CHAPTER III
MATERIALS AND METHODS

3.1 Study areas

Based on the previous study on fish-borne zoonotic trematodes (FZT) in fish farms in Vientiane, Lao PDR (Sithithaworn et al, unpublished), two FZT positive farms were selected for this study. The first farm is Sangsavang pun pla farm (SSPP) which performed combined hatchery and nursery activities located in Vientiane municipality area associated with the Mekong River. The other farm is Laos-viet farm (LV) located within the Nam Ngum River wetland at Tha Lad district, Vientiane Province (Figure3.1).

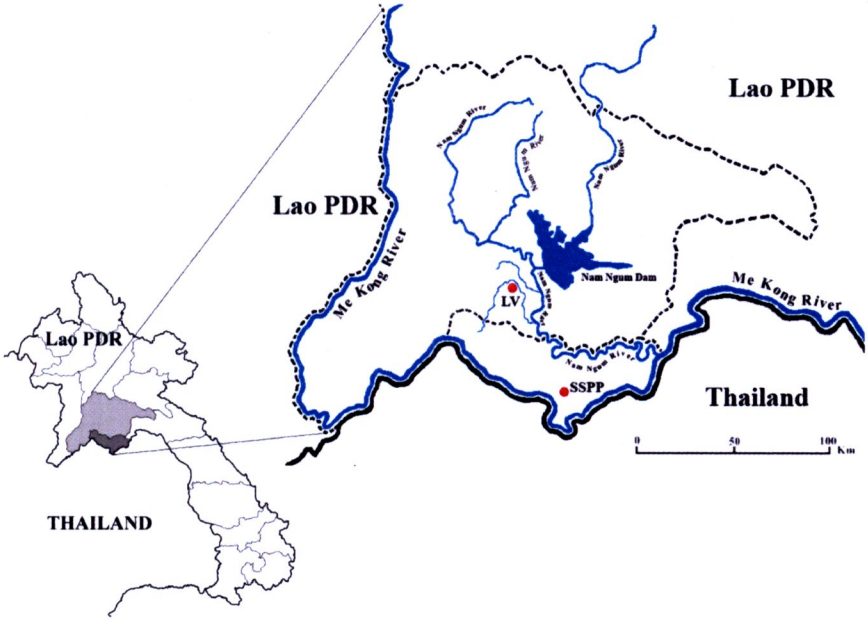
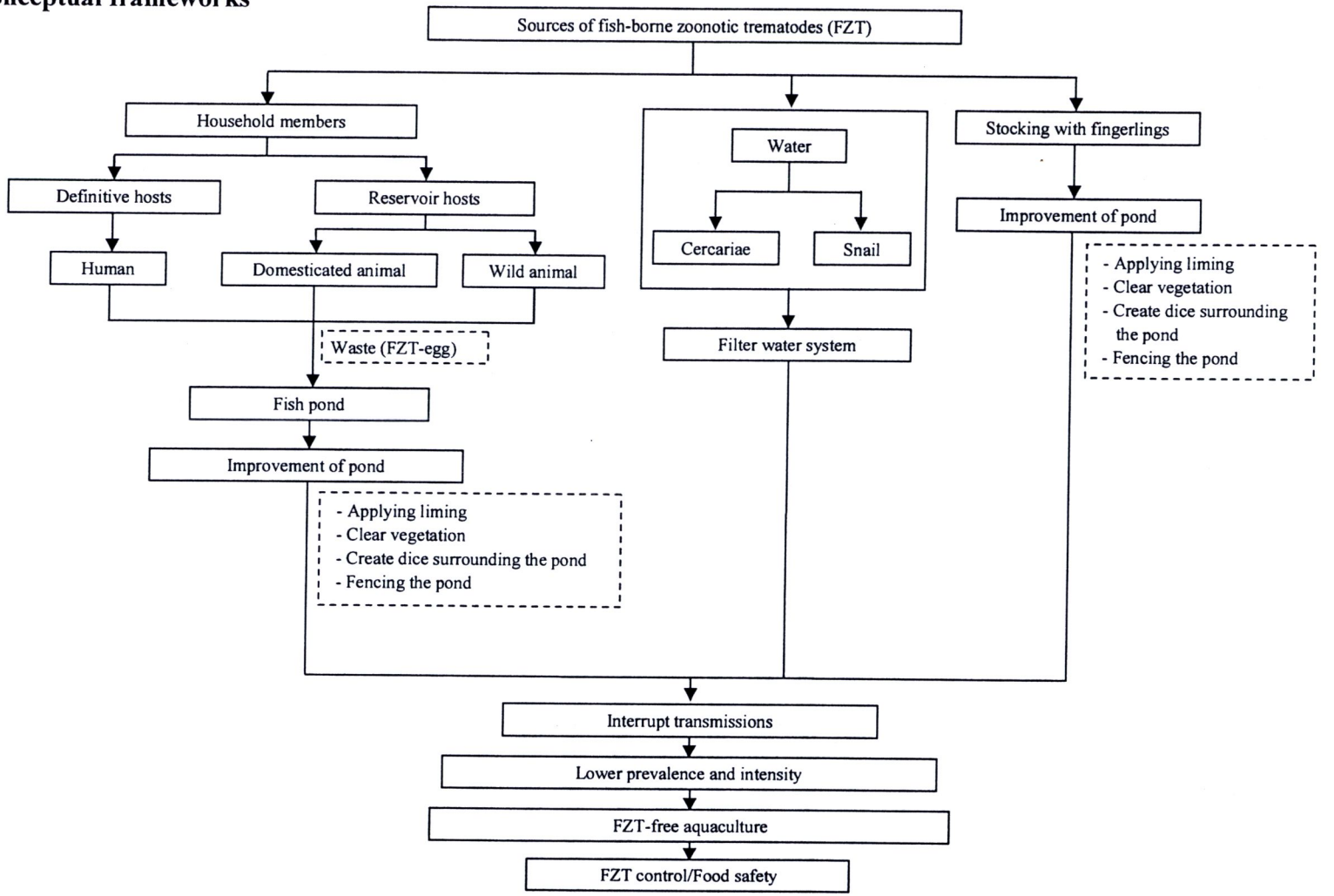


Figure 3.1 Studies area for control trial to reduce contamination of fish-borne zoonotic trematodes in aquaculture farms in Lao PDR. (LV): Laos-viet farm, (SSPP): Sangsavang pun pla farm.

Conceptual frameworks



3.2 Experimental design

Applications of preventive measures in the treated ponds included pond preparation by draining the water out and drying the pond completely for about 2-4 weeks or more, to kill wild fish, snails and other aquatic animals prior to restocking with fish. A basal dose of 100 to 150 kg / ha of good quality lime is applied to disinfect the pond and maintain an alkaline pH that facilitates growth of plankton. The lime is carefully scatter to cover the bottom area of the pond and vegetation within and surrounding area are removed. Creates dice surrounding the pond and erects fencing around the pond to prevent animal passage and pond contamination. The second action was an installation of a sand-based water filter system to supply inlet water. Just like water in underground aquifers is made pure by going through various layers of sand and porous rock and use filter water into the culture pond to get rid of snail and parasite contamination. Thirdly, determine fish fry quality by per examination for the presence of FZT before stocking. The treated and untreated ponds were approximately 10 meters in width and 10-15 meters in length. The dept is approximately 1.5 meter.

FZT infection in fish will be monitored at monthly intervals for 6 months. In each farm, two ponds are used i.e. as treated and untreated pond. While in untreated pond, the conventional practice in farm management is allowed but in the treated pond, preventive measures concerning the prevention of FZT are implemented. The main species stocked are silver barb (*Barbonymus gonionotus*), grass carp (*Ctenopharyngodon idellus*), silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*), rohu (*Labeo rohita*), nile tilapia (*Oreochromis niloticus*), bighead carp (*Aristichthys nobilis*), red finned pecu (*Piaractus brachypomus*) and mrigal (*Cirrhinus mrigala*). Nine species of culture fish commonly produce in the hatchery farms will be used in the poly culture system.

