



Monogenean Parasite Infections in Asian Seabass, *Lates calcarifer* (Bloch, 1790) during Crop Production in Earthen Pond Culture at Surat Thani, Thailand

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Abstract

This study aimed to identify monogenean parasites in Asian seabass (*Lates calcarifer*) and to assess infection rates in the host fish in an earthen pond culture during crop production in Surat Thani province, Thailand. One hundred fish were examined for parasite infections from July to November, 2017. Two species of monogeneans, *Laticola paralatesi* and *Laticola seabassi*, were detected on gill filaments. The highest 75% prevalence was observed in September. The highest mean intensity of *L. paralatesi* was recorded in September (5.8 parasites per fish). *L. seabassi* was detected at the highest mean intensity in November (2.6 parasites per fish), which was significantly different among the five months ($p < 0.05$). Moreover, the fish size was not significantly correlated with *L. paralatesi*, but there was a significant positive correlation between the weight of fish and the number of *L. seabassi* ($r = 0.928$; $p < 0.05$). This study corroborates that *Laticola* spp. is commonly found in Asian seabass, with parasite transmission from fish to fish, and the larval stage from wild infected fish live in natural water sources and is spread to fish ponds. The present work reveals monthly profiles of infestations by monogenean parasites in earthen pond culture and the results can be applied in fish health management. This is the first report on monogenean parasite infections in Asian seabass, in earthen pond cultures in southern Thailand.

Introduction

Lates calcarifer is a catadromous fish that is found in freshwater, brackish water and marine environments, including streams, lakes, billabongs, estuaries and coastal waters (Khrukhayan et al., 2016). They are born in seawater, then living in freshwater, then brackish water and adults again migrate to the sea to breed. This

species is widely distributed throughout Southeast Asia including Thailand and in some other Pacific countries (northern Australia and Indo-West Pacific) (Mohamed-Jawad et al., 2012). The Asian seabass is a commercially important fish species grown in aquaculture. The culture systems are either pond or cage cultures. This species is tolerant of a wide range of salinities from freshwater to seawater, with the latter type of cultures used in fresh,

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brackish and marine water ecosystems (Thirunavukkarasu et al., 2009). These fish have been cultivated in brackish water as well as in freshwater ponds and marine cages in many Southeast Asian countries (Philipose et al., 2010). Also, earthen ponds are practiced on most farms because of the low production cost and the fast growth due to various kinds of natural food in the pond environment. Ponds are prepared traditionally and are also used for shrimp cultures (Jerry, 2013). In Thailand, Asian seabass cultures are found in eastern, central, and southern parts. In the southern part of Thailand, Songkhla and Nakhonsithammarat provinces are important to Asian seabass production. Asian seabass is an economically important fish species in Thailand and other countries of Asia and Pacific regions. Thailand has been the top producer of both fingerlings and marketable fish. The production has increased from 9,700 tons to 16,900 tons in 2014 (Department of Fisheries, 2015). The global production of Asian seabass had increased to 71,581 tons in 2014 (FAO, 2017; Joerakate et al., 2018). Moreover, Surat Thani province of Thailand also produces a substantial amount of Asian seabass along its coastal areas. Interestingly, Thai fishery organizations are promoting fish production in earthen ponds and have successfully initiated culture programs with the aid of a group of fish farmers at Klong Chanak sub-district, in Muang district, Surat Thani province.

The fish production is affected by diseases, especially parasites, viz., protozoans, monogeneans, digeneans, cestodes, nematodes, acanthocephalans and crustaceans (Yang et al., 2006; Ruckert et al., 2008; Petchsupa & Nilrat, 2008; Reed et al., 2009; Jerry, 2013; Mama et al., 2014). Parasite infections are an important consideration in animal health management, especially in aquaculture (Hutson et al., 2012). Many species of monogenean parasites in Asian seabass have been reported from Southeast Asia and other Pacific countries. The four species *Laticola lingaoensis*, *L. latesi*, *L. paralatesi* and *Diplectanum penangi* are reportedly found in the gills of fish from the South China Sea (Yang et al., 2006). Also, parasites in Asian seabass have been reported in Indonesia, in the context of severe parasite and disease outbreaks, when the fish were examined from Lampung Bay, South Sumatra. These fish were infected with monogeneans, such as *Pseudorhabdosynochus epinepheli*, *P. lantauensis*, *Benedenia epinepheli* and *Neobenedenia melleni* (Ruckert et al., 2008). Additionally, the ectoparasitic monogenean *Neobenedenia* sp. attaches to the body

surface of its host using attachment organs. Histopathological changes in the fish associated with *Neobenedenia* sp. include epidermal damage. Cutaneous mucus secreted by mucous cells is an important component of teleost immune responses. *Neobenedenia* sp. infected fish had changes in epidermal thickness and decreased numbers of mucous cells (Trujillo-Gonzalez et al., 2015).

