Seasonality of Gill Monogeneans in Red Hybrid Tilapia (*Oreochromis* sp.) Cage Culture Systems in Central Thailand

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ABSTRACT

A seasonal study of monogenean gill parasites in the cage culture of red hybrid tilapia (*Oreochromis* sp.) in three fish farms was conducted between June 2010 and May 2011. The farms represented three different areas and conditions of study, namely, freshwater culture in Khwae Noi River, Kanchanaburi province (Farm A), an urban residential area (Farm B), and brackish water culture (Farm C). Farms B and C are located in Samut Songkhram province. During the study period, water quality was kept within acceptable ranges. This study investigated the occurrence of five gill monogenean species. *Cichlidogyrus sclerosus* was the most dominant parasite followed by *C. tubicirrus*, *C. thurstonae*, *Scutogyrus longicornis* and *C. tilapiae*. The highest prevalence and mean intensity of these parasitesin red hybrid tilapia cultured in cages occurredin Farm B followed by Farm A then Farm C. The gill parasites showed a 100% prevalence and the highest mean intensity in April at all study sites. The results showed that majority of the fish sampled had higher infestations by gill monogenean parasites duringthe dry season.

Key words: Seasonality, Gill monogenean, red hybrid tilapia

INTRODUCTION

Red hybrid tilapia was introduced in Thailand in the late 1990s by the large agri-business company, Charoen Pokphand (CP), creating a new strain of red tilapia called "Tabtim" (ruby) to be raised in cage systems (Mariojouls *et al*., 2004). An intensive system for cage farming of red tilapia has been developed through joint trials with the Thai Department of Fisheries (DOF) based on stocking all male fry at high densities. Recently, cage culture of red hybrid tilapia has increased rapidly both in fresh and marine waters, particularly because of its rapid growth and it can be effortlessly produced in many confined water bodies throughout the country. However, tilapia farms are infested with various species of the gill monogenean, especially the genera *Cichlidogyrus*, causing cichlidogyriasis. Some epizootic outbreaks of cichlidogyriasis have been associated with crowded and poor water quality conditions, which increase the extent of parasite

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transmission (Khalil, 1971; Paperna, 1980; Bondad-Reantaso and Arthur, 1990; Jiménez-García *et al*., 2001). These outbreaks can be concurrent with secondary infection by opportunistic pathogens because of mechanical damage in the lamellae produced by the special posterior-positioned attachment organs of the monogenean flukes. In order to provide a predictive capability for possible parasitic outbreaks and an opportunity to design preventive management actions, detailed information of the proliferation and species of *Cichlidogyrus* spp. on the gills of apparently infected fish through every seasons is required. Seasonal patterns of monogenean infections in fish are poorly known, but several pertinent papers have been published (Chubb, 1977; Boungou *et al*., 2008; Koyun, 2011). The present seasonal study aimed to examine the prevalence and mean intensity of monogenean infesting the gills of red hybrid tilapia (*Oreochromis* sp.) from three different cage fish farms in the central part of Thailand.

MATERIALS AND METHODS

Experimental study

A total of 720 specimens of infected red hybrid tilapia were randomly sampled by using gill nets or cast nets to examine the presence of gill monogenean parasites from June 2010 to May 2011. In this study, we divided the season of Thailand to two seasons. The rainy or wet season is from June to October and the dry season is from November to May (Climate center, Bureau of Meteorology, 2011). Twenty specimens were collected each month from three different farms in the central part of Thailand, namely: Farm A located on the Khwae Noi River, Kanchanaburi province, which represented the fresh water conditions; Farm B situated in a residential area in the city; and, Farm C which represented the brackish water conditions, influenced by salinity fluctuation from the Gulf of Thailand. Farms B and C are situated on the Mae Klong River, Samut Songkhram province.