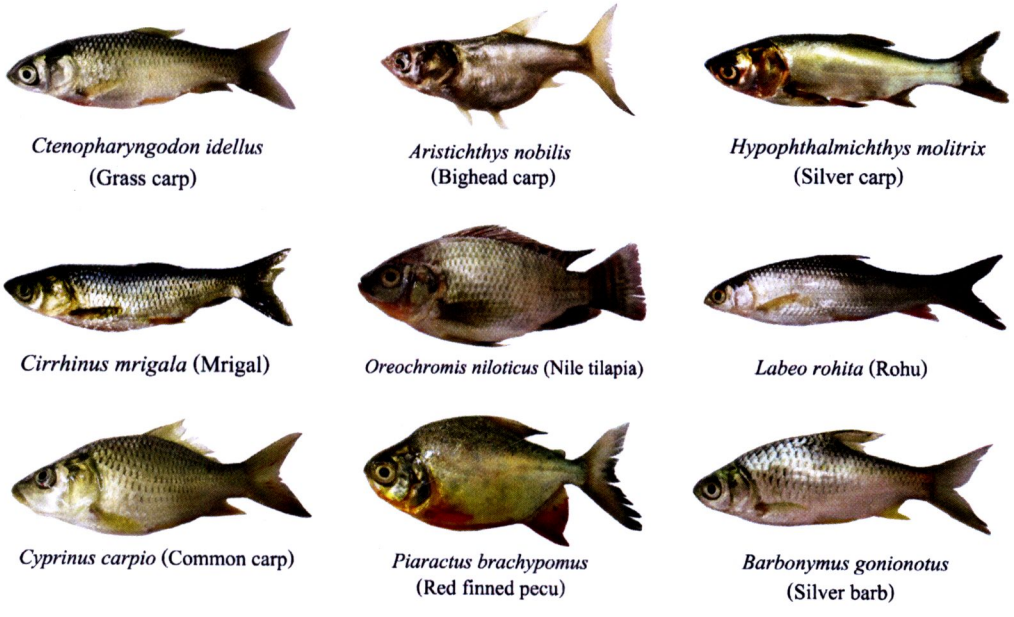
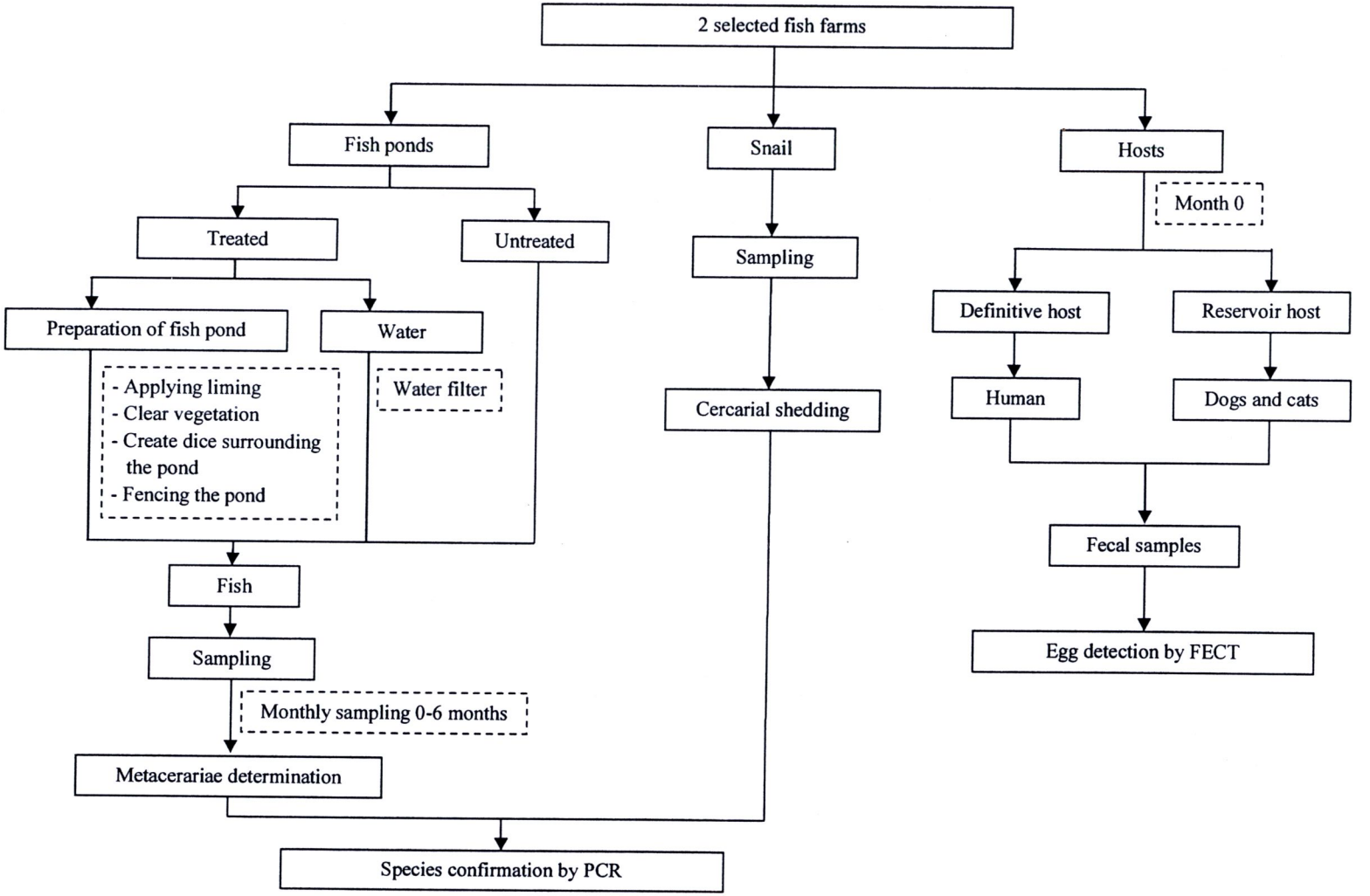
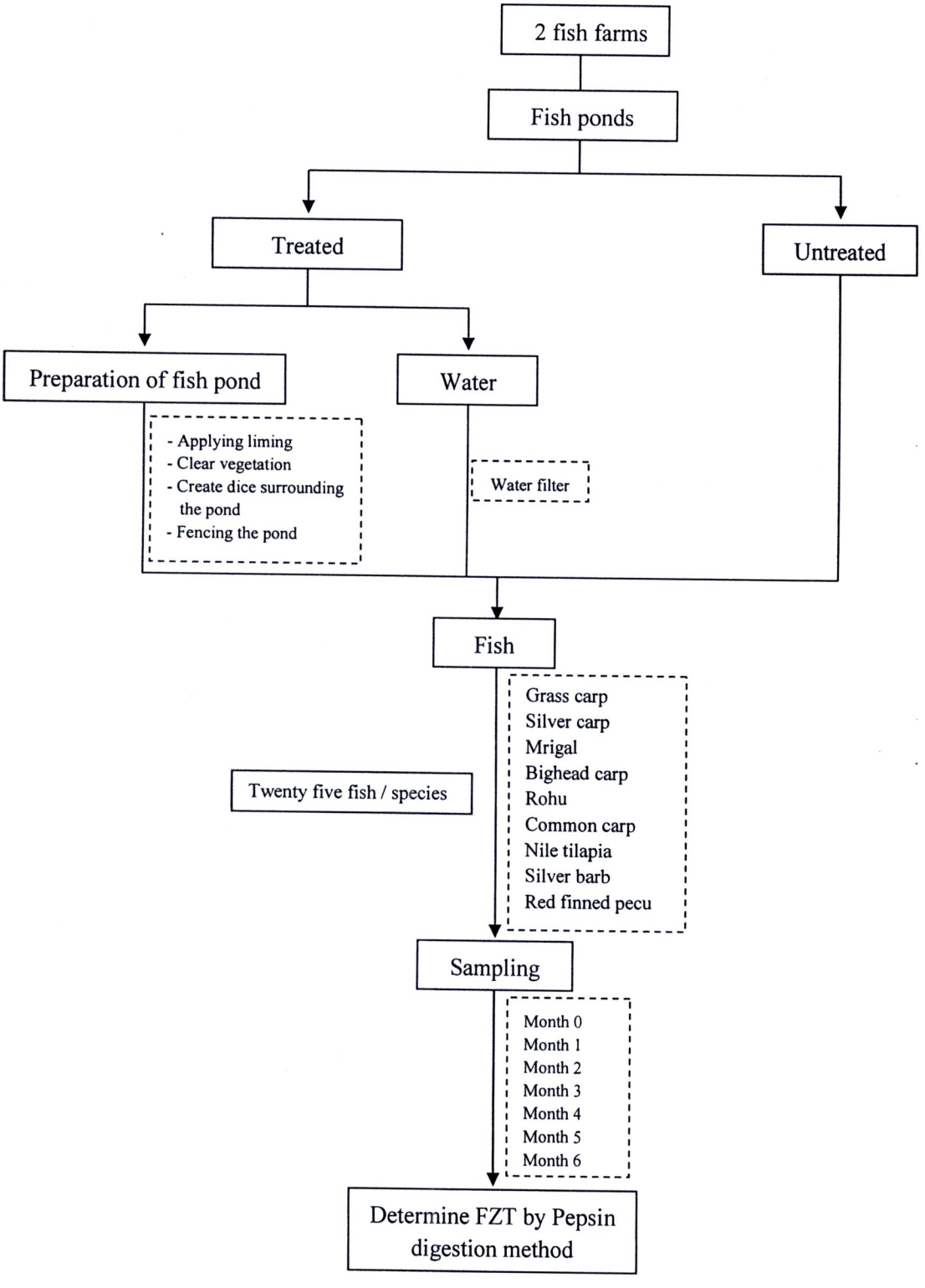


Figure 3.2 Nine species of culture fish available in the studied farms in Lao PDR.

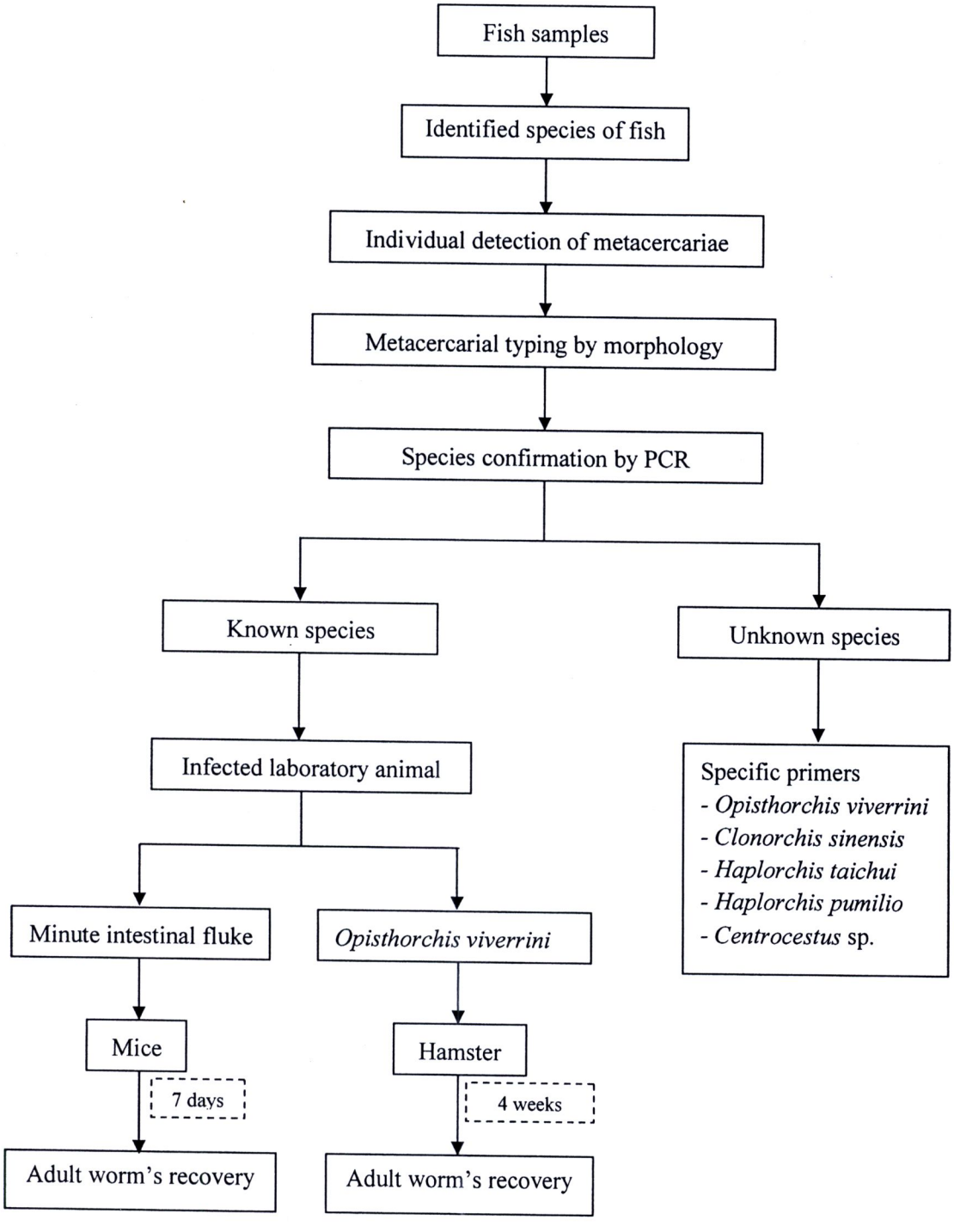
Research designs



Experimental design: control trial



The infection rate of trematodes in fish farm



3.3 Fish sampling and determination of metacercariae

The monthly samplings of fish were performed during the period of June 2010 to December 2010 from both studied farms. Twenty five fish / species were randomly selected for individual examination. After collection, the fish were placed on ice and transported to the laboratory. Fish were held at 4 °C in a refrigerator until processed, within 1 week of collection. In the laboratory the body size and weight of each fish was measured and recorded. Small fish (less than 150 g in weight) were individually ground and digested whole. For larger fish (>150 g), 2-3 parts (50% or 70-100 gm) of the fish were taken for metacercariae recovery (Figure 3.3). The protocol for metacercariae digestion and enumeration was done by using standard pepsin digestion technique (Sithithaworn et al., 1997). Briefly, individual fish was minced by electric blender and digested with pepsin A (BDH). The ratio of pepsin per fish-homogenate was 4:1 and the mixture solution was incubated at 37 °C in shaking water bath for 1 hour. Thereafter, the mixture was strained only once though the 250-micrometer apertures. The final filtrate was sediment in conical jars with several changes of 0.85% saline until the supernatant was clear and examined using a dissecting microscope. Identification of the metacercariae was assisted by use of morphological criteria detailed with published keys (Schell, 1970; Velasquez, 1973a, 1973b; Pearson and Ow-Yang, 1982; Scholz et al., 1991; Kaewkes, 2003).

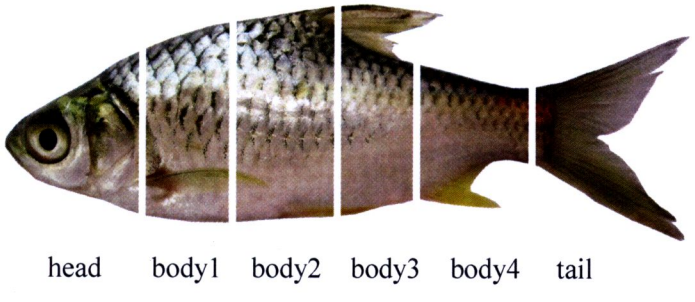


Figure 3.3 Partition of large fish (>150 g) into 6 portions and 3 portions (head, body2, body4) of each fish were sampled for recovery of metacercariae.

3.4 Preparation of adult worm

The adult stages of FZT were obtained from experimental infection of Swiss albino mice or hamsters. These laboratory animals (2-4 animal/fish species) were fed with metacercariae by intragastric intubation under slight anesthesia with ether. After infection, the animals were kept at room temperature, fed with commercial pellet and water *ad libitum* (C.P. Thailand) for 1 week in case of mice or 4 weeks in case of hamsters. After the animals were sacrificed, adult worms were recovered from the intestine or liver of the infected animals. The worms were cleaned several times with NSS and kept at –20°C and some were processed for staining for morphological identification.

3.5 Snail sampling

Snails were collected 2 times at month 0 and month 1 sampling from SSPP farm and we collected 6 times in month 0, 1, 2, 3, 4 and 5 sampling from LV farm. Methods of snail collection were handpicking from objects and solid surfaces and by dredging the sediment with a scoop. Snail sampled included *Bithynia siamemsis goniomphalos*, *Melanoides tuberculata*, *Tarebia granifera*, *Filopaludina* sp.

