Seasonal Variation of Diplectanid Monogeneans in Cage Cultured Seabass from Bangpakong River, Thailand

Patcharee Khrukhayan*, Chalor Limsuwan and Niti Chuchird

ABSTRACT

In this study, monogenean gill parasites in cage cultured seabass (*Lates calcarifer*) in three farms were investigated between June 2010 to June 2011. The farms (A, B, C) represent the three salinity zones in seabass cage culture in Thailand. Three species of diplectanid monogenean, *Laticola lingaoensis*, *L. paralatesi* and *Diplectanum penangi*, were isolated from 691 seabass. The prevalence values of parasites from the three farms were more than 90%. *L. lingaoensis* was the most dominant parasite followed by *L. paralatesi* and *D. penangi*, respectively. The mean intensity of monogenean parasite species was highest in Farm B which was statistically different ($p<0.05$) followed by Farm A then Farm C. The prevalence of gill parasites was high in all farms in both dry and rainy seasons. In addition, the mean intensity of gill parasites in Farms A and B showed a high rate of infection during the dry season.

Keywords: diplectanid monogeneans, gill parasites, seabass, *Lates calcarifer*, seasonal variation

INTRODUCTION

Seabass (*Lates calcarifer* Bloch) inhabits a wide variety of freshwater, brackish water and marine habitats including streams, lakes, billabongs, estuaries and coastal water (Grey 1987). This species is an economically important fish in Thailand, especially with cage culture systems. Most of the cages are located in coastal areas in the Gulf of Thailand and the Andaman Sea.

The Bangpakong River is an important river of eastern Thailand. It is located in Prachin Buri and Chachoengsao Province. The name of the river is different in each province. It is named the Prachin Buri River where it flows through Prachin Buri Province and the Bangpakong River flows through Chachoengsao province. This river flows into the Gulf of Thailand at Tambon Tha kham, Amphor Bangpakong, Chachoengsao province. This area is the largest seabass farming area in Thailand.

The cage culture of seabass has been faced with pollution in the water and the severity of disease has been increasing. Recently, the number of fish farmers and culture cages in Bangpakong River has

Aquaculture Business Research Centre, Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Chatuchak, Bangkok 10900, Thailand
*Corresponding author, e-mail: ffisprk@ku.ac.th

expanded rapidly. Moreover, the farmers have been encouraged to intensify their farming systems. The impact on captive fish in more crowded conditions had problems because the captive fish are confined within a limited space thereby allowing hatching monogeneans to easily find their host fish. In addition, stressors found in the captive environment make the stocked fish more susceptible to disease. Mortality rates increased while productivity decreased. The aim of this investigation was to determine the seasonal variation, composition, prevalence and mean intensity of monogeneans infesting fish commonly found in Bangpakong River, Chachoengsao Province, Thailand.

MATERIALS AND METHODS

This study was conducted along the Bangpakong River, Chachoengsao Province which is situated in eastern Thailand (Fig.1) from June 2010 to June 2011 in three consecutive seasons (rainy season 2010: June-October 2010; dry season 2010/2011: November 2010-April 2011, and rainy season 2011: May-June 2011). The river has brackish water and flows into the Gulf of Thailand. The study was designed to collect specimens bi-weekly from 3 different farms (A, B, C) in seabass cage farming zones along the Bangpakong River. Farm A is located in Thakham subdistrict, Bangpakong district, about 10 km from the mouth of the Gulf of Thailand. This farm was affected by salinity fluctuations, and represented the mouth of the river of the Gulf of Thailand and high intensity farming. This area has the highest number of cages. Farm B is located in Thasa-an subdistrict, Bangpakong district, about 25 km away from the sea. This farm represented the middle zone of farming area. Farm C is located in Klongna subdistrict, Muang district, about 55 km away from the sea, with few cages. This farm represented the last zone of farming areas.

