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# Water Quality, Bacterial Counts and Mussel Growth in Four Mussel Culture Areas in the Upper Gulf of Thailand

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## Abstract

Four mussel culture areas in the Upper Gulf of Thailand were monitored for 1.5 years to assess environmental pollution in terms of bacterial levels, water quality and mussel growth. Contamination with bacteria including fecal coliforms was worse in Mae Klong and Chao Phraya estuaries than Ang Sila and Bang Pakong. More serious contamination in those estuaries usually occurred during the rainy season (July-October) and at low tides. Bacterial contamination of mussels occurred 10-40% of the sampling time, whereas the culture water was contaminated 20-55% of the time. Salinity fluctuated (1-35 ppt) during the rainy season, but dissolved oxygen (3.3-9.8 mg·l<sup>-1</sup>), temperature (26-33°C) and pH (6.5-8.7) were suitable for mussel culture. Obvious eutrophication in the gulf leads to frequent algal blooms, which may have caused the occasional large-scale mussel die-offs. At the four sites, mussel growth in terms of meat weight gain was 8-26% in 1994 and 60-140% in 1995, condition factor was 97-110 (good to excellent), and density settlement was 17-59 cm<sup>-1</sup> cross section.

## Introduction

With an annual production of about 50,000 tonnes, valued at US\$ 7 million, the cultured green mussel (*Perna viridis*) is one of the major economic shellfish products in Thailand (Department of Fisheries, in press). Although mussels are produced in many areas along the Thai coast, more than 90% of production comes from the Upper Gulf of Thailand.

Most mussel beds are concentrated in estuaries of four major rivers that flow through the heartland of Thailand, where major industries, farmlands and population centers are located. Contamination of the Upper Gulf of Thailand by hazardous chemical compounds and microorganisms in wastewaters has been previously reported (Menasveta and Cheevaparanapiwat 1981; Ruddle 1982; Hungspreugs and Yuangthong 1984; Phillips and Muttarasin 1985; Chalermwat

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and Lutz 1989; Hungspreugs et al. 1989; Phillips and Tanabe 1989; Siriwong et al. 1991). Mussels are filter feeders able to accumulate waterborne contaminants through lower food chain organisms, and are an ideal organism for monitoring environmental pollution (Viarengo and Canesi 1991; Widdows and Donkin 1992). However, contamination of mussels, a food commodity, poses a danger to public health (Hackney et al. 1992; Shumway 1992). Pollution in mussel culture areas would also have adverse economic consequences.

The present report describes the water quality, bacterial levels and mussel growth in the estuaries of four rivers over a period of 1.5 years.

### Materials and Methods

The four study sites were Ang Sila estuary in Chonburi, Bang Pakong estuary in Chachoengsao, Chao Phraya estuary in Samut Prakarn, and Mae Klong estuary in Samut Songkhran (Fig. 1). Water and mussel samples were col-

