



Original Article

Comparison of helminth fauna from three different habitats in the Andaman Sea coastal ecosystem, southern Thailand

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ABSTRACT

Helminths of marine fish are highly diverse, impact food safety and can reflect an environmental change. Some helminths have been recognized as biological markers for coastal ecosystem evaluation. Recently, the coastal area near Tarutao Island, Satun province, southern Thailand, has been reported to be impacted by an ecological change. However, information regarding helminth biodiversity in this area is limited. Therefore, this study investigated helminths in commercially caught marine fish from three ecological areas of the coastal ecosystem near Tarutao Island. Physicochemical factors that may impact helminth diversity were also recorded. In total, 1660 marine fish (36 species) were caught using trapping and netting. Among them, at least 11 species were infected with helminths. In the parasite assemblages of these fish populations, *Ligophorus* spp. and *Metamicrocotyla* sp. specifically infected gills of *Mugil cephalus*, which was found in both mangrove and seagrass areas. *Ligophorus* spp. had the highest prevalence of infection in *M. cephalus* and may be a predominant species and potential biological indicator for the mangrove area. The results also revealed that helminth diversity and physicochemical factors significantly differed between the areas studied; therefore, physicochemical factors may play an important role as contributing factors that affect helminth diversity in the Andaman Sea coastal ecosystem.

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Introduction

Helminths of marine fish negatively affect food safety and security, and they have been reported to be biological indicators of environmental factors such as food chain structure, heavy metal contamination, environmental pollution, global climate change, and fish stock assessments (Quiazon, 2015). Although many helminths of marine fish have been reported, only a small proportion of parasites in marine ecosystems have been studied; consequently, the number of marine fish parasites is considered to still be underestimated (Poulin, 2014).

Helminths that infect marine fish include nematodes, cestodes, digenean and monogenean trematodes and acanthocephalans (Cortés, 2012). Among known species of helminths in marine fish, some are reported to be human pathogens (Adams et al., 1997),

such as anisakid nematodes (*Pseudoterranova decipiens*, *Contracaecum osculatatum*, and *Anisakis* spp.), cestodes (*Diphyllobothrium*), and digenean trematodes (*Heterophyes*, *Stellanchamus*, and *Metagonimus*). All of these have been recognized as parasites that may impact food safety and sustainability, particularly *Anisakis simplex* (Adams et al., 1997; Purivirojkul, 2009). Moreover, some helminths have been identified as biological indicators of heavy metal pollution for aquatic resource management (Quiazon, 2015), such as nematodes (*Hysterothylacium*, *Anisakis*, *Anguillicola*, and *Philometra*), acanthocephalans (*Pomphorhynchus* and *Acanthocephalus*), and cestodes (*Bothriocephalus*, *Monobothrium*, and *Ligula*). Some helminth parasites have also been recognized as biological indicators of anthropogenically induced stresses (Fajer-Ávila et al., 2006; Hechinger et al., 2007; Palm, 2011; El Hafidi et al., 2013), including monogenean trematodes (*Ligophorus* spp. and *Pseudorhabdosynochus*), digenean trematodes (*Didymodictynus*), nematodes (*Spirophilometra*, *Philometra*, and *Raphidascaris*), and acanthocephalans (*Serrasentis*). Because of the important roles of helminths in marine fish described above, parasite diversity should be estimated.

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In Thailand, information regarding helminth parasites in marine fish is limited; most has been obtained from the Gulf of Thailand marine ecosystem (Upatham et al., 1989; Nootmorn et al., 2002; Nuchjangreed et al., 2006; Bussarawit et al., 2008; Purivirojkul and Areechon, 2008; Purivirojkul, 2009). A few studies have been conducted and reported on some helminths in deep-sea fish, such as yellowfin tuna and deep-sea sharks from the Andaman Sea in Thailand (Purivirojkul and Areechon, 2008; Purivirojkul et al., 2009). However, there is no information regarding helminths from marine fish in the Andaman Sea coastal areas (Bussarawit et al., 2008).