3.6 Cercarial shedding

The samples from each site were sorted by species, cleaned and examined for trematode infection by cercarial shedding. The cercarial shedding were done by placing individual snails in a small plastic container (3 cm×2.5 cm) half-filled with dechlorinated water (5 ml) and exposed to a 60 W light for five hours at room temperature. The emerged cercariae were examined and identified by morphological criteria (Schell, 1970)

3.7 Polymerase chain reaction (PCR)

Unknown species of metacercariae and cercariae are confirmed for their taxa by specific PCR protocol as follows;

3.7.1 Specific primer and PCR condition

specific primers	Forward and reverse primer (5' ⟶ 3')	Amplicon	PCR condition
O. viverrini **primer** (Wongratanacheewin et al., 2001)	Forward primer: OV-6F- CTGAATCTCTCGTTTGTTCA Reverse primer: OV-6R- GTTCCAGGTGAGTCTCTCTA	330 bp	94 °C 5 min 94 °C 30 sec; 52 °C 30 sec; 72 °C 45 sec (30 cycle) 72 °C 10 min 4 °C α
C. sinensis **primer** (Parvathi et al., 2007)	Forward primer: CS1F- CCAACCGAGTTGGTCAAGTT Reverse primer: CS1R- CAATCCAACGCACTCTCTGA	283 bp	94 °C 3 min 94 °C 1 min; 62 °C 1 min; 72 °C 1 min (41 cycle) 72 °C 5 min 4 °C α
H. taichui **primer** (Unpublished)	Forward primer: LC1- CGAGTATCGATGAAGAACGCAGC Reverse primer: HT4sp- GTGCACAAAGAATTGCATGG	558 bp	94 °C 1 min 94 °C 1 min; 55 °C 1 min; 72 °C 3 min (30 cycle) 72 °C 5 min 4 °C α
H. pumilio **primer, specific to ITS2 gene.** (Sato et al., 2009)	Forward primer: ITS 2- CTTGAACGCACATTGCGG CCATGGG Reverse primer: ITS2- GCGGGTAATCACGTC TGAGCCGAGG	380 bp	94 °C 4 min 94 °C 1 min; 60 °C 30 sec; 72 °C 2 min (30 cycle) 72 °C 2 min 4 °C α
Centrocestus **sp. primer** (unpublished)	Forward primer: LC1- CGAGTATCGATGAAGAACGCAGC Reverse primer: Centsp- CCAATGCCGAGATCACAGACAAG	367 bp	94 °C 1 min 94 °C 1 min; 55 °C 1 min; 72 °C 3 min (30 cycle) 72 °C 5 min 4 °C α

3.7.2 Preparation of genomic DNA

Pools of frozen metacercariae or cercariae stored in 1.5 ml microcentrifuge tube were boiled with 100 µl of distilled water at 95 °C for 10 min. Sodium dodecyl sulphate (SDS) and proteinase K were subsequently added to the final concentration of 1% and 0.1 mg/ml, respectively (Maniatis et al., 1982). The sample was incubated at 65°C for 1 hr. After the removal of all protein components from the lysate, DNA was removed by extraction with phenol. The solution was transferred to 1.5 ml microcentrifuge tube, and then centrifuged at 10,000 rpm for 10 min. The supernatant was added with phenol-chloroform. The mixture was centrifuged at 10,000 rpm for 10 min. The total DNA was precipitated with 2 volumes of absolute ethanol, and it was chilled at-20°C for 3-4 hrs or overnight. After centrifugation, the pellet was washed with 70% ethanol and dried at 37 °C. The DNA pellet was re-suspended in 50 µl of TE buffer and kept at –20 °C until used for PCR.

3.7.3 Measurement of DNA

The concentration of purified nucleic acid was measured by calculation of the absorption value at 260 nm using spectrophotometer (NanoVue, GE Healthcare, USA). An O.D. of 1 corresponds to approximately 50 µg/ml of doublestranded DNA. The DNA concentration was calculated from the following equation.

$$\text{DNA concentration } (\mu g/\mu l) = \frac{OD260 \times 50 \times \text{dilution of solution}}{1000}$$

The ratio between the absorbance of 260 nm and 280 nm provided estimation for purity of DNA. Purified DNA should have 260/280 O.D. ratio of 1.6-2.0.

3.7.4 Agarose gel electrophoresis

Agarose gel electrophoresis is a standard method used to separate, identify and purify DNA fragments according to their molecular sizes. This study 1.5% agarose gel was prepared in the horizontal plastic chamber. After the gel become solid, 5 µl of amplified DNA from each sample was mixed with 2 µl of dye and loaded into slots of

gel. The electrophoresis was operated using 60 volt/cm until loading dye nearly reach the end of the gel. The gel was then stained in 0.1 mg % of ethidium bromide solution for 20 min, destained in distilled water. The nucleic acid in the gel was visualized as fluoresced bands under UV light (254 nm), and the photograph was taken with UVP Transluminator (Biodoc-IT™ Imaging System, UVP Trans-illuminator, Cambridge, UK).

3.8 Faecal examination

Fecal samples were collected from human and domestic animals in the selected farms. The samples included 12 human, 2 dogs, 3 cats from SSPP farm and 20 human and 4 dogs from LV. Samples were stored in an ice box and transferred to the laboratory. Two grams of faeces were fixed with 10% formalin and processed for formalin-ethyl acetate concentration technique (FECT) (Elkins et al., 1990). The procedure began with a thorough mixing of faecal suspension in 10% formalin and stained through gauze into a test tube and 3 ml ethyl acetate was added and vigorously shacked. After centrifugation for 5 min, discarded the supernatant and re-suspend in 10% formalin. Two drops of final faecal suspension were placed onto a slide, covered with a cover slip and examined. The number of eggs per gram of faeces (epg) was calculated to measure intensity of infection.

3.9 Statistical analyses

Data from this study were entered into Microsoft Excel and analyzed by SPSS V.16. Data on prevalence were validated by Chi-squares test and intensity data were compared by non parametric two sample test. Chi-squares test were used to compare prevalence between sampling times, species of fish, size of fish. Mann-Whitney *U*-test was used to compare intensity of metacercariae with times, species and size of fish.

CHAPTER IV
RESULTS

4.1 *Haplorchis taichui* infections and species of fish

A total of 3,478 fishes were collected from two studied farms for FZT examination. In Laos-viet farm (LV), the samples included 1808 fish (939 fish from the untreated pond and 869 fish from the treated pond) while those from Sangsavang pun pla farm (SSPP), there were 1670 fish (837 fish from the untreated pond and 833 fish from the treated pond).

The FZT prevalence and intensity of *H. taichui* for each fish species in LV farm are compared and shown in Figure 4.1. The prevalence and intensity varied significantly between the species of fish examined ($P < 0.001$). Seven out of 9 species of fish harbored *H. taichui* metacercariae with the prevalence from 4.0 to 100%. Red finned pecu and nile tilapia were the only two species that had no *H. taichui* metacercariae. Silver barb and silver carp were highly susceptible to *H. taichui* as they have higher intensity of metacercariae than other infected species.

Three species (silver carp, mrigal and rohu) of the treated pond had significantly lower prevalence of *H. taichui* than those in the untreated pond ($p<0.05$). Trends of lower intensities were also seen with no statistical significance in silver carp, mrigal and rohu. Overall, silver barb had the highest prevalence and infection intensities of *H. taichui*.

In SSPP farms, only silver barb had *H. taichui* metacercariae. But *Opisthorchis viverrini* and *Centrcestus* sp. metacerariae were also detected in this species of fish. In common carp, *Centrocestus* sp. metacercariae were also detected.

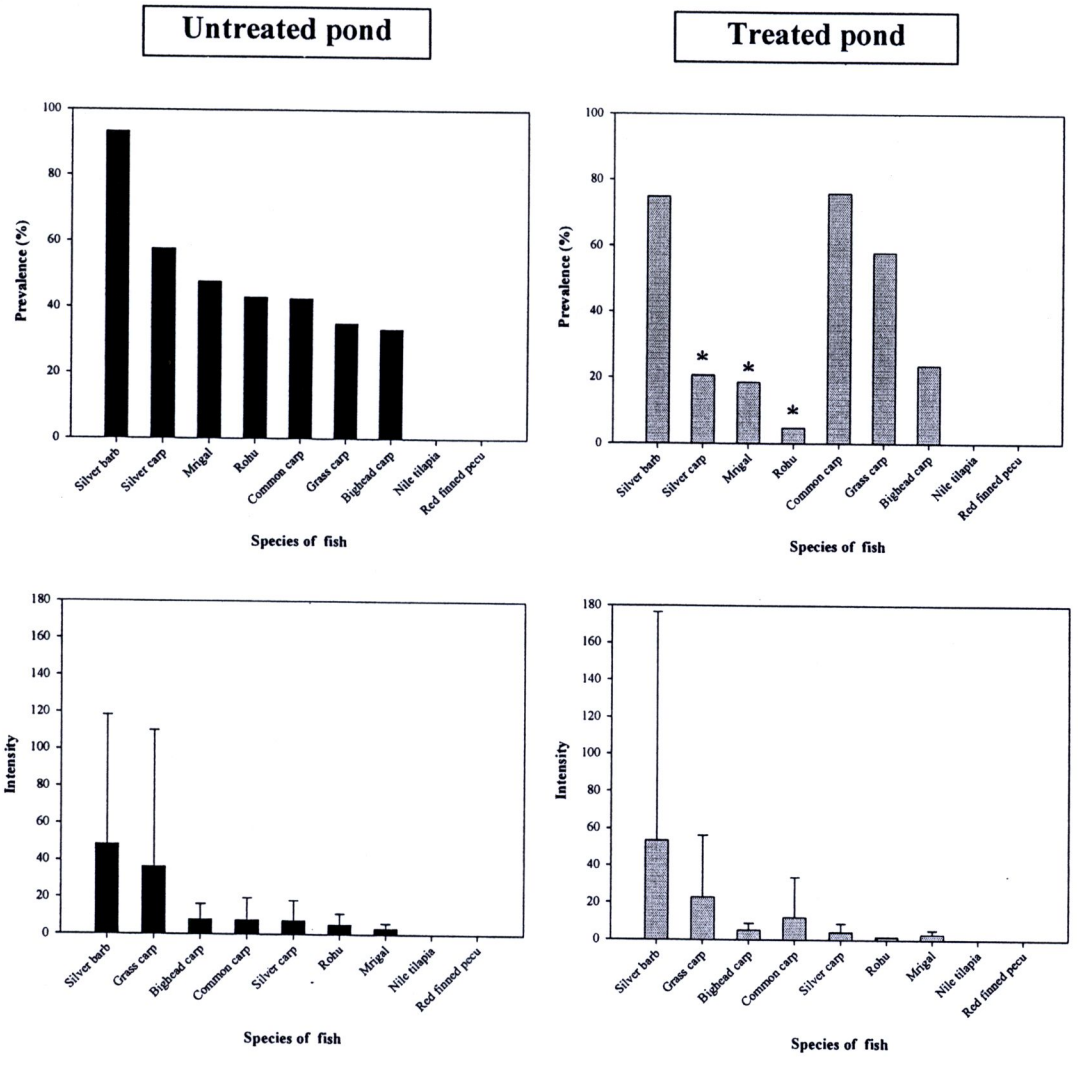


Figure 4.1 Prevalence and intensity of *H. taichui* metacercariae in different species of culture fish from Laos-viet farm. * Represent statistical significance between the treated and untreated ponds ($p<0.05$).

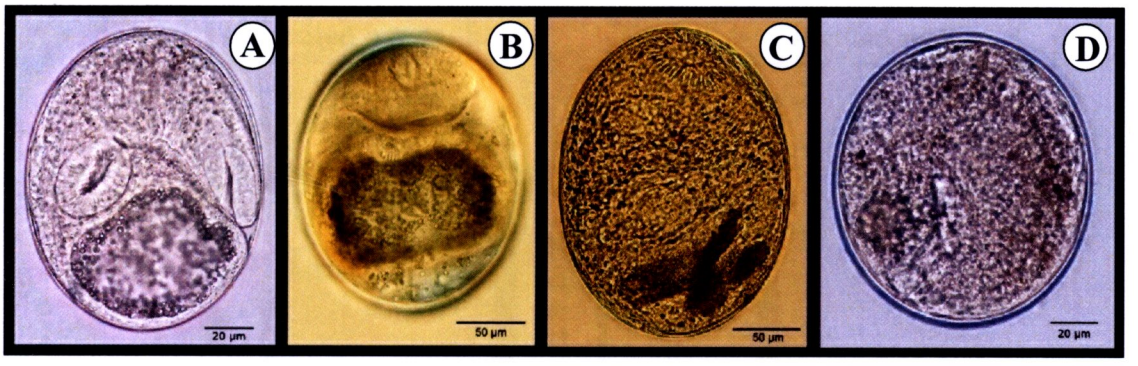


Figure 4.2 Images of trematode metacercariae from fish. A: *O. viverrini*; B: *H. taichui*; C: *Centrocestus* sp.; D: Unknown metacercariae (probable *Haplorchis pumilio*).