Fish and parasites sampling

A total of 691 seabass specimens were randomly sampled from 3 farms in Bangpakong River. These fish were collected from each farm every 2 weeks from June 2010 to June 2011. After fish were caught from the cages, they were measured for total length and body weight (Table 1). Consequently, they were transported dead in a

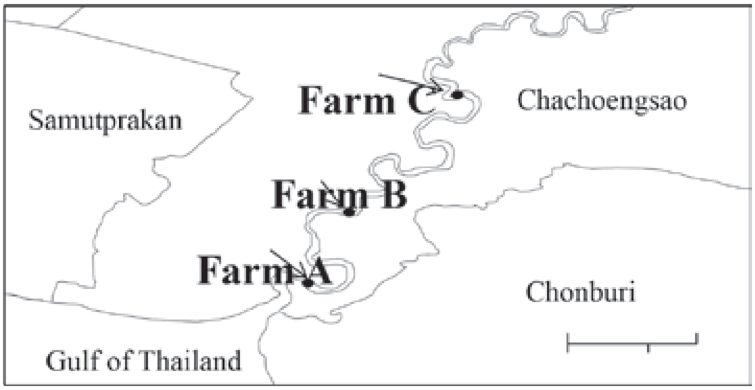

Figure 1. Location of studied fish farms along the Bangpakong River, Chachoengsao Province.

Table 1. Characteristics of *Lates calcarifer* sampled from cage farms along Bangpakong River, Thailand.

Farm	n	TL (cm)	TW (g)
A	249	16.3 (4.1-42.0)	96 (3-915)
B	200	20.8 (7.9-36.0)	153 (5-930)
C	242	13.3 (6.2-37.5)	40 (3-590)

n, number; TL, total length, TW, total weight.

cool box to Aquaculture Business Research Center (ABRC) laboratory, Kasetsart University, Bangkok, Thailand. Gill arches of each fish were separated and scraped into a petri dish filled with clean water to remove the monogeneans. An examination under a stereo microscope was then performed. Detected parasites were fixed in 70% ethanol and stained in Heidenhain's iron hematoxyline, dehydrated in ethanol series, cleared in xylene and mounted in permount solution.

Measurement of water quality

Water quality parameters were measured every other week with 3 water samples (replicates) collected from each farm. *In situ*, temperature, dissolved oxygen (DO) and salinity were measured with YSI meter model 85, pH with pH meter, and transparency by Secchi disc. Water samples for total ammonia-nitrogen were collected from 30 cm depth in a volume of 500 ml and preserved at 4ºC for analysis by the phenate method at ABRC laboratory.

Data analysis

Parasites were magnified 40x - 100x by using a compound microscope and identified at different levels, as described by Yamaguti (1963), Liang and Leong (1991) and Yang *et al*. (2006). The prevalence mean intensity and mean abundance of parasites were determined according to Bush *et al*. (1997). The water quality parameters, mean intensity and mean abundance of each parasite in each farm were compared using ANOVA, then by Turkey's post hoc for multiple comparisons. The statistical software SPSS was used to analyze the data, with a level of significance of $p<0.05$. Seasonal population dynamics of parasites were calculated from total parasites.

RESULTS

Parasitological study

Prevalence and mean intensity of parasites

Parasitological analysis of 691 specimens of *L. calcarifer* cultured in cages from three different farms in Bangpakong River were found to have two genera and 3 species of diplectanid monogenean from the gills of fish. The diplectanid monogenean species were identified as *Laticola lingaoensis*, *L. paralatesi* and *Diplectanum penangi* (Figures 2 to 4).

