

ศักยภาพของไส้เดือนทะเล *Prionospio membranacea*
ในการบำบัดทางชีวภาพของดินตะกอนอินทรีย์สารสูงใต้แพเลี้ยงหอยแมลงภู๋
บริเวณอ่าวศรีราชา จังหวัดชลบุรี

Potential of the Spionid Polychaete *Prionospio membranacea* for Bioremediation of
Organically Enriched Sediments Beneath Green Mussel Rafts in Sriracha Bay,
Chon Buri Province

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บทคัดย่อ

การสะสมอินทรีย์สารในดินตะกอนใต้แพเลี้ยงหอยแมลงภู๋เป็นปัญหาสำคัญของระบบเพาะเลี้ยงชายฝั่งเนื่องจากอาจทำให้เกิดสภาพดินตะกอนเสื่อมโทรม การสะสมของซัลไฟด์ และการเปลี่ยนแปลงของประชากรสัตว์ทะเลหน้าดิน การบำบัดทางชีวภาพโดยใช้สิ่งมีชีวิตท้องถิ่นจึงเป็นแนวทางที่มีศักยภาพในการลดผลกระทบต่อสิ่งแวดล้อม การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาศักยภาพของไส้เดือนทะเล *Prionospio membranacea* ในการบำบัดทางชีวภาพของดินตะกอนอินทรีย์สารสูงใต้แพเลี้ยงหอยแมลงภู๋บริเวณอ่าวศรีราชา จังหวัดชลบุรี โดยเก็บรวบรวมไส้เดือนทะเลตัวเต็มวัยจากดินตะกอนที่มีอินทรีย์สารสูง กระตุ้นให้สืบพันธุ์ในห้องปฏิบัติการ และเพาะเลี้ยงในปริมาณมากจนได้ระยะ early benthic stage จากนั้นปล่อยลงในดินตะกอนใต้แพเลี้ยงหอยแมลงภู๋ที่ความหนาแน่น 400 ตัวต่อตารางเมตร เปรียบเทียบกับพื้นที่ควบคุมที่ไม่มีการเติมไส้เดือนทะเล และติดตามผลเป็นเวลา 30 วัน โดยประเมินการเจริญเติบโต การเพิ่มมวลชีวภาพ ปริมาณอินทรีย์สาร ปริมาณซัลไฟด์ และความชุกชุมของสัตว์ทะเลหน้าดิน ผลการศึกษพบว่า *P. membranacea* สามารถดำรงชีวิตเจริญเติบโต เพิ่มมวลชีวภาพ และสร้างกลุ่มประชากรในดินตะกอนใต้แพเลี้ยงหอยแมลงภู๋ได้ โดยพื้นที่ที่เติมไส้เดือนทะเลมีปริมาณอินทรีย์สารลดลง 38.53–46.05% และปริมาณซัลไฟด์ลดลง 6.29–16.18% ในระยะเวลา 30 วัน พื้นที่ที่ไม่มีการคลุมตาข่ายมีแนวโน้มให้ประสิทธิภาพการลดอินทรีย์สารและซัลไฟด์สูงกว่าพื้นที่ที่คลุมตาข่าย ผลการวิจัยแสดงให้เห็นว่า *P. membranacea* มีศักยภาพเป็นสิ่งมีชีวิตท้องถิ่นสำหรับใช้บำบัดทางชีวภาพในระบบเพาะเลี้ยงหอยแมลงภู๋ชายฝั่ง อย่างไรก็ตาม ควรมีการศึกษาซ้ำในหลายฤดูกาล เพิ่มระยะเวลาการทดลอง และทดสอบความหนาแน่นของการปล่อยไส้เดือนทะเลในหลายระดับ เพื่อพัฒนาแนวทางการประยุกต์ใช้ในระดับพื้นที่จริงอย่างมีประสิทธิภาพ

คำสำคัญ : *Prionospio membranacea*, การบำบัดทางชีวภาพ, ดินตะกอนอินทรีย์สารสูง, แพเลี้ยงหอยแมลงภู๋, อ่าวศรีราชา

Abstract

Organic enrichment in sediments beneath green mussel rafts is an important environmental problem in coastal aquaculture areas because it can lead to sediment deterioration, sulfide accumulation, and changes in benthic faunal communities. Bioremediation using native benthic organisms is therefore a promising approach for reducing environmental impacts in coastal culture systems. This study aimed to evaluate the potential of the polychaete *Prionospio membranacea* for bioremediation of organically enriched sediments beneath green mussel rafts in Sriracha Bay, Chon Buri Province, Thailand. Adult polychaetes were collected from organic-rich coastal sediments, induced to spawn under laboratory conditions, and mass-cultured until the early benthic stage. The early benthic stages were then released into sediments beneath green mussel rafts at a density of 400 individuals/m² and compared with a control area without polychaete addition. Bioremediation performance was evaluated after 30 days based on growth, biomass increase, organic matter content, sulfide concentration, and benthic faunal abundance. The results showed that *P. membranacea* exhibited apparent recovery and establishment, grew, increased biomass, and established a population in sediments beneath green mussel rafts. The addition of *P. membranacea* reduced organic matter content by 38.53–46.05% and sulfide concentration by 6.29–16.18% within 30 days. Non-netted plots tended to show higher reduction efficiency than netted plots. These findings indicate that *P. membranacea* has potential as a native bioremediation organism for improving organically enriched sediments in coastal mussel culture areas. Further studies under different seasons, longer experimental periods, and varying stocking densities are recommended to optimize its practical application in coastal aquaculture management.

Keywords: *Prionospio membranacea*, bioremediation, organically enriched sediment, green mussel raft, Sriracha Bay

1. Introduction

Coastal aquaculture plays a vital role in supporting local economies and ensuring food security in coastal communities. In Thailand, green mussel (*Perna viridis*) culture is widely practiced in several coastal areas. However, intensive coastal aquaculture may affect marine environmental quality through the accumulation of organic matter derived from mussel biodeposits, settled plankton, and other organic materials beneath culture areas. Excessive organic enrichment in sediments can lead to sediment deterioration, oxygen depletion, sulfide accumulation, and changes in benthic faunal communities, which are important indicators of coastal ecosystem degradation (Pearson & Rosenberg, 1978; Diaz & Rosenberg, 1995; Rosenberg, 2001).