4.2 Prevalence and intensity of FZT metacercariae

Details of time-profiles in prevalence and intensity of *H. taichui* infection in fish samples were compared between the treated and untreated ponds. For silver carp reduction in prevalence and intensity were seen in the treated pond compared to the untreated pond (Figure 4.3). For mrigal, slight reductions in prevalence occurred at month 1 and no difference was seen thereafter (Figure 4.4). For grass carp, the prevalence at month 1-3 were lower and the intensity was lower at month 6 in the treated pond than the untreated pond (Figure 4.5). No obvious difference between ponds was obtained in common carp (Figure 4.6). In silver barb, the overall prevalence at month 6 were similar but the intensities were lower in the treated compared with the untreated pond at month 5 and 6 (Figure 4.7). In the bighead carp, although samples were not available at all sampling points, there were trends of lower prevalence and intensity in the treated than those in the untreated pond (Figure 4.8). In rohu, there were limited samples and no difference between ponds was evident (Figure 4.9).

Results from SSPP farm revealed three types of FZT metacercariae, namely *O. viverrini* in silver barb (at month 1, 4) from the treated pond and *H. taichui* in silver barb from both ponds. Metacercariae of *Centrocestus* sp. were found in common carp (at month 4) from the untreated pond.

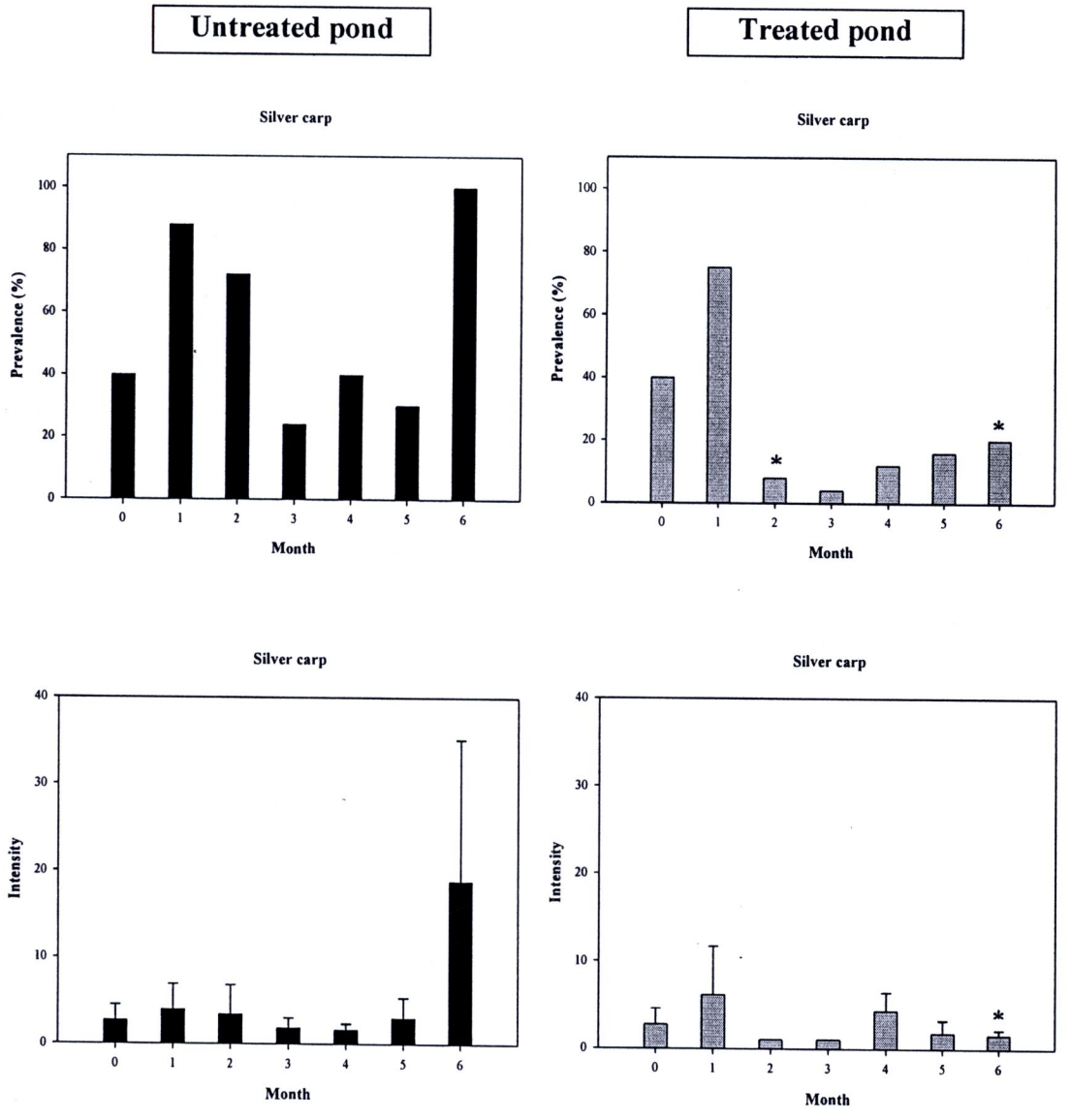


Figure 4.3 The prevalence and intensity of *H. taichui* metacercariae in silver carp sampling at monthly intervals during the study period from Laos-viet farm. * Represent statistical significance between the treated and untreated ponds ($p<0.05$).

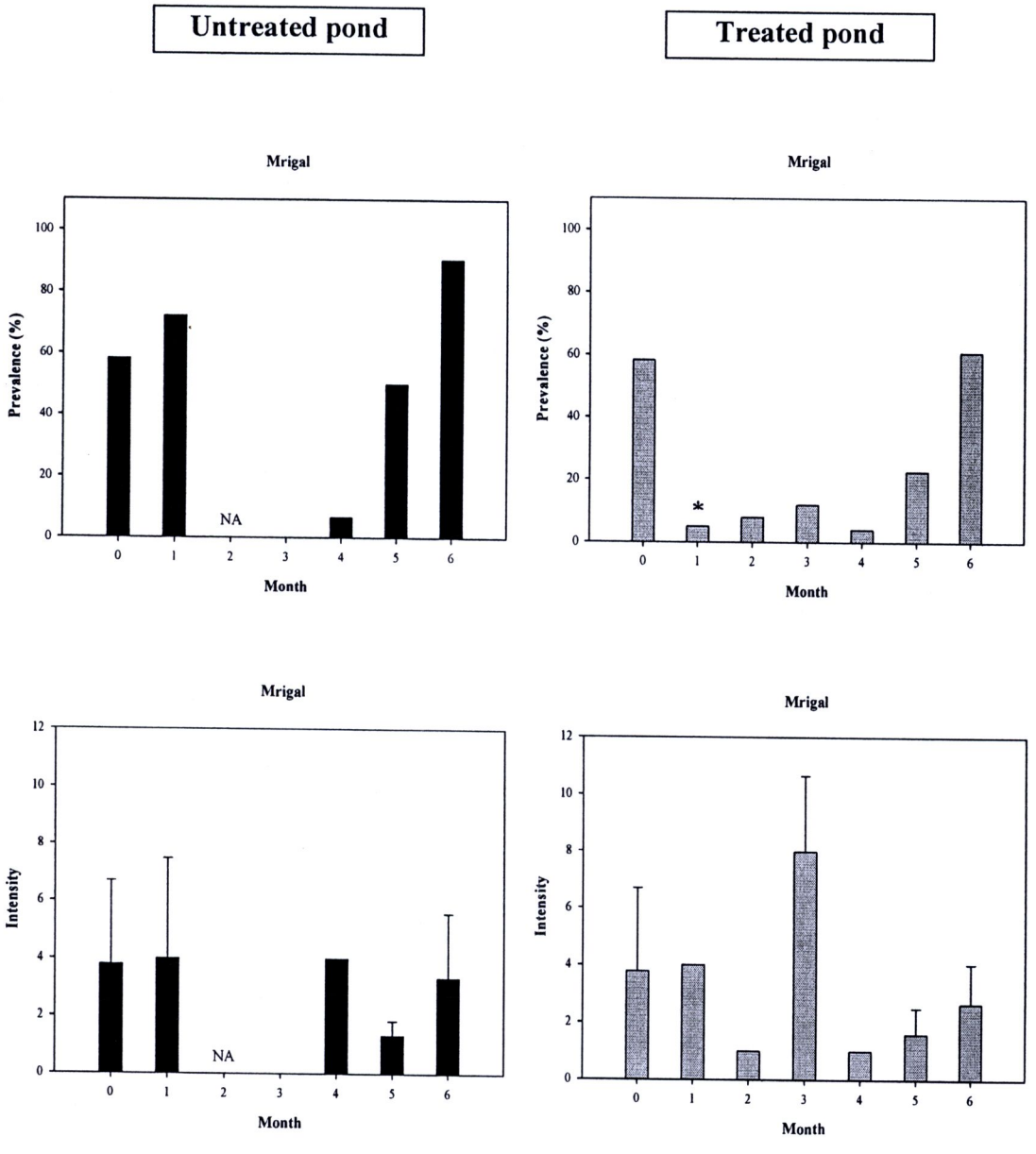


Figure 4.4 The prevalence and intensity of *H. taichui* metacercariae in mrigal sampling at monthly intervals during the study period from Laos-viet farm. * Represent statistical significance between the treated and untreated ponds ($p<0.05$).

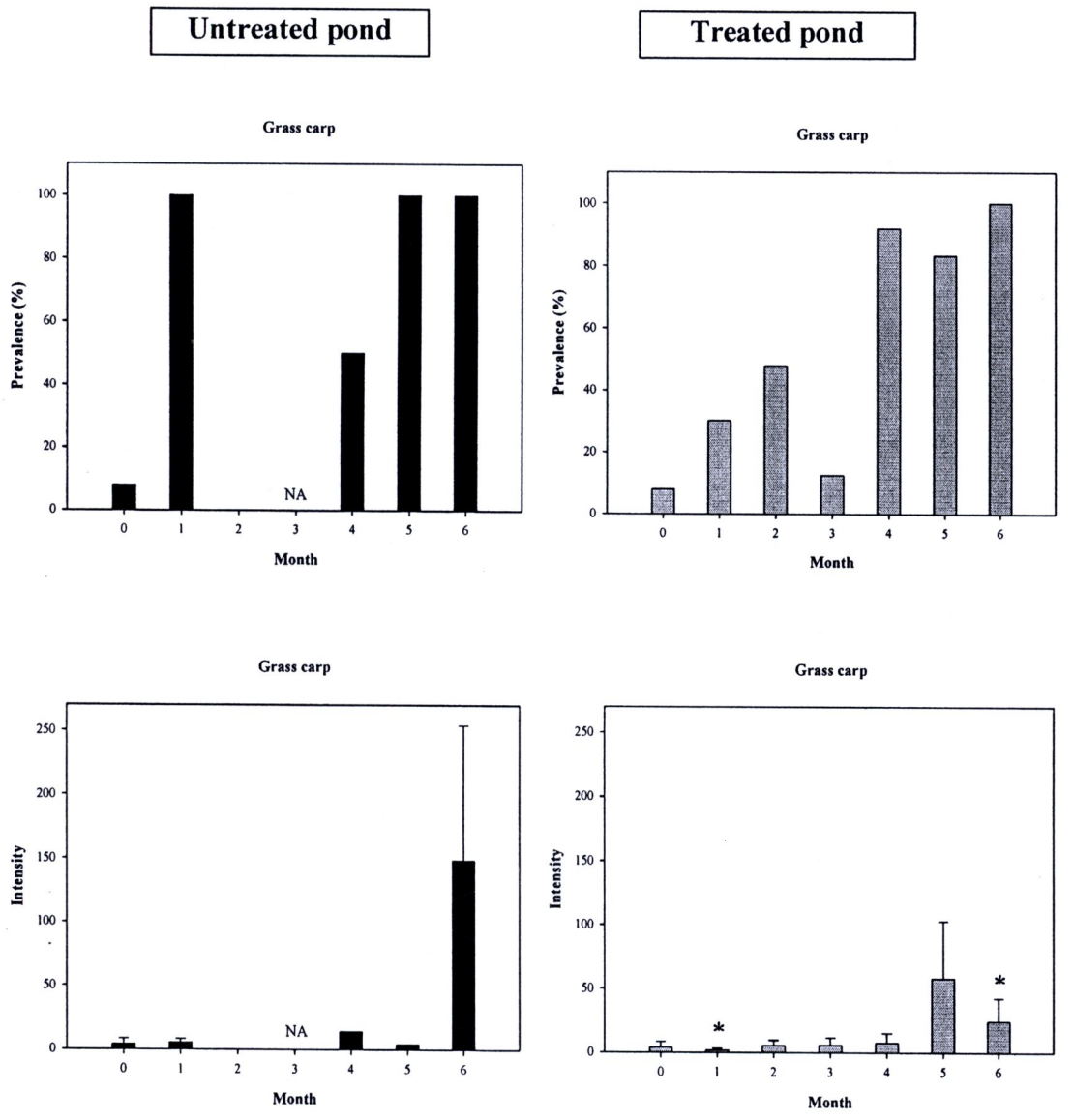


Figure 4.5 The prevalence and intensity of *H. taichui* metacercariae in grass carp sampling at monthly intervals during the study period from Laos-viet farm. * Represent statistical significance between the treated and untreated ponds ($p<0.05$).