Gills monogenean parasite sampling

Fish samples were placed in plastic bags and transported in a foam box with ice to the Aquaculture Business Research Center Laboratory, Kasetsart University, Bangkok, Thailand. Consequently, they were dissected by separating the gill from both sides into a petri dish, which have been filled with clean water (distilled water for freshwater monogenean samples or saline water for brackish water monogenean samples). The gills were then examined under a stereo microscope. Parasites found in the gills were removed using a needle and transferred with a dropper onto a glass slide and then covered with a cover slip. Parasites were then fixed and preserved with ammonium picratum glycerine. Specimens were flattened gently under the cover glass (Malmberg, 1957).

Water quality measurement

Water quality parameters such as salinity, dissolved oxygen, transparency, pH, temperature and total ammonia were measured at the fish collection sites every month. Salinity, pH and temperature were measured using YSI DO 200-4M meter. Dissolved oxygen and transparency were measured with a DO meter and a secchi disc, respectively. A 500 ml water sample was collected at each site on each sampling day, and samples were frozen for further ammonia analysis according to APHA *et al*. (1995).

Data analysis

With a microscope, each parasite specimen was identified up to species level and classified on the basis of morphological characteristics, according to the existing keys and species descriptions (Pariselle and Euzet, 1995a; Pariselle and Euzet, 1995b; Paperna, 1996; Arthur and Lumanlan-Mayo, 1997; Pariselle *et al*., 2003; Sirikanchana, 2003). The total number of parasites found in each sampling was taken into account for calculating the prevalence and intensity. The prevalence (%) was estimated as the ratio between the number of infected fish and the number of examined fish expressed in percentages, while the intensity of infection was defined as the mean number of parasites per infected fish (Margolis *et al*., 1982). The comparison of prevalence and mean intensity of each parasite in each farm were calculated using one-way analysis of variance (ANOVA). In the analysis, confidence level was held at 95% and $p<0.05$ was set for significance.

RESULTS

Water quality parameters from the three red hybrid tilapia cage farms measured between June 2010 and May 2011are shown in Table 1. Salinity was stable in Farms A and B, while it varied in range from 0.86 to 16.95 psu in Farm C, during the whole study period. The highest water temperature was observed in July 2010, while the lowest water temperature was recorded in January 2011, in all farms.

Parasitological analysis was performed on 720 red hybrid tilapia samples (*Oreochromis* sp.) from cage farms in the central part of Thailand. Two genera and five species of monogeneans were found in the gills of fish. These are identified as: *Cichlidogyrus* spp. (*C. sclerosus*, *C. tubicirrus*, *C. thurstonae* and *C. tilapiae*) and *Scutogyrus* sp. (*S. longicornis*) (Figure 1).

Prevalence and mean intensity of gill parasites in red hybrid tilapia

The prevalence and intensity of gill monogenean parasites in red hybrid tilapia cultured in cages from three different farms are given in Table 2. The highest prevalence of gill monogenean parasites was observed in the samples collected from Farm B which was 90.41% ($p<0.05$). The mean intensities ($p<0.05$) of this parasite were significantly higher in fish from Farms A and B than in Farm C, especially for the genus *Cichlidogyrus* sp.

The highest number of gill monogeneans was recorded for *C. sclerosus*, which was present in all farms. The prevalence of *C. thurstonae* in Farm B was the highest at 71.59%, which was statistically different ($p<0.05$). Conversely, the lowest prevalence and mean intensity ($p<0.05$) for *C. tilapiae* were found in fish samples collected from Farm C ($p<0.05$). Fish samples from Farm C also showed the lowest mean intensity for the gill monogeneans when compared to Farms A and B ($p<0.05$). The prevalence and mean intensity of *C. tubicirrus* and *S. longicornis* were highest in Farm B and lowest in Farm C, which were statistically different ($p<0.05$).

Seasonal study

The parasites were isolated from the gills of red hybrid tilapiato determine seasonal prevalence and mean intensity between June 2010 and May 2011. The data were analyzed graphically on percentage of prevalence (%) and mean intensity against the sampling period of three different farms, respectively. Results are shown in Figure 2.