Parasite infections in Asian seabass have been reported in eastern and southern Thailand. In Pattani bay, fish rearing cages are affecting both wild and cultured fish species. Two species of monogenean parasites were identified, i.e. *Diplectanum latesi* and *D. papaverensis*. Histopathological changes in the fish included hemorrhage, edema, hyperplasia, inflammation, degeneration and necrosis (Petchsupa & Nilrat, 2008). In cage cultures in Nathap canal (Songkhla) and Saiburi canal (Pattani) 7 species of parasites were identified, namely *Trichodina japonica*, *Laticola latesi*, *L. lingaoensis*, *L. paralatesi*, *Lernanthropus latis*, *Caligus epidermicus* and *C. rotundigenitalis*. Histopathological changes in gill, liver, kidney and spleen included degeneration, edema, hyperplasia, necrosis and melanomacrophagy (Mama, 2015). Moreover, three species of monogeneans, *Laticola lingaoensis*, *L. paralatesi* and *Diplectanum penangi*, were isolated from cage cultures in Bangpakong River, Chachoengsao province (Khrukhayan et al., 2016). Parasite infections can affect animal health in aquaculture and require management responses. Therefore, parasites in fish have been considered an indicator of environmental health and important for the development of aquaculture (De Carvalho-Souza et al., 2009). Regarding pond cultures, knowledge of parasites affecting Asian seabass is poor. The present study aimed to identify parasitic monogeneans during crop production and to assess the infection rates in an earthen pond culture at Klong Chanak sub-district, Muang district, in Surat Thani province of Thailand.

Materials and methods

The study was conducted in the earthen pond culture during crop production from July to November 2017 at Klong Chanak sub-district, Muang district, Surat Thani province, in southern peninsular Thailand (9°10'56''N, 99°20'16''E) (Fig. 1). The fish pond is rectangular in shape with 6,400 square meter size and 150-200 centimeter water depth. A total of 15,000 fish

were raised in the earthen pond with a stocking density of 2.34 fish per square meter. Pond bottom is flat and slopes towards the drainage gate. The Asian seabass fry with size grading at proximately (n=20) 9.22 ± 0.61 centimeter (8.0-10.0) and 9.14 ± 1.56 gram (6.84-11.57) were obtained from a hatchery in Chachoengsao province, central Thailand. The worm-free fish were raised in an earthen pond and fed with chopped trash fish. After one month, the fish were sampled randomly by using a dip net and hook. Twenty specimens were examined once a month for 5 months. The live fish were transported to the laboratory in the Prince of Songkla University, Surat Thani campus, for examination of parasite infections. The fish were killed with 200 ppm clove oil (modified from Mladenio et. al, 2013). The total length and weight of fish were measured.