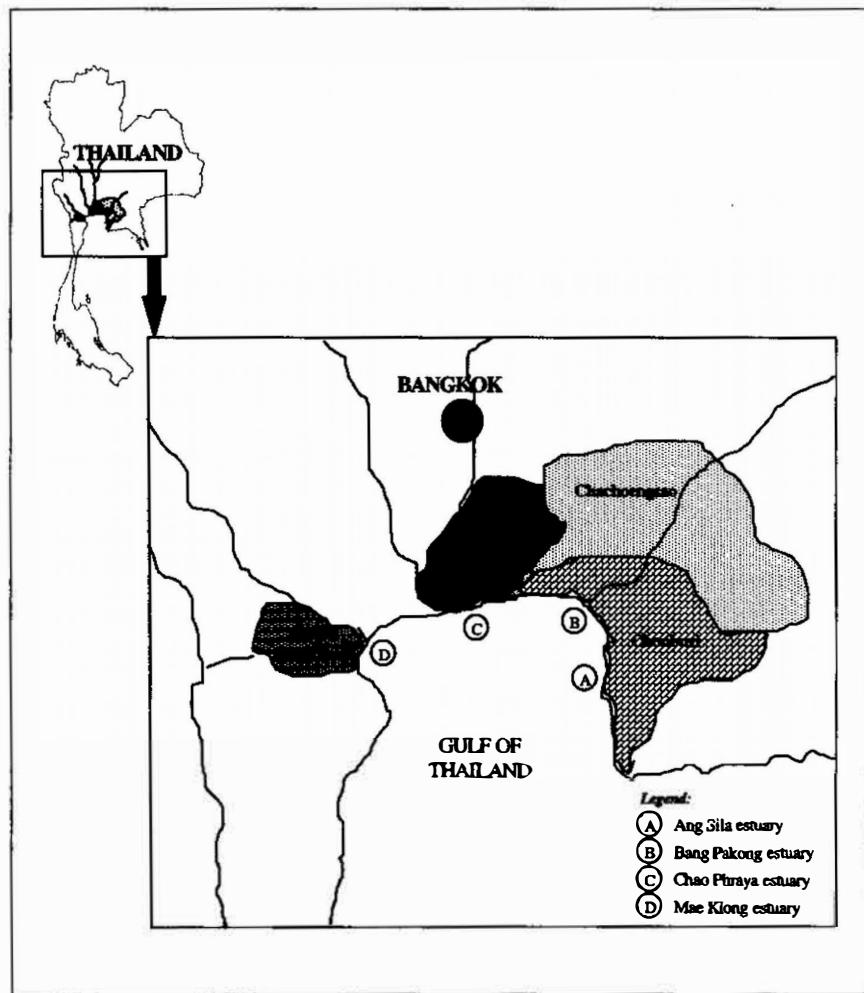


Fig. 1. Location of sampling sites at mussel-growing areas in the Upper Gulf of Thailand.

lected monthly from three locations (replicates) at each site. The water quality variables monitored were dissolved oxygen (DO), temperature, pH and salinity. DO and temperature were measured with an oxygen meter (WTW Oxymeter Microprocessor Oxi 96), pH with a Labotec Weisbaden pH96, and salinity with an Atago refractometer.

Mussel growth was based on measurements of shell length, width, thickness, total weight and meat weight. Condition index (CI), which measures the plumpness of the meat, was determined by displacement method (Quayle and Newkirk 1989). Condition index was computed as:

$$\frac{\text{total dry meat weight}}{(\text{whole volume}) - (\text{shell volume})} \times 1,000$$

This criteria classifies CI >120 as plump, 100-119 as excellent, 80-99 as good, and <79 as fair.

An estimate of mussel settling density on bamboo poles was made. A 15-cm section of the pole was randomly selected, and the mussels detached and counted. Density was estimated at per centimeter of pole in cross section (diameter about 7 cm).

Bacterial analysis of water and mussel samples included standard heterotrophic plate count (SHPC), fecal coliform (FC) and total coliform (TC). The Spread Plate Method using Difco Plate Count Agar was used for SHPC. The 3-tube Most Probable Number (MPN) fermentation test was used for coliform determination. The media used were Difco Lauryl Tryptose Broth for presumptive TC test, Difco Brilliant Green Bile 2% medium for confirmative TC test and Difco EC medium for FC test. Analysis followed APHA (1970) procedures.

Growth data was analyzed by linear regression. Statistical tests for site and seasonal differences included multi-factor analysis of variance (ANOVA) and least significant difference (LSD) test. Association of bacterial indicator organisms in water and mussel were tested by correlation analysis. Significance was tested at 95% confidence level. The Statgraphics software version 7.0 was used.

## Results

In the Upper Gulf of Thailand, mussels were cultured mostly on bamboo poles. Culture sites had water depths greater than 4 m. Barnacles were the dominant fouling organisms. Initial mussel settlement density tended to be high but thinned out naturally over time (Fig. 2). Average mussel density ( $\pm$  SE) per centimeter of pole in cross section in the different sites are  $59 \pm 33$  in Chao Phraya,  $56 \pm 12$  in Ang Sila,  $30 \pm 7$  in Mae Klong, and  $17 \pm 3$  in Bang Pakong.

Water quality was generally suitable for the culture of green mussels (Table 1). However, salinity fluctuation during the rainy season (July-October) may stress the mussels and cause mortality.