Table 1. Water quality parameters in red hybrid tilapia cages measured each month in three farms in central Thailand

Parameters	Jun-10	Jul-10	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11	Mar-11	Apr-11	May-11	Mean±SD
							Farm A						
Salinity	0	0	0	0	0	0	0	0	0	0	0	0	0
Dissolved oxygen (mg/L)	7.70	6.73	7.60	7.18	3.78	4.95	5.10	5.95	5.72	6.00	6.00	5.40	6.01±1.16
Transparency	67.00	32.85	38.85	22.10	10.00	38.00	69.15	72.50	43.35	60.56	40.00	25.00	43.28±20.09
pH	8.75	8.52	8.80	8.76	7.90	6.95	7.30	8.55	8.10	7.70	7.65	7.50	8.04±0.63
Temperature (°C)	30.85	31.65	30.35	29.23	27.80	27.65	27.15	25.90	27.10	27.96	28.70	30.80	28.76±1.81
Ammonia (mg/L)	0.145	0.205	0.195	0.153	0.415	0.155	0.048	0.102	0.087	0.107	0.132	0.270	0.168±0.098
							Farm B						
Salinity	0.10	0.15	0.20	0.20	0.20	0.25	0.15	0.75	0.60	0.13	0.10	0.10	0.24±0.21
Dissolved oxygen (mg/L)	4.90	3.72	4.77	4.27	5.13	6.72	5.37	6.08	4.91	4.08	5.34	6.89	5.18±0.98
Transparency	81.65	108.35	60.85	42.00	21.65	44.85	56.65	80.85	68.35	72.76	75.20	70.00	65.26±22.38
pH	8.15	8.45	8.05	8.33	8.00	7.10	7.20	8.05	8.20	7.60	7.80	8.00	7.91±0.42
Temperature (°C)	31.40	31.65	31.35	30.73	29.05	28.05	27.50	26.90	28.00	28.63	29.15	30.80	29.43±1.68
Ammonia (mg/L)	0.155	0.140	0.250	0.240	0.400	0.490	0.052	0.080	0.058	0.107	0.415	0.813	0.266±0.228
							Farm C						
Salinity	1.40	1.75	0.95	0.86	1.50	11.25	8.40	16.95	11.30	5.13	3.90	1.20	5.30±5.44
Dissolved oxygen (mg/L)	4.52	4.63	2.27	4.44	5.00	6.10	5.65	5.03	4.76	4.13	6.00	7.25	4.98±1.23
Transparency	43.85	61.25	47.15	31.00	25.00	68.35	73.35	86.00	62.50	51.66	42.00	27.70	51.65±19.13
pH	8.35	8.45	8.00	8.26	8.00	7.25	7.60	7.90	7.95	7.40	7.87	8.10	7.93±0.36
Temperature (°C)	31.30	30.90	30.75	30.53	28.85	27.50	27.35	25.85	27.50	28.40	28.90	30.90	29.06±1.79
Ammonia (mg/L)	0.315	0.120	0.250	0.213	0.300	0.655	0.043	0.507	0.623	0.143	0.222	0.760	0.346±0.233

Table 2. Prevalence and mean intensity of monogenean gill parasites of cage cultured red hybrid tilapia (*Oreochromis* sp.) at three different culture conditions