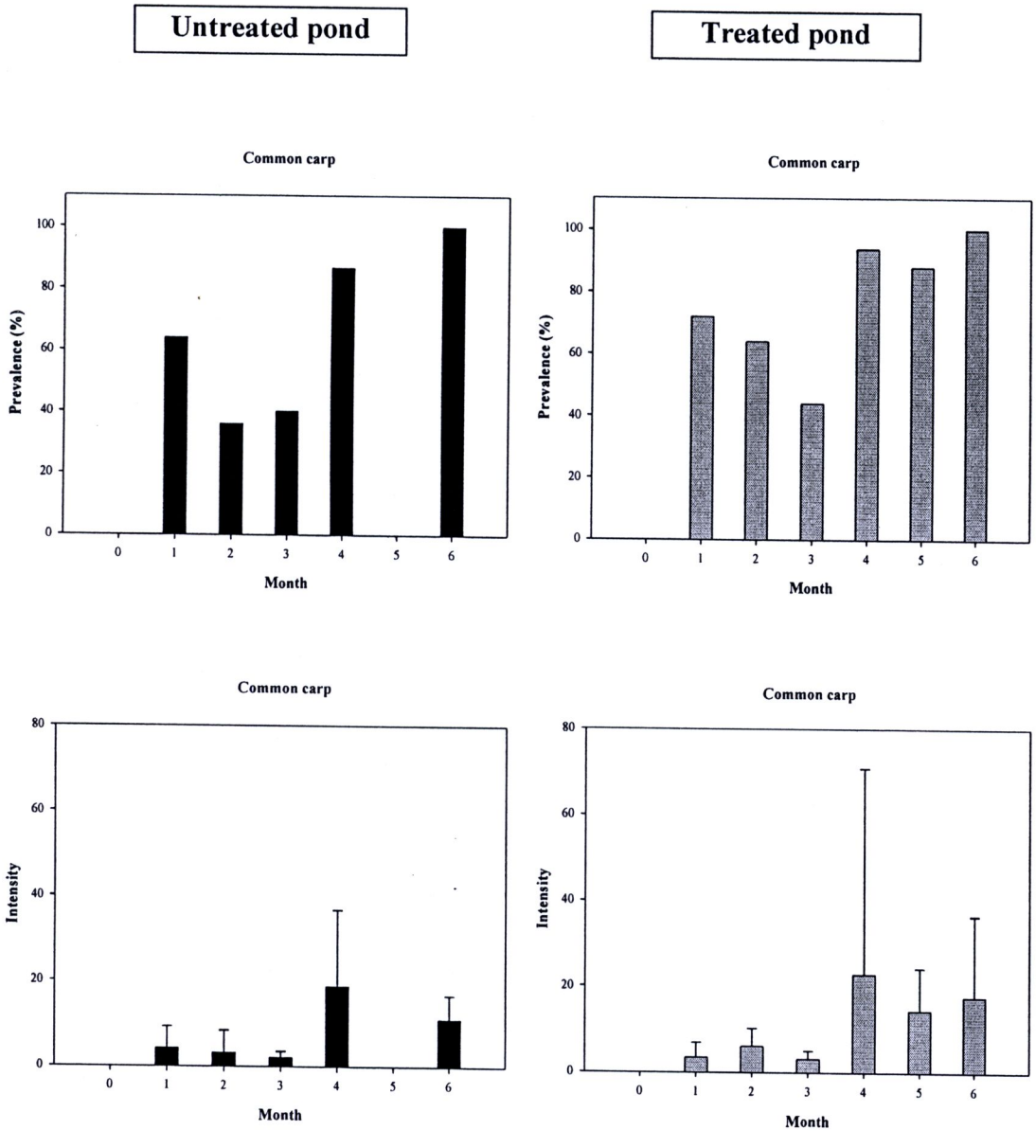


Figure 4.6 The prevalence and intensity of *H. taichui* metacercariae in common carp sampling at monthly intervals during the study period from Laos-viet farm.

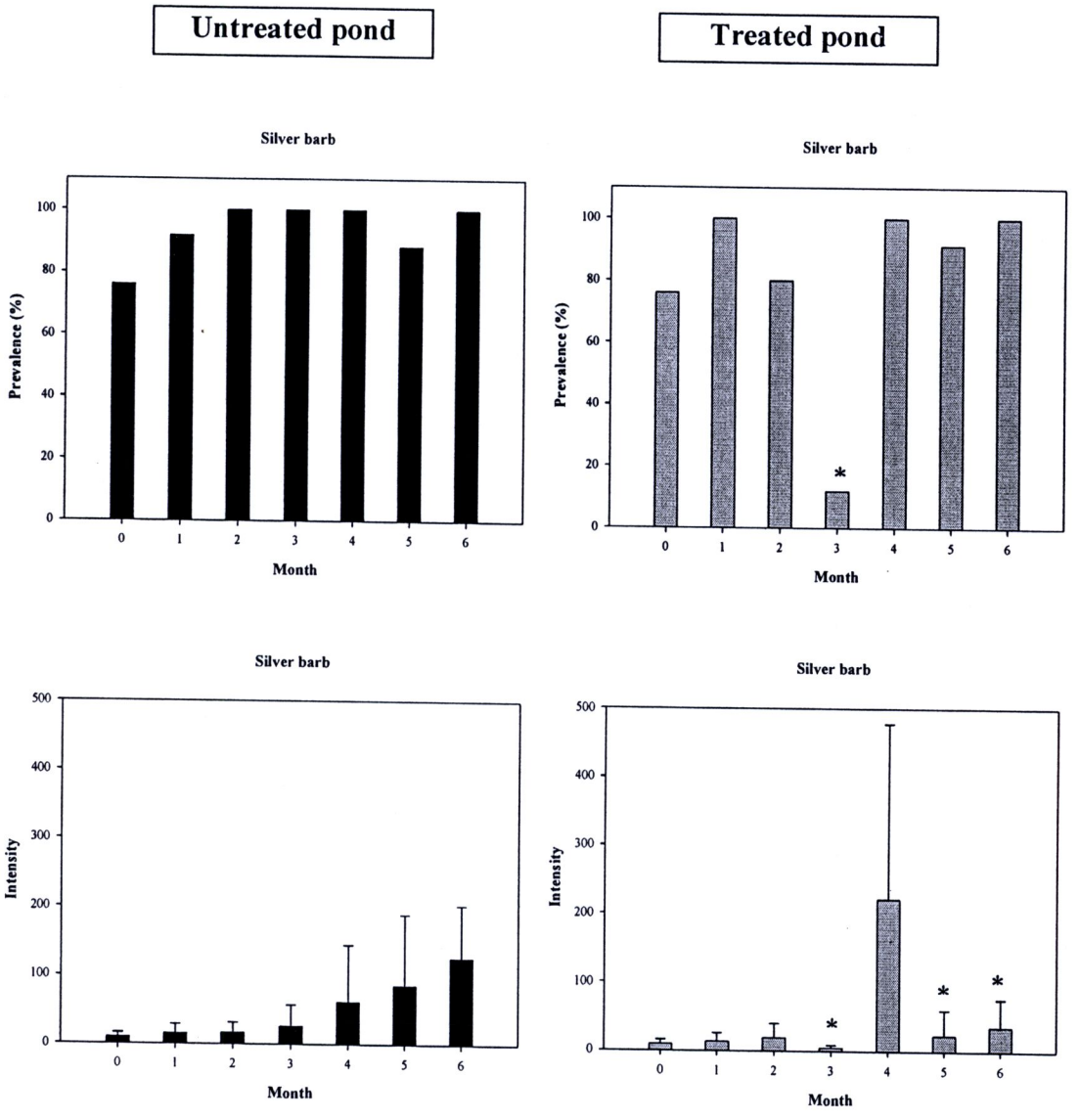


Figure 4.7 The prevalence and intensity of *H. taichui* metacercariae in silver barb sampling at monthly intervals during the study period from Laos-viet farm. * Represent statistical significance between the treated and untreated ponds ($p<0.05$).

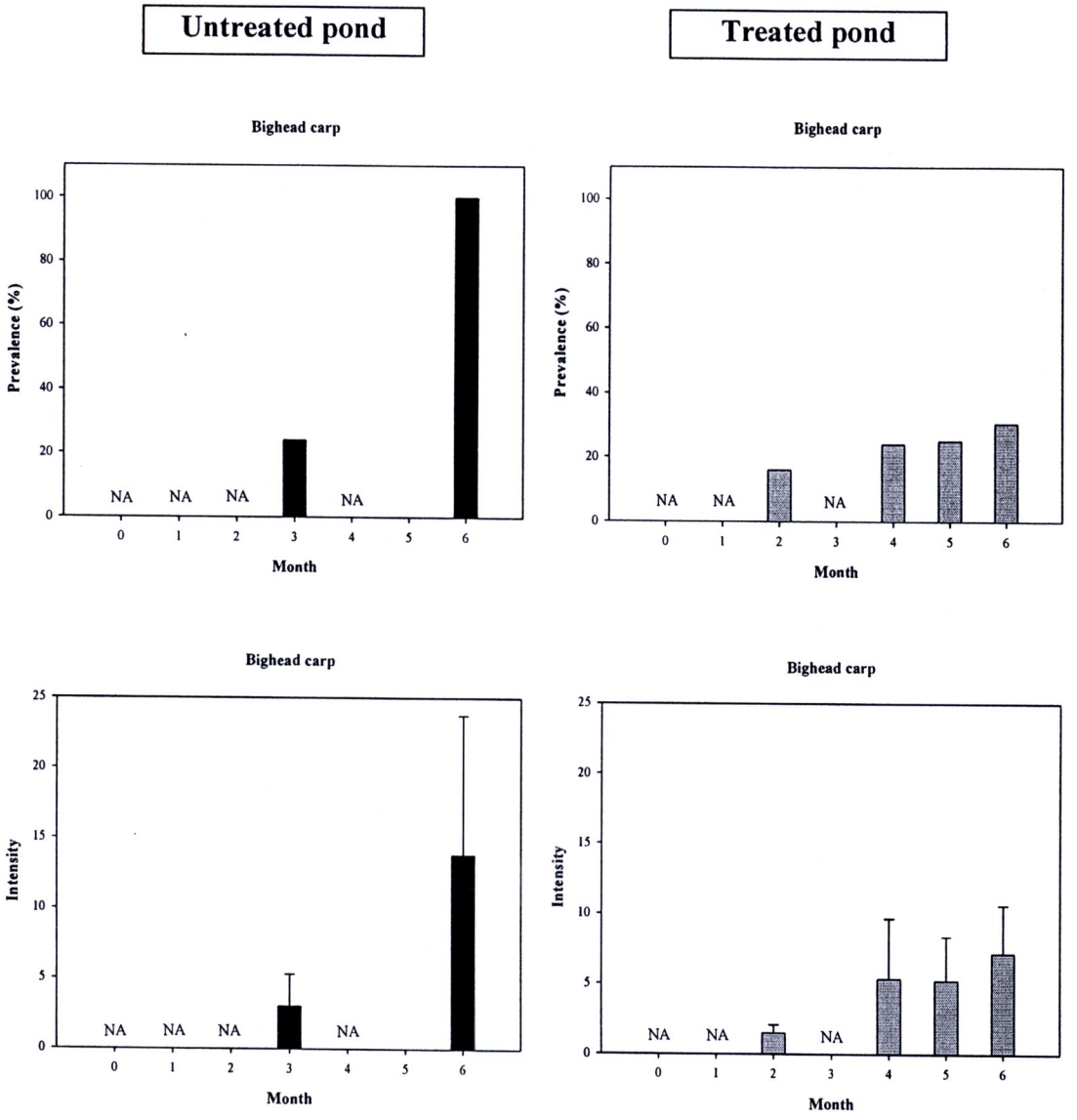


Figure 4.8 The prevalence and intensity of *H. taichui* metacercariae in bighead carp sampling at monthly intervals during the study period from Laos-viet farm.

