ครั้งที่ 4

Fund for Sustainable Education (FUSE)
SVITA Foundation

Proceedings of
การประชุมวิชาการ
The 4th National Meeting on Biodiversity Management in Thailand
การบริหารจัดการความหลากหลายทางชีวภาพแห่งชาติ

Science and Emerging Technology for Biodiversity Management

21-23 มิถุนายน 2560
โรงแรมนภาลัย จ.อุดรธานี

BiOD4

Proceedings the 4th National Meeting on Biodiversity Management in Thailand
การประชุมวิชาการการบริหารจัดการความหลากหลายทางชีวภาพแห่งชาติ ครั้งที่ 4 (2017): 23–33

Distribution and abundance of benthic dinoflagellates in seagrass areas of the eastern coast of the upper Gulf of Thailand

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Abstract: Seagrass ecosystems are very important nursery areas for larvae of both marine vertebrates and invertebrates. The system provides food and refuge areas for these larvae. Leaves of seagrass also can be good substrates for microorganism attachment, especially for benthic phytoplankton. Among very important epiphytic microalgae species include toxic benthic dinoflagellates that can cause ciguatera fish poisoning (CFP) symptoms. Toxins of toxic benthic dinoflagellates can transfer through the food chain from the small herbivorous fish that graze on the seagrass leaves and then to carnivorous fish and finally to humans. Little is known about toxic benthic dinoflagellates in seagrass ecosystems. In Thailand, two cases of CFP have been reported; patients had severe ciguatera like symptoms. To understand the distribution and abundance of benthic dinoflagellates in seagrass ecosystems, this study was conducted in four seagrass areas of the eastern coast of the upper Gulf of Thailand: Samaesan Island in Chonburi Province, Ban Phe coastal and Rock Garden Village in Rayong Province, and Kung Krabaen Bay in Chanthaburi Province, during the southwest and the northeast monsoon. Three substrates in the seagrass areas including sediment, seagrass leaves, and screen trap, were collected for dinoflagellate species identification. A total of five species of benthic dinoflagellates were observed in the four study areas. The highest abundance and diversity of benthic dinoflagellates was at Samaesan Island during the northeast monsoon which might be due to higher salinities and seagrasses diversity at this site relative to the other areas.
Keywords: distribution, benthic dinoflagellates, seagrass, upper Gulf of Thailand, monsoon

Introduction

Seagrass ecosystems are very important for marine larvae of both vertebrates and invertebrates in coastal areas. This ecosystem provides a permanent habitat, a temporary nursery area for larvae and juvenile stages, refuge (Jackson *et al*., 2001) and provides relatively stable environments in term of reduced wave action that is suitable for attachment of small organism such as algae, epiphytes, and especially for benthic phytoplankton. Benthic phytoplankton in seagrass areas can be 20 times more abundant than those in the areas without seagrass (Mcroy and Mcmillan, 1977). Nevertheless, benthic phytoplankton are associated with the grazers that consume them as a food source in seagrass areas (Orth and Montfrans, 1984).

Benthic dinoflagellates are one type of benthic phytoplankton that attach on substrates under water such as rocks, dead corals, seaweeds, and seagrasses. They belong to Class Dinophyceae, as do other dinoflagellates, but most dinoflagellates float in the water column while benthic dinoflagellates attach on substrates. Benthic dinoflagellates can be divided into non-toxic and toxic groups. At present, studies of toxic benthic dinoflagellates are increasing because toxins accumulate in seafood that negatively affects human health. Toxins can transfer through the food chain from the small herbivorous fish that graze toxic benthic dinoflagellates on the seagrass leaves (or other substrates) and then to carnivorous fish, and finally to humans. Some species of benthic dinoflagellates cause human syndromes such as Ciguatera Fish Poisoning (CFP) from consumption of contaminated marine fish. CFP can accumulate in small herbivorous fish grazing on the coral reefs into bigger carnivorous fish (Burgess and Shaw, 2001). CFP symptoms can be either gastrointestinal or neurological. Gastrointestinal symptoms involve vomiting, diarrhea, nausea and abdominal pain. Neurological symptoms include tingling of the lips, hands, and feet, severe itch of the skin, and unusual temperature perception disturbances where cold objects give a dry-ice sensation (Ansdell, 2015).

