การใช้ไส้เดือนทะเลสกุล *Prionospio* ในการบำบัดทางชีวภาพของตะกอนที่มีสารอินทรีย์สูงใต้แพเชือกเลี้ยงหอยแมลงภู่

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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USE OF SPIONID POLYCHAETES GENUS *Prionospio* IN BIOREMEDIATION
OF ORGANICALLY ENRICHED SEDIMENT
UNDER GREEN MUSSEL RAFTS

Mr. Natthakitt To-orn

A Dissertation Submitted in Partial Fulfillment of the Requirements
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ณัฐกิตติ์ โตอ่อน : การใช้ไส้เดือนทะเลสกุล *Prionospio* ในการบำบัดทางชีวภาพของตะกอนที่มีสารอินทรีย์สูงใต้แพเชือกเลี้ยงหอยแมลงภู่ (USE OF SPIONID POLYCHAETES GENUS *Prionospio* IN BIOREMEDIATION OF ORGANICALLY ENRICHED SEDIMENT UNDER GREEN MUSSEL RAFTS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ณิฎฐารัตน์ ปภาวสิทธิ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร. วากะ ซาโต้-โอโคชิ, 169 หน้า.

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาศักยภาพของไส้เดือนทะเลสกุล *Prionospio* เพื่อนำมาใช้บำบัดทางชีวภาพของตะกอนที่มีสารอินทรีย์สูงใต้แพเชือกเลี้ยงหอยแมลงภู่ในอ่าวศรีราชา จังหวัดชลบุรี เกณฑ์การคัดเลือกชนิดของไส้เดือนทะเลที่เหมาะสมมีหลายประการ จากผลการศึกษาการกระจายและความหนาแน่นของไส้เดือนทะเลสกุล *Prionospio* บริเวณอ่าวศรีราชา พบว่าไส้เดือนทะเลชนิด *Prionospio (Prionospio) membranacea* เป็นชนิดเด่นเกินกว่าร้อยละ 70 ของไส้เดือนทะเลในสกุลนี้ สามารถพบได้ทั่วไปในบริเวณที่มีอินทรียสารสูง เช่นบริเวณใต้แพเลี้ยงหอยแมลงภู่และบริเวณชายฝั่ง ไส้เดือนทะเลชนิด *Prionospio (Minuspio) pulchra* พบมากรองลงมาในบริเวณเดียวกัน การปรับตัวของโครงสร้างอวัยวะที่ใช้ในการหายใจของไส้เดือนทะเลสกุลนี้เป็นเกณฑ์หนึ่งที่มีความสำคัญ การศึกษาครั้งนี้แสดงให้เห็นถึงความแตกต่างในการปรับตัวของโครงสร้างอวัยวะที่ใช้ในการหายใจในไส้เดือนทะเลชนิดเดียวกันที่สัมพันธ์กับสภาพปริมาณอินทรียสารในดิน ไส้เดือนทะเลที่อาศัยอยู่ในบริเวณที่มีปริมาณอินทรียสารสูงจะมีความยาวของคู่เหงือกสูงกว่าที่พบได้ในบริเวณที่มีสารอินทรีย์ต่ำ นอกจากนี้จำนวนของ pinnules บนคู่เหงือกของไส้เดือนทะเลที่อาศัยอยู่ในบริเวณอินทรียสารสูงมีมากกว่า ความยาวของคู่เหงือกและจำนวน pinnules บนคู่เหงือกมีความสัมพันธ์กับขนาดตัวของไส้เดือนทะเล ความแตกต่างในการปรับตัวของโครงสร้างอวัยวะที่ใช้ในการหายใจที่สัมพันธ์กับสภาพปริมาณอินทรียสารในดินพบได้ตั้งแต่ระยะแรกลงเกาะของไส้เดือนทะเล รูปแบบการสืบพันธ์และพัฒนาการของไส้เดือนทะเลนับเป็นเกณฑ์การคัดเลือกอีกเกณฑ์หนึ่ง พบว่ารูปแบบการสืบพันธ์และพัฒนาการของไส้เดือนทะเล *P. membranacea* และ *P. pulchra* ตรงกับรูปแบบการสืบพันธุ์และพัฒนาการของไส้เดือนทะเลกลุ่มบุกเบิก การเพิ่มปริมาณอินทรียสารช่วยเพิ่มศักยภาพในการสืบพันธุ์ของไส้เดือนทะเลในด้านความดกไข่และการลดช่วงระยะเวลาในพัฒนาการของไส้เดือนทะเลโดยเฉพาะช่วงเวลาในการลงเกาะ นอกจากนี้การเพิ่มปริมาณอินทรียสารยังส่งผลต่อความสำเร็จในการลงเกาะของตัวอ่อนไส้เดือนทะเล จากการศึกษาประสิทธิภาพการบำบัดทางชีวภาพของไส้เดือนทะเลทั้งสองชนิดพบว่ามีประสิทธิภาพในการเปลี่ยนสารอินทรีย์ในดินให้กลายเป็นมวลชีวภาพและสามารถลดปริมาณอินทรียสารในดิน เมื่อเปรียบเทียบผลตามเกณฑ์การคัดเลือกไส้เดือนทะเลเพื่อเป็นตัวบำบัดทางชีวภาพพบว่า *P. membranacea* เป็นไส้เดือนทะเลที่มีศักยภาพสูงสุดในการบำบัดทางชีวภาพของตะกอนที่มีสารอินทรีย์สูงใต้แพเชือกเลี้ยงหอยแมลงภู่ในอ่าวศรีราชา จังหวัดชลบุรี ผลการศึกษาพบว่าการบำบัดทางชีวภาพโดยใช้ตัวอ่อนไส้เดือนทะเลระยะลงเกาะที่ได้จากการเพาะเลี้ยงปริมาณมากมีศักยภาพในการเปลี่ยนสารอินทรีย์ในดินให้กลายเป็นมวลชีวภาพ นอกจากนี้ยังมีประสิทธิภาพในการลดปริมาณอินทรียสารและปริมาณซัลไฟด์ในดิน ความหนาแน่นของไส้เดือนทะเล *P. membranacea* ที่เพิ่มขึ้นจะช่วยเพิ่มประสิทธิภาพการบำบัดทางชีวภาพ

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NATTHAKITT TO-ORN: USE OF SPIONID POLYCHAETES GENUS *Prionospio* IN BIOREMEDIATION OF ORGANICALLY ENRICHED SEDIMENT UNDER GREEN MUSSEL RAFTS. ADVISOR: ASSOC. PROF. NITTHARATANA PAPHAVASIT, CO-ADVISOR: ASSOC. PROF. WAKA SATO-OKOSHI, Ph.D., 169 pp.

Spionids, *Prionospio* spp., in this study were proposed as the potential bioremediators of organically enriched sediment under green mussel rafts in Sriracha Bay, Chonburi Province. Key criteria as bioremediator in selecting the potential *Prionospio* species were focused. The distribution and abundance of spionid polychaetes in Sriracha Bay revealed that *Prionospio (Prionospio) membranacea*, dominated of 70% of the spionids found in the area, was widely distributed in the high organic content area in the mussel raft culture area and nearshore station. *Prionospio (Minuspio) pulchra*, the second most abundant species, also found distributed in high organic content area but in low density. Morphological adaptation of respiratory structure in spionid polychaetes were considered one of the key criteria for selecting potential polychaete species. The present study revealed the presence of intraspecies morphological differences in respiratory structures in spionids as related to different organic conditions. In the high organic content area, branchial pairs were significant longer than those in the area of low organic content. The pinnules were more numerous in the high organic area. In addition, branchial length and pinnule numbers showed the tendency to increase in relation to body size. These morphological differences of these spionids under different organic condition occurred earlier in their early benthic juvenile stage. Reproductive and development pattern in spionid polychaetes in organically enriched sediment is another key criteria for potential bioremediator. The characteristic of reproduction and larval development patterns of the two dominant spionids, *P. membranacea* and *P. pulchra* fit the life history of opportunistic species. Organic enriched sediment increased their reproductive potential in term of increase fecundity and decrease time to metamorphosis and settlement. Organic enriched sediment also increased the settlement success in the larvae of these polychaete species. From the bioremediation efficiency assessment, the two spionids, *P. membranacea* and *P. pulchra* were efficiently converted the organic waste into biomass and reduced the organic matter in the sediment. From the results of key criteria as bioremediator, *P. membranacea* was selected as the potential species for the treatment of organically enriched sediment under green mussel rafts in Sriracha Bay, Chonburi Province. The artificially mass culture of selected spionid was efficiently bioremediate the enriched sediment through the process of converting organic waste into biomass and reduced the organic matter and sulphide. High density of *P. membranacea* increased the bioremediation efficiency.

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CHAPTER I
INTRODUCTION

A. General Background and Hypothesis

The rapid expansion of coastal aquaculture coupled with the adverse impacts on the coastal environment are the major concerns and challenges for the utilization of coastal resources. The major impacts included direct destruction of coastal habitats, organic enrichment/eutrophication, alteration of biodiversity in marine food webs and alteration of oxygen consumption in the coastal ecosystem (Chua, 1992). Mussel culture is one of aquaculture activities that demonstrated the extent of impacts depend on the farm size, cultivation and harvesting techniques as well as the hydrodynamic of the coastal area (Carlsson et al., 2009;Christensen et al., 2003; Crawford et al., 2003; da Costa & Nalesso, 2006; Grant et al., 1995; Stenton-Dozey et al., 1999). Organic enrichment derived from localized biodeposition of faeces and psuedofaeces, forming thick layers of silt with high concentration of organic matter underneath and around the mussel beds. Aggregations of filter feeding bivalves also regulated nutrient fluxes, sedimentation and primary production in coastal ecosystem. The depth of oxygenated layer of the sediment decreased and bottom oxygen may be depleted, leading to anoxia of the sediment and the overlying waters (Carlsson et al., 2009; Christensen et al., 2003; da Costa & Nalesso, 2006; Grant et al., 1995; Kaspar et al., 1985; Tsutsumi H., 1983). Tantanasarit and Babel (2014) reported the removal rate of carbon, nitrogen and phosphorus by green mussel (*Perna viridis*) base on the calculation from the mussel filtration rate were 3302, 380 and 124 mg/year/individual, respectively. Therefore, the nutrients as carbon, nitrogen and phosphorus were released as mussel faeces were estimated to 621, 135 and 13 mg/month/individual.

The organic enrichment to the environment was the dramatic changes within the benthic community structure by reducing the species to only few tolerant species and lowering both the diversity and community richness (Pearson & Rosenberg, 1978). Benthic community changes under mussel farms has been extensively documented (Christensen et al., 2003; da Costa & Nalesso, 2006; Grant et al., 1995; Kaspar et al., 1985; Stenton-Dozey et al., 1999). The organic enriched sediments were usually dominated by opportunistic or pioneer species such as polychaetes belong to families Capitellidae and Spionidae. These family are small polychaetes, short-lived, prolific and capable of exploiting suboptimal environment. Stenton-Dozey et al. (1999) indicated the shift in macrofauna community structure from suspension to deposit feeders. The abundance of enriched organic matter and dislodged mussels attracted opportunistic deposit feeders and carnivores. This results supported by Grant et al. (1995) conclusion that the impact of mussel falling to the sediments was more noticeable in benthic community structure than was any impact due to organic sedimentation or hypoxia. Christensen et al. (2003) showed the organic enrichment below the mussel farms at Beatrix bay, New Zealand was due to the intensified sedimentation of organic matter. The sedimentation change resulted in the increase in benthic community structure due to an enhancement of small surface deposit-feeding polychaetes, typical of organically enriched sediments including *Dorvillea incerta*, *Capitella capitata* and *Prionospio* spp. accompanied by the displacement of fewer bioturbating macroinvertebrates such as subsurface deposit-feeding polychaetes, *Hetermastus filiformis*, Cirratulidae, Cossuridae, Lumbrineridae and Maldanidae and suspension-feeding bivalves. However, da Costa and Nalesso (2006) reported that the mussel culture had no negative impact on the macrobenthic community at Coqueiro's Beach, Anchieta, Southeast Brazil. In spite of polychaetes being the most diverse group, the majority of polychaetes were predators (*Goniada* sp., *Lumbrinereis* sp.) common in the organic enrichment area. Species referred as indicator species in organic enriched area such as spionid polychaetes, *Apoprionospio pygmacea*, *Prionospio heterobranchia* and *Polydora cornuta*, or the capitellids *Mediomastus* and nephtids, *Nephtys* sp. were too scarce to suggest any impact.

Bioremediation involved the use of living organisms to remove or detoxify pollutions within a given environment. Many animals in aquatic ecosystems hyperaccumulated, stabilized or degraded pollutants. The use of animals for bioremediation can be achieved in three ways: pollutants can be extracted from an area by harvesting wild populations; through the introduction, culture and harvest of animals- a form of aquaculture, and supplementation or maintenance of wild animal population, leading to stabilization or degradation of pollutions (Alexander, 1994; Amin et al., 2013; Gifford et al., 2007) Bioremediation is currently in use for improving water quality and maintaining the health and stability of aquaculture system and coastal areas. There have been extensive studies on the use of deposit feeding polychaetes, *Capitella* species for bioremediation of organic wastes from fish farms in southern Japan. The biological activities of the these deposit feeding polychaetes accelerated the decomposition of organic matter and oxidation of reduced substances in the organically enriched sediment (Chareonpanich et al., 1993; Chareonpanich et al., 1994; Tsutsumi et al., 1990; Tsutsumi et al., 2002) had developed a method to treat enriched sediment with artificially cultured colonies of *Capitella* sp. They also conducted the experiments to measure the efficiency of *Capitella* sp.1 to decompose the organic matter daily added to the sediment in the outdoor pools. They also used *Capitella* to treat the organically enriched sediment below the fish farm in enclosed coastal sea of Kusuura Bay, Kyushu, Japan (Kinoshita et al., 2008; Tsutsumi et al., 2005). Several species from the family Nereididae have been intensively cultured and integrated in the shrimp maturation and culture system. The aim was to simultaneously remediate wastewater and produce harvestable polychaetes biomass without supplement feeding (Palmer, 2010, 2011). Van Bruggen (2012) conducted the bioremediation of particulate sea bass waste by the polychaetes *Capitella* sp. and *Nereis diversicolor* in a recirculating aquaculture system. Bioturbation activities by these polychaetes were important in the bioremediation processes including feeding, reworking the sediment, construction of burrows/ tubes and irrigation process. Cuny et al. (2007) showed that the presence of the polychaetes *Nereis diversicolor* favored the development of bacteria that play an active role in natural bioremediation processes of oil polluted environment. The presence of *N. diversicolor* induced significant changes in the composition of oil.

Coastal area of Sriracha bay, Chonburi province, eastern coastline of Thailand, is one of the major green mussel (*Perna viridis*) culture areas. The ecological impacts from the mussel farms in the area were evidenced by organic enriched sediment of high total organic matter (TOM) of 3.91-19.16% and high acid volatile sulfides (AVS) of 0.0508-1.2773 mgg^{-1} dry weight (Intarachart & Khaodon, 2009). In addition, the macrofauna composition in this area indicated polluted condition with the dominant polychaete species such as *Prionospio* spp., *Magelona* sp., *Scoloplos* sp., *Ophelina* sp., *Notomastus* sp., *Heteromastus* sp. and *Euclymene* sp.

((To-orn & Intarachart, 2010; To-orn & Paphavasit, 2003). Spionidae, especially the genera *Prionospio* are abundant in high organic polluted area with low dissolved oxygen and high sediment sulphide content ((Diaz & Rosenberg, 1995; Pearson & Rosenberg, 1978). The polychaete, *Prionospio* spp. were one of the opportunistic polychaetes living in organically enriched condition. They were usually small size and high tolerance which enable them to rapidly colonize areas with high levels of organic matters, due to their adaptations in feeding, respiration and reproduction (Blake & Arnofsky, 1999; Fauchald & Jumars, 1979; Lamont & Gage, 2000; Yokoyama, 2007; Yokoyama & Tamai, 1981).

Marine Biotic Index (AMBI) has been proposed as the ecological indicator based on soft-bottom benthos in particular polychaetes for organic enrichment assessment. Polychaetes were categorized into five groups according to their sensitivity to increasing organic enrichment (Borja et al., 2000; Carvalho et al., 2006; Cheung et al., 2008; D. M. Dauer, 1993; Grall & Glémarec, 1997). The spionid polychaetes as well as the cirratulids were classified into Group IV as the second-order opportunistic species (slight to pronounced unbalanced situations). These were the small species with a short life cycle, adapted to life in reduced sediment where they can proliferate. They were subsurface deposit-feeders. These spionids can proliferate in organic enrichment sediment, such as *Prionospio caspersi, P. cirrifera, P. ehlersi, P. fallax, P. malmgreni, P.multibranchiata* and *P. steenstrupi* (Borja et al., 2000; Cheung et al., 2008; D. M. Dauer, 1993; Grall & Glémarec, 1997).

Spionids, *Prionospio* spp. in this study were proposed as the potential bioremediators of organically enriched sediment under green mussel rafts in Sriracha bay, Chonburi Province. The organically enriched sediment under green mussel rafts would be treated by exploiting the biological activity of these spionid polychaetes which were abundant in the area. This would avoid the risk of introducing invasive species in the coastal areas (Gifford et al., 2007; Tsutsumi et al., 1990). The introduction, culture and harvest of animal taxa as bioremediators required detailed knowledge of the biological requirements for successful husbandry practices (Gifford et al., 2007). Key criteria as bioremediator in selecting the potential *Prionospio* species were focused. Comparative study on respiratory structure in spionids, *Prionospio* spp. both in adult and larval to settlement stages in organically enriched sediment were conducted as the assessment of respiratory adaptation in enriched sediment. Effect of organically enriched sediment on reproductive and development patterns in spionids, *Prionospio* spp. was another key criteria as bioremediator. The assessment on the bioremediation efficiency of spionids, *Prionospio* spp. in organically enriched sediment was also carried out. After the potential spionid species was selected, the process of rapid population growth or culture of selected spionid would be carried out in the laboratory. Introduction of the post-settlement stage of the selected spionids from the mass culture in the laboratory to the sediment under the green mussel rafts would be conducted to assess the bioremediation potential of spionid polychaete. The selected polychaete *Prionospio* species as potential bioremediator can be cultured and integrated into the aquaculture system and in the treatment of organically polluted sediment in the coastal areas.

B. General Account of Spionid Polychaetes Genus *Prionospio*

1. General Characteristic

Spionid polychaetes are found distributed in large scale around the world (Blake & Arnofsky, 1999; Fauchald & Jumars, 1979; Rouse, 2001). The spionid genus *Prionospio* was abundance in high organic pollution area (Diaz & Rosenberg, 1995; Pearson & Rosenberg, 1978). In Thailand, spionid genus *Prionospio* were also common in high organic content areas (Angsupanich, 1999; Benjabanpot, 2007; Chatananthawej, 2001; Nootcharoen, 2009; Paphavasit, 2006; To-orn & Intarachart, 2010; To-orn & Paphavasit, 2003).

Blake and Kudenov (1978) described the dominant characteristics of the genus *Prionospio* Malmgren, 1867 as follows; prostomium more or less straight, medially incised or rounded on anterior margin, without frontal horns; caruncle variously elongated. Peristomium fused in varying degree with chaetiger 1, often forming low lateral wings. Parapodia of chaetiger 1 reduced, notopodia on branchial bearing segments enlarged; postbranchial notopodia becoming smaller, inconspicuous; dorsal folds or crests present or absent on postbranchial segments, rarely on branchial segments. Anterior setae all capillaries; hooded hooks in posterior noto- and neuropodia; hooks bi- tri- or multidentate; inferior saber setae present.

Pygidium with 1 long medial cirrus and 2 short ventrolateral cirri or thickened lobes as in Figure1.1.

The polychaetes genus *Prionospio* has three subgeneric grouping as recognized by Blake and Kudenov (1978), namely *Prionospio, Minuspio* and *Aquilaspio.* The subgenus *Prionospio* has both pinnate and apinnate branchiae on anterior part while the *Minuspio* bears only apinnate branchiae. The subgenus *Aquilaspio* has only pinnate branchiae on the anterior part.

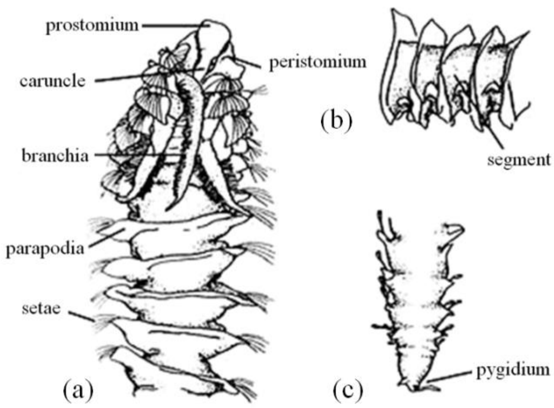

Figure 1. 1 *Prionospio.* (a) Anterior part, dorsal view. (b) Segments 6-9 showing dorsolateral skinfolds. (c) Pygidium, dorsal view.
(after (Sigvaldadóttir, 1996)

2. Distribution of *Prionospio* in Organically Enriched Sediment

The spionid polychaete *Prionospio* are one of the polychaetes that can be found in abundance in high organic pollution area (Diaz & Rosenberg, 1995; Pearson & Rosenberg, 1978). In Thailand, Chatananthawej (2001) and Nootcharoen (2009) concluded that *Prionospio japonica* can be used as indicator species for moderated organic enrichment area, because they were tolerant to organic enrichment condition (organic content 3.69-4.05%). Tolerance limits to organic enrichment condition in spionids, *Prionospio* is presented in Table 1.1.

One of the key criteria for selecting potential bioremediation species is choosing the native species in avoiding the risk of introducing invasive species in the coastal areas (Gifford et al., 2007; Tsutsumi et al., 1990). The coastal area of Sriracha Bay, Chonburi Province, was affected by human activities due to urbanization, fishing port and the expansion of green mussel raft cultures. The problem of organic enrichment in the sediment has arisen. In this study area, *Prionospio (Prionospio) membranacea* was dominant in term of abundant that found in high organic content area (4.47-9.55%TOC) and low organic content area (1.56-2.22%TOC), other *Prionospio* species were observed (Table1.2). While *Paraprionospio inaequibranchia* was very rare, found abundance in estuary riched in fine clay sediment (Paphavasit, 2006).

Table 1. 1Spionid polychaetes, *Prionospio* found in organic enrichment condition both Thailand and other country.

(after [1](Ansari et al., 1986); [2](Grall & Glémarec, 1997); [3](Angsupanich, 1999); [4](Borja et al., 2000); [5](Moreira et al., 2000), [6](Chatananthawej, 2001); [7](To-orn & Paphavasit, 2003); [8](Tsutsumi et al., 2005); [9](Paphavasit, 2006); [10](Benjabanpot, 2007); [11](Cheung et al., 2008); [12](Shin et al., 2008); [13](Nootcharoen, 2009); [14](To-orn & Intarachart, 2010)

Organic Matter (%)	**Sediment type**	**Species**	**References**
0.11-2.80	fine sand	*Prionospio depauperata*	6, 8
	fine sand	*Prionospio* cf. *malayensis*	6, 8
	fine sand	*Prionospio multibranchiata*	6, 8
	fine sand	*Prionospio* cf. *neilsoni*	6
	mud	*Prionospio polybranchiata*	1
	mud	*Prionospio tridentata*	
3.0-4.0	mud and very fine sand	*Prionospio japonica*	6,8
	mud	*Prionospio malmgreni*	2, 4, 13
	fine sand and very fine sand	*Prionospio sexoculata*	6
	very fine sand	*Prionospio multibranchiata*	6, 4
5.7-16.64	mud to sand	*Prionospio cirrifera*	3, 4
	mud to sand	*Prionospio cirrifera*	2, 4
	fine sand to medium sand	*Prionospio cirrobranchiata*	13
	mud to sand	*Prionospio ehlersi*	4
	mud to sand	*Prionospio fallax*	4
	mud to sand	*Prionospio malmgreni*	4, 13
	sandy mud	*Prionospio membranacea*	7, 13
	sandy mud, muddy sand	*Prionospio pulchra*	5, 7, 8,14
	mud to sand	*Prionospio saccifera*	10
	mud to sand	*Prionospio steenstrupi*	4
	mud to sand	*Prionospio* spp.	3, 4, 7

Table 1. 2Species and density (ind.m^{-2}) of spionid polychaetes, genera *Prionospio* and *Paraprionospio* from Sriracha Bay, Chonburi Province during April 2013 to January 2014.

	Coastal town	**Mussel raft culture**	**Low organic enriched area far from human activities**
Prionospio (Prionospio) membranacea	252±211.20	315±344.60	20±29.38
Prionosio (Minuspio) pulchra	85±79.01	11±23.31	16±50.21
Prionospio (Minuspio) multibranchiata	9±13.68	0±0.00	1±4.85
Prionospio (Minuspio) japonica	6±11.11	0±0.00	0±0.00
Prionospio (Aquilaspio) sexoculata	41±58.94	11±13.63	28±43.74
Paraprionospio inaequibranchia	0±0.00	0±0.00	2±6.34

Since the experiments in the dissertation required large sample of spionids and in the artificially cultured colonies of the selected spionid polychaetes to treat enriched sediment under the green mussel rafts in the final stage. *P. membranacea*, the most dominant spionids and *P. pulchra*, the next species in term of abundance in the area, were used as the two potential candidates for bioremediation of organically enriched sediment under green mussel rafts in Sriracha Bay, Chonburi Province.

Both *P. membranacea* and *P. pulchra* are small size polychaetes, ranging from 6.5-14.0 and 6.0-15.0 mm, respectively as in Figure 1.2. They usually share the same habitat of organically enriched sediment. Both spionids live in mucous tubes and being the subsurface deposit feeders.

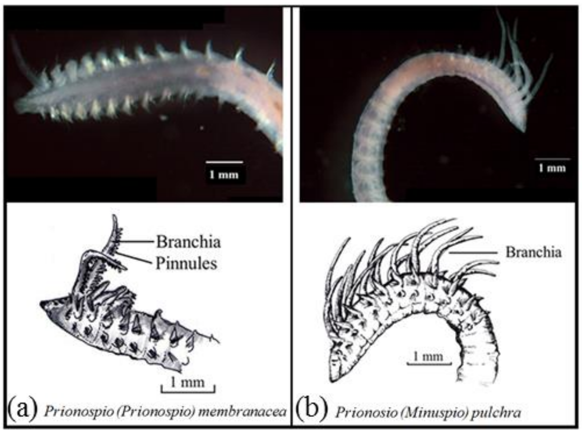

Figure 1. 2 General characteristics of (a) *Prionospio (Prionospio) membranacea* and (b) *Prionospio (Minuspio) pulchra*. (Drawing by To-orn)

3. Adaptation of *Prionospio* in Organically Enriched Sediment

Spionid polychaetes can be found in abundant in many areas from intertidal zone to deep sea (Blake & Arnofsky, 1999; Fauchald & Jumars, 1979; Rouse, 2001). They can adapt themselves being tolerant to environmental changes especially the organic enrichment areas where low dissolved oxygen and high sulfide content in the sediment (Diaz & Rosenberg, 1995; Pearson & Rosenberg, 1978). These spionid polychaetes have succeed in term of evolution and occupied several habitats through their adaptations in feeding and diversed patterns of reproduction and development (Blake & Arnofsky, 1999; Fauchald & Jumars, 1979).

3.1 Respiratory adaptation

In polychaetes, the most simple adaptation to improve the exchange of respiratory gases is by increasing the surface or volume ratio through the development of gills or other surface specialized for gas exchange (Weber, 1978). The spionids, in particular species of *Prionospio*, showed high tolerance to the conditions of organic enrichment and hypoxia in coastal waters (Lamont & Gage, 2000). In organic enrichment condition, these spionids were persistent to hypoxia due to the morphological adaptation of respiratory structures. Lamont and Gage (2000) found that the undescribed polychaete, *Prionospio (Minuspio)* sp.A would develop more branchial pairs in the low oxygen conditions. In spionids, *Paraprionospio patiens*, with the enlarged respiratory structure, is known as the biological indicator of heavy organic pollution and/or oxygen-depleted water. Lamellae of this species, being bifoliate shape with the first pair of branchiae is the longest and each has more than 50 pairs of lamellar plates, are kept separate from each other in order to enhance O_2 diffusion (Yokoyama, 2007).

3.2 Reproductive pattern and life cycle

Adaptation of these spionids to unsuitable condition including the adaptations in life cycle, feeding and reproductive pattern for rapid colonization in the area. In organic enrichment condition, adaptation of reproductive patterns in polychaetes were important for the recruitment. In spionid polychaetes, both asexual and sexual reproduction were found (Blake & Arnofsky, 1999; Rouse, 2001). However asexual reproduction has not been reported in genus *Prionospio*. Sexual reproduction in spionids are usually gonochoric. Oogenesis occurs in the segments of the mid-region of the body and both intra-and extra-ovarian oogenesis (Rouse, 2001). Blake and Arnofsky (1999) noted that three different types of mature eggs occur in spionids: (1) eggs have thick honeycomb membranes with cortical alveoli; (2) eggs have thick smooth membranes that lack alveoli; and (3) eggs have thin envelopes and lack alveoli. The 3-layered egg envelopes lacking cortical alveoli were the type of egg envelope found in *Prionospio.* The reproductive biology in spionids differed according to developmental types, including (1) pure broadcast spawners having planktotrophic or lecithotrophic larval development; (2) brooding in capsules and cocoons; and (3) viviparity. For the genus *Prionospio* being broadcast spawners species with planktotrophic larval development (Table 1.3).

Table 1. 3Reproductive patterns and life cycle of spionid polychaetes, *Prionospio* spp. in organic enrichment condition.
(after (Blake & Arnofsky, 1999)

Species	**Egg size (μm)**	**Egg envelope structure**	**Type of development**	**Shape of Nectochaete**	**Size at metamor-phosis and length of planktonic life**
Prionospio caspersi	-	-	-	Long, narrow	22 chaetigers, 2100 μm
Prionospio cirrifera	180	-	Plankto-trophic	Long, narrow	15-19 chaetigers, 1200 μm
Prionospio fallax	100	Thick, sculptured	-	Long, narrow	24 chaetigers, 2400 μm; 30 days
Prionospio lighti	-	thick	-	Long, narrow	28 chaetigers, 2100 μm;>30 days
Prionospio saldanha	-	-	-	Long, narrow	23 chaetigers, 1668 μm
Prionospio steenstrupi	-	thick	Plankto-trophic	Long, narrow	19-21chaetigers, 1400-1700 μm

The life cycles of spionid polychaete, *Prionospio* was shown in Figure 1.3. According to Blake and Arnofsky (1999), *Prionospio* species was broadcast spawner. Spawned oocytes were fertilized in water column. Newly hatching larvae from fertilized oocytes developed into pelagic planktotrophic larvae. The larva of *Prionospio* was usually long and thin, with numerous segments. Ciliated pits were well developed. Provisional larval chaetae were longest on chaetiger 1, which became the first adult chaetiger except in *Paraprionospio*. The planktotrophic larvae grow into metamorphosed stage within 30 days or more. This stage has the characteristics similar to those for the adult such as branchiae, palpi, parapodial lamellae and hooded hooks. The metamorphosing larvae had the body segments of 6-28 chaetigers and larval size ranged 360-2100 μm. Metamorphosing larvae developed into the early benthic stage juvenile, which several characteristics in adult stage developed well and formed the tubes in the sediment (Blake & Arnofsky, 1999; Yokoyama, 1981).

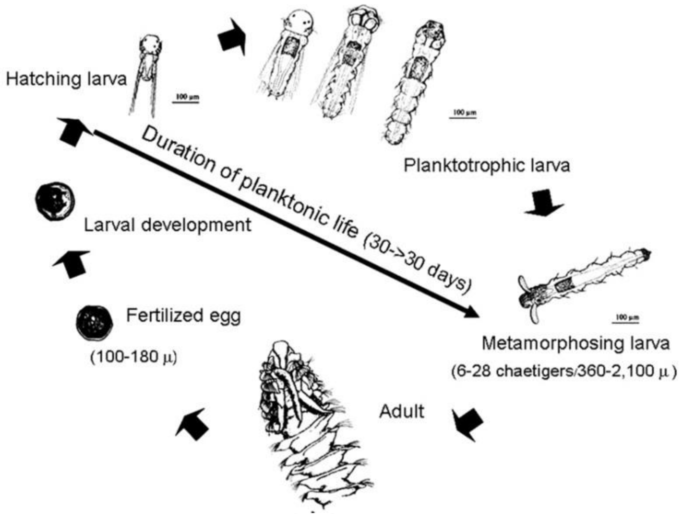

Figure 1. 3 Life cycle of spionid polychaete, *Prionospio*
(after (Blake & Arnofsky, 1999; Radashevsky et al., 2006; Sigvaldadóttir, 1996; Yokoyama, 1981)

3.3 Feeding adaptation

Most polychaetes, living in organic enrichment area, were usually deposit feeders and opportunistic feeders. Most spionids live in tubes or burrows in the soft bottom and feed at the sediment-water interface by using a single pair of tentaculate palps. Spionid can alter both the mode and rate of feeding in response to the presence of suspended particles (D. M Dauer et al., 1981; Yokoyama, 1988). Fauchald and Jumars (1979) had proposed the feeding mode categories for polychaetes that include aspects of functional morphology, degree of motility, trophic origin of food and the stratum of food collection. They recognized two feeding modes based upon whether the organism handles food particles singly as macrophages or in bulk as microphages. In the latter, it is further divided into three submodes according to the stratum where food was collected (in the water column, at the sediment surface, and below the sediment surface). They reported that spionids were generally considered as surface deposit feeders, using their ciliated palps to select food particles from the surrounding medium. D. M Dauer et al. (1981) proposed the fourth submode of microphages previously defined by Fauchald and Jumars (1979) as the interface-feeding for species which utilize particles from the sediment surface, in suspension and resuspended in the bedload. This interface-feeding submode separated the Spionidae from exclusively filter-feeding polychaete taxa, such as the Sabellariidae and Serpulidae, and from exclusively surface deposit-feeding taxa such as the Cirratulidae and Ampharetidae. Having the ability to utilize a wide variety of food resources, spionid polychaetes were found to have broad spatial distribution. *Paraprionopio pinnata*, fed on both suspended (including resuspended) and deposited particles and increased the feeding rate in the presence of a current transporting suspended particles. In the absence of suspended particles, small *P. pinnata* fed on deposit with one palp or with two palps in large *P. pinnata*. When particles were resuspended by a current, large individuals *P. pinnata* changed their palp orientation by arching the middle region of the palp into the current to collect suspended particles while the tip of the palp continued to collect deposited particles at its distal end. From the laboratory observations of feeding behavior in *P. pinnata*, Yokoyama (1988) reported that worms fed on the surface deposits using a pair of ciliated tentacles. As a result of feeding, a circular area appeared on the surface of the sediment around the opening of the tube. The radius of such a feeding scar was approximately equal to the length of tentacle which was approximately 30-40% of the body length in a relaxed live specimen worms lacking their feeding tentacles lost their ability to ingest food particles. However, they regained their feeding ability within 3-5 days with the rapid regeneration of the tentacles.

3.4 Polychaetes as bioremediators

Bioremediation is the use of living organisms to remove or detoxify pollutants within a given environment (Alexander, 1994). In aquaculture system such as fish farms, shrimp farms and mussel farms, polychaetes can be used as bioremediators converting nutrients and organic matter released in the culture system into biomass which can be easily removed and harvested as the valuable by-product (Alexander, 1994; Gifford et al., 2007; Kinoshita et al., 2008; Palmer, 2010, 2011; Tsutsumi et al., 2005; Tsutsumi et al., 2002; Van Bruggen, 2012). The bioremediation process of organic enrichment by polychaetes can be shown in Figure 1.4. Bioturbation in polychaetes are importance activities for bioremediation process, which is related including deposit feeding, reworking, construction of burrows and tubes and irrigation in the sediment (Rosenberg, 2011).

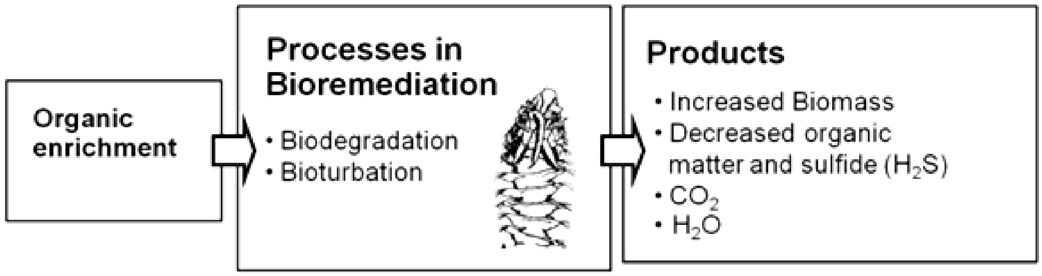

Figure 1. 4 Bioremediation of organic enrichment by polychaetes.
(after (Alexander, 1994; Sigvaldadóttir, 1996)

The utilization of polychaetes in bioremediation in aquaculture system were widely introduced. There have been extensive studies on the use of deposit feeding polychaetes, *Capitella* species for bioremediation of organic wastes from fish farms in Southern Japan (Chareonpanich et al., 1993; Chareonpanich et al., 1994; Tsutsumi et al., 1990). Artificially culture colonies of *Capitella* sp.1 had been introduced to treat enriched sediment in the outdoor pool (Tsutsumi et al., 2002) and in enriched sediment under the fish farms (Kinoshita et al., 2008; Tsutsumi et al., 2005). Several species from the family Nereididae have been studied to simultaneously remediate wastewater and produce harvestable polychaete biomass without supplemental feeding (Palmer, 2010, 2011). Van Bruggen (2012) studied bioremediation of particulate sea bass waste by the polychaetes *Capitella* sp. and *Nereis diversicolor* in a recirculating aquaculture system. The deposit feeding polychaete, *Capitella* sp. reduced 0.008% of total organic matter/ind./day/m^2, while the large polychaete, *N. diversicolor* reduced total organic matter of 0.027%/ind./day/m^2.

Filter feeding polychaetes were also used as bioremediators in the aquaculture system. Giangrande et al. (2005) studied the filter feeder sabellid, *Sabella spallanzanii* as biofilter in the treatment of wastes from intensive aquaculture system. They found that the single worm of 0.6 mg dry weight, was able to remove within the first hour of activity more than 50% of the particulate organic matter from the water column with initial concentration of 22.76 mg L^{-1}. A biomass of 1 mg dry weight of worm can remove 4.93 mg L^{-1} of suspended solids within 3 h from the water column. This sabellid polychaete *S. spallanzanii* was introduced as potential bioremediator in remediation of waste fish farms with recycled water. Parandavar and Kim (2014) studied the improvement of outlet water quality in fish culture by rockworm *Marphysa sanguinea* in the semi-recirculating system. They found that the rockworm *M. sanguinea* with the density of 4000 inds/m^2 of less than 0.5 g was an excellent potential candidate for integrated aquaculture and nutrient recycling including the removal of organic wastes in land-based systems

4. Polychaete Culture

The polychaetes species are cultivated, as a food item, commercial and research purposes. The culture of nereidid polychaete is being developed for several reasons such as baits for fishing, and as supplementary feed to increase the reproductive fitness of crustacean brood-stock, thus generating a synergism between basic scientific research and the aquaculture of these worms (Olive, 1999). These nereid polychaetes are used as potential bioremediators in the aquaculture system in particular shrimp farm and simultaneously produce harvestable polychaetes biomass (Palmer, 2010, 2011). Bischoff et al. (2009) studied the potential of solid waste originating from a recirculated fish culture system, i.e. faecal material, uneaten food pellets and bacterial biofilms as food sources for the polychaete *Nereis diversicolor*. The result found that these nereid polychaetes could be a valuable food for fish as the solid waste provided essential fatty acids to the fish. This indicated that a recycling or even an upgrade of excreted feed nutrients such as fatty acids, which were otherwise discharged, can be achieved through integrated aquaculture combining fish and worm cultures.

Brown et al. (2011) demonstrated that the production of the polychaete worm, *Nereis virens* from a marine recirculating fish culture system was highly efficient. This species was proposed as an excellent candidate for integrated aquaculture and waste recycling. *Platynereis dumerilii*, a marine polychaete, showed several characteristics that promoted its use as a model laboratory animal in reproduction and ecotoxicology studies (Garcia-Alonso et al., 2013).

C. Conceptual Framework of Research

A bioremediation technique has been developed successfully using artificially mass-cultured colonies of *Capitella*, an opportunistic deposit feeding polychaete in the treatment of organically enriched sediment in the fish farms and enclosed bay (Kinoshita et al., 2008; Tsutsumi et al., 2005; Tsutsumi et al., 2002). These opportunistic polychaetes in the families Capitellidae and Spionidae were usually found to dominate the organic enriched sediments. The spionids, *Prionospio* spp., were commonly found in the organic enriched sediment under the green mussel farms in Sriracha Bay, Chonburi Province. They were proposed as the bioremediators of organically enriched sediment under green mussel raft. This would avoid the risk of introducing invasive species by using the native species already common in the area. Certain key criteria as bioremediator in selecting the potential *Prionospio* species were focused prior to the introduction of artificially mass-cultured colonies of the selected species of *Prionospio* in the treatment of organically enriched sediment under the green mussel rafts in the field. Figure 1.5 outlined the conceptual framework of this study on "Use of Spionid Polychaetes in Genus *Prionospio* in Bioremediation of Organically Enriched Sediment under Green Mussel Rafts".

As the spionid polychaetes have succeed in occupying the organic enrichment habitat through their adaptations in respiration, diversed patterns of reproduction and development and feeding (Blake & Arnofsky, 1999; Fauchald & Jumars, 1979; Lamont & Gage, 2000; Pearson & Rosenberg, 1978; Rouse, 2001; Yokoyama, 1988, 2007). These adaptations in respiratory structure and reproductive and development patterns as well as the bioremediation efficiency in these spionids were used as the key criteria for selecting the potential *Prionospio* species.

1) Comparative study on the morphological adaptation of respiratory structure in spionid genus *Prionospio* in organically enriched sediment is the first criteria for selection of spionids as potential bioremediator. Seasonal distribution and abundance of these spionids in the study area was also conducted.