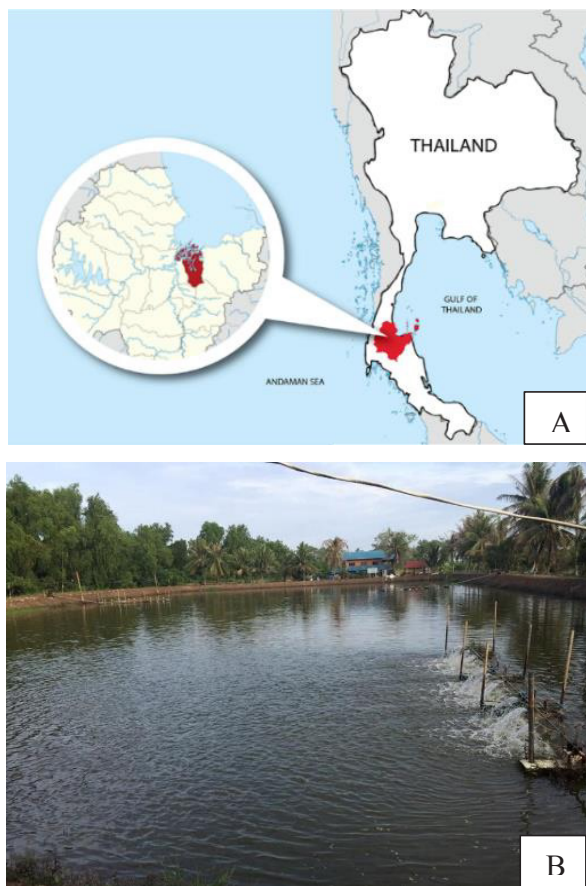


Fig. 1 Location of study site (A) Map of Klong Chanak sub-district, Muang district, Surat Thani province, southern Thailand; and (B) the earthen pond culture of Asian seabass

1. Water quality in fish pond

This study monitored the key water quality parameters, namely temperature, salinity, and pH. Water samples were measured for temperature using a thermometer; Hydrogen ion concentration using a pH meter with platinum electrode, calibrated with standard buffer solution prior to use; and salinity using a hand refractometer. The temperature varied in the range 30.6-31°C. The pH varied in the range 6.86-7.43, during the observed five months (July-November 2017). The salinity varied within 2-6 ppt, and the lowest and highest values in the pond culture were observed in September and July 2017 (Table 1).

Table 1 Water quality parameters in earthen fish pond during crop production

Month (2017)	Water quality parameters		
	Temperature (°C)	Salinity (ppt)	pH
July	31±0	6±0	6.86±0.03
August	0.6±0.57	3±0	7.94±0.1
September	31±0	2±0	7.55±0.22
October	31 ±0	4±0	7.24±0.25
November	31 ±0	4±0	7.43±0.29

2. Parasite examination

Monogeneans were observed in Asian seabass reared in an earthen pond culture at Klong Chanak sub-district, Muang district, Surat Thani province. Gills were removed and each gill arch was cut out and gill filaments were separated; dorsal fin, pectoral fin, pelvic fin, anal fin and caudal fin were separated; scales were removed from both sides; and each part of the body was placed in a Petri dish containing 0.85% saline solution. The body surface of fish was scraped from anterior to posterior part using a cover slip. By examining under a stereomicroscope and a compound microscope, the counts of parasites were recorded (modified from Ruckert et al., 2008; Sonthi et al., 2016). In addition, internal organs including the stomach, intestine, liver, and gall bladder were observed from fish samples. No endoparasites were infecting these fish. Monogenean specimens were removed from the gill filaments using fine forceps and needles or pipettes under a dissecting microscope. Specimens were transferred to glass slides, covered with cover slips, fixed in ammonium picrate glycerine and sealed with nail varnish (modified from Moreira et al., 2015). To prepare permanent slides, some specimens were fixed in 4% formalin under a cover slip, then stained with Semichon's acetic-carmin. The stained slides were dehydrated through a graded ethanol series,

cleared in xylene, and mounted in permount (modified from Ruckert et al., 2008). Fresh specimens and permanent slides were observed under a compound microscope. Line drawings were done on fresh and stained materials with a drawing tube (UDA60) attached to a compound microscope. Monogenean parasites were identified based on genus *Laticola* spp. in Asian seabass (Yang et al., 2006; Chotnipat et al., 2015). Measurements are reported in micrometers with the means followed by ranges in parenthesis.

3. Data analysis

The prevalence and mean intensity were compared between the observed 5 months, following Bush et al. (1997). Prevalence was calculated as the number of infected hosts divided by the total number of examined hosts (expressed as a percentage):

$$\text{Prevalence} = \frac{\text{No. of infected hosts} \times 100}{\text{No. of examined hosts}}$$

Mean Intensity is the total number count of a particular parasite divided by the number of infected hosts:

$$\text{Mean Intensity} = \frac{\text{Total no. of a particular parasite}}{\text{No. of infected hosts}}$$

The data were subjected to correlation analysis conducted using statistical analysis programs. The mean intensity of each parasite in each month were compared using analysis of variance (ANOVA) then, by LSD post hoc test for multiple comparisons. Pearson's correlation was assessed between weight, length of fish and number of parasite infections. Statistical analysis used the significance threshold level of 0.05 ($p < 0.05$).