All CFP events have been reported in tropical and temperate zones that are related to the bloom of benthic dinoflagellates. There are many cases of CFP reported. In the Philippines human deaths have been reported by ingestion of the crab *Demania reynaudii* contaminated by palytoxin from *Ostreopsis,* a benthic dinoflagellate species (Alcala *et al*., 1988). There has been a report from Italy that macroalgae, invertebrates, and fish larvae died after blooms of a benthic dinoflagellate, *Ostreopsis ovata* (Vale and Ares, 2007; Totti *et al*., 2010). In Thailand, two patients from Bangkok in 2007, and four patients from Phuket in 2009, were reported from two small outbreaks. All patients consumed the marine fish and had severe gastrointestinal symptoms like ciguatera fish poisoning (CFP) symptoms. One patient had acute ventilatory failure requiring intubation whereas the remaining had neurological disturbances consisting of paresthesia, severe vertigo and ataxia (Saraya *et al*., 2014). While the reported causes (consumption of certain fish) and symptoms reported were consistent with CFP toxins, there is no laboratory in Thailand for confirmation of CFP toxins, hence these events must be classified as putative CFP (Thanakitjaroenkul *et al.,* 2013).

Benthic dinoflagellates have been reported in many coastal tropical and temperate zones mainly on coral reefs and macroalgae in reef areas, but there have been only a few studies on seagrass areas. Marasigan *et al*. (2001) studied populations of *Prorocentrum* on Taklong Island, Philippines, and reported six species of *Prorocentrum* on leaves of *Enhalus acoroides*. *Prorocentrum lima* was the dominant species in this study. Mohammad-noor *et al*. (2004) mainly focused on the diversity of benthic species in Malaysia. Six genera including 16 species of benthic dinoflagellates were found on seagrass leaves; six species were shown to be potentially toxic by the *Artemia* bioassay test. *Prorocentrum lima* was found in all stations and was shown to be potentially toxic. Rodriguez *et al*. (2010) surveyed benthic dinoflagellates associated with *Thalassia testudinumin* on San Andres Island, Colombia. Three genera with eight species of benthic dinoflagellates were found on seagrass leaves; six of eight species belonged to the genus *Prorocentrum*. Mounir *et al*. (2013) studied seasonal variation of organisms on *Posidonia oceanica* leaves and in the water column at Kerkennah Islands, Tunisia. Nine species from four genera were found on seagrass leaves. *Prorocentrum lima* was the most abundance species of benthic dinoflagellates. Mabrouk *et al*. (2014) studied epiphytic microalgae on the leaves of *Posidonia oceanica* and *Cymodocea nodosa* in Tunisia; eleven species from five genera were found on seagrass leaves. *Cymodocea nodosa* exhibited the highest density of epiphytic microalgae on the leaves while *Posidonia oceanica* had the highest leaf area index.

The first description of a benthic dinoflagellate was *Ostreopsis siamensis* Schmidt (1902). It was surveyed and collected for classification at Chang Island in 1902 to 1903. However, this first discovery was from plankton samples, not from benthic substrates. *Ostreopsis* was considered a member of the tycoplankton community (i.e., pseudo-plankton). Subsequently, Plumsomboon *et al*. (2002) studied benthic microalgae in mangroves and coastal ecosystems in Thailand and reported *Ostreopsis* sp., *Gambierdiscus toxicus*, and *Coolia tropicalis* (only *C. tropicalis* was found on seagrass leaves). The seagrass areas in Thailand cover 255 square kilometers, which are distributed along the Andaman Sea and the Gulf of Thailand in 13 provinces with 13 species of seagrasses have been reported (Department of Marine and Coastal Resources of Thailand, 2015). Jarulakkhana and Sriwoon (2012) studied the diversity of benthic dinoflagellates on *Enhalus acoroides* leaves and reported four genera of benthic dinoflagellates including *Coolia*, *Gambierdiscus*, *Prorocentrum*, and *Sinophysis* at Lem Yong Lum, Trang Province. Tawong (2014) reported that *Ostreopsis* was the most abundant species, with 174 cell/g macrophyte, at Kung Wiman Bay, Chanthaburi Province; *Gambierdiscus* and *Coolia* were the most abundant in Andaman sites with 3.230 and 47.040 cell/g macrophyte at Tangkhen Bay, Phuket Province. In another study of benthic dinoflagellates in Thailand, Somboon *et al*. (2016) compared two sampling methods (screen vs. examination of natural materials; *Amphiroa* sp. and *Haliclona* sp.) at Rin and Jan Island, Chonburi Province. Three benthic dinoflagellate genera were observed, *Ostreopsis*, *Prorocentrum*, and *Gambierdiscus,* with *Ostreopsis* as the dominant genus; the screen sampling method provided higher estimates of benthic dinoflagellate abundance than sampling from natural materials.