Farm	*C. sclerosus*		*C. tubicirrus*		*C. thurstonae*		*C. tilapiae*		*Scutogyrus longicornis*		Total parasites	
	Prevalence (%)	Intensity	Prevalence (%)	Intensity	Prevalence (%)	Intensity	Prevalence (%)	Intensity	Prevalence (%)	Intensity	Prevalence (%)	Intensity
A	57.78^{a}	34.07^{A}	42.96^{ab}	12.71^{A}	42.22^{a}	8.55^{A}	14.44^{a}	2.38^{A}	40.37^{ab}	7.33^{AB}	67.04^{a}	47.82^{A}
B	67.16^{a}	73.02^{A}	49.82^{a}	22.24^{A}	71.59^{b}	9.55^{A}	29.15^{a}	4.57^{A}	45.02^{a}	9.49^{A}	90.41^{b}	80.26^{A}
C	44.81^{a}	11.52^{B}	21.11^{b}	1.74^{B}	20.37^{a}	1.96^{B}	4.07^{b}	1.00^{B}	16.30^{b}	3.61^{B}	57.78^{a}	11.35^{B}

* The total number of prevalence and the intensity with different superscripts are significantly different at $P<0.05$

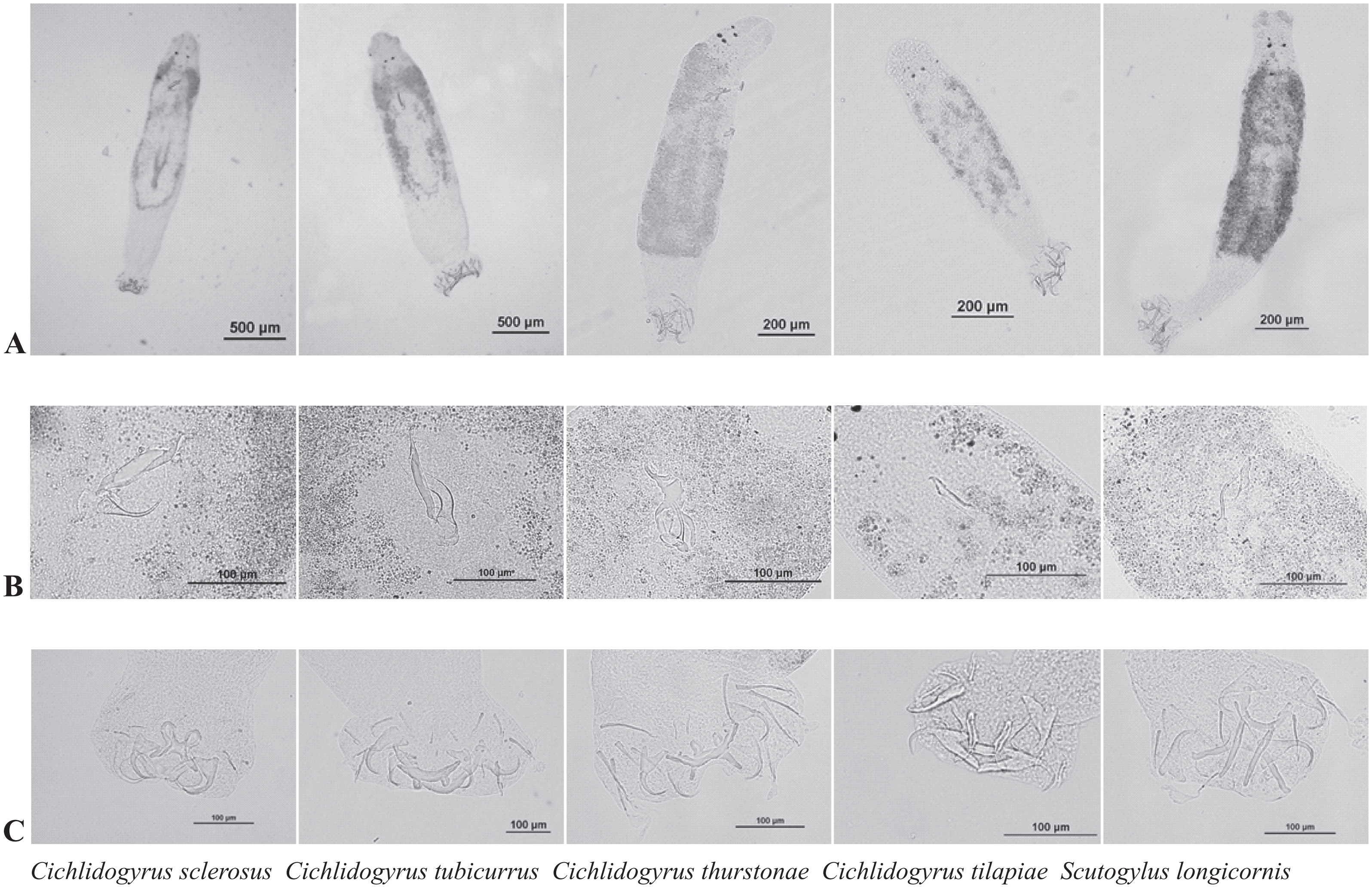

Figure 1. A) general view, B) copulatory organ, and C) opisthaptor, of five gill monogenean parasites from cage cultured red hybrid tilapia (*Oreochromis* sp.)