This coastal ecosystem is composed of mangrove, seagrass and coral reef, which have significantly different physicochemical properties (Sridhar et al., 2008). Helminth parasite diversity in this ecosystem may be related to environmental changes (Doney, 2013). Some physicochemical factors may be associated with the parasite diversity and parasite maturity, particularly water temperature, salinity and concentrations of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions (Ohashi et al., 2007).

The coastal ecosystem near Tarutao Island, Andaman Sea, Satun province, southern Thailand is an area that is affected by environmental problems. Mangrove areas have been destroyed by drainage for agriculture and aquaculture and excavation for fish and shrimp farming (Barbier, 2003). Coral bleaching around the island has also occurred due to water pollution from shrimp farming on nearby land and also from snorkeling and coastal fishery activity (Barbier, 2003; Akase et al., 2014). The elucidation of the helminth diversity of marine fish in the coastal area near Tarutao Island would provide useful preliminary information to evaluate the effect of environmental change. Therefore, this study first estimated the helminth diversity of commercially caught marine fish collected from three different ecological areas (mangrove, seagrass and coral reef) in the coastal ecosystem around Tarutao Island. Commercial marine fish species and physicochemical factors associated with helminth parasite diversity were also analyzed.

Materials and methods

Study areas and fish collection

Dead marine fish were purchased from local fishermen. The fish had been caught from three areas in this coastal ecosystem: 1) the mangrove-intertidal area at 6°67'N and 99°90'E in La-Ngu district, Satun province, southern Thailand; 2) the seagrass area at 6°40'N and 99°60'E; and 3) the coral reef area of Tarutao National Park, in Mueang district, Satun province, southern Thailand at 6°43'N and 99°32'E. Water temperature, depth of water, salinity level and Ca^{2+} and Mg^{2+} concentrations were recorded in each area. Fish were caught eight times between May 2014 and April 2015 by trap and net. The collected samples were kept on ice and transferred after approximately 12 hr to the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. There, the fish were identified to the species level and the body length and weight were measured.

Helminth parasite collection

Fish were examined for both ectoparasites and endoparasites. The fish scales, gill, abdominal organs (intestinal cavity, liver, visceral, stomach, gonads), tail, fish muscle, and head of each fish were separated in a Petri dish. The intestinal cavity of each fish was examined by cutting from the oral cavity through the esophagus behind the oral cavity. The gut was pulled out and straightened, then gradually detached with scissors along the whole length up to the anus. Intestinal organs were opened longitudinally with

scissors in a Petri dish, and parasites that were visible by the naked eye were removed from the host using fine-point forceps. The minute parasites were separated and removed under a light microscope. All parasites were washed using 0.85% saline solution and kept in 10% formaldehyde. Helminths were stained with Semichon's acetocarmine, mounted in Canada balsam, then morphologically identified using light microscopy according to the method described by Yamaguti (1963), Yamaguti (1971), Kabata (1979) and Moravec (1994). The prevalence and mean intensity of each helminth species were then determined.

Physicochemical factor data

During each sampling time, the physicochemical parameters were recorded. Water levels were determined using a lead line for mangrove and seagrass areas but using a Hawkeye handheld sonar system (H22Px; NorCross; Orlando, FL, USA) for the coral reef area. Water temperatures of mangrove and seagrass areas were measured using a thermistor (109SS-L stainless-steel temperature probe for harsh environments; Campbell Scientific; Logan, UT, USA), whereas coral reef water temperatures were measured while recording the water level with a Hawkeye handheld sonar system (H22Px; NorCross; Orlando, FL, USA). Seawater was collected monthly using a submerged container to note the salinity and Ca^{2+} and Mg^{2+} concentrations. The salinity values of the collected water were recorded using a salinity meter (YK-31SA; Lutron Electronics Co. Inc.; Taipei, Taiwan) and the Ca^{2+} and Mg^{2+} concentrations were measured using a calcium and magnesium test kit for irrigation water (HI 38081; Hanna Instruments; Carrollton, TX, USA).

Statistical analysis

The physicochemical factors that were recorded in the three study areas (water temperature, water depth, salinity level, Ca^{2+} and Mg^{2+} concentrations) were statistically analyzed to determine their impact on helminth parasite diversity. Correlations between these factors and parasite species diversity were determined using Pearson's correlation coefficient (r) from 24 paired data (eight sampling times for each of the three study areas) and the statistical differences of factors between study areas were analyzed using nonparametric Kruskal-Wallis one-way analysis of variance facilitated by the computer program SPSS v.22.0 (Green et al., 1996).