However, the diversity and distribution of benthic dinoflagellates in the seagrass areas of Thai waters are still limited. Thus, this study was carried out to investigate the distribution, diversity, and abundance of benthic dinoflagellates in seagrass ecosystems in the eastern coast of the upper Gulf of Thailand.

Materials and Methods

Study sites

Four seagrass areas along the eastern coast of the upper gulf of Thailand were sampled including: Samaesan Island, Chonburi Province; Ban Phe Coastal, Eastern Marine Fisheries Research and Development Center, Rayong Province (12°36’30.0’’N; 101°25’33.2’’E); Rock Garden Village, Makampom Bay, Rayong Province (12°39’46.2’’N; 101°39’28.2’’E); and Kung Krabaen Bay, Chanthaburi Province (12°35’27.9’’N; 101°53’47.2’’E) (Figure 1). Studies were conducted during the southwest and northeast monsoon during the period of July-August 2016 and December 2016 - January 2017, respectively.

Field sampling

Three line transects were sampled with nine quadrats (50 cm^2) in the four seagrass areas (Figure 2). Seagrass density in each quadrat of the four areas was determined by visual examination and photographs followed the Seagrass Monitoring Field Guide (PMBC).

Nine screen traps (20 cm^2) were fixed with a steel rod by cable tie; the rods were implanted in each quadrat and left for 24 hrs. After 24 hrs, three substrates of which surface sediment, seagrass leaves, and the screen traps were sampled from each quadrat. Seawater samples in each of the four areas was also collected.

At first, Seagrass leaves and sediment samples were measured in gram wet weight and then the benthic dinoflagellates were separated from the substrates by vigorously shaking with the filtered seawater in the plastic bottles for 1 minute (Tester *et al*., 2014). Then poured the extracted seawater samples through a 100 µm mesh size sieve to remove the large particles and again passed those seawater samples through a 20 µm mesh size sieve (Shah *et al*., 2013). The samples retained on the 20 µm mesh sieve were kept in small bottles and fixed for dinoflagellate species identification with 2-3% of iodine Lugol’s solution.

Identification and Quantitation

Benthic dinoflagellates were observed with a Nikon TMS inverted microscope and counted by using a Sedgewick Rafter counting chamber. For morphological identification, unarmored species (naked benthic dinoflagellates) were identified based on shape and position and torsion of the cingulum. The armored species (thecate benthic dinoflagellates) were identified based on shape and difference in thecal plate arrangement according to Fukuyo (1981), Steidinger and Tangen (1997), Faust and Gulledge (2002) and Hoppenrath *et al*. (2014).

Result and Discussion

Identification of Benthic Dinoflagellates

Unarmored species

Genus *Amphidinium* Claparede and Lachmann 1859 (Figure 3A) belongs to Order Gymnodiniales, Family Gymnodiniaceae. *Amphidinium* spp. motlile cells were ellipsoid to spheroid in ventral view and dorsoventrally flattened; non-motile cells were spheroid. The epicone was reduced with a tongue-like shape. The starch sheath pyrenoid having a ring-like shaped and located in the middle of the cell body. The nucleus was spheroid to ellipsoid and located in the posterior of the hypocone with yellow-brown chloroplasts. The sulcus originated near the mid-ventral line and near the origin cingulum.

Armored species

Genus *Coolia* Meunier 1919 (Figure 3B) belongs to Order Peridiniales, Family Thoracosphaeraceae. *Coolia* spp. cells were spherical in dorsoventral view and ellipsoid in lateral view with brown chloroplasts. The epitheca was a little flattened relative to the hypotheca in altitude. The cingulum was narrow and lined along the cell body while the sulcus was deep, short and wide in ventral view. The thecal surface was smooth and irregularly scattered with trichocyst pores. Thecal plate arrangement was similar to the genus *Ostreopsis*.