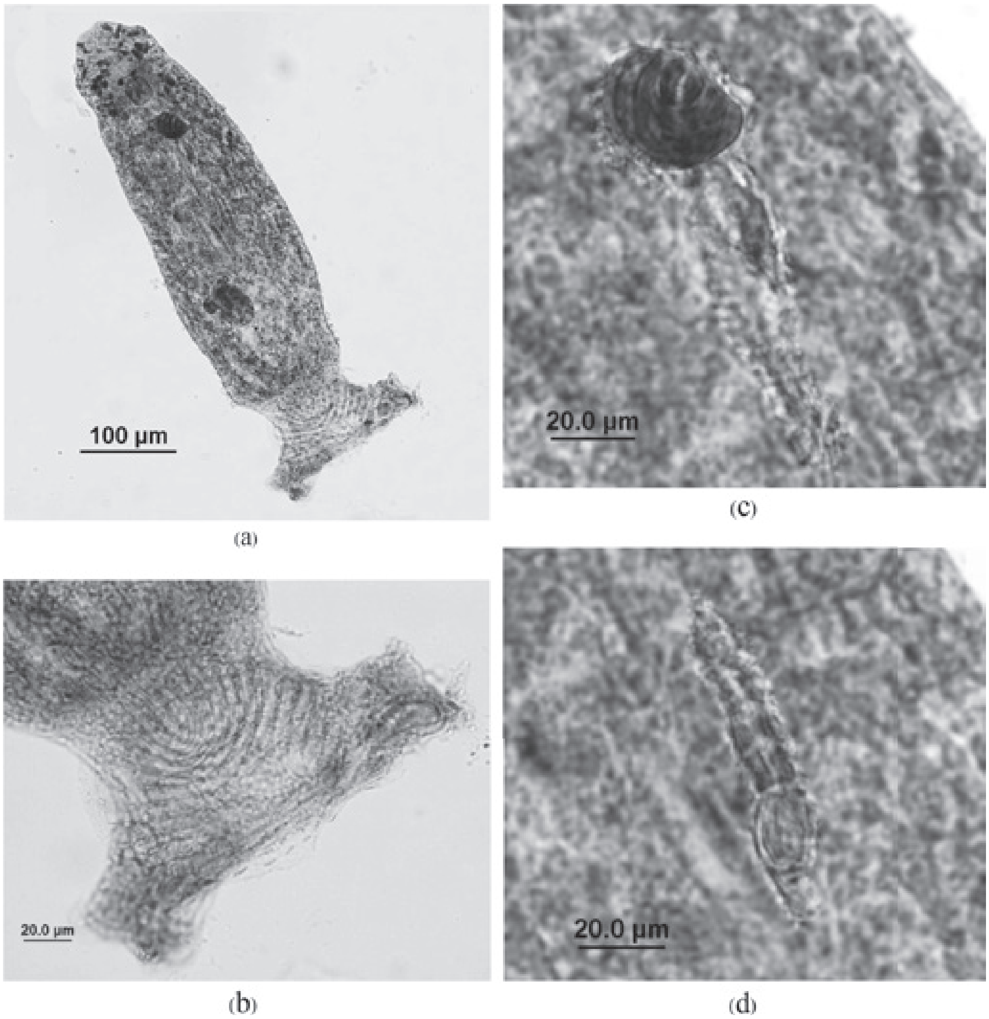

Figure 2. *Laticola lingaoensis*. (a) Entire worm, ventral view. (b) Haptor. (c) Male copulatory organ. (d) Vagina.