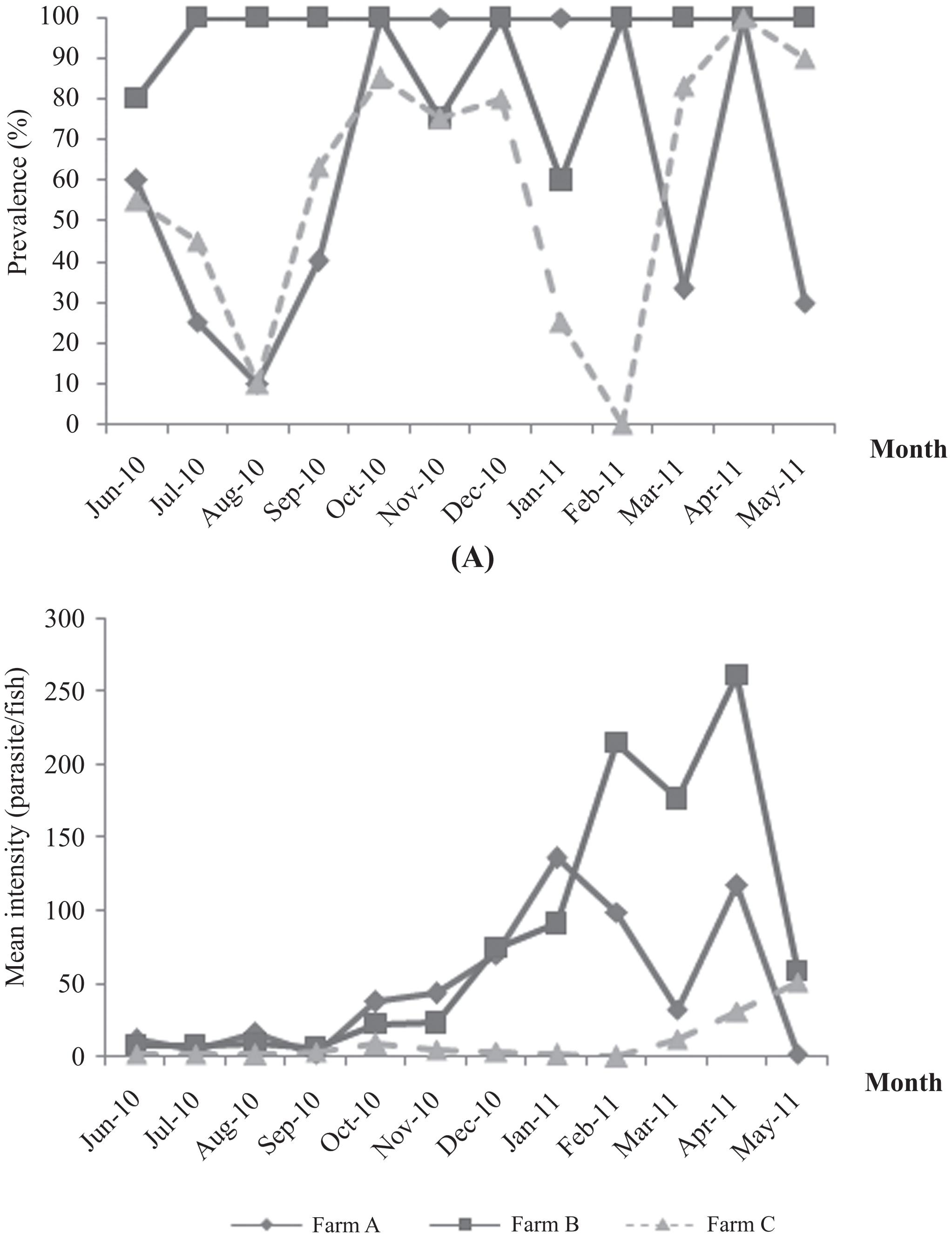

Figure 2. The seasonality of the prevalence (A) and mean intensity (B) of gill monogenean parasites of cage cultured red hybrid tilapia (*Oreochromis* sp.) in three farms in the central part of Thailand.

Concerning seasonal dynamics, Figure 2 shows that the highest seasonal prevalence and mean intensity of gill monogeneans in fish samples were recorded in January for Farm A and in April for Farms B and C. The prevalence and mean intensity of gill monogeneans increased since September and were highest in April, in all the sites. Fish from Farm A showed the highest prevalence of gill monogeneans during October to February at 100%, then dropped in August and March. Likewise, prevalence declined sharply in Farm C in August and February. Thereafter, it increased again to a peak in April at 100%. The infestation of monogenean parasite in Farm B revealed the prevalence of 100% almost throughout the year, except for the month of January, June and November. However the highest value of prevalence of monogenean parasite was recorded up to 60%. On the contrary, the prevalence and the mean intensity of gill parasites from Farm C were lower than in the other farms. In addition, the parasite was not found in February.

On the other hand, further analysis of each species of gill monogeneans showed that the seasonal prevalence of all species of parasite, except *C. tilapiae*, increased over 50% during October to February in Farm A, and during October to December in Farm B. Whereas for Farm C, only *C. sclerosus* increased up to 50% between October and December. *C. sclerosus* exhibited the highest prevalence and mean intensity in all sampled farms. It also presented the 100% prevalence rate in the fish samples collected from Farms A and B during October and February, but not in Farm C where it was not found in the month of February.

DISCUSSION

Water quality parameters during the study period were within the acceptable ranges for tilapia, which can tolerate a wide range of environmental conditions as described by El-Sayed (2006). The salinity was higher and had a wide range in Farm C due to salinity fluctuations in the Gulf of Thailand. This makes Farm C different from the other two farms. The higher dissolved oxygen readings observed in all farms were related to water movement at the air-water interface that makes oxygen transfer across the water. The water temperature levels were in the suitable range for tilapia culture, with an optimum range from 27 to 32°C (El-Sayed, 2006). The variation in water quality parameters could present severe consequences on fish health in high temperature climate (Tavares-Dias *et al*., 2008).