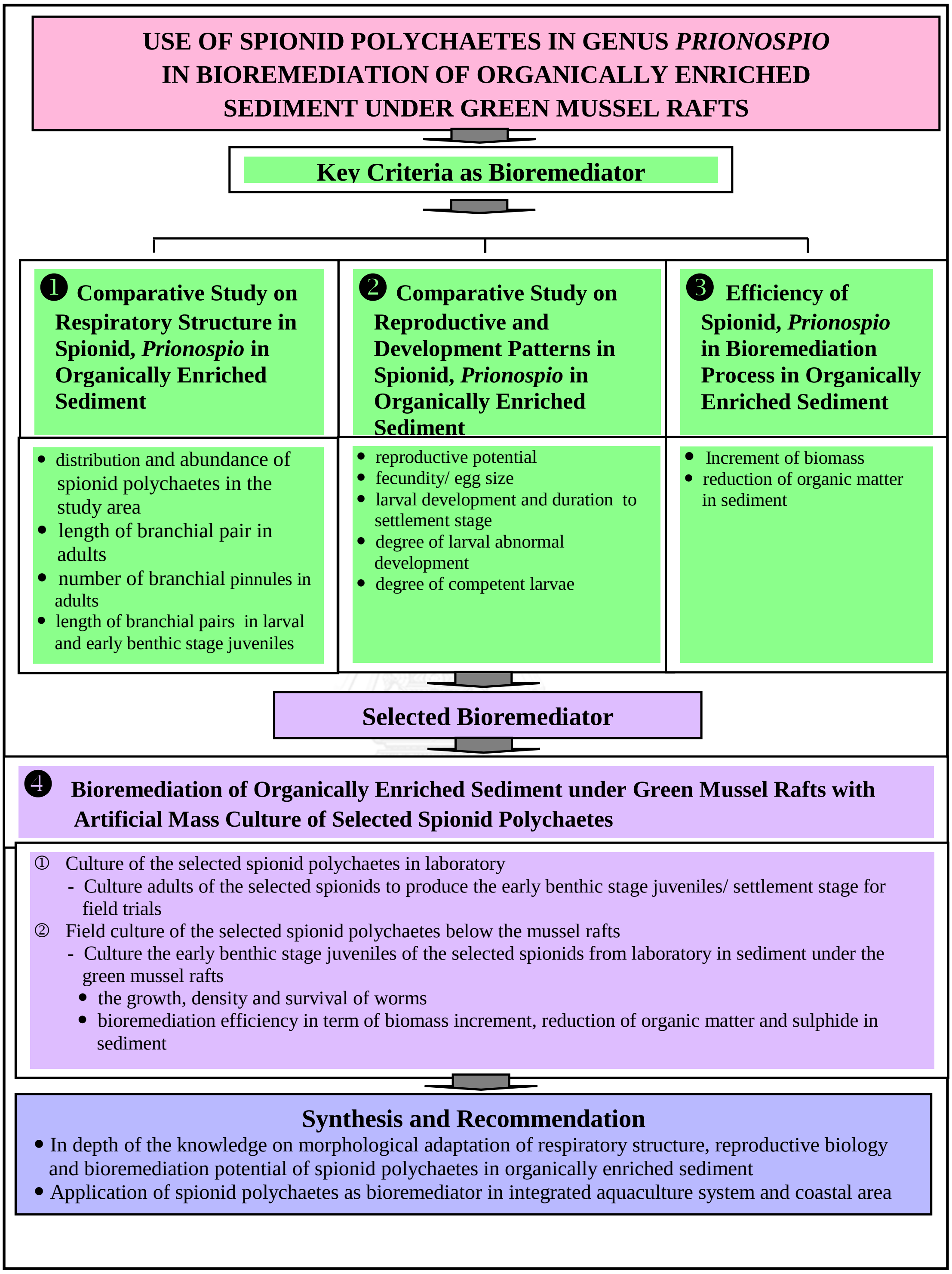

Figure 1. 5 Conceptual framework of research on " Use of Spionid Polychaetes in Genus *Prionospio* in Bioremediation of Organically Enriched Sediment under Green Mussel Rafts"

Adults *Prionospio* sampled from the field from areas of high organic content and low organic content, were compared in term of increased branchial area such as the length of brancial pairs and the number of branchial pinnules. The respiratory structures in larva and settlement juvenile stages of spionid, *Prionospio*, which were cultured in the laboratory in different levels of organic enriched sediment were also compared in term of the length of brancial pairs and the number of branchial pinnules.

2) Comparative study on reproductive and development pattern in spionids, *Prionospio* spp. in organically enriched sediment is another key criteria for potential bioremediator. Adult spionids, *Prionospio* sampled from the high organic content area in Sriracha Bay, Chonburi Province were cultured in organic enriched condition in the laboratory. Reproduction potential such as fecundity rate, the larval development and duration to settlement stage as well as the degree of abnormality in the larval development and degree of competent larvae were compared. These data were important for the elucidation of these spionids to proliferate in organically enriched sediment.

3) In order to study the potential of *Prionospio* in bioremediation in organically enriched sediment, adults *Prionospio*, sampled from the field in high organic content area were compared in term of bioremediation efficiency in the laboratory in term of increased biomass and the reduction rate of organic matters in the sediment were also carried out.

The selected *Prionospio* species was chosen as the potential bioremediator from the results from above experiments. The artificially mass-culture of the selected *Prionospio* was to be produced and used in the bioremediation of organically enriched sediment under green mussel raft.

4) In order to produce mass culture of selected spionid polychaete, culture of adults of the selected species in the laboratory was conducted to produce the early benthic stage juveniles/ settlement stage. After the early benthic stage juveniles stage had been reached, these juveniles from the laboratory were harvested and introduced into the sediment under the green mussel raft. Growth and density as well as survival rate of the worms in the field culture were monitored. Bioremediation efficiency in term of biomass increment, reduction of organic matter and sulphide in sediment were also assessed.

D. Description of Study Area

1. Description of Survey Area

Sriracha Bay is located at Latitude 13º10′-13º11′N and Longtitude 100º54′-100º56′E in Sriracha District, Chonburi Province, on the eastern coast of the Gulf of Thailand (Figure 1.6). The bay is a small semi-open coastline about 900 km^2 and the depth of 15-20 m. The eastern coastline of the bay line with mud flat, sandy beaches and rocky shores (Anongponyoskun & Meksumpun, 2007). The sampling stations are located in an area of high organic content, with mussel raft culture installations and the presence of municipal waste effluents. The sediment in this area characteristic by 3.25-4.90% organic matter and the texture of 6.91-22.22% in the silt-clay fraction. The second area is from an area with low organic content, at a distance from mussel raft culture and with reduced human coastal activities. The sediment in the low organic area consisted of 2.22% organic matter and 0.46% in the silt-clay fraction.

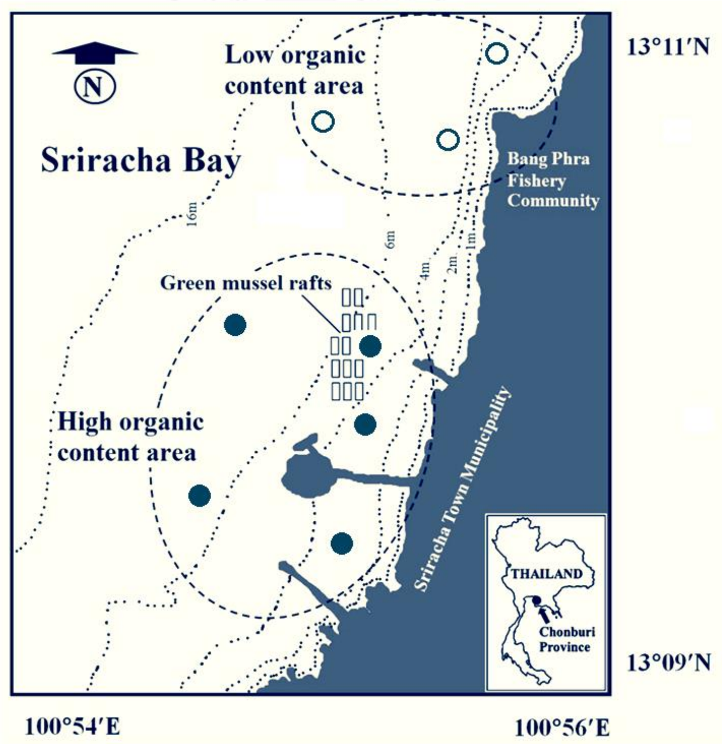

Figure 1. 6Study sites in Sriracha Bay, Chonburi Province, eastern coastline of Thailand.

2. Description of Mussel Raft Culture

Sriracha Bay has a large area of nearshore green mussel (*Perna viridis*) culture. There are two types of green mussel culture namely bamboo poles and floating rafts. The bamboo poles culture is the traditional mussel culture with numerous bamboo pole lined into the sediment to support the fouling mussel colonies. The bamboo poles culture is usually carried out offshore in the 4-6 m depth. The floating rafts were usually varied in size of 10x10, 20x20 or 40x40 m^2. Each raft consisted of small floating buckets served as buoys binded together by ropes. Each raft was kept in place by anchoring with cement blocks as in Figure 1.7. Because of high growth rates and good taste coupled with good market price in green mussel, the floating rafts culture has greatly increased in the coastal areas. In Sriracha Bay alone, the area of floating rafts of green mussel has increased 3 times from 0.5 km^2 to 1.5 km^2 during 2001-2004 (Anongponyoskun & Meksumpun, 2007; M. S. Carlsson et al., 2009).

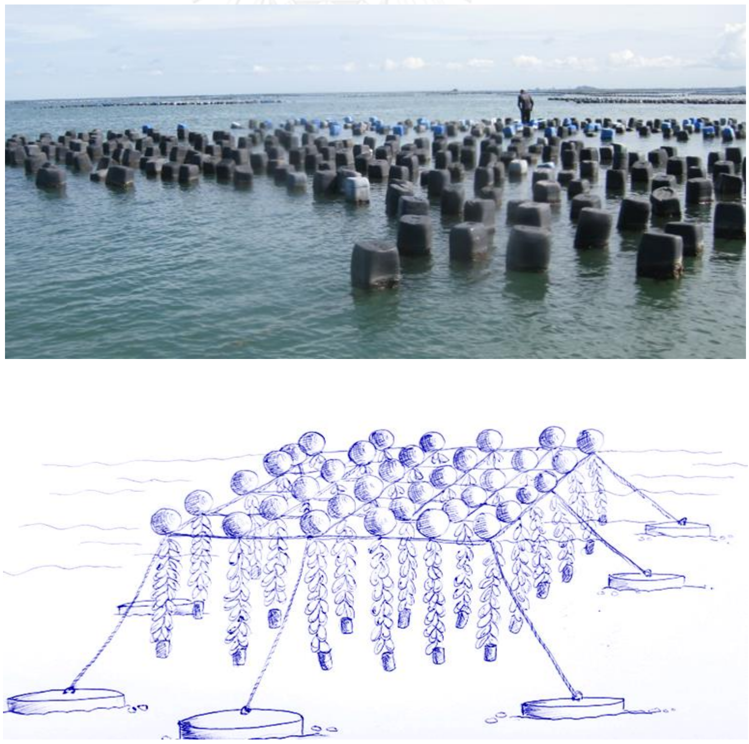

Figure 1. 7Green mussel rafts in Sriracha Bay, Chonburi Province, eastern coastaline of Thailand. (Drawing by To-orn)

E. Research Methodology

Research Attributes	Methodology
1. Comparative study on respiratory structure in spionid polychaete genus *Prionospio* in organically enriched sediment	• Distribution in spionid polychaetes - Specimens were collected by using Ekman grab (0.0225 m^2) from an area of high and low organic content. Samples were sieved through a 0.5 mm mesh and fixed in 10% buffered formalin for later sorting and identification of *Prionospio* following the procedures of M Imajima (1990a, 1990b); M. Imajima (1990) - Environmental parameter: Sediment sample was taken at each site to analyze total organic matter by the loss on ignition (Nelson, 1982) and particle size composition by dry sieving using a 63 µm sieve to separate the coarse (sand) and fine (silt-clay) fractions. Water quality including temperature, salinity, pH and dissolved oxygen (DO) were measured *in situ* - Data analysis: One-way ANOVA was carried out to see the variation in the abundance of spionid polychaetes at different stations. The Pearson's correlation co-efficient was used to analyze the relationship between polychaete abundance and environmental variables. • Comparative study of morphological adaptation on respiratory structure - Adult spionid, *Prionospio* sampled from high and low organic content areas were measured the length of each branchial pair following Yokoyama (1988) procedure, and counted the number of digitiform branchial pinnules using an ocular micrometer. -Larvae and juveniles of spionid, *Prionospio* were cultured in the laboratory in different organic ecrichment condition for comparative study on the length of brancial pairs and the number of branchial pinnules. The length of each branchial pair was measured using an ocular micrometer. Counts were made on the number of digitiform branchial pinnules

Research Attributes	Methodology
	- Data analysis: The relationships between the length of branchial pairs and body size, and between the number of branchial pinnules and body size were analyzed using linear regression. The length of branchial pairs and number of branchial pinnules of *Prionospio* from the two sites were compared using a t -test (Zar, 1999).
2. Comparative study on reproductive and development patterns in spionid polychaete, *Prionospio* in organically enriched sediment	• Adults *Prionospio* were collected from high organic content area by using Ekman grab. Samples were sieved through a 0.25 mm mesh and sorted under stereo-microscope. Sediment for the experiment was collected from (1) near Bang Phra fishery community outside of mussel raft, representing the low organic content area (2) under the floating mussel rafts in the bay, representing the high organic content, and (3) from Sriracha port, near Sriracha Town Municipality under the influence of domestic waste effluents, representing the high level of organic enrichment in the sediment using Ekman grab, and sieved through a 1.0 mm mesh. Each sediment sample was freeze in the laboratory at least for 24 h to kill the living organisms. The seawater was filtered through a 1.0 micrometer filter bag for the experimental use. • Fecundity of spionid polychaetes: Spionids were added to the cultured boxes (20x25x15cm), with the treated sediment at the level of 3 cm from the bottom with the filtered seawater level 3 cm above the surface sediment, in three different organic enriched treatments: (1) normal level of organic matter in the sediment from Bang Phra fishery community outside of mussel raft, (2.75%TOC); (2) high level of organic matter in the sediment from under the green mussel rafts, (6.17%TOC); and (3) high level of organic enrichment in the sediment from Sriracha port, near Sriracha Town Municipality under the influence of domestic waste effluents, (12.94%TOC). For each treatment, four cultured boxes were used as replicates. The experiments were carried out for 30 days.

Research Attributes	Methodology
	- Fecundity of spionid polychaetes were compared from the number of oocytes in the total number of reproductive segments in maturing females at the end of the 30 days experiment following Yokoyama (1990) procedure. • Larval development of spionid polychaetes; Pelagic planktotrophic larvae of spionid plychaetes with 2 to 3 chaetiger were added to the cultured boxes (33x42x28cm), with the treated sediment at the level of 3 cm from the bottom with the filtered seawater level 10 cm above the surface sediment, in three different organi enriched treatments. For each treatment, four cultured boxes were used as replicates. • Larval development and duration to settlement stage, degree of larval abnormal development and degree of competent larvae were compared.
3. Efficiency of spionid, *Prionospio* in bioremediation process in organically enriched sediment	• Adults *Prionospio* were collected from high organic content area by using Ekman grab. Samples were sieved through a 0.25 mm mesh and sorted under stereo-microscope. Sediment for the experiment was collected from under the floating mussel rafts using Ekman grab, and sieved through a 1.0 mm mesh. Each sediment sample was freeze in the laboratory at least for 24 h to kill the living organisms. The seawater was filtered through a 1.0 micrometer filter bag for experimental use. - Spionid polychaetes were added to the cultured boxes (20x25x15cm), with the treated sediment at the level of 3 cm from the bottom with the filtered seawater level 3 cm above the surface sediment, in three different density treatments: (1) control (without spionid polychaetes), (2) 10 individuals of spionids (normal density of 200 inds.m^{-2}) and (3) 20 individuals of spionids (twice the normal density, 400 inds.m^{-2}). For each treatment, four cultured boxes were used as replicates.

Research Attributes	Methodology
	The experiments were carried out for 30 days. - The bioremediation efficiency in spionid polychaetes were compared in term of biomass increament and the reduction of organic matter in sediment at the end of the 30 days experiment. The growth in term of biomass increment, the body length and the body size (width of the 5th chaetiger, excluding parapodia), and the number of chaetigers of the spionids were measured and counted by using an ocular micrometer following Yokoyama (1988) procedure. The wet weights of the spionids were measured. The sediment from each cultured boxes were analyzed for organic content by Ignition loss (Nelson, 1982)
4. Bioremediation of organically enriched sediment under green mussel rafts with artificial mass culture of selected spionid polychaetes	• The selected spionid, *Prionospio* species with high potential in respiratory adaptation, reproduction and bioremediation in organically enriched sediment was cultured in laboratory with sediment from under the floating mussel rafts and field culture under the green mussel rafts. • Culture of the selected polychaete *Prionospio* species in laboratory: Culture matured adults of the selected spionids in order to produce the early benthic stage juveniles/ settlement stage. The early benthic stage juveniles were harvested as mass culture to be introduce into the sediment under the green mussel rafts for field trials. • Themass culture of early benthic stage juveniles were added to the cages (50 cm x 50 cm x 30 cm) that covered with net of 1 cm mesh screen inorder to prevent predators placed on the surface sediment under green mussel rafts, in two different density treatments; (1) the normal density of 200 inds.m^{-2} and (2) 400 inds.m^{-2} at twice the normal density. For each treatment, four cultured boxes were used as replicates.

Research Attributes	Methodology
	The experiments were carried out for 30 days. - Bioremediation efficiency in term of reduction of organic matter and sulphide in sediment were compared. The total organic content in sediment were analyzed by Ignition loss (Nelson, 1982) and acid volatile sulphide content (H2S + FeS: AVS) of the sediment were determined with AVS test column (Gastec, Model 201H) (Chareonpanich et al., 1993; Chatananthawej, 2001). - Growth, density and survival of the spionids were monitored at the end of the 30 days experiment following Yokoyama (1988) procedure.

F. Objectives

1. To compare the morphological adaptation on respiratory structure in adult and larval stages of spionid genus *Prionospio*.

2. To study the reproductive and development patterns in spionid, *Prionospio* in organically enriched sediment.

3. To study the efficiency of spionid, *Prionospio* in bioremediation process in organically enriched sediment.

4. To introduce the bioremediation technique using artificial mass culture of the selected spionid polychaete *Prionospio* in organically enriched sediment under the green mussel rafts.

G. Expected Results

This research results will contribute to the knowledge on morphological adaptation of comparative respiratory structure, reproductive biology and bioremediation potential of spionid polychaetes in organic enrichment sediment. These can be further used in the application of spionid polychaetes as bioremediator of the organic waste in the aquaculture system and in coastal area.

CHAPTER II
COMPARATIVE STUDY OF MORPHOLOGICAL ADAPTATION ON RESPIRATORY STRUCTURE IN SPIONIDS GENUS *Prionospio*

A. Introduction

Spionid polychaetes are common and widely distributed around the world in both sandy and muddy sediments from intertidal zones to the deep sea (Rouse, 2001). Spionids have succeeded in completing their life cycle in several habitats in coastal areas because of their adaptations in feeding and diverse patterns of reproduction and development (Blake & Arnofsky, 1999; Fauchald & Jumars, 1979). The spionids in genera *Prionospio Malmgren*, 1867 and *Paraprionospio* Caullery, 1914 are abundance in highly organic polluted areas with low dissolved oxygen and high sediment sulfide content (Diaz & Rosenberg, 1995; Pearson & Rosenberg, 1978; Yokoyama & Tamai, 1981), tolerance which enables them to rapidly colonize areas with high levels of organic matters. Several spionids were known as indicator species for organic-rich areas. *Paraprionospio pinnata* could maintain its population in organically polluted area as a result of its resistence to hypoxia (D.M. Dauer, 1985; Yokoyama, 1995). *Paraprionospio pinnata* is known as a biological indicator for heavy organic pollution and/ or oxygen-depleted waters, while *Paraprionospio cordifolia* for semi-enclosed, eutrophicated environment (Yokoyama, 2007). In Thai waters, Chatananthawej (2001) and Nootcharoen (2009) concluded that *Prionospio japonica* can be used as indicator species for moderated organic enrichment area.

Tolerance to hypoxia and high sulfide content in sediment is one of the key criteria for selection of potential polychaetes as bioindicator for aquacultural wastes. Lamont and Gage (2000) noted that benthic invertebrates are able to tolerate permanent hypoxia by overcoming powerful constraints on morphological adaptation towards enhancing O_2 diffusion by increasing their respiratory area relative to body size. In polychaetes, the simplest adaptation to improve exchange of respiratory gases is by increasing the surface or volume ratio for gas exchange through development of gills or other respiratory surfaces (Weber, 1978). Elaborate branchiae in many polychaetes, are developed as extensions of the body wall, thereby greatly increasing the surface area available for gas exchange, and the dorsal branchiae in particular may take a variety of shapes that affect respiration (Rouse, 2001; Storch & Alberti, 1978). Morphological responses to hypoxic conditions, such as enlargement of respiratory structures, have been observed in several families of polychaetes ranging from intertidal to abyssal polychaetes (Hourdez et al., 2002; Lamont & Gage, 2000; Nkwoji, 2012; Storch & Alberti, 1978). In annelids, moderate hypoxia is known to induce physiological responses such as increased ventilation, while first behavioral responses to severe hypoxia are exhibited through elongation, tube construction or migration from burrows, and raising respiratory structures higher and above the sediment or water interface to access faster moving water containing more oxygen (L.A. Levin, 2003).

In spionids of the genus *Prionospio*, respiratory structures (in particular sites of the branchiae, number of branchial pairs, relative lengths of branchial pairs, number and shape of branchial pinnules) show one of the important diagnostic values of the species (Blake & Kudenov, 1978; Hylleberg & Nateewathana, 1991; M Imajima, 1990a, 1990b; M. Imajima, 1990; Yokoyama, 2007; Yokoyama & Tamai, 1981). However, there is little information available concerning the morphological and physiological adaptive features of these polychaetes to hypoxia. Yokoyama and Tamai (1981) noted the presence of elongated branchiae with numerous and well-separated lamellar plates in *Paraprionospio* sp. form A in oxygen-depleted and organic-rich coastal areas of Japan although intraspecific variation in branchial length and number of branchial pinnules had not been reported previously. In revising the genus *Paraprionospio* (Yokoyama, 2007), different morphological enlargement of respiratory structure were described according to the coastal habitats found. These morphological characteristic were utilized to enhance O_2 diffusion.

Morphological adaptations of respiratory structure in spionid polychaetes were considered as one of the key criteria for selecting potential polychaete species as bioremediator in treating high organic sediment under mussel rafts in Sriracha Bay, Chonburi Province. Specific objectives of this study were to determine: (1) distribution and abundances of spionid polychaetes in Sriracha Bay, Thailand, (2) comparative study on respiratory structure in spionid genus *Prionospio* as within-species responses between high and low organic content areas of Sriracha Bay and (3) whether these morphological differences of these spionids under different environmental conditions occurred earlier in their planktonic larval stages to settlement.

B. Literature Review

1. Distribution of Spionid Polychaetes

Spionids in the genera *Prionospio* and *Paraprionospio* can be found in organic enrichment areas in Upper Gulf of Thailand shown in Table 2.1. Paphavasit (2006) reported *Prionospio* spp. were dominant group of polychaetes as high as 1164 ind m^{-2} in high organic sediment of 7.45% in coastal area of Samut Sakhon Province. In organic enrichment sediment from the shrimp farm effluents at Kung Krabaen Bay, Chanthaburi Province, Chatananthawej (2001) found that *Prionospio (Minuspio) japonica* was tolerant to organic enrichment condition and can be used as indicator species for moderated organic enrichment area. *P. japonica* was also proposed as the indicator of organic enrichment area in Pak Panang Estuary, Nakhon Si Thammarat Province, where organic enrichment resulting from domestic wastes ((Nootcharoen, 2009). *Prionospio cirrobranchiata* and *Paraprionospio pinnata* were found high abundance in organic enriched sediment of 5.78-16.64% in Bangpakong Estuary, Chon Buri Province (Benjabanpot, 2007).

Table 2. 1Distribution of spionid polychaetes, *Prionospio* and *Paraprionospio* in organic enrichment areas in Gulf of Thailand.
(after [1]Paphavasit (2006); [2]Benjabanpot (2007); [3]To-orn and Intarachart (2010); [4]Chatananthawej (2001); [5]Angsupanich (1999); [6]Nootcharoen (2009))

Spionid polychaetes	**Upper Gulf of Thailand [1]**				**Bangpakong Estuary, Chon Buri [2]**	**Sriracha Bay,Chon Buri [3]**	**Kung Krabaen Bay, Chanthaburi [4]**	**Songkhla Lagoon [5]**	**Pak Panang Estuary, Nakhon Si Thammarat [6]**
	Samut Sakhon	**Samut Songkharm**	**Phetchaburi**	**Offshore**					
Paraprionospio pinnata	✓	✓			✓		✓		
Prionospio caspersi							✓		
Prionospio cirrifera								✓	
Prionospio					✓				
Prionospio depauperata							✓		✓
Prionospio japonica							✓		✓
Prionospio cf. *malayensis*							✓		✓
Prionospio membranacea							✓		
Prionospio							✓		✓
Prionospio cf. *neilsoni*							✓		
Prionospio pulchra							✓		
Prionospio sexoculata							✓		
Prionospio (Prionospio)	✓	✓	✓	✓		✓		✓	
Prionosio (Minuspio) sp.	✓		✓	✓		✓			

2. Respiratory Structure in Spionid Polychaetes

Blake and Kudenov (1978) reviewed spionid genus *Prionospio* with three subgeneric groupings, namely *Prionospio* Malmgren, 1867, *Minuspio* Foster, 1971 and *Aquilaspio* Foster, 1971, which various combinations of branchiae including apinnate (smooth) or with digitiform pinnules, beginning on chaetiger 2. The subgenus *Prionospio* possessed both pinnate and apinnate branchiae on anterior part while the *Minuspio* bears only apinate branchiae. The subgenus *Aquilaspio* has only pinnate branchiae on the anterior part. M Imajima (1990b) M. Imajima (1990) described the branchial characteristic of spionid polychaetes; genus *Prionospio (Prionospio)* Malmgern, 1867, the branchiae from chaetiger 2, numbering 4-5 pairs in various arrangements of pinnate with digitiform pinnules and apinnate branchae; genus *Prionospio (Minuspio)* Foster, 1971, branchiae all pinnate beginning on chaetiger 2, varying from four to forty pairs; genus *Prionospio (Aquilaspio)* Foster, 1971, all branchiae with digitiform pinnules, two to four pairs, beginning on chaetiger 2 (Figure 2.1).

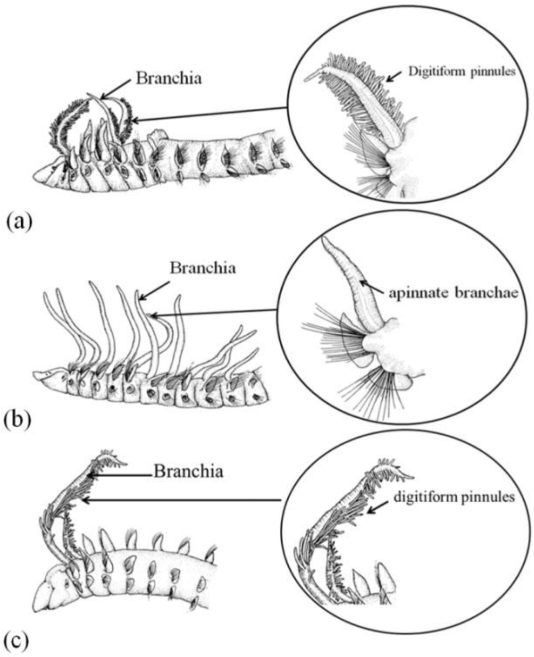

Figure 2. 1 Spionids genus *Prionospio.* (a) *Prionospio (Prionospio).* (b) *Prionospio (Minuspio).* (c) *Prionospio (Aquilaspio).*
(after (M Imajima, 1990a, 1990b) (M. Imajima, 1990))

Few research studies had focused on the respiratory structures in larval and juvenile stages in polychaetes. Yokoyama (1981) noted that branchiae first developed as small protuberances on segment 2 in the 11-segment larva in *Paraprionospio* pinnata. As the larva grew, they become larger and bipinnate similar to the characteristic form of the adult. A second pair of branchiae become visible first on segment 3in the 22- segment larva. In metamorphosed larvae of 35 segments, the anterion two pairs of branchiae were well developed to form the pinnation. The third pair of branchiae were found on segment 4. In the early benthic stage juvenile, the dorsal ridge connecting the bases of the first pair of branchiae was apparented. The branchial pinnation developed well in the anterior two pairs, but the third pair of branchiae was not fully differentiated. Small protuberances as buds of the thread-like filaments in the adult, were found in front of the bases of the third branchiae. In *Prionospio patagonica*, only reported from Southern Chile and the Ross Sea in Antractica, has four pairs of branchiae on segments 2-5, of which pairs 1 and 4, pinnate and pairs 2 and 3 smooth. After settlement, as growth proceeds, branchiae appear first on segment 2 and then on following segments, until segment 15 (Radashevsky et al., 2006). Yokoyama (1995) observed that all developmental stages *Paraprionospio* sp. form A larvae were distributed in anoxic or hypoxic water all day long. These larvae have the ability to use oxygen at very low partial pressures and/or an anaerobic energy metabolism. He furthered observed from larval distribution in Kumihama Bay, Japan Sea that dissolved oxygen was the possible environmental cue triggering the appearance and settlement of larvae.

3. Adaptation of Respiratory Structure in Polychaetes to Hypoxia and Anoxia

In many polychaetes, elaborate branchiae are developed as extensions of the body wall, thereby greatly increasing the surface area available for gas exchange, and the dorsal branchiae in particular may take a variety of shapes that affect respiration ((Rouse, 2001; Storch & Alberti, 1978). Morphological responses to hypoxic conditions, such as enlargement of respiratory structures, have been observed, particular in abyssal polychaetes. Jouin and Gaill (1990) investigated the distinctive features of hydrothermal- vent polychaetes in the Family Alvinellidae with respect to the low oxygen content of the ambient seawater. The specific gill surface areas in *Alvinella pompejana*, and *Paralvinella grasslei*, are the largest measured to date: 12 and 47 cm^2 per g wet weight, respectively. *Alvinella pompejana* and *P. grasslei* bears numerous flat sickle-shaped lamellae and cylindrical filaments in each gill, respectively, where both lamellae and filaments have a ciliated mucous epidermis. These extensive, abundantly ciliated respiratory surfaces are clearly adapted enable these deep-sea polychaetes to extract oxygen under conditions of low ambient concentrations. Similar adaptations, including enlargement of respiratory structures, were observed in specimens of Spionidae, Cossuridae and Paraonidae from the oxygen minimum zone on the Oman margin (Lamont & Gage, 2000). The apparent responses to hypoxic condition consisted of enlargement in size and branching of the branchae relative to similar species living in normal levels of dissolved oxygen. This study was one of the first to observe within-species variability in number and development of branchiae in relation to degree of hypoxia. The study had concluded that inorder for benthic invertebrates able to tolelate permanent hypoxia, there are powerful constraints for morphological adaptation aimed towards enhancing O_2 diffusion by increasing body area/ mass ratio. A deep-sea orbiniid polychaete, *Methanoaricia dendrobranchiata* Blake, 2000 from the Gulf of Mexico also was observed to have an enlarged gill surface area (Hourdez et al., 2002).

Intertidal polychaetes, such as the spionid *Malacoceros fuliginosus* and the orbiniid *Scoloplos armiger* are common in organic enriched habitats (Pearson & Rosenberg, 1978). Broad rows of cilia on the gills of these two polychaetes facilitate the continuous flow of water over the gill surface (Storch & Alberti, 1978). *Nereis diversicolor*, which is also found in organic enriched areas, has its greatest specific branchial surface area in the notopodia, another adaptation to increase the efficiency of gaseous exchange (Nkwoji, 2012).

There is little information available concerning the morphological adaptation features of spionids in the genus *Prionospio* to hypoxia. Yokoyama and Tamai (1981) noted the presence of elongated branchiae with numerous and well-separated lamellar plates in *Paraprionospio* sp. form A in oxygen-depleted and organic-rich coastal area of Japan. This species, later in the revision of the genus was assigned to *Paraprionospio patiens* with three pairs of branchiae with bifoliate-shape lamellae. The first branchial pair is the longest and each has more than 50 pairs of lamellar plates (Yokoyama, 2007). Another species with enlarged respiratory structures, *Paraprionospio cordifolia*, assigned to previous described as *Paraprionospio* Form B, possess the flabellate instead of bifoliate lamellae. These two spionids were considered as the biological indicator for enriched coastal area (Yokoyama, 2007). Intraspecific variation in branchial length and number of branchial pinnules had not been reported previously.

C. Materials and Methods

1. Study Area

Study sites were located in Sriracha Bay, Chonburi Province, on the eastern coast of the Gulf of Thailand (Figure 2.2). Eight sampling sites represented high organic enriched area with mussel raft culture area and domestic waste effluents and low organic contents area where coastal activities were less and away from mussel raft culture. Based on the sediment quality standards of the Land Development of Thailand, an organic content value exceeding 4% is highly organic enriched. Thus high organic content would indicate organic enriched conditions in the sediment, although in open coast and sandy area, the range of oxygen content may not be low but the sulfide content could be high making it a high organic content area. Data collected from April to October represented the wet season, while data from November to March represented the dry season.

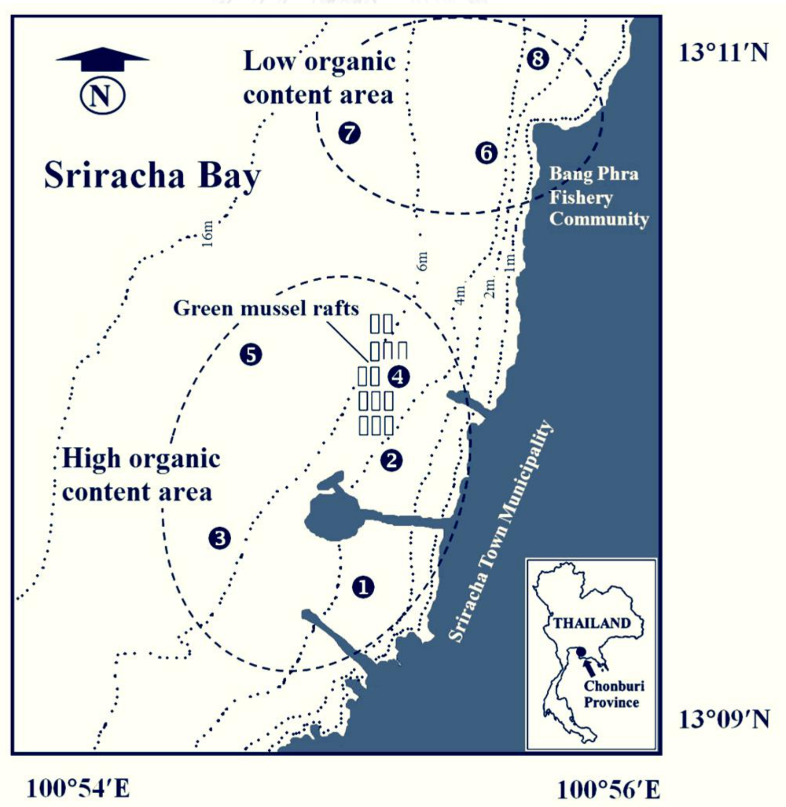

Figure 2. 2Study sites in Sriracha Bay, Chonburi Province, eastern coastline of Thailand.

Station 1, 2, 3, 4 and 5 were classified the areas of high organic content, with mussel raft culture installations and the presence of municipality waste effluents. Station 1 and 2 were located near shore at 500 m from shore with 2-5.8 m depth, near Sriracha Town Municipality. Another nearshore Station 4 was at 2-5.5 m depth, located in the green mussel raft area. Sediment in these stations were black loamy sand with sulphide odor. Two offshore station about, 1,500 m from shore, Station 3 and 5 were located at 4-7.7 m depth. Sediment in two offshore stations were green black sandy loam sediment with strong odor of sulphide. due to organic enrichment from traditional green mussel bamboo poles culture. Sediment in these sites were mainly characteristic by very light fine sediment, easily resuspended into the water column. Station 6 and 8 were located nearshore at 0.6-4.8 m depth, near Bang Phra fishery community. Station 7 was located at 0.5-5.2 m depth, far from the fishing community. These stations were classified the areas with low organic content, at a distance from mussel raft culture and where human coastal activities are reduced. Sediment in these sites were coarse sand to fine sand.

2. Distribution and Abundance of Spionid Polychaetes

Specimens of spionid polychaetes *Prionospio* were collected from 8 stations quantitatively during April 2013 to March 2014. Three replicates were taken at each sampling site, using a Ekman grab (0.0225 m^2). Samples were sieved through a 0.5 mm mesh and fixed in 10% buffered formalin for later sorting and identification of the fauna. An additional sediment sample was also taken at each site to analyze total organic matter and particle size composition. The total organic matter (TOM) was determined by the loss on ignition (Nelson, 1982). The percent silt-clay in sediment samples was calculated by dry sieving using a 63 µm sieve to separate the coarse (sand) and fine (silt-clay) fractions, which were then dried at 105℃ for 24 h, weighed, and expressed as percent of the initial weight (Buchanan, 1971). Salinity, temperature, pH and dissolved oxygen were measured *in situ* at the bottom water with DO meter (YSI Model 57 Oxygen Meter).

3. Comparative Study of Morphological Adaptation on Respiratory Structure

3.1 Study areas and collection of adult spionid specimens

Specimens of spionid polychaetes were collected from both areas of high organic content and low organic content in Sriracha Bay, Chonburi Province. Samples were taken by Ekman grab and sorted through a 0.5 mm mesh sieve, and fixed in 10% formaldehyde buffer with seawater.

3.2 Morphological characteristics of respiratory structures

Spionids of the genera *Prionospio* and *Paraprionospio* were identified from the specimens following the procedures of M Imajima (1990a, 1990b) M. Imajima (1990) and Yokoyama (2007), respectively. The width of the 5th chaetiger including parapodia in the spionid specimens were measured under a stereo-microscope with an ocular micrometer and expressed as body size.

In the genus *Prionospio*, the length of each branchial pair and the length of the shortest and longest pinnules were measured using an ocular micrometer. Counts were made of the number of digitiform branchial pinnules for branchial pairs 1 and 4 in *P. membranacea* and for branchial pairs 1 and 2 in *P. sexoculata*. Values are expressed as the mean number of pinnules per branchia. Figure 2.3 showed the measurement of the length of branchial pairs and number of branchial pinnules.

In the genus *Paraprionospio*, the length of each branchial pair was also measured, and the number of branchial pinnules that are lamellar plates in branchial pair 1-3 was counted and expressd as the mean number per one branchia.

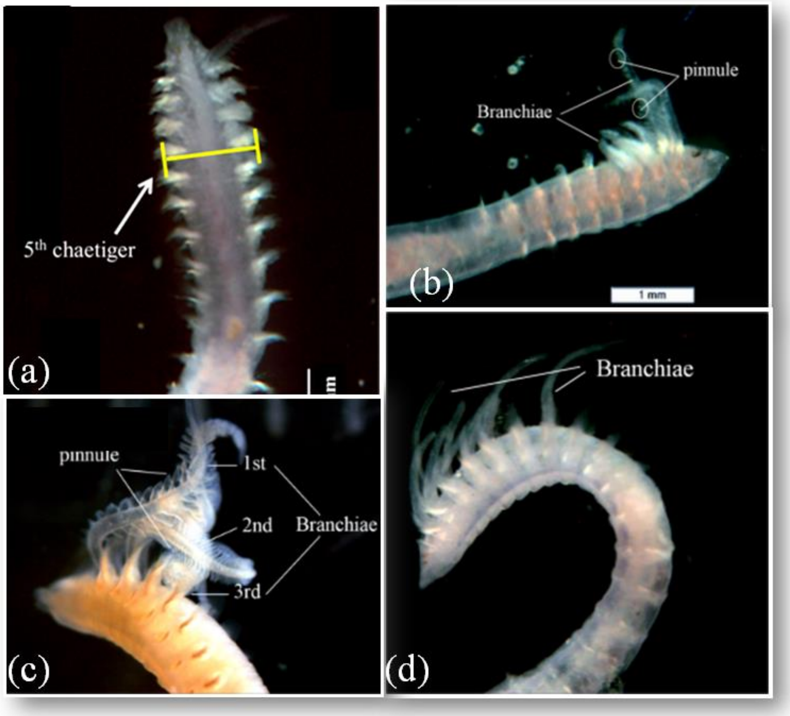

Figure 2. 3Measurement of the length of each branchial pair and the number of branchial pinnules in spionids of the genera *Prionospio* and *Paraprionospio*. (a) width of the 5th chaetiger (b) branchiae and pinnules in *Prionospio (Prionospio) membranacea* (c) branchiae and pinnules in *Paraprionospio* (d) apinnate branchiae in *Prionospio (Minuspio) pulchra*.

3.3 Morphological characteristic of respiratory structures in larvae and juvenile of spionid polychaetes

In order to determine whether the organic enriched condition affected the morphological differences in respiratory structure at very small size stages of polychaetes, specimens of larvae and juveniles of *P. membranacea* and *P. pulchra* were used from the experiments on the effect of organically enriched sediment on reproductive and developmental patterns in spionid, *Prionospio*. The mature females and males were selected under a stereo-microscope from the acclimated polychaetes in the aquaria. Fifty mature individuals of spionids, 25 of females and 25 of males were used in the the induced spawning experiment. These mature spionids were introduced into a 500 ml beakers already filled with 100 ml filtered seawater. The 50 ml of freshwater was then added into the beaker to induce the sudden salinity change. Adult spionids released gametes into water column where fertilization and larval development took place within 24 hrs. In order to harvest newly hatched larvae for culture, the beaker was decanted through a 63 μm mesh.

The newly hatched larvae were added to the two set of cultured boxes (33x42x28cm) at the density of 30 ind. These culture boxes were with treated sediment of different organic condition at the level of 3 cm form bottom and the filtered seawater level 10 cm above the surface sediment. The filtered seawater in each box was gently aerated to create the resuspension in form of cloudy snowball in the water column. Two different levels of organic conditions were used in rearing these larvae: (1) low organic content in the sediment (2.75%TOM) and (2) high organic content in the sediment (6.17%TOM). Each treatment were with 4 replications. The planktonic stage larvae were sampling by using 63 μm hand net for the assessment on the development of respiratory structure. The larval settiement stage/ early benthic stage juveniles were sampled by using a 2.5 cm diameter plastic hand core. The length of each branchial pairs and the number of branchial pinnules in the larval stages were measured and counted under the stereo-microscope.

4. Data Analyses

The comparison of abundance of spionids among sampling sites and their seasonal variation, were tested using a one way analysis of variance (ANOVA). The Pearson Correlation between the environmental parameters and abundance of spionid polychaetes were analysed. The correlation coefficient were compared at significant level of p<0.05. Differences among sites and season were analysed with cluster analysis and multidimensional nonparametric scaling ordination (MDS) based on Bray-Curtis similarities to determine the population structure of spionid polychaetes in Sriracha Bay.

The relationships between the length of branchial pairs and body size, and between the number of branchial pinnules and body size were analyzed using linear regression. The length of branchial pairs and number of branchial pinnules of *Prionospio* of the same size range from the two sites were compared using a t -test (Zar, 1999).

D. Results and Discussion

1. Distribution and Abundance of Spionid Polychaetes in Sriracha Bay

1.1 Environmental factors

Differences in temperature, salinity, dissolved oxygen among sites were minimal (Table 2.2). However water depth, the silt-clay fraction in the sediment, and total organic matter varied strongly with sites and season. Water depths in the high organic content area were higher than those in the low organic content area. . Water depths in both areas increased during the dry season as in Figure 2.4. Silt-clay fraction in the sediment was statistically significant difference between high and low organic content areas (p<0.05). However the seasonal differences in the silt-clay fractions were minimal. The nearshord sites, station 1, 2 and 4, in the high organic content area were mostly loamy sand. The silt-clay fraction were high in the offshore stations of station 3 and 5. Sediment in these area was sandy loam in both season. The sediment was very fine greenish black sandy loam with sulfides odor. During the dry season, the silt-clay content increased in the high organic content in November in particular station 5 offshore. This was due to the land reclamation for public park in the Sriracha Municipality. Silt-clay fractions were low in the low organic content areas, station 6, 7 and 8. Sediment in the low organic area was mostly sand. These sites were far from mussel raft culture and coastal activities.