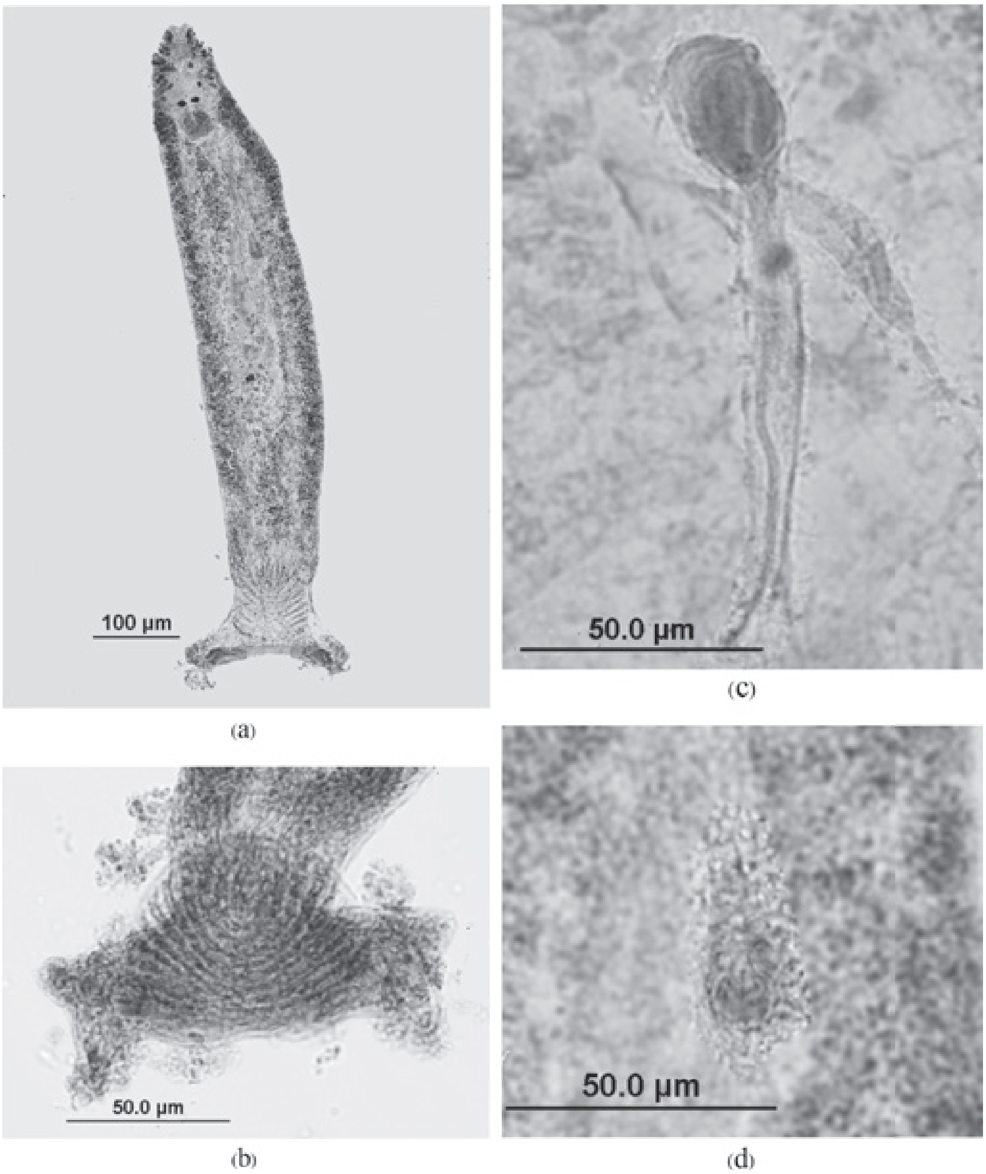

Figure 3. *Laticola paralatesi*. (a) Entire worm, ventral view. (b) Haptor. (c) Male copulatory organ. (d) Vagina.

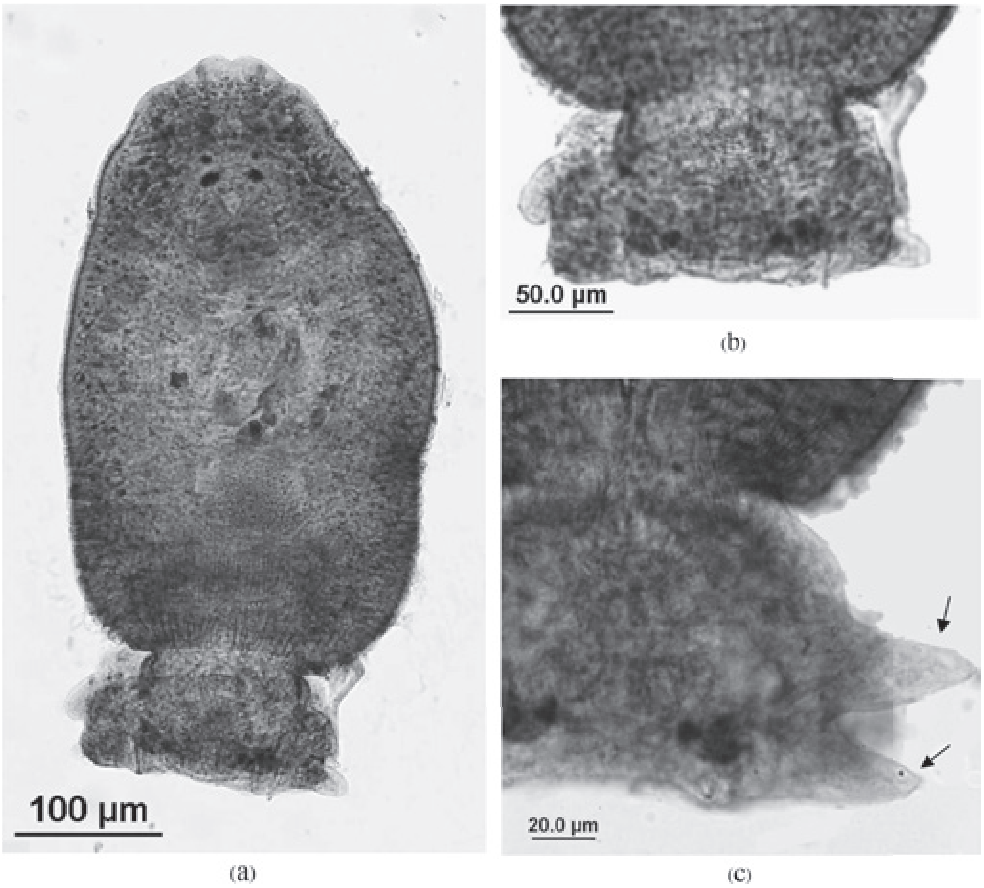

Figure 4. *Diplectanum penangi*. (a) Entire worm, ventral view. (b) Haptor. (c) Haptoral finger-like projections