Results and discussion

A total of one-hundred live specimens were collected during crop production (July to November 2017) from the earthen pond culture. Fish total length ranged from 14.74 to 41.01 centimeters (28.9 ± 3.07) and weight was from 44.66 to 1,013.81 grams (464.74 ± 133.17) (Table 2). Thirty-nine of the collected fish were infected with 2 species of monogeneans on the gill filament (Fig. 2), *Laticola paralatesi* and *L. seabassi*, seen in the illustrations in Fig. 3 and 4. The study describes the total

length-weight relationship with the two species monogeneans in the earthen pond culture. A comparison between fish size and monogenean infections were analyzed. A positive correlation was not statistically significant between the total length ($r = 0.626$; $p > 0.05$), weight ($r = 0.518$; $p > 0.05$) of fish and the number of *L. paralatesi*. In addition, fish infections with *L. seabassi* were not significantly correlated with total length ($r = 0.857$; $p > 0.05$). A significant positive existed between the weight of fish and the number of *L. seabassi* ($r = 0.928$; $p < 0.05$).

Table 2 Measurements of Asian seabass from earthen pond in Surat Thani province: mean length and mean weight (range in parentheses)

Month (2017)	Fish size (Mean±SD)	
	Length (cm)	Weight (g)
July	14.74±1.75 (11-17.5)	44.66 ± 15.56 (17.39-72.69)
August	24.02±2.35 (18-28)	208.55 ± 55.68 (81.53-316.93)
September	29.32±3.65 (21.5-33.7)	399.59 ± 144.81 (120.78-521.8)
October	35.4 ± 3.38 (27.5-41.5)	657.06 ± 180.12 (287.72-939.56)
November	41.01 ± 4.24 (30-47)	1013.81 ± 269.67 (402.32-1458.08)

1. Description of *Laticola* spp.

Laticola paralatesi (n=20) (synonyms *Diplectanum paralatesi* Nagibina, 1976; *Pseudorhabdosynochus yangjiangensis* Wu & Li, 2005). Body 466.5 µm (300-600) long; greatest width 151.5 µm (140-170). *Laticola seabassi* (n=10) (synonyms *Pseudorhabdosynochus seabassi* Wu et al., 2005; *L. lingaoensis* Yang et al., 2006). Body 671 µm (500-880) long, with short peduncle; greatest width 179 µm (120-270) at level of gonads. Body surface was covered with tegumental scales which extend from peduncle to level of copulatory organ. Prohaptor moderately developed; consists of cephalic lobes; each head organ comprises 2-3 groupings of terminations of cephalic-gland ducts. Two pairs of eyespots, the anterior pair is smaller than the posterior pair. Pharynx oval shaped. Intestinal caeca terminate posterior to gonads near peduncle. Testis crescent shaped. Copulatory organ spoon shaped, distal tubular portion elongate. Oval shaped ovary. *L. paralatesi* presents a vase shaped sclerotized vaginal valve, with proximal duct extending from the vaginal valve to a seminal receptacle. *L. seabassi* presents vaginal valve shaped as blossoming flower of thistle with 2 central distally directed lobes. Vitellarium dense. Vitellarium extends posteriorly from the level of posterior pair of eye-spots to terminations of intestinal caeca. Opisthaptor, armed with dorsal and ventral pairs of anchors. Ventral squamodisc horseshoe shaped with

rows of rodlets; rodlets delicate, dumbbell shaped. Haptor consists of 14 marginal hooklets (Table 3, Fig. 3-4).

Table 3 Characteristics measured for two monogenean parasites (*Laticola* spp.)