Average shell growth at all the sites is low for tropical conditions ( $<1 \text{ cm} \cdot \text{mo}^{-1}$ ). Comparison of mussel growth based on shell length at the four sites is shown in Fig. 3. Two growth curves were plotted for the three sites, Ang Sila, Bang Pakong and Mae Klong, which suffered mussel mortality in October

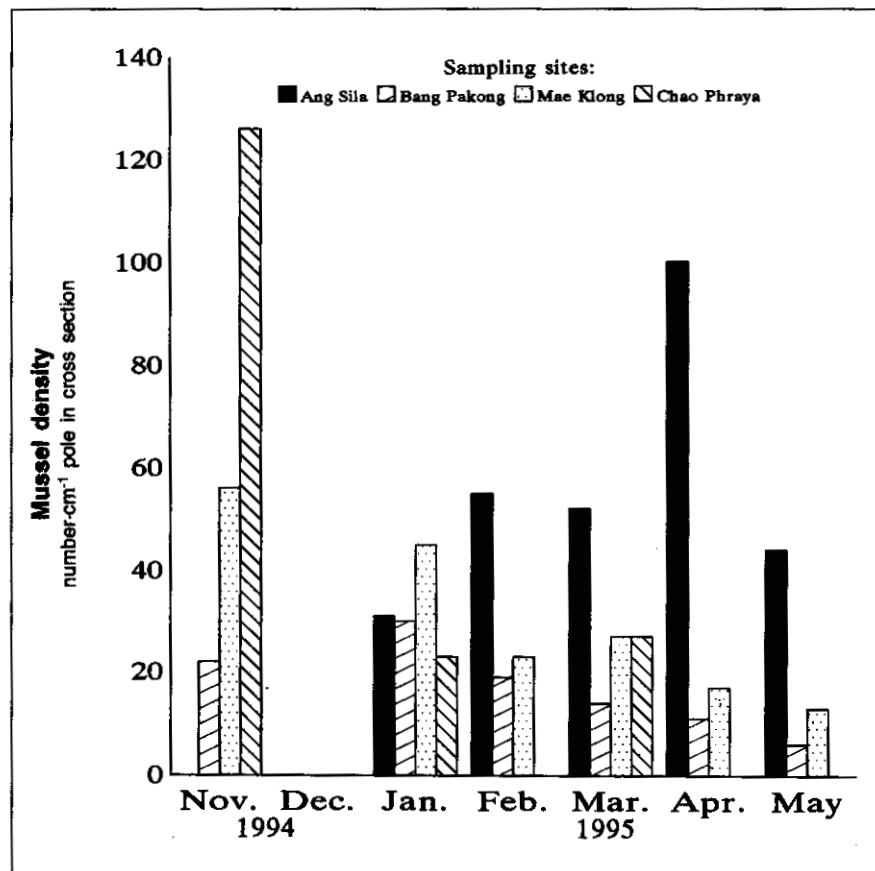


Fig. 2. The mean mussel settlement density on bamboo poles (diameter about 7 cm) at the four mussel culture areas in the Upper Gulf of Thailand.

Table 1. Water quality at four estuaries in the Upper Gulf of Thailand, October 1993-May 1995.

Water parameters	Statistics	Sampling sites				Level suitable for mussel culture
		Ang Slia	Bang Pakong	Mae Klong	Chao Phraya	
DO (mg·l⁻¹)	Mean	5.8	6.9	6.7	5.9	>2.5
	SE	±0.2	±0.4	±0.6	±0.2	
	Range	4.6-7.8	3.4-8.9	3.3-9.8	4.9-6.9	
Temperature (°C)	Mean	29.4	30.2	29.5	29.3	26-32 <sup>a</sup>
	SE	±0.3	±0.4	±0.6	±0.4	26-32 <sup>b</sup>
	Range	26.7-31.7	28.1-32.5	26.6-31.0	27.7-32.7	
pH	Mean	7.8	7.9	7.8	8.1	6-8.2 <sup>b</sup>
	SE	±0.2	±0.2	±0.3	±0.2	
	range	6.7-8.7	6.5-8.6	7.5-8.3	6.7-8.6	
Salinity <sup>c</sup> (ppt)	Mean	29	26	26	26	27-33 <sup>a</sup>
	SE	±1.9	±2.4	±2.8	±1.5	27-65 <sup>b</sup>
	Range	6-35	1-35	7-34	10-32	

<sup>a</sup> According to Vakily (1989)

<sup>b</sup> According to Sivalingam (1977)

<sup>c</sup> Normally, salinity levels fall within the suitable range but during the rainy season, salinity may decline (see range values), this results to mean levels slightly below the suitable range.