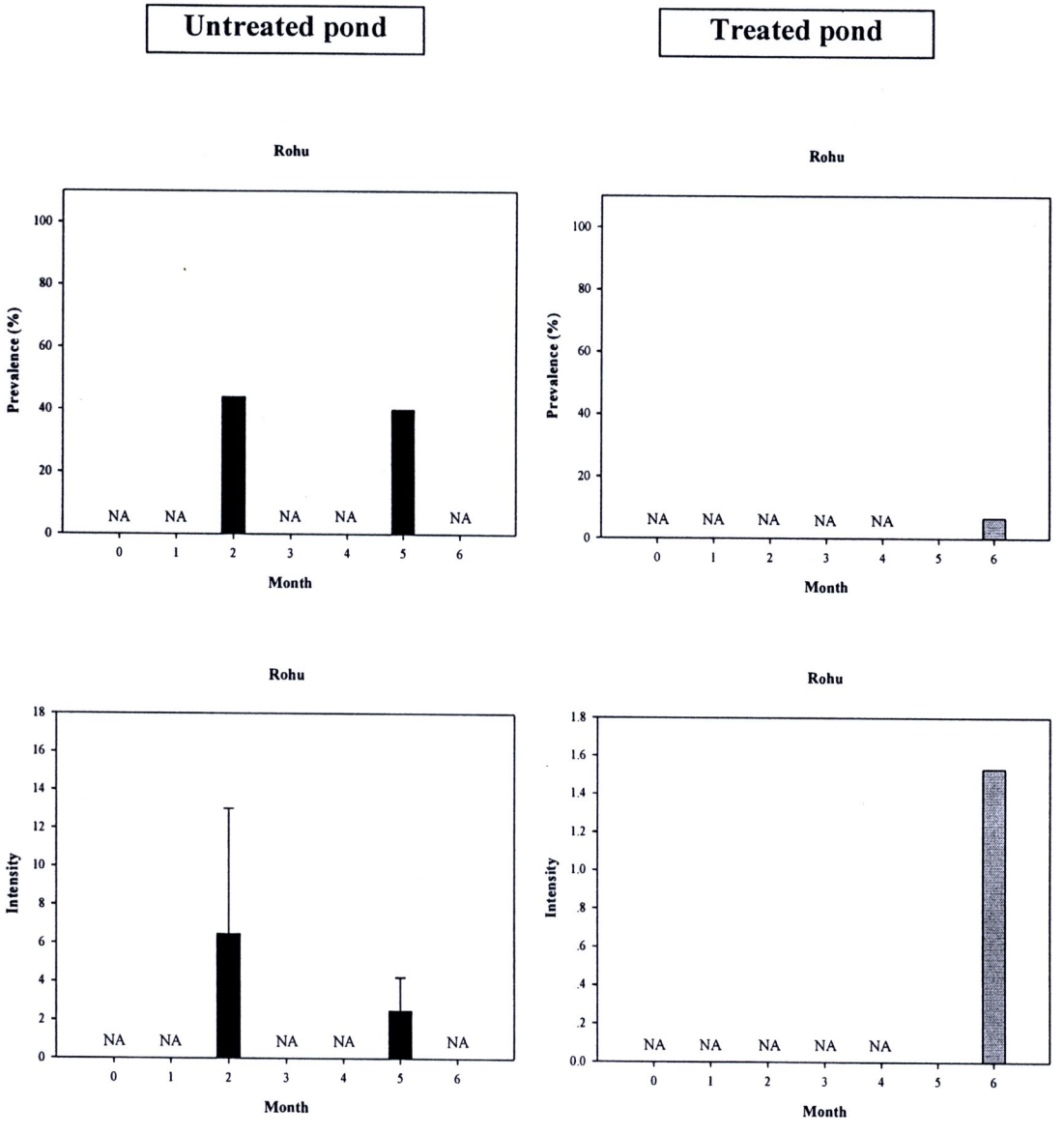


Figure 4.9 The prevalence and intensity of *H. taichui* metacercariae in rohu sampling at monthly intervals during the study period from Laos-viet farm.

4.3 Influence of fish size on prevalence and intensity of *H. taichui* infection

In order to explore effects of body size or age of fish on transmission pattern of FZT metacercariae, data of monthly samplings of both treated and untreated ponds from LV farm were combined for the analyses. The relations between body length, body width and body weights on prevalence of infection and intensity of *H. taichui* were shown in Figure 4.8-4.11. All of the parameters i.e. body length, width and weight showed positive relations with the prevalence of infection in silver barb, silver carp, common carp, grass carp ($p<0.05$) (Figure 4.10, 4.11). Similar patterns of size–related intensity were observed in silver barb, silver carp, common carp, grass carp where body length, width and body weight significantly correlated with intensity of infection ($p<0.05$) (Figure 4.12-4.13). There was no effect of size on infection status in mrigal, rohu, nile tilapia, bighead carp and red finned pecu (data not shown).

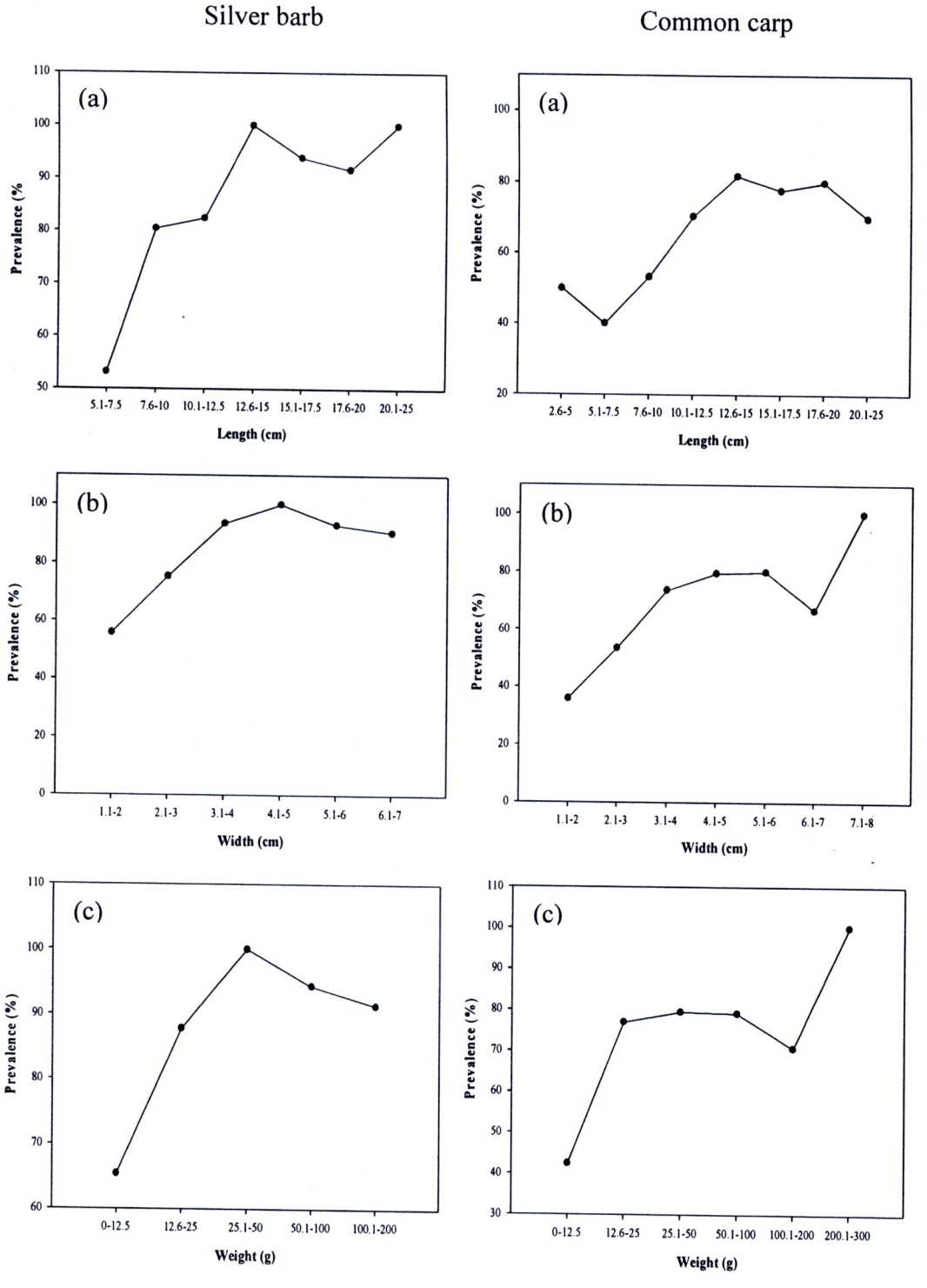


Figure 4.10 Size-related prevalence of *H. taichui* metacercariae in silver barb and common carp from Laos-viet farm. (a): length, (b): width and (c): weight.

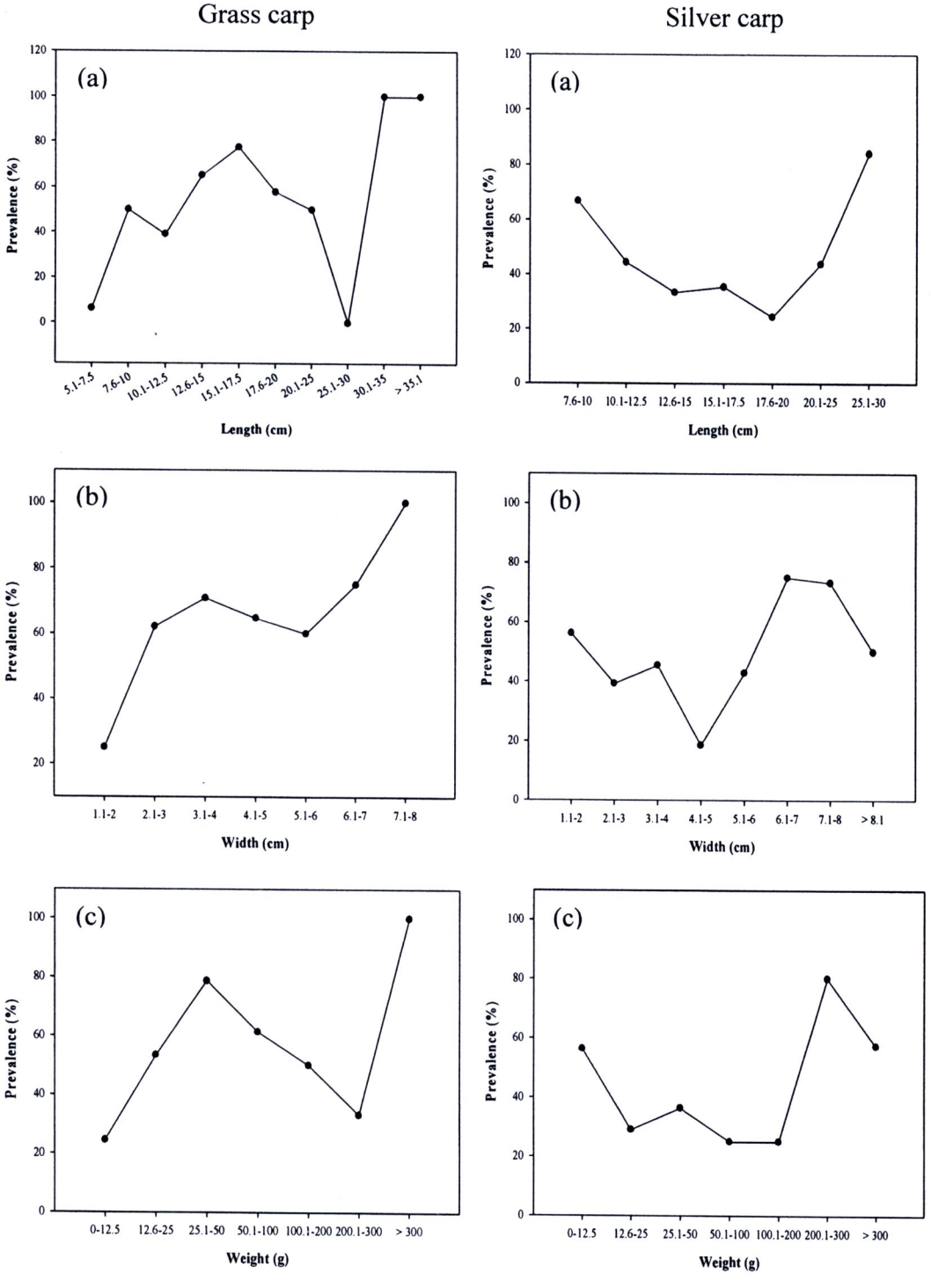


Figure 4.11 Size-related prevalence of *H. taichui* metacercariae in grass carp and silver carp from Laos-viet farm. (a): length, (b): width and (c): weight.