Fish-parasite interactions

The interactions between marine fish and helminth parasites, and the parasite species assemblages within fish populations were evaluated using a bipartite graph in the "bipartite packages" implemented in R freeware v.3.0.3 (Dormann et al., 2009). Host-parasite interaction presence/absence matrices that combined all habitats were visualized using "plotweb" functions.

Ethics statement

This study did not require approval by an ethics committee as all fish in this study were bought as a part of normal commercial catches in the study areas.

Results

Fish species diversity

The commercial marine fish were caught from mangrove, seagrass and coral reef areas in this coastal ecosystem. Only three

species (*Anodontostoma chacunda*, *Mugil cephalus*, and *Lates calcarifer*) were collected from the mangrove area. These three species were also found in the seagrass area. Seventeen species were caught from the seagrass area, whereas 22 species were caught in the coral reef area (Table 1); five species were common to both these areas (*Ellochelon* sp., *Epinephelus diacanthus*, *L. calcarifer*, *Otolithes ruber*, *Parastromateus niger*). Only *L. calcarifer* existed in all three areas, but its size differed significantly among the different areas. The fish that lived in the mangrove area were smaller than those in the seagrass and coral reef areas (Table 1). All fish species infected by helminth parasites are shown in Fig. 1.

Physicochemical factors and helminth diversity

The water level, temperature, salinity and Ca^{2+} and $^{+}\text{Mg}^{2+}$ concentration data are shown in Table 2. All physicochemical factors significantly differed among the three areas in this coastal ecosystem (Table 2). The water level, temperature, salinity, and Ca^{2+} and $^{+}\text{Mg}^{2+}$ concentrations recorded in the coral reef area were relatively higher than the corresponding values measured in the seagrass and mangrove areas. Numbers of helminth species were negatively correlated with water level ($r = -0.838$; $p < 0.001$), temperature ($r = -0.4913$; $p = 0.007$), salinity ($r = -0.7789$;

$p < 0.001$), Ca^{2+} concentration ($r = -0.8694$; $p < 0.001$) and Mg^{2+} concentration ($r = -0.8915$; $p < 0.001$).

Helminth diversity and parasite assemblages in fish populations

Helminths were collected from each fish species in the mangrove, seagrass and coral reef areas. The fish species that had parasite infections in internal and external organs are reported in Table 3. *Mugil cephalus* caught from both mangrove and seagrass areas had *Ligophorus* spp. and *Metamicrocotyla* sp. in their gills (Fig. 2, Table 3). However, *Ligophorus* spp. was the predominant species that infected *M. cephalus* in the mangrove area but had a low prevalence of infection in *M. cephalus* from the seagrass area (Table 3). *Intuscirrus aspictti* (a digenean trematode) was also discovered in *M. cephalus* gills, but this species was found in low prevalence and very low intensity (Table 3). Metacercariae of *Stel-lanchesmus* sp. (a digenean trematode) were collected from the tail of *M. cephalus*. The rest of the metacercariae were of unknown digenean trematode species. No parasite was discovered in an internal organ or muscle.

The fish collected from the seagrass area had three nematode species (Table 2). *Procamallanus* sp. was the major species that infected fish intestines, whereas *Philometra* sp. was found in the

Table 1
Species of commercial marine fish collected from three ecological areas in the coastal ecosystem near Tarutao Island, Satun province, southern Thailand during May 2014 to April 2015.