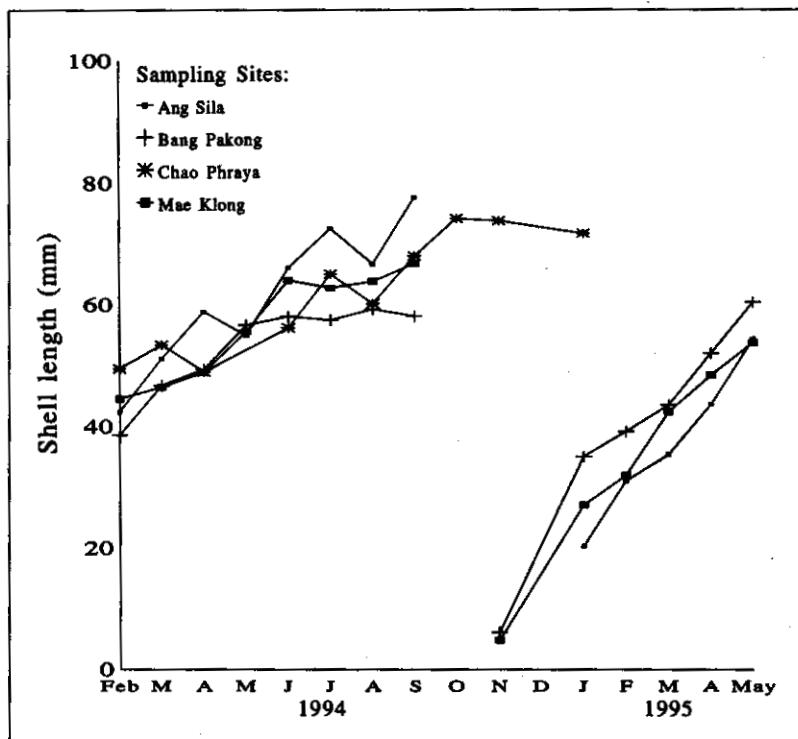


Fig. 3. Mussel growth based on mean shell length at the four mussel-growing areas in the Upper Gulf of Thailand during 1994, and the succeeding populations which thrived after the October 1994 mortality.

Table 2. Mean mussel growth and percentage gain at four estuaries in the Upper Gulf of Thailand in 1994 and 1995.

Site	Period	Shell length (Y) (mm)	Total weight (X) (g)	Meat weight (g)	Regression curve
Ang Sila	1994	5.0 (9%)	2.1 (26%)	0.76 (26%)	$Y=3.66 + (0.22)X$ ( $r^2=91\%$ )
	1995	8.5 (29%)	1.8 (100%)	1.1 (140%)	$Y=1.99 + (0.46)X$ ( $r^2=97\%$ )
Bang Pakong	1994	2.7 (6%)	0.71 (16%)	0.23 (12%)	$Y=2.60 + (0.38)X$ ( $r^2=94\%$ )
	1995	6.3 (15%)	2.1 (46%)	1.4 (60%)	$Y=2.68 + (0.29)X$ ( $r^2=99\%$ )
Chao Phraya	1994	2.4 (4%)	1.2 (11%)	0.4 (8%)	$Y=3.68 + (0.19)X$ ( $r^2=89\%$ )
Mae Klong	1994	3.2 (6%)	1.1 (15%)	0.52 (17%)	$Y=3.02 + (0.29)X$ ( $r^2=97\%$ )
	1995	6.7 (19%)	1.5 (56%)	0.68 (60%)	$Y=2.27 + (0.42)X$ ( $r^2=97\%$ )

Mussel growth in 1994 covered February to September at Ang Sila, Bang Pakong and Mae Klong estuaries; sampling was interrupted by mussel die-offs in October 1994; 1995 covered the growth of reestablished mussels from January to May. Mussel growth at Chao Phraya covered February 1994 to January 1995, sampling was discontinued due to strong water current.