Table 2. 2Environmental characteristics of the two sampling areas in Sriracha Bay, during April 2013 to March 2014.

Habitat characteristics	High organic content area		Low organic content area	
	Wet season	**Dry season**	**Wet season**	**Dry season**
Sediment type	loamy sand-	loamy sand-	sand	sand
Silt-clay (%)	21.42±4.67	22.89±1.47	0.79±0.40	1.03±0.43
Total organic matter (%)	6.94±0.48	6.88±0.32	3.02±0.48	2.54±0.53
Dissolved oxygen (mg L^{-1})	5.09±0.29	5.71±0.06	5.15±0.38	5.73±0.87
Salinity (psu)	29.18±0.18	32.80±0.00	29.26±2.86	32.80±1.10
Temperature (°C)	30.31±0.11	28.60±0.08	30.72±1.39	28.65±0.74
pH	8.11±0.15	8.12±0.01	8.11±0.28	8.13±0.09
Water depth (m)	4.07±0.18	5.81±0.11	1.90±0.84	3.58±0.63

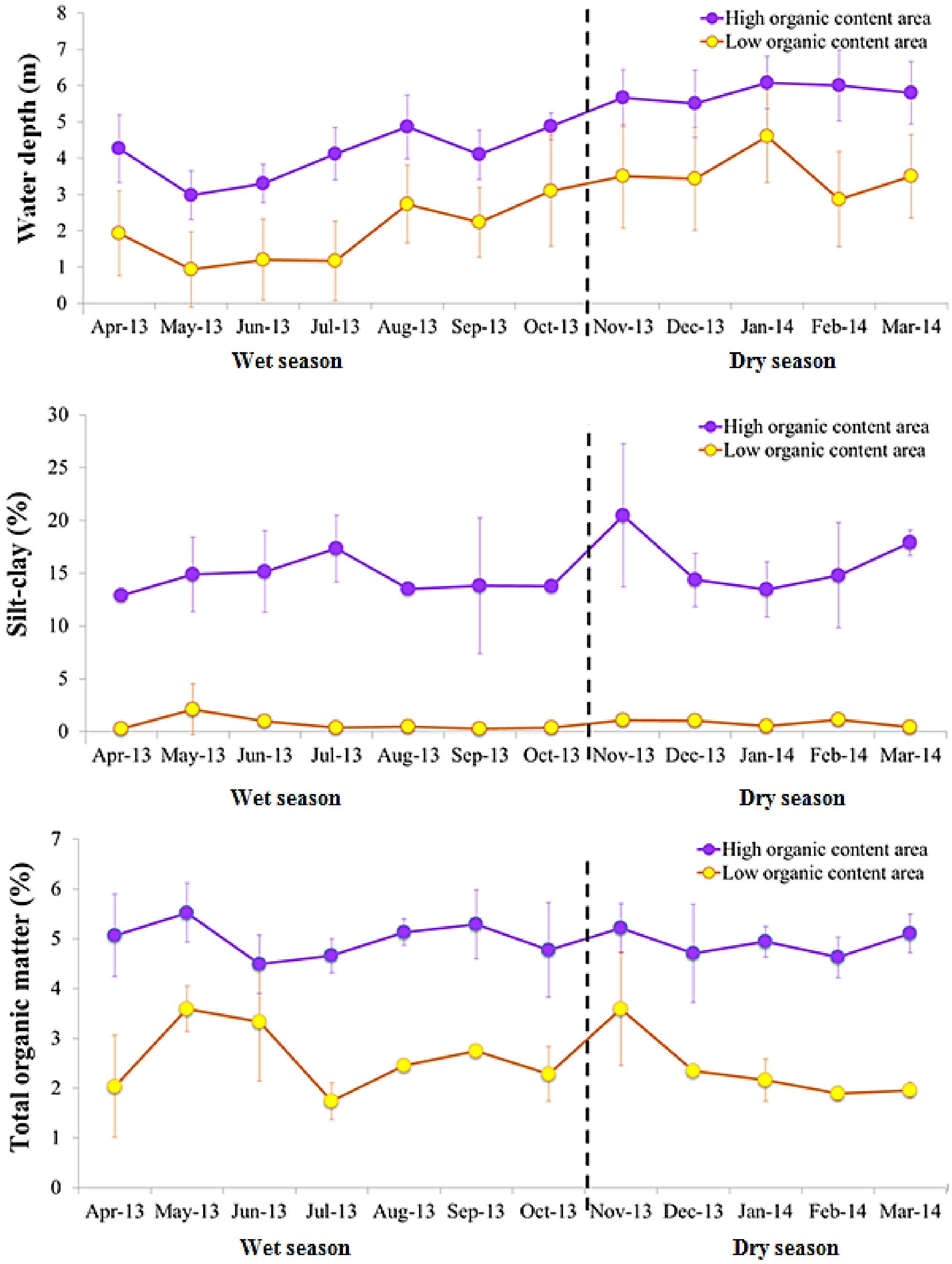

Figure 2. 4Seasonal variations in water depth, particlesize composition and total organic matter in Sriracha Bay, Chonburi Province from April 2013 to March 2014.

The total organic matter showed the positive relationship with the silt-clay fraction. The total organic matter in the high organic area were in the range of 3.85-12.36%. The area under the mussel raft culture, station 4 was enriched with the total organic matter of 4.01-5.04%. Total organic matter in sediment were high in the offshore area of station 3 and 5 in the range of 6.28-10.85 and 9.00-11.77%. The enriched sediment in these area was fine greenish black sandy loam with sulphide odor. This was due to the effect of traditional mussel cultures on bamboo poles. The low organic matter in the range of 1.31-5.19% observed in the low organic content area at stations 6, 7 and 8. Mussel culture is known to have a severe local environmental impact due to increased sedimentation of biodeposits (feces and pseudo feces) contributing to organic enrichment in the sediment inside the farming area (M. Carlsson et al., 2009; Christensen et al., 2003; Grant et al., 1995; Kaspar et al., 1985; Ysebaert et al., 2008) showed the mud fraction in the sediment underneath and surrounding bottom and suspended mussel cultures greatly elevated. In Sriracha Bay, Intarachart (2008) reported the total organic content in the area of green mussel raft culture was in the range of 3.64-10.50%. The total organic matter in the offshore area of previously traditional mussel cultures on bamboo poles was in the range of 10.19-14.7%.

The environmental parameters in Sriracha Bay, Chonburi Province were within the Thailand National Water Quality Classification for aquaculture purposes as in Figure 2.5. Dissolved oxygen content were not statistically significant between high and low organic content area. The similar trend showed for salinity, temperature and pH. However seasonal variations in environmental parameters were observed. The dissolved oxygen content were in the range of 3.64-6.16 mg/l during wet season and 4.80-7.80 mg/l during dry season in the high organic content area.

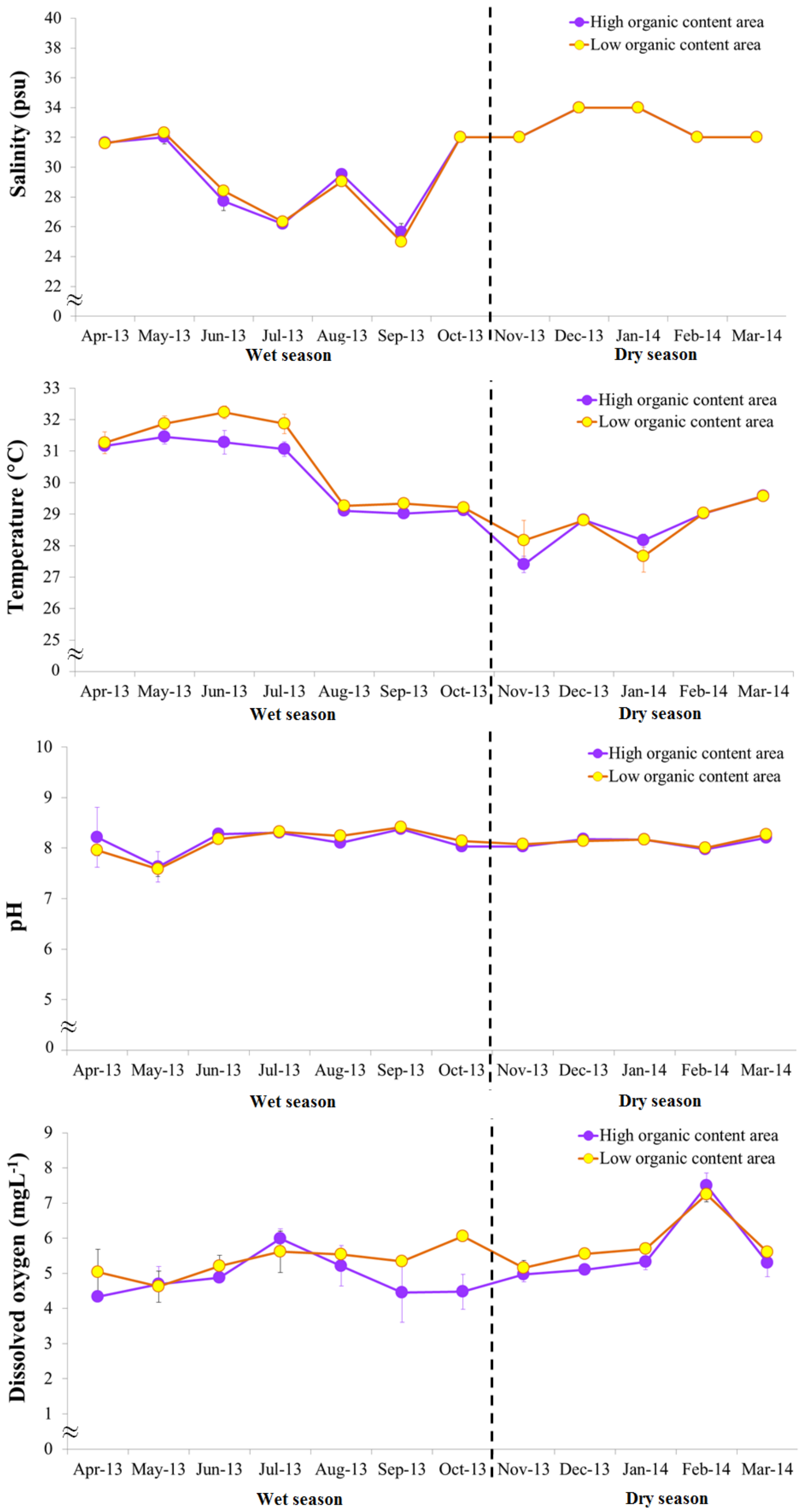

Figure 2. 5Seasonal variations in environmental parameters in Sriracha Bay, Chonburi Province from April 2013 to March 2014.

In the low content area, the dissolved oxygen content in the wet season were in the range of 4.01-6.14 mg/l. High dissolved oxygen content observed in the dry season in the range of 4.7-7.60 mg/l. Salinity variations between the two area were minimal. High salinity observed during the dry season corresponding to the increased water depth. The temperature variations between the two areas followed the same trend as the salinity. However low temperature observed during the dry season. pH values showed minimal differences among sites and season.

1.2 Species composition and abundance

Six species of spionid polychaetes, *Prionospio* and *Paraprionospio* were found in the study sites of Sriracha Bay, including *Prionospio (Prionospio) membranacea* Imajima, 1990*, Prionospio (Minuspio) pulchra* Imajima, 1990, *Prionospio (Aquilaspio) sexoculata* Augener, 1918*, Prionospio (Minuspio) multibranchiata* Berkeley, 1927, *Prionospio (Minuspio) japonica* Okuda, 1935 and *Paraprionospio inaequibranchia* (Caullery, 1914) (Figure 2.6). *P. membranacea* dominated the high organic content area characterized by mussel raft culture and effluents from Sriracha Municipality. This species comprised 70% of the spionids found in the area. *P. pulchra* and *P. sexoculata* were the second and third commonest species in term of abundance (Table 2.3).

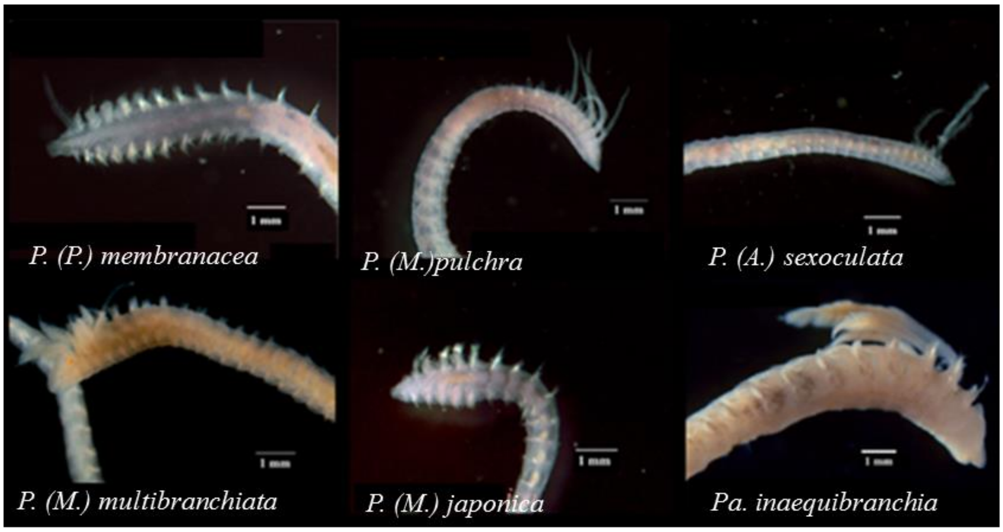

Figure 2. 6Spionid polychaetes in Sriracha Bay, Chonburi Province.

Table 2. 3Abundance (average number of individuals $m^{-2} \pm$ SD) of spionid polychaetes in Sriracha Bay, Chonburi Province.

Spionid polychaetes	High organic content area		Low organic content area	
	Wet season	Dry season	Wet season	Dry season
P. membranacea	246±130.28	29±19.78	28±19.03	0±0.00
P. pulchra	58±30.28	12±11.51	20±43.53	3±7.45
P. sexoculata	24±31.25	12±13.37	34±29.13	13±19.18
P. multibranchiata	5±4.38	0±0.00	2±3.19	0±0.00
P. japonica	4±3.78	0±0.00	0 ± 0.00	0±0.00
Pa. inaequibranchia	0±0.00	0±0.00	2±4.07	0±0.00

Spionid polychaetes abundance showed significantly difference between high and low organic content areas ($p<0.05$), and between wet and dry season ($p<0.05$). Density of spionids in high organic content area was higher than low organic content area due to abundance of dominant spionid, *P. membranacea.* Clear relationships between the silt-clay fractions and the total organic matter in the sediment and the *P. membranacea* distribution have been demonstrated as in Table 2.4. From this study, temperature was the major factor determining the distribution of *P. pulchra*. This was also true for the distribution of other spionids, *P. multibranchiata*, *P. japonica and Pa. inaeqnibranchia*. Salinity showed the inversed relationship with the density of *P. multibranchiata* and *P. japonica*. Spionid polychaetes in Sriracha Bay were more abundant in the wet season, where high temperature and low salinity observed.

Table 2. 4Pearson Correlation test between environmental parameters and the spionid polychaetes density. Significant difference (p<0.05).

	Temp.	Sal.	pH	DO	Depth	TOM	Silt-clay
P. membranacea	0.048	-0.349	0.053	-0.215	0.162	0.531*	0.513*
P. pulchra	0.398*	-0.378	0.007	-0.252	-0.170	0.336	0.270
P. sexoculata	-0.003	-0.230	0.240	-0.148	-0.085	0.033	0.015
P. multibranchiata	0.465*	-0.534*	0.034	-0.048	-0.173	0.271	0.269
P. japonica	0.060*	-0.586*	0.369	-0.081	0.101	0.324	0.368
Pa. inaequibranchia	0.399*	0.136	-0.545*	-0.208	-0.457*	-0.270	-0.287

Prionospio (Prionospio) membranacea

Spionid polychaetes abundanace in Sriracha Bay were shown in Figure 2.7 for *P. membrancea, P. pulchra, P. sexoculata, P. multibranchia* and *P. japonica.* The most abundant species in the area was *P. membrancea*, which widely distributed in the area except for the offshore station 5 as in Table 2.5. Maximum abundances were observed during the wet season from July to October in particularly in the mussel raft area of station 4 and nearshore area of station 1 and 2.

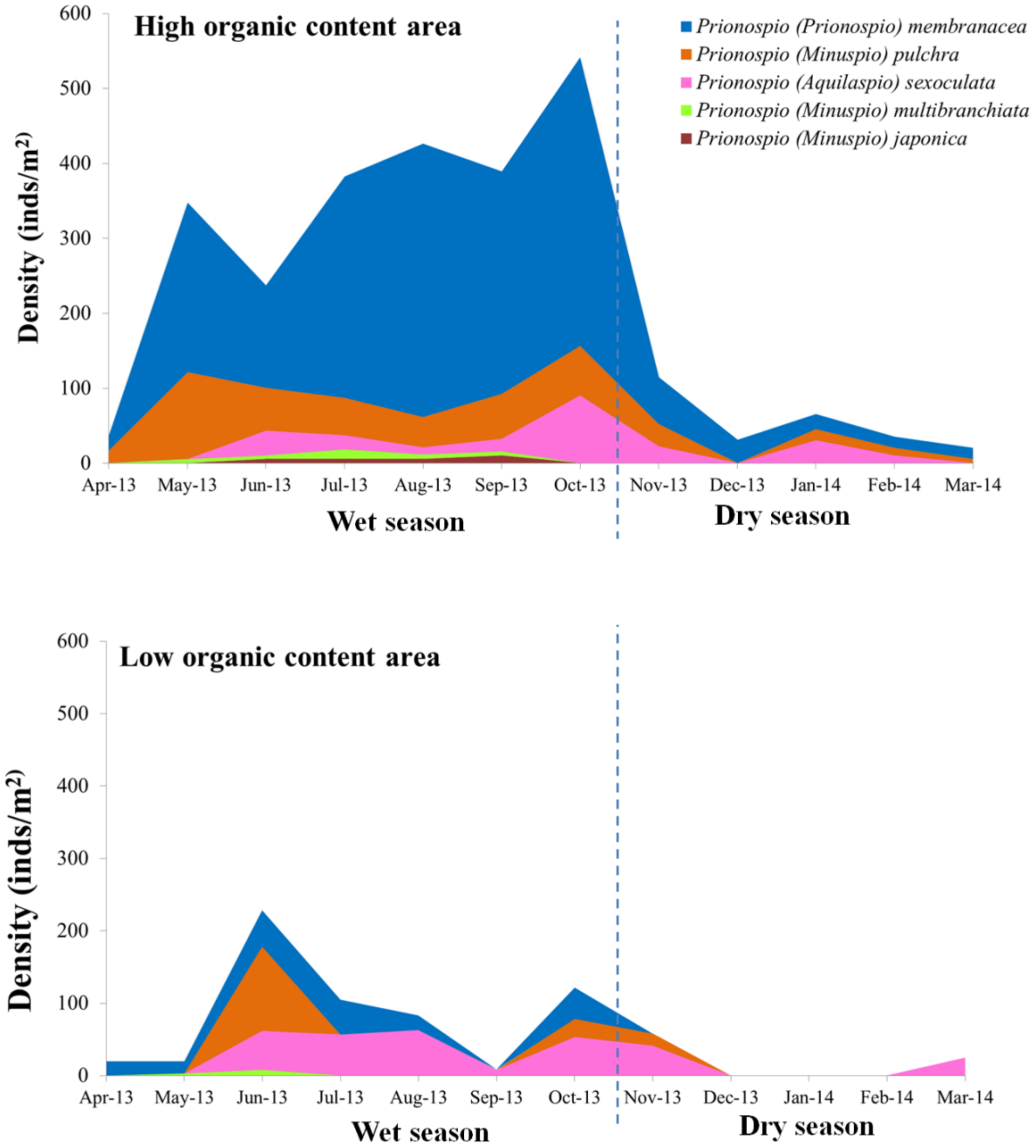

Figure 2. 7Seasonal variations in the abundance of the spionid polychaetes in Sriracha Bay, Chonburi Province.

Table 2. 5 Distribution and Abundance (average number of individuals m^{-2}± SD) of spionid polychaetes in Sriracha Bay, Chonburi Province.

	High organic content area					**Low organic content area**		
	Station1	**Station2**	**Station3**	**Station4**	**Station5**	**Station6**	**Station7**	**Station8**
Wet season								
P. membranacea	361	286	144	442	0	29	32	24
	(219.78)	(218.83)	(171.68)	(338.30)	(0.00)	(36.25)	(31.34)	(31.55)
P. pulchra	169	41	0	62	18	36	25	0
	(61.06)	(31.15)	(0.00)	(139.76)	(47.25)	(94.49)	(43.30)	(0.00)
P. sexoculata	24	57	25	15	0	46	46	9
	(34.97)	(88.83)	(66.14)	(14.14)	(0.00)	(40.28)	(57.40)	(22.68)
P. multibranchiata	19	6	0	0	0	5	0	0
	(12.82)	(15.12)	(0.00)	(0.00)	(0.00)	(9.57)	(0.00)	(0.00)
P. japonica	11	7	0	0	0	0	0	0
	(13.36)	(12.20)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Pa. inaequibranchia	0	0	0	0	0	7	0	0
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(12.20)	(0.00)	(0.00)
Dry season								
P. membranacea	50	83	0	10	0	0	0	0
	(17.68)	(64.09)	(0.00)	(22.36)	(0.00)	(0.00)	(0.00)	(0.00)
P. pulchra	50	10	0	0	0	0	10	0
	(53.03)	(13.69)	(0.00)	(0.00)	(0.00)	(0.00)	(22.36)	(0.00)
P. sexoculata	32	30	0	0	0	5	35	0
	(46.04)	(27.39)	(0.00)	(0.00)	(0.00)	(11.18)	(54.77)	(0.00)
P. multibranchiata	0	0	0	0	0	0	0	0
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
P. japonica	0	0	0	0	0	0	0	0
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Pa. inaequibranchia	0	0	0	0	0	0	0	0
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)

Note.-The numbers in the parentheses are standard deviation (±SD).

Clear relationships between the silt-clay fractions and the total organic matter in the sediment and the *P. membrancea* distribution. This species was found abundant in high organic content area as compared to lower density observed in low organic content area. However, *P. membrancea* was not found in station 5, the offshore area in the high organic content area where enriched condition was observed. Other polychaetes were not recorded from this station except for *P. pulchra* only in the month of October. Such unexpected trend could have been possible caused by the physical characteristics of the sediment in this station being very fine greenish black sandy loam with sulphide odor. Larval habitat selection maybe affected. The presence of some pollutants might have reduced the number of larval metamorphosis in the sediment such as the presence of higher concentration of hydrogen sulphide (Thiyagarajan et al., 2005). Although the absolute hydrogen sulphide concentration at this station had not been measured, but, Intarachart (2008) reported the AVS sulphide concentrations in the nearshore mussel raft culture of 0.15-0.45 mg/g and the offshore in the mussel culture on bamboo poles of 0.13-0.27 mg/g. High concentration of ammonia also observed in the area. In this study, the experiments on the effect of organically enriched sediment on the larval development also support this. The lower percentage of metamorphosed and competent larvae of *P. membrancea* as well as *P. pulchra* were observed in the low and enriched organic sediment as compared to the high organic sediment treatment. The percentage of metamorphosed larvae of *P. membrancea* in the low organic, high organic and enriched sediment treatments were 42, 68 and 64%. The percentage of competent larvae of *P. membrancea* in respective treatments were 31, 60 and 55%. Similar trend was observed for *P. pulchra*. Water depth in this station of 4.5-7.7 m. may also hindered the larval dispersal and settlement of coastal larvae due to short planktonic duration. The planktonic phase in *P. membranacea* as revealed from this study was only 8-14 days. The metamorphosed larvae need to swim closed to the bottom surface to explore for places to settle.

In Kung Krabaen Bay, Chantaburi Province, eastern coastline of Thailand, *P. membrancea* was less common spionid polychaetes found at offshore stations. They were reported from very fine sand to sandy loam area with the total organic matter in the range of 0.83-2.19% (Chatananthawej, 2001). In Pak Panang Bay, Nakhon Si Thammarat Province, southern Thailand, *P. membrancea* the rare species in the area, was reported from the degraded mangrove forest in the shrimp farm area. The sediment was loam to sandy clay loam with the high organic content in the range of 4.05-5.915 (Nootcharoen, 2009).

Prionospio (Minuspio) pulchra

This species was the second most abundant species in the area. It can be found distributed in the high organic content area except for the offshore station 3. High abundance were observed at the nearshore station 1 and the mussel raft culture area-station 4. In low organic content area, this species was found only at station 6 and the offshore station 7. *P.pulchra* can not be found at station 8. Other spionids than *P. membrancea* and *P. sexoculata*, recorded in the wet season, also absent from this station. Station 8 was the nearshore station in the vicinity of the Bang Phra fishery community. The sediment was mainly coarse sand with the total organic matter of 1.31-2.46%. The abundance of this species in the wet season was higher than in the dry season in both areas of high and low organic. The density of *P. pulchra* showed the close correlation with water temperature. M Imajima (1990b) reported that *P. pulchra* was found in intertidal to 67 m in Japan. Kinoshita et al. (2008) *P. pulchra* in hugh organic content sediment of fish farm in Kusuura Bay, Japan. Chatananthawej (2001) also reported *P. pulchra* from the offshore areas in Kung Krabaen Bay Chantaburi Province same as *P. membranacea*

Prionospio (Aquilaspio) sexoculata

This spionid polychaete was the third most abundant species in Sriracha Bay. The abundance of *P.sexoculata* in the wet season was higher than those in the dry season both in the area of high and low organic. In the area of high organic content, this species was found in abundance ranging from 25-250 ind.m^{-2} during the density of *P. sexoculata* ranged from 25-120 ind.m^{-2} in the low organic content area. M Imajima (1990a) reported thedistribution of *P. sexoculata* from South West Africa and Japan, in intertidal to 20 m. In Kung Krabaen Bay, Chantaburi Province Thailand, *P. sexoculata* was found in the area of total sediment of 2.71-3.69% (Chatananthawej, 2001).

Prionospio (Minuspio) multibranchiata

The less common spionid can be found in both high organic content area and low organic content area *P. multibranchiata* was found only in wet season, from May to September in the nearshore station 1 and 2 in the high organic area and at station 6 in the low organic area. M Imajima (1990b) reported the distribution of *P. multibranchiata* from Vancouver Island, Gulf of Mexico, Florida in North America to Japan. They were found from the intertidal to 83 m. This species was low in density at Kung Krabaen Bay, Chantaburi Province, where sediment type was sandy loam with 2.51% organic content (Chatananthawej, 2001). Nootcharoen (2009) also reported the distribution of *P. multibranchiata* in Pak Phanang Bay, Nakhon Si Thammarat Province in low organic content sediment ranging from 0.76-2.84%. The sediment type was sandy clay to sandy loam.

Prionospio (Minuspio) japonica

Although *P. japonica* has been proposed as the indicator species for moderated organic enrichment area in Thai waters as the species was dominant species in high organic sediment (2.71-3.69%) of Kung Krabaen Bay, Chantaburi Province (Chatananthawej, 2001) and Pak Phanang Bay, Nakhon Si Thammarat Province in the organic sediment of 2.00-3.14% (Nootcharoen, 2009). This species was found only in the nearshore station of 1 and 2 in high organic content area and the nearshore station 6 in the low organic area in Sriracha Bay. They were found only the month of May to September in the wet season. In Japan, M Imajima (1990b) reported the distribution of *P. japonica* from intertidal area to the depth of 5 m in the brackish area.

Paraprionospio inaequibranchia

Pa. inaequibranchia was rare in terms of abundance in Sriracha Bay, only at sandy area of station 6, located in the low organic content area during April and May in the wet season. In Upper Gulf of Thailand, Paphavasit (2006) found that *Pa. inaequibranchia* (reported as *Paraprionospio* sp.) was most abundant in coastal area of Samut Sakhon and Samut Songkharm Province. In Samut Sakhon, their abundant was 1,164 inds.m^{-2} in enriched organic mud sediment of 7.45%.

1.3 Population structure of spionid polychaetes in Sriracha Bay

MDS ordination revealed three significantly different groups of spionids (Figure 2.8, Table 2.6). The first grouping (Group I) consisted of species from nearshore stations in the high organic content area, near Sriracha town municipality (station 1 and 2) and the green mussel raft area (station 4) at a similarity level of 72.25%. The percent silt-clay in sediment ranged from 5.93-40.91%, and total organic matter from 3.84-12.36%. The dominant spionid species of this group was *P. membranacea* about 72.54% of total abundance and followed by *P. pulchra* (16.36%) and *P. sexoculata* (8.53%), respectively.

The second group (Group II) was represented by in high organic content area of offshore stations (stations 3 and 5), where the sediment in these areas were deep black and smelly sediment to organic enrichment from traditional green mussel bamboo poles culture. Silt-clay fraction and total organic matter in sediment were high ranging from 3.01-61.57% and 4.01-11.77%, respectively. The dominant spionid polychaetes was *P. membranacea* about 71.01%, followed by *P. pulchra* (9.58%).

The third group was represented by nearshore stations in the low organic content area in the vicinity of Bang Phra fishery community (stations 6 and 8) and the offshore station 7. This group had a similarity level of 45.31%. The percent silt-clay and total organic matter in sediment were low ranging from 0.07-3.80% and 1.31-5.19%, respectively. The dominant spionid of group II was *P. sexoculata* contributing 43.96% and followed by *P. membranacea* (28.88%) and *P. pulchra* (23.06%), respectively.

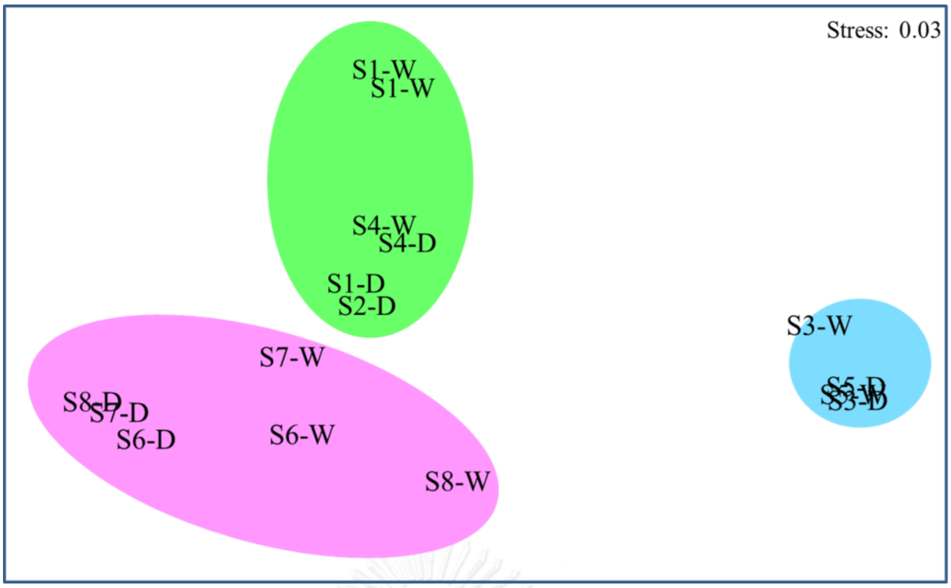

Figure 2. 8Multidimensional scaling (MDS) ordination plot of the spionid polychaetes cluster groups in Sriracha Bay, Chonburi Province.

Table 2. 6Population structure of spionid polychaetes in Sriracha Bay, Chonburi Province.

Sites	Dominant species	Environmental parameters
Group I (S1, S2 and S4)	*P. membranacea* (72.54%) *P. pulchra* (16.36%) *P. multibranchiata* (1.48%) *P. japonica* (1.09%) *P. sexoculata* (8.53%)	Silt-clay= 5.93-40.91% TOM = 3.84-12.36% DO = 3.64-7.80 mg L^{-1} Salinity = 25.20-4.00psu Temperature = 27.31-31.60°C pH = 7.42-8.43 Water depth = 2.10-7.30m
Group II (S3 and S5)	*P. membranacea* (71.01) *P. pulchra* (9.58%)	Silt-clay= 3.01-61.57% TOM = 4.01-11.77% DO = 4.38-7.60 mg L^{-1} Salinity = 25.20-34.00psu Temperature = 27.50-31.90°C pH = 7.40-8.90 Water depth = 2.10-7.70m
Group III (S6, S7 and S8)	*P. membranacea* (28.88%) *P. pulchra* (23.06%) *P. multibranchiata* (1.70%) *P. sexoculata* (43.96%) *Pa. inaequibranchia* (2.42%)	Silt-clay= 0.07-3.80% TOM = 1.31-5.19% DO = 4.01-7.40mg L^{-1} Salinity = 24.90-34.00psu Temperature= 27.20-32.40°C pH = 7.48-8.42 Water depth = 0.50-5.20m

The results suggested the existence of three spionid groups: (1) high organic content area, near Sriracha town municipality and the green mussel raft area station (2) high organic content area of offshore station and (3) the low organic content area, probably due to the silt-clay fraction and total organic matter in sediment. Tamai (1985) stated that mud content and organic content were important factors affecting the distribution of marine deposit feeders. Mud content indicated hydrographical conditions, and total nitrogen content the indicator of organic materials in sediment which provided food for surface and subsurface deposit feeders. In addition, water depth is one of the most important factors affecting the distribution of coastal organisms due to the larval development and the duration of planktonic larval phase (Blake & Arnofsky, 1999; Lisa A Levin, 1984; Thiyagarajan et al., 2005).

2. Comparative Study of Morphological Adaptation on Respiratory Structures in Spionids Genus *Prionospio*

2.1 Morphological characteristics of respiratory structures in adult spionids

Prionospio (Prionospio) membranacea

This species has four pairs of branchiae on chaetigers 2-5: pairs 1 and 4 are pinnate with digitiform pinnules; and pairs 2 and 3 are apinnate. A morphological response to habitat was observed through the increased length of branchial pairs 1 and 4 in relation to their body size in specimens from high organic content (r=0.66, p<0.05, n=35; r=0.51, p<0.05, n=35, respectively). The length of branchial pairs 2 and 3 (which was approximately similar at 0.4 mm) was only weakly related to body size (r=0.44, p<0.05, n=35; r=0.46, p<0.05, n=35, respectively) as shown in Figure 2.9. In the low organic content area, increase in the length of branchial pairs 1 and 4 was significantly proportional to the increase in body size (r=0.83, p<0.05, n=30; r=0.74, p<0.05, n=30, respectively), while the length of branchial pairs 2 and 3 also increased with body size (r=0.85, p<0.05, n=30; r=0.84, p<0.05, n=30, respectively) as shown in Figure 2.9. A comparison of the length of each branchial pair in specimens from high organic content areas (n=35) indicates that branchial pairs 1 and 4 are significantly longer than pairs 2 and 3 by approximately 1.8 and 1.5 times, respectively (Figure 2.10); and pairs 1 and 4 were approximately twice as long in the low organic content area (n=30; Figure 2.10).

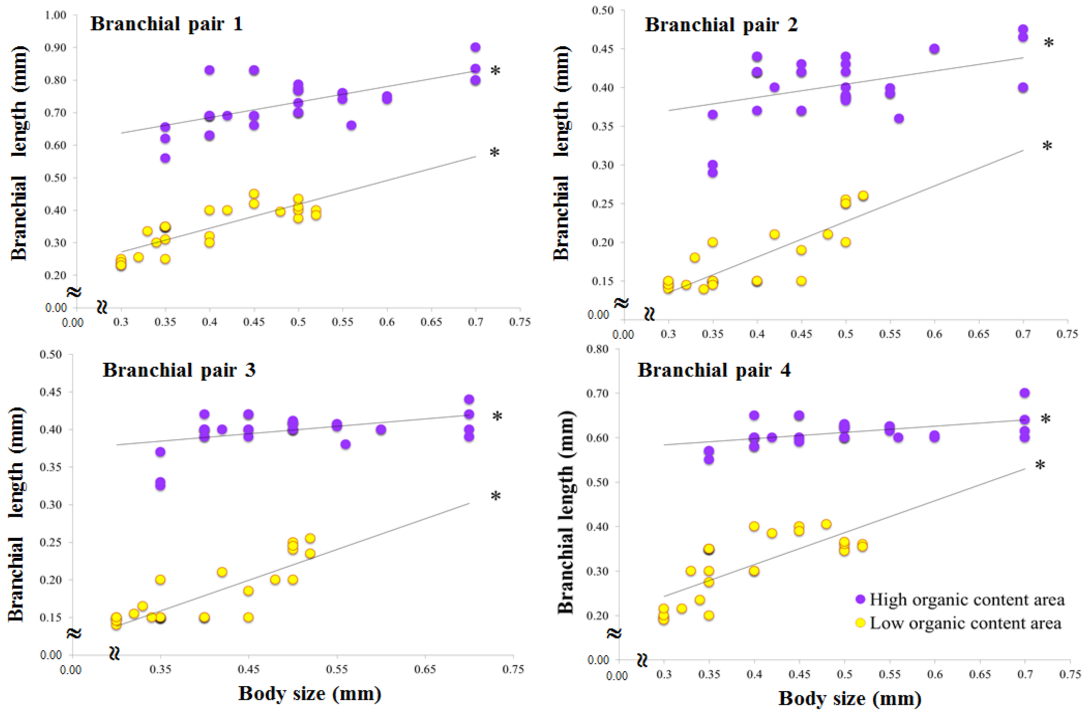

Figure 2. 9Length of branchial pairs according to body size in *Prionospio (Prionospio) membranacea* in areas of high and low organic content from Sriracha Bay, Chonburi Province. *Significant difference (p<0.05) between areas.

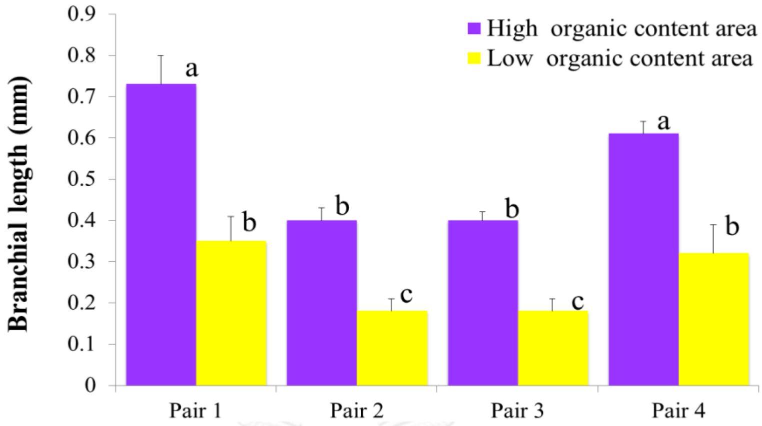

Figure 2. 10Mean length of branchial pairs in *Prionospio (Prionospio) membranacea* in areas of high and low organic content from Sriracha Bay, Chonburi Province. Different letters above bars indicate significant differences (p<0.05) within the same pair of chaetiger and between branchial length from different pair of chaetigers. Error bars represent SD.

The number of branchial pinnules in pairs 1 and 4 in specimens from high organic content increased with the body size (r=0.80, p<0.05, n=35; r=0.60, p<0.05, n=35, respectively) as shown in Figure 2.11. The number of branchial pinnules of pairs 1 and 4 in *P. membranacea* specimens from this low organic content area also increased in relation to body size (r=0.79, p<0.05, n=30; r=0.89, p<0.05, n=30, respectively) as shown in Figure 2.11. There were 31-54 branchial pinnules in pair 1 and 20-28 in pair 4 in specimens from the high organic content area; approximately double the number in the low organic content area, 18-30 and 9-16, respectively (Figure 2.12).

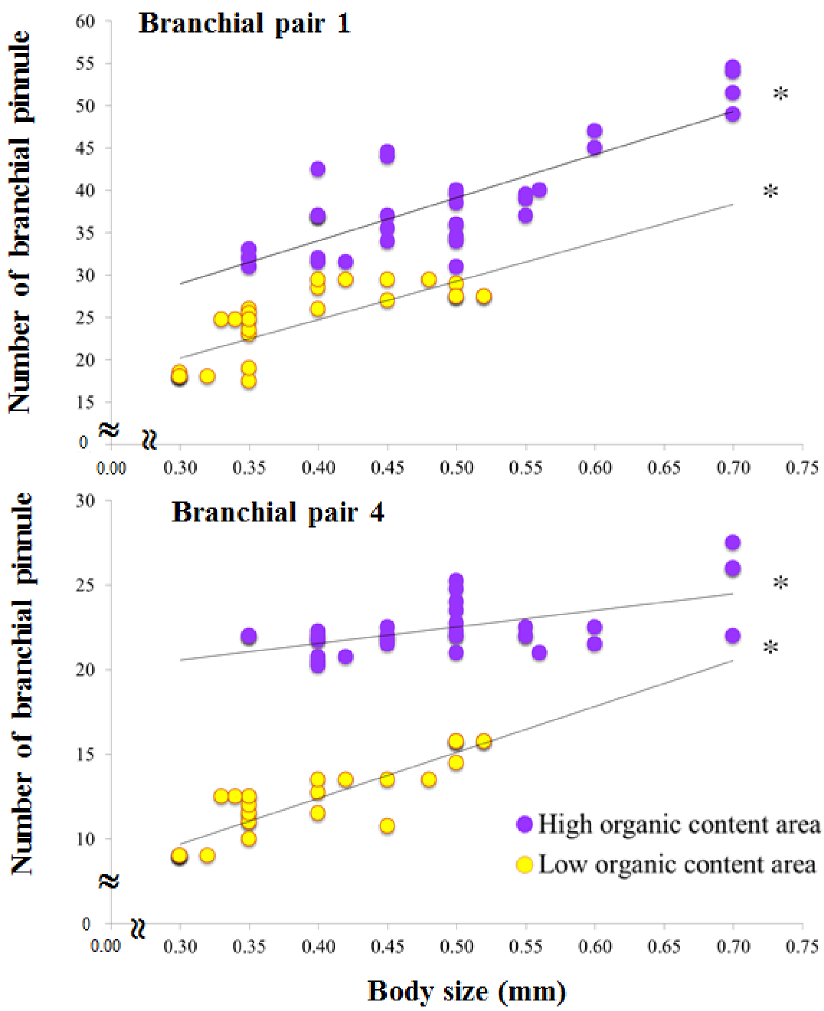

Figure 2. 11Number of branchial pinnules of pairs 1 and 4 in *Prionospi (Prionospio) membranacea* in areas of high and low organic content from Sriracha Bay, Chonburi Province. *Significant difference (p<0.05) between areas.