Genus *Ostreopsis* Schmidt 1901 (Figure 3C) belongs to Order Peridiniales, Family Ostreopsidaceae. *Ostreopsis* spp. cells were flattened and tear shaped with many golden-brown chloroplasts. The epitheca and hypotheca were equal in size and shape. The cingulum was inside view along the cell body. The thecal surface was smooth with evenly scattered trichocyst pores. Thecal plate arrangement was Po, 3', 7'', 5''', 2'''', 1p, which is common for this genus.

Genus *Prorocentrum* Ehrenberg 1833 (Figure 3D) belongs to Order Prorocentrales, Family Prorocentraceae. *Prorocentrum* spp. cells were flattened in dorsoventral view and oval shaped with many golden-brown chloroplasts. The epitheca and hypotheca were equal in size. This genus lacks a cingulum and sulcus. The periflagellar of the epithecal was V-shaped. Both thecal plates were concave and had trichocyst pores evenly scattered except for the central area.

Distribution of Seagrasses and Sediment Types

The species composition of seagrasses and sediment types at four study sites have shown the same pattern in both southwest and northeast monsoon. *Enhalus acoroides* has been found at Samaesan Island and Kung Krabaen Bay, while *Halophila minor* was observed only at Samaesan Island where the sediment types were sand and sandy clay, respectively. *Halodule pinifolia* could be observed only at Ban Phe Coastal and Rock Garden Village where sediment type of both areas was sandy clay. The percent coverage of seagrasses in each study sites were also shown in (Table 1).

Distribution and Abundance of Benthic Dinoflagellates

Samaesan Island, Chonburi Province

Southwest monsoon

Four genera of benthic dinoflagellates were present; *Amphidinium* spp., *Coolia* spp., *Ostreopsis* spp. and *Prorocentrum* spp. *Ostreopsis* spp. was the most abundant species, with 41 cells/100 cm^2

Northeast monsoon

Four genera of benthic dinoflagellates were present. Highest abundance of benthic dinoflagellate was *Ostreopsis* spp. with 408 cells/100 cm^2.

The greatest overall abundance of benthic dinoflagellates was present at Samaesan Island on all three substrates; surface sediments, seagrass leaves (*Enhalus acoroides* and *Halophila minor*) and the screen traps (Fig. 4). *Ostreopsis* spp. was the most abundant species in this area. These data are consistent with an earlier study of Tawong (2014) and Somboon *et al*. (2016) who worked in this area.

Ban Phe Coastal, Eastern Marine Fisheries Research and Development Center, Rayong Province

Southwest monsoon

Benthic dinoflagellates were present at very low cell density. *Amphidinium* spp. was found on *Halodule pinifolia* and the screen traps with 1 cells/gram wet weight and 2 cells/1000 cm^2 respectively (Figure 4).

Northeast monsoon

Three genera of benthic dinoflagellates; *Amphidinium* spp., *Coolia* spp. and *Ostreopsis* spp. were observed. The highest abundance and diversity of benthic dinoflagellate was *Ostreopsis* spp. with the maximum density of 132 cells/gram wet weight. *Prorocentrum* spp. was not observed in this area.

Rock Garden Village, Makampom Bay, Rayong Province

Southwest monsoon

Benthic dinoflagellates were not detected.

Northeast monsoon

Four genera of benthic dinoflagellates; *Amphidinium* spp., *Coolia* spp., *Ostreopsis* spp. and *Prorocentrum* spp. were found with high *Coolia* spp. cell density of 63 cells/100 cm^2.

Kung Krabaen Bay, Chanthaburi Province

Southwest monsoon

Benthic dinoflagellates were present at very low cell density. Only *Prorocentrum* spp. was found during the southwest monsoon on *Enhalus acoroides* and the screen traps with 1 cells/gram wet weight and 2 cells/1000 cm^2, respectively. The study of Piumsomboon *et al.* (2002) showed similar distribution pattern and cell density.

Northeast monsoon

Four genera of benthic dinoflagellates; *Amphidinium* spp., *Coolia* spp., *Ostreopsis* spp. and *Prorocentrum* spp. were observed. *Amphidinium* spp. had the highest cell density of 43 cells/100 cm^2.

Hydrography and Chemical Ecology

Water temperatures at the four study sites were somewhat higher during the southwest monsoon than during the northeast monsoon (Table 2). These small differences were likely related to the tidal cycle.

pH varied from a low of 7.30 to 8.27 (Table 2). pH tended to be higher during the northeast monsoon at each of the four sites. Dissolved oxygen (DO) had varied within a narrow range from 6.20 to 6.31.