Coastal ecological area	Species	Number of fish	Average values (mean \pm SD)	
			Body length (cm)	Body weight (g)
Mangrove	<i>Anodontostoma chacunda</i>	40	12.3 \pm 0.7	39.6 \pm 5.5
	<i>Mugil cephalus</i>	40	13.6 \pm 1.0	46.7 \pm 14.7
	<i>Lates calcarifer</i>	36	12.8 \pm 1.2	40.6 \pm 16.6
Seagrass	<i>Anodontostoma chacunda</i>	20	12.8 \pm 0.8	39.3 \pm 7.6
	<i>Mugil cephalus</i>	40	15.1 \pm 1.5	51.2 \pm 17.8
	<i>Cynoglossus</i> sp.	40	31.5 \pm 1.5	147.4 \pm 20.1
	<i>Dasyatis zugei</i>	40	16.9 \pm 1.7	114.7 \pm 37.9
	<i>Dendrophysa russelli</i>	40	15.3 \pm 2.6	50.5 \pm 25.9
	<i>Ellochelon</i> sp.	30	15.8 \pm 1.7	53.5 \pm 26.9
	<i>Epinephelus diacanthus</i>	30	16.7 \pm 4.6	129.9 \pm 55.5
	<i>Gerres filamentosus</i>	36	13.3 \pm 3.7	43.2 \pm 28.2
	<i>Johnius coitor</i>	40	13.0 \pm 2.7	47.1 \pm 24.5
	<i>Johnius carouna</i>	40	13.6 \pm 2.7	47.9 \pm 26.5
	<i>Lates calcarifer</i>	38	14.2 \pm 2.9	145.1 \pm 30.1
	<i>Leiognathus</i> sp.	76	11.3 \pm 2.1	50.1 \pm 15.5
	<i>Otolithes ruber</i>	39	15.6 \pm 2.8	60.7 \pm 23.1
	<i>Pampus argenteus</i>	24	11.9 \pm 3.0	48.7 \pm 26.1
	<i>Parastromateus niger</i>	14	17.5 \pm 3.3	108.0 \pm 31.9
	<i>Pomadasys argenteus</i>	53	15.6 \pm 2.7	71.1 \pm 25.7
	Coral reef	<i>Nemipterus hexodon</i>	15	20.9 \pm 4.2
<i>Atule mate</i>		80	14.4 \pm 3.1	66.7 \pm 22.0
<i>Caranx leptolepis</i>		80	14.6 \pm 3.3	60.0 \pm 21.5
<i>Caranx sexfasciatus</i>		80	13.9 \pm 2.5	59.8 \pm 20.7
<i>Chirocentrus nudus</i>		19	34.7 \pm 10.5	219.6 \pm 48.5
<i>Decapterus russelli</i>		80	21.2 \pm 3.7	165.2 \pm 38.1
<i>Dussumieria hasselti</i>		80	20.1 \pm 2.3	52.5 \pm 26.2
<i>Ellochelon</i> sp.		40	20.4 \pm 3.9	60.4 \pm 22.6
<i>Epinephelus diacanthus</i>		20	30.1 \pm 9.1	722.6 \pm 310.2
<i>Epinephelus bleckeri</i>		20	36.6 \pm 7.4	362.7 \pm 263.1
<i>Epinephelus bruneus</i>		20	24.8 \pm 7.0	359.5 \pm 242.5
<i>Epinephelus coioides</i>		14	27.1 \pm 7.2	372.6 \pm 260.9
<i>Lates calcarifer</i>		14	17.1 \pm 5.9	147.8 \pm 55.6
<i>Lutjanus lineolatus</i>		18	21.7 \pm 2.1	203.2 \pm 41.1
<i>Lutjanus vitta</i>		60	25.5 \pm 3.2	359.0 \pm 106.3
<i>Megalaspis cordyla</i>		60	20.5 \pm 2.8	56.3 \pm 36.5
<i>Otolithes ruber</i>		40	22.1 \pm 5.1	63.2 \pm 32.1
<i>Parastromateus niger</i>		11	19.4 \pm 5.6	128.1 \pm 55.2
<i>Rastrelliger neglectus</i>		60	19.4 \pm 4.8	62.1 \pm 35.6
<i>Sardinella gibbosa</i>	60	17.5 \pm 4.2	37.2 \pm 19.2	
<i>Secutor ruconius</i>	40	13.6 \pm 2.7	37.0 \pm 15.7	
<i>Siganus juvus</i>	17	14.9 \pm 2.2	41.1 \pm 18.4	
<i>Trichiurus lepturus</i>	16	45.3 \pm 10.2	255.5 \pm 44.0	