This study showed the prevalence and mean intensity of monogenean gill parasites in red hybrid tilapia (*Oreochromis* sp.) cultured in cages in three different farms. The five monogenean species found in this study belong to two genera: the genus *Cichlidogyrus* with four species and the genus *Scutogyrus* with one species. Similarly, Boungou *et al*. (2008) studied the infestation of five parasitic monogenes (*Cichlidogyrus tilapiae*, *C. halii*, *C. thurstonae*, *C.rognoni* and *Scutogyrus longicornis*) of *Oreochromis niloticus* in the Dam of Loumbila and their parasites were observed throughout the year. In Zambia, the monogeneans of the genus *Cichlidogyrus* in three species of cichlids are found the whole year round in an artificial lake (Batra, 1984). *Cichlidogyrus* sp. was a common monogenean parasite found in different species of tilapia and displayed a vast geographic distribution (Pariselle and Euzet, 1995a; Pariselle *et al*., 2003). Five species of *Cichlidogyrus* were introduced though the importation of tilapia (*Oreochromis mossambicus* and *O. niloticus*) to the Philippines (Natividad *et al*., 1986; Bondad-Reantaso and Arthur, 1990) and two species of *Cichlidogyrus* were introduced to Malaysia (Shaharom, 1985). In Thailand, some researchers found these parasites in tilapia farms cultured in different waters. Lerssutthichawal (2008) found three species of *Cichlidogyrus* spp. in red tilapia from natural waters and cultured areas (ponds, net cages and ditches) of Nakhon Srithammarat, Southern Thailand, namely, *C. sclerosus*, *C. thurstonae* and *C. tilapiae*. Elsewhere, these monogeneans had not caused any detectable damages to fish, even in heavy infestations of confined juveniles (Paperna *et al*., 1984). In the present study, the gill monogenean *C. sclerosus* had the highest count per infected fish found in all sites. Khalil (1971) and Kabata (1985) also reported this species from cultured *Oreochromis* spp. in Southeast Asia. Sanchez-Ramirez *et al*. (2007) noted that this species was also considered a chemical pollution biomarker.

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 total gill monogeneans in Farm B were highest, followed by Farm A, and the lowest in Farm C. This means that higher infestation of gill parasites occurs in red tilapia cage farms in freshwater conditions (Farms A and B) more than in brackish water conditions (Farm C).The high parasite load in the fish from Farm B may be caused by the over crowding of cages where a couple of small cages were placed in a large cage, which was similar to the findings of Akoll *et al*. (2012). Farm C showed the lowest gill parasite infection because of salinity levels which ranged widely from 0.86 to 16.95 psu. Increased salinity was able to control monogeneans in freshwater fish (Reed *et al*., 2012).

Regarding the effect of seasonal variation on the prevalence and mean intensity of gill monogenetic trematodesin the present study, the highest rate of infection was during the month of January for Farm A, and the month of April for Farms B and C (Figure 2). The number of gill parasites in Farms A and B increased from October to April. It showed the high rate of infection during the dry season. Akoll *et al*. (2012) found that the prevalence and mean intensity of parasites (*Cichlidogyrus tilapiae* and *C. sclerosus*) were higher during dry season than in the wet season. The results of El-Seify *et al*. (2011) showed that the peak of seasonal dynamics of monogeneanson *Oreochromis niloticus* was during autumn, followed by summer. For all seasonal studies, water quality could influence the 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). Some monogeneans can increase their number at higher water temperatures. This was also reported by Flores-Crespo *et al*. (1992), who verified that higher intensity of dactylogyrids in tilapia were found at higher temperatures.

Koyun (2011) showed that three dactylogyrid species, namely *D. fraternus*, *D. alatus* and *D. minutes*, reached the maximum infection level during summer (June to August) when the water temperature was highest (23°C). In addition, *D. anchoratus* on crucian carp reached the maximum infection level in spring (March to May) and summer (June to August) with no infection level in winter.

This seasonal study showed that the prevalence and mean intensity of five gill monogeneans in three different farm locations were higher during the dry season. The farm locations had an effect on the occurrence of gill monogenean parasites, as they are dependent on water quality conditions such as salinity and temperature. Although water quality parameters in fish cage culture could be difficult to control as they are in open waters i.e. in rivers and reservoirs, farm management practices should consider water quality conditions in order to maintain the health of cage cultured tilapia. It was evident that the parasitological assessment was influenced by seasonality, even though the water quality parameters were within their recommended range of tilapia culture. Hence, the information obtained from this research can be used for more effective control measures of monogenean gill parasite infestation in cage culture systems.