The prevalence values of all parasites from the three farms were more than 90%, i.e. 94.78, 99.00 and 92.15% in Farms A, B and C, respectively. The mean intensity values were 185.3±28.3, 353.4±48.1 and 35.9±5.7 in Farms A, B and C, respectively. The mean intensity of monogenean parasite species was highest in Farm B which was statistically different (p<0.05).

The highest number of parasites was recorded for *L. lingaoensis*, which was present in all farms. Farm B had the highest value (99.00% of *L. lingaoensis*) while Farm C had the lowest value (24.79%. of *D. penangi*). The highest mean intensity value was found in Farm B for *D. penangi* (310.4±90.2) while, the lowest value was found in the same species from Farm C (10.9±1.8). The results of mean intensity were highest in Farm B in all monogenean parasite species when compared with Farm A and Farm C. The prevalence and mean intensity of gill monogenean parasites in seabass cultured in cages from Bangpakong River from the three farms are given in Table 2.

Table 2. Total number of prevalence (*P%*) and mean intensity (MI±SE) of monogenean gill parasites in cage cultured seabass from three farms.

Farm	*Laticola lingaoensis*		*L. paralatesi*		*Diplectanum penangi*		overall monogeneans	
	P (%)	MI±SE	P (%)	MI±SE	P (%)	MI±SE	P (%)	MI±SE
A	94.78	159.7±22.4^{b}	60.64	19.8±3.6^{ab}	25.30	48.5±32.1^{a}	94.78	185.3±28.3^{b}
B	99.00	246.8±35.3^{c}	66.50	20.9±2.6^{b}	29.50	310.4±90.2^{b}	99.00	353.4±48.1^{c}
C	86.36	28.8±6.0^{a}	50.41	10.9±1.3^{a}	24.79	10.9±1.8^{a}	92.15	35.9±5.7^{a}

Different superscripts in a column represent statistically significant differences at $p<0.05$

Seasonal comparison

Concerning seasonal dynamics of overall parasites, Figure 5 shows that the seasonal prevalence values from all three farms exhibited high values of more than 90% almost all year round. However, it decreased to 71.43% in Farm A in July 2010, and in Farm C in July and August 2010 when it decreased to 78.57 and 78.95%, respectively, and in April 2011 (decreased to 60%).