Sriracha Bay, Chon Buri Province, is a coastal area that supports multiple human activities, including coastal fisheries, urban development, industrial activities, and green mussel raft culture. Organic matter accumulation beneath green mussel rafts has been observed in

this area, where sediments are often characterized by fine muddy texture, dark color, unpleasant odor, and high organic content. Under such conditions, some benthic fauna may decline, whereas tolerant taxa, particularly certain polychaetes, often become dominant in organically enriched sediments.

Bioremediation has received increasing attention as an environmentally friendly approach for reducing the impacts of organic enrichment in marine ecosystems and aquaculture systems. This approach relies on the biological activities of organisms that can utilize organic matter, enhance decomposition processes, and modify sediment conditions through movement, burrowing, tube construction, and sediment reworking. These activities can improve oxygen exchange and promote biogeochemical processes in organically enriched sediments (Alexander, 1994; Rosenberg, 2001; Gifford et al., 2007).

Polychaetes, particularly deposit-feeding species, are considered promising organisms for sediment bioremediation. Polychaetes in the families Capitellidae, Nereididae, and Spionidae are commonly found in organically enriched areas and play important roles in organic matter decomposition and sediment bioturbation. Previous studies have demonstrated that *Capitella* spp. can effectively reduce organic matter accumulation in sediments affected by fish farming and coastal aquaculture activities (Tsutsumi et al., 1990; Tsutsumi et al., 2002; Tsutsumi et al., 2005; Kinoshita et al., 2008). Nevertheless, the selection of organisms for bioremediation should prioritize native species that are locally available, well adapted to organic-rich conditions, and unlikely to create ecological risks associated with the introduction of non-native species.

The polychaete *Prionospio membranacea* Imajima, 1990, formerly referred to as *Prionospio* (*Prionospio*) *membranacea* Imajima, 1990, belongs to the family Spionidae and is commonly found in organically enriched coastal sediments in Sriracha Bay, particularly beneath green mussel rafts and in areas influenced by domestic wastewater. Previous work also indicated that *Prionospio* spp., particularly *P. membranacea*, were abundant in high-organic-content sediments beneath green mussel rafts, supporting their potential use as locally available organisms for sediment bioremediation (To-orn, 2016). This species possesses several biological characteristics suitable for bioremediation, including local occurrence, a relatively short life cycle, the ability to reproduce and increase in abundance under organic-rich sediment conditions, and feeding behavior associated with surface sediment organic particles and suspended particles near the sediment-water interface (Fauchald & Jumars, 1979; Dauer et al., 1981; To-orn, 2016).

A recent review on polychaete aquaculture has emphasized the increasing global interest in culturing polychaetes for industrial aquaculture, alternative feed resources, biotechnology products, and applications in integrated aquaculture and bioremediation systems (Herrera-Perez & Ferrando, 2026). In this context, the development of mass-culture techniques for locally available polychaete species is important for supporting practical applications in environmental management. Previous laboratory-based studies have shown that spionid polychaetes of the genus *Prionospio*, particularly *P. membranacea*, have potential

for the bioremediation of organically enriched sediments beneath green mussel rafts by converting sedimentary organic matter into biomass and reducing organic matter content in sediment (To-orn, 2016; To-orn & Paphavasit, 2017). However, To-orn & Paphavasit (2017) focused mainly on a laboratory-based experiment under controlled conditions, whereas the present study extends this previous work to a field application using mass-cultured early benthic stages of *P. membranacea* under actual green mussel raft conditions. Therefore, the novelty of the present study lies in its field-based evaluation of mass-cultured *P. membranacea*, considering not only polychaete biomass and sediment organic matter reduction, but also sulfide concentration and benthic faunal composition after field application. This study aimed to evaluate the potential of *P. membranacea* for bioremediation of organically enriched sediments beneath green mussel rafts in Sriracha Bay, Chon Buri Province, Thailand.

2. Materials and methods

2.1 Study area and experimental organism

This study was conducted from November to December 2016 in Sriracha Bay, Sriracha District, Chon Buri Province, Thailand, a coastal area where green mussel (*Perna viridis*) raft culture has been continuously practiced. The experimental site was located beneath green mussel rafts approximately 800 m from the shoreline. Prior to the field experiment, baseline conditions of the study area were recorded, including sediment appearance, sediment texture, total organic matter, sulfide concentration, and benthic faunal composition. Basic water quality conditions during the study period were also recorded, with water temperature ranging from 27.11 to 27.70°C, salinity from 30.95 to 31.26, water pH from 8.18 to 8.55, dissolved oxygen from 3.95 to 5.56 mg/L, and water depth from 4.80 to 5.51 m. The sediments beneath the rafts were characterized by fine muddy texture, yellowish-green to dark coloration, and an odor associated with the accumulation of organic matter from mussel culture activities.

The experimental organism used for bioremediation was the spionid polychaete *P. membranacea* Imajima, 1990, which belongs to the phylum Annelida, class Polychaeta, and family Spionidae. This species is commonly found in organically enriched coastal sediments in Sriracha Bay. Species identification was confirmed based on diagnostic morphological characteristics following Imajima (1990) and To-orn (2016), particularly the branchial arrangement and chaetal characteristics of *P. membranacea*. Adult polychaetes were collected from organic-rich coastal sediments and selected as broodstock for laboratory mass culture prior to field application.

2.2 Mass culture of polychaetes

Mature individuals of *P. membranacea* were sexed, and reproductively mature males and females were selected for induced spawning under laboratory conditions. The mass-culture procedure followed the approach previously developed for *Prionospio* polychaetes by To-orn (2016). After fertilization, the larvae were collected and reared continuously for

approximately 30 days until they reached the early benthic stage, which was considered suitable for release into sediments beneath green mussel rafts for bioremediation purposes. In this study, the “early benthic stage” refers to post-larval *P. membranacea* that had completed the planktonic larval phase, settled onto the substrate, and begun a benthic mode of life. At this stage, the individuals were capable of crawling or remaining on the sediment surface and were considered suitable for field release.

The apparent recovery and establishment of *P. membranacea* in the experimental plots were evaluated 30 days after release based on the abundance of *P. membranacea* recorded in the treatment plots in relation to the initial release density of 400 individuals/m², the pre-experimental baseline, and the control raft. Because *P. membranacea* naturally occurs in Sriracha Bay and the released individuals were not individually marked, the abundance recorded after the experiment was not interpreted as a true survival rate of released individuals alone.

2.3 Field experimental design

The field experiment was conducted beneath three green mussel rafts. Each raft covered an approximate sediment area of 1,600 m². Raft A was designated as the control area without polychaete addition, whereas rafts B and C were used as experimental areas where *P. membranacea* was added to the sediments beneath the mussel rafts.

Rafts B and C were selected as separate experimental rafts located within the same green mussel culture area and were subjected to the same field treatment. These rafts were used to represent field treatment areas under comparable raft-culture conditions rather than fully independent randomized replicates.

Each treatment raft was divided into two field plots of approximately 800 m² each. In raft B, plot B1 was assigned as the non-netted plot, whereas plot B2 was assigned as the netted plot covered with a 1.0-mm mesh net over the sediment surface. Similarly, in raft C, plot C1 was assigned as the non-netted plot, whereas plot C2 was assigned as the netted plot (Figure 1). The distance among rafts was approximately 20 m. No formal buffer strips were established within each raft; however, the non-netted and netted plots were arranged as separate halves of the sediment area beneath each raft to reduce direct interference between treatments. The field layout was constrained by the raft structure, diver accessibility, and sediment conditions beneath the green mussel rafts.

The netted plots were established to reduce predator access and to evaluate whether physical protection could enhance the establishment and apparent recovery of the released *P. membranacea* under field conditions. Potential predators or organisms that may disturb the released polychaetes in the study area included predatory polychaetes, such as *Nereis* sp. and *Diopatra* sp., nemerteans, amphipods, and other mobile benthic or demersal organisms that may consume or disturb small benthic polychaetes. A netted control plot without polychaete addition was not included because the main objective of the study was to evaluate the field application of mass-cultured *P. membranacea* with and without net

protection. However, it is acknowledged that netting itself may influence water circulation, oxygen exchange, sediment deposition, predator access, and sediment conditions. Therefore, the effects observed in the netted plots should be interpreted as the combined effects of *P. membranacea* addition and net protection, rather than the effect of polychaete addition alone.

Early benthic stages of *P. membranacea* were released into the sediments by scuba diving at an initial density of 400 individuals/m². The initial release density of 400 individuals/m² was selected based on previous laboratory findings and field observations of spionid polychaetes in Sriracha Bay. Previous studies reported that *P. membranacea* naturally occurred in organically enriched sediments in Sriracha Bay, with densities ranging from 13 to 655 individuals/m², and that a density equivalent to 400 individuals/m² showed high potential for organic matter reduction and biomass production under laboratory conditions (To-orn & Paphavasit, 2017). Therefore, this stocking density was selected for the present field application trial.

Because each treatment plot covered approximately 800 m², the estimated number of polychaetes released into each plot was approximately 320,000 individuals. Therefore, approximately 320,000 individuals were released into each of B1, B2, C1, and C2, corresponding to approximately 640,000 individuals per treatment raft and approximately 1,280,000 individuals in total across all treatment plots. The experiment was monitored for one month after release.

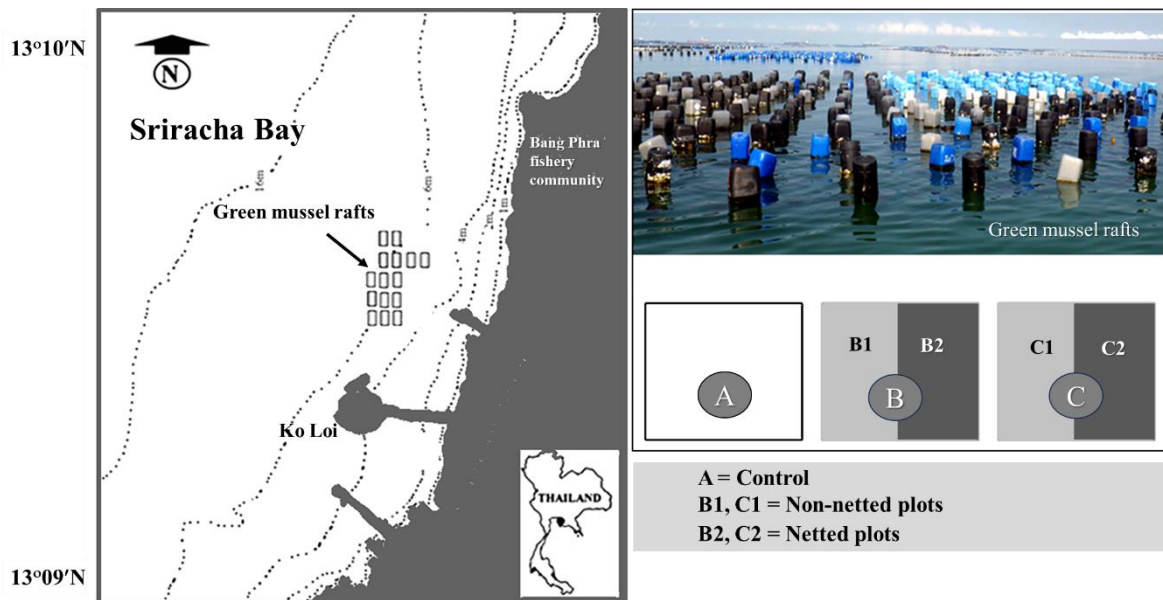


Figure 1 Study area and schematic diagram of the field experimental design beneath green mussel rafts in Sriracha Bay, Chon Buri Province. A = control raft without polychaete addition; B and C = treatment rafts receiving *Prionospio membranacea* addition; B1 and C1 = non-netted plots; B2 and C2 = netted plots covered with a 1.0-mm mesh net over the sediment surface. Adapted from To-orn and Intarachart (2016).

2.4 Sediment and benthic fauna sampling

Sediment and benthic fauna samples were collected from the control and experimental areas before the experiment to establish baseline conditions. Sampling was repeated after one month to compare changes in sediment quality and benthic faunal communities. Sediment and benthic fauna samples were collected using an Ekman grab with an opening size of 15 × 15 cm, corresponding to a sampling area of 225 cm² or 0.0225 m². Initial observations indicated that the sediments beneath the control and experimental rafts were generally comparable in terms of muddy texture, organic enrichment, sulfide accumulation, and dominance of deposit-feeding polychaetes. However, because the experiment was conducted under actual green mussel raft conditions and included limited raft-level replication, baseline comparability among rafts was interpreted descriptively rather than as evidence from a fully randomized experimental design.

For benthic fauna analysis, three grab samples were collected from each sampling area. The collected sediments were sieved through a 0.5-mm mesh sieve to retain macrobenthic organisms. The retained organisms were preserved in 10% neutral formalin and transported to the laboratory for further analysis. In the laboratory, benthic fauna samples were sorted, washed, counted, and identified to appropriate taxonomic groups under a stereomicroscope. Benthic faunal abundance was calculated as individuals per square meter by dividing the number of individuals collected in each grab sample by the sampling area of the Ekman grab (0.0225 m²), and the results were expressed as individuals/m².

For sediment quality analyses, three additional grab samples were collected from each sampling area. Surface sediment from the upper approximately 3 cm of each grab sample was subsampled and used for the analysis of total organic matter and acid volatile sulfide. Sediment subsamples were placed in clean containers and transported to the laboratory for subsequent analysis. Acid volatile sulfide (AVS) was analyzed using an AVS Test Column following the manufacturer's procedure for sediment sulfide analysis. Wet sediment subsamples were placed into the test column, and sulfide concentration was determined according to the reading procedure provided with the test kit. AVS concentrations were calculated on a dry-weight basis and expressed as mg/g dry weight. The composition and abundance of benthic fauna, as well as sediment quality variables, were then evaluated before and after the experiment.

2.5 Analysis of organic matter and sulfide in sediment

Total organic matter (TOM) in sediment was analyzed using the ignition loss method following Nelson and Sommers (1982). Prepared sediment samples were dried and combusted at high temperature, and the loss in sample weight after combustion was calculated as the percentage of total organic matter in the sediment. Sediment sulfide was assessed as acid volatile sulfide (AVS), which was used as an indicator of sediment conditions affected by organic matter accumulation and reducing conditions. Changes in TOM and AVS before and after the experiment were used to evaluate the bioremediation efficiency of *P. membranacea*.

2.6 Growth and biomass assessment

After 30 days, *P. membranacea* individuals were collected from the experimental plots to evaluate apparent recovery, establishment, growth, and biomass increase. Body length and body width were measured, the number of chaetigers was counted, and body weight or biomass was assessed for polychaetes collected from each experimental plot. These parameters were used as indicators of population establishment and utilization of organic matter in sediments beneath green mussel rafts.

2.7 Evaluation of bioremediation efficiency

The bioremediation efficiency of *P. membranacea* was evaluated using four main indicators: (1) increases in body length, body width, number of chaetigers, and biomass of the polychaetes; (2) reduction in total organic matter (TOM) in the sediment; (3) reduction in sediment sulfide concentration; and (4) changes in the composition and abundance of benthic fauna. The use of multiple indicators allowed the assessment of bioremediation performance in terms of both sediment quality improvement and benthic faunal response.

2.8 Statistical analysis

Quantitative data, including body length, body width, number of chaetigers, polychaete biomass, total organic matter, sulfide concentration, and benthic faunal abundance, were presented as mean $\pm$ standard deviation (mean $\pm$ SD). Statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). For statistical analyses, sampling plots within each raft were treated as field sampling units for comparing sediment quality and benthic faunal responses among the control and treatment areas. However, because the field design included one control raft and two treatment rafts without randomized raft-level replication, these sampling plots should not be interpreted as fully independent experimental units at the raft level. Therefore, the results should be interpreted as a field application trial rather than a fully replicated randomized experiment.

Data from B1 and C1, as well as B2 and C2, were not pooled as single replicated treatment groups for statistical inference. Instead, these plots were presented separately to show the response patterns of the non-netted plots (B1 and C1) and the netted plots (B2 and C2) under comparable treatment conditions. Because the field experiment was conducted under actual green mussel raft conditions and did not include fully randomized raft-level replication, pooling data from different rafts could overestimate the degree of replication. Therefore, comparisons were interpreted cautiously as trends observed in a field application trial rather than as results from fully independent pooled experimental replicates.

Differences in variables were analyzed separately for relevant comparisons, including comparisons between the control and experimental areas and comparisons between pre- and post-experimental conditions within the limitations of the field design. When data satisfied the assumptions of normality and homogeneity of variance, one-way analysis of variance (ANOVA)

was applied, followed by Tukey's Honestly Significant Difference (HSD) test for post-hoc multiple comparisons. Statistical significance was set at $p < 0.05$.

Two-way ANOVA or repeated measures analysis was not applied because the experimental design was not a fully balanced factorial design with randomized raft-level replication, and the before–after observations were not structured as repeated measurements of fully independent experimental units at the raft level. Therefore, the statistical results were used as supporting evidence for evaluating the field application of *P. membranacea* rather than as inference from a fully replicated factorial or repeated-measures experiment.

3. Results and discussion

3.1 Growth and biomass increase of *Prionospio membranacea* in sediments beneath green mussel wafts

The results showed that, after the release of early benthic stages of *P. membranacea* into sediments beneath green mussel rafts for 30 days, the polychaetes were able to establish, grow, and increase their biomass in both non-netted and netted sediment plots. Body length, body width, number of chaetigers, and body weight significantly increased after the experiment compared with the initial values ($p < 0.05$) (Figure 2). The growth responses were presented according to plot condition, with B1 and C1 representing non-netted plots and B2 and C2 representing netted plots. The average increase in body length ranged from 11.30 to 11.32 mm, while body width increased by 0.365–0.370 mm. The number of chaetigers increased by 37–38 chaetigers, and body weight increased by 0.0020–0.0022 g within 30 days.

When comparing non-netted and netted plots, the growth parameters showed similar increasing trends. This indicates that sediment conditions beneath green mussel rafts in both treatments were suitable for the growth of *P. membranacea* individuals recorded after release. These results suggest that this polychaete species can utilize organic matter accumulated in the sediment as a food source and convert it into its own biomass, which is an important characteristic of benthic deposit feeders.

The increase in the number of chaetigers, together with increases in body length, body width, and body weight, indicates that *P. membranacea* established after release and continued to grow, maintain its presence, and increase biomass in the experimental areas within 30 days. This characteristic is important for its potential use as a bioremediation organism, as biomass increase in polychaetes is associated with the removal of organic matter from sediments through feeding, growth, and sediment-reworking activities. These processes are further linked to the reduction of organic matter and sulfide concentrations in the following sections.

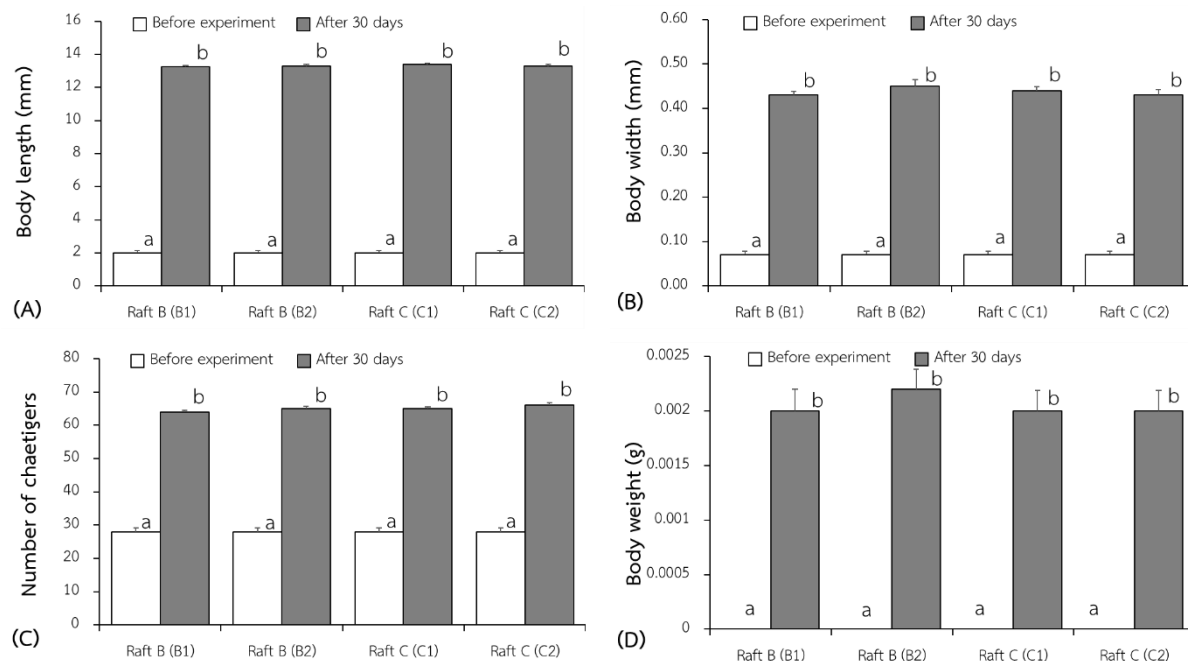


Figure 2 Growth and biomass increase of *Prionospio membranacea* after 30 days in sediments beneath green mussel rafts: (A) body length, (B) body width, (C) number of chaetigers, and (D) body weight in plots B1, B2, C1, and C2 before the experiment and after 30 days. B1 and C1 represent non-netted plots, whereas B2 and C2 represent netted plots. Values are presented as mean $\pm$ SD. Different letters above bars indicate significant differences between before experiment and after 30 days within each plot ($p < 0.05$).

At the end of the 30-day field experiment, the abundance of *P. membranacea* in the treatment plots indicated apparent recovery and establishment after release. Because *P. membranacea* occurs naturally in Sriracha Bay and the released individuals were not individually marked, the observed abundance after the experiment should not be interpreted as a true survival rate of released individuals alone. Instead, the establishment of *P. membranacea* was interpreted in relation to the initial stocking density, the pre-experimental baseline, and the control raft. Although the apparent recovery under field conditions was not high, the individuals recorded in the treatment plots were able to grow and increase biomass during the experimental period.

3.2 Reduction of organic matter content in sediments beneath green mussel rafts

The mean organic matter content in sediments beneath green mussel rafts before the experiment was $5.25 \pm 0.04\%$. After 30 days, the control raft without the addition of *P. membranacea* (raft A) had a mean organic matter content of $4.63 \pm 0.06\%$, which was not significantly different from the initial value ($p > 0.05$). In contrast, the experimental rafts B and C, where *P. membranacea* was added, showed a significant reduction in sediment organic matter content after 30 days ($p < 0.05$) (Figure 3A). The organic matter contents in B1, B2, C1, and C2 were $2.45 \pm 0.23\%$, $2.80 \pm 0.24\%$, $2.32 \pm 0.21\%$, and $2.72 \pm 0.19\%$, respectively,

corresponding to reductions of 44.01%, 38.53%, 46.05%, and 39.78%, respectively. These results indicate that the addition of *P. membranacea* was associated with a reduction of organic matter in sediments beneath green mussel rafts within 30 days. The non-netted plots tended to show greater reductions in organic matter content than the netted plots.

The reduction in organic matter content may be attributed to the feeding activity, movement, and sediment-reworking behavior of the polychaetes. These activities can increase contact between organic particles, oxygen, and sediment-associated microorganisms, thereby enhancing organic matter decomposition in the sediment (Fauchald & Jumars, 1979; Alexander, 1994; Rosenberg, 2001). The present findings are consistent with previous studies by Tsutsumi et al. (2002), Tsutsumi et al. (2005), and Kinoshita et al. (2008), which reported that deposit-feeding polychaetes can reduce organic matter accumulation in aquaculture sediments.

3.3 Reduction of sulfide concentration in sediments beneath green mussel rafts

The mean sulfide concentration in sediments beneath green mussel rafts before the experiment was 0.37 ± 0.01 mg/g dry weight. After 30 days, the control raft without the addition of *P. membranacea* (raft A) had a mean sulfide concentration of 0.34 ± 0.06 mg/g dry weight, which was not significantly different from the initial value ($p > 0.05$). In contrast, the experimental rafts B and C, where *P. membranacea* was added, showed a significant reduction in sediment sulfide concentration after 30 days ($p < 0.05$) (Figure 3B). The sulfide concentrations in B1, B2, C1, and C2 were 0.28 ± 0.01 , 0.31 ± 0.01 , 0.29 ± 0.01 , and 0.32 ± 0.01 mg/g dry weight, respectively, corresponding to reductions of 16.18%, 8.09%, 14.38%, and 6.29%, respectively. These results indicate that the addition of *P. membranacea* was associated with a reduction in sulfide concentration in sediments beneath green mussel rafts within 30 days. The non-netted plots tended to show greater reductions in sulfide

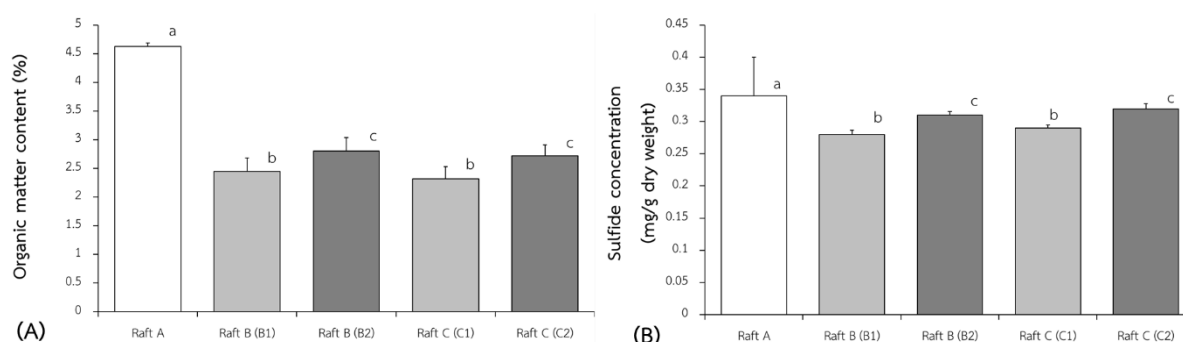


Figure 3 Changes in sediment quality beneath green mussel rafts after bioremediation using mass-cultured *P. membranacea*: (A) organic matter content in sediment (%) and (B) sulfide concentration in sediment (mg/g dry weight). A = control; B1 and C1 = non-netted plots; B2 and C2 = netted plots. Values are presented as mean $\pm$ SD. Different letters above bars indicate significant differences among sampling areas after 30 days ($p < 0.05$).

concentration than the netted plots, which was consistent with the trend observed for sediment organic matter reduction.

The initial sediment organic matter content in this study was $5.25 \pm 0.04\%$, suggesting substantial accumulation of organic material in the muddy sediments beneath green mussel rafts. Because sediment organic matter thresholds vary among coastal environments, sediment types, and analytical methods, the organic matter value was interpreted together with sediment appearance, odor, sulfide concentration, and benthic faunal composition. The initial sulfide concentration was 0.37 ± 0.01 mg/g dry weight, indicating sulfide accumulation in the organically enriched muddy sediments. When interpreted together with sediment characteristics, organic matter content, and benthic faunal composition, the sediments beneath the green mussel rafts in the present study can be interpreted as organically enriched and moderately sulfide-accumulated sediments rather than severely degraded sediments. The reductions in organic matter and sulfide concentrations after the addition of *P. membranacea* suggest that this species was associated with sediment quality improvement under organically enriched conditions.

Excessive organic matter accumulation in sediments can increase microbial oxygen consumption and promote hypoxic or anoxic conditions. Under such conditions, anaerobic sulfate reduction may lead to the production and accumulation of hydrogen sulfide, which is toxic to many benthic organisms. These changes can alter benthic community structure by reducing sensitive taxa and favoring tolerant or opportunistic deposit feeders. Therefore, the reductions in organic matter and sulfide concentrations observed after the addition of *P. membranacea* may represent an improvement in sediment conditions and benthic habitat quality beneath green mussel rafts.

The reduction in sulfide concentration after the addition of *P. membranacea* may be associated with the burrowing, movement, and bioirrigation activities of the polychaetes. These activities can enhance oxygen diffusion from the sediment surface into deeper sediment layers. Increased oxygen penetration can reduce strongly reducing conditions in the sediment, thereby decreasing sulfide accumulation. In organically enriched sediments, sulfide is mainly produced through microbial sulfate reduction under anaerobic conditions, where organic matter serves as an important substrate for microbial decomposition. The burrowing and bioirrigation activities of *P. membranacea* may enhance the transport of oxygenated water into the sediment, thereby promoting the re-oxidation of reduced sulfur compounds such as sulfide to more oxidized sulfur forms. At the same time, the reduction of organic matter may limit the substrate available for anaerobic sulfate reduction. These processes suggest that *P. membranacea* may contribute to sulfur cycling in the sediment by reducing sulfide production and enhancing sulfide oxidation through bioturbation and bioirrigation. These mechanisms are consistent with the roles of deposit-feeding and burrowing polychaetes in improving sediment conditions through bioturbation, bioirrigation, and changes in sediment redox conditions (Pearson & Rosenberg, 1978; Hutchings, 1998; Rosenberg, 2001).

The present findings are consistent with previous laboratory-based studies on spionid polychaetes, particularly *P. membranacea* and *P. pulchra*, which demonstrated their ability to convert sedimentary organic matter into biomass and reduce organic matter content in organically enriched sediments beneath green mussel rafts (To-orn & Paphavasit, 2017). These findings support the role of spionid polychaetes as surface or interface deposit feeders that can utilize organic particles in sediments and contribute to sediment reworking. In addition, spionid feeding and burrowing activities may influence sediment conditions through particle reworking, tube construction, and bioirrigation, although direct evidence for sulfide reduction by spionid polychaetes remains limited. Therefore, the reduction in sulfide concentration observed in the present field trial may be indirectly related to the combined effects of organic matter reduction, sediment reworking, and enhanced oxygen penetration associated with the activity of *P. membranacea*. Previous studies on other deposit-feeding polychaetes, particularly *Capitella* spp., have also shown that polychaete biological activities can reduce the impacts of organic matter accumulation and improve sediment chemical characteristics in aquaculture systems (Chareonpanich et al., 1993; Tsutsumi et al., 2002; Kinoshita et al., 2008). However, the percentage reduction in sulfide concentration in this study was lower than the reduction in organic matter content, suggesting that sulfide may respond more slowly to changes in sediment redox conditions, oxygen availability, and anaerobic decomposition processes. Therefore, longer monitoring periods and further studies focusing specifically on sulfur dynamics are required to clearly evaluate sediment improvement through *P. membranacea*-mediated bioremediation.

3.4 Abundance of benthic fauna beneath green mussel rafts

The abundance of benthic fauna beneath green mussel rafts was examined before and after the application of mass-cultured *P. membranacea* for bioremediation. The benthic faunal community in the study area reflected organically enriched sediment conditions, with deposit-feeding polychaetes being the dominant group. The major taxa included *P. membranacea*, *Scoloplos* sp., *Aricidea* sp., *Cirratulus* sp., and *Notomastus* sp. Predatory polychaetes, such as *Nereis* sp. and *Diopatra* sp., as well as other benthic fauna, including Nemertea, Amphipoda, and bivalves, were present in relatively low abundance.

Before the experiment, the total abundance of benthic fauna beneath the green mussel rafts was 1,439 individuals/m², and the community was dominated by several polychaete taxa, particularly *P. membranacea*, *Scoloplos* sp., and *Aricidea* sp. In terms of benthic faunal diversity, the community was composed mainly of several polychaete taxa with different feeding and ecological functions, including deposit-feeding spionids, orbinids, cirratulids, and capitellids, together with predatory polychaetes and other benthic invertebrates. Although deposit-feeding polychaetes were dominant, the occurrence of multiple taxa indicates that the sediments beneath green mussel rafts still supported a benthic faunal assemblage composed of both tolerant and opportunistic groups.

Because *P. membranacea* occurs naturally in Sriracha Bay, the individuals recorded after the experiment may have included both naturally occurring individuals and released individuals that established in the treatment plots. Therefore, the abundance of *P. membranacea* was interpreted in relation to the pre-experimental baseline and the control raft, rather than as the survival of released individuals alone.

After 30 days, the control raft without *P. membranacea* addition (raft A) had a total benthic faunal abundance of 1,567 individuals/m². In contrast, the experimental plots B1, B2, C1, and C2 had total abundances of 2,983, 1,733, 2,883, and 1,717 individuals/m², respectively (Figure 4). When *P. membranacea* was considered separately, its abundance clearly increased in the plots where the polychaetes were added. The abundances of *P. membranacea* in B1, B2, C1, and C2 were 1,583, 1,417, 1,750, and 1,333 individuals/m², respectively, whereas only 267 individuals/m² were recorded in the control raft. The increase in total benthic abundance was mainly associated with the establishment and growth of *P. membranacea* in the treatment plots, while other benthic taxa remained present at lower abundance. This pattern suggests that the bioremediation treatment was associated with increased abundance of the target deposit-feeding polychaete without completely replacing the existing benthic faunal assemblage.

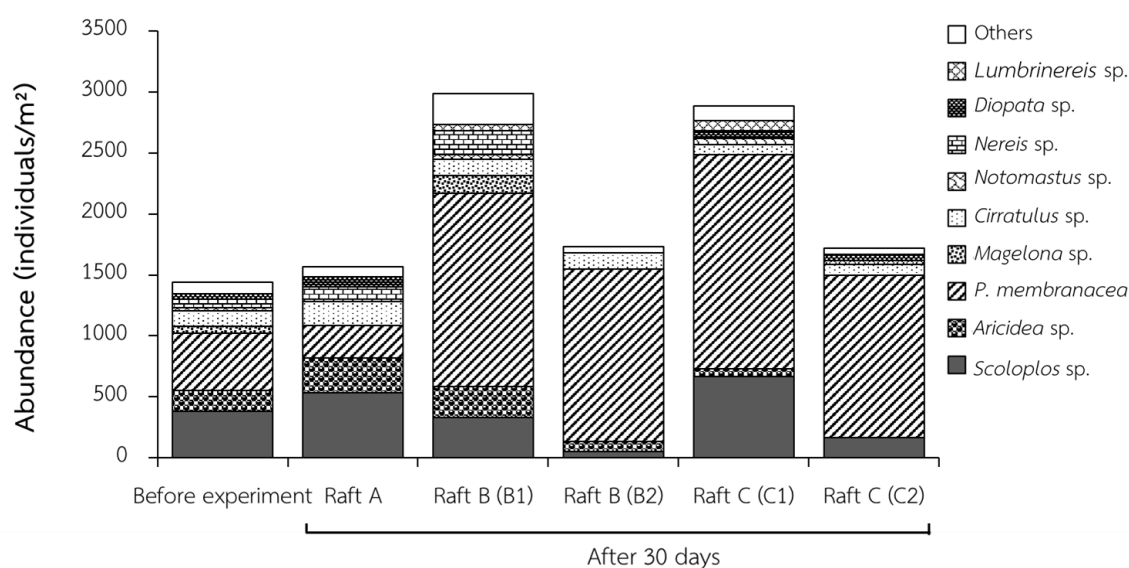


Figure 4 Abundance and composition of benthic fauna beneath green mussel rafts before the experiment and after 30 days of bioremediation using mass-cultured *Prionospio membranacea*. A = control raft; B1 and C1 = non-netted plots; B2 and C2 = netted plots. Abundance is expressed as individuals/m².

These results indicate that mass-cultured *P. membranacea* showed apparent establishment, increased abundance, and growth in sediments beneath green mussel rafts within 30 days. In addition, the non-netted plots, B1 and C1, tended to show higher total benthic faunal abundance and higher abundance of *P. membranacea* than the netted plots, B2 and C2. This pattern may be related to better water circulation and oxygen exchange in

the non-netted plots, which may have provided more favorable conditions for the establishment and biological activities of the polychaetes in the sediment.

The composition of benthic fauna observed in this study was consistent with the characteristics of organically enriched habitats, where opportunistic polychaetes and deposit feeders often become dominant (Pearson & Rosenberg, 1978; Dauer, 1993; Diaz & Rosenberg, 1995). In particular, *P. membranacea*, *Scoloplos* sp., and *Aricidea* sp. are taxa that can tolerate organic-rich sediment conditions. Some of these taxa have also been reported from coastal areas of Sriracha Bay affected by organic matter accumulation in sediments (To-orn & Paphavasit, 2003). The increase in *P. membranacea* in the experimental plots therefore supports the potential of this species as a native benthic organism capable of utilizing sedimentary organic matter and contributing to bioremediation beneath green mussel culture systems.

However, the changes in benthic faunal communities observed in this study should be interpreted as short-term responses within a 30-day field application trial. This period was sufficient to detect initial changes in the abundance and establishment of *P. membranacea*, as well as associated reductions in sediment organic matter and sulfide concentrations. Nevertheless, it was not long enough to evaluate long-term bioremediation performance, population persistence, seasonal variation, or successional changes in benthic community structure. Longer-term monitoring over several months and across different seasons is therefore needed to determine whether the increase in *P. membranacea*, together with reductions in organic matter and sulfide, can lead to sustained sediment quality improvement and broader recovery of benthic community structure beneath green mussel rafts.

3.5 Overall bioremediation efficiency and mechanisms of *Prionospio membranacea*

The overall results indicate that the biological activities of *P. membranacea* were associated with reductions in organic matter and sulfide concentrations in sediments beneath green mussel rafts. The bioremediation efficiency appeared to be related to the increase in abundance, apparent establishment, and biomass of this species in the experimental plots. After the addition of *P. membranacea*, the experimental rafts B and C showed reductions in sediment organic matter and sulfide concentrations relative to the control raft A, where no polychaetes were added ($p < 0.05$). These results suggest that the establishment of *P. membranacea* in the sediment may enhance bioremediation processes under field conditions.

Relative to the control raft A, the non-netted plot in raft B (B1) showed 1.89-fold and 1.21-fold reductions in organic matter and sulfide concentrations, respectively, whereas the netted plot (B2) showed 1.65-fold and 1.05-fold reductions, respectively. In raft C, the non-netted plot (C1) showed 2.00-fold and 1.17-fold reductions in organic matter and sulfide concentrations, respectively, whereas the netted plot (C2) showed 1.70-fold and 1.01-fold reductions, respectively. These results are consistent with the trends observed in the previous sections, in which non-netted plots tended to show higher reduction efficiency than netted plots. This pattern may be related to better water exchange and oxygen availability at the

sediment surface, as well as less restriction on polychaete movement and sediment-reworking activities in the non-netted plots.

The main mechanisms underlying the bioremediation potential of *P. membranacea* are likely related to its feeding activity, movement, burrowing behavior, and tube construction in the sediment. Deposit-feeding polychaetes can utilize organic particles in sediments as food and convert part of the sedimentary organic matter into their own biomass. At the same time, their movement and burrowing activities promote sediment bioturbation and bioirrigation, which enhance oxygen exchange between the sediment surface and deeper sediment layers. These processes can stimulate microbial activity associated with organic matter decomposition and reduce strongly reducing conditions that favor sulfide accumulation in the sediment (Fauchald & Jumars, 1979; Dauer et al., 1981; Rosenberg, 2001).

The present field study is consistent with previous laboratory findings, which demonstrated that *P. membranacea* was more efficient than *Prionospio pulchra* in converting organic waste from enriched sediments into biomass and had potential as a bioremediator for organically enriched sediments beneath green mussel rafts (To-orn & Paphavasit, 2017). Therefore, the present study provides additional field-based evidence for the potential application of mass-cultured *P. membranacea* under actual green mussel raft conditions in Sriracha Bay.

When bioremediation efficiency was evaluated in relation to the apparent recovery, establishment, and growth of *P. membranacea* in the experimental plots, the initial stocking density of 400 individuals/m² resulted in the occurrence and increased abundance of *P. membranacea* after 30 days. Because *P. membranacea* naturally occurs in Sriracha Bay and the released individuals were not individually marked, the abundance recorded after the experiment should not be interpreted as a true survival rate of released individuals alone. Instead, the abundance of *P. membranacea* after the experiment was interpreted in relation to the pre-experimental baseline and the control raft. Under these field conditions, sediment organic matter decreased by 38.53–46.05%, while sulfide concentration decreased by 6.29–16.18% in the experimental plots. Although the apparent recovery of *P. membranacea* under field conditions was not high, the individuals recorded in the treatment plots were able to grow, increase biomass, and perform biological activities that contributed to sediment improvement. The relatively low apparent recovery may be associated with several field-related factors, including predation, movement or dispersal of individuals away from the release plots, hydrodynamic disturbance beneath the rafts, and variation in sediment conditions. These findings suggest that *P. membranacea* has potential as a native bioremediator for organically enriched sediments beneath green mussel rafts.

Nevertheless, the practical application of *P. membranacea* at the field scale should consider several factors, including apparent recovery, establishment success, stocking density, season, water circulation, thickness of the organic-rich sediment layer, and the abundance of predators or benthic organisms that may interfere with population establishment. Future studies should evaluate different stocking densities, extend the monitoring period beyond 30

days, and compare seasonal conditions in order to optimize the use of *P. membranacea* as a sediment management tool for coastal green mussel culture systems.

It should be noted that the present study was conducted as a field-based application trial under actual green mussel raft conditions. The experimental design had limitations in terms of raft-level replication and randomization, as one raft was used as the control area and two rafts were used as treatment areas receiving *P. membranacea* addition. Therefore, some degree of pseudoreplication may be present, and the plots within each raft should be interpreted as field sampling plots within the same raft environment rather than fully independent randomized replicates. Although the observed reductions in sediment organic matter and sulfide concentration, together with the increase in *P. membranacea* abundance and biomass, provide useful field-based evidence on the potential of mass-cultured *P. membranacea* for improving organically enriched sediments beneath green mussel rafts, the results should be interpreted with caution. Further studies with greater raft-level replication, randomized treatment allocation, and repeated trials across different seasons and environmental conditions are needed to confirm the effectiveness and broader applicability of this bioremediation approach.

Another limitation of the field design was the absence of a netted control plot without *P. membranacea* addition. The netted plots were intended to reduce access by potential predators or disturbing organisms, including predatory polychaetes such as *Nereis* sp. and *Diopatra* sp., nemerteans, amphipods, and other mobile benthic or demersal organisms. However, netting may also affect water circulation, oxygen exchange, sediment deposition, and sediment redox conditions. Therefore, the responses observed in the netted plots may reflect the combined effects of polychaete addition and net protection, as well as possible changes in sediment conditions caused by the net itself. Future studies should include both netted and non-netted controls to separate the effects of netting from those of *P. membranacea* bioremediation.

4. Conclusion

This study demonstrated that the mass-cultured polychaete *P. membranacea* has potential as a bioremediation organism for organically enriched sediments beneath green mussel rafts in Sriracha Bay, Chon Buri Province. The polychaetes showed apparent recovery and establishment, grew, and increased biomass in the sediments within 30 days after release. The initial stocking density of 400 individuals/m² was associated with reductions in sediment organic matter and sulfide concentrations in the experimental plots. The non-netted plots tended to show higher bioremediation efficiency than the netted plots, although this pattern should be interpreted with caution because a netted control plot without *P. membranacea* addition was not included. These findings suggest that the feeding, movement, burrowing, and sediment-reworking activities of *P. membranacea* were associated with the improvement of sediment quality beneath green mussel culture systems. Therefore, this species may be considered for application as a native benthic organism for managing environmental impacts

from coastal aquaculture. However, because this study was conducted as a field application trial with limited raft-level replication and without randomized treatment allocation, the findings should be interpreted with caution. Further replicated and randomized field studies across different seasons and environmental conditions, longer monitoring periods, and varying stocking densities are recommended to confirm the bioremediation potential of *P. membranacea* and support its practical application beneath green mussel rafts.

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