จุฬาลงกรณ์มหาวิทยาลัย
ทุนวิจัยรัชดาภิเษกสมโภช

รายงานผลการวิจัย

การหาความจำเพาะต่อโฮสท์ของโกลคิเดียมของหอยมุกน้ำจืด *Hyriopsis (Limnoscapha) myersiana* (Lea, 1856)

โดย

สมศักดิ์ ปัญหา

กรกฎาคม 2534

กิตติกรรมประกาศ

ผู้วิจัยต้องขอแสดงความขอบพระคุณต่อจุฬาลงกรณ์มหาวิทยาลัย และบุคคลต่าง ๆ ที่ได้ช่วยจัดสรรเงินผลประโยชน์ของมหาวิทยาลัยส่วนหนึ่งจัดตั้งเป็นเงินทุนวิจัยรัชดาภิเษกสมโภช ซึ่งทำให้ผู้วิจัยซึ่งเป็นผู้สอนและผู้วิจัยท่านอื่น ๆ ได้มีโอกาสได้ทำงานให้บรรลุผล ซึ่งจะทำให้เกิดประโยชน์ต่อผู้วิจัยและส่วนรวม ผู้วิจัยก็ต้องขอขอบพระคุณเป็นอย่างสูงต่อคณะกรรมการประจำสาขาชีวภาพที่ได้พิจารณาให้ได้รับทุนครั้งนี้ และยังได้ช่วยเสนอแนะในทุกเรื่องของงานวิจัย

ขอกราบขอบพระคุณ คุณบัญชันว์ มหาวรรณ ผู้อำนวยการมูลนิธิแพทย์อาสาสมเด็จ-พระศรีนครินทราบรมราชชนนี ที่ให้ความกรุณาเอื้อเฟื้อสถานที่ทำงานวิจัย ณ บริเวณไร่ทับสาธร ติดแม่น้ำแควน้อย จังหวัดกาญจนบุรี และยังได้ให้กำลังใจมาโดยตลอด

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ขอขอบพระคุณพนักงานขับรถของคณะฯ คือ คุณบุญชลอ พลอยงาม และ คุณวรา นิ่มงาม ที่ได้ช่วยให้งานวิจัยนี้ลุล่วงไปได้ดี

ที่จะลืมเสียมิได้คือ คุณศักดา นามวงษ์, คุณสุรินทร์, คุณเกตุ, คุณเจริญ ต้นตระกูล ชาวบ้านอำเภอบ้านหมอ จ.สระบุรี, ชาวบ้าน ต.ทาแค จ.ลพบุรี, ลุงเฟื่อง ขำปั้น อำเภอชุมแสง จ.นครสวรรค์, นายติ่ง, นายระเบิด จากไร่ทับสาธร ที่ได้ช่วยดำน้ำเก็บตัวอย่างหอยและปลา ผู้วิจัยขอกราบขอบพระคุณมา ณ. โอกาสนี้

ท้ายสุดนี้ ขอขอบพระคุณเจ้าหน้าที่ฝ่ายวิจัย จุฬาฯ ทุกท่านที่ได้อำนวยความสะดวกในทุกเรื่อง และขอขอบคุณ คุณศิริลักษณ์ นาคฉาย และ คุณเอกสิทธิ์ พุ่มมิตร ที่ช่วยพิมพ์รายงานวิจัย

ชื่อโครงการวิจัย	การหาความจำเพาะต่อโฮสท์ของโกลคิเดียมของหอยมุกน้ำจืด *Hyriopsis (Limnoscapha) myersiana* (Lea, 1856)
ชื่อผู้วิจัย	นายสมศักดิ์ ปัญหา
เดือนและปีที่ทำวิจัยเสร็จ	มีนาคม 2534

บทคัดย่อ

ได้ทำการศึกษาวงจรสืบพันธุ์และศึกษาความจำเพาะต่อโฮสท์ของโกลคิเดียมของหอยมุกน้ำจืด *Hyriopsis (Limnoscapha) myersiana* ในระหว่างเดือนเมษายน 2533 ถึงเดือนพฤษภาคม 2534 มาสุเปียเกิดขึ้นตั้งแต่เดือนพฤศจิกายน จะพบสูงสุดในเดือนธันวาคมและมกราคม โกลคิเดียมจะเกิดขึ้นราวปลายเดือนพฤศจิกายน การศึกษาในห้องปฏิบัติการพบว่าหอยจะปล่อยโกลคิเดียมออกหมดในระยะเวลา 24 ชั่วโมง

ได้ทำการทดลองเรื่องความจำเพาะต่อโฮสท์ในลูกปลา11ชนิดโดยใช้ความเข้มข้นของโกลคิเดียม 4 ระดับ แล้วแสดงกราฟเปอร์เซ็นต์การตายในโกลคิเดียมแต่ละระดับด้วยค่า LE_{50} (ค่าความเข้มข้นโกลคิเดียมที่ทำให้ปลาตาย 50 %) ที่ 30 วัน หลังจากการเกาะติดของโกลคิเดียมของปลาแขยงข้างลาย *Mystus vittatus* ปลาสวาย *Pangasius sutchi* ปลากดเหลือง *Mystus nemurus* มีค่า 15,000, 20,000, 50,000 ตามลำดับ ปลาหมอช้างเหยียบและปลาหมอตาลนั้น มีการตายน้อยมาก ปลาที่มีความต้านทานต่อการเกาะของโกลคิเดียมและให้ผลผลิตเป็นหอยมุกวัยอ่อนสูงคือ ปลาบู่ *Oxeleotris marmoratus* และปลาหมอช้างเหยียบ *Pristolepis fasciatus* ปลาที่ไม่มีความต้านทานเลยคือปลาซิว *Rasbora* sp.

Project Title: Investigation on the Host Specificity of the Glochidium of Freshwater Pearl Mussel, *Hyriopsis (Limnoscapha) myersiana* (Lea, 1856)

Name of Investigator: Somsak Panha

Year: 1991

Abstract

The reproductive cycle and the host specificity of the freshwater pearl mussel, *Hyriopsis (Limnoscapha) myersiana* were studied from April 1990 to May 1991. Marsupia were found already in early November but, the peak occurred between December and January. The glochidia will emerge from late November till early March. In the laboratory, mussel took less than 24 hours to release all the glochidia.

The experiment on host specificity of the glochidia on the fry of 11 species of fish was carried out, using 4 levels of glochidium concentration. The mortality of the fish after infection was plotted against these exposure levels. After 30-days, the interpolated LE_{50} values (exposure concentration of glochidia that killed 50 % of the fry) for Iridescent mystus *(Mystus vittatus)*, Striped catfish *(Pangasius sutchi)*, Yellow Mystus *(M. nemurus)*, were 15,000, 20,000, 50,000 respectively. The mortalities were very low in Striped tiger nandid *(Pristolepis fasciatus)* and Temminck's kissing gourami *(Helostoma temmincki)*. The most resistant were Sand Goby *(Oxyeleotris marmoratus)* and Striped tiger nandid, which also gave the highest production of juveniles. Species of *Rasbora* were not resistant at all, all specimens died soon after infection.

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Investigation on the Host Specificity of the Glochidium of Freshwater Pearl Mussel, *Hyriopsis (Limnoscapha) myersiana* (Lea, 1856)

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Introduction

Freshwater pearl mussels have a very interesting life-cycle. In the larval stage (glochidium), it needs to parasitise (glochidiosis) with fish or some amphibians (Lefevre and Curtis, 1910; Seshaiya, 1941; Howard, 1951; Walker