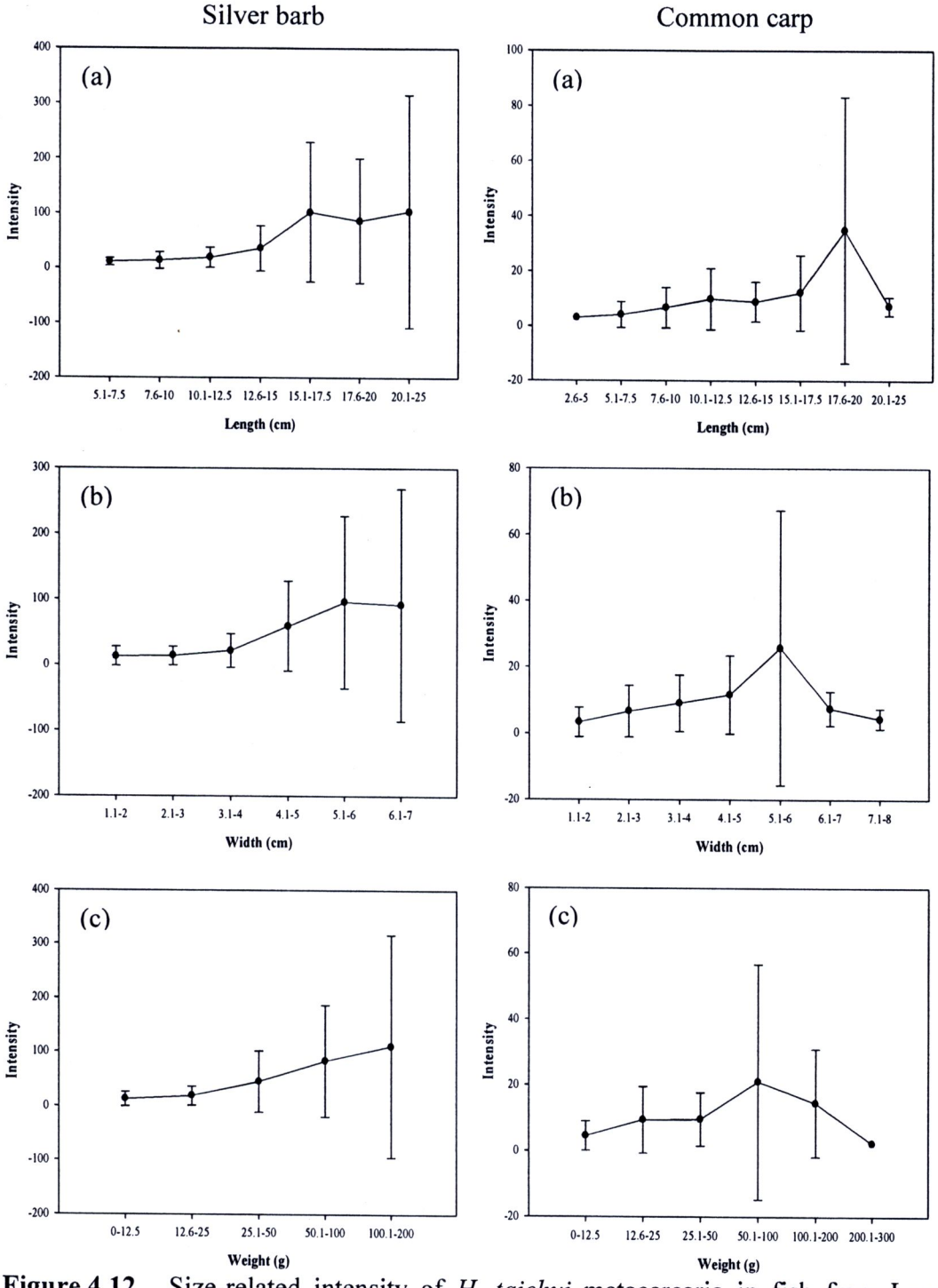


Figure 4.12 Size-related intensity of *H. taichui* metacercaria in fish from Laos-viet farm. Data shown are mean ± SD of cyst/fish. (a): length, (b): width and (c): weight.

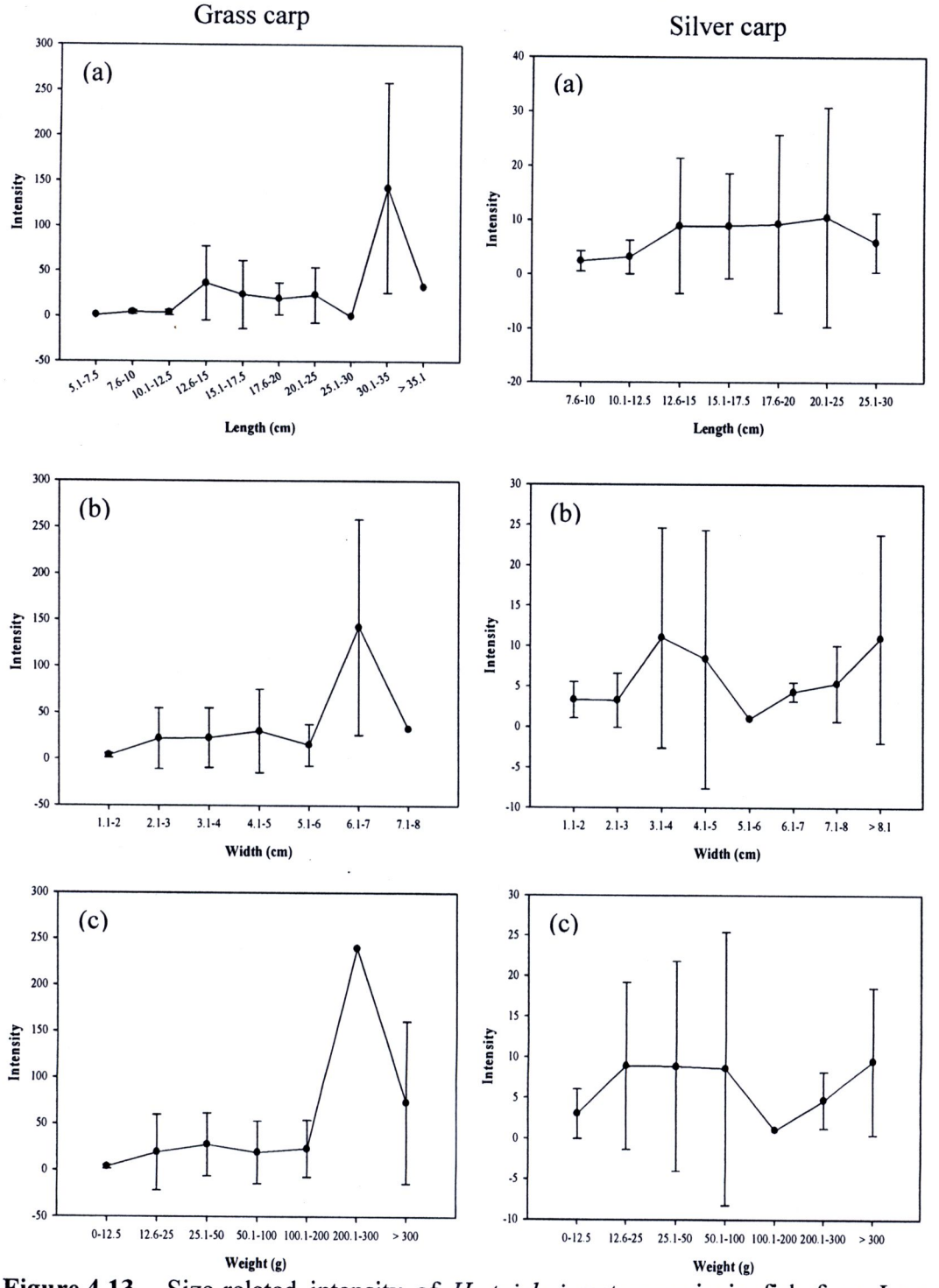


Figure 4.13 Size-related intensity of *H. taichui* metacercaria in fish from Laos-viet farm. Data shown are mean ± SD of cyst/fish. (a): length, (b): width and (c): weight.

4.4 Identification of FZT metacercariae and cercariae by PCR method

Unknown metacercariae recovered from each species of fish (at month 0 and 1), were pooled and subjected to PCR for analyses.

Based on specific PCR protocols analyses of pooled metacercariae revealed that *H. taichui* were found in 4 species of fish (mrigal, common carp, grass carp and silver carp) (Figure 4.14). *H. pumilio* DNA was detected in 3 species of fish (mrigal, grass carp, silver barb) (Figure 4.15). In case of *Centrocestus* sp. only silver barb from SSPP farm showed positive PCR (Figure 4.16).

Analyses of cercariae by PCR indicated that *H. taichui* were found in *Tarebia granifera* from LV but not in snails from SSPP farm (Figure 4.14). In case of *H. pumilio*, positive PCR results were observed in *Melanoides tuberculata* form both LV and SSPP farms (Figure 4.15 and Table 4.3).

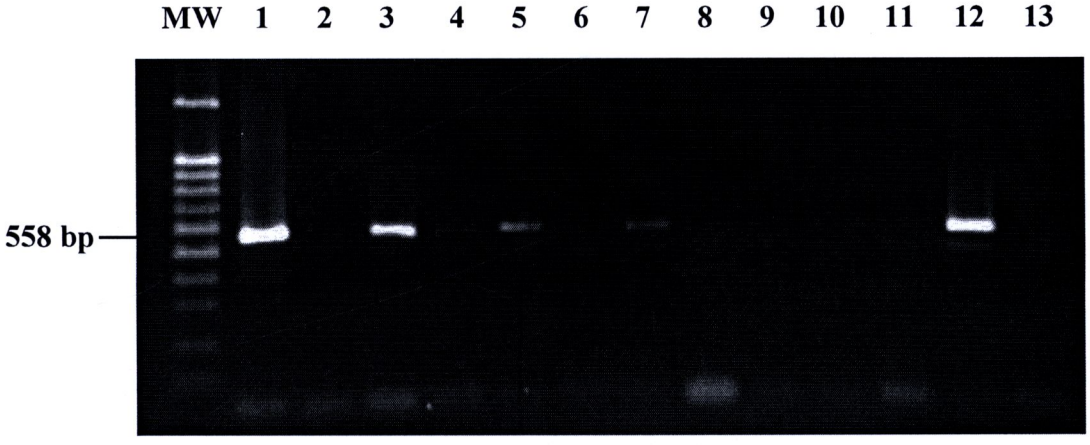


Figure 4.14 The DNA fragments resulted from PCR analyses using *H. taichui*- specific primer. Lane MW is DNA size markers (100 bp). Plasmid DNA of adult *H. taichui* as positive control (lane 1) and negative control (lane 2). The DNA products in lane 3, 4, 5, 6, 7, 8, 9, 10 are unknown metacercariae from mrigal, common carp, grass carp, silver barb, silver carp, bighead carp, rohu and red finned pecu, respectively. Lane 11, 12, 13 are cercariae from *M. tuberculata* from SSPP farm, cercariae from *T. granifera* from

LV farm, cercariae from *M. tuberculata* from LV farm, respectively. The DNA bands were visible in lane 3, 4, 5, 7 and 12. No bands were seen in lane 6, 8, 9, 10, 11 and 13.