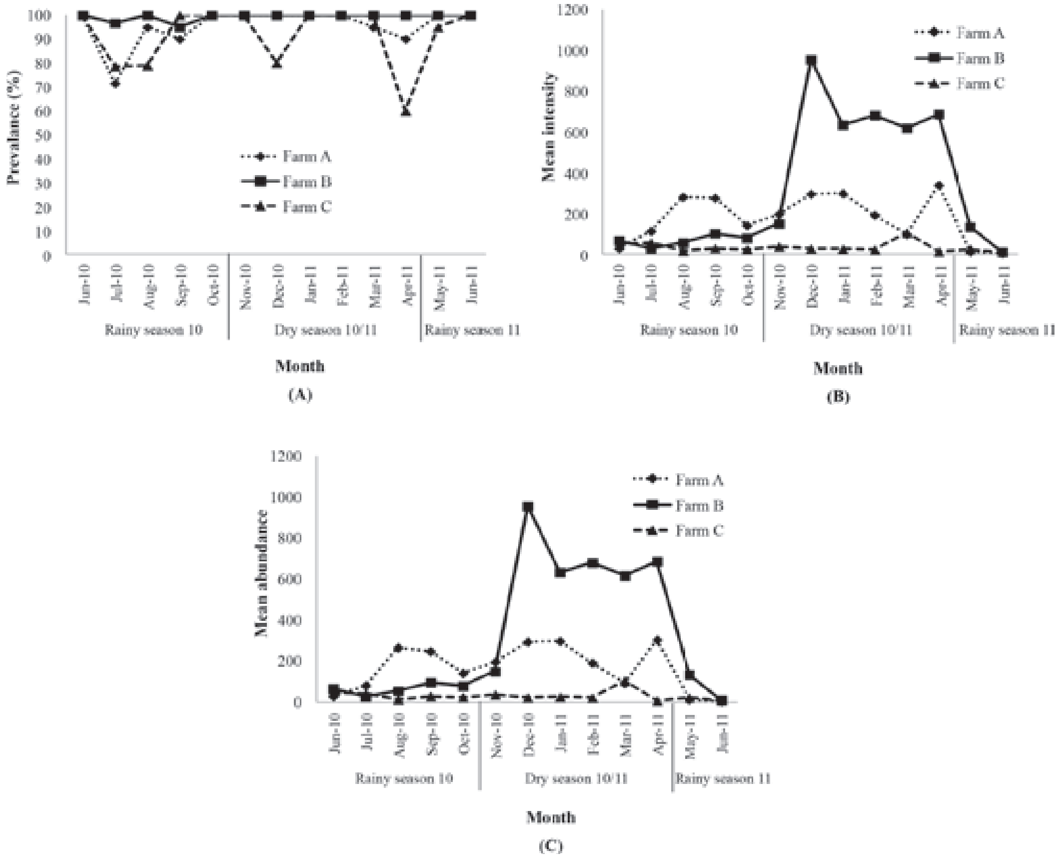

Figure 5. Seasonal change in prevalence (A), mean intensity (B), and, mean abundance (C) of overall diplectanid monogeneans in cage cultured seabass.

In Farm A, the mean intensity and mean abundance were low in June and July 2010. After that, from August 2010 to February 2011, high levels of infection were observed, but then dropped to lower levels in March 2011. After which, it rose again to a peak level in April 2011 and dropped to low levels again in the next 2 months. On the other hand, Farm B had low levels from June to October 2010 and increased to moderate in November 2010. During December 2010 to April 2011, it showed peak levels with the highest value found in December 2010, with May being moderate and June 2011 dropping to a low level. This cycle showed low, moderate and peak values. Meanwhile, Farm C had low levels of infection of monogeneans in fish and were lower than the other farms throughout the year.

Water quality of Bangpakong River

The status of water quality parameters in Bangpakong River are shown in Table 3. Comparison on water quality parameters showed that there were no significant differences (p> 0.05) among the three farms during one year. The river exhibited low dissolved oxygen (DO) concentrations (< 5 mg/l), with Farm A having the lowest concentration. Temperature and pH levels in all the three farms were nearly 30°C and 7.1, respectively. Water salinity exhibited a wide range, with Farm C salinities ranging from 0.10 to 26.23 psu and levels of <0.5 psu or freshwater for a period of 5 months (August-November, 2010 and June 2011). Transparency was dependent on rainfall, with lower values in the rainy season and higher values in the dry season. Total ammonia-nitrogen levels in all the sampling sites had similar values.

Table 3. Physicochemical parameters (Mean±SD) of water samples from different sites in Bangpakong River.

Physicochemical parameters	Sampling sites		
	Farm A	Farm B	Farm C
DO (mg/l)	2.8±1.2	3.4±2.1	3.4±1.9
Temperature (°C)	30.7±2.3	30.5±1.7	29.9±1.6
pH	7.1±0.9	7.1±0.6	7.1±0.6
Salinity (psu)	15.4±13.5	13.6±12.7	8.6±9.4
Transparency (cm)	24±16	23±11	17±10
Total ammonia-nitrogen (mg/l)	2.48±2.15	2.19±1.95	1.78±1.58

DISCUSSION

Three monogenean species were found from gills of 691 host fish samples. These three species belong to two genera: i.e. genus *Laticola* with two species and genus *Diplectanum* with one species. All three species are members of the family Diplectanidae. The diplectanid monogeneans were found only on the gills of fish hosts, feeding on mucus and they are very host-specific which means that they did not infect other fish species (Leong and Colorni, 2006). This study found *Laticola lingaoensis*, *L. paralatesi* and *Diplectanum penangi*. Similarly, Yang *et al*. (2006) studied the infestation of *L. lingaoensis*, *L. latesi*, *L. paralatesi* and *D. penangi* in *Lates calcarifer* from South China Sea. Whereas, Leong and Wong (1990) reported that the most frequently encountered parasites infecting juvenile seabass imported from Thailand were *Trichodina* sp. and *Pseudorhabdosynochus latesi*.

