was positive significant correlation between TP content and total organic carbon content[28]. Furthermore, phosphorus content was affected by salinity[29] and the salinity in water over mudflats was higher than that in the river way in the estuary. All these led to the spatial distribution characteristics of phosphorus content in Daguhe Estuary.

The temperature in May, August and November was higher than that in February and there were fewer nutrients including phosphate releasing from the sediment-water interface to water body in February in the Jiaozhou Bay[30]. Adsorption or desorption of phosphorus was influenced by salinity and the salinity order was autumn > winter > spring > summer in the estuary. Enhancements of algae and bacteria were promoted by increased temperature, which implied that more nutrients were used in May, August and November. Moreover, the mineralization of organic matters and the excretion of shellfish increased as increased temperature[31]. All these led to the seasonal distribution characteristics of phosphorus content in the estuary.

Benthic shellfish culture Zhuanghe led to TP accumulation in sediments[10], however, benthic shellfish was also used to reduce nutrients loads[32]. OP was easily mineralized[33]and mudflats were exposed to air periodically and the maximum water depth was 4m, which meant that oxygen concentration in the estuary was high enough. As oxygen with high concentration promoted mineralization of OP[34], the primary form of phosphorous in the estuary was IP. Just like other shellfish culture zones, the benthic shellfish in the estuary led to high IP content in sediments[35]. There were complex interactions among execration of phosphate, biomass of shellfish, biomass of algae and phosphate concentration[36]. Biomass of benthic shellfish in August and November was higher than that in other months in the estuary[37]. Thus, there might be abundant benthic algae in the estuary which meant that there might be no food limitation to benthic shellfish culture with the present scale.

In the uncultured mudflat and the river way, there was arenaceous sediment with larger particulate size. There was slimy and sandy sediment with smaller particulate size in the cultured mudflat. The bio-disturb of benthic shellfish led to higher water content. There was high organic matter content in the cultured mudflat. Moreover, the benthic shellfish culture reduced phosphorous accumulation by increasing water content and organic matter content. Thus, it was inappropriate to assess impact of benthic shellfish culture on sediment by TP content only. Phosphorous accumulation included impacts of benthic shellfish on phosphorous content, water content and dry bulk density of sediment. Thus, it was adequate to assess impacts of bottom-sowing culture on sediments by phosphorus accumulation.

Conclusion

Whereas the bottom-sowing culture for Manila clam in the estuary trended to increase TP and IP content in sediments, it trended to reduce Ex-P content. The benthic shellfish culture reduced phosphorous accumulation by increasing water content and organic matter content. The culture in the estuary had no significant effects on IP accumulation and it could reduce TP or Ex-P accumulation. It was inappropriate to assess impact of benthic shellfish culture on sediment by TP content only and it was adequate to assess the impacts culture on sediments by phosphorus accumulation.

Acknowledgement

The research was supported by Specialized Research Program for Marine Public Welfare Industry from the State Oceanic Administration, P.R. China (No. 200805066), National "Twelfth Five-Year" Plan for Science & Technology Support (No.2011BAD13B02), and Independent Innovation Foundation of Shandong University.

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