EFFICACY OF DIGESTION METHOD FOR COLLECTING METACERCARIAE FROM THE FISH BODY

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In the Far East, particularly in Japan and in China, several kinds of fish have been proved taking the part of second intermediate host for various larval trematodae, namely, *Clonorchis sinensis*, *Metorchis orientalis*, *Echinostomus perfoliatus* and *E. japonicus*, several kinds of Cyathocotyle, Centrocestus and others, and we have to collect and examine so very often various metacercariae from fish bodies. The digestion method is one of the most convenient collection means of various metacercariae from the tissue of fish-bodies, and we adopted the digestion method for such purposes for years. The routine procedure of this method is as follows. The portion of fish to be examined is minced to fine pieces; put them into a flask and add them an adequate amount of artificial gastric juice after putting them in an incubator, regulate at ca. 37°C for one to four hours; pour them into a large watch glass; add them an adequate amount of 0.7% saline; after a few minutes, remove the major part of supernatant with a pipette; after repeating such procedure several times and metacercariae found in the residual sediment are finally collected using a small pipette under a dissecting microscope.

However, during this procedure, a certain number of various metacercariae with fine floating substances are usually lost on removing the supernatant. The purpose of this work is to determine how much part of the total metacercariae in the tissue of fish body could be collected when adopting such a routine method.

Materials: As materials, authors used *Pseudorasbora parva* and *Sarcocheilichthys sinensis*, five in number respectively; their body length ranging from three to ten cm. These were obtained from a Chinese market in Shanghai. These two species of fish in this area are usually infested with various species of metacercariae.

Method: Each fish was separated into five different parts, head, fins, gills, scales and the remains. On each part, the above mentioned routine procedures were applied respectively, and all the metacercariae found in residual sediment were collected. The number of metacercariae

thus collected is signified as "collected" metacercariae in the following Table. Then, the same procedures were performed with all the supernatant repeatedly and the metacercariae found were collected on each time until no such were found in its residual sediment. All the metacercariae thus found in these procedures are signified as "lost" metacercariae in the following Table.

Results: Table 1 shows the number of total and collected metacercariae in each five *Pseudorasbora*, and Table 2 in each five *Sarcocheilichthys*. In both Tables, the total number of metacercariae is represented as the total sum of collected and lost metacercariae.

As seen in Table 1, in the case of *Pseudorasbora*, average ratio of collected metacercariae to the total number is ca. 60%. Among the species of metacercariae, that of *Clonorchis sinensis* showed the highest ratio of the collected metacercariae, ca. 70% of the total number. The ratio of collected metacercariae of *Exorchis oviformis*, *Metacercaria hasegawai* and *Metorchis orientalis* were ca. 66%, 69% and 63% of the total number respectively, whereas that of *Cyathocotyle orientalis* showed the lowest ratio of the collected metacercariae among various species of metacercaria. In the case of *Sarcocheilichthys*, an average ratio of collected metacercariae to the total number of metacercaria showed ca. 81%, which is higher than that in the case of *Pseudorasbora*. Among the various metacercariae, that of *Clonorchis* shows the highest, and that of *Cyathocotyle* the lowest ratio as in the case of *Pseudorasbora*. Although cysts of both of *Clonorchis* and *Cyathocotyle* metacercaria are found mostly in subcutaneous and muscle tissues, that of the former are usually encircled with thicker outer cyst wall produced by the host, which difference is thought to be one of the reason of relatively lower ratio of collecting the metacercaria of *Cyathocotyle*.

On the other hand, the relatively higher efficacy in collecting metacercariae of *Clonorchis* indicates the usefulness of this method when we examine the relative incidence of *Clonorchis* in its various second intermediate fish hosts.