1994. The first curve is prior to the mortality, while the second is for reestablished mussels. At Ang Sila estuary, the regression curves based on mussel shell length (Y) and total weight (X) were  $Y = 3.66 + (0.22)X$  for 8 months in 1994, and  $Y = 1.99 + (0.46)X$  for 5 months in 1995. The mean monthly growth increments were 5.0 mm (9%) in shell length, 2.1 g (26%) in total weight, and 0.76 g (26%) in meat weight in 1994 and 8.5 mm (29%), 1.8 g (100%) and 1.1 g (140%), respectively, in 1995. These data for Ang Sila estuary and the three other sites are shown in Table 2. The mean mussel condition (plumpness) index was 97 in Ang Sila, 110 in Bang Pakong, 105 in Mae Klong, and 103 in Chao Phraya, classified as good to excellent.

The levels of coliform (total and fecal) and heterotrophic plate count bacteria in water differed significantly between sites and sampling months. Comparison by LSD showed the bacterial levels in water at Mae Klong and Chao Phraya to be significantly ( $P < 0.05$ ) higher than at Ang Sila and Bang Pakong. Based on the criteria of  $TC < 70 \text{ MPN} \cdot 100 \text{ ml}^{-1}$  and  $FC < 14 \text{ MPN} \cdot 100 \text{ ml}^{-1}$ , the levels set by the National Shellfish Sanitation Program (1993) for areas approved for shellfish harvesting, the mussel farms at the four study sites were contaminated during the rainy season (July-October). Mae Klong and Chao Phraya had the higher coliform densities and were the most frequently contaminated (Table 3). Ang Sila was the least polluted in terms of coliform density and frequency of contamination.

Bacterial level in mussels also varied significantly among sampling periods but not among sites. The bacterial counts in mussels infrequently reached the unacceptable level for safe consumption,  $> 230 \text{ MPN FC} \cdot 100 \text{ g}^{-1}$ . During the study period, mussels were contaminated once at Ang Sila and Bang Pakong, twice at Mae Klong and four times at Chao Phraya. Mussel contamination did not always coincide with high loads of bacteria in water, as shown by a poor correlation of indicator organisms. Where coliform levels were higher in mussel than in water, concentration factors (count in mussel/count in water) may reach several thousandfold.

Levels of TC and FC were significantly ( $P < 0.001$ ) and positively correlated in water ( $r = 0.64$ ) and mussel ( $r = 0.70$ ). Standard heterotrophic plate count (SHPC) bacteria were usually present in greater numbers in mussels than in water at the same site.

A heavy mussel mortality occurred at three of the four monitored sites in October 1994. Mussel farmers from Ang Sila, Bang Pakong and Mae Klong suffered severe economic losses. Examination of water showed heavy blooms of dinoflagellates, dominated by species of *Ceratium* and *Noctiluca*. The mussels recolonized after the mortalities. However, it took 7 months to resume harvest and bring in income.

## Discussion

The incidence of mussel mortality in three culture areas in the Upper Gulf of Thailand shows that environmental degradation had occurred. Though exact causes cannot be pinpointed, it was more likely due to a combination

Table 3. Means, standard errors, ranges of bacterial density in water and mussels, and frequency of contamination at the four mussel growing areas in the Upper Gulf of Thailand, during February 1994 - May 1995.

Site	Sample	SHPC (CFU•g <sup>-1</sup> or ml <sup>-1</sup> )	Total coliform (MPN•100 g <sup>-1</sup> or ml <sup>-1</sup> )	TC concentration factor (mussel/water)	Fecal coliform (MPN•100 g <sup>-1</sup> or ml <sup>-1</sup> )	FC concentration factor (mussel/water)
Ang Sila	Mussel	8,400 ±2,200 (180-41,000)	898 ±448 (3.6-11,000)	141 ±117 (0.04-3.056)	459 ±422 (1.8-11,000)	125 ±117 (0.18-3.056)
	Water	780 ±160 (10-3,000)	93 ±31 (1.8-1,100)	21 ±8 (1.8-240)	21 ±8 (1.8-240)	21 ±8 (1.8-240)
Bang Pakong	Mussel	20,000 ±12,000 (50-290,000)	4/15 <sup>1</sup> 1,712 ±713 (3-11,000)	241 ±147 (0.02-3.667)	70 ±44 (1.8-1,100)	4.3 ±2.1 (0.03-47.8)
	Water	800 ±160 (20-3,000)	121 ±34 (3-1,100)	21 ±5 (1.8-150)	21 ±5 (1.8-150)	21 ±5 (1.8-150)
Chao Phraya	Mussel	7,500 ±1,800 (630-37,000)	5/15 <sup>1</sup> 917 ±491 (3-11,000)	4.6 ±2.2 (0.13-47.8)	75 ±20 (1.8-240)	2.1 ±0.8 (0.07-16)
	Water	1,600 ±330 (30-7,900)	501 ±228 (3.6-4,600)	4/9 <sup>1</sup> 118 ±50 (1.8-1,100)	4/9 <sup>1</sup> 118 ±50 (1.8-1,100)	4/9 <sup>1</sup> 118 ±50 (1.8-1,100)
Mae Klong	Mussel	7,800 ±1,900 (150-30,000)	2,049 ±767 (9.1-11,000)	51 ±21 (0.02-367)	1,351 ±698 (1.8-11,000)	31 ±19 (0.01-478)
	Water	1,100 ±270 (5-10,000)	365 ±73 (1.8-1,100)	2/10 <sup>1</sup> 144 ±44 (1.8-1,100)	2/10 <sup>1</sup> 144 ±44 (1.8-1,100)	7/15 <sup>1</sup>