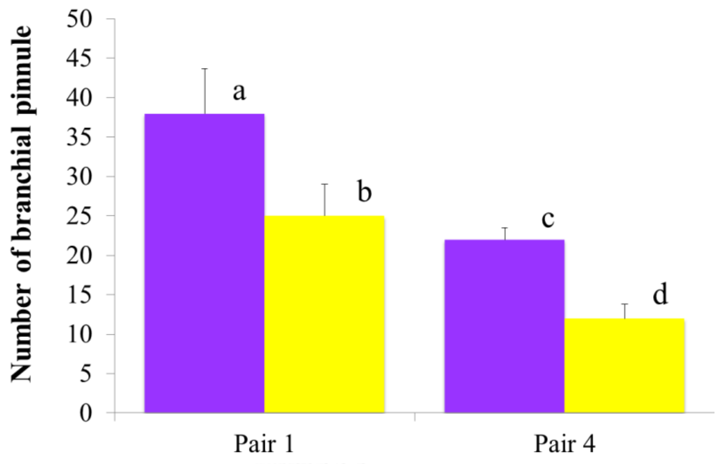

Figure 2.12Mean number of branchial pinnules of *Prionospio (Prionospio) membranacea* in areas of high and low organic content from Sriracha Bay, Chonburi Province. Different letters above indicate significant differences (p<0.05) within the same pair of chaetiger and between number of branchial pinnule from different pair of chaetigers. Error bars represent SD.

A comparison of the length of branchial pairs and the number of branchial pinnules in *P. membranacea* from the two areas in Sriracha Bay indicates that the length of branchial pairs 1, 2, 3 and 4 was significantly longer in specimens from high organic content area with the number of branchial pinnules of pairs 1 and 4 more numerous than those from the low organic content area (p<0.05). Moreover, the size and number also differed even at small-size stages which had the trendency to increase with size.

The arrangement of branchial pinnules of *P. membranacea* from high and low organic content areas in Sriracha Bay was the same, where the branchial pinnules were irregularly arranged from the brachial base up to the tip of branchiae. There were no differences in the length of branchial pinnules of the same body size (0.3-0.39, 0.4-0.49, 0.5-0.59 mm) of the specimens from these two areas at 0.01-0.03, 0.01-0.05 and 0.02-0.11 mm, respectively.

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The arrangement of branchial pinnules of *P. membranacea* from high and low organic content areas in Sriracha Bay was the same, where the branchial pinnules were irregularly arranged from the brachial base up to the tip of branchiae. There were no differences in the length of branchial pinnules of the same body size (0.3-0.39, 0.4-0.49, 0.5-0.59 mm) of the specimens from these two areas at 0.01-0.03, 0.01-0.05 and 0.02-0.11 mm, respectively.

Prionospio (Minuspio) pulchra

This species has apinnate cylindrical branchiae located from chaetiger 2. All specimens examined from the two study areas in Sriracha Bay had 9 pairs of apinnate branchiae. Length of the branchial pairs 1-9 in specimens (n=30) from the high organic content area was 0.53-0.98, 0.48-1.00, 0.48-0.99, 0.47-0.90, 0.43-0.85, 0.35-0.80, 0.30-0.70, 0.30-0.65 and 0.20-0.41 mm, respectively and in specimens (n=25) from the low organic content area, the length was 0.43-0.83, 0.33-0.88, 0.38-0.84, 0.43-0.81, 0.37-0.68, 0.29-0.63, 0.23-0.53, 0.20-0.43 and 0.20-0.33 mm, respectively. The lengths of branchial pairs 1, 2, 3 and 4 were longer than in pairs 5, 6, 7, 8 and 9 for specimens from the high organic content area by 1-3 times, and from the low organic content area by 1.0-2.8 times (Figure 2.13). The length of each branchial pair from high organic content area showed a positive correlation to the body size with correlation value (r) between 0.66 (length of branchial pair 8) and 0.85 (length of branchial pair 1) (p<0.05, n=30). In the low organic content area, the length of each branchial pair showed an increasing trend in relation to body size with a correlation value (r) between 0.83 (length of branchial pair 6) and 0.64 (length of branchial pair 9) (p<0.05, n=25).

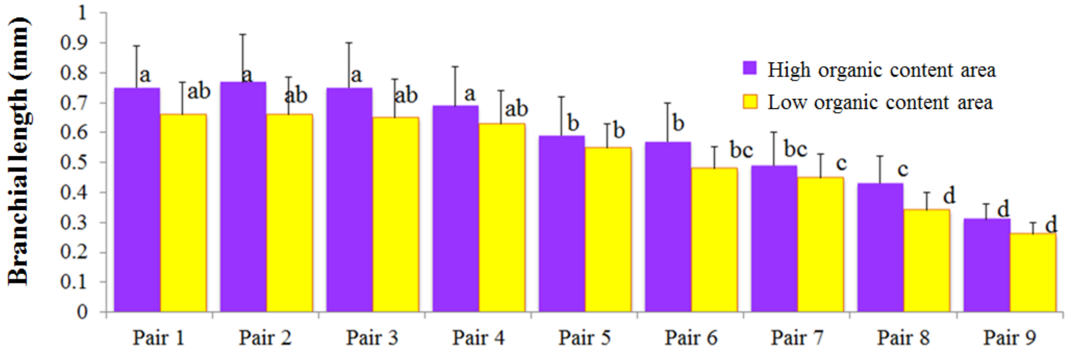

Figure 2.13Mean length of branchial pairs in two species of *Prionospio (Minuspio) pulchra* in areas of high and low organic content from Sriracha Bay, Chonburi Province. Different letters above bars indicate significant differences (p<0.05) within the same pair of chaetiger and between branchial length from different pair of chaetigers. Error bars represent SD.

Comparing the length of branchial pairs among specimens from the two sample areas, *P. pulchra* of the same body size of 0.3-0.39, 0.4-0.49 mm, 0.5-0.59 and 0.6-0.69 mm, the mean length of branchial pairs of specimens from the high organic content area (n=30) from 0.25±0.03 to 0.55±0.03, 0.29±0.02 to 0.76±0.08, 0.29±0.02 to 0.80±0.10 and 0.35±0.05 to 0.95±0.04 mm, was longer by about 1.08-1.41, 1.00-1.32, 1.05-1.38 and 1.16-1.37 times than that of those in the low organic content area (n=25) which was between 0.20±0.00 to 0.48±0.04, 0.25±0.05 to 0.59±0.06, 0.27±0.02 to 0.70±0.06 and 0.29±0.03 to 0.80±0.06 mm.

Prionospio (Aquilaspio) sexoculata

This species has two pairs of branchiae on chaetigers 2 and 3 with digitiform pinnules. The length of branchial pairs 1 and 2 of specimens (n=20) from the high organic content area was 0.50-1.55 and 0.30-0.70 mm, respectively. In specimens (n=3) from the low organic content area, the respective lengths of branchial pairs 1 and 2 were 0.35-0.60 and 0.21-0.30 mm. In the specimens from both study areas, the mean length of branchial pair 1 was approximately twice as long as pair 2 (Figure

2.14). Increases in length of branchial pairs 1 and 2 in the high (r=0.50, p<0.05, n=20; r=0.79, p<0.05, n=20, respectively) and low organic content areas (r=0.95, p<0.05, n=3; r=0.98, p<0.05, n=3, respectively) were proportionate to the increase in body size. This characteristic was also observed in the number of branchial pinnules in branchial pairs 1 and 2.

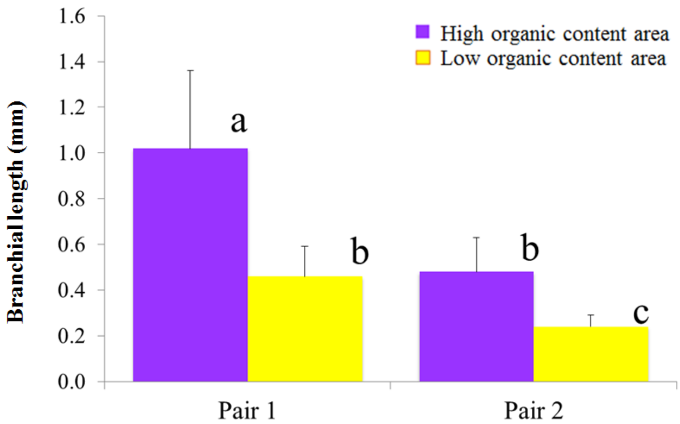

Figure 2.14Mean length of branchial pairs in *Prionospio (Aquilaspio) sexoculata* in areas of high and low organic content from Sriracha Bay, Chonburi Province. Different letters above bars indicate significant differences (p<0.05) within the same pair of chaetiger and between branchial length from different pair of chaetigers. Error bars represent SD.

The number of branchial pinnules of pairs 1 and 2 of specimens (n=20) from the high organic content area was 41-88 and 17-57, respectively; while for specimens (n=3) from the low organic content area, the respective numbers of branchial pinnules were 22-48 and 14-28. The mean number of branchial pinnules of pair 1 of specimens from both study areas showed that the number of branchial pinnules of pair 1 was more numerous than that of pair 2 by approximately two times (Figure 2.15).

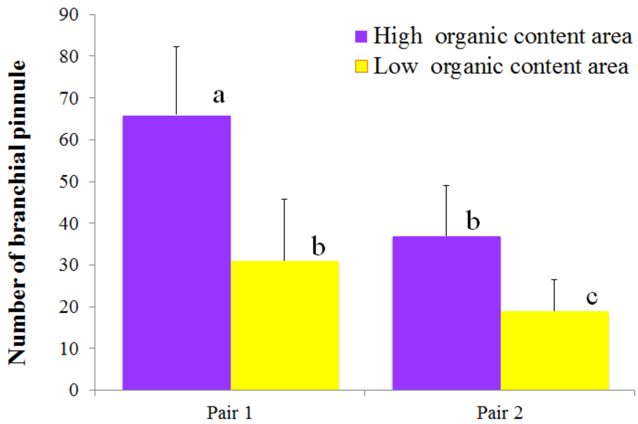

Figure 2.15Mean number of branchial pinnules in *Prionospio (Aquilaspio) sexoculata* in areas of high and low organic content from Sriracha Bay, Chonburi Province. Different letters above bars indicate significant differences (p<0.05) within the same pair of haetiger and between number of branchial pinnule from different pair of chaetigers. Error bars represent SD.

Specimens from the low organic content area were limited to only three individuals, which is insufficient for a statistical comparison of the branchial lengths of specimens from the two areas. However, for *P. (A.) sexoculata* of the same body size (0.4-0.49 mm), the mean length of branchial pairs 1 and 2 was 0.81±0.21 and 0.36±0.10 mm, respectively (n=9), in the high organic content area, longer by about 1.2-1.4 times those of specimens from the low organic content area (0.60±0.00 and 0.30±0.00 mm, respectively; n=1). The mean number of branchial pinnules of pairs 1 and 2 in species from the high organic content area (53±6.81 and 30±8.89, respectively; n=9) seemed to be slightly more numerous than those in low organic content area (37-57 and 25-33, respectively; n=1).

The branchial pinnules in specimens from the two study areas were similarly arranged from the branchial base up to the tip of the branchiae. With the two body sizes of 0.40-0.49 and 0.5-0.59 mm, the length of the branchial pinnules was 0.02-0.13 and 0.03-0.13 mm, respectively.

Paraprionospio inaequibranchia

Only one individual, body size of 0.92 mm, was found in the low organic content area of Sriracha Bay. This specimen had three pairs of flabellate branchiae on chaetigers 1–3. Branchial pair 1 was the largest and branchial pair 3 the smallest. A filament was present at the base of branchial pair 3. The length of branchial pair 1 (2.0-2.15 mm) was longer than pair 2 (2.0-2.03 mm) and pair 3 (1.23-1.26 mm). The number of branchial pinnules of pairs 1, 2 and 3 were 68-82, 43-47 and 64-74, respectively.

The respiratory structures in *Prionospio* and *Paraprionospio* are in the form of branchia with pinnules in highly branched gills and apinnate branchia as short fingerlike protrusions of the body wall. Through the increased size and number of pinnules of branched gills, the surface area available for gas exchange will be increased. Mangum (1976) concluded that the branched gills of the bloodworm *Glycera americana* Leidy, 1855, increase its gaseous exchange surface by 28% of its total body surface area compared with *Glycera dibranchiate* Ehlers, 1868, which has simple unbranched gills which can increase its exchange surface area by 21% of its total body surface area. Apart from increased gill surface area, the effectiveness of oxygen uptake could also be increased through increased ventilation rates, increased efficiency of O_2 removal from the bloodstream, elevated circulation capacity, reduced blood/water diffusion distances, and increased blood pigment affinity for oxygen (Childress and Siebel 1998 cited by L.A. Levin (2003).

The present study has established the presence of intraspecies morphological differences in respiratory structures in the spionids *P. membranacea, P. pulchra* and *P. sexoculata*, related to different environmental conditions. In the high organic content area, branchial pairs were significantly longer than those in the area of low organic content. Also, for the two species *P. membranacea* and *P. sexoculata* possessing pinnules, the pinnules were more numerous. In addition, branchial lengths and pinnule numbers showed a tendency to increase in relation to body size. These spionids are usually found at a depth of 1-2 cm from the sediment surface, so they are surface deposit feeders with adequate access to oxygen in the overlying water. D. M

Dauer et al. (1981) proposed that spionid polychaetes were interface-feeder species that utilized particles from the sediment surface, in suspension and resuspended in the sediment. Spionids altered both the mode and rate of feeding in response to the presence of suspended particles. When particles were resuspended by a current, the polychaetes changed their palp orientation modes and increased their feeding upon suspended particles. This also help to increase ventilation in polychaetes.

Morphological adaptations of respiratory structures in the spionids, which allow them to tolerate low oxygen conditions in organically-rich sediments, have rarely been observed. However, the results of the present study concurred with those on respiratory organs (especially in spionid polychaetes) showing enhanced O_2 diffusion through enlarged branchiae, increased brachial length and increased number of branchial pinnules and branchial forms. Lamont and Gage (2000) reported that low concentrations of dissolved oxygen and permanent hypoxia were related to enlargement of respiratory surface areas through elongation, proliferation and increased in number of branchial pinnules: *Prionospio (Minuspio)* sp. A has a greater branchial length in relation to body size at shallower depths (417 m) and low oxygen (0.14 ml L^{-1}); and the number of branchial lamellar plates present on the pairs of branchiae in *Paraprionospio* sp.A in relation to body size is significantly different at different depths. The latter was described as *Paraprionospio patiens* Yokoyama, 2007, a species with enlarged respiratory structures which could be considered as a biological indicator for heavy organic pollution and/or oxygen-depleted waters. *Pa. patiens* has three pairs of branchiae with bifoliate-shaped lamellae. The first branchial pair is the longest and each has more than 50 pairs of lamellar plates. These morphological characteristics are utilized to enhance O_2 diffusion. *Paraprionospio cordifolia* Yokoyama, 2007, and *Paraprionospio coora* Wilson, 1990, as assigned to *Paraprionospio* sp. Form B and Form CI, respectively, possess the flabellate instead of bifoliate lamellae (Yokoyama, 2007). *Pa. cordifolia* is known as an indicator for semi-enclosed, eutrophic environments; *Pa. coora* is widely distributed in muddy environments with high organic matters as well as sandy substrates in open coasts.

2.2 Morphological characteristics of respiratory structures of larvae and juveniles

The branchial structures in spionid polychaetes, *P. membranacea* and *P. pulchra* in laboratory treatments of high and low organic content were fully developed in the early benthic stage juveniles. Significant differences in the periods from planktonic larval stages to settlement in both species in the laboratory treatments of high and low organic content. The planktonic larval stage to metamorphosed larval stage and to early benthic stage juvenile in *P. membranacea* in high organic content treatment were 2-8, 8-10 and 26-30 days respectively. It took longer developmental period in the low organic treatments as in Table 2.7. The early benthic stage juveniles period was 40-44 days. The development duration in *P. pulchra* also showed the similar trend of more extended period in the low organic content treatments (Table 2.8).

The respiratory structures of *P. membranacea* larvae and juvenile consisted of branchial pairs 1 developed first in 19-21 chaetigers larvae as small protuberances on chaetiger 2. The development of respiratory structure was related to the growth of the larvae. Larvae in the high organic content area showed rapid development compared to those in low organic content area. A fourth pair of branchiae appeared on chaetiger 5 in 20-21chaetigers larvae. A second pair of branchiae developed laters in 22-24 chaetigers larvae on chaetiger 3. The third pair of branchiae later appeared on chaetiger 4 of 25-27 chaetigers larvae. The branchial pinnation developed well in the branchial pairs 1 and 4 at the size of 24-26 chaetigers larvae. The branchial pinnules in the 1st branchial pair appeared as protuberances in the 22-25 chaetigers larvae. The branchial pinnules first appeared on the 4th branchial pairs in the 25-28 chaetigers larvae. The metamorphosed larval stage began at 8-10 chaetigers larvae. Appearance of palps was detected. During this time the metamorphosed larvae would swim near the bottom. The respiratory structure began to developed not until the early benthic stage was reached that the respiratory structure fully developed as in adult.

Table 2. 7 Development of respiratory structure from larval stage to early benthic stage juvenile/ settlement stage in spionid polychaetes, *Prionospio (Prionospio) membranacea* reared in laboratory treatments of high and low organic content.

	High organic content	**Low organic content**
Larval stage (2-7 chaetigers)	2-8 days	2-16 days
• branchial length (mm)	absent	absent
• number of brachial pinnules	absent	absent
Metamorphosed larval stage (8-10 chaetigers)	8-10 day	16-17 days
• appear of palps	8-12 day (8-10 chaetigers)	16-17 days (8-10 chaetigers)
• 1st branchial pair first developed	18-24 days (19-26 chaetigers)	31-35 days (20-25 chaetigers)
• 2nd branchial pair first developed	21-25 days (22-26 chaetigers)	32-36 days (22-26 chaetigers)
• 3rd branchial pair first developed	23-27 days (25-29 chaetigers)	35-40 days (25-29 chaetigers)
• 4th branchial pair first developed	21-25 days (20-25 chaetigers)	32-35 days (21-25 chaetigers)
• number of brachial pinnules		
• pinnules as protuberances on 1st pair	21-24 days (22-25 chaetigers)	32-35 days (21-25 chaetigers
• pinnules as protuberances on 4th pair	23-25 days (25-28 chaetigers)	34-28 days (26-30 chaetigers)
The early benthic stage juvenile (28-	26-30 days	40-44 days
• branchial pairs and number of branchial pinnules fully developed		
• branchial length (mm)		
• 1st branchial pair	0.19±0.03	0.16±0.01
• 2nd branchial pair	0.09±0.02	0.07±0.01
• 3rd branchial pair	0.08±0.02	0.07±0.01
• 4th branchial pair	0.15±0.02	0.12±0.01
• number of brachial pinnules		
• 1st branchial pair	10±2.24	6±2.88
• 4th branchial pair	6±2.61	4±1.66

Table 2. 8 Development of respiratory structure from larval stage to early benthic stage juvenile/ settlement stage in spionid polychaetes, *Prionospio (Minuspio) pulchra* reared in laboratory treatments of high and low organic content.

	High organic content	**Low organic content**
Larval stage (2-7 chaetigers)	2-18 days	2-29 days
• branchial length (mm)	absent	absent
• number of brachial pinnules	absent	absent
Metamorphosed larval stage (8-12 chaetigers)	19-21 days	30-31 days
• appear of palps	20-24 days	30-33 days
	(10-13 chaetigers)	(10-15 chaetigers)
• 1^{st} branchial pair first developed	30-35 days	36-43 days
	(18-22 chaetigers)	(18-24 chaetigers)
• 2^{nd} branchial pair first developed	30-35 days	36-43 days
	(18-22 chaetigers)	(18-24 chaetigers)
• 3^{rd} branchial pair first developed	30-35 days	36-43 days
	(18-22 chaetigers)	(18-24 chaetigers)
• 4^{th} branchial pair first developed	30-35 days	36-43 days
	(18-22 chaetigers)	(18-24 chaetigers)
• 5^{th} branchial pair first developed	32-37 days	40-45 days
	(20-25 chaetigers)	(21-25 chaetigers)
• 6^{th} branchial pair first developed	34-37 days	41-47 days
	(22-25 chaetigers)	(22-28 chaetigers)
• 7^{th} branchial pair first developed	36-38 days	43-48 days
	(24-27 chaetigers)	(24-28 chaetigers)
• 8^{th} branchial pair first developed	38-40 days	45-50 days
	(26-30 chaetigers)	(26-32 chaetigers)
• 9^{th} branchial pair first developed	39-42 days	47-52 days
	(28-33 chaetigers)	(28-33 chaetigers)
The early benthic stage juvenile (26-	38-42 day	46-50 days
• branchial pairs fully developed		
• branchial length (mm)		
• 1^{st} branchial pair	0.21±0.019	0.2±0.017
• 2^{nd} branchial pair	0.21±0.019	0.2±0.019
• 3^{rd} branchial pair	0.2±0.011	0.19±0.020
• 4^{th} branchial pair	0.19±0.009	0.17±0.021
• 5^{th} branchial pair	0.15±0.012	0.14±0.022
• 6^{th} branchial pair	0.13±0.015	0.12±0.022
• 7^{th} branchial pair	0.11±0.017	0.1±0.018
• 8^{th} branchial pair	0.08±0.015	0.07±0.019
• 9^{th} branchial pair	0.05±0.009	0.45±0.012

The development of branchiae in of *P. (M.) pulchra*, began with the branchial pairs 1–4 appeared first on chaetiger 2-5in 18-20 chaetigers larva. Branchial pairs 5, 6 and 7 developed later in 21-23, 22-23 and 24-26 chaetigers larvae, respectively. The branchial pairs 8 and 9 developed later in 26-28 and 28-31 chaetigers larvae, respectively. Both spionid polychaetes once settled, they formed tubes in the sediment. The early benthic stage juvenile of *P. membranacea* and *P. pulchra* as they formed mucous tubes in sediment as shown in Figure 2.16. As compared to the development of respiratory structures in *Paraprionospi pinnata* larval and juveniles stages as described by (Yokoyama, 1981), the respiratory structures in the two spionids, *P. membranacea* and *P. pulchra* were more rapid and fully developed as in adult in the early benthic stage juvenile/ settlement stage while in *Pa. pinnata*, the branchial pairs and pinnation had not yet fully developed.

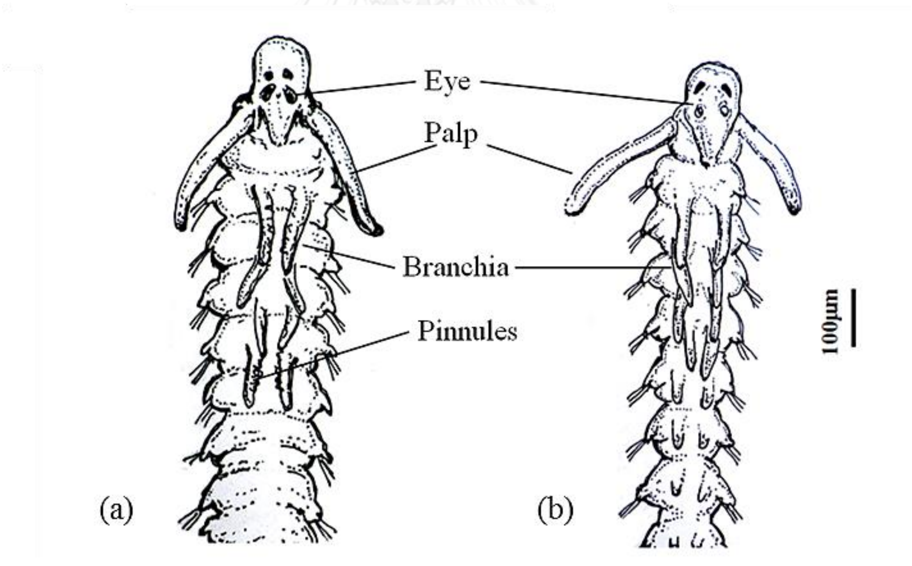

Figure 2.16Early benthic stage juvenile. (a) *Prionospio (Prionospio) membranacea* (b) *Prionospio (Minuspio) pulchra*

Prionospio (Prionospio) membranacea

A morphological response to different organic habitat in the early benthic stage juvenile/ settlement stage was observed through the increased length of branchial pairs 1 and 4 in relation to their body size in specimens from high organic content (*r*=0.90, *p*<0.05, *n*=25; *r*=0.94, *p*<0.05, *n*=25, respectively). The length of branchial pairs 2 and 3 (which was approximately similar at 0.08 mm) was also related to body size (*r*=0.81, *p*<0.05, *n*=25; *r*=0.79, *p*<0.05, *n*=25, respectively) as shown in Figure 2.17. In the low organic content, increase in the length of branchial pairs 1 and 4 was significantly proportional to the increase in body size (*r*=0.89, *p*<0.05, *n*=25; *r*=0.90, *p*<0.05, *n*=25, respectively), while the length of branchial pairs 2 and 3 also increased with body size (*r*=0.83, *p*<0.05, *n*=25; *r*=0.87, *p*<0.05, *n*=25, respectively) as shown in Figure 2.17.

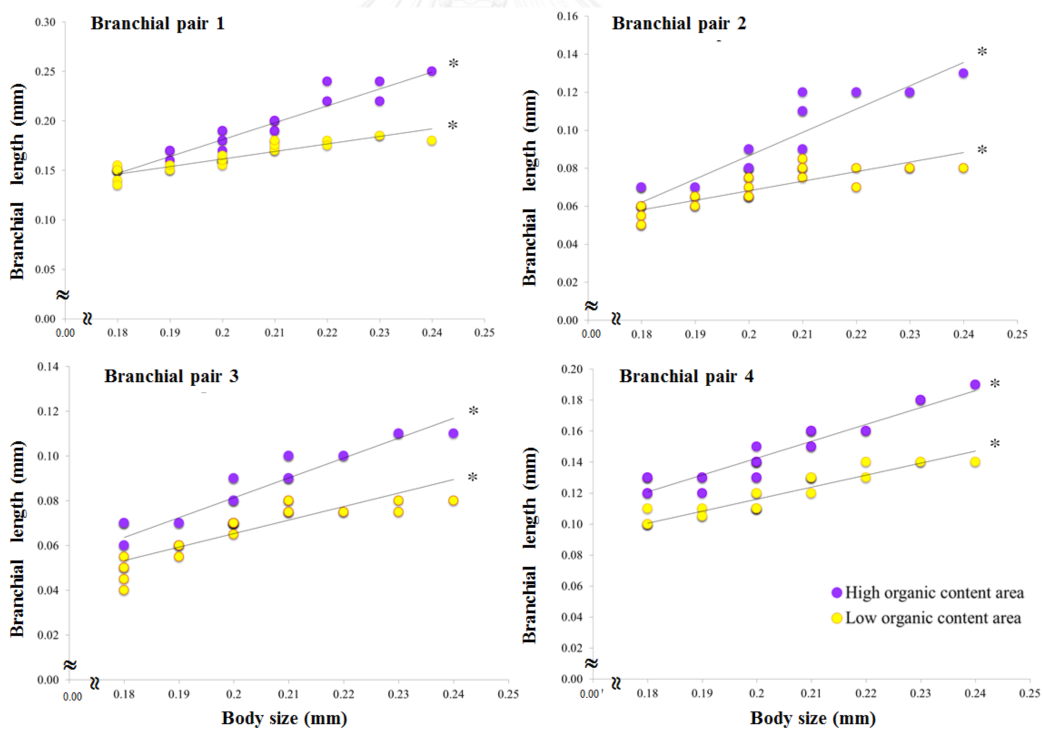

Figure 2. 17Length of branchial pairs according to body size in the early benthic stage juvenile of *Prionospio (Prionospio) membranacea* in laboratory treatments of high and low organic content. *Significant difference (*p*<0.05) between treatments.

A comparison of the length of each branchial pair in the early benthic stage juvenile from high organic content (n=25) indicates that branchial pairs 1 and 4 are significantly longer than pairs 2 and 3 by approximately 2 and 1.7 times, respectively (Figure 2.15) and pairs 1 and 4 were longer approximately 1.7 times in the low organic content (n=25; Figure 2.18).

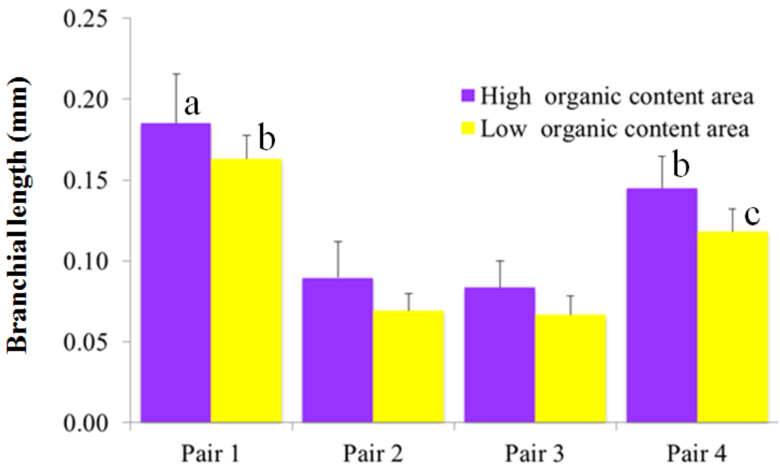

Figure 2. 18Mean length of branchial pairs in the early benthic stage juvenile of *Prionospio (Prionospio) membranacea* in laboratory treatments of high and low organic content. Different letters above bars indicate significant differences (p<0.05) within the same pair of chaetiger and between branchial length from different pair of chaetigers. Error bars represent SD.

The number of branchial pinnules in pairs 1 and 4 in specimens from high organic content increased with the body size (r=0.85, p<0.05, n=25; r=0.82, p<0.05, n=25, respectively) as shown in Figure 2.18. The number of branchial pinnules of pairs 1 and 4 in *P. membranacea* specimens from this low organic content also increased in relation to body size (r=0.94, p<0.05, n=30; r=0.91, p<0.05, n=30, respectively) as shown in Figure 2.19. There were 6-14 branchial pinnules in pair 1 and 2-10 in pair 4 in specimens from the high organic content; approximately 1.7 and 1.5 times the number in the low organic content, 2-11 and 1-8, respectively (Figure 2.20).

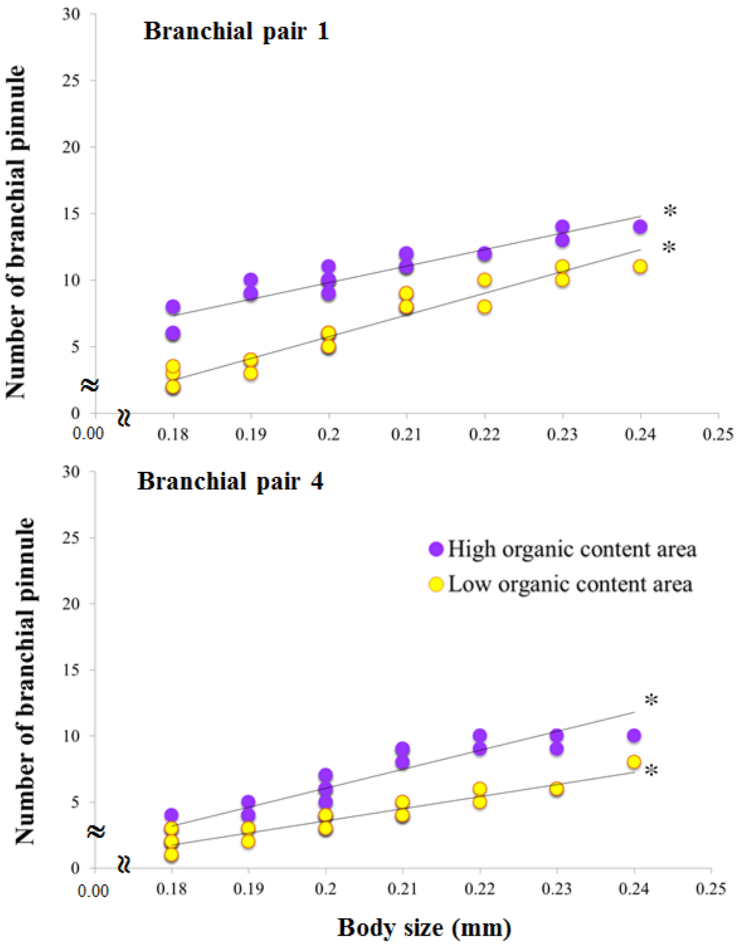

Figure 2. 19Number of branchial pinnules of pairs 1 and 4 in the early benthic stage juvenile of *Prionospio (Prionospio) membranacea* in laboratory treatments of high and low organic content. *Significant difference (p<0.05) between treatments.

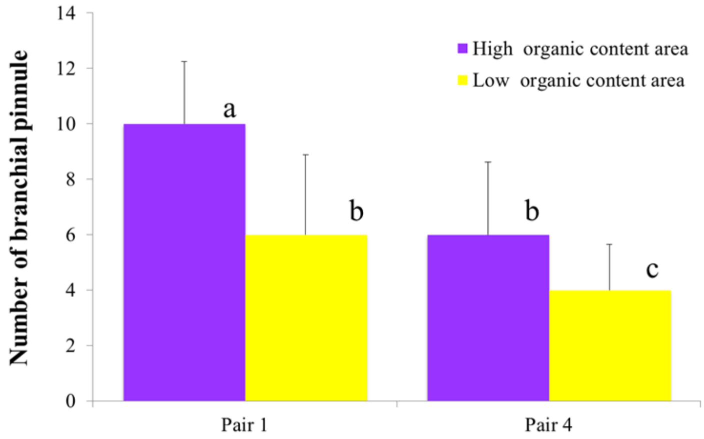

Figure 2. 20Mean number of branchial pinnules in the early benthic stage juvenile of *Prionospio (Prionospio) membranacea* in laboratory treatments of high and low organic content. Different letters above bars indicate significant differences (p<0.05) within the same pair of chaetiger and between branchial length from different pair of chaetigers. Error bars represent SD.

A comparison of the length of branchial pairs and the number of branchial pinnules in the early benthic stage juvenile of *P. membranacea* from the two areas in Sriracha Bay indicates that the length of branchial pairs 1, 2, 3 and 4 was significantly longer in specimens from high organic content with the number of branchial pinnules of pairs 1 and 4 more numerous than those from the low organic content (p<0.05).

Prionospio (Minuspio) pulchra

Length of the branchial pairs 1-9 in in the early benthic stage juvenile of *P. pulchra* (n=25) from the high organic content was 0.53-0.98, 0.48-1.00, 0.48-0.99, 0.47-0.90, 0.43-0.85, 0.35-0.80, 0.30-0.70, 0.30-0.65 and 0.20-0.41 mm, respectively and in specimens (n=25) from the low organic content area, the length was 0.43-0.83, 0.33-0.88, 0.38-0.84, 0.43-0.81, 0.37-0.68, 0.29-0.63, 0.23-0.53, 0.20-0.43 and 0.20-0.33 mm, respectively.

The lengths of branchial pairs 1, 2, 3 and 4 were longer than in pairs 5, 6, 7, 8 and 9 for specimens from the high organic content by 0.6-2.1 times, and from the low organic content by 0.8-1.7 times (Figure 2.21). The length of each branchial pair from high organic content showed a positive correlation to the body size with correlation value (*r*) between 0.70 (length of branchial pair 8) and 0.90 (length of branchial pair 6) ($p<0.05$, $n=25$). In the low organic content, the length of each branchial pair showed an increasing trend in relation to body size with a correlation value (*r*) between 0.65 (length of branchial pair 6) and 0.87 (length of branchial pair 1) ($p<0.05$, $n=25$).

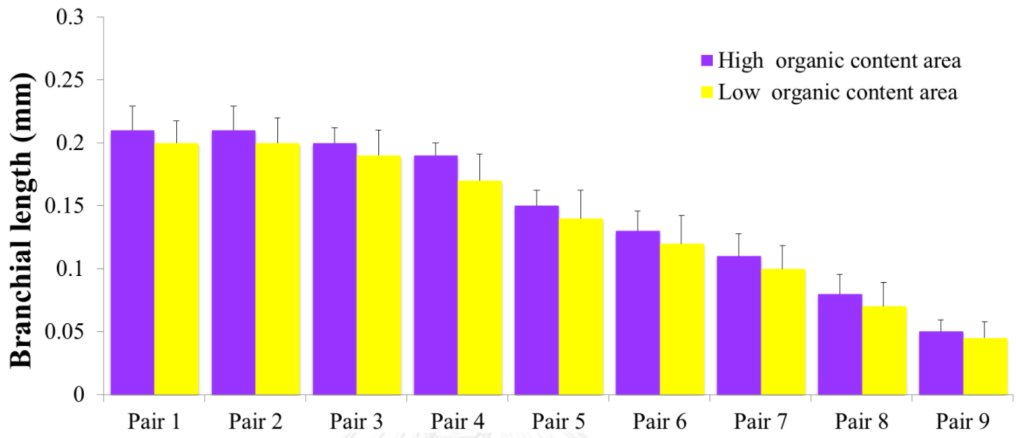

Figure 2. 21Mean length of branchial pairs in the early benthic stage juvenile of *Prionospio (Minuspio) pulchra* in laboratory treatments of high and low organic content. Error bars represent SD.

Comparing the length of branchial pairs among the early benthic stage juvenile specimens from the two sample, *P. pulchra* of the same body size of 0.10-0.19 and 0.2-0.29 mm, the mean length of branchial pairs of specimens from the high organic content ($n=25$) from 0.02±0.00 to 0.20±0.02 and 0.03±0.00 to 0.22±0.02 mm, was longer by about 1.03-1.62 and 0.99-1.38 times than that of those in the low organic content area ($n=25$) which was between 0.01±0.00 to 0.19±0.01 and 0.02±0.01 to 0.21±0.02 mm.

The respiratory structure in spionid polychaetes, *P. membranacea* and *P. pulchra* began to develop early in the metamorphosed larval stage of 8-10 chaetigers larvae in *P. membranacea*. The respiratory structure first noted as the appearance of palps in coiled position not fully extended in 10-13 chaetigers larvae. The branchial pairs gradually developed as the larvae grew. The number of pinnules on the branchial pairs developed in the 22-25 chaetigers larvae. The respiratory structure in *P. membranacea* was fully developed in the early benthic stage juvenile/ settlement stage as in adult. In *P. pulchra*, the first appearance of palps was found in the metamorphosed larval stage of 10-13 chaetigers larvae. The branchial pairs gradually developed as the larvae grew. The branchial pairs were fully developed in the early benthic stage juvenile of 26-30 chaetigers larvae. From the study of respiratory structures in larvae and juvenile of spionid polychaetes. it was evidenced that the morphological differences of these spionids reflected phenotype differences to different environmental conditions.It is noted that the length of branchial pairs and number of branchial pinnules were observed be different in the high and low organic areas at the early benthic stage juvenile and even in very small individuals in both *P. membranacea* and *P. pulchra*.

It is interesting that the spionid larvae from the metamorphosed larval stage and the early benthic stage juveniles were able to detect and discriminate the different organic condition in the sediment in order to adapt accordingly. The metamorphosed larvae were observed to swim and crawl on the surface of the sediment. It took the metamorphosed larvae only 18-20 days to become competent to settle as the early benthic stage juveniles. The spionid larvae must be responding to the biological cues in term of organic bound to particulates in the sediment (Cohen & Pechenik, 1999; Qian & Chia, 1993). Once the respiratory structure fully developed in the early benthic stage juveniles, the morphological differences to different organic conditions were evidenced. As Cohen and Pechenik (1999) concluded that the cue for metamorphosis larvae was most likey associated with organic sediment. Therefore, the reliance of larvae on an organic, sediment-associated cue coupled with increased juvenile fitness on substrates of high organic content should decrease the likelihood that larvae would metamorphose in response to substrates of low organic content. It should be interesting to conduct the experiments to test this by collecting gravid females and males polychaetes from high organic content area and artificially induced spawning to produce newly hatch larvae. The larvae would be transfer to the culture boxes of low organic sediment. The assessment on the morphological adaptation of respiratory structure in these larvae should be carried out. Also in vice versa, the gravid females and males polychaetes collected from the low organic content should be carried out in the same procedure to be reared in the high organic sediment.

E. Conclusion

Morphological adaptations of respiratory structure in spionid polychaetes were considered as one of the key criteria for selecting potential polychaete species as bioremediator in treating high organic sediment under mussel rafts in Sriracha Bay, Chonburi Province. Comparative study of morphological adaptation in spionid genus *Prionospio* revealed the presence of intraspecies morphological differences in respiratory structures in *P. membranacea, P. pulchra* and *P. sexoculata* related to different environmental conditions. In the high organic content area, branchial pairs were significantly longer than those in the area of low organic content. Also, for the two species *P. membranacea* and *P. sexoculata* possessing pinnules, the pinnules were more numerous. In addition branchial lengths and pinnule numbers showed a tendency to increase in relation to body size. These morphological differences of these spionids under different environmental conditions occurred earlier in their early benthic juvenile stage.

The distribution and abundance of spionid polychaetes in Sriracha Bay revealed six species including *P. membranacea, P. pulchra, P. sexoculata, P. multibranchiata, P. japonica* and *Pa. inaequibranchia. P. membranace,* dominated of 70% of the spionids found in the area, was widely distributed in the area in particular the high organic content area in the mussel raft culture area and nearshore stations. Maximum abundances were observed during the wet season from July to October *P. pulchra*, the second most abundant species, also found distributed in the high organic content area. Sediment type, organic matter as well as the water depth determined the distribution and abundance of spionid polychaetes. The distribution and abundance study revealed large natural stocking density of potential spionids as the bioremediator for enriched sediment under the mussel raft culture in Sriracha Bay.

CHAPTER III
COMPARATIVE STUDY ON REPRODUCTIVE AND DEVELOPMENT PATTERNS IN SPIONID, *Prionospio* IN ORGANICALLY ENRICHED SEDIMENT

A. Introduction

Organic enrichment in the coastal sediment could cause dramatic changes in the benthic community structure, reducing the species to only few tolerant species, lowering both the diversity and community richness (Pearson & Rosenberg, 1978). The macrofauna were found to dominate numerically by opportunistic species, mostly polychaetes. The organic enriched sediments were usually dominated by opportunistic species such as *Capitella* spp. in the Family Capitellidae and *Prionospio* spp. in the Family Spionidae. Species composition in marine benthic communities changes along the gradient of increasing organic enrichment. Marine Biotic Index (AMBI) has been proposed as the ecological indicator based on soft-bottom benthos in particular polychaetes for organic enrichment assessment. Polychaetes were categorized into five guoups according to their sensitivity to increasing organic enrichment. The spionid polychaetes as well as the cirratulids were classified into Group IV as the second-order opportunistic species under slight to pronounced unbalanced situations. The capitellid polychaetes *Capitella capitata* and spionid, *Scolelepis fuliginosa* were classifield into Group V as the first-order opportunistic species (Borja et al., 2000; Carvalho et al., 2006; Cheung et al., 2008; D. M. Dauer, 1993; Grall & Glémarec, 1997). Opportunistic life-history characteristics in these pollution-indicator species included being small size, short generation time, high reproductive rate and high mortality (J. Grassle & Grassle, 1974; Yokoyama, 1990). Adaptation on reproductive patterns and larval development in these polychaetes were important for the recruitment and population dynamic in organic enrichment condition where low oxygen concentration and high sulfide concentration. Opportunistic polychaetes are among the early colonizers of disturbed area or organic enrichment area due to the life history characteristics such as prolonged breeding season, high recruitment, high growth rate, short interval between fertilization and maturity, and short life span.

Although they usually have high subsequent mortality ((J. Grassle & Grassle, 1974; Lisa A Levin, 1984; Yokoyama, 1990).