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Table 1
Collected metacercaria form *Pseudorasbora parva*

Species of metacercaria	Number of metacercaria	No. of fish examined					Total Sum
		No. 1	No. 2	No. 3	No. 4	No. 5	
1. <i>Clonorchis sinensis</i>	Total number	18	317	41	26	5	407
	Collected number	14	232	32	18	3	299
	Percentage of collect. number to total	77.8	73.2	78.0	69.2	60.0	73.5
2. <i>Exorchis oviformis</i>	Total number	122	6553	892	880	806	9253
	Collected number	64	4600	509	446	559	6178
	Percentage of collect. number to total	52.5	70.2	57.1	50.7	69.3	66.8
3. <i>Metacercaria hasegawai</i> a	Total number	0	204	0	1	0	205
	Collected number	0	140	0	1	0	141
	Percentage of collect. number to total	0	68.6	0	100.0	0	68.8
4. <i>Metorchis orientalis</i>	Total number	381	3307	1738	438	3415	9279
	Collected number	175	2020	979	242	2463	5879
	Percentage of collect. number to total	45.9	61.1	56.3	55.2	72.1	63.4
5. <i>Cyathocotyle orientalis</i>	Total number	4386	3812	11149	3126	10712	33185
	Collected number	1524	1748	6289	1853	7389	18803
	Percentage of collect. number to total	34.7	45.9	56.4	59.3	69.0	56.7
6. <i>Echinocasmus japoniscu</i>	Total number	330	1056	746	34	175	2341
	Collected number	246	774	236	29	147	1432
	Percentage of collect. number to total	74.5	73.3	31.6	85.3	84.0	61.2
7. <i>Echinocasmus perforiatus</i>	Total number	0	3	2	0	7	12
	Collected number	0	3	2	0	7	12
	Percentage of collect. number to total	0	100.0	100.0	0	100.0	100.0
8. <i>Proisorhynchus echinatus</i>	Total number	1	1	4	1	0	7
	Collected number	1	1	1	0	0	3
	Percentage of collect. number to total	100.0	100.0	25.0	0	0	42.9
9. Unspecified	Total number	0	0	3	0	2	5
	Collected number	0	0	3	0	2	5
	Percentage of collect. number to total	0	0	100.0	0	100.0	100.0

Total Sum	Total number	5238	15253	14575	4506	15122	54694
	Collected number	2024	9518	8051	2589	10570	32752
	Percentage of collect. number to total	38.6	62.4	55.1	57.5	69.9	59.9

Table 2
Collected metacercaria from *Sarcocheilichthys sinensis*

Species of metacercaria	Number of metacercaria	Number of fish examined					Total Sum
		No. 1	No. 2	No. 3	No. 4	No. 5	
1. <i>Clonorchis sinensis</i>	Total number	623	1557	81	2020	46	4327
	Collected number	537	1517	58	1454	30	3596
	Percentage of collect. number to total	86.2	97.4	71.6	72.0	65.2	83.1
2. <i>Exorchis oviformis</i>	Total number	45	43	5	35	18	146
	Collected number	34	36	3	14	10	97
	Percentage of collect. number to total	75.6	83.7	60.0	40.0	55.6	66.4
3. <i>Metacercaria hasegawai</i> a	Total number	55	89	23	2	285	454
	Collected number	36	74	12	2	195	319
	Percentage of collect. number to total	65.4	83.1	52.2	100.0	68.4	70.3
4. <i>Matorchis orientalis</i>	Total number	6	0	3	0	0	9
	Collected number	6	0	3	0	0	9
	Percentage of collect. number to total	100.0	0	100.0	0	0	100.0
5. <i>Metorchis taiwanensis</i>	Total number	0	0	0	15	0	15
	Collected number	0	0	0	8	0	8
	Percentage of collect. number to total	0	0	0	53.3	0	53.3
6. <i>Cyathocotyle orientalis</i>	Total number	1	0	0	0	0	1
	Collected number	6	0	0	0	0	1
	Percentage of collect. number to total	100.0	0	0	0	0	100.0
7. <i>Echinochasmus perfoliatus</i>	Total number	4	0	3	47	0	54
	Collected number	4	0	3	30	0	37
	Percentage of collect. number to total	100.0	0	100.0	63.8	0	68.5

8. <i>Prosorhynchus echinatus</i>	Total number	4	0	0	0	0	4
	Collected number	3	0	0	0	0	3
	Percentage of collect. number to total	75.0	0	0	0	0	75.0
9. Unspecified	Total number	6	2	2	2	0	12
	Collected number	0	1	2	2	0	5
	Percentage of collect. number to total	0	50.0	100.0	100.0	0	41.7
Total Sum	Total number	744	1691	117	2121	350	5023
	Collected number	621	1658	81	1510	235	4075
	Percentage of collect. number to total	83.5	96.3	69.2	71.2	67.1	81.1