Characteristic	Measurement (μm)	
	<i>L. paralatesi</i> (n=20)	<i>L. seabassi</i> (n=10)
Body length	466.5 (300-600)	671 (500-800)
Body width	151.5 (140-170)	179 (120-270)
Pharynx length	38.5 (25-50)	44.25 (35-62.5)
Pharynx width	29.12 (22.5-40.0)	41.75 (27.5-50)
Testis length	29.25 (25-32.5)	39.25 (25-50)
Testis width	36 (32.5-37.5)	45.75 (32.5-62.5)
Ovary length	82.5 (62.5-112.5)	79.75 (50-125)
Ovary width	37.25 (25-45)	42.75 (30-62.5)
Copulatory organ length	82.12 (75-85)	89.5 (75-112.5)
Haptor length	87.75 (75-105)	107.5 (80-137.5)
Haptor width	176.12 (155-200)	175.25 (137.5-212.5)
Number of rodlet rows	11-13	11-13
Ventral squamodisc length	76 (52.5-100)	84 (70-125)
Ventral squamodisc width	103.5 (80-125)	99 (87.5-112.5)
Dorsal anchor length	41.62 (37.5-50)	31.5 (22.5-45)
Ventral anchor length	47.75 (40-52.5)	35.25 (25-42.5)
Dorsal bar length	50.62 (50-52.5)	45.5 (32.5-55)
Ventral bar length	105 (95-125)	115 (100-137.5)
Number of marginal hooklets	14	14
Marginal hooklet length	10 (10-10)	10 (10-10)

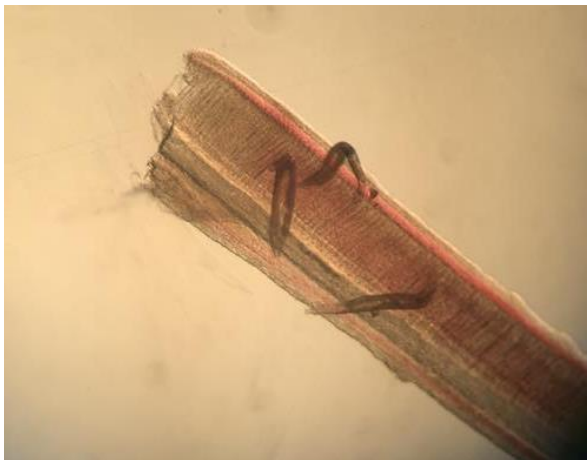


Fig. 2 *Laticola* spp. infecting gill filament of Asian seabass

The prevalence of parasite infections from July to November 2017 is shown in Fig. 5. The highest prevalence of *L. paralatesi* was observed in September with 75% (15/20) infection rate. *L. seabassi* was found in November with 40% (8/20) infection rate. The mean intensity of *L. paralatesi* was higher than of *L. seabassi* species, as seen in Table 3. The highest mean intensity of *L. paralatesi* were recorded in September with

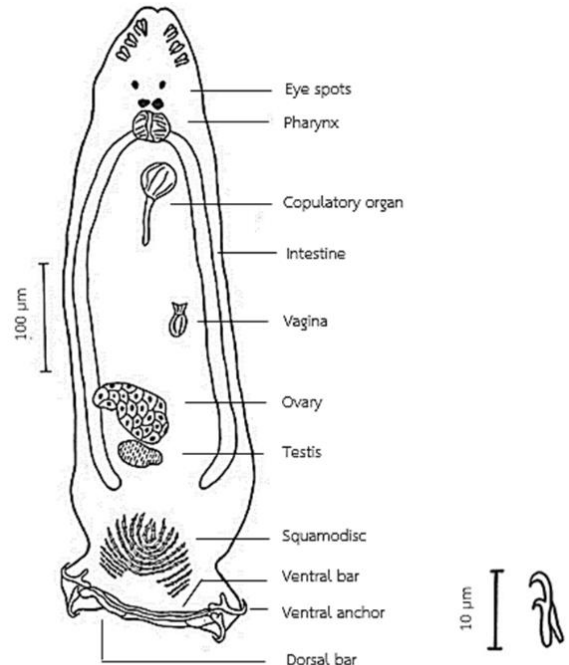


Fig. 3 Line drawing of *Laticola paralatesi* (ventral, composite) and marginal hooklet