Salinity showed large changes from site to site and during the monsoon seasons. Salinity was invariably high at all four sites during the northeast monsoon (31-33 psu). In contrast, salinity was greatly decreased at three sites (Ban Phe Coastal, 16 psu; Rock Garden Village, 5 psu; Kung Krabaen Bay, 17 psu) during the southwest monsoon; salinity remained high (34 psu) at Samaesan Island during the southwest monsoon (Table 2).

In general, higher salinities tend to be required for growth of marine benthic dinoflagellates (Morton *et al*., 1992; Pistocchi *et al*. 2011; Yamaguchi *et al*., 2012; Sparrow *et al*., 2013; Tawong *et al*., 2016). In contrast, the small changes in water temperature, pH and DO are not likely to have a significant impact on growth of marine benthic dinoflagellates.

Substrates

Three substrate types were sampled as part of this study: screen traps, seagrasses and sediments. The highest recovery of benthic dinoflagellate cells was from screen traps, seagrass leaves, and sediments, respectively, at Samaesan Island, Kung Krabaen Bay and Rock Garden Village; at Ban Phe Coastal seagrass leaves had a greater abundance of benthic dinoflagellates than the other substrates tested (Fig.4). The high recovery of benthic dinoflagellates from screen traps observed in this study is consistent with earlier results of Somboon *et al*. (2016); an explanation for the exception in Ban Phe Coastal, where higher recovery from seagrass leaves was recorded, is probably related to the low water turbulence at Ban Phe Coastal relative to the other three study sites (i.e., high water turbulence would resuspend epiphytic benthic dinoflagellates from their natural substrates such that they would be available for capture in the screen traps.

Conclusion

Four genera of benthic dinoflagellates were found in seagrass areas in the eastern coast of the Upper Gulf of Thailand including *Amphidinium* spp., *Coolia* spp., *Ostreopsis* spp. and *Prorocentrum* spp. The brown macrophyte *Padina* sp. was also found in the seagrass area at Samaesan Island; this macrophyte may provide more attachment habitat for benthic dinoflagellates and could, in part, explain the high diversity of benthic dinoflagellates observed at this study site. The northeast monsoon had higher abundances and diversity of benthic dinoflagellates than the southwest monsoon, which might be due to some physical factors, most likely salinity (i.e., salinity was 31-33 psu during the northeast monsoon at all four sites but decreased substantially at three sites during the southwest monsoon). Salinity is an important factor for many benthic dinoflagellates, with higher salinities generally more suitable for growth of marine benthic dinoflagellates.

The four seagrass areas examined during this study are sites that support local fishery activities. Hence, the presence of potentially toxic benthic dinoflagellates is cause for concern. For instance, the genus *Amphidinium* (which was observed in this study at all four sites), can produce many bioactive compounds, including some that may act as ichythotoxins that cause massive fish kills (Murray *et al*., 2015) and other compounds with hemolytic activity (Yasumoto, 1990) that may be implicated in CFP events (Tindall and Morton, 1998). *Prorocentrum* spp. can produce okadaic acid and its congeners. Furthermore, genera of benthic dinoflagellates that can cause ciguatera fish poisoning were found in this study (*Coolia* spp. and *Ostreopsis* spp.). The effects of toxic benthic dinoflagellate blooms can impact a variety of economic sectors, loss of product and lost revenue from tourists, marine ecosystem harm, and especially the public health. Thus, it is important to monitor benthic dinoflagellates that may be harmful to human health and/or ecosystem function. Further study is needed to investigate aspects of toxins produced by the five benthic dinoflagellate species observed in this work.

Acknowledgement

The authors are grateful to Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn – Chulalongkorn University, the Royal Thai Navy, Chulalongkorn University and JSPS-NRCT Core to Core Program: Harmful Algal Blooms for all supports and collaboration. We gratefully acknowledge, Asst. Prof. Dr. Thamasak Yeemin and LCdr. Puttipipat Srijunboon for providing diving assistance and diving equipment during this study. We thank Dr. Francis Gerald Plumley for proofreading and commenting on this manuscript. Thanks also to Ingon Thongcomdee, Kamonphorn Patthanasiri, Wichuda Yentua, Thirapha Praradorntham, Chanoknard Karnjanapak, Satianpong Keangsuphar, Patiphan Phumphoung and Sucharat Suksai for support in field studies.

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Table 1 Summary of seagrasses and sediment types.