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Guide for Authors

Contributions

Kasetsart University Fisheries Research Bulletin publishes articles on problems and issues in fisheries science and related topics. Acceptable topics include ecosystem and population dynamics, resource assessment, fishing gear technology, fish processing, socioeconomics, farming systems, breeding, nutrition, fish health, pollution and aquatic resources management. Articles may be research papers, short communications or invited reviews.

- Research papers must not exceed 20 manuscript pages including Tables and Figures.
- Short communications are results of brief but significant work. Manuscripts must not exceed 8 pages, must have an abstract, but may omit the usual major headings of full papers.
- Invited reviews will be solicited by the Editor and the Editorial Board. Manuscripts must have an abstract and must not exceed 40 pages.

Only original, unpublished manuscripts not under consideration for publication elsewhere may be submitted. Articles must be technically sound and written in English. The editors will assist authors for whom English is a second language.

Authors must display good knowledge of the primary scientific literature. Authors must also prepare manuscripts according to the journal's standards and instructions in order to facilitate prompt review and processing of papers.
Tables, Figure captions and Figures

Preparation of Manuscripts

1. Organize the research paper logically and clearly, with sections complementing but not repeating each other, as follows:

- Title page - with concise but specific title and author's name and affiliation
- Abstract - summarizes the study in not more than 200 words, on a page by itself
- Introduction - lays out the problem addressed, current level of knowledge, the aims of the study, and the hypotheses tested
- Materials and methods - includes all crucial information to allow replication of the study
- Results - gives concise summary of data in Tables and Figures
- Discussion - places the study in the larger context of fisheries science and literature
- Conclusion - encapsulates the scientific contribution of the study
- Acknowledgement - credits contributors of thoughts, expertise and funds
- References - must substantially include the peer-reviewed primary literature

2. Type the manuscript using Microsoft Office Word software using letter size page (215 cm x 280 cm) with 2.5 cm margins all around. Double-space the manuscript throughout, including references, Tables and Figure legends. Use Times New Roman font with font size of 10 or 12 points.

3. Leave a triple space before and after all headings. Use capital and lower case letters, never all capitals. Avoid footnotes, addenda or appendices; if they are really important, incorporate them briefly in the text. Underline only the words to be italicized. Define acronyms or unfamiliar abbreviations at first mention in the text. Do not give any acronym in parenthesis if it is not used later again in the text.

4. Give the Latin name and family of the species at first mention in the manuscript. Subsequent references may use the common name. Italicize (or underline) Latin names. Example: Asian sea bass (*Lates calcarifer*, Centropomidae).

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6. Use metric units or the International System of Units (with base units meter, gram, second, liter, mole, joule, etc.). Common units such as day, tons, hectare, watts, horsepower, °C and ppt salinity may be acceptable. Use abbreviations of units only beside numerals (e.g., 5 m); otherwise spell out units (e.g.,

only meters away). Do not use plural forms or periods for abbreviations of units. Use superscripts and subscripts instead of the bar (/) for compound units; for example, 2 $t \cdot ha^{-1} \cdot year^{-1}$ instead of 2/t/ha/year, 10 $g \cdot m^{-2}$ instead of 10 g/m^2.

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(proceedings)	Ronquillo, I.A. 1974. A review of the roundscad fishery of the Philippines. Proceedings Indo-Pacific Fisheries Council 15 (3):351-375.
(book chapter)	Smith, I. and K.C. Chong. 1984. Southeast Asian milkfish culture: economic status and prospects. In: Advances in milkfish biology and culture (ed. J.V. Juario, R.P. Ferraris and L.V. Benitez), pp. 1-20. Island Publishing House, Manila.
(journal article)*	Widodo, J. 1991. Maturity and spawning of shortfin scad (Decapterus macrosoma) (Carangidae) of the Java Sea. Asian Fisheries Science 4:245-252. * Journal titles should be given in full.

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