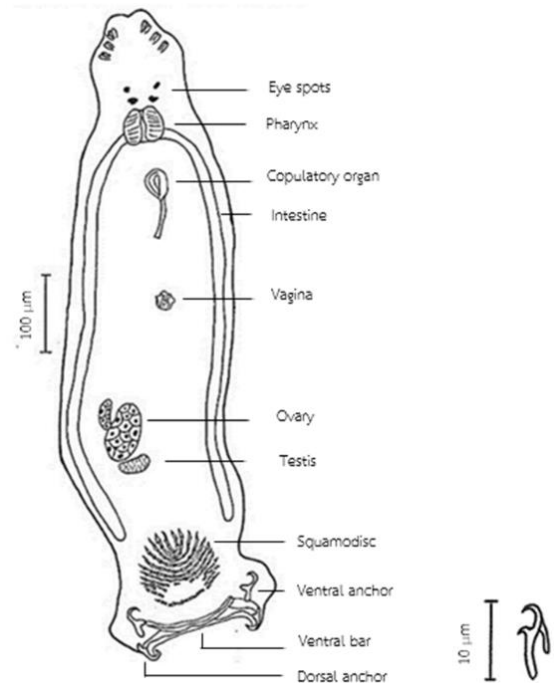


Fig. 4 Line drawing of *Laticola seabassi* (ventral, composite) and marginal hooklet

respective infection rates of 5.8 (87/15) parasites per fish, which was statistically significantly different from others ($p < 0.05$). Meanwhile, *L. seabassi* had a high level in November, when the infection rate was 2.6 (21/8) parasites per fish (Table 3).

Table 3 Mean intensities of parasite infections by *L. paralatesi* and *L. seabassi*

Month (2017)	Infected fish/Examined fish	Mean intensity (\pm SD)	
		<i>L. paralatesi</i>	<i>L. seabassi</i>
July	0/20	0 ^a	0 ^a
August	0/20	0 ^a	0 ^a
September	15/20	5.8 \pm 3.16 (3-10) ^b	0 ^a
October	12/20	4.45 \pm 2.94 (1-8) ^b	2 \pm 0.69 (1-3) ^b
November	12/20	2.9 \pm 1.69 (1-5) ^{bc}	2.6 \pm 1.57 (1-5) ^b

Remark: a,b,c Different superscripts indicate significant differences according to LSD post hoc test for multiple comparisons with $\alpha = 0.05$

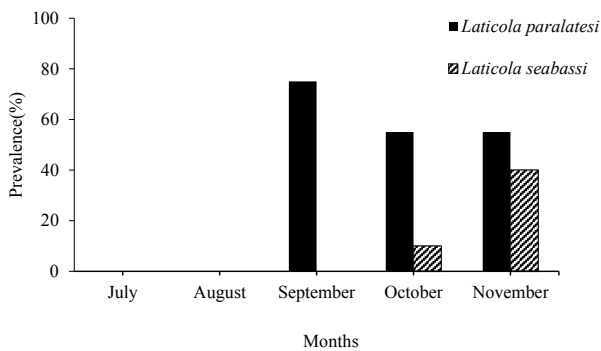


Fig. 5 Prevalence of monogenean infections in Asian seabass during crop production

Two monogenean species, *Laticola paralatesi* and *L. seabassi* were found in the gill filaments of infected fish. These two species belong to the genus *Laticola* and are members of the family Diplectanidae. The results showed that the prevalence and mean intensity of parasites per fish were highest in September 2017. In Thailand, two genera of monogenean parasites, *Laticola* spp. and *Diplectanum* spp., have been reported in cage cultures of Asian seabass. Parasite infestations in southern and eastern Thailand were found by *Diplectanum* sp., *D. papaverensis*, *D. latesi*, *D. penangi*, *L. latesi*, *L. lingaensis* and *L. paralatesi* (Petchsupa & Nilrat, 2008; Mama, 2015; Sonthi et al., 2016; Khrukhayan et al., 2016). Similarly, Yang et al. (2006) reported 4 species of monogeneans in Asian seabass viz. *L. lingaensis*, *L. latesi*, *L. paralatesi* and *D. penangi* infecting fish gills in the South China Sea. Also, four species of parasites were found, namely *Pseudorhabdosynochus epinepheli*, *P. lantauensis*, *Benedenia epinepheli* and *Neobenedenia*