The distribution of parasites varied from one habitat to another which could be due to host-parasite relationship and abiotic factors such as dissolved oxygen, temperature and pH (Anderson, 1992). The result of this study showed that the prevalence and mean intensity of overall gill monogeneans were highest in Farm B ($p<0.05$), followed by those in Farm A, and the lowest in Farm C. Differences in intensity and abundance of monogeneans were due to types of cage culture. Farm A had floating cages, while Farms B and C had fixed cages. Floating cages allowed easier water flow than fixed cages resulting in cleaner cage bottoms. In addition, rearing density in Farm B was higher. The most abundant parasite in examined fish samples was *L. lingaoensis*. This study showed that the overall prevalence of parasites was > 90% in all farms showing that there was an outbreak of monogenean parasites. This could be due to high density of fish in cages, and the cage culture itself was in a limited area. Moreover, the fish were reared continuously in cages without a break for the next culture period. As a consequence, new fish introduced into the farm would likely be infected with one or more species of parasites which already existed in the farm. Therefore, the fish farm itself could be a reservoir for parasites (Leong *et al*., 2006).

This study was carried out in consecutive seasons. Regarding the effect of seasonal variation on prevalence, mean intensity and mean abundance of diplectanid monogeneans, this study showed that all farms had higher prevalence in both dry and rainy seasons. In contrast, mean intensity and mean abundance values of gill parasites in Farms A and B showed higher rate of infection during the dry season when salinity levels were high. This was also reported by Yufa *et al*. (2010), who verified that the overall prevalence of *Diplectanum grouperi* was slightly higher in autumn than in summer under wild conditions. The mean intensity and variance were highest in summer, decreasing slightly in autumn to the lowest levels in winter and spring. For all seasonal studies, water quality could influence monogenoidea parasitism. Temperature is commonly regarded as one of the most important factors determining the existence and abundance of monogenean parasites (Koskivaara *et al*., 1991) while some of them tend to produce more at higher water temperatures, others prefer a cool water temperature (Hanzelova and Zitnan, 1985). In addition, many scientists insisted on the importance of temperature as one of the factors in controlling parasitic infections (Hopkins, 1959; Chubb, 1963; Manter, 1966; Kennedy, 1971; Muralidhar, 1989; Rohde, 1993; Rodrigues and Saraiva, 1996; Turner, 2000; Wang *et al*., 2001 and Yufa *et al*., 2010). Bangpakong River is located next to the Gulf of Thailand and is affected by salinity fluctuations. Thus, farm locations have an effect on the occurrence of diplectanid monogenean, as they are dependent on water quality conditions such as salinity and temperature. Water quality variations in fish cage culture could be difficult to control, because they are located in open waters such as rivers and reservoirs. Therefore, farmers should focus on the health of the fish and use good farming practices and consider water quality conditions. It was evident that the parasitological assessment was influenced by seasonality, although the water quality parameters were within their recommended range for culture. Henceforth, the information obtained from this research can be used for more effective control measures of monogenean parasite infestation in cage culture systems.

ACKNOWLEDGEMENT

The authors would like to thank the fish farmers from Bangpakong River for fish samples, the National Science and Technology Development Agency, Ministry of Science and Technology (NSTDA/MOSTE), and the Aquaculture Business Research Center, Department of Fishery Biology, Faculty of Fisheries, Kasetsart University (ABRC) for research support.

LITERATURE CITED

Anderson, R.C. 1992. **Nematodia Parasites of Vertebrates, Their Development and transmission.** CAB International, Willingford. pp 99.

Bush, A.O.; K.H. Lafferty, J.M. Lotz, A.W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis *et al*. revisited. **J. Parasitology.** 83, 575-583

Chubb, J.C. 1963. **On the characterization of parasite fauna of the fish of Llyn Tegid,** Proc. Zool. Soc. London, 141:609-621.

Grey, D.L. 1987. An overview of *Lates calcarifer* in Australia and Asia, pp. 15-21. In J.W. Copland and D.L. Grey, eds. **Management of Wild and Cultured Seabass/Barramundi (*Lates calcarifer*):** Proceedings of an international workshop. 24-30 September 1986. Australian Centre for International Agriculture Research (ACIAR). Darwin, Australia.

Hanzelova, V. and R. Zitnan. 1985. Epizotiologic importance of the concurrent monogenean invasion in carp. **J. Helminthologia**, 22: 277 -283.