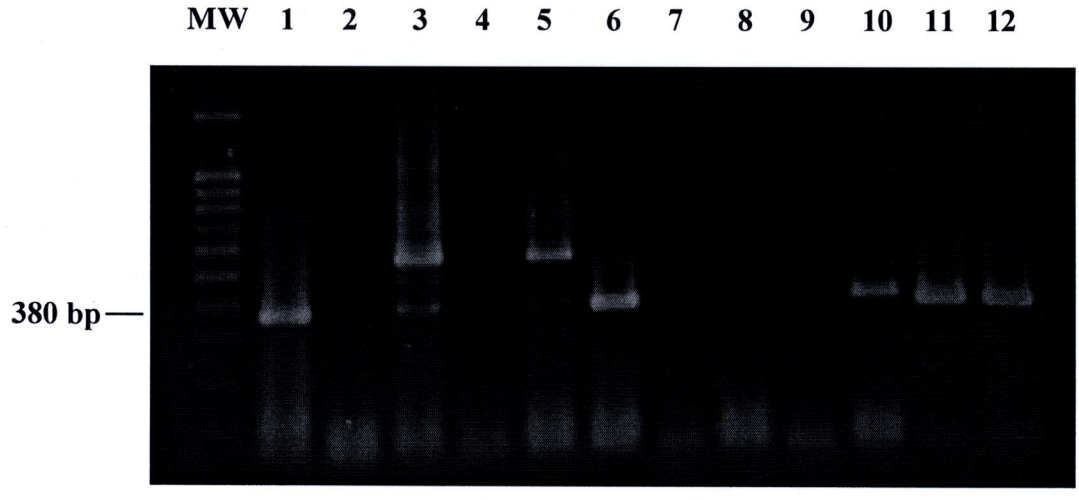


Figure 4.15 The DNA fragments resulted from PCR analyses using *H. pumilio*-specific primer, MW is DNA size markers (100 bp). DNA of the cercariae *H. pumilio* as positive control (lane 1) and negative control (lane 2). The DNA products in lane 3, 4, 5, 6, 7, 8, 9, 10 are unknown metacercariae from mrigal, common carp, grass carp, silver barb, silver carp, bighead carp, rohu and red finned pecu, respectively. Lane 11 and 12 are cercariae from *M. tuberculata* from SSPP farm, cercariae from *M. tuberculata* from LV farm, respectively. The DNA bands were visible in lane 3, 5, 6, 10, 11 and 12. No bands were seen in lane 4, 7, 8, and 9

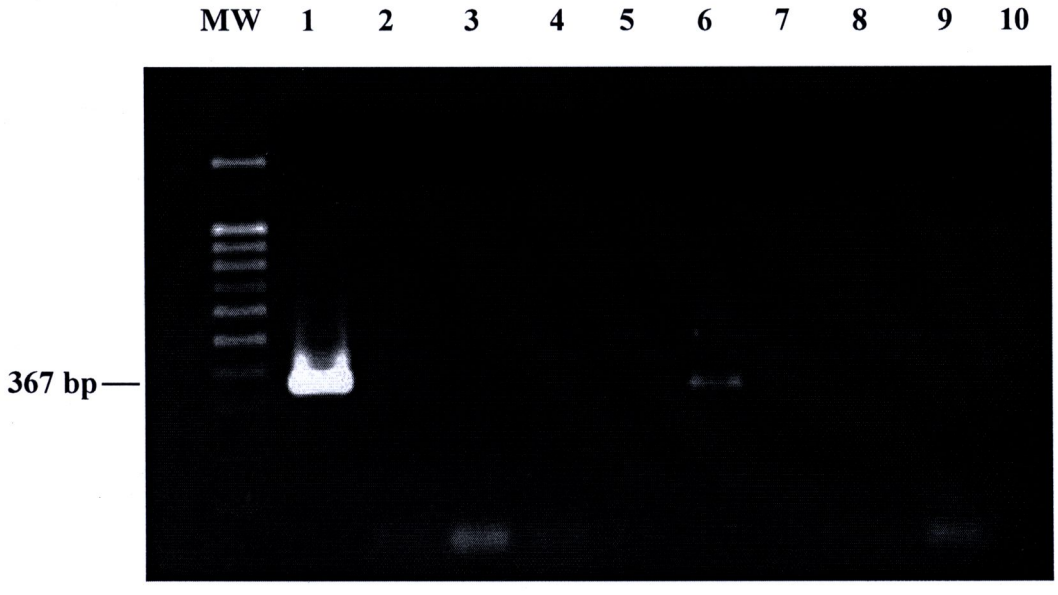


Figure 4.16 The DNA fragments resulted from PCR analyses using *Centrocestus* sp.-specific primer, MW is DNA size markers (100 bp). Plasmid DNA of the adult *Centrocestus* sp. as positive control (lane 1) and negative control (lane 2). The DNA templates in lane 3, 4, 5, 6, 7, 8, 9, 10 are unknown metacercariae from mrigal, common carp, grass carp, silver barb, silver carp, bighead carp, rohu and red finned pecu, respectively. The DNA band was visible in lane 6. No bands were seen in lane 3, 4, 5, 7, 8, 9 and 10.

4.5 Summary of FZT metacercariae detected by morphological and molecular methods

When using result from both morphological and PCR analyses of metacercariae, there are 8 out of 9 species of fish from LV farm harbored FZT (Table 4.1). *H. taichui* are found in 7 species of fish and *H. pumilio* is found in 4 species of fish.

In case of SSPP farm, only two species of fish namely silver barb and common carp harbored FZT metacercariae (Table 4.2). Most importantly, *O. viverrini*, *H. taichui* and *Centrocestus* sp. are found in silver barb while common carp had only *Centrocestus* sp. metacercariae.

Table 4.1 Summary of FZT metacercariae detected by morphological and molecular methods from Laos-viet farm.

Common name	Species of FZT metacercariae							
	Morphological			Molecular methods*				
	OV	HT	CT	OV	HT	HP	CT	CS
Silver barb	-	+	-	-	-	+	-	-
Mrigal	-	+	-	-	+	+	-	-
Grass carp	-	+	-	-	+	+	-	-
Silver carp	-	+	-	-	+	-	-	-
Common carp	-	+	-	-	+	-	-	-
Bighead carp	-	+	-	-	-	-	-	-
Red finned pecu	-	-	-	-	-	+	-	-
Rohu	-	+	-	-	-	-	-	-
Nile tilapia	-	-	-	-	-	-	-	-

* FZT unidentified metacercariae detected by PCR

OV: *Opisthorchis viverrini*, HT: *Haplorchis taichui*, HP: *Haplorchis pumilio*, CT: *Centrocestus* sp., CS: *Clonorchis sinensis.*

Table 4.2 Summary of FZT metacercariae detected by morphological and molecular methods from Sangsavang pun pla farm.

Common name	Species of FZT metacercariae							
	Morphological			Molecular methods*				
	OV	HT	CT	OV	HT	HP	CT	CS
Silver barb	+	+	-	+	-	-	+	-
Mrigal	-	-	-	-	-	-	-	-
Grass carp	-	-	-	-	-	-	-	-
Silver carp	-	-	-	-	-	-	-	-
Common carp	-	-	+	-	-	-	-	-
Bighead carp	-	-	-	-	-	-	-	-
Red finned pecu	-	-	-	-	-	-	-	-
Rohu	-	-	-	-	-	-	-	-
Nile tilapia	-	-	-	-	-	-	-	-

* FZT unidentified metacercariae detected by PCR

OV: *Opisthorchis viverrini*, HT: *Haplorchis taichui*, HP: *Haplorchis pumilio*, CT: *Centrocestus* sp., CS: *Clonorchis sinensis*.

Table 4.3 Trematodes cercariae identified by PCR method from the studied farms in Lao PDR.

Species of snail	Locality	Type of cercariae	Species of FZT metacercariae detected by PCR				
			OV	HT	HP	CT	CS
Melanoides tuberculata	SSPP	Parapleurolophocercous cercariae	-	-	+	-	-
Melanoides tuberculata	LV	Parapleurolophocercous cercariae	-	-	+	-	-
Tarebia granifera	LV	Parapleurolophocercous cercariae	-	+	-	-	-

4.6 Prevalence of cercariae in snail samples from the studied farms

The samples consisted of 3 species of snails from SSPP farm and 4 species of snails from LV farm. Examinations of snails within the SSPP farm revealed two types of trematode cercariae in *Bithynia siamemsis goniomphalos* namely monostome cercariae (0.20%) and xiphidiocercariae (6.45-8.33%). *M. tuberculata* shed two types of cercariae, namely parapleurolophocercous cercariae (1.76%) and xiphidiocercariae (6.15-10.00%). *Filopaludina* sp. harbored no trematode cercariae (Table 4.4).

Within the snail samples from LV farm, the majority being *T. granifera* while the other two species were fewer in numbers namely *M. tuberculata* and *Filopaludina* sp. As detailed in Table 1, 3 types of cercariae namely parapleurolophocercous cercariae (1.24-10.25%), xiphidiocercaria (0.17-1.19%) and monostome cercariae (0.20%) were detected in *T. granifera*. Although with relatively smaller sample size, parapleurolophocercous cercariae (3.85-7.69%) and xiphidiocercariae (6.34%) were found in *M. tuberculata*. No cercariae was found in *Filopaludina* sp. For parapleurolophocercous cercariae in *T. granifera* is known as type 1 which represented *H. taichui* (Kumchoo et al., 2005) and for those in *M. tuberculata*, they are known as type 2 which represented *H. pumilio* (Díaz et al., 2008).

Identifications of cercariae into 3 major types and their morphologies are shown in Figure 4.16 and confirmations by PCR were presented in Table 4.3.

Table 4.4 Prevalence rates of trematode cercariae in snail samples from the studied farms in Lao PDR.

Farm	Month sampling	Snail species	Snail sample size	FZT cercariae prevalence (%)			
				Monostome	Xiphidiocercariae	Parapleuro-lophocercous Type 1	Parapleuro-lophocercous Type 2
SSPP	0	*M. tuberculata*	130	-	6.15	-	-
		B. s. goniomphalos	492	0.20	8.33	-	-
	1	*M. tuberculata*	170	-	10	-	1.76
		B. s. goniomphalos	31	-	6.45	-	-
		Filopaludina sp.	25	-	-	-	-
LV	0	*T. granifera*	365	0.20	-	1.37	-
	1	*T. granifera*	923	-	1.19	2.93	-
		M. tuberculata	30	-	-	-	6.67
		Filopaludina sp.	39	-	-	-	-
	3	*T. granifera*	1205	-	0.17	1.24	-
		M. tuberculata	13	-	-	-	7.69
	4	*T. granifera*	517	-	0.58	10.25	-
		M. tuberculata	205	-	6.34	-	5.85
	5	*T. granifera*	1182	-	0.42	3.55	-
		M. tuberculata	26	-	-	-	3.85

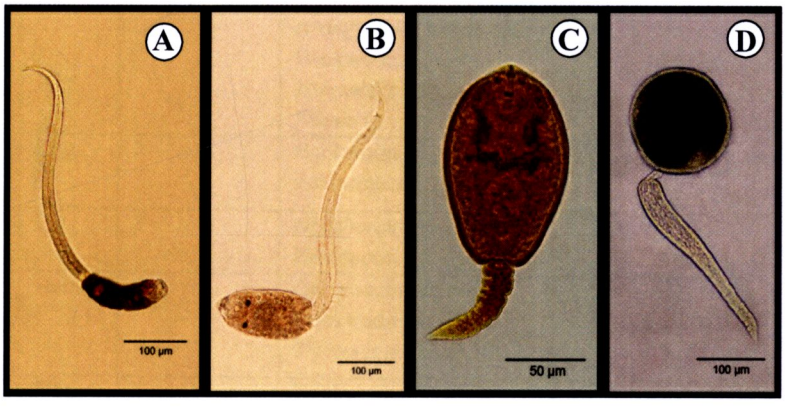


Figure 4.17 Images of trematode cercariae observed in snails. A: Parapleuro-lophocercous cercariae (heterophyidae type 1: *H. taichui*); B: Parapleuro-lophocercous cercariae (heterophyidae type 2: *H. pumilio*) (A and B are human and mammal trematodes); C: Xiphidiocercariae (Virgulate type) (trematode of invertebrate, amphibians and reptile); D: Monostome cercariae (trematode of cattle, horse or frog).

4.7 Prevalence of parasitic infection in human and animal hosts

A list of prevalence of parasites detected in stool samples from human, dog and cat in the two farms is presented in Table 4.5. The parasites found in the two farms consisted of FZT, soil transmitted nematodes and protozoa. Among the FZT, the majority were the liver fluke, *O. viverrini*, (41.67% in human and 100% for cat from SSPP farm and 30% for human from LV farm). Minute intestinal flukes (MIF) were also present in human (33.33%) form SSPP farm. Echinostomes and tape worms were also detected in human and dog from SSPP farm. The soil transmitted nematodes *Trichuris trichiura*, hookworm and *strongyloides stercoralis* were also present in both farms. For protozoa, *Isospora* sp. was observed in LV farm with prevalence rates of 25%.

Table 4.5 Prevalence rates of parasite infection in humans and animal reservoir hosts from the studied farms measured by stool examination.

Farm	Host	Sample size	Parasite	No. of infected	% Prevalence
SSPP	Human	12	*Opisthorchis viverrini*	5	41.67
			minut intestinal fluke	4	33.33
			strongyloides stercoralis	3	25
			Hookworm	2	16
			Hymenolepis sp.	1	8.3
			Taenia sp.	1	8.3
	Dog	2	Hookworm	1	50
			Echinostome	1	50
	Cat	3	*Opisthorchis viverrini*	3	100
			Hookworm	3	100
LV	Human	20	*Opisthorchis viverrini*	6	30
			Hookworm	2	10
			Trichuris trichiura	7	35
	Dog	4	Hookworm	1	25
			Toxocara sp.	1	25
			Isospora sp.	1	25