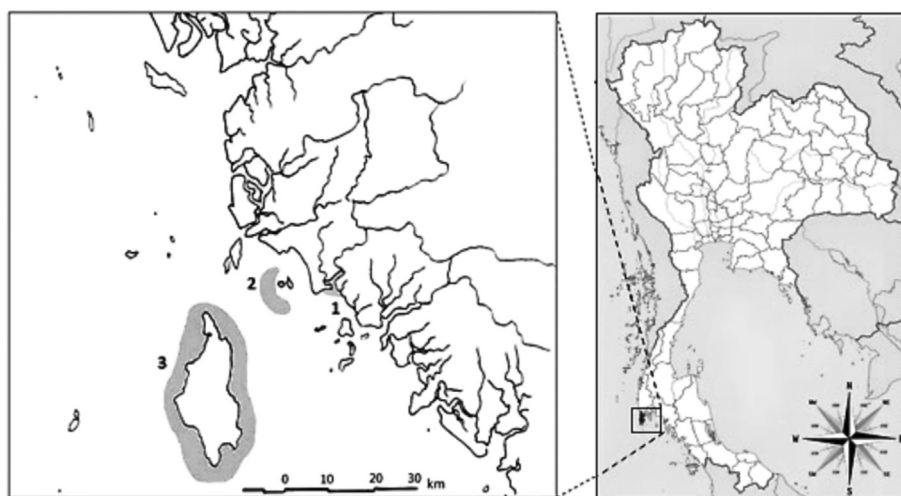


Fig. 1. Fish collection sites (gray shading) in three areas near Tarutao Island (1 indicates the mangrove area, 2 indicates the seagrass area, 3 indicates the coral reef area).

Table 2

Average values of physiochemical factors recorded eight times from May 2014 to April 2015 from each ecological area.

Coastal ecological area	Water level (m)	Temperature (°C)	Salinity (ppt)	Ca ²⁺ concentration (ppm)	Mg ²⁺ concentration (ppm)
Mangrove	0.33 ± 0.11	25.23 ± 0.83	29.13 ± 1.05	230.62 ± 6.23	520.75 ± 4.03
Seagrass	4.55 ± 0.81	26.19 ± 0.68	31.11 ± 0.62	250.87 ± 5.89	534.25 ± 5.40
Coral reef	23.49 ± 2.97	27.05 ± 0.80	35.85 ± 0.43	413.50 ± 6.57	927.13 ± 5.33
Mean ± SE	9.46** ± 2.97	26.16** ± 0.80	32.03** ± 2.97	298.33** ± 83.83	660.71** ± 192.58

ppt = parts per trillion.

** = highly significant ($p < 0.01$) difference of average values among coastal ecological areas.

Table 3

Species of infected fish from each ecological area and species of helminth found (black shading indicates percentage of prevalence of infection and different levels of gray indicates ranges for mean parasite intensity).

Coastal ecological area	Fish species (N)	Helminth	% Prevalence of infection (mean of parasite intensity)				
			Gill	Tail	Intestine	Muscle	Others
Mangrove	<i>Mugil cephalus</i> (40)	<i>Ligophorus</i> spp.	100 (120.7)				
		<i>Metamicrocotyla</i> sp.	25 (1.2)				
		<i>Intuscirrus aspicotti</i>	15 (0.4)				
		<i>Stellanchasmus</i> sp.		47.5 (8.1)			
		unknown		32.5 (5.4)			
Sea grass	<i>Mugil cephalus</i> (40)	<i>Ligophorus</i> spp.	35 (30.8)				
		<i>Metamicrocotyla</i> sp.	12.5 (0.9)				
		<i>Johnius carouna</i> (40)			30 (0.8)		
		<i>Johnius coitor</i> (40)			47.5 (1.4)		
		<i>Dendrophysa russelli</i> (40)			82.5 (8.0)		
Coral reef	<i>Lutjanus vitta</i> (36)	<i>Bucephaloides</i> sp.	22.5 (1.2)				
		<i>Gonopodasmuis</i> sp.	10 (0.7)				
		<i>Didymosystis reniformis</i>	30 (1.5)				
		<i>Philometra</i> sp.					10 (0.4)