Study Sites	Seagrass Samples			Sediment Types
	Species	% cover		
		SW	NE	
Samaesan Island	*Enhalus acoroides* and *Halophila minor*	68	72	Sand
Ban Phe Coastal	*Halodule pinifolia*	68	69	Sandy clay
Rock Garden Village	*Halodule pinifolia*	68	69	Sandy clay
Kung Krabaen Bay	*Enhalus acoroides*	51	73	Sandy clay

Table 2 Summary of temperature, salinity, pH, depth, and dissolved oxygen (DO) during Southwest and Northeast monsoon in the four study areas.

	Samaesan Island (Chonburi)		Ban Phe Coastal (Rayong)		Rock Garden Village (Rayong)		Kung Krabaen Bay (Chanthaburi)	
	SW	NE	SW	NE	SW	NE	SW	NE
Temp (ºC)	31±0	28±0.3	32±0	28±0	32±0.3	27±0.1	30±0.6	28.5±0.1
Salinity (psu)	34±0	34±0	16±0	31±0	5±0	31±0	17±0	31±0
pH	8.2±0.1	8.3±0.2	7.80±0.1	8.24±0.2	7.30±0.1	8.03±0.2	7.60±0.2	8.12±0.1
Depth (m)	0.3±0.1	2.5±0.2	0.5±0	1.5±0.1	0.2±0	1.3±0	0.2±0	2.0±0.2
DO (mg/L)	6.2±0	6.3±0.1	6.2±0.1	6.20±0.1	6.28±0	6.31±0.1	6.28±0	6.28±0

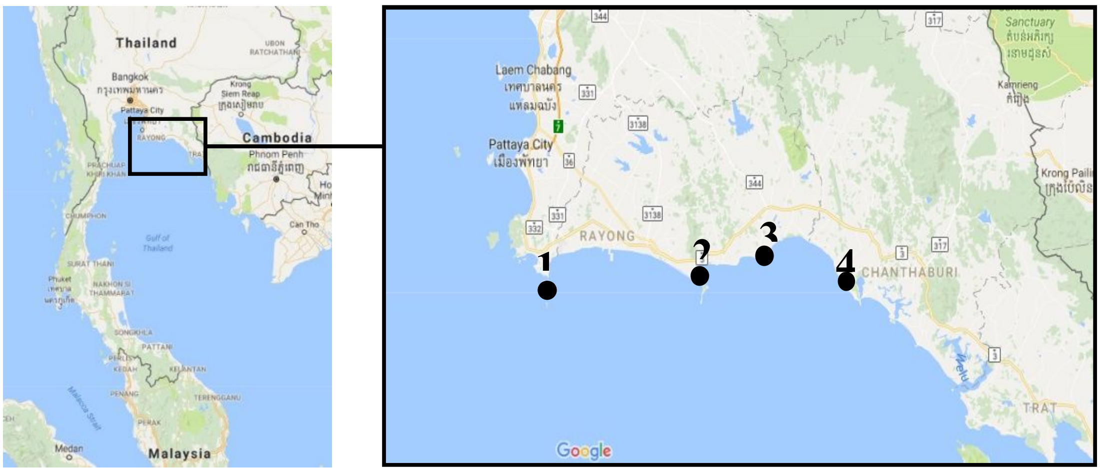

Figure 1 Study sites; 1= Samaesan Island, Chonburi Province, 2= Ban Phe Coastal, Eastern Marine Fisheries Research and Development Center, Rayong Province, 3= Rock Garden Village, Makampom Bay, Rayong Province, and 4= Kung Krabaen Bay, Chanthaburi Province.

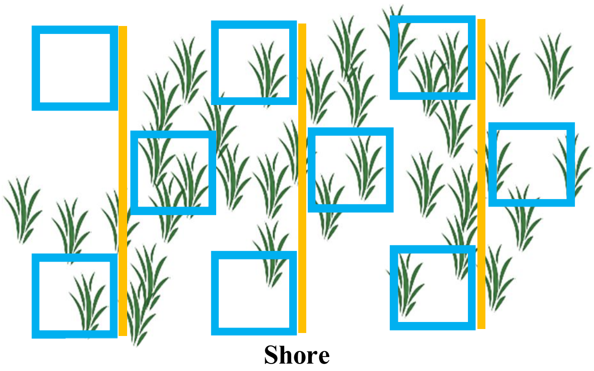

Figure 2 Line transects and quadrats sampling used in the study sites.