melleni, in the gills and on body surfaces of fish in Indonesia (Ruckert et al., 2008). This study was performed on fish samples from an earthen pond culture. Two species of diplectanid monogeneans were examined. However, monogeneans are mostly ectoparasites with a direct life cycle (Saraiva et al., 2015). This study indicated that *Laticola* spp. infected wild fish in this area, and the free-living larval stage (oncomiracidium) can contaminate water and enter a fish pond. This parasite managed to maintain transmission successfully in the culture system. Interestingly, free-swimming oncomiracidium of diplectanid monogenean can swim continuously for 4-8 hr to find definitive hosts under laboratory conditions (Erazo-Pagador & Cruz-Lacierda, 2010). This suggests that diplectanids enter farming systems through intake of natural water resources containing eggs and oncomiracidia or infected wild broodstock (Whittington et al., 1999). These parasites can cause gill damage. The gills are among the most delicate structures of a fish, and exposure outside the body makes them liable to damage by any irritant materials (Saraiva et al., 2015). It is well-known that these parasites are considered a threat to fish cultures and cause health status reduction and mortality in many species of farmed fish (Saraiva et al., 2015). Diplectanid parasite infections may cause damage to respiratory organs and histopathological changes in the gills, inducing darkened body, lethargy, loss of appetite, excess mucus production, lamellar fusion, hemorrhage, inflammation, degeneration, necrosis, hyperplasia, edema, swollenness and paleness (Petchsupa & Nilrat, 2008; Reed et al., 2009; Jerry, 2013; Mama et al., 2014; Mama, 2015). A comparison between fish size and number of parasite infections indicated that the larger fish tend to be more infected by monogeneans than the smaller fish. No parasites infected fish during the two first months of their culture, but parasites were observed in fish in the earthen pond after two months. While worm-free fingerlings were received from the hatchery, the parasite was initially observed in fish two months post-transfer into the earthen pond. This suggests that disease in farmed fish emerge from interactions between host, environment and pathogens. Generally, diseases are often caused by widespread pathogens that are commonly found in the culture environment (Gibson-Kuch, 2012). Some organisms, including eggs or larval stage monogenean parasites, contaminated the water source of the fish pond. On the other hand, the salinity was lowest in September 2017 matching the highest infection rate.

The effect of salinity on ectoparasite distribution of catadromous fish is such that the fish lose their ectoparasites after they migrate to marine water (Esch & Fernandez, 2013). However, parasites tend to increase in numbers in the larger fish, according to Ravi & Yahaya, (2016). *Neobenedenia melleni* monogenean species were analyzed for associations between fish length and parasitic intensity in infected fish, at Jerejak Island, Penang, Malaysia. A positive relationship between fish length and parasite diversity was found, because the larger fish show a larger infection surface area for parasitic colonization. Monogenean parasites affecting gills and body surface of fish can cause gill fluke disease and skin fluke disease. In contrast, there was no relationship between parasite infection and size of fish in another prior study (Olurin & Somorin, 2006; Amaechi, 2015). The problem of parasites infecting fish could be best handled through proper management procedures that eliminate the conditions favoring parasite infestation (Amaechi, 2015). The treatment of monogenean parasites has been investigated by Hutson et al. (2012), who reported that aqueous extracts from tropical seaweeds were evaluated for effects on the life cycle of an ectoparasite, *Neobenedenia* sp., infecting farmed *L. calcarifer*. The extracts of two seaweeds, *Ulva* sp. and *Asparagopsis taxiformis*, delayed embryonic development and inhibited egg hatching. The established treatment against monogeneans involves recurrent acute bathing of infected stock primarily in either formalin or in freshwater solutions (Jerry, 2013). Therefore, the rates of parasite infections are important to fish health management. Pond culture is more convenient for the control of parasite infections than a cage culture. However, parasite infections are important factors affecting fish health. Management begins with the prevention of disease rather than treatment. Prevention of fish disease is accomplished through water quality management, nutrition and sanitation. Hence, aquaculture system should consider using re-circulated water and fine filtration to reduce parasite transmission (Whittington et al., 1999). However, *Laticola* spp. are commonly found in Asian seabass in both pond culture and cage culture. Therefore, good management practices are required to promote the health status and fish production of earthen pond cultures in Thailand.

Conclusion

In this study, diplectanid monogenean parasites

infecting Asian seabass in an earthen pond culture in Klong Chanak sub-district, Muang district, Surat Thani province, were assessed from July to November, 2017. Two monogenean species, *Laticola paralatesi* and *L. seabassi* were found from the gill filaments. The overall prevalence of parasites was dominant in September among the five months observed. The mean intensity of *L. paralatesi* was highest in September, while *L. seabassi* was highest in November. While there were no parasite infections in fingerlings from the hatchery, infected fish were found after two months in an earthen pond. This information supports developing methodologies to produce an integrated health management system of Asian seabass in earthen pond cultures.

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