Hopkins, C.A. 1959. Seasonal variations in the incidence and development of cestode *Proteocephalus fillicolis* (Rud, 1810) In Gasteosteus aculeatus L. I1766). **Parasitology,** 49: 529-542.

Kennedy, C.R. 1971. The effect of temperature upon the establishment and survival of the cestode *Caryophyllaeus laticeps* in Orfe, *Leuciscus indus*. **Parasitology,** 63:59-66.

Koskivaara, M., E.T. Valtonen and M. Prost. 1991. Dactylogyrids on the gills of roach in central Finland: Features of infection and species composition. **Int. Parasitology,** 21(5); 565-572.

Koyun, M. 2011. Seasonal distribution and ecology of some *Dactylogyrus* species infecting *Alburnus alburnus* and *Carassius carassius* (Osteichthyes: Cyprinidae) from Porsuk River, Turkey. **African Journal of Biotechnology,** 10(7): 1154-1159.

Leong, T.S. and S.Y. Wong. 1990. Parasites of healthy diseased juvenile grouper (*Epinephelus malabaricus* (Bloch and Schneider)) and Asian seabass (*Lates calcarifer* (Bloch)) in floating cages in Penang, Malaysia. **Asian Fisheries Science,** 3: 319-327.

Leong, T.S. and A. Colorni. 2006. Infectious diseases of warmwater fish in marine and brackish waters. In: Woo, P.T.K., Bruno, D.W. and Lim, L.H.S. (eds), **Diseases and Disorders of Finfish in Cage Culture.** CAB International 2002. pp. 193-230.

Leong, T.S., T. Zilong and J.E. William. 2006. **Important parasitic diseases in cultured marine fish in the Asia-Pacific Region.** Featured articles. In: http://www.thefishsite.com

Liang, K.S. and T.S. Leong.1991. A redescription of *Pseudorhabdosynochus latesi* (Tripathi, 1955) and description of Diplectanum penangi n.sp. (Monogenea: Diplectanidae) from Lates calcarifer cultured in floating cages in Malaysia and Thailand. **Journal of Bioscience,** 2(1&2): 77-84.

Manter, H.W. 1966. Parasites of fishes as biological indicators of recent and ancient conditions. In: **Host parasite relationships,** McCauley, J.E. (Ed.). Oregon state University Press, USA. pp: 59-71.

Muralidhar, A. 1989. Seasonal variation of helminth parasites in marine fishes at East coast of India. **Ind. J. Helminthol,** 41: 1-4.

Rodrigues, A.A. and A. Saraiva. 1996. Spatial distribution and seasonality of *Pseudodactylogyrus anguillae* and *P. bini* (Monogenea) on the gills of the European eel *Anguilla Anguilla* from Portugal. **Bull of the European Association of Fish Pathologists,** 63(3): 85-88.

Rohde, K. 1993. **"Ecology of marine parasites"** An introduction to marine parasitology. Department of Zoology, Unit of New England, Armidale, NSW, 2351, Australia. CAB International.

Tuner, E.T. 2000. Seasonality of *Alloglossoides cardicola* (Trematoda: Macroderiodea) infection in the cray fish, *Procambarus acutus*. **South western Naturalist,** 45(1): 69-71.

Wang, G.T., W.J. Yao and P. Nie. 2001. Seasonal occurrence of *Dollfustrema vaneyi* (Digenea: Buccephalidae) metaceraria in the bull head cat fish *Pseudobagnus fluvidraeo* in a reservoir in China. **Disease of Aquatic Organisms,** 44(2): 127-131.

Yamaguti, S. 1963. **Systema Helminthum,** Vol. IV. Monogenea and Aspidocotylea. Interscience publishers, a division of John Wiley & Sons, New York and London.

Yang, T., D.C. Kritsky, D.C., Y. Sun, J. Zhang, S. Shi and N. Agrawal. 2006. Diplectanids infesting the gills of the barramundi *Lates calcarifer* (Bloch) (Perciformes: Centropomidae), with the proposal of *Laticola* n.g. (Monogenoidea: Diplectanidae). **Systematic Parasitology,** 63:127-141.

Yufa, L., C.L. Brown and T. Yang. 2010. Seasonal dynamics of *Diplectanum grouperi* parasitism on wild versus culture groupers, *Epinephelus* sp., and the linkage between infestation and host species phylogeny. **J. Parasitol,** 96(3): 541-546.