CHAPTER 2

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Fresh-water fish

According to current information, the metacercariae of *O. viverrini* are frequently found in fresh-water fish in the Suborder Cyprinoidei. The present study therefore focused on this group of fish. Fish from natural habitats, known by interviewing the sellers, were purchased directly from the markets located in Amphoe Muang, Amphoe Hangdong, Amphoe Sarapee, Amphoe Sansai and Amphoe Doisaket, Chiang Mai province. Reared fish were purchased from fish farms located in the five districts above.

All fish were identified to the genus or species using the taxonomic characters described by The Provincial Department of Fisheries, located in Chiang Mai, and by Taki (1974).

The numbers of fish required for this study were estimated as follows:

$n = (z_1+z_2)^2 \times 2\bar{p}\,(1-\bar{p}) / (p_1-p_2)^2$ (Smith and Morrow, 1991)

where n = minimum number of fish in each collecting site

z = standard value, 95% confidence interval (z_1 =1.96, z_2 = 1.64)

p_1 = prevalence of *O. viverrini* metacercariae in cyprinoid fish from natural habitats, average 51% (Sujjanun and Thitasut, 1971) or roughly 0.5

p_2 = prevalence of *O. viverrini* metacercariae in cyprinoid fish from fish farms, expected to be half of the lowest prevalence in cyprinoid fish from natural habitats being recorded (i.e. 24.4%, Sujjanun and Thitasut, 1971) or 0.12

$\bar{p}$ = average of p_1 and p_2 = 0.38

By this calculation the minimum number of fish required from each collecting site was 39. However, the numbers of fish examined in the present study were as follows :

natural habitats	**species of fish**	**number**
Amphoe Muang	*Puntius leiacanthus*	50
Amphoe Hangdong	*Cyclocheilichthys repasson*	50
	Puntius leiacanthus	50
	Osteocheilus species	81
Amphoe Sarapee	*Puntius leiacanthus*	51
Amphoe Sansai	*Puntius leiacanthus*	47
	Esomus metallicus	48
	Osteocheilus species	50
Amphoe Doisaket	*Puntius leiacanthus*	51
		total 478
fish farms		
Amphoe Muang	*Osteocheilus* species	50
	Puntius gonionotus	50
Amphoe Hangdong	*Puntius gonionotus*	50
Amphoe Sarapee	*Puntius gonionotus*	47
Amphoe Sansai	*Puntius gonionotus*	50
Amphoe Doisaket	*Puntius gonionotus*	50
		total 297

2.1.2 Raw-fish products

Two types of raw-fish products, "*pla-jom*" and "*pla-som*", were studied. Generally, "*pla-jom*" is made from small fish, mainly cyprinoid, mixed with salt (about 1 : 10, salt : fish) and kept in a bottle at room temperature for about 1-3 months before being eaten. "*Pla-som*" is made from medium and large sizes of fresh cyprinoid fish or others, mixed with boiled rice, salt and garlic, and kept at room temperature for at least 24 hours before being eaten. The product samples were purchased from the markets, and about 100 g of each of samples were examined. Details of number and collecting site of the samples are shown below:

places	**number of samples**		**number of samples**	
Amphoe Muang	"*pla-jom*"	6	"*pla-som*"	16
Amphoe Hangdong	"*pla-jom*"	7	"*pla-som*"	4
Amphoe Sarapee	"*pla-jom*"	4	"*pla-som*"	3
Amphoe Sansai	"*pla-jom*"	8	"*pla-som*"	2
Amphoe Doisaket	"*pla-jom*"	10	"*pla-som*"	5
	total	**35**		**30**

2.1.3 Essential equipment

Shaking water bath

Blender

Light microscope

Stereoscopic microscope

Petri dishes

Flasks 250 ml, 500 ml, 1,000 ml

Sediment flasks (Conical cylinders)

Mesh screens 600 μm, 300 μm

Gauze

Pasture pipettes

Slides and Cover glasses

Tray, size 30x30 cm

Films and Camera

2.1.4 Major chemicals and reagents

0.85% NaCl

1% hydrochloric acid

Pepsin (1:10,000, SIGMA Co., Ltd.)

10% formalin solution

2.2 METHODS

2.2.1 Collection of metacercariae from fresh-water fish

The collected fish were washed with water several times before their abdomens were opened and then the internal organs removed. The weight and length of dissected fish were recorded. Metacercariae were recovered and collected by a digestion method described by Pattanapanyasat *et al.* (1983). Briefly, the individual fish was homogenized with acid pepsin solution (1% hydrochloric acid 1 ml : pepsin 1 g : 0.85% sodium chloride solution 100 ml) in a blender, with the ratio of fish and the solution of 1 g per 10 ml. The mixtures were incubated in a shaking water bath at 37°C for 1.5 h. The whole digested content was filtered through 600 μm and 300 μm mesh screens into a conical cylinder and

allowed to stand for sedimentation (about 20 min). The sediment was washed with 0.85% sodium chloride several times until the supernatant was clear. It was then poured into a petri dish and examined under stereoscopic microscope for searching for metacercariae.

Identification of metacercariae was made under a light microscope by using criteria described by Vajrasthira *et al.* (1961); Pearson (1964); Pearson and Ow-Yang (1982) and Radomyos *et al.* (1983). Those of *O. viverrini* can be recognized by the morphological features described in section 1.2.4.

This morphological identification method appears to be simple and practical and has been used in many studies, e.g. Kruatachue *et al.* (1982); Vichasri *et al.* (1982); Tesana *et al.* (1985) and Scholz *et al.* (1991).

2.2.2 Collection of metacercariae from raw-fish products

The raw-fish products were also examined by the digestion method as described above. About 100 g of samples were digested individually. The recovered metacercariae were observed for their morphology and movement under the microscope.