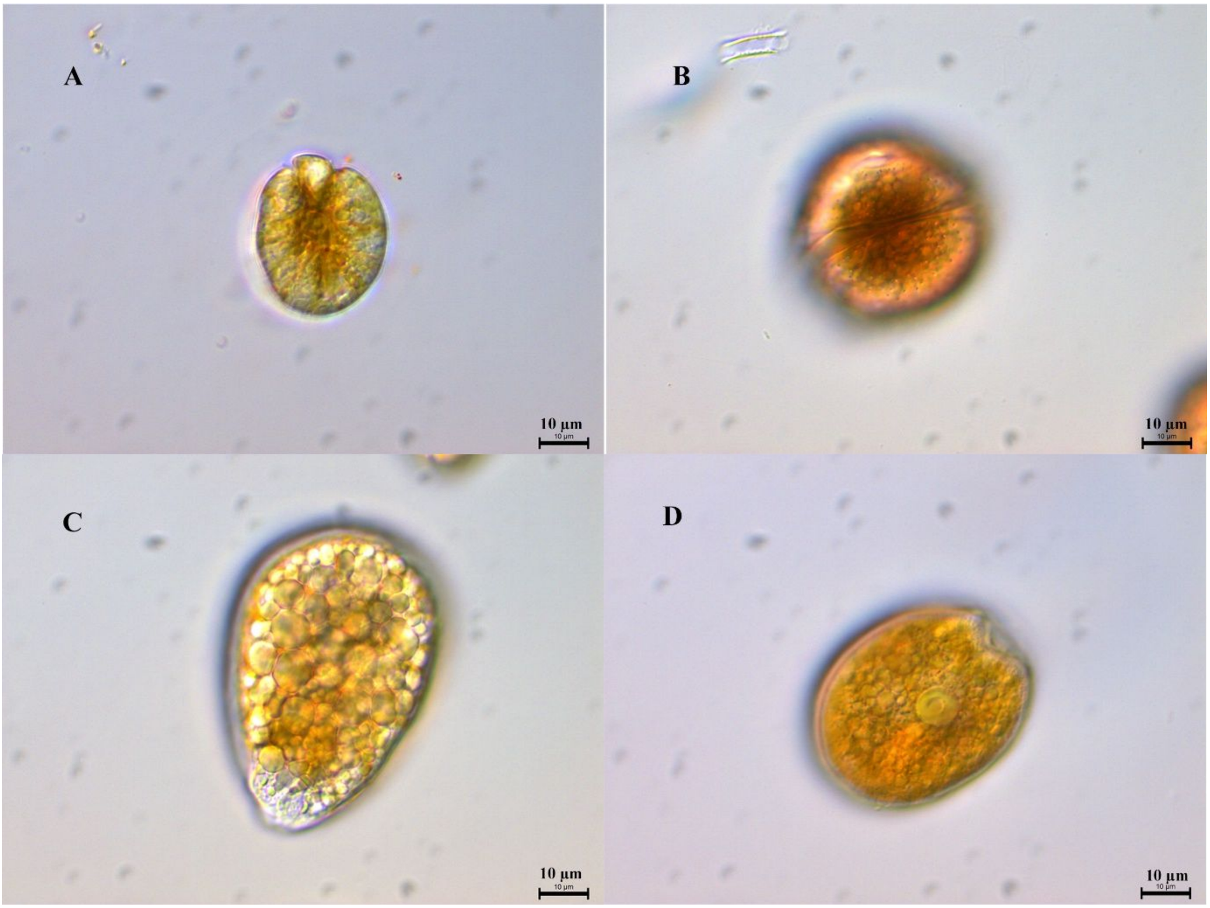

Figure 3 Light micrographs showing the morphology of the five species of benthic dinoflagellates found in this study. Species included *Amphidinium* sp. (A), *Coolia* sp. (B), *Ostreopsis* sp. (C), and *Prorocentrum* sp. (D). Scale bar = 10 µm

Figure 4 Abundance and diversity of benthic dinoflagellates on each substrate in each of four study areas. *Amphidinium* spp. *Coolia* spp. *Ostreopsis* spp. and *Prorocentrum* spp. *Cell units; Screen = cells/100 cm^2, Sediment and Seagrass = cells/gram wet weight. SW= southwest monsoon, NE= northeast monsoon.

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