Effect of organic enrichment on larval development in polychaetes have been well documented in term of modes of larval development, fecundity, larval mortality and development and larval habitat selection ((Bridges, 1996; Cohen & Pechenik, 1999; L. Levin & Creed, 1986; Lisa A Levin, 1984; Mok et al., 2008; Thiyagarajan et al., 2005; Tsutsumi et al., 1990; Tsutsumi et al., 2001; Yokoyama, 1990, 1995; Zajac, 1986). The success of larval development and larval habitat selection in a habitat plays a central role in the dynamics of adult population (Cohen and Pechenik, 1999).

Reproductive and development pattern in spionid polychaetes in organically enriched sediment is one key criteria for potential bioremediator in treating high organic sediment under mussel rafts in Sriracha Bay, Chonburi Province. The objectives of this study were to determine: (1) whether the reproductive and development pattern in spionid genus *Prionospio,* fit the opportunistic life-history strategy (2) comparative study on reproduction responses in spionid genus *Prionospio* to organically enriched sediment.

B. Literature Review

1. Reproductive Patterns in Spionid Polychaetes

Spionid polychaetes are common and widely distributed around the world in both sandy and muddy sediment from intertidal zones to the deep sea (Rouse and Pleijel, 2001). Spionids have succeeded in completing their life cycle in several habitats in coastal areas because of their adaptations in feeding and diverse patterns of reproduction and development (Blake & Arnofsky, 1999; D. M Dauer et al., 1981; Fauchald & Jumars, 1979). Two types of asexual reproduction occur in spionids: (1) architomy as fragmentation of the body into individual segments as in *Pygospio elegans* and (2) paratomy, involving the division of the body into two distinct halves, reported in *Polydora tetrabranchia* (Blake & Arnofsky, 1999). Diverse sexual reproduction and larval development found in spionids (Blake & Arnofsky, 1999; Wilson, 1991). Wilson (1991) classified the sexual reproductive modes in polychaetes based on the fate of ova and type of larval development. The ova fate classes were categorized 1) free spawning, 2) brooding on the body, 3) brooding within the body (viviparity), 4) brooding in capsules within the tube, 5) brooding along the lining of the tube and 6) encapsulation in a gelatinous mass. The type of larval development consisted of; (1) planktotrophic (having planktonic feeding larvae), (2) lecithotrophic (having planktonic, non-feeding larvae; and (3) direct development (having no free-swimming larval phase). He found that the family of Spionidae was notably diverse exhibiting 10 reproductive modes. All spionids appear to have paired ovaries with two patterns for their structure and oogenesis. A single pair of ovaries is found in each middle body segment as in *Marenzelleria viridis*. Oocytes remain in the ovary until a late stage of oogenesis and are thus intraovarian. The second pattern of ovaries attaching to the muscles near the ventral midline as in most other spionids such as *Polydora* and *Spio*. Oocytes are released into coelom where they continue to grow by extra ovarian oogenesis (Blake & Arnofsky, 1999).

Three different types of eggs noted by Blake and Arnofsky (1999) occur in spionids: (1) eggs with complex thick, often highly ornamented egg envelopes (=membranes) resembling honeycombs that contain prominent and numerous cortical alveoli (=membrane vesicles) ; (2) eggs with thick egg envelopes, probably formed of several glossy layers that have a reticulated, but not honeycombed surface and that lack cortical alveoli; and (3) eggs with thin envelopes consisting of a single, never ornamented layer, and that also lack cortical alveoli. Eggs of spionids, *Prionospio*, classified as the second egg type, with a 3-layered egg envelope and appeared to be intermediate between the highly ornamented types and those with thin, single-layered egg envelopes.

Blake and Arnofsky (1999) noted that most spionids are polytelic, i.e. reproducing more than once in a season, which many species are capable of establishing dense populations during the times they reproduce because a single female can produce sequential sets of gametes (Blake & Arnofsky, 1999; Wilson, 1991). Some spionid are monotelic, producing no more than one brood per year as *Spio martinensis* (Gudmundsson, 1985). In spionid *Pronospio* species, these species are polytelic. Yokoyama (1990) noted that the population of *Paraprionospio* which is closely related to *Prionospio* species, is polytelic. He reported that *Paraprionospio* sp. (form A) later assigned as *Paraprionospio patiens*, breed from Kumihama Bay, Japan at a low level throughout the year. Two distinct peaks, in spring and in late summer through autumn were observed. In other opportunistic polychaetes were also reported being polytelic such as *Capitella capitata*, *Polydora ligni*, *Steblospio benedicti* and *Scolelepis fuIiginosa* mostly spawning period occurred most of the year (Lisa A Levin, 1984; Yokoyama, 1990). *Paraprionospio patiens*, found in silty tubes in the estuarine environment in Southern Chile, demonstrated the two peaks of planktotrophic larvae in the beginning of summer in October-November and the end of summer in March (Radashevsky et al., 2006).

2. Patterns of Larval Development in Spionid Polychaetes

Full range of larval development types were demonstrated among the spioniform polychaetes Blake and Arnofsky (1999) including pure broadcast spawners having planktotrophic or lecithotrophic larval development, brooding in capsules and cocoons, and viviparity. In brooders, development may be direct or continue in the plankton. Free spawning with planktotrophic larvae pattern, represented the primitive means of reproduction, found in more families and orders in polychaetes than any other mode (Wilson, 1991). He also noted that spionids, *Prionospio cirrifera*, *P. malmgreni* and *P. steenstrupi* were free spawning with planktotrophic larvae. According to the larval development in *Paraprionospio* spp., these spionids produced entirely pelagic planktonic larvae as in *Paraprionospio pinnata* (Yokoyama, 1981) and in *Paraprionospio patiens* (Yokoyama, 1990). Several spionid polychaetes exhibit multiple development modes, poecilogony, with either planktotrophic and lecithotrophic development, in *Polydora ligni* (Zajac, 1986) and in *Streblospio benedicti* (L. Levin & Creed, 1986).

As in most of the opportunistic polychaetes, length of planktonic phase varied according to species from several hours to 2 months or more. The larvae are long, thin, and have numerous segments. Size at metamorphosis in *Prionospio* species, such as *Prionospio caspersi, P. cirrifera, P. fallax, P. lighti, P. saldanha* and *P. steenstrupi* ranged in 15 to 28 chaetiger and 1400 to 2400 μm, and length of planktic life in these spionids were 30 to >30 days (Blake & Arnofsky, 1999). Yokoyama (1981) concluded that the numbers of segments and the lengths at the time of metamorphosis in the spionid *Prionospio* species including *Prionospio caspersi*, *P.*

cirrifera, P. fallax, P. lighti, P. saldanha and *P. steenstrupi* which have the planktotrophic type of development, range in length from 0.75-0.50 mm and in the number of segments from 19-25. In larger spionid, *Paraprionospio patiens* the length of planktonic phase was longer of 2 months at the size of settlement of 3,800-5,380 µm. The time to maturity was 3-6 months (Yokoyama, 1990).

3. Effect of Organic Enrichment on Reproduction and Larval Development Pattern

The availability and quality of the organic rich sediment is closely related to the growth, survival and reproduction most deposit feeding polychaetes in particular *Capitella* spp., the important indicator of organic pollution (Cohen & Pechenik, 1999; Thiyagarajan et al., 2005; Tsutsumi et al., 2001). Larvae of *Capitella* sp. selectively metamorphosed in organic rich natural sediment. Cohen and Pechenik (1999) found that the chemical cues that induced metamorphosis in *Capitella* was probably organic and bound to fine particulates. Larvae of *Capitella* sp. would preferentially metamorphose into sediment of high organic content. Delayed metamorphosis occurred in the low organic content sediment. Tsutsumi et al. (1990) concluded that in order to efficiently utilize organic materials in the sediment, *Capitella* species may required either (1) some specific microorganism, the levels of which increase with levels of the organic materials or (2) concluded that *Capitella* species demonstrated the physiological requirement for hydrogen sulfide as an important resource for metabolic activity. Thiyagarajan et al. (2005) further concluded that type of sediment organic matter may play more important role in the larval habitat selection in *Capitella* sp. I than the concentration of organic matter alone. Their study showed a precise relationship between sediment biochemical properties and larval habitat selection. Larvae tend to choose sediments with low carbohydrate/protein ratio.

Level of organic content in the sediment had significant effect on the survivorship, reproductive activity and egg production in polychaetes. Zajac (1986) found that decreasing food density had negative effects on growth and reproduction in spionid polychaete *Polydora ligni*. Decrease in the gametogenic segments, egg capsules and fecundity was pronounced. Longer maturation time also occurred in smaller worm size. Longer time for females to deposit the first brood of eggs was observed as well as the longer inter-brood period. L. Levin and Creed (1986) found that in general, higher food level increased survivorship, reproductive activity and egg production in *Streblospio benedicti* in particular in adults with lecithotrophic development. Cohen and Pechenik (1999) also found that juveniles of *Capitella* sp. metamorphosed more slowly in decreased organic content. Availability of larval food determined rate of larval development, settlement and condition of newly settled juveniles. Qian and Chia (1993) demonstrate the effect of food limitation on larval development in two polychaetes, *Capitella* sp. and *Polydora ligni*. They found that starved juveniles of both species had lower settling rates and had prolonged larval life span. This would consequently increase the probability of other mortality sources in natural habitats such as predation risks and advection away from suitable habitat.

C. Material and Methods

1. Collection of Polychaetes and Sediments

Two spionids polychaetes *P. membranacea* and *P. pulchra* were collected from August to September 2013 from Sriracha Bay, Chonburi Province (13°11′N, 100°55′E), on the east coast of the Gulf of Thailand as in Figure 3.1. Adult specimens of two spionid species were collected from an area of high organic content at station 1 (74.66-6.08%TOC) near Sriracha Town Municipality under the influence of domestic waste effluents.

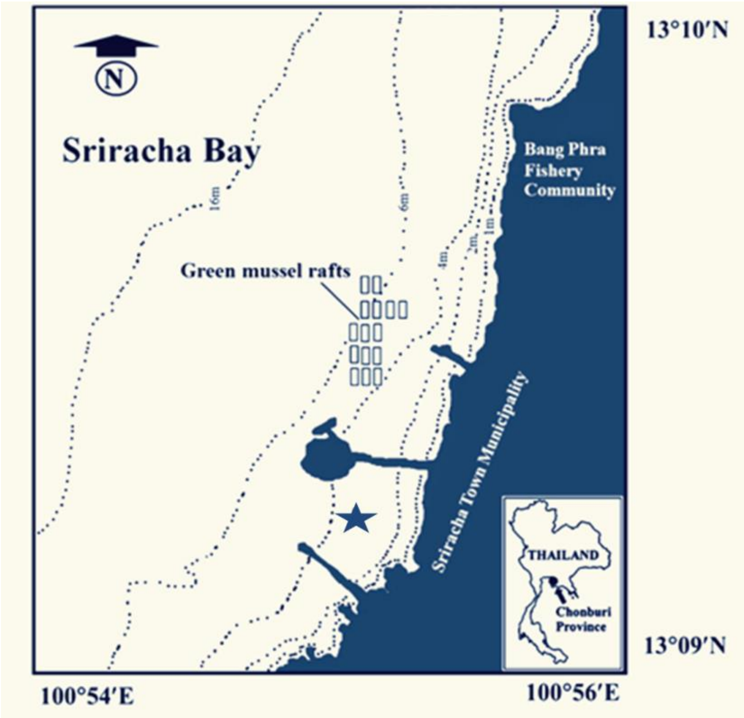

Figure 3 1 Study area, Sriracha Bay, Chonburi Province, the eastern coastline of Thailand. ★: sampling site for spionid polychaetes.

Samples were taken by Ekman grab and washed through a 0.25 mm mesh sieve. Specimens were transported afterward to the laboratory for sorting and identification. Sediment for the experiments was collected from different sites. Sediment used in the study on life cycle and larval development of spionid polychaetes was collected from near shore at 200 m from shore with 1-1.5 m depth, in front of Sriracha Fishery Research Station (3.19-3.59%TOM). For the experimental studies the reproductive responses in *Prionospio* polychaetes to organically enriched sediment, the sediment for the experiment was collected from: (1) from station 6, near Bang Phra fishery community outside of mussel raft representing low organic content, (2.00-2.84%TOM), (2) from under the floating mussel rafts in the bay representing high organic content, (6.13-9.33%TOM) and (3) from Sriracha port, near Sriracha Town Municipality under the influence of domestic waste effluents representing high level of organic enriched sediment, (10.39-13.92%TOM).The level of organic matter was twice the level of the organic matter from sediment under the green mussel rafts. Each sediment sample was sieved through a 1.0 mm mesh and freezed in the laboratory at least for 24 h to kill living organisms. The seawater from the mussel rafts was filtered through a 1.0 micrometer filter bag for the experimental use.

2. Preparation of Experimental Animals and Sediments

In the laboratory, spionid specimens were sorted under a stereomicroscope. The spionid polychaetes, *P. membranacea* and *P. pulchra* were identified following the procedures of M Imajima (1990b); M. Imajima (1990). Both spionids were acclimated in aquaria, which added the treated sediment and filtered seawater. The aquaria was provided with mild aeration. The acclimated polychaetes were individually measured for estimation the body size and body weight at the beginning of the experiment. The body length and the width of the 5^{th} chaetiger (including the parapodia) of mature specimen were also measured under a stereomicroscope with an ocular micrometer and expressed as body size. The numbers of chaetigers in each worm were counted. Worms were weights to determine the wet. Total organic matter in the sediment was determined by the loss on ignition ((Nelson, 1982) prior to the experiment.

3. Studies on Reproductive Pattern and Larval Development in *Prionospio* Polychaetes

3.1 Induced spawning

Life cycle and larval development of spionid polychaetes experiment, the mature females and males from the acclimated polychaetes were selected under a stereo-microscope for the experiment. Fifty mature individuals of spionids, 25 of females and 25 of males were introduced into a 500 ml beaker which added in the 100 ml filtered seawater. The sudden change of salinity in the beaker was by adding in the 50 ml of freshwater. This technique of induced spawning followed observations of the high density of spionid polychaetes and spawning peak during the wet season. Adult spionids released gametes into water where fertilization and larval development occurred within 24 hrs. The beaker was then decanted through a 63 μm mesh to harvest newly hatched larvae for the experiment. Induced spawning of spionid polychaetes in laboratory as shown in Figure 3.2.

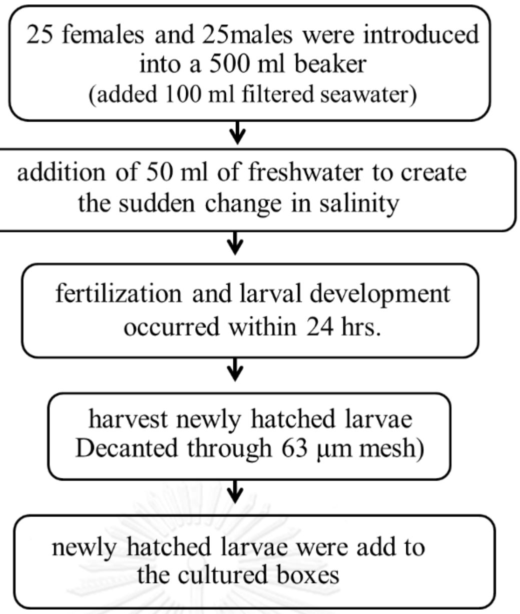

Figure 3 2 Diagram of induced spawning of spionid polychaetes in laboratory experiment.

3.2 Larval culture

The newly hatched larvae were added to the cultured boxes (33x42x28cm), that filled the treated sediment (3.50%TOM) at the level of 3 cm from the bottom with the filtered seawater level 10 cm above the surface sediment. The filtered seawater in each box was strongly aerated to create the resuspension of sediment in form of cloudy snowballs. The larval development of the spionid larvae were assessed by sampling for planktonic stage using 63 μm hand net. The early benthic stage juvenile/ settlement stage were sampled by using a 2.5 cm diameter plastic hand core

4. Studies on Reproductive Responses in *Prionospio* Polychaetes to Organically Enriched Sediment

4.1 Fecundity in *Prionospio* polychaetes

Four sets of culturing boxes (20x25x15 cm) were prepared for this study. Four different levels of organic enrichment of the sediment were used: (1) low level of organic matter in the sediment from station 6, near Bang Phra fishery community outside of mussel raft (2.75%TOM), (2) normal level of organic condition in the sediment in front of Sriracha Fishery Research Station (3.50%TOM); (3) high level of organic matter in the sediment from under the floating mussel rafts in the bay (6.17%TOM) and (4) enriched in the sediment from Sriracha port, near Sriracha Town Municipality under the influence of domestic waste effluents (12.94%TOM). Each treated sediment was filled into cultured boxes at the level of 10 cm from the bottom with the filtered seawater level 3 cm above the surface sediment. The filtered seawater in each box was gently aerated. The immature spionid polychaetes were added to the cultured boxes of 10 individuals of spionids to represent the normal density based on the average density of the spionid, *P. (P.) membranacea* from high organic content area and the mussel rafts area in Sriracha Bay, 200 inds.m^{-2} observed by To-orn and Intarachart (2010). For each treatment, four cultured boxes were used as replicates. The experiments were carried out for 30 days.

For the assessment of reproductive potential in spionid polychaetes, at the end of the 30 days experiment, sediment from each cultured boxes washed through a 0.25 mm mesh sieve. Specimens were examined for the presence of the gametes in coelom under a stereo-microscope. The total number of oocytes per worm was estimated by following the procedures of Tamai (1985). All oocytes in the coelom of the female were cleared into the cylinder with 50 cc of tap water. Three subsamples of 1 cc of oocytes were taken for counting. The number of oocytes in each subsample was counted under the microscope, and the mean value obtained from the three subsamples was used to calculate the total number of oocytes per worm. The oocyte diameter was shown by the average value of the shortest and longest diameter measured with an ocular micrometer.

4.2 Larval development

Pelagic planktonic larvae of spionid polychaetes with 2-3 chaetiger from breeding females with eggs and males with sperm in coelom were added to the cultured boxes (33x42x28cm), that filled the treated sediment at the level of 3 cm from the bottom with the filtered seawater level 10 cm above the surface sediment, in three different treatments: (1) low level of organic matter in the sediment (2.75%TOM); (2) normal level of organic matter in the sediment (2.75%TOM); (3 high level of organic matter in the sediment (6.17%TOM); and (4) enriched organic enrichment in the sediment (12.94%TOM) as in the reproductive potential experiment in adult spionids. The planktotrophic larvae of the spionid polychaete were added to the cultured boxes of 30 individuals of spionids (200 inds.m^{-2}). For each treatment, four cultured boxes were used as replicates.

The assessment of organically enriched sediment on larval development of spionid polychaetes were compared based on, larval development and duration to settlement stage, degree of larval abnormal development and degree of competent larvae (Figure 3.3).

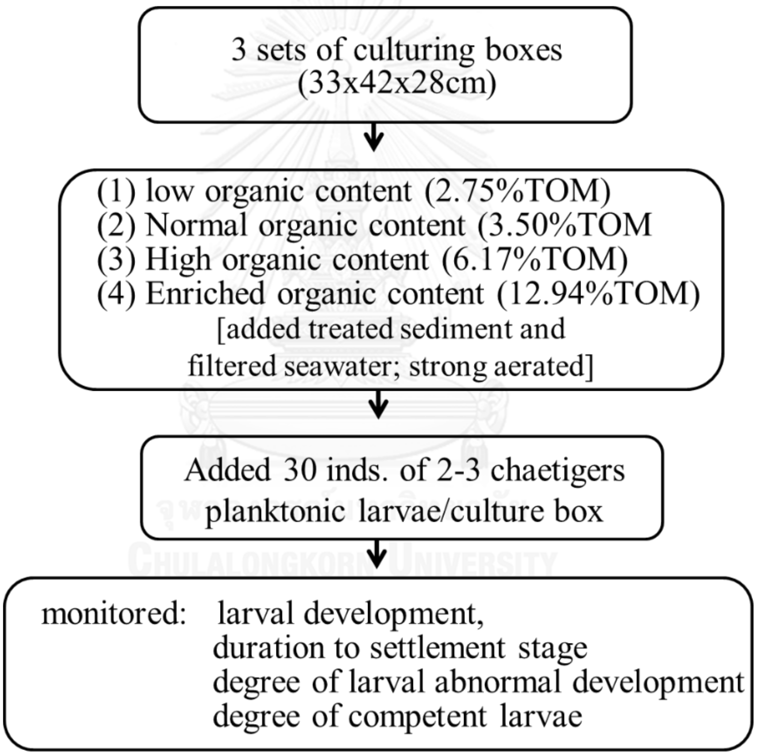

Figure 3 3 Diagram of larval development of spionid polychaetes in laboratory experiment.

5. Statistical Analysis

One-way analysis of variance (ANOVA) at the 95% confidence level (p<0.05) was applied to analyze the differences of fecundity, oocytes size and duration of spionid polychaetes. The fecundity, oocytes size and duration of two spionid species were compared using a t-test ((Zar, 1999).

D. Results and Discussion

1. Reproductive Patterns and Larval Development in *Prionospio (Prionospio) membranacea*

1.1 Life cycle

The reproduction in spionid polychaete *P. membranacea* is gonochoric. A single pair of ovaries found in each body segment oogenesis of the intraovarian pattern. Gametes occurs on 11-54 chaetigers in matured polychaetes. If in large size polychaetes, the gametes can be found from the 11th segment to the whole body except for the last 5-6 segment. The fecundity of spionids was 503-552 oocytes. Fecundity is closely correlated to the body size of polychaetes spawned oocytes were 80-100 μm in diameter with thick smooth membranes that lack alveoli. The spionid *P. membranacea* is broadcast spawner species with planktotrophic larval development. The life cycles of *P. (P.) membranacea* was shown in Figure 3.4.

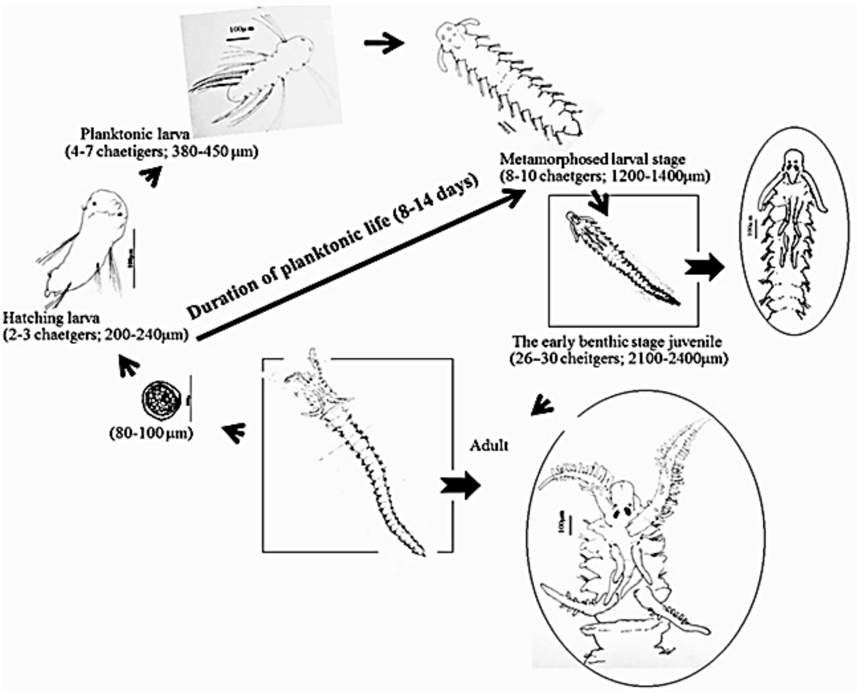

Figure 3 4 Life cycle of spionid polychaete, *Prionospio (Prionospio) membranacea*.

The planktonic larvae developed from fertilized oocytes within 24 hrs, 2-3 chaetiger, 200-240 μm long, having very long provisional chaetae on chaetiger 1. As in the larval development in Table 3.1. During planktonic phase, *P. membranacea* larvae had the body segments of 4-7 chaetigers and the larval size ranged 380-450 μm. Length of pelagic phase was 8-14 days. The length of pelagic phase in *P. membranacea* was shorter than thos found in the temperate species of *Prionospio* (Blake & Arnofsky, 1999; Radashevsky et al., 2006; Yokoyama, 1981). Metamorphosing larvae, with 8-10 chaetiger and a length of 1200-1400 μm beared which adult capillary chaetae and palps becoming longer and directed anteriorly. Metamorphosing larvae would swim and crawl on the bottom. During this phase, the respiratory structure started to develop. The respiratory structure branchial pairs 1 developed first in 19-21 chaetigers larva as small protuberances on chaetiger 2. The fourth pair of branchiae appeared on chaetiger 5 in 20-21chaetigers larva. A second pair of branchiae developed in 22-24 chaetigers on chaetiger 3. And the third pair of branchiae was found on chaetiger 4 of 25-27 chaetigers larva. The branchial pinnation developed well in the branchial pairs 1 and 4 when the larvae with 24-26 chaetigers at the early benthic stage juvenile. The early benthic stage juvenile was usually with 26-30 chaetiger, 2100-2400 μm long with fully developed respiratory structure. The duration of the early benthic stage juvenile was 28-32 days. The early benthic stage juveniles would settle into the sediment and built mucous tubes. The juveniles would protrude the palps in the water column. Adult size of *P. membranacea* was 34-70 chaetiger at the length of 6.5-14.5 mm. The time of maturity was 38-58 days (Table 3.1). Immature adult size started at 5.5-6.5 mm. in size. Form the observation in the field, the spionid, *P. membranacea* was with prolonged breeding season in particular the wet season. From the induced spawning experiment, the gravid females were able to produce 6 or more egg batches within 6 months.

Table 3. 1 Larval development of spionid polychaetes, *Prionospio (Prionospio) membranacea* in enriched sediment under the mussel rafts from Sriracha Bay, Chonburi Province, eastern coast of Thailand.

Characteristics in larval development	**Low organic content**	**Normal condition**	**High organic content**	**Enriched organic content**
Oocytes size (μm)	60-90	80-100	80-100	80-100
Fecundity	152-463	503-552	826-848	853-894
Type of development	plankto-trophic	plankto-trophic	plankto-trophic	plankto-trophic
Stage at release (no. chaetigers)	2-3	2-3	2-3	2-3
Size at release (μm)	200-240	200-240	200-240	200-240
Size at metamorphose (no. chaetigers)	9-10	8-10	8-11	9-10
Length of planktonic phase (days)	16-17	8-14	8-10	8-12
Sized at settlement				
• number of chaetiger (chaetigers)	26-30	26-30	26-30	26-32
• Length (μm)	1900-2100	2100-2400	2100-2500	2000-2400
• Duration (days)	40-44	28-32	26-30	25-28
Adult size (mm)	5.5-6.3	5.5-6.5	4.8-6.0	5.5-7.0
Size at maturity				
• number of chaetiger (chaetigers)	56-70	44-70	50-76	54-77
• Length (mm)	11.3-14.3	6.5-14.5	9.1-13.8	11.1-14.0
Time to maturity (days)	60-80	38-58	46-60	48-58

1.2 Fecundity

In all treatments of different organic content, higher fecundities were realized with increasing size in *P. membranacea*. The number of oocytes produced by a mature female in low organic condition, high organic condition and enriched sediment were positively correlated to the body length in term of number of chaetiger (r=0.81, p<0.05, n=8; r=0.76, p<0.05, n= 18; r=0.72, p<0.05, n= 20, respectively) as shown in Figure 3.5. Such a relationship was easily understandable from the fact that there were more gametic chaetigers in a longer worm. This corresponded to the finding in *Paraprionospio pinnata* (Yokoyama, 1981), and in *Polydora ligni* (Zajac, 1986). L. Levin and Creed (1986) found that body size, egg size and fecundity were strongly correlated in female, *Streblospio benedicti* in both individuals with planktotrophic and lecithtrophic development. Average fecundity of mature females of *P. membranacea* in high organic matter and enriched organic matter condition after 30 days were higher than those in low organic matter condition *(p*<0.05*)* which average fecundity of mature females in high organic matter (837±9.12 oocytes/ind.) and enriched organic matter condition (869±18.85 oocytes/ind.) were 2.19 and 2.27 times, as compared with those in low organic matter condition (383±153.81 oocytes/ind.) Effect of organic enrichment in the sediment on the fecundity of *P. membranacea* as in the

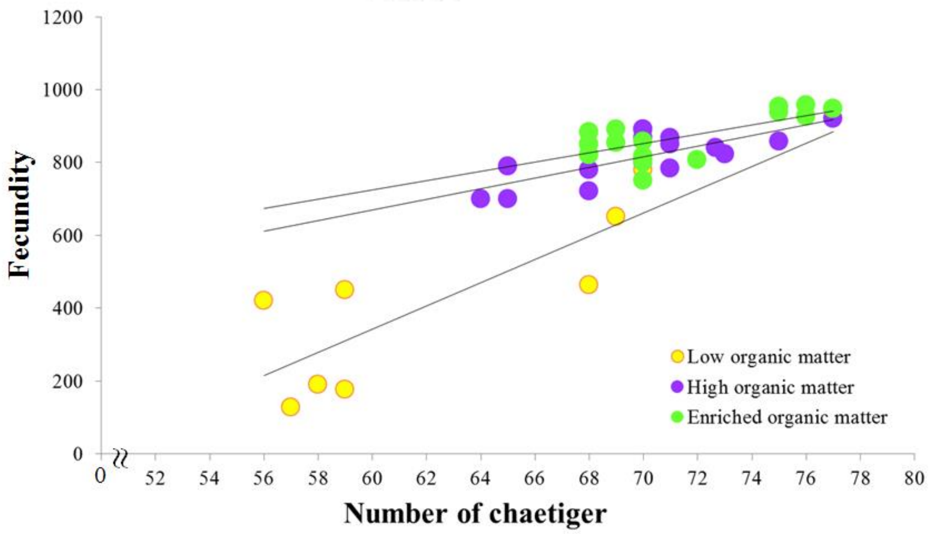

Figure 3 5 Relationship between fecundity of mature female *Prionospio (Prionospio) membranacea* in different organic condition treatments and the body length (numbers of chaetiger)**.**

Average values of oocyte diameter of a mature female of *P. membranacea* from experiment after 30 days between the treatment showed no significant different (*p*>0.05). However, the of oocyte diameter of a mature female in high organic matter and enriched organic matter condition showed the trend of larger oocyte of 80-100 μm when compared to those of 60-90 μm in low organic matter condition. Higher food levels in term of organic matter for deposit feeding polychaetes increased survivorship, reproductive activity and egg production (L. Levin & Creed, 1986; Zajac, 1986).

1.3 Larval development and duration to settlement stage

Larval habitat selection is important to place organisms into substrates that will allow for rapid growth and increased reproductive success (Cohen & Pechenik, 1999). In this study, the size of *P. membranacea* at metamorphosis and settlement among low, high and enriched organic matter condition showed no significant different (p<0.05). Bridges (1996) invertigated the effects of organic addition to size and maturity in two polychaetes, *Capitella* sp.I and *Streblospio benedicti*. He found that organic additions in terms of sewage and algae had no effect on the size of gravid females and fecundity in *Capitella* sp.I. However the high percentage of 40% of juveniles collected from the organic addition treatment was twice larger in term of body length than those juveniles in mud. No significant changes observed in *S. benedicti*. However the level of organic condition showed the pronounced effect on time to metamorphosis and settlement and the settlement success as in Figure 3.6 and Table 3.1.

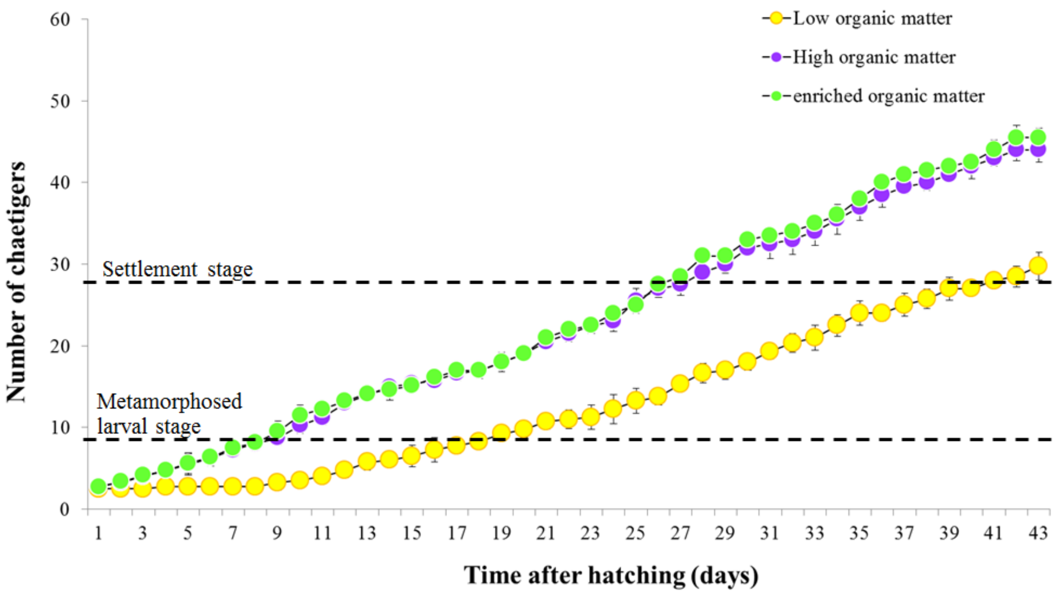

Figure 3 6 Size (number of chaetigers) and time after hatching to metamorphosis and settlement (day) in larvae of spionid polychaetes, *Prionospio (Prionospio) membranacea* in different organic conditions.

Cohen and Pechenik (1999) showed the relationship between sediment organic content, metamorphosis and postlarval performance in the deposit-feeding polychaete *Capitella* sp.I. They found that the mud of low organic content lowered juveniles growth rate and increased time to reproductive maturity. Metamorphosis occurred more slowly in decreased organic content. The larvae of *Capitella* sp.I would prefentially metamorphose into sediment of high organic content, supporting rapid growth and high fecundity. In their study, the larvae of *Capitella* sp.I postphoned metamorphosis in sediments of lowest organic content tested (0% and 5%). Larvae delayed metamorphosis for several days. In this study, *P. membranacea* showed the similar response to different sediment organic content as in *Capitella* sp. I. The lower percentage of metamorphosed in low organic matter condition as in Figure3.7. The percentage of metamorphose larvae of *P. membranacea* in the low organic, high organic and enriched sediment treatments were 42±4.00, 68±4.61 and 64±5.65 respectively. The percentage of competent larvae of *P. membranacea* in respective treatments were 31±2.00, 60±9.79 and 55±6.83 respectively. The duration from the time of hatching to metamorphosis decreased as the sediment organic content increased. Time to metamorphosis in the larvae of *P. membranacea* in high organic and enriched organic conditions were 9 and 9.5 days as compared to the prolonged period of 16.5 days in low organic condition. Time to settlement in *P. membranacea* larvae also shortened in high organic and enriched organic conditions of 27 and 26 days. The time to settlement in *P. membranacea* larvae also extended to 41 days. The prolonged larval life span would increase the predation risks and advection away from suitable habitat in natural habitats (Qian & Chia, 1993).

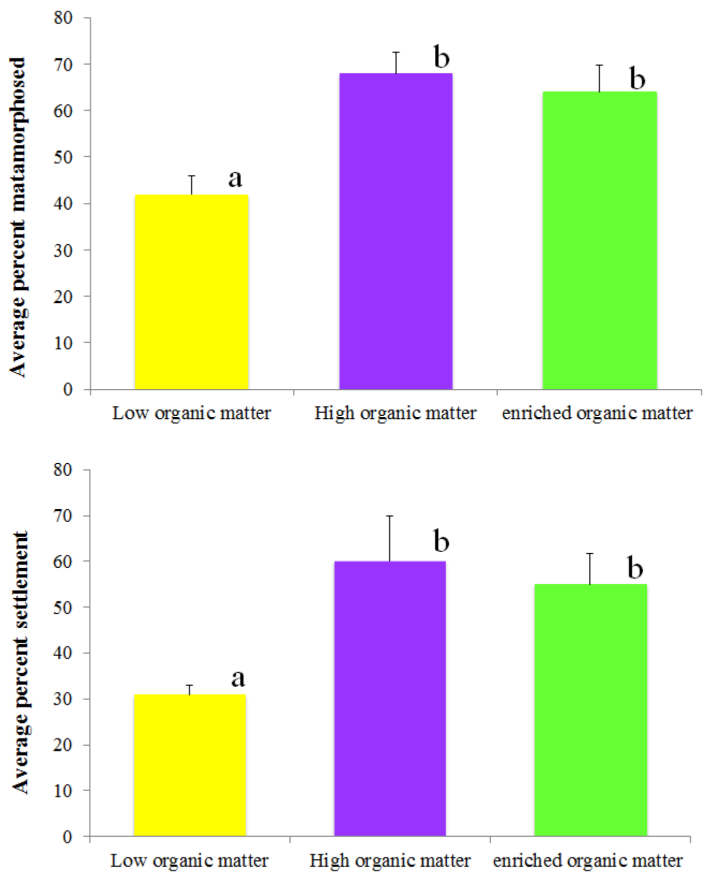

Figure 3 7 Success settlement of *Prionospio (Prionospio) membranacea* larvae in different organic conditions. Different letters above bars indicate significant differences (p<0.05). Error bars represent SD.

2. Reproductive Patterns and Larval Development in *Prionospio (Minuspio) pulchra*

2.1 Life cycle

P. pulchra shared the similar traits of reproductive pattern and larvae development as *P. membranacea*. *P. pulchra* is gonochoristic which oocytes occurs in the segments of the mid-region of the body of mature females from 11-12 segments toward the last 5-6 segments as in *P. membranacea.* Fecundity size of this spionids was 463-520 oocytes. Spawned oocytes were 80-100 µm in diameter with thick smooth membranes that lack alveoli. *P. pulchra* is also another broadcast spawner species with planktotrophic larval development. The life cycles of *P. pulchra* was shown in Figure 3.8.

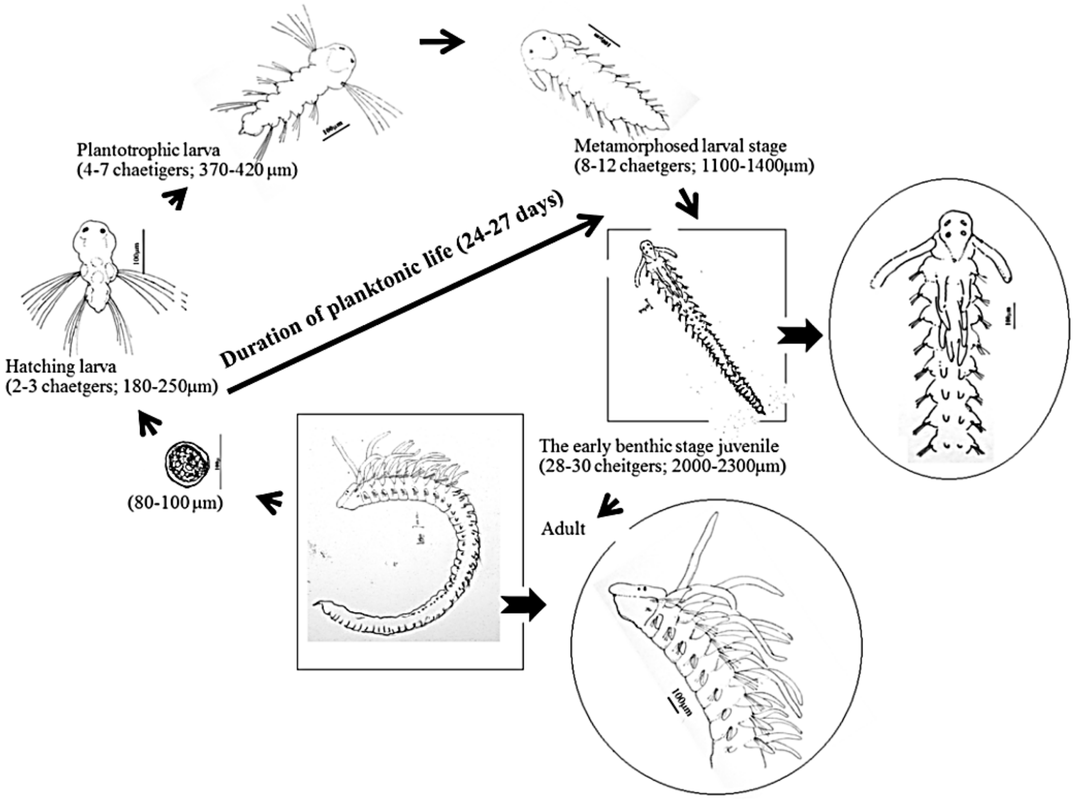

Figure 3 8 Life cycle of spionid polychaete, *Prionospio (Minuspio) pulchra*

The planktonic larvae of *P. pulchra* developed from fertilized oocytes within 24 hrs, with size at release 2-3 chaetiger and 200-240 µm long. The 2-3 chaetigers stage had very long provisional chaetae on chaetiger 1. During planktonic phase, *P. pulchra* larvae grew into 4-7 chaetigers stage larvae with size range of 370-420 µm. Size at metamorphosis in *P. pulchra* wqas with 8-12 chaetigers and a length of 1,100-1,400 µm. Adult capillary chaetae presented during this stage with palps becoming longer and directed anteriorly. The larvae crawled to explore the bottom. The duration of planktonic phase in this species was 9-12 days. The development of respiratory structure in *P. pulchra* progressed as branchial pairs 1–4 appeared first on chaetiger 2-5 in 18-20 chaetigers larva. Branchial pairs 5, 6 and 7 developed in 21-23, 22-23 and 24-26 chaetigers larva, respectively. The branchial pairs 8 and 9 further developed in

26-28 and 28-31 chaetigers larva, respectively. All branchial pair developed well in the early benthic stage juvenile, 26-30 chaetiger, 2,100 -2,400 μm in length. The duration from hatching to the early benthic stage juvenile was 38-43 days. Adult size of *P*. *pulchra* was 4.8-5.2 mm. in length with 27-33 chaetigers. Size at maturity was those worm of 50-60 chaetigers and a length of 6.0-9.8 mm. Time to maturity in *P*. *pulchra* was 58-65 days (Table 3.2). The pelagic phase in *P*. *pulchra* was shorter than those temperate spionid polychaetes (Blake & Arnofsky, 1999; Radashevsky et al., 2006; Yokoyama, 1981). From the observation in the field, the breeding season in *P*. *pulchra* coincided with the low salinity in the wet season. However the breeding season in *P*. *pulchra* was found to be a month delayed comparing to *P*. *membranacea*. From the induced spawning experiment, the gravid females in *P*. *pulchra* would produce 3-4 egg batches within 6 months.

Table 3. 2 Larval development of spionid polychaetes, *Prionospio (Minuspio) pulchra* in enriched sediment under the mussel rafts from Sriracha Bay, Chonburi Province, eastern coast of Thailand.