N = number of each fish species. Percentages of prevalence of infection are listed: □ = 0, □ = 1 to 25, □ = 26 to 50, □ = 51 to 75, □ = 76 to 100

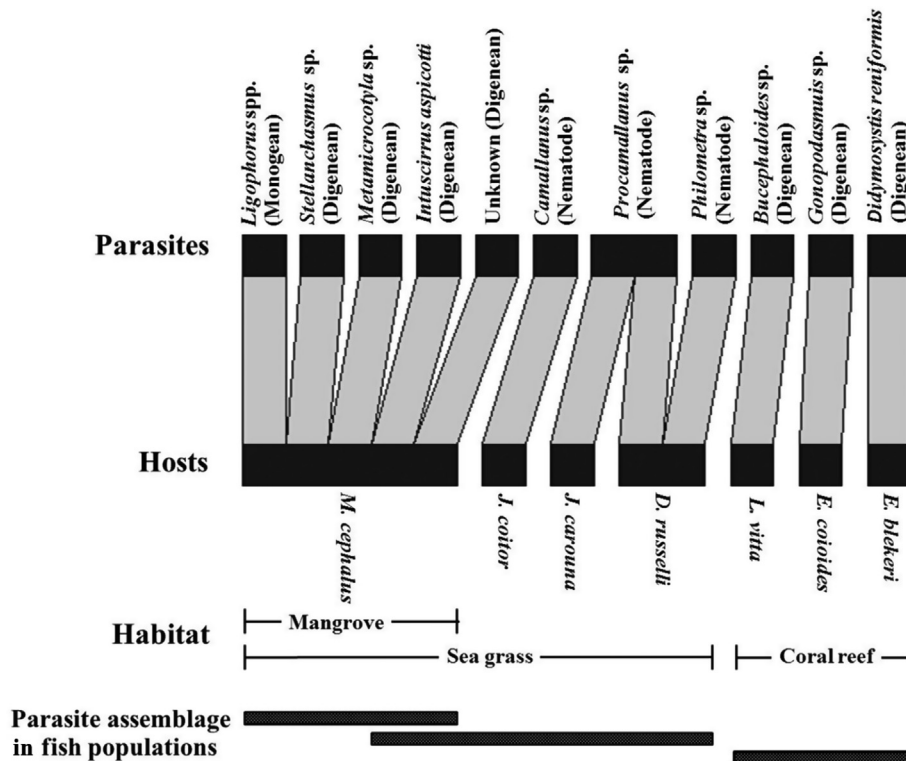


Fig. 2. Interactions between fish host and helminth species, and parasite assemblages of fish populations in each habitat. The shaded boxes show the interactions between fish host species and helminth species. Each box represents the habitat of the collected fish. Parasite assemblages in fish populations show the areas in which each species of helminth was found in each fish.

gonads of *Dendrophysa russelli*. *Procammallanus* sp. and *Camallanus* sp. were found in two fish species, *Johnius carouna* and *J. coitor*, respectively. Two species of ectoparasites, *Metamicrocotyla* sp. and *Ligophorus* spp., were found in *M. cephalus* gills. There was no overlap of fish species infected by parasites between the seagrass and coral reef areas. Only digenean parasites were found in the gills of *Lutjanus vitta*, *Epinephelus coioides* and *E. blekeri* (Fig. 2).

Discussion

This study was the first report of helminth diversity in marine fish from the coastal ecosystem of the Andaman Sea near Tarutao Island, Satun province, southern Thailand. The number of helminth species was negatively correlated with physicochemical factors recorded from the mangrove, seagrass and coral reef areas. Additionally, the physicochemical factors (water level, temperature, salinity, Ca^{2+} and Mg^{2+} concentrations) significantly differed among the mangrove, seagrass and coral reef areas.