<sup>1</sup>Frequency of contamination expressed as: number of times found contaminated/number of samplings, based on the National Shellfish Sanitation Program (1993) standard for safe shellfish growing/harvesting waters of TC <70 MPN•100 ml<sup>-1</sup> and FC <14 MPN•100 ml<sup>-1</sup>; for shellfish products, FC <230 MPN•100 g<sup>-1</sup>.

and interaction of factors associated with algal blooms. Indeed, there is reason for concern where economic losses have burdened both the farmers and the mussel industry.

Public health is a major concern where culture sites are subject to high coliform loads, especially during the rainy season and during low tides, and mussels are contaminated. Mussels generally have higher levels of SHPC, TC and FC than water because they filter-feed on micro-particles including bacterial contaminants. Contamination during the rainy season and at low tide is a consequence of greater levels of run-off and flushing of domestic and agro-industrial wastes in the area at these times. Similar findings were earlier reported in Thai waters by Saitanu (1989, 1992). Mussels may also be contaminated through unhygienic practices during harvest and handling (for example, washing with foul water in mussel landing areas).

Depuration of mussels is an uncommon practice in Thailand, but the relay method or the transfer of mussels grown from polluted to clean natural growing waters may be more appropriate and should be encouraged among farmers of Mae Klong and Chao Phraya to ensure bacterially safe mussels.

Many factors can cause mussel mortality, for example, environmental stress due to undesirable water quality, presence of harmful compounds, or a combination and interaction of several unfavorable conditions. But the mass die-off observed in the present study coincided with a heavy plankton bloom. Occurrence of mussel die-offs coinciding with algal blooms has been reported elsewhere (Tracey 1988; Shimuzu 1989; Mahoney et al. 1990). The causes of mussel mortality during algal blooms may be due to oxygen depletion and physical disturbances (Shimuzu 1989). Tracey (1988) also reported effects on feeding inhibition due to reduced filtration rates and starvation stress induced by noxious properties of the bloom alga. Lesser and Shumway (1993) noted biotoxin effects on physiological processes such as changes in feeding and respiration rates, shell valve closure, mucous production and altered cardiac activity.

Reports of *Ceratium* and *Noctiluca* blooms have been previously reported in Thai waters (Piyakarnchana et al. 1986; Brohmanonda 1987; Suvapepun 1989; Chua et al. 1989). *Noctiluca* blooms cause fish mortality due to release of large amounts of ammonia (Okaichi and Nishio 1976), and affect mussel production by inhibiting settlement of mussel larvae on poles (White et al. 1984 cited in Chua et al. 1989).

## Conclusions

There is a need for regular monitoring of the environmental status of the Upper Gulf of Thailand. With rapid population growth and industrial development in the area, there is an increasing threat of hazardous contamination in mussels with bacteria, viruses and toxic substances.

Assessment of waste loading in relation to sustainability of mussel culture in the Upper Gulf of Thailand is another aspect worthy of investigation. Biodeposition in mussel beds may be an important nutrient source for algal blooms, which occurs in increasing frequency and is adversely affecting aquatic communities in the gulf. We hypothesize that nutrient loading in the Upper Gulf of Thailand might elucidate the seasonality of plankton blooms which coincided with the mass mortality of mussels. The occurrence of algal blooms and its effects are supported by this study and other cited papers.

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