Characteristics in larval development	**Low organic content**	**Normal condition**	**High organic content**	**Enriched organic content**
Oocytes size (μm)	65-100	80-100	72-196	70-105
Fecundity	177-349	463-520	650-890	783-857
Type of development	plankto trophic	plankto trophic	plankto trophic	plankto trophic
Stage at release (no. chaetigers)	2-3	2-3	2-3	2-3
Size at release (μm)	180-250	180-250	180-250	180-250
Size at metamorphose (no. chaetigers)	8-12	8-12	8-12	8-12
Length of planktonic phase (days)	30-31	24-27	19-21	16-19
Sized at settlement • number of chaetiger (chaetigers) • Length (μm) • Duration (days)	 27-33 2100-2400 49-56	 27-33 2000-2300 38-43	 28-35 2000-2400 37-38	 26-36 2200-2400 37-40
Adult size (mm)	5.0-5.2	4.8-5.2	5.0-5.5	5.2-5.5
Size at maturity • number of chaetiger (chaetigers) • Length (mm)	 50-62 7.0-9.0	 50-66 6.0-9.8	 54-62 7.5-8.7	 53-70 7.3-15.0
Time to maturity (days)	63-70	58-65	51-60	53-60

2.2 Fecundity

High fecundity were also observed with increasing size in *P. pulchra*. The fecundity of a mature female in low organic matter condition, high organic matter condition and enriched organic matter condition were positively correlated with number of chaetigers (r=0.97, p<0.05, n=5; r=0.89, p<0.05, n= 9; r=0.62, p<0.05, n=14, respectively) as shown in Figure 3.9. Average fecundity of mature females of *P. pulchra* in high organic matter and enriched organic matter condition after 30 days were higher than those of low organic matter condition (p<0.05). The average fecundity of mature females of *P. pulchra* in high organic matter (808±33.18 oocytes/ind.) and enriched organic matter condition (823±10.50 oocytes/ind.) were 3.06 and 3.12 times, as compared with those in low organic matter condition (264±68.71 oocytes/ind.).

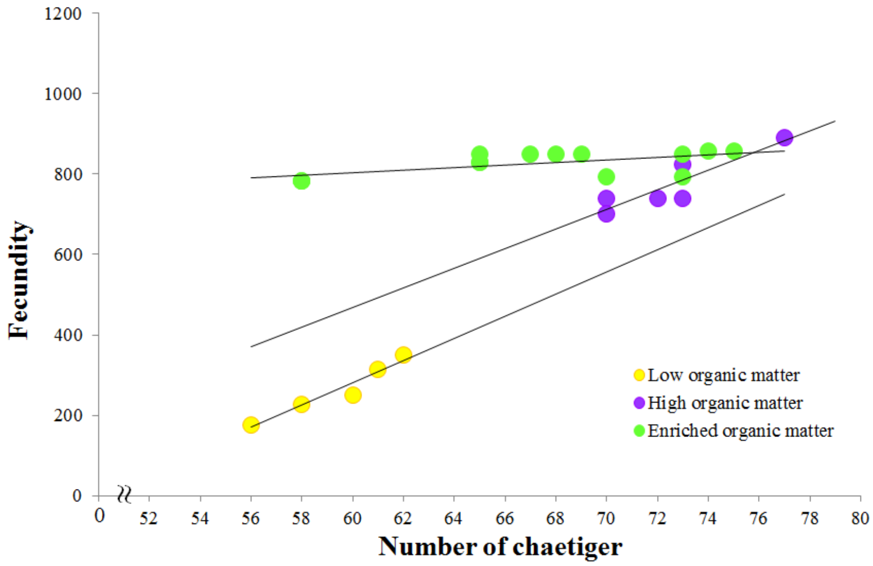

Figure 3 9Relationship between fecundity of mature female *Prionospio (Minuspio) pulchra* in different organic condition treatments and the body length (number of chaetiger).

The level of organic enrichment did not showed the pronounced effect on oocyte size. Average values of the oocyte diameter of a mature female of *P. pulchra* from experiment after 30 days between low, high and enriched organic matter condition showed no significant different (p>0.05). However oocyte size did showed the trends of larger oocytes in the high and enriched organic conditions as in *P. membranacea.*The average oocyte diameter in a mature female of *P. pulchra* in high and enriched organic conditions were 72-196 and 70-105 µm, respectively. The average oocyte diameter in the low organic condition was 65-100 µm.

2.3 Larval development and duration to settlement stage

Size at metamorphosis and settlement of *P. pulchra* did not showed significant differences among low, high and enriched organic sediment (p>0.05) as in Figure 3.10. Time to metamorphosis and settlement decreased as the sediment organic increased. *P. pulchra* larvae metamorphosed in high organic and enriched organic condition within 16-21 days, respectively. The larvae took longer time of 30 days to metamorphosis in low organic condition. Time from hatching to settlement in the three treatments also followed the same trend. The duration of larval settlement in high organic and enriched organic condition was 37-38 days.

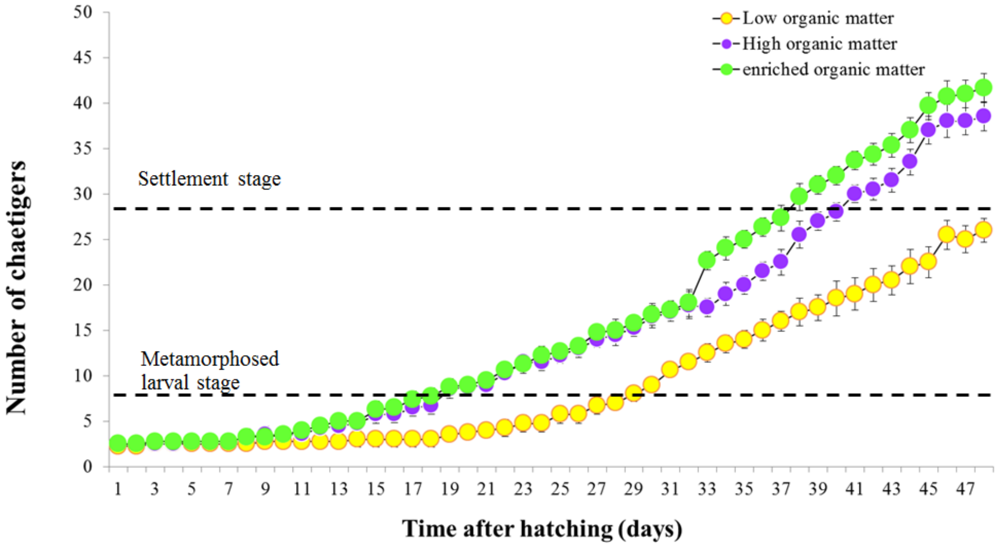

Figure 3 10 Size (number of chaetigers) and time after hatching to metamorphosis and settlement (day) in larvae of spionid polychaetes, *Prionospio (Minuspio) pulchra* in different organic conditions.

The lower percentage of metamorphosed and competent larvae at settlement in *P. pulchra* was also observed in the low organic condition. The percentage of metamorphose larvae of *P. pulchra* in the low organic, hugh organic and enriched sediment treatments were 36±4.62, 49±8.24 and 57±6.83, respectivety as in Figure 3.11. The percentage of competent larvae of *P. pulchra* in respective treatments were 12±4.2, 40±6.53 and 48±6.53, respectively. This finding corresponded to Cohen and Pechenik (1999) in *Capitella* sp. I.

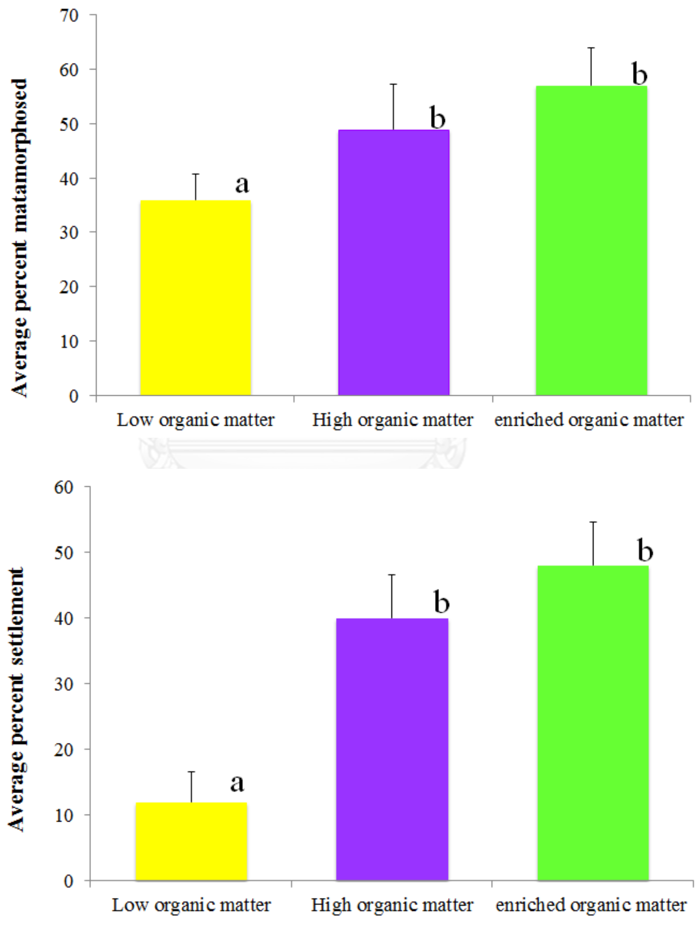

Figure 3 11 Success settlement of *Prionospio (Minuspio) pulchra* larvae in different organic conditions. Different letters above bars indicate significant differences (p<0.05). Error bars represent SD.

3. Comparative Study on Reproduction Responses in *Prionospio* Polychaetes to Organically Enriched Sediment

3.1 Fecundity/ oocytes size

The two spionids polychaetes, *P. membranacea* and *P. pulchra* shared the same reproductive traits of being broadcast spawners with planktotrophic development. In the treatments of different organic conditions after 30 days, there were no significant different in the average fecundity size of mature females of two spionids in low organic matter and high organic matter ($p<0.05$). But in the enriched sediment, the average fecundity size in mature females of *P. membranacea* was 869±18.85 oocytes/ind, which was higher than those of *P. pulchra* being 823±10.50 oocytes/ind ($p<0.05$). The oocyte size in the two spionids were in the same range in the three treatments.

3.2 Larval development and duration to settlement stage

The size at metamorphosis and size at settlement in the two spionids, *P. membranacea* and *P. pulchra* showed no significant different ($p>0.05$). However the two spionids showed the different responses in term of time to metamorphosis and time to settlement as well as the success in larval settlement in different organic conditions. Time to metamorphosis and time to settlement in *P. membranacea* larvae were shorten as compared to those in *P. pulchra* in different organic condition. The percentage of metamorphosed larvae in *P. membranacea* larvae in high organic and enriched organic condition were significantly higher by 1.39 and 1.12 times of those in *P. pulchra* larvae. Early maturation even under favorable conditions such as organic enriched sediment may be quickly to more favorable conditions and colonized the area (Zajac, 1986). In organic enrichment condition, *P. membranacea* demonstrated the higher reproductive potential than *P. pulchra*. This also help to explain why *P. membranacea* can be found as the most abundant species widely distributed in the coastal area of Sriracha Bay as compared to five other spionids. Reproduction and larval development pattern as well as the mud content and total organic matter in the sediment played the important roles in the recruitment of *P. membranacea.*

The reproduction pattern in spionid polychaete *P. membranacea* and *P. pulchra*, the two dominant spionids in Sriracha Bay, matched the description of *Prionospio* given by Blake and Arnofsky (1999) and Rouse (2001), which they are gonochoric. Their oocytes were thick smooth membranes that lack alveoli, and occured in the segments of the mid-region of the body. Most of the pollution-indicator species displayed opportunistic life-histiry characteristics such as small size, short generation time, high reproductive rate and high mortality as in Table 3.3. Such as characteristics allow these species to quickly colonize a marine habitat during the early seral stage that follow the environmental disturbance such as low oxygen condition or the generation of hydroden sulfide in the enriched organic condition (Blake & Arnofsky, 1999; J. P. Grassle & Grassle, 1976; H. & Kikuchi. T, 1983; Lisa A Levin, 1984; Radashevsky et al., 2006). This study also demonstrated *P. membranacea* and *P. pulchra*, also shared the opportunistic life history characteristics.

The spionid *P. membranacea* and *P. pulchra* were broadcast spawner species with planktotrophic larval development. Their larvae were long and with thin, numerous segments. *P. membranacea* and *P. pulchra* have the short planktonic phase of 8-14 and 24-27 days, respectively. Size at settlement for these two spionids respectively were 2,100-2,400 and 2,000-2,300 µm. This characteristics were found in other *Prionospio* species such as *Prionospio caspersi, P. cirrifera, P. malmgreni, P. fallax, P. lighti, P. saldanha* and *P. steenstrupi* ranged in 15 to 28 chaetiger and 1400 to 2400 µm, and length of planktonic life in these spionids were 30 to>30 days (Blake & Arnofsky, 1999; Wilson, 1991). The characteristic of reproductive pattern of two spionids were fit in Group IV as the second-order opportunistic species (slight to pronounced unbalanced situations) of Marine Biotic Index (AMBI), with the small species with a short life cycle, adapted to life in reduced sediment where they can proliferate (Borja et al., 2000; Carvalho et al., 2006; Cheung et al., 2008; D. M. Dauer, 1993; Grall & Glémarec, 1997). The fecundity size in the two spionids *P. membranacea* and *P. pulchra* were 503-552 and 463-520 oocytes/ind. These fecundity size were the characteristic of opportunistic species such as *Capitella* sp. I (J. P. Grassle & Grassle, 1976), *Polydora ligni* and *Streblospio benedicti* (Lisa A Levin, 1984).

Table 3. 3 Life history traits of pollution-indicator polychaetes in organic enriched sediment (Adapted from Yokoyama, 1990)

Species	Time to maturity	Spawning period	Oocyte size (µm)	Length of planktonic phase	Trophic mode of larva	Size at settlement	Adult size	Life span	Reference (Study area)
Capitella sp. I	30-40 days	Most of the year	260	several hours	lecithotrophic		3-12 mm		J. P. Grassle and Grassle (1976) (North America)
Capitella capitata	46-48 days	Most of the year	300	< 24 hours	lecithotrophic	350-460 µm			H. and Kikuchi. T (1983) (Japan)
Capitella sp.			220	several hours	lecithotrophic	11 chaetigers			Lisa A Levin (1984) (North America)
Marenzelleria viridis			200-260	45 days	planktotrophic	750 µm			Blake and Arnofsky (1999) (North America)
Paraprionospio patiens	3-6 months		140	2 months	planktotrophic	3,800-5,380 µm	60 mm.	> 1 yr.	Yokoyama (1990) (Japan)
Prionospio membranacea	38-58 days	Most of the year	80-100	8-14 days	planktotrophic	2,100-2,400 µm	55-65 mm.	> 1 yr.	This study (Thailand)
Prionospio pulchra	58-65 days	Most of the year	80-100	24-27 days	planktotrophic	2,000-2,300 µm	48-52 mm.	> 1 yr.	This study (Thailand)
Prionospio patogonica		Summer	82-92		planktotrophic	360-570 µm	31 mm. -128 chaetigers		Radashevsky et al. (2006) (South America)
Streblospio benedict	2-3 months	Most of the year	100-200	0-7 days	lecithotrophic		20 mm.	1 yr.	Lisa A Levin (1984) (North America)
Streblospio benedict	2-3 months	Most of the year	70-90	7-45 days	planktotrophic		20 mm.	1 yr.	
Polydora ligni		Most of the year	70	7-14vdays	adelphophagia planktotrophic				Lisa A Levin (1984) (North America)

In *Paraprionospio patiens*, the large brood size of 3,000-4,300 oocytes/ind at the size of settlement 3,800-5,380 µm. The planktonic phase was 2 months. (Yokoyama, 1990). He concluded from his study that *Pa. patiens* has an annual life cycle represented by seasonally defined reproduction and recruitment. More than three months are required before the individual reaches maturity. It lives for 1 year or more with relatively low mortality after settlement. These characteristics fit well of the equilibrium species. According to Lisa A Levin (1984), polychaetes with small adult size, brood protection, small brood size, and reduced planktonic larval phases as in *Capitella* spp. and *Streblospio benedict* were adapted to small-scale disturbances, while polychaetes with larger brood sizes and longer-lived larvae, whose planktonic abundance was highly seasonal and variable from year to year such as *Polydora ligni* were adapted to large disturbances. The small-scale dispersal abilities of the former species induced rapid colonization of disturbed patches and that the latter species colonized successfully if the timing of the disturbance coincided with periods of peak larval availability. From this study, *P. membranacea* and *P. pulchra* will fall into the first category as assigned by Lisa A Levin (1984).

Increase organic content in the sediment showed the close correlation with the increase in fecundity size of the two spionids. The enriched sediment condition under the mussel raft culture would provide favorable habitat for these spionid polychaetes. The time to metamorphosis and settlement also showed pronounced increases in the high and enriched organic sediment. Of the two spionid polychaetes, *P. membranacea* demonstrated the higher reproductive potential and can rapidly proliferate in the organically enriched sediment.

E. Conclusion

The characteristic of reproduction and larval development patterns of the two dominant spionids, *P. membranacea* and *P. pulchra* in Sriracha Bay, fit the life-history of opportunistic polychaete species in organic enriched sediment. They also demonstrated the Marine Biotic Index (AMBI) of GroupIV as the second-order opportunistic cycle. Organic enriched sediment increased their reproductive potential in term of increase fecundity and decrease time to metamorphosis and settlement. Organic enriched sediment also increased the settlement success in the larvae of these polychaete species. Of the two spionids, *P. membranacea* demonstrated the higher reproductive potential and can rapidly proliferate in the organically enriched sediment.

CHAPTER IV
EFFICIENCY OF SPIONIDS GENUS *Prionospio* IN BIOREMEDIATION PROCESS IN ORGANICALLY ENRICHED SEDIMENT

A. Introduction

Mussel aquaculture in marine waters has seen a dramatic increase worldwide. The mussel culture required a fairly productive coastal environment with an adequate production of phytoplankton with the advantage of no artificial feed requirement (M. Carlsson et al., 2009; Christensen et al., 2003; da Costa & Nalesso, 2006; Grant et al., 1995). Mussel culture is known to have a severe local environmental impact due to increased sedimentation of biodeposits (faeces and pseudofaeces) contributing to organic enrichment in the sediment inside the farming area. In some cultures, where currents are not strong enough to transport this material, the depth of the oxygenated layer of the sediment decreased and bottom oxygen may be depleted, leading to anoxia of the sediment and the overlying water. Aggregations of mussels regulated nutrient fluxes, sedimentation and primary production in the coastal ecosystems (M. S. Carlsson et al., 2009; Christensen et al., 2003; Grant et al., 1995; Kaspar et al., 1985; Ysebaert et al., 2008). Tantanasarit and Babel (2014) reported the removal rate of carbon, nitrogen and phosphorus by green mussel (*Perna viridis*) which calculated based on the mussel filtration rate were 3302, 380 and 124 mg yr^{-1} ind^{-1}, respectively. Nutrient released, as carbon, nitrogen and phosphorus from mussel faeces were calculated to be 621, 135, 13 mg yr^{-1} ind^{-1}. This organic enrichment could cause dramatic changes in the benthic community structure, reducing the species to few tolerant species, lowering both the diversity and community richness (Pearson & Rosenberg, 1978). Changes in benthic communities under the mussel farms has been extensively documented (Christensen et al., 2003; da Costa & Nalesso, 2006; Grant et al., 1995; Kaspar et al., 1985; Stenton-Dozey et al., 1999; Ysebaert et al., 2008). The macrofauna were found to dominate numerically by opportunistic species, mostly polychaetes. Opportunistic polychaetes, such as *Capitella* spp. in the Family Capitellidae and *Prionospio* spp. in the Family

Spionidae, were usually small, short-lived, prolific and capable of exploiting the organically enriched sediment (Borja et al., 2000; D. M. Dauer, 1993; Diaz & Rosenberg, 1995; Pearson & Rosenberg, 1978).

Many polychaete species were used in the bioremediation process in the integrated aquaculture system and in the coastal area. These polychaete species are resistant to toxicity and have the ability to generate an economic return following remediation activities such as polychaetes in the Family Nereididae. Polychaete sand filters were usually integrated in the shrimp and fish farming systems to simultaneously remediate wastewater and produce harvestable polychaete biomass without supplemental feeding (Brown et al., 2011; Palmer, 2010, 2011; Van Bruggen, 2012). These polychaetes, used as bait for sport and professional fishing also as a food source in aquaculture, Polychaete worms have been identified as source of essential fatty acids and have an important role in the development of the gonads (Narciso & da Fonseca, 2000). Capitellid polychaete, *Capitella* spp., small deposit feeders, have been successfully used as the bioremediator of organic waste from fish farms in Southern Japan (Chareonpanich et al., 1994; Tsutsumi et al., 1990; Tsutsumi et al., 2005; Tsutsumi et al., 2002). Tsutsumi and his team had developed the technique of introducing the artificially mass culture of *Capitella* sp.I on the organically enriched sediment below a net pen in the fish farm. Kinoshita et al. (2008) had applied the same method of bioremediation of organically enriched sediment deposited below fish farms in Japan enclosed bay. They were able to demonstrate that this method of applying mass–culture technique of deposit-feeding polychaete *Capitella* sp.I was promising to enhance the decomposition rate of organic matter markedly in organically enriched sediment below fish pens.

The coastal area of Sriracha Bay, Chonburi Province, eastern coast of the Gulf of Thailand, is one of the important areas for green mussel (*Perna viridis*) rafts culture. Organic enriched sediment in this bay resulted from mussel rafts culture and the domestic effluents from the municipal town of Sriracha. Spionid polychaetes, *Prionospio* are one of the dominant polychaetes in the organically enriched areas of Sriracha Bay (To-orn & Intarachart, 2010). These spionids similar to the capitellids were found abundant in highly organic polluted areas with low dissolved oxygen and high sediment sulphide content, tolerance which enables them to rapidly colonize areas with high levels of organic matters, due to their adaptation in feeding and diverse patterns of reproduction and development (Blake & Arnofsky, 1999; Borja et al., 2000; Fauchald & Jumars, 1979). Two dominant spionids, *P. membranacea* and *P. pulchra*, were found in the area. The former species was abundant of 13-655 inds.m^{-2} while the latter species was rare of 0-68 inds.m^{-2}.

As the bioremediation of organically enriched sediment from fish farms by the deposit feeder polychaete, *Capitella* have been proven successful, this present study aims to assess the possibility of two surface deposit-feeding polychaetes, *P. membranacea* and *P. pulchra* to remediate organically enriched sediment from green mussel rafts. Effect of polychaete density on the bioremediation efficiency is also evaluated. The results can be used as the guideline in the application of mass culture of spionid polychaetes in the bioremediation of organic enriched sediment from aquaculture system.

B. Literature Review

Bioremediation is the use of living organisms to remove or detoxify pollutants within a given environment (Alexander, 1994). In aquaculture system such as fish farms, shrimp farms and mussel farms, polychaetes can be used as bioremediator that nutrients and organic matter released in the culture system could be converted into biomass which can be easily removed and harvested as the valuable by-product (Alexander, 1994; Gifford et al., 2007; Kinoshita et al., 2008; Palmer, 2010, 2011; Tsutsumi et al., 2005; Tsutsumi et al., 2002; Van Bruggen, 2012). Bioturbation of polychaetes are importance activities to enhance the bioremediation process, including deposit feeding, reworking of sediment, construction of burrows and tubes and irrigation process (Rosenberg, 2011).

The utilization of polychaetes in bioremediation in aquaculture system were widely introduced. There have been extensive studies on the use of deposit feeding polychaetes, *Capitella* species for bioremediation of organic wastes from fish farms in Southern Japan (Chareonpanich et al., 1993; Chareonpanich et al., 1994; Tsutsumi et al., 1990). Artificially culture colonies of *Capitella* sp.1 had been introduced to treat enriched sediment in the outdoor pool (Tsutsumi et al., 2002) and in enriched sediment under the fish farms (Kinoshita et al., 2008; Tsutsumi et al., 2005).

Several species from the family Nereididae have been studied to simultaneously remediate wastewater and produce harvestable polychaete biomass without supplemental feeding (Palmer, 2010, 2011). Van Bruggen (2012) studied bioremediation of particulate sea bass waste by the polychaetes *Capitella* sp. and *Nereis diversicolor* in a recirculating aquaculture system. The deposit feeding polychaete, *Capitella* sp. reduced 0.008% of total organic matter/ind./day/m^2, while the large polychaete, *N. diversicolor* reduced total organic matter of 0.027%/ind./day/m^2. Cuny et al. (2007) showed that the presence of the polychaetes *N. diversicolor* favored the development of bacteria that play an active role in natural bioremediation processes of oil polluted environment. The presence of *N. diversicolor* induced significant changes in the composition of oil.

Filter feeding polychaetes were also used as bioremediators in the aquaculture system. Giangrande et al. (2005) studied the filter feeder sabellid, *Sabella spallanzanii* as biofilter in the treatment of wastes from intensive aquaculture system. They found that the single worm of 0.6 mg dry weight, was able to remove within the first hour of activity more than 50% of the particulate organic matter from the water column with initial concentration of 22.76 mg L^{-1}. A biomass of 1 mg dry weight of worm can remove 4.93 mg L^{-1} of suspended solids within 3 h from the water column. This sabellid polychaete *S. spallanzanii* was introduced as potential bioremediator in remediation of waste fish farms with recycled water. Parandavar and Kim (2014) studied the improvement of outlet water quality in fish culture by rockworm *Marphysa sanguinea* in the semi-recirculating system. They found that the rockworm *M. sanguinea* with 4000 inds/m^2 of less than 0.5 g was an excellent potential bioremediator for the integrated aquaculture and nutrient recycling including the removal of organic wastes in land-based systems.

C. Materials and Methods

1. Collection of Polychaetes and Sediments

The spionids polychaetes *P. membranacea* and *P. pulchra* were collected from October to November 2013 at Sriracha Bay, Chonburi Province (13°11′N, 100°55′E), on the east coast of the Gulf of Thailand as in Figure 4.1. Adult specimens of two spionid species were collected from the area of high organic content at station 1 (4.66-6.08%TOC) near Sriracha Town Municipality under the influence of domestic waste effluents.

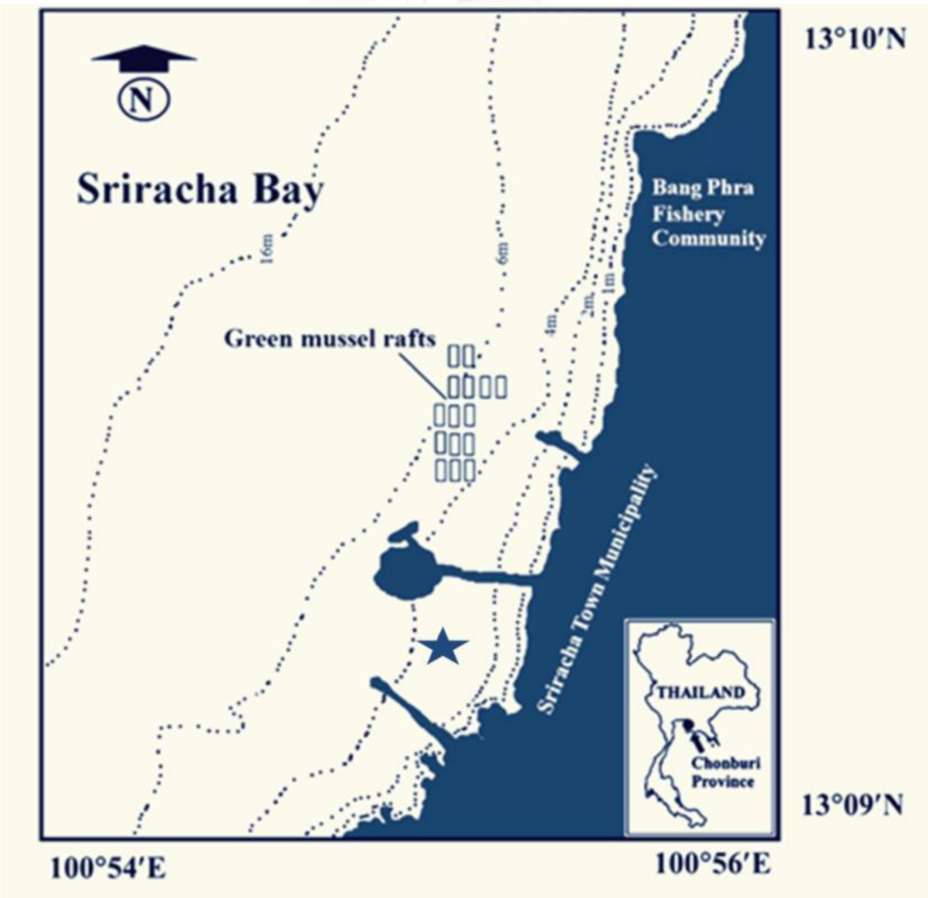

Figure 4. 1Study area in Sriracha Bay, Chonburi Province, the eastern coastline of Thailand. ★: sampling for spionid polychaetes.

Samples were taken by Ekman grab and washed through a 0.25 mm mesh sieve. Specimens were transported afterward to the laboratory for sorting and identification. The sediment used in the experiment was collected from under the floating mussel rafts in the bay using Ekman grab. Each sediment sample was sieved through a 1.0 mm mesh and freeze in the laboratory at least for 24 h to kill the living organisms. The seawater from the mussel rafts was filtered through 1.0 micrometer filter bag for the experimental use.

2. Preparation of Experimental Animals and Sediments

In the laboratory, spionid specimens were sorted under a stereo-microscope. The spionid polychaetes, *P. membranacea* and *P. pulchra* were identified following the procedures of M Imajima (1990b); M. Imajima (1990). Both spionids were acclimated in aquaria, which added the treated sediment and filtered seawater. The aquaria was provided with mild aeration. The acclimated polychaetes were individually measured the body size and body weight at the beginning of the experiment. The body length and the width of the 5th chaetiger (including the parapodia) of immature specimen were also measured under a stereo-microscope with an ocular micrometer and expressed as body size. The numbers of chaetigers in each worm were counted. The wet body weight was determined. Total organic matter in the sediment was determined by the loss on ignition (Nelson, 1982) prior to the experiment.

3. Studies on Bioremediation of Organically Enriched Sediment by Spionid Polychaetes

Twelve cultured boxes (20x25x15 cm) were each prepared with treated sediment at the level of 3 cm from the bottom with the filtered seawater level 10 cm above the surface sediment. The filtered seawater in each box was gently aerated. Spionid polychaetes were then added to the cultured boxes in three different density treatments: (1) control (without spionid polychaetes), (2) 10 individuals of spionids (normal density of 200 inds.m^{-2} based on the average density of the spionid, *P. membranacea* from high organic content area and the mussel rafts area in Sriracha

Bay, observed by To-orn and Intarachart (2010) and (3) 20 individuals of spionids (twice the normal density of 400 inds.m^{-2}).

For each treatment, four cultured boxes were used as replicates. Figure 4.2 demonstrated the setup for the experiment on bioremediation of organically enriched sediment by spionid polychaetes. The experiments were carried out for 30 days.

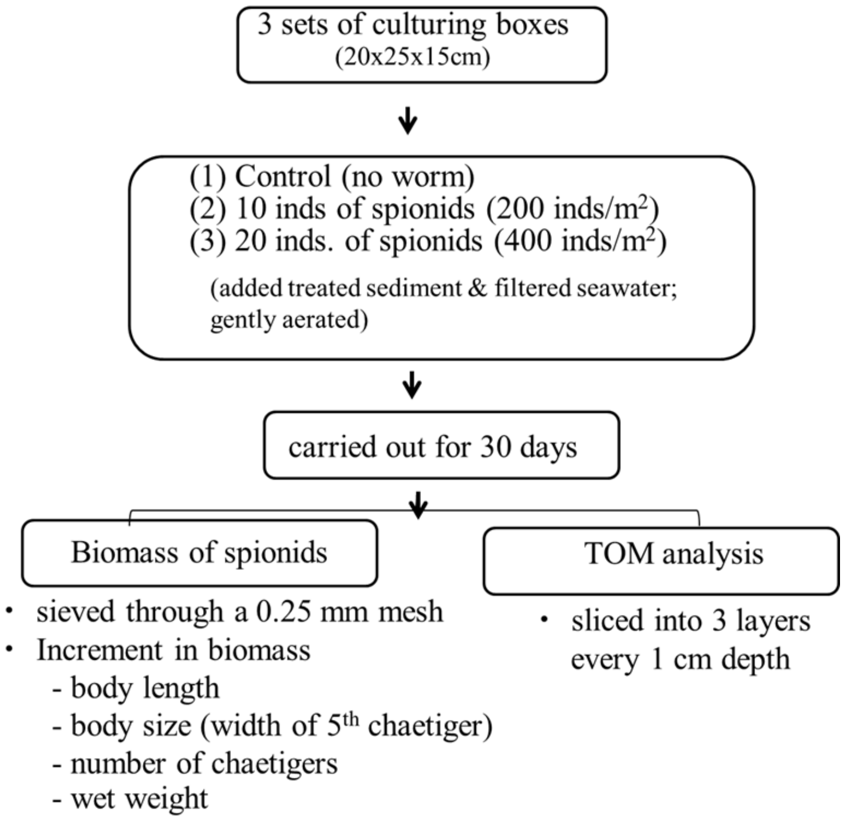

Figure 4. 2 Experimental set up for the bioremediation of organically enriched sediment by spionid polychaetes, *Prionospio (Prionospio) membranacea, Prionospio (Minuspio) pulchra.*

4. Assessment of Potential Spionid Polychaetes in Bioremediation

At the end of the 30 days experiment, the sediment from each cultured boxes were sampled by using a plastic hand core with an area of 0.55 cm^2. The sediment were sliced into three layers every 1 cm depth from surface (0-1, 1-2 and 2-3 cm in depth) for the analysis of total organic content by Ignition loss (Nelson, 1982). After that, the remaining sediment in each cultured boxes were washed through a 0.25 mm mesh sieve for the biomass study of the spionid polychaetes. The body length and the body size (width of the 5^{th} chaetiger, excluding parapodia), and the number of chaetigers of the spionds were measured and counted by using an ocular micrometer. The wet weights of the spionids were measured.

Products in term of increased polychaetes biomass and the ability to reduce the organic matter in the enriched sediment from mussel raft culture were assessed as the bioremediation efficiency of the spionid polychaetes. Increased of polychaete biomass in percentage were calculated from body length, body size, number of chaetiger and body wet weight at the initial and final phases of the experiments. The bioremediation efficiency of spionid polychaetes were enumerated from the percentage of total organic matter reduction at the initial and final phases of the experiments (Figure 4.2).

5. Statistical Analysis

One-way analysis of variance (ANOVA) at the 95% confidence level ($p<0.05$) was applied to analyze the differences of spionid polychaetes biomass and total organic matter in sediment between treatments. The biomass increment and the total organic matter reduction rate in sediment among the initial and final phases of the experiment, and among three layers between two spiond species were compared using a t-test (Zar, 1999).

D. Results and Discussion

1. Increment in Biomass in Spionid Polychaetes

The growth in term of biomass increment in spionid polychaete, *P. membranacea* was evidenced with the growth in term of average body length, body size and number of chaetigers in the initial and final phase of the experiment were significantly different (p<0.05). The average body length, body size and number of chaetigers of the spionid after 30 days of the experiment were higher than those in the initial phase about 1.76, 1.27 and 1.34 times in experimental treatment of 10 individuals. The body weight showed the same pattern of increase at the end of the experimental with final body weight was 2.34 times comparing to the initial phase. In the high density treatment of 20 individuals, the lower rate of increased biomass was observed (p<0.05). The average body length, body size and the number of chaetigers in the spionid after 30 days of experiment were 1.60, 1.17 and 1.28 times. While the final body weight of the polychaetes in the high density treatment was only 1.76 times the initial weight (Figure 4.3, Table 4.1).

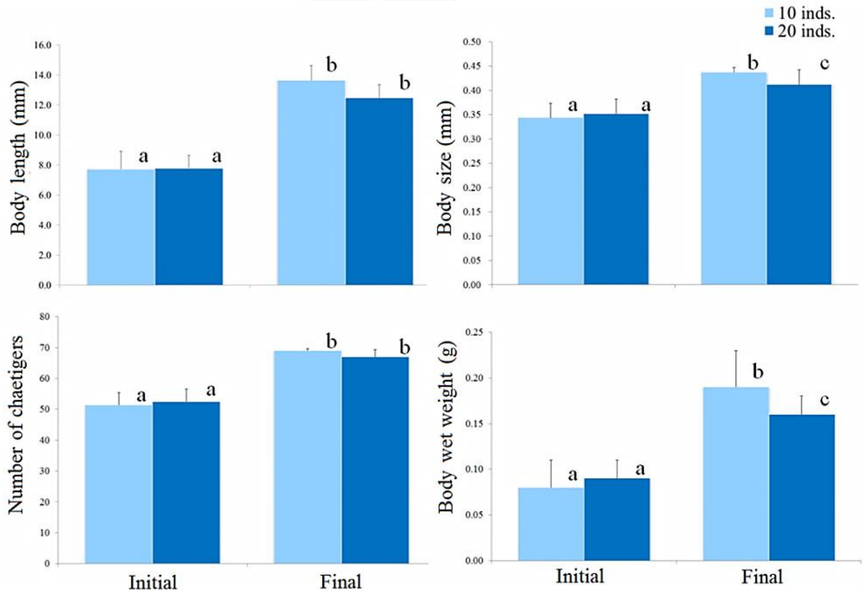

Figure 4. 3Average of biomass of *Prionospio (Prionospio) membranacea* in the bioremediation of enriched sediment under the mussel rafts from Sriracha Bay, Chonburi Province, eastern coastline of Thailand. Different letters above Bars indicate significant differences (p<0.05). Error bars represent SD.

Table 4. 1 Increment in biomass of spionid polychaetes, *Prionospio (Prionospio) membranacea* in the bioremediation of enriched sediment under the mussel rafts from Sriracha Bay, Chonburi Province, eastern coastline of Thailand.

Increment in biomass	**10 individuals**		**20 individuals**	
	Total increased biomass (30 days)	**Increment growth rate per day**	**Total increased biomass (30 days)**	**Increment growth rate per day**
Body length (mm)	5.920±1.07	0.020±0.03	4.676±1.05	0.156±0.03
Body size (mm)	0.094±0.02	0.003±0.00	0.061±0.03	0.002±0.00
Number of chaetiger (chaetigers)	17.500±1.00	0.583±0.03	14.750±2.217	0.492±0.07
Body wet weight (g)	0.00110±0.00	0.00004±0.00	0.00070±0.00	0.00002±0.00

The biomass increment in *P. pulchra* in the bioremediation experiments also showed the similar trends as in *P. membranacea*. The growth in term of average body weight, average body size and the number of chaetigers in the normal density treatment of 10 individuals after 30 days were 1.79, 1.31 and 1.32 times the initial values. The final body weight was 1.85 times the initial weight. Low growth also observed in the high density treatment of 20 individuals (p<0.05). The spionid growth was significantly different between the treatments. The average body weight, average body size and the number of chaetigers after the 30 days in the high density of 20 individuals were 1.57, 1.18 and 1.25 times the initial values. The final body weight was 1.17 times the initial weight (Figure 4.4, Table 4.2).

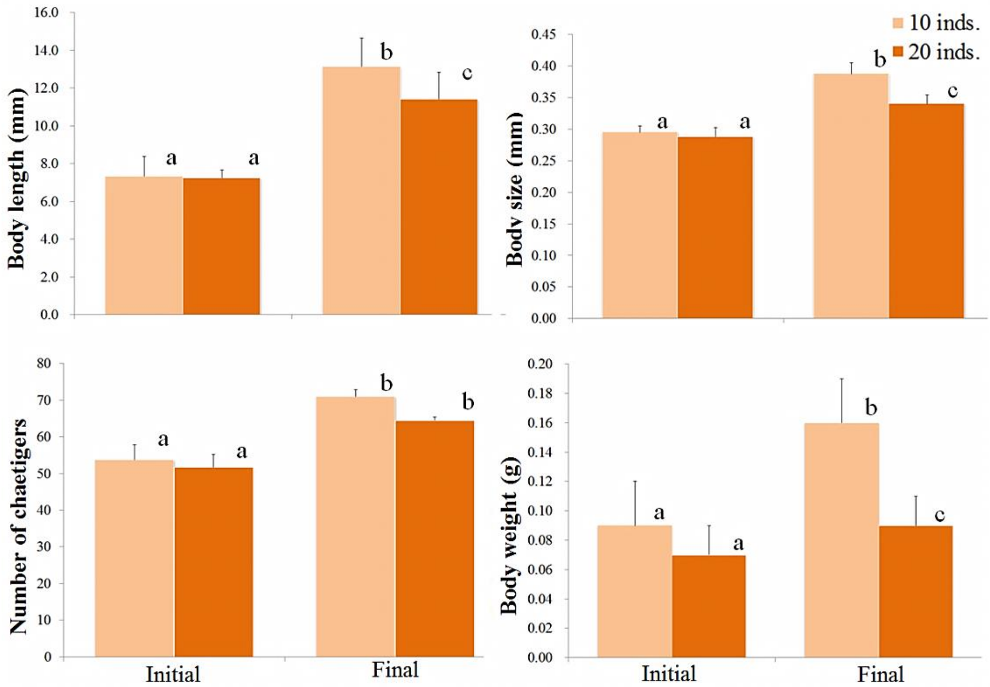

Figure 4. 4 Average of biomass of *Prionospio (Minuspio) pulchra* in the bioremediation of enriched sediment under the mussel rafts from Sriracha Bay, Chonburi Province,eastern coast of Thailand. Different letters above bars indicate significant differences (p<0.05). Error bars represent SD.

Table 4. 2 Increment in biomass of spionid polychaetes, and *Prionospio (Minuspio) pulchra* in the bioremediation of enriched sediment under the mussel rafts from Sriracha Bay, Chonburi Province, eastern coast of Thailand.

	10 individuals		**20 individuals**	
Increment in biomass	**Total increased biomass (30 days)**	**Increment growth rate per day**	**Total increased biomass (30 days)**	**Increment growth rate per day**
Body length (mm)	5.820±0.80	0.194±0.29	4.155±0.29	0.139±0.00
Body size (mm)	0.093±0.00	0.003±0.00	0.053±0.00	0.002±0.00
Number of chaetiger (chaetigers)	17.250±0.95	0.575±2.62	12.750±2.63	0.425±0.08
Body weight (g)	0.00070±0.00	0.00003±0.00	0.00010±0.00	0.000004±0.00

The rate of biomass increment in term of average body length, average body size and the number of chaetigers in the two spionids were not significantly different (p>0.05). However the increment growth rate in term of increased body weight in both treatments of 10 and 20 individuals of *P. membranacea* were significantly higher than *P. pulchra* (p<0.05) as in Figure 4.5.