This study also explored which fish species were infected by helminths, and revealed that helminths were specific to fish species, organs infected and the habitat of the fish host, except for *Ligophorus* spp. in *M. cephalus* (Table 3). *Ligophorus* spp. was found in both mangrove and seagrass areas. The percentage prevalence of infection and parasite assemblages in *M. cephalus* revealed that *Ligophorus* spp. was the predominant species in the mangrove area and also existed in the seagrass area but in low prevalence. A study in the waters of the Mediterranean and Atlantic coasts of Morocco revealed that *Ligophorus* spp. in *M. cephalus* was suggested to be a biological indicator (El Hafidi et al., 2013). The current study revealed that *Ligophorus* spp. is a potential biological indicator of environmental health for other living organisms in the coastal environment near Tarutao Island. The increase or decrease of parasite populations (as a

biological indicator) reflect that these parasites are facing environmental changes, which affect their life cycle and immunological defenses. Pollution may result in a decrease in the abundance and prevalence of parasites (Sasal et al., 2007).

In addition to *Ligophorus* spp. as a potential biological indicator found in *M. cephalus*, the metacercariae-infective stage of *Stellan-chasmus* sp. was also found in the fish in the mangrove area. *Stellan-chasmus* sp. causes food-borne trematodiasis (Johansen et al., 2010). The ecological environment of the mangrove area is suitable for completing the life cycle of *Stellan-chasmus* sp., because this parasite requires brackish water snails, such as *Stenomelania newcombi* or *Thiara granifera*, which act as the first intermediate host (Noda, 1959).

The fish that lived in the coral reef area showed the lowest number of helminth species and percentage prevalence of helminth infections. Helminths found in large fish that lived in the coral reef area were all ectoparasitic digenean trematodes such as *Bucephaloides* sp., *Gonopodasmius* sp., and *Didymosystis* sp. In Thailand, *G. epinepheli* has been investigated in the Gulf of Thailand and the Andaman Sea (Tudkaew et al., 2008). This parasite was found in *E. coioides* and preferentially infected medium-sized fish (200–700 g), and infected a relatively high percentage of *E. coioides* caught from the Andaman Sea (Tudkaew et al., 2008). Helminth diversity was the highest in the mangrove area with regard to numbers of groups (digenean and monogean) and species. However, helminths were only found in *M. cephalus*. In the seagrass area, most parasites collected belonged to one group (nematodes), which were found in *Johnius carouna* and *J. coitor*, which belong to a niche in the seagrass area. *Dendrophysa russelli* was found in the coral reef area but presented with no infection.

The physicochemical factors (water level, temperature, salinity, Ca^{2+} and Mg^{2+} concentrations) were reported to be contributing

factors that affect parasite diversity (Ohashi et al., 2007). Changes in those factors may affect the life cycles of helminths in coastal ecosystems (Frimeth, 1987; Moser, 1991; Sasal et al., 2007). In the current study, these factors significantly differed among the areas studied. However, the salinity of the seagrass and coral reef areas were in the range of euhaline seawater (30–35 parts per trillion; ppt). Salinity was noted as an important physical factor that increased the prevalence of the monogenean trematode, *Metamicrocotyla mecracantha* (Baker et al., 2008). However, the results of the current study differed. *Metamicrocotyla* sp. in *M. cephalus* caught in the mangrove area (salinity 29.1 ppt) represented a higher percentage of infections than those in the seagrass area (salinity 31 ppt). However, small sample sizes of fish may have caused underestimation or overestimation.

Although this study might have been limited based on the number of fish examined, the overlap of helminth species in the three areas in this coastal ecosystem of the Andaman Sea in Satun province depended on the fish host distribution, such as *M. cephalus* distribution. The fish species with specific ecological niches in the seagrass and coral reef areas had specific helminth species. Therefore, the physicochemical factors listed above may play an important role as contributing factors that affect helminth diversity in the Andaman Sea (Rohde, 1984, 1993; Bussarawit et al., 2008).

Species diversity of helminth parasites in commercial marine fish from different ecological areas of the coastal ecosystems of the Andaman Sea of Thailand were explored in this study. *Ligophorus* spp. was the predominant species in the mangrove area but was also present in the seagrass area. This species was previously suggested to be a potential biological indicator (El Hafidi et al., 2013). However, larger sample sizes of *M. cephalus*, enhanced fish examination and increased frequency of physicochemical and environmental data collection should be performed in future studies to confirm that *Ligophorus* is a suitable biological indicator for the coastal ecosystem near Tarutao Island.

Conflict of interest

None declared.

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