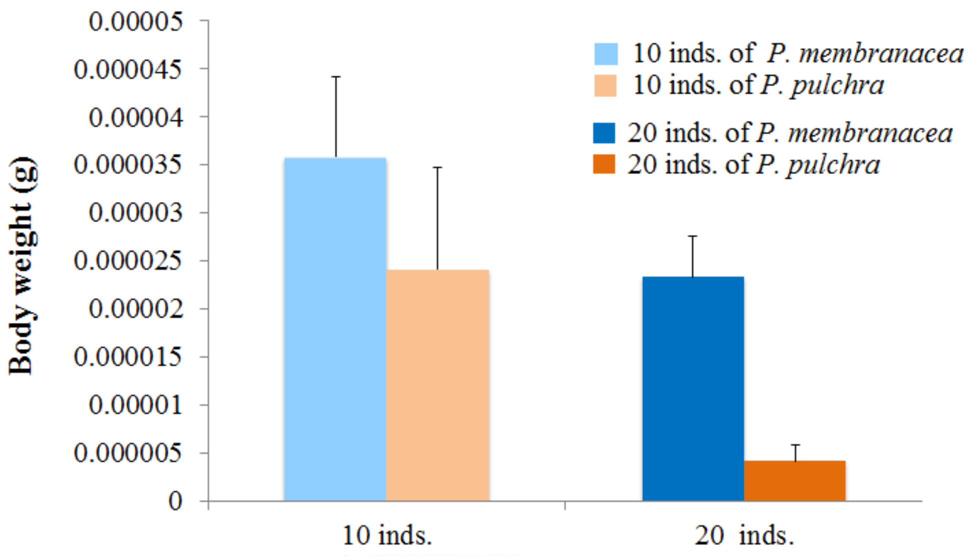

Figure 4. 5 Increment growth rate (g wet weight per a day) of spionid polychaetes, *Prionospio (Prionospio) membranacea* and *Prionospio (Minuspio) pulchra* in the bioremediation of enriched sediment under the mussel rafts from Sriracha Bay, Chonburi Province, eastern coastline of Thailand.

The biomass increment in the two spionid polychaetes, *P. membranacea* and *P. pulchra* in the bioremediation process revealed that *P. membranacea* was more efficient in converting the organic waste from the enriched sediment to biomass than *P. pulchra*. It was noted that in the normal density treatment, *P. membranacea* was observed with oocytes. As stated, *P. membranacea*, the dominant spionid polychaetes, widely distributed in Sriracha Bay in particular near the Sriracha Municipality and mussel raft culture. *P. pulchra* can also be found in the same area but in low density. These spionid polychaetes are small in the same size range. From this study, *P. membranacea* proved to be more reproductive potential in organic enriched sediment as compared to *P. pulchra*. The fecundity rate of mature females in *P. membranacea* of 869±18.85 oocytes/ind. was higher than those in *P. pulchra* of 823±10.50

oocytes/ind. The duration of larval metamorphosed and settlement in *P. membranacea* were more rapid than those in *P. pulchra*. The recruitment of spionid polychaetes, *P. membranacea* can be rapid in short period.

Spionid polychaetes help to stabilize and reworking of the surface layers of soft mud and sand bottom through their feeding and burrowing activities. These biological activities enhance the bioremediation process in the enriched sediment. Increased biomass in spionids indicated that organic matter in the sediment was consumed as food. Fauchald and Jumars (1979) reported that spionids were generally considered surface deposit feeders. D. M Dauer et al. (1981) further proposed that spionid polychaetes were interface-suspension and resuspended in the sediment. Spionid polychaetes have a pair of grooved, ciliated palps that arise from the peristomium. Palps that came in direct contact with the sediment surface picked up particles in the food groove and transported them by ciliary action to the everted pharynx. When particles were resuspended by a current, the polychaetes changed their palp orientation modes and increased their feeding upon suspended particles Yokoyama (1988) demonstrated the feeding bahavior of *Paraprionospio* sp. from A. in the laboratory. The worms extended their tentacles and two pairs of branchiae onto the surface of the sediment, while liying in their tubes. Worms fed on the surface of the sediment throughout the day using a pair of ciliated tentacles. As a result of feeding, a circular area appeared on the surface on the sediment around the opening of the tube. The radius of the feeding scar was approximately equal to the length of the tentacle, which was approximately 30-40% of the body length. As demonstrated from our result that the biomass increment in the normal density treatment was higher than the high density treatment. The density of the spionid polychaetes affected the food supply and the feeding efficiency. This was true in several deposit feeding polychaetes. Miller and Jumars (1986) reported that spionid polychaete *Pseudopolydora kempi japonica* in laboratory experiment had the mean value of 1.4 cm for feeding radius (a circular feeding area of 6.2 cm), which at population densities more than 0.16 cm^{-2} neighboring worms' feeding areas would overlap. In laboratory experiments of the spionid polychaete *Polydora ligni*, Zajac (1986) found that differences in intra-specific density and food supply effected growth and reproduction. In large eunicid polychaete, ragworm *Marphysa sanguinea*, Parandavar and Kim (2014) found that *M. sanguinea* with 2000 inds.m^{-2} had a 0.8 g final weight which was lower than 1000 inds.m^{-2} and higher than 4000 inds.m^{-2}. Van Bruggen (2012) found that biomass (g dry weight) of 4 inds. of *Capitella* sp. with feed sea bass waste was higher than 37 and 372 inds.

2. Reduction of Organic Matter in Sediment

The total organic matter in sediment at the initial phase of *P. membranacea* experiments were 7.187±0.702%. No significant changes occurred in the no worm treatment. In spionid *P. membranacea*, the reduction of organic matter in sediment in experimental treatment of 10 and 20 individuals in the initial and final phase of the experiment were significantly different (p<0.05). The total organic matter in sediment at the end of the experimentas were 1.61, 1.59 and 1.65 times in 0-1, 1-2 and 2-3 cm layer, respectively comparing to the initial phase. The reduction of total organic matter in sediment 0-1, 1-2 and 2-3 cm layer in the final phase were not significantly difference (p>0.05). In the high density treatment of 20 individuals, the reduction of total organic matter in final phase were higher than those in initial phase of 2.14, 2.09 and 2.08 in 0-1, 1-2 and 2-3 cm layer, respectively. The reduction of total organic matter in three layers in final phase of the high density treatment were not significantly difference (p>0.05) (Figure 4.6).

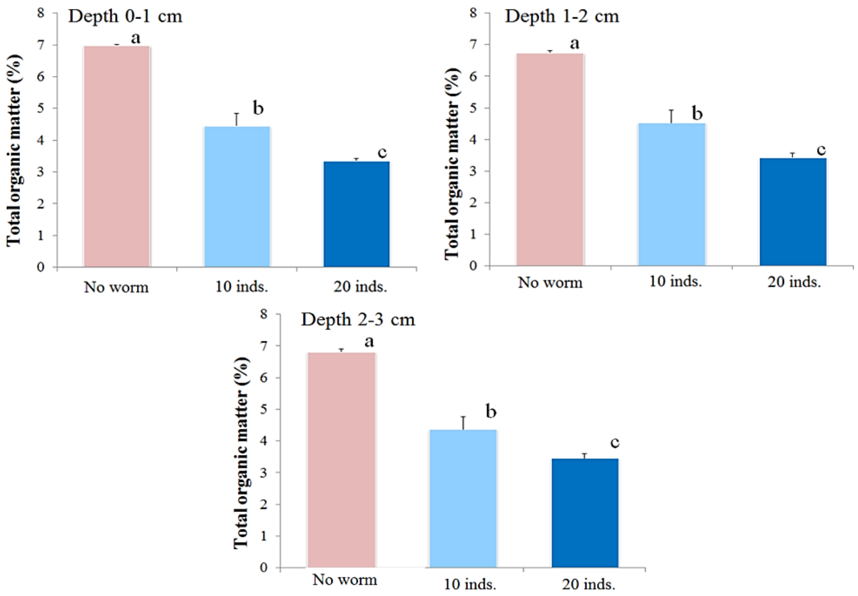

Figure 4. 6Reduction of total organic matter (%) in three layers of sediment in bioremediation of organically enriched sediment under green mussel rafts spionid polychaete, *Prionospio (Prionospio) membranacea* in Sriracha Bay, Chonburi Province, eastern coastline of Thailand.

The total organic matter in sediment at the initial phase of the *P. pulchra* experiments were also 7.187±0.702%. No significant changes was detected in the no worm treatment. The reduction in total organic matter in sediment in *P. pulchra* in the bioremediation experiments showed the similar trends as in *P. membranacea*. The decreasing in the total organic matter in sediment in the normal density treatment of 10 individuals after 30 days were 1.63, 1.60 and 1.57 times compared to the initial values in 0-1, 1-2 and 2-3 cm layer, respectively. High reduction in organic matter in sediment also observed in the high density treatment of 20 individuals (p<0.05). The reduction of total organic matter in sediment were significantly different between the treatments. The reduction in total organic matter in sediment after the 30 days in the high density of 20 individuals were 2.05, 1.87 and 2.00 times the initial values (Figure 4.7).

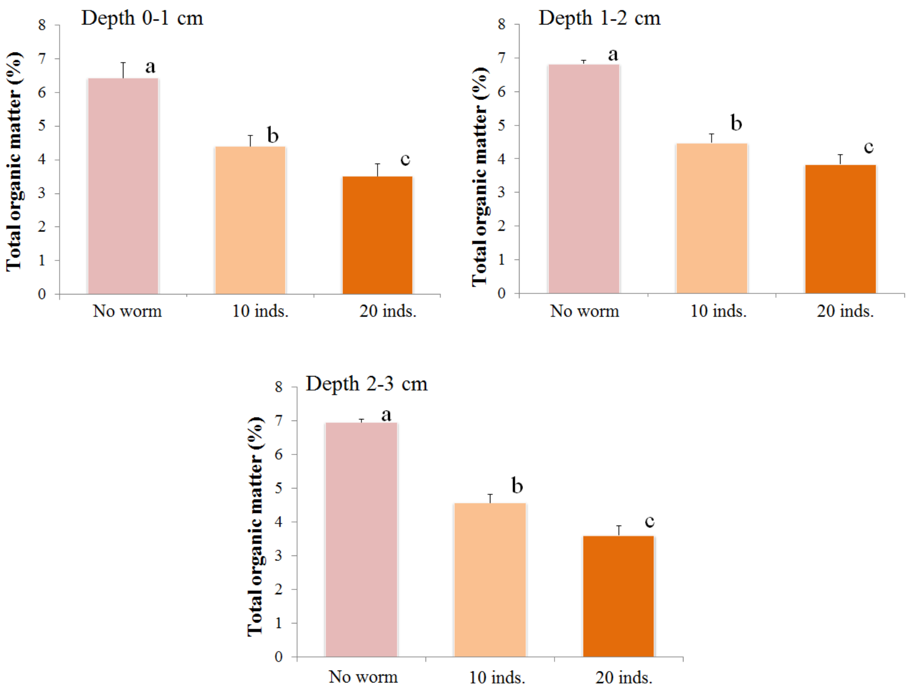

Figure 4. 7 Reduction of total organic matter (%) in three layers of sediment in bioremediation of organically enriched sediment under green mussel rafts spionid polychaete, *Prionospio (Minuspio) pulchra* in Sriracha Bay, Chonburi Province, eastern coastline of Thailand.

Comparision on the initial total organic matter in the sediment and the final total organic matter at the end of the bioremediation experiment showed that the two spionid polychaetes had potentially decreased the level of organic matter in the sediment through their biological activities as in the Figure 4.8. In *P. membranacea*, the decrease of organic matter after 30 days of the bioremediation experiment was highest in the high density treatment at the reduction rate of 52.48% from the initial total organic matter. The organic reduction rate in the normal density treatment in *P. membranacea* was 38.16% after 30 days. Similar trend was observed in the reduction of organic matter in the sediment in the bioremediation experiment in *P. pulchra*. The ability of the two spionid polychaetes to decrease the level of organic matter in the enriched sediment under the green mussel rafts were similar (p<0.05). After 30 days, the organic matter reduction rate in the high density treatment of *P. pulchra* was

49.30%, while in the normal density treatment was 37.60% compared to the initial values.

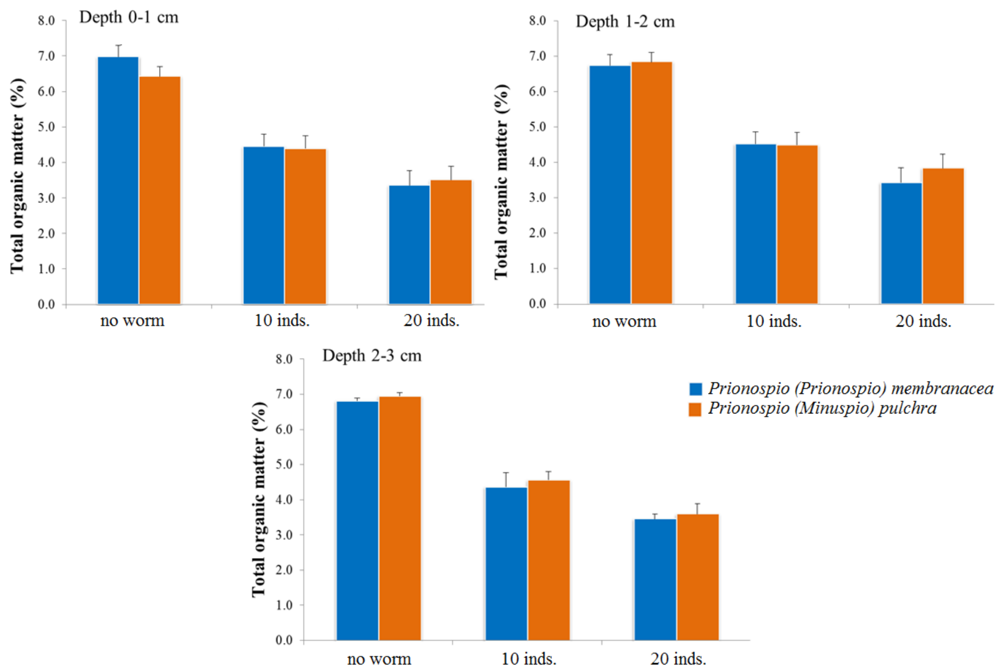

Figure 4. 8Reduction of total organic matter (%) of three layer of sediment in bioremediation of organically enriched sediment under green mussel rafts by spionid polychaetes, *Prionospio (Prionospio) membranacea* and *Prionospio (Minuspio) pulchra*

Biological activities of deposit feeding polychaetes such as feeding and reworking can apparently promote the decomposition of organic matter in the sediment (Chareonpanich et al., 1994; D. M Dauer et al., 1981; Tsutsumi et al., 1990; Yokoyama, 1988). These spionid polychaetes excreted fecal pellets which were placed outside their tubes in a neat pile. The bottom currents further eroded and transported fecal pellets that accumulated within the worm's foraging area (D. M Dauer et al., 1981). Kinoshita et al. (2008) concluded from the application of artificially mass-cultured colonies of a deposit-feeding polychaete, *Capitella* sp. as bioremediator of organically enriched sediment deposited below fish farms that the dense culture of *Capitella* sp. increased the decomposition of organic matter. The reworking activities of the sediment by dense patches of *Capitella* including feeding on the subsurface sediment and excreting fecal pellets on the sediment surface as well as burrowing in the sediment, and spouting the subsurface sediment onto the sediment surface had promoted sediment oxidation and provided an oxygen-rich environment suitable for aerobic bacteria in the deeper subsurface sediment.

This present study showed the potential of two interface deposit feeding polychaetes, *P. membranacea* and *P. pulchra* to remediate the organically enriched sediment from green mussel rafts. The potential in bioremediation of organic matter in sediment of these two spionid polychaetes were high when compared to *Capitella* sp. commonly used in the bioremediation of enriched sediment under fish farms in Southern Japan as in the Table 4.3. Chareonpanich et al. (1994) concluded the efficiency of *Capitella* sp. as bioremediator that 0.5 g DW (or 33,139 inds.) of *Capitella* had the ability to decompose organic matter with 1 g of TOC in 1 m^2 of sediment. Tsutsumi et al. (2002) conducted the experiments with outdoor pools to measure how efficiently the artificially cultured colonies of *Capitella* introduced to the pools were able to decompose the loaded organic matter to the sediment in those outdoor pools of 3x9 m each. They found that within 16 weeks of experiment period, the outdoor pools with artificially cultured *Capitella* showed the apparent decreased levels of TOC relatively to those of control. During the last nine weeks (from week 7 to week 16) of experimental period, the artificially cultured *Capitella* patches in the experimental pools were able to reduce the TOC ranged from 16.29-30.6%.

Table 4. 3 Bioremediation efficiency in polychaetes.

Polychaetes as bioremediatore	Reduction of Total organic Matter	Reference
Capitella sp.1	16.29-30.2% (9 weeks in outdoor pools)	Tsutsumi et al. (2002)
P. membranacea	38.12% (200 inds/m^2) 52.43% (400inds/m^2) (4 weeks in experiment)	This study
P. pulchra	37.55% (200inds/m^2) 49.24% (400inds/m^2) (4 weeks in experiment)ks in)	This study

The selected spionid polychaete for the bioremediation of organically enriched sediment under green mussel raft in Sriracha Bay, Chonburi Province should be *P. membranacea*. This species were more abundant in the coastal area of Sriracha Bay, while *P. pulchra* was rare. Although the two spionids showed the similar potential in the reduction of organic matter in the sediment, *P. membranacea* was more efficient in converting the organic waste from the enriched sediment to biomass than *P. pulchra*. It can be predicted that for further application of this result by the introduction of mass culture of spionid polychaetes in the enriched sediment under the green mussel rafts, the *P. membranacea* population would increase in density and biomass rapidly. This inturn would increase the decomposition rate of organic matters in the sediment. As shown from the result, the high density of *P. membranacea*, twice the normal density, would increase the reduction of organic matter in the sediment 1.38 times compared to normal density. The methods on the future application of mass-cultured of spionid polychaetes to treat the organically enriched sediment under the green mussel rafts and in the coastal area will be investigated through mass culture of the selected potential bioremediator species following the technique developed for *Capitella* sp. by Tsutsumi et al. (2005); Tsutsumi et al. (2002). Mass culture of selected spionid polychaetes in the laboratory for the production of metamorphosed larvae (settlement stage). These larvae will further be introduced in sediment under the green mussel rafts as field culture of post-settlement stage at different density. Growth, density, survival of worms as well as the efficiency of bioremediation will be monitored.

E. Conclusion

The spionid polychaete *P. membranacea* and *P. pulchra* can be used as bioremediators for the treatment of organically enriched sediment under the green mussel rafts in Sriracha Bay, Chonburi Province. The two spionids were efficiently converted the organic waste into biomass and reduced the organic matter in the sediment. The spionid polychaetes *P. membranacea* is the potential species for the treatment of organic waste in sediment from aquaculture system.

CHAPTER V
BIOREMEDIATION OF ORGANICALLY ENRICHED SEDIMENT UNDER GREEN MUSSEL RAFTS WITH ARTIFICIALLY MASS CULTURE OF SELECTED SPIONID, *Prionospio (Prionospio) membranacea*

A. Introduction

Polychaetes are commonly used as bioremediator in the aquaculture system such as fish farms, shrimp farms and mussel farms. Through bioremediation, these polychaetes can converted nutrients and organic matter released in the culture system into biomass which can be easily removed and harvested as the valuable by-product (Alexander, 1994; Gifford et al., 2007; Kinoshita et al., 2008; Palmer, 2010, 2011; Tsutsumi et al., 2005; Tsutsumi et al., 2002; Van Bruggen, 2012) The utilization of polychaetes in bioremediation in aquaculture system were widely introduced particular in the deposit feeding polychaetes, *Capitella* species and several species in the family Nereididae. The introduction culture and harvest of selected polychaetes as bioremediators required detailed knowledge of biological requirements for successful husbandry pratices (Gifford et al., 2007). Most importantly the selected species should already be in abundant and widely distributed in the area. This would avoid the risk of introducing invasive species into the coastal area or in the system (Gifford et al., 2007; Tsutsumi et al., 1990).

The polychaete, *Prionospio* spp. were one of the opportunistic polychaetes living in organically enriched condition of low dissolved oxygen and high sediment sulphide content (Diaz & Rosenberg, 1995; Pearson & Rosenberg, 1978). These spionids *Prionospio* spp. were proposed as the potential bioremediator of organically enriched sediment under green mussel rafts in Sriracha Bay, Chonburi Province. Key criteria as bioremediator in selecting the potential *Prionospio* species were focused in this study.

Of the six spionid polychaetes, *P. membranacea* was the most abundant and widely distribution in the coastal areas of Sriracha Bay, Chonburi Province. This species was found in the area of high organic content in the mussel raft culture area and nearshore stations. Adaptation in feeding, respiration and reproduction of *P. membranacea* can be enables them to rapidly colonize in high organically enriched condition. Adaptation in respiratory structure, *P. membranacea*, living in high organic content areas, has longer of branchial pairs and more numerous of the pinnules. These enlarged respiratory surface area enhanced O_2 diffusion. Their feeding and burrowing activities also facilitated the respiration in low O_2 condition. *P. membranacea* was one of the inter-face deposit feeding polychaetes (D. M Dauer et al., 1981; Fauchald & Jumars, 1979). This spionid *Prionospio* has a pair of grooved, ciliated palps that arise from the peristomium. Palps that came in direct contact with the sediment surface picked up particles in the food groove and transported them by ciliary action to the everted pharynx. When particles were resuspended by a current, the polychaetes changed their palp orientation modes and increased their feeding upon suspended particles (D. M Dauer et al., 1981).

In organic enrichment condition, the reproductive adaptation of *P. membranacea* was important for the recruitment. The life history characteristics of opportunistic species allow them to respond to disturbances at any time of the year and they can rapidly exploit an open environment. These characteristic features were with small size, prolonged breeding season, high recruitment, high growth rate, short interval between fertilization and maturity, and short life span, although they usually have high subsequent mortality (J. Grassle & Grassle, 1974; Yokoyama, 1990). These opportunist life history features were found in *P. membranacea*. This species was broadcast spawner with planktotrophic larval development. *P. membranacea* has the short planktonic phase of 8-14 days. The life span was 38-58 days. Organic enriched sediment increased their reproductive potential in term of increase fecundity of 869±18.85 oocytes/ind. The organic enriched condition also shorten the time to metamorphosis and settlement. The percentage of metamorphosed larvae and percentage of competent larvae in *P. membranacea* were high of 64-68 and 55-60%, respectively in enriched condition. *P. membranacea* shared the same life-history trait with *Capitella*, one of the important pollution indicator. *Capitella* spp. have been successfully used as the bioremediator of organic waste from fish farms in Southern Japan ((Chareonpanich et al., 1994; Kinoshita et al., 2008; Tsutsumi et al., 1990; Tsutsumi et al., 2005; Tsutsumi et al., 2002). In term of bioremediation efficiency, *P. membranacea* was efficiently converted the organic waste into biomass and reduced the organic matter in the sediment. High density of this spionid polychaete also increased the bioremediation efficiency.

As the bioremediation of organically enriched sediment from fish farms by the artificially mass culture of deposit feeding polychaete, *Capitella*, have been proven successful, the specific objective of this study to produce artificially mass culture of selected spionid polychaete, *P. membranacea* in the laboratory. The artificially mass culture of this species would later be introduced into organically enriched sediment under the green mussel rafts for the treatment of organic waste. Growth and density as well as survival rate of the worms in the field experiments will be monitored. Bioremediation efficiency of the mass culture of *P. memebranacea* will be assess in term of biomass increment, reduction of organic matter and sulphide in sediment.

B. Materials and Methods

1. Collection of Polychaetes and Sediments

Specimens of *P. membranacea* were collected during May 2014 from Sriracha Bay, Chonburi Province (13°11′N, 100°55′E), on the east coast of the Gulf of Thailand. Adult specimens were collected at station 1, an area of high organic content (4.66-6.08%TOC), near Sriracha Town Municipality under the influence of domestic waste effluents as in Figure 5.1. The worms were collected by the Ekman grab and washed through a 0.25 mm mesh sieve. Specimens were transported afterward to the laboratory for sorting and identification.

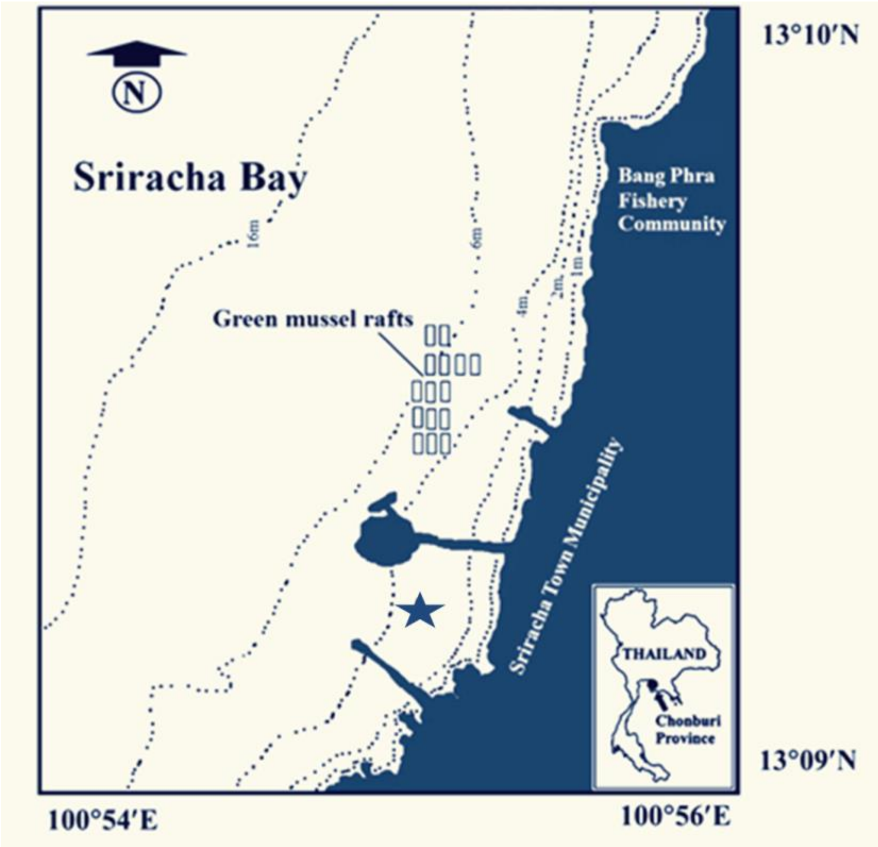

Figure 5. 1 Study area, Sriracha Bay, Chonburi Province, the eastern coastline of Thailand. ★: sampling for spionid polychaetes.

The sediment was collected from under the floating mussel rafts in the bay using Ekman grab. Each sediment sample was sieved through a 1.0 mm mesh and freeze in the laboratory at least for 24 h to kill living organisms. The seawater from the mussel rafts was filtered through a 1.0 micrometer filter bag for the experimental use.

2. Culture of Spionid Polychaetes

2.1 Induced spawning

The spionids were maintained in stock cultures under laboratory conditions in aquarium containing a 3 cm layer of the treated sediment, 10 cm of aereated seawater (34 psu salinity) above the surface sediment. The aquaria were maintained at 28-30 °C. The worms were sampling for the maturity examination under a stereo-microscope. The oocytes and sperm in the coelom of mature females and male, respectively, were clearly visible. Fifty mature individuals of spionids, 25 of females and 25 of males were introduced into a 500 ml beaker which added in the 100 ml filtered seawater. The sudden change of salinity in the beaker was by adding in the 50 ml of freshwater. This technique of induced spawning followed observations of the high density of spionid polychaetes and spawning peak during the wet season. Adult spionids released gametes into water where fertilization and larval development occurred within 24 hrs. The beaker was then decanted through a 63 µm mesh to harvest newly hatched larvae for the experiment. Induced spawning of spionid polychaetes in laboratory as shown in Figure 5.2.

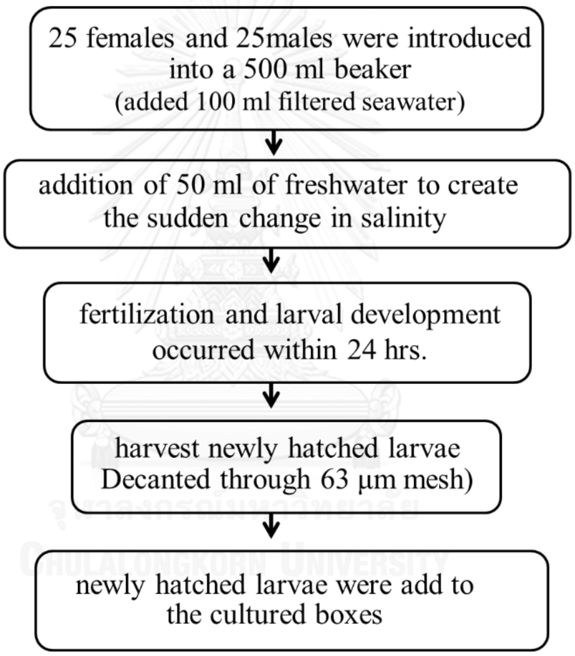

Figure 5. 2 Diagram of induced spawning of spionid polychaetes in laboratory experiment.

3.2 Larval culture

The newly hatched larvae were added to the cultured boxes (33x42x28cm), that filled with the treated sediment at the level of 3 cm from the bottom with the filtered seawater level 10 cm above the surface sediment. The filtered seawater in each box was strongly aerated to create the resuspension of sediment in form of cloudy snowballs. The larval development of the spionid larvae were assessed by sampling for planktonic stage using 63 μm hand net. The early benthic stage juveniles/ settlement stage were sampled by using a 2.5 cm diameter plastic hand core (Figure 5.3). After the early benthic stage juveniles had been reached, these juveniles from the laboratory were harvested and introduced into the organic enriched sediment under the green mussel rafts, for bioremediation experiment in the field.

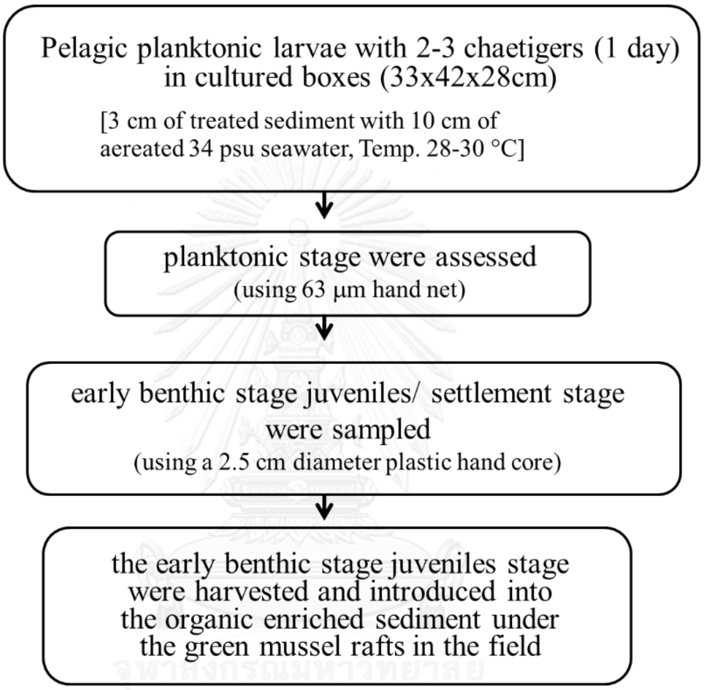

Figure 5. 3 Diagram of larval culture of spionid polychaetes in laboratory experiment.

3. Studies on Bioremediation of Organically Enriched Sediment under Green Mussel Rafts with Artificially Mass Culture of *Prionospio (Prionospio) membranacea*

The experiments were carried out under the green mussel raft in the Sriracha Bay, Chonburi Province. The floating rafts were 40x40 m^2 in size, at 2-5.5 m depth, located near shore at 500 m from shore. Six cages (50x50x30 cm) were used for the experiment under the green mussel rafts. Mesh screen net of 1 cm was used to cover these cages inorder to prevent predators of these polychaetes on the surface sediment. Three treatments with different levels of the density of the early benthic stage juveniles stage of *P. membranacea* were prepared for field experiment; (1) control (without spionid polychaetes), (2) 50 individuals of spionids (normal density of 200 inds.m^{-2} based on the average density of the spionid, *P. membranacea* from high organic content area and the mussel rafts area in Sriracha Bay, as observed by To-orn and Intarachart (2010) and (3) 100 individuals of spionids (twice the normal density of 400 inds.m^{-2}). For each treatment, three cages were used as replicates. Figure 5.4 and 5.5 demonstrated the experimental setup for the bioremediation of organically enriched sediment under the green mussel rafts with artificially mass culture of *P. membranacea*. The experiments were carried out for 30 days.

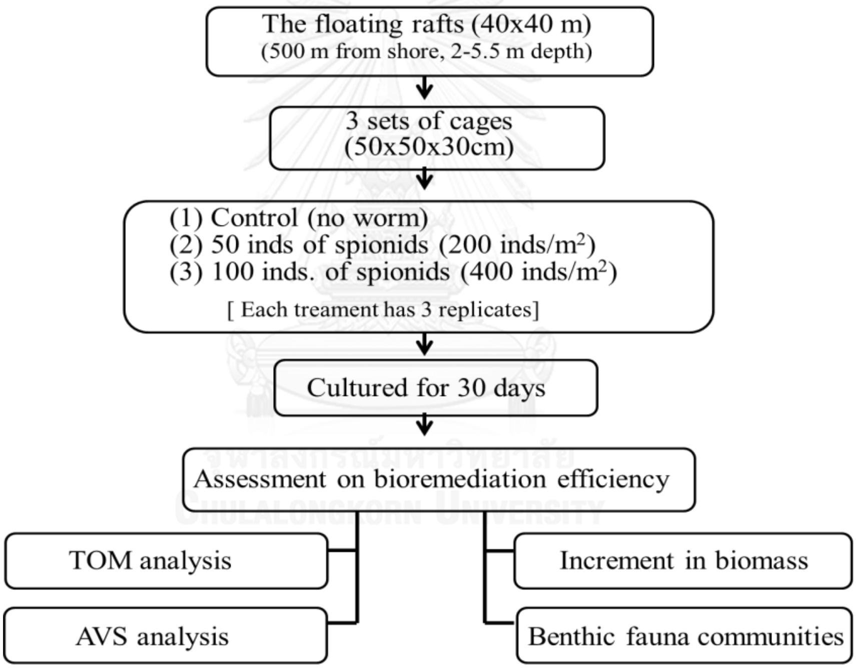

Figure 5. 4 Experimental set up for the bioremediation of organically enriched sediment under mussel rafts with artificially mass culture of *Prionospio (Prionospio) membranacea*

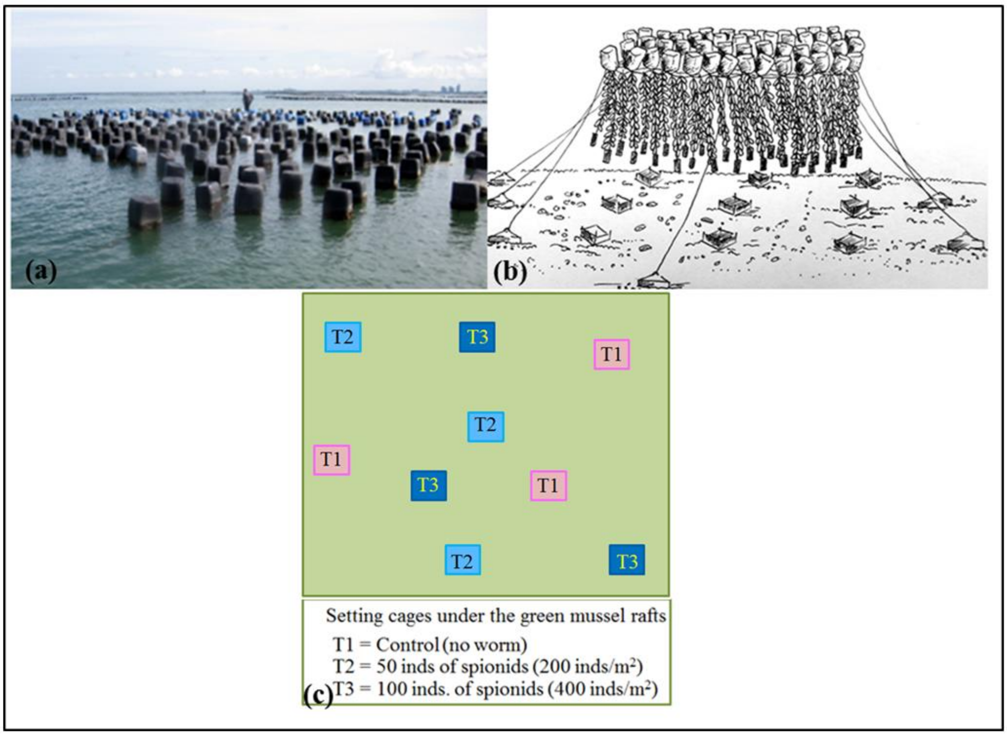

Figure 5. 5 Bioremediation of organically enriched sediment under green mussel rafts with artificially mass culture of selected spionid, *Prionospio (Prionospio) membranacea* in Sriracha Bay, Chonburi Province, the eastern coastline of Thailand. (a) floating green mussel raft (b) drawing of setting cages under the green mussel raft (c) model of setting cages under the green mussel raft. (Drawing by To-orn)

4. Assessment of Bioremediation Efficiency in *Prionospio (Prionospio) membranacea* in Organically Enriched Sediment under Green Mussel Rafts

At the end of the 30 days experiment, the sediment from each cage were sampled by using an Ekman grab. After that the sediment were sampled by using a plastic hand core with an area of 0.55 cm^2. The sediment were then sliced into three layers every 1 cm depth from surface (0-1, 1-2 and 2-3 cm in depth) for the analysis of the total organic matter and the levels of Acid Volatile Sulphide (AVS) in the sediment.

The remaining sediment were washed through a 0.25 mm mesh sieve and fixed in 10% buffered formalin for later sorting and identification for the biomass study of the spionid *P. membranacea* and the macrobenthic community. The body length and the body size (width of the 5th chaetiger, excluding parapodia), and the number of chaetigers of the spionds were measured and counted by using an ocular micrometer. The wet weights of the spionids were measured.

Products in term of increased polychaetes biomass and the ability to reduce the organic matter in the enriched sediment from mussel raft culture were assessed as the bioremediation efficiency of the spionid polychaetes. Increment of polychaetes biomass in percentage were calculated from body length, body size, number of chaetiger and body wet weight at the initial and final phases of the experiments.

The bioremediation efficiency of spionid polychaetes were enumerated from the percentage of total organic matter and the levels of AVS in the sediment reduction at the initial and final phases of the experiments. The levels of AVS in the sediment were measured with an hydrogen sulphide absorbent column tube (Hedorotec, 201H). The total organic matter (TOM) was determined by the loss on ignition (Nelson, 1982).

Macrobenthic faunas were collected from sediment under green mussel rafts prior to the experiment and after the 30 days experiment, using a Ekman grab (0.0225 m^2) for assessment of benthic communities. Samples were sieved through a 0.5 mm mesh and fixed in 10% buffered formalin for later sorting and identification of the fauna.

5. Data Analyses

One-way analysis of variance (ANOVA) at the 95% confidence level ($p<0.05$) was applied to analyze the differences of biomass of spionid polychaetes, and total organic matter and the levels of AVS in the sediment between treatments. The increasing of biomass of the spionids, and decreasing of total organic matter and the levels of AVS of the sediment among the initial and final of the experiment, and among three layers between two spiond species were compared using a t-test (Zar, 1999).

C. Results and Discussion

1. Mass culture of the Spionid *Prionospio (Prionospio) membranacea*

The introduced spawning of spionid, *P. membranacea* was successful with 65-80% of newly hatched larvae within 24 hours of fertilization. Table 5.1 shows the duration and development stage of *P. membranacea* from the laboratory culture. The newly hatched planktonic larvae of 2-3 chaetigers larvae with 200-240 μm in length transformed into the metamorphosed larvae of 8-10 chaetigers and 1.2-1.4 mm in length within 8-12 days. Within 26-30 days, metamorphosed larvae developed into the settlement larval size of 26-30 chaetigers and 2.1-2.5 mm. The survival rate of metamorphosed larvae and competent larvae at settlement stage were 58-65 and 50-55%., respectively. With the induced spawning and larval culture techniques used in this study, it is possible to produce and maimtain the artificially mass culture of this spionid polychaetes. From the artificially induced mating in 10 pairs of mature females and males, the newly hatched larvae were produced in the range of 1,400-5,840 individuals. The numbers of metamorphosing larvae were 1,532-3,387 individuals. Number of competent larvae at settlement stage were in the range of 1,284-2,803 individuals.

Table 5. 1The larval development in *Prionospio (Prionospio) membranacea* in the artificially mass culture in the laboratory.

Development stage	Age	Size	Survival rate (%)
Fertilized egg	30 min.	80-100 μm	-
Newly hatched larvae	24 hr.	200-240μm 2-3 chaetigers	65-80% (% hatching)
Planktonic larvae	1-9 d.	300-850 μm 3-8 chaetigers	
Metamorphosing larvae	8-12 d.	1.2-1.4 mm 8-10 chaetigers	58-65%
Sized at settlement	25-34 d.	2.1-2.5 mm. 26-30 chaetiges	50-55%

2. Bioremediation Efficiency of Artificially Mass Culture of Organically Enriched Sediment under Green Mussel Rafts

2.1 Increment of biomass in spionid *Prionospio (Prionospio) membranacea*

The growth in term of biomass increment in spionid, *P. membranacea* was evidenced with the increment of average body length, body size and number of chaetigers. The average body length, body size and number of chaetigers of the early benthic stage juveniles stage of *P. membranacea* at the start of the experiment were 2.31±0.13 mm, 0.09±0.08 mm and 30±1.10 chaetigers. At the end of the 30 days experiment, the average body length, body size and number of chaetigers of adults *P. membranacea* were 13.77±0.09 mm, 0.47±0.09 mm and 69±0.69 chaetigers in the experimental treatment of 50 individuals, respectively. The average body length, body size and number of chaetigers were 13.26±0.15 mm, 0.46±0.02 mm and 67±0.73 chaetigers in experimental treatment of 100 individuals, respectively. No statistically significant difference (p>0.05) in the average body length, body size and number of chaetigers of *P. membranacea* between the experimental treatment of 50 and 100 individuals.

The body weight of the early benthic stage juveniles stage at the start the experiment can not be measured due to their small size of 2.1-2.5 mm. However, the average body weight of adult at the end of the experimental was 0.0025±0.0002 and 0.0024±0.0001 g in the normal density treatment of 50 individuals and the high density treatment of 100 individuals, respectively. No significant differentbetween the two treatments ($p>0.05$) (Figure 5.6). Increment in biomass of spionid polychaetes, *P. membranacea* from the early benthic stage juveniles stage at the start the experiment to adult stage at after 30 days of experiment under green mussel raft as shown Table 5.2.

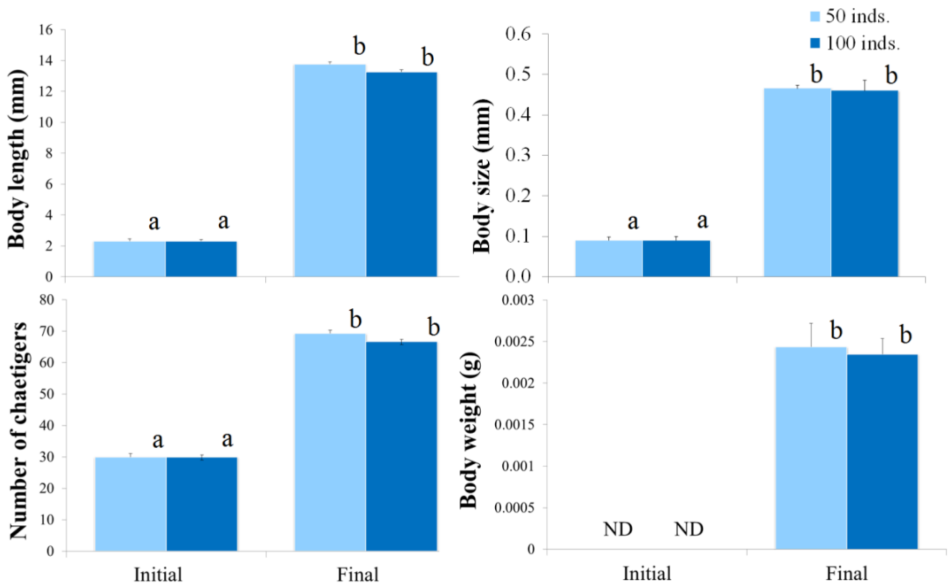

Figure 5. 6Average of biomass of *Prionospio (Prionospio) membranacea* in the bioremediation of enriched sediment under the mussel rafts by artificially mass culture. Different letters above bars indicate significant differences (p<0.05). Error bars represent SD.

Table 5. 2 Increment in biomass of spionid polychaetes, *Prionospio (Prionospio) membranacea* in the bioremediation of enriched sediment under the mussel rafts by artificially mass culture.

Increment in biomass	**50 individuals**		**100 individuals**	
	Total increased biomass (30 days)	**Increment growth rate per day**	**Total increased biomass (30 days)**	**Increment growth rate per day**
Body length (mm)	10.99±0.46	0.37±0.15	11.41±0.26	0.38±0.01
Body size (mm)	0.35±0.014	0.012±0.0004	0.39±0.04	0.013±0.001
Number of chaetiger chaetiger)	35.72±2.79	1.19±0.09	39.83±0.67	1.33±0.02

2.2 Survial rate of spionid *Prionospio (Prionospio) membranacea*

The average number of adults of spionid *P. membranacea* in experimental treatment of 50 and 100 individuals after the 30 days of the experiment from the early benthic stage juveniles stage to adult stage were 23±3.51 and 54±7.55 individuals, respectively. The survival rates calculated were 47±7.02 and 54±6.56%, respectively. No significant difference in the survival rates of *P. (P.) membranacea* in experimental treatment of 50 and 100 individuals at after the 30 days of experiment (p>0.05).

The result demonstrated that the juveniles of *P. membranacea* (26-30 chaetigers) from artificially mass culture in the laboratory can grow and survived to adult stage (67-69 chaetigers) in organically enriched sediment under green mussel rafts. Biomass increment of this spionids under green mussel rafts indicated that organic matter in the sediment was consumed as food source for *P. (P.) membranacea*, a thread-like small interface deposit feeder (Fauchald and Jumars, 1979; Dauer *et al.*, 1981). Density also increased in organically enriched sediment under green mussel rafts. From this study, *P. membranacea* responded to the enriched sediment by increased fecundity and decreased time to metamorphosis ans settlement. Moreover the increase percentage of metamorphosed larvae and competent larvae also correlated to the increase organic condition. High density of *P. membranacea* resulted in the high bioremediation efficiency in term of the reduction of total organic matter and increment of biomass. Tsutsumi et al. (2002) studied bioremediation of organic matter loaded on the sediment in outdoor pools with a polychaete, *Capitella* sp.1. They concluded that organic loading from daily addition 1.82 g C/m^2 of the organic matter to the sediment of the outdoor pools, was balanced with the decomposition rate of organic matter in the sediment when the patches of *Capitella* of 50 g WW/m^2 established. *Capitella* was able to establish further denser patches within a short period, due to its extraordinarily large potential for population increase. Tsutsumi et al. (2002) also estimated the potentials of *Capitella* colonies for the decomposition of the organic matter in the sediment in laboratory experiments and showed that colonies with approximately 100 g WW/m^2 in biomass were required to balance the decomposition of the organic matter by the biological activities of *Capitella* with organic loading of 3g C/m^2/day on the sediment under the temperature conditions of 18°C.

2.3. Reduction of Organic Matter in Sediment

The total organic matter level of the sediment under the mussel rafts at the start of the experiment was 6.38±0.24%. After 30 days of the experiment, the total organic matter levels of the three layers of the sediment in experimental treatment of 50 and 100 individuals markedly decreased. No significant change was detected in the total organic matter levels in the control or no worm treatment. The total organic matter levels of the three layers of the sediment ranged 4.62-4.67% and 3.45-3.56% in experimental treatment of 50 and 100 individuals, respectively. The total organic matter level of the sediment among these experiment were also significantly different (p<0.05). High density of spionid polychaetes resulted in higher bioremediation efficiency. The total organic matter level of the sediment of the three layers of the sediment in these experiments showed no statistically significant differences (p>0.05) (Figure 5.7). It was estimated that the reduction of total organic matter in the enriched sediment under the mussel rafts by mass culture of *P. membranacea* in normal and high density treatments were 26.23% and 45.81% within 30 days.

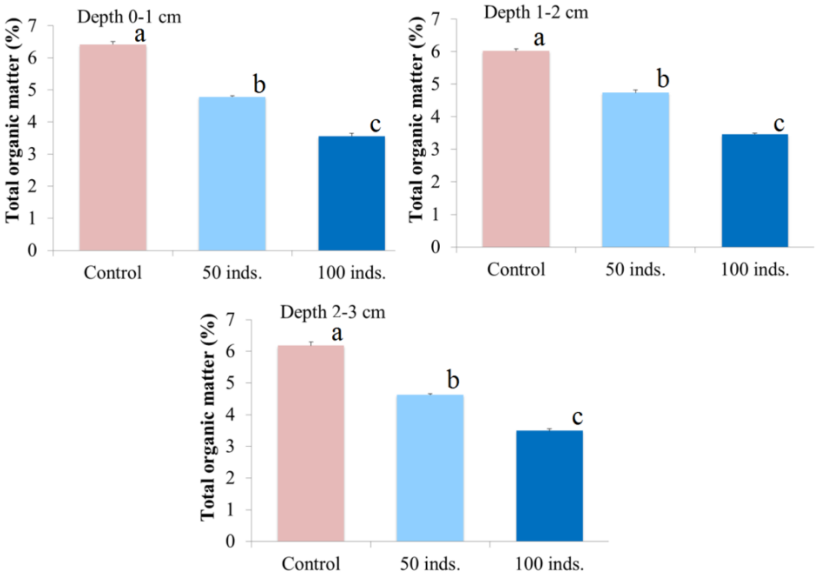

Figure 5. 7Reduction of total organic matter (%) in three layers of sediment in bioremediation of enriched sediment under the mussel rafts by artificially mass culture of *Prionospio (Prionospio) membranacea***.** Different letters above bars indicate significant differences (p<0.05). Error bars represent SD.

2.4. Reduction of Acid Volatile Sulphide in the sediment

Figure 5.8 shows the levels of AVS of the three layers of the sediment under the mussel rafts after 30 days of the experiments. At the start of the experiments, the levels were 0.32±0.02 mg/g dry weight. After 30 days of the experiment, the levels of AVS of the sediment in control treatment remained unalter in the three layers within the range of 0.31-0.32 mg/g dry weight. The levels of AVS of the sediment in experimental treatment of 50 and 100 individuals markedly decreased compared to the beginning of the experiment. The levels of AVS of the sediment in all three layers of experimental treatment of 100 individuals ranging 0.21-0.23 mg/g dry weight was significantly higher than the normal density of 50 individuals in the range of 0.24-0.26 mg/g dry weight (p<0.05). No significant differences among the levels of AVS in the three layers of the sediment in all treatment (p>0.05). The reduction of AVS in the sediment in percentage by the mass culture of *P. membranacea* at the normal density and high density treatments were 11.99 and 17.92, respectively.

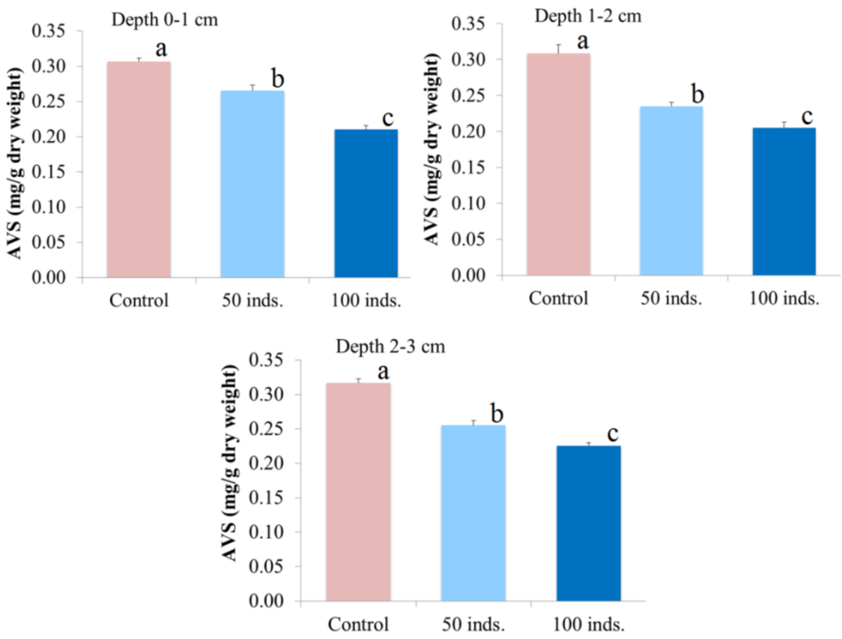

Figure 5. 8Reduction of AVS (mg/g dry weight) in three layers of sediment in bioremediation of enriched sediment under the mussel rafts by artificially mass culture of *Prionospio (Prionospio) membranacea*. Different letters above bars indicate significant differences ($p<0.05$). Error bars represent SD.

2.5 Abundance of the Macrobenthic Communities

The species composition and abundance of the macrobenthic communities were monitored at the start of the experiment and after 30 days of the experiment in all treatments under green mussel rafts. The species composition of dominant species in the bioremediation experiments by artificially mass culture of *P. membranacea* is shown in Table 5.3 and Figure 5.9. The macrofauna composition under the mussel raft indicated the enriched condition dominated by deposit feeding polychaetes. Dominant polychaetes such as *P. membranacea*, *P. pulchra*, *Scoloplos* sp. *Aricidea* sp., *Notomastus* sp., *Euclymene* sp., *Cossura* sp. and *Ophelina* sp. were common corresponding to those reported for Sriracha Bay ((To-orn & Intarachart, 2010); To-orn and Paphavasit (2003). Filter feeding terebellid, *Loimia* sp., commonly found in carbonate tubes among the dead mussel shells. Predatory polychaetes, *Lumbrinereis* sp., *Nereis* sp. and *Eunice* sp. were found in lesser number. Amphipod crustacean was another dominant detritus feeder under the mussel raft. Nemertean was another invertebrate predator found in the area. Filter feeding bivalves, *Gafarium divaricatum*, found buried in the sediment.

At the beginning of the experiment, the macrobenthic communities consisted mainly of four species of polychaetes namely, *Scoloplos* sp. (Orbiniidae), *Aricidea* sp. (Paraonidae), *P. membranacea* (Spionidae) and *Loimia* sp. (Terebellidae). Small crustaceans, Amphipoda also common. These five species comprised 73.57% of the abundance of the macrobenthos collected in the sediment under the mussel raft area. Spionid, *P. membranacea* showed the drastically increased with 30 days of experiments with high density 1,167 individuals/m^2 in normal density treatment of 50 individuals. Highest density of 2,700 inds/m^2 was observed in the high density treatment of 100 individuals.

Table 5.3 The species composition of the dominant species (ind./m^2) of the macrobenthic communities in bioremediation of enriched sediment under the mussel rafts by artificially mass culture of *Prionospio (Prionospio) membranacea*.

Benthic composition	**Before the experiment (ind./m^2)**	**After 30 days of the experiment**		
		No worm (ind./m^2)	**50 ind. (ind./m^2)**	**100 ind. (ind./m^2)**
Phylum Nemertea	83	17	50	83
Phylum Annelida				
Class Polychaeta				
Family Orbiniidae				
Scoloplos sp.	183	167	83	250
Family Paraonidae				
Aricidea sp.	133	83	167	133
Family Spionidae				
P. membranacea	283	417	1167	2700
P. pulchra	50	117	167	250
Family Capitellidae				
Notomastus sp.	83	150	167	67
Family Maldanidae				
Euclymene sp.	67	83	33	133
Family Lumbrinereidae				
Lumbrinereis sp.	50	83	33	67
Family Terebellidae				
Loimia sp.	417	500	300	333
Family Cossuridae				
Cossura sp.	17	0	33	17
Family Opheliidae				
Ophelina sp.	0	33	0	50
Family Nereidae				
Nereis sp.	17	17	33	50
Family Eunicidae				
Eunice sp.	50	33	0	50
Phylum Mollusca				
Class Bivalvia				
Family Veneridae				
Gafarium divaricatum	33	33	0	0
Phylum Arthropoda				
Class Crustacea				
Order Amphipoda	233	133	167	83
Total	**1700**	**1867**	**2400**	**4267**

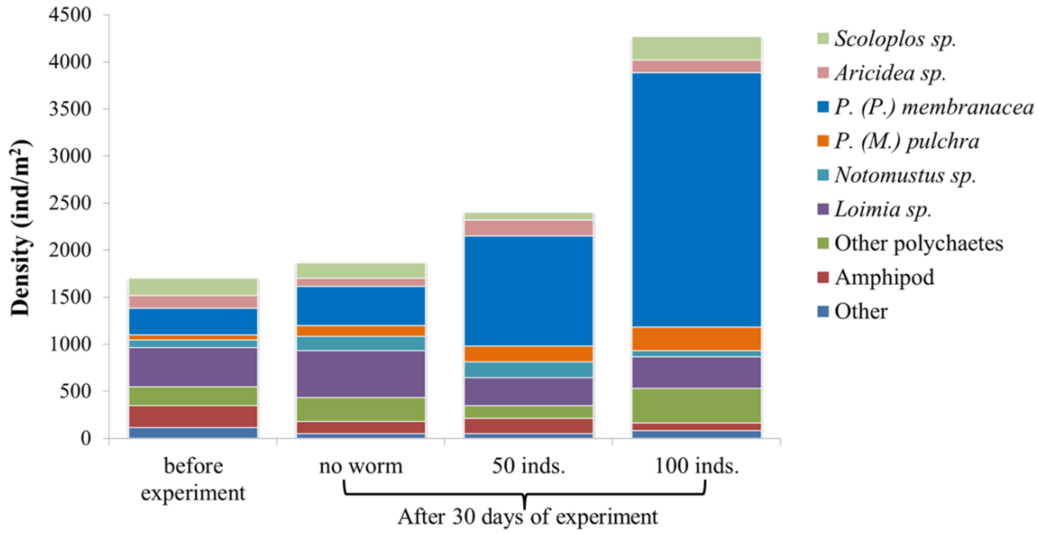

Figure 5. 9Abundance of the macrobenthic communities in bioremediation of enriched sediment under the mussel rafts by artificially mass culture of *Prionospio (Prionospio) membranacea*.

These biological activities of *P. membranacea* can thus promote the decomposition of organic matter and oxidation of sulphides in the sediment. The bioremediation efficiency of *P. membranacea* increased with on the population size. In this study, the reduction of total organic matter and AVS in the sediment in high density of 100 individuals of *P. membranacea* were 1.8 and 1.4 times, respectively compare to control experiment. The treatment of normal density of 50 individuals showed the reduction of total organic matter and AVS in the sediment were 1.3 and 1.2 times, respectively when compare to control experiment. Burrowing and feeding activities of polychaetes played the important roles in recycling and reworking of benthic sediments, bioturbating sediments and in the burial of organic matter (Hutchings, 1998). Increased in the spionid population also increase the bioturbation activities. Tsutsumi et al. (2002) concluded that the *Capitella* species was the potential polychaetes for the bioremediation of organically enriched sediment. They burrow into the sediment and creating numerous burrows in the sediment. *Capitella*, being the deposit feeder, feed on the sediment and excreted fecal pellets and piled them like a tube on the sediment. As the density of *Capitella* species increased in the sediment, the larger space suitable for the bacterial activities were created in the subsurface sediment by the burrowing activities of this polychaetes. From the macrobenthic community change, other deposit feeders, *Scoloplos* sp., *Aricidea* sp., *Loimia* sp. and small crustaceans, Amphipoda also increased under the mussel raft during the field experiment, but to lesser degree. The increase in spionid polychaete, *P. membranacea* outnumbered other deposit feeders in particular in the high density of *P. membranacea* treatment. The reduction of the organic matter and AVS in the sediment in particular in the high density of 100 individuals of *P. membranacea* should resulted mainly from the bioturbation activity of *P. membranacea* alone.

P. membranacea can be used as the potential bioremediator of organically enriched sediment under green mussel rafts in artificially mass culture as in the method developed to treat enriched sediment with artificially culture of *Capitella* sp. ((Kinoshita et al., 2008; Tsutsumi et al., 2005; Tsutsumi et al., 2002). From his experiment of introducting the artificially cultured colonies of *Capitella* into the sediment in the outdoor pools, Tsutsumi et al. (2002) concluded that within 16 weeks of experimental period, the outdoor pools with artificially cultured *Capitella* showed the apparent decreased levels of TOC relatively to those of control. During the last nine weeks (from week 7 to week 16) of experimental period, the artificially cultured *Capitella* patches in the experimental pools were able to reduce the TOC ranged from 16.29-30.60% This study revealed the high bioremediation efficiency of *P. membranacea* in the experiment of introducing the artificially mass culture of this species in the enriched sediment under the mussel rafts. It can be extrapolated from this study that the mass culture of *P. membranacea* at the density of 640,000 individuals need to be introduced into the sediment under the mussel raft, each approximately 1,600 m^2 in size inorder to reduce the total organic matter in the sediment 45.81% within 30 days as in Table 5.4. This calculation based on the survival rate of *P. membranacea* in the field experiment of 54%.

Table 5. 4 Density of the selected spionid, *Prionospio (Prionospio) membranacea* in the artificially mass culture bioremediation introduced into the organically enriched sediment under the mussel rafts. Area under each mussel raft is approximately 1,600 m^2.

Density in mass culture (ind.)	**Bioremediaition efficiency (30 days period)**		**Survival rate in field (%)**
	Reduction in TOC (%)	**Reduction in AVS (%)**	
320,000	26.23	11.99	47
640,000	45.81	17.92	54

The mass culture of spionid polychaete, *P. membranacea* can easily be achieved by the induced spawning and larval culture techniques used in this study. Mass culture of *P. membranacea* can efficiently bioremediate the organically enriched sediment under the mussel rafts.

D. Conclusion

P. membranacea was selected as the potential bioremediator of organically enriched sediment under green mussel rafts in Sriracha Bay, Chonburi Province. The artificially mass culture of selected spionid were efficiently bioremediate the enriched sediment under green mussel rafts through the process of converting organic waste into biomass and reduced the organic matter and sulfide. High density of *P. membranacea* increased the bioremediation efficiency. During the 30 days experiment of introducing the mass culture of *P. membranacea* in the enriched sediment under the green mussel rafts, the spionid polychaete rapidly proliferated in organically enriched sediment.

CHAPTER VI

SYNTHESIS AND RECOMMENDATION

A. Research Synopsis

The rapid expansion of coastal aquaculture coupled with the adverse impacts on the coastal environment are the major concerns and challenges in the utilization of coastal resources. Coastal area of Sriracha Bay, Chonburi Province, eastern coastline of Thailand, is one of the major green mussel (*Perna viridis*) culture areas. Organic enrichment was evidenced by the high total organic matter of 3.85-12.36% in the sediment in the mussel raft culture area and nearshore station. Polychaetes are commonly used as bioremediators in the aquaculture system such as fish farms, shrimp farms and mussel farms (Alexander, 1994; Gifford et al., 2007; Kinoshita et al., 2008; Palmer, 2010, 2011; Tsutsumi et al., 2005; Tsutsumi et al., 2002; Van Bruggen, 2012). The culture and harvest of selected polychaete as bioremediators required detailed knowledge of the biological requirement for successful husbandry practices (Gifford et al., 2007). Most importantly the selected species should already abundant and widely distributed in the area. This would avoid the risk of introducing invasive species into the coastal area or in the system (Gifford et al., 2007; Tsutsumi et al., 1990).

The spionids, *Prionospio* spp., were commonly found in the organic enriched sediment under the green mussel farms in Sriracha Bay, Chonburi Province. They were proposed as the bioremediators of organically enriched sediment under green mussel raft. Key criteria as bioremediator in selecting the potential *Prionospio* species were focused prior to the introduction of artificially mass-cultured colonies of selected species of *Prionospio* in the treatment of organically enriched sediment under the green mussel rafts in the field.

The distribution and abundance of spionid polychaetes in Sriracha Bay revealed six species including *Prionospio (Prionospio) membranacea*, *Prionospio (Minuspio) pulchra*, *Prionospio (Aquilaspio) sexoculata*, *Prionospio (Minuspio) multibranchiata*, *Prionospio (Minuspio) japonica* and *Paraprionospio* *inaequibranchia.* The spionids, *P. membranace,* dominated of 70% of the spionids found in the area, was widely distributed in the area in particular the high organic content area in the mussel raft culture area and nearshore stations. The abundance of *P. membranace* was found in high organic content area of 50-1,000 ind.m^{-2} as compared to lower density observed in low organic content area of 40-80 ind.m^{-2}. Sediment type, organic matter as well as the water depth determined the distribution and abundance of spionid polychaetes. Clear relationships between the silt-clay fractions and the total organic matter in the sediment in the *P. membrancea* distribution. However *P. membrancea* was not found at the offshore area where high enriched condition was observed in the traditional bamboo poles culture area. The possible explanation for such unexpected trend could cause by the physical characteristics of the sediment. Larval habitat selection may also be affected by the presence of some pollutants which reduced the number of metamorphosed and settlement larvae. The chemical cues for larval habitat selection and settlement for *P. membrancea* should be further investigated. *P. pulchra*, the second most abundant species, also found distributed in the high organic content area but in low density of 25-375 ind.m^{-2}. The distribution and abundance study revealed large natural stocking density of potential spionids as the bioremediator for enriched sediment under the mussel raft culture in Sriracha Bay.

Morphological adaptations of respiratory structure in spionid polychaetes were considered as one of the key criteria for selecting potential polychaete species as bioremediator in treating enriched organic sediment under mussel rafts in Sriracha Bay, Chonburi Province. Comparative study on respiratory structure in spionids, *Prionospio* spp. both in adult and larval to settlement stages in organically enriched sediment were conducted as the assessment of respiratory adaptation in enriched sediment. The present study has established the presence of intraspecies morphological differences in respiratory structures in the spionids *P. membranacea, P. pulchra* and *P. sexoculata*, related to different environmental conditions. In the high organic content area, branchial pairs were significantly longer than those in the area of low organic content. Also, for the two species *P. membranacea* and *P. sexoculata* possessing pinnules, the pinnules were more numerous. In addition, branchial lengths and pinnule numbers showed a tendency to increase in relation to body size. These morphological differences of these spionids under different environmental conditions occurred earlier in their early benthic juvenile stage. The length of branchial pairs and number of branchial pinnules were observed to be different in the high and low organic areas at the early benthic stage juvenile and even very small individuals in both *P. membranacea* and *P. pulchra*. The finding reflected phenotypic differences to different environmental condition in the spionid polychaete genus *Prionospio.* It is interesting that the spionid larvae from the metamorphosed larval stage and the early benthic stage juveniles were able to detect and discriminate the different organic condition in the sediment in order to adaptation accordingly. Enlargement of respiratory structure as morphological response to hypoxic conditions have been observed in several families of polychaetes ranging from intertidal to abyssal polychaetes (Hourdez et al., 2002; Lamont & Gage, 2000; Nkwoji, 2012; Yokoyama, 2007). In addition, these spionids are usually found at a depth of 1-2 cm from the sediment surface, being the interface-feeders that utilized particles from the sediment surface, in suspension and resuspended in the sediment (D. M Dauer et al., 1981; Yokoyama, 1988). Their feeding activities also help to increase ventilation in these polychaetes.

Reproductive and development pattern in spionid polychaetes in organically enriched sediment is one key criteria for potential bioremediator in treating high organic sediment under mussel rafts in Sriracha Bay, Chonburi Province. The fecundity rate of polychaetes in the high organic content treatment were higher than those in the low organic content treatment. The fecundity rate of mature females in *P. membranacea* of 869±18.85 oocytes/ind. was higher than those in *P. pulchra* of 823±10.50 oocytes/ind. The number of oocytes produced by a mature female of two spionid polychaetes were positively correlated to number of chaetigers.

The duration of larvae metamorphosed and settlement in *P. membranacea* were rapidly than those in *P. pulchra.* The duration of larval development of two spionid polychaetes in the high organic content treatment were higher than those in the low organic content treatment. Survival rate of larvae metamorphosed and settlement in *P. membranacea* were higher than those in *P. pulchra.* The survival rate of larvae metamorphosed and settlement of two spionids in the high organic content treatment were higher than those in the low organic content treatment. Reproductive and development pattern in spionid polychaetes fit to Marine Biotic Index (AMBI). The spionid polychaetes *P. membranacea* and *P. pulchra* were classified into Group IV as the second-order opportunistic species (slight to pronounced unbalanced situations). The species in this group were the small species with a short life cycle, adapted to life in reduced sediment where they can proliferate (Borja et al., 2000; Carvalho et al., 2006; Cheung et al., 2008; D. M. Dauer, 1993; Grall & Glémarec, 1997).

The potential of *Prionospio* in bioremediation in organically enriched sediment is one of key criteria for selection of polychaete species as potential bioremediator. The ability of the two surface deposit-feeding polychaetes, *P. membranacea* and *P. pulchra* to remediate organically enriched sediment from green mussel rafts were assessed. The results of the biomass increment of the two spionid polychaetes in the bioremediation process revealed that *P. membranacea* was more efficient in converting the organic waste from the enriched sediment to biomass than *P. pulchra*. The decreased organic matter in the sediment of two spionid polychaetess in the high density treatment of 20 individuals were higher than those in the normal density treatment of 10 individuals. The spionid polychaete *P. membranacea* and *P. pulchra* can be used as bioremediators for the treatment of organically enriched sediment under the green mussel rafts in Sriracha Bay, Chonburi Province. The two spionids were efficiently converted the organic waste into biomass and reduced the organic matter in the sediment. The former species was abundant of 40-1,000 inds.m^{-2} while the latter species was rare of 25-375 inds.m^{-2}. The spionid polychaetes *P. membranacea* is the potential species for the treatment of organically enriched sediment under the green mussel rafts due to they can be collected for culture in the laboratory.

Bioremediation of organically enriched sediment under green mussel rafts with artificial mass culture of selected spionids *P. membranacea* was carried out by the mass culture of selected spionid *P. membranacea* in the laboratory. The post-settlement stage of the selected spionids *P. membranacea* from the mass culture were introduced in the sediment under the green mussel rafts for assessment the bioremediation potential of spionid polychaete. The result revealed the survival rate of larvae settlement stage of 50-55%. The number of settlement stage from artificial breeding of 10 pairs of gravid males and females were 1,284-2,803 individuals, average 2,079±631.10 individuals that enough for culture in the field. The results from field demonstrated that the early benthic stage juveniles stage of the selected spionid *P. (P.) membranacea* of 26-30 chaetigers from artificially mass culture can grow and survived to adult stage of 67-69 chaetigers in organically enriched sediment under green mussel rafts as survival rate 47-54%. The selected spionid *P. membranacea* were efficiently converted the organic waste into biomass, and reduced the organic matter and the levels of AVS of the sediment under the green mussel rafts. The potential in bioremediation of organic matter in sediment of the selected spionid *P. membranacea* were high when compared to *Capitella* sp. commonly used in the bioremediation of enriched sediment under fish farms in Southern Japan (Chareonpanich et al., 1994; Tsutsumi et al., 2002).

B. Application of Spionid Polychaetes as Bioremediator of Organic Waste in the Aquaculture

From this study, *P. membranacea* was selected as the potential bioremediator of organically enriched sediment under green mussel raft in Sriracha Bay, Chonburi Province. The artificially mass culture of this species of spionids were efficiently bioremediate the enriched sediment under the mussel raft through the process of converting organic waste into biomass and reduced the organic matter and sulfide. It can be calculated that inorder to bioremediate the enriched sediment under the average size mussel raft of 1,600 m^{-2}, the mass culture of *P. membranacea* at the density of 640,000 individuals need to be introduced into the enriched sediment to yield the reduction of total organic matter in the sediment to 45.8% and the reduction of sulfides in from of AVS 17.92%. The refer, there is the need to maintain a large scale of mass culture of this spionid.

In the laboratory, the method for the induced spawning for *P. membranacea* had been conducted by trials and errors until the method with most successful spawning and produced the highest hatching larvae was found and used in this experiment. The culture of the spionid larvae was also through trials and errors. Figure 6.1 showed the induced spawning and the culture of *P. membranacea* larvae to early benthic stage juveniles/ settlement stage in the laboratory to produce mass culture. During the larval development period, no addition of larval food is needed. The strong aeration to create suspened sediment in form of suspended snowball in the water column in the culture tank is necessary. This spionid was interface-feeding species that utilized organic particles from the sediment surface, in suspension and resuspended in the sediment (D. M Dauer et al., 1981; Yokoyama, 1988).

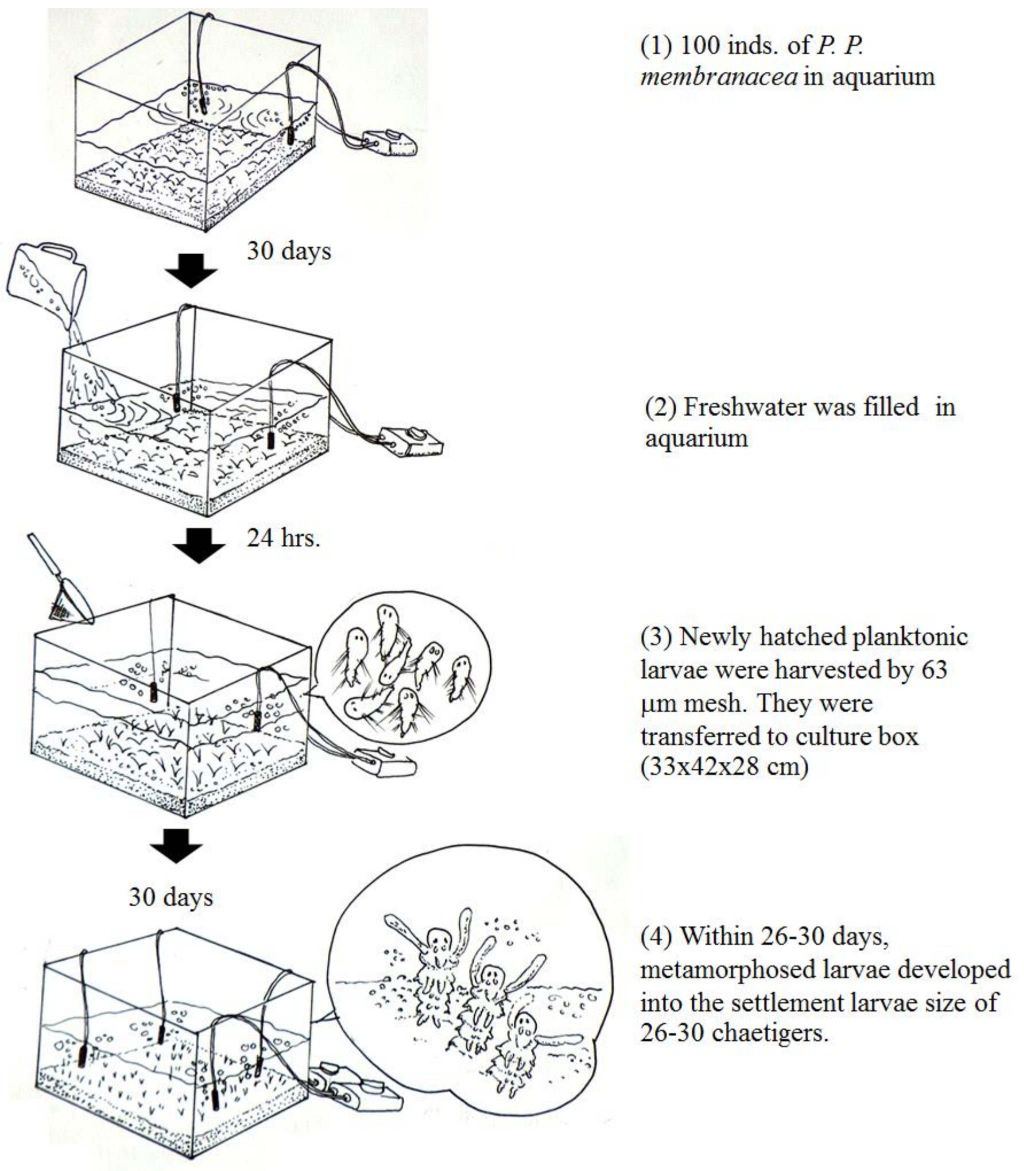

Figure 6. 1 Experimental set up for induced spawning and the culture of *Prionospio (Prionospio) membranacea* larvae to settlement stage in the laboratory to produce mass culture.

The larvae feed on the suspended organic matter in the water column to ensure their development and settlement. Unlike the maintenance of mass culture in *Capitella* and polychaetes in the Family Nereididae, there was the need to add larval food to ensure the larval development and settlement (Palmer, 2010, 2011; Tsutsumi et al., 2005; Tsutsumi et al., 2002). This is an advantous for producing mass culture of this small spionid polychaetes. To increase the production of mass culture of the settlement stage larvae of *P. membranacea,* increased in settlement space is needed. Food supply is not the major problem in the larval development in the laboratory. Increase in food supply during this phase may possibly enhance the production of the settlement larva. This need to be further investigated.

In order to apply the results from the experiment of bioremediation efficiency of organically enriched sediment under green mussel rafts with artificially mass culture of *P. membranacea* in a larger scale, the experimental set up is proposed. Three mussel rafts of 1,600 m^{-2} in the nearshore area of the same water depth were designed for the application of the bioremediation of organic waste by artificially mass cultured of *P. membranacea*. In Figure 6.2, the mussel raft A is used as control without the introduction of mass culture of *P. membranacea*. Mussel raft B and C are the experimental rafts with the introduction of the artificially mass culture of the early benthic stage juveniles of *P. membranacea* into the sediment for bioremediation.

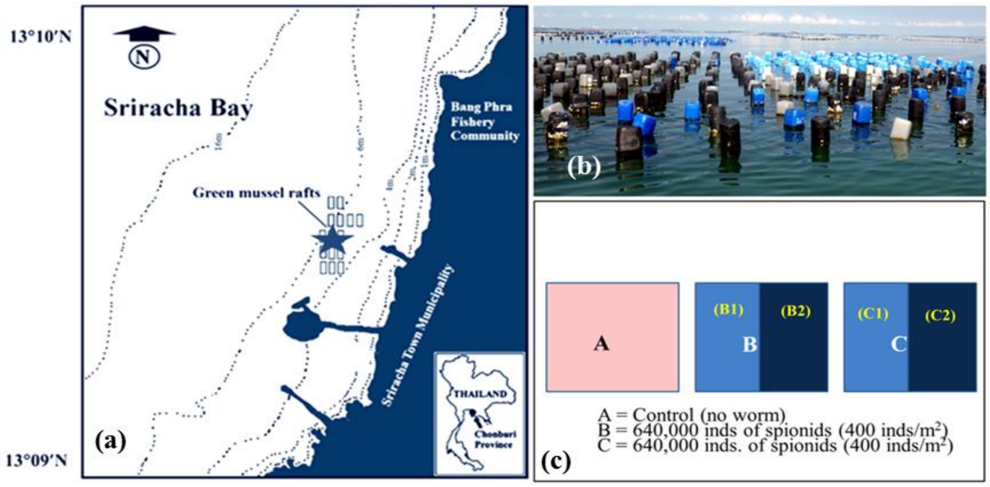

Figure 6.2 Bioremediation of organically enriched sediment under mussel raft in Sriracha Bay, Chonburi Province, with artificially mass culture of selected spionid, *Prionospio (Prionospio)*. (a) study site (b) green mussel raft (c) model of study.

In sediment area under the experimental raft, B is divided into equal area of B1 and B2. The B1 area is covered with net of 1cm mesh screen to prevent predator while the remaining area of B2 not covered. The sediment area under the experimental raft, C is set up in the same procedure. Prior to the experiment, the macrobenthic and sediment samplings will be carried out. The mass culture of the early benthic stage juveniles of *P. membranacea* at the density of 400 inds/m2 will be introduced into the sediment under the experimental rafts, B1, B2, C1 and C2 by scuba diving. The experiments are to be carried out for 30 days. At the end of the 30 days experiment, the bioremediation efficiency of spionid polychaetes will be assessed from biomass increment in spionid, *P. membranacea* and reduction of total organic matter and sulphide concentration in the sediment. Changes in benthic animals after 30 days of experiment were also monitored.

For the long term bioremediation of organic waste in the sediment under the mussel rafts, it is interesting to add the artificially mass cultured of the early benthic stage juveniles of *P. membranacea* every month to maintain the bioremediation efficiency and the stable population of spionid polychaete as follow Tsutsumi et al. (2002). As most spionids, they showed the wide salinity range from tidal freshwater to hypersaline marine condition (Dix et al., 2005). The potential selected spionids, *P.*

membranacea can be used as bioremediator in other aquaculture system such as fish farms and shrimp farms.

C. Application of Spionid Polychaetes as Bioremediator of Organic Waste in Coastal Area

Bioremediation of organic enriched sediment in the coastal area of Sriracha Town Municipality under the influence of domestic waste effluents can easily be carried out in the same way by introducing the mass culture of *P. membranacea* settlement stage into the enriched sediment as in Figure 6.3. The spionid polychaetes can rapidly proliferated in the enriched sediment and increased the bioremediation efficiency. The feeding behavior of the spionid polychaetes being deposit feeders enhanced the organic aggretion and incorporated certain pollutants into the trophic pathway of bioaccumulation (D. M Dauer et al., 1981; Muschenheim, 1987; Yokoyama, 1988). The sediment in the Sriracha nearshore area is usually black with sulphide order. Dominant benthic animals in the area were *P. membranacea* and capitellid polychaetes, *Notomastus* sp. and *Hetermastus* sp. indicating high organic enriched condition in the area.

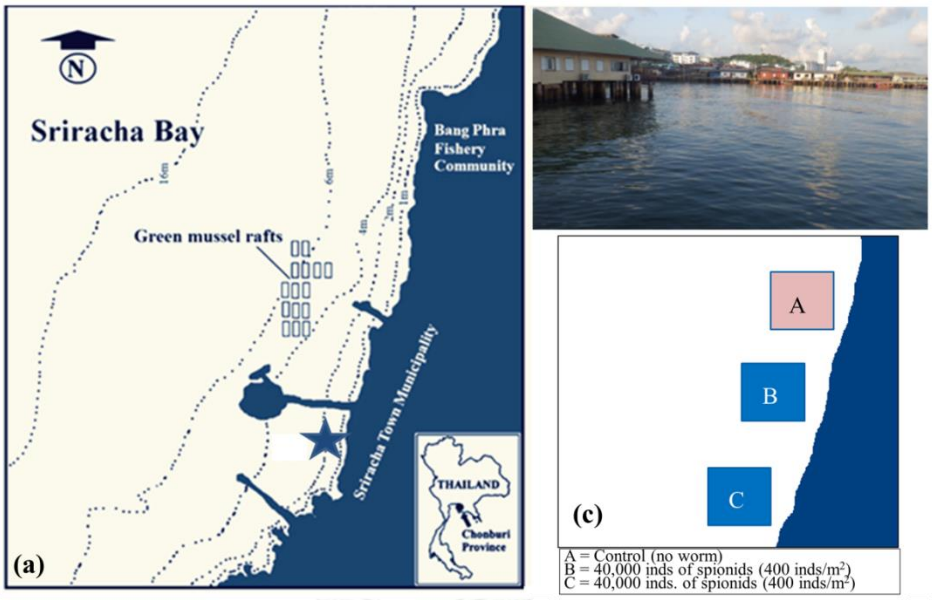

Figure 6.3 Bioremediation of organically enriched sediment in coastal area of Sriracha Bay with artificially mass culture of selected spionid, *Prionospio (Prionospio)*. (a) study site (b) coastal area of Sriracha Bay, Chonburi Province (c) model of setting plots.

Three plots of 100x100 m (Figure 6.3) at the water depth of 0.5-1 m were designed for the bioremediation of organic waste by *P. membranacea*. It is not necessary to cover the plots by net of 1 cm mesh screen in order to prevent predators due to the low diversity of benthic fauna. Plot A is the control area without the introduction of mass culture of spionid polychaetes. Plots B and C are the areas of bioremediation by introducing the artificially mass culture of the early benthic stage juveniles of *P. membranacea* into the sediment. Prior to the experiment, benthic communities and sediment quality in each plot will be carried out. The mass culture, 40,000 individuals of the early benthic stage juveniles of spionid polychaetes (400 inds/m^2) are then added into the sediment of plots A and B. The experiments are to be carried out for 30 days. At the end of the 30 days experiment, the bioremediation efficiency of spionid polychaetes will be assessed from biomass increment in spionid, *P. membranacea* and reduction of total organic matter and sulphide concentration in the sediment. Changes in benthic animals after 30 days of experiment will also be monitored.

D. Promotion of Spionid Polychaetes as Potential Feed for Aquarium Ornamental Fish

Most of the polychaetes used as bioremediator that nutrients and organic matter released in the culture system could be converted into biomass which can be easily removed and harvested as the valuable by-product ((Gifford et al., 2007; Palmer, 2011; Tsutsumi et al., 2005; Tsutsumi et al., 2002; Van Bruggen, 2012). Several species from the family Nereididae have been studied to simultaneously remediate wastewater and produce harvestable polychaete biomass without supplemental feeding. These polychaetes were important supplementary feed for brood stock of shrimps and also as valuable food for fishes.

As the selected spionid, *P. membranacea*, small size polychaete, can be the potential candidate for fish feed in particular the juveniles of economically marine fish and ornamental fishes. Ontogenetic niche shift in winter flounder, *Pseudapleuronectes americans* revealed that small size spionid polychaetes, calanoid copepods and ampeliscid amphipods were the main diet in newly settled and small juvenile winter flounder. Large size fish feed on various species of polychaetes, amphipods and siphons of the bivalves (Stehlik & Meise, 2000).

The aquarium ornamental fish export industry in Thailand was a well developed and growing industry with increasing export revenue. Suitable, high-quality feed significantly improved the growth and survival raft of ornamental fish. Live feeds have been shown to significantly improve the growth and survival of many ornamental fish compared to conventional feed. Common live feed in the Thai ornamental fish market include, freshwater fleas, brine shrimps, blood worms and oligochaetes (Chittapun et al., 2013). The highest energy and protein content in feed were found in the oligochaete, *Limnodrilus hoffmeisteri* of 57.42 Kcalx100g^{-1} and 59.13% respectively. The high amount of astraxanthin, the promoter of pigmentation in fish, was also observed in this oligochaete of 10.90 mg per gram of sample (Chittapun et al., 2013).

L. hoffmeisteri, is one of the most widespread and abundant aquatic oligochaetes in the world. It is widely reconized as an effective indicator of organically polluted aquatic environment. The life history of this oligochaete varied according to local condition and worms could breed throughout the year. *L. hoffmeisteri* can be collected from the sediment of Chao Phraya River (Prajongsak, 2013). The field collected populations may reach the completion of their life cycle after establishing in laboratory culture sufficiently supplied with organic food source (Kanchana-Aksorn & Petpiroon, 2008). Nutritive value of the spionid polychaete need to be further investigated to support as the potential candidate as feed for aquarium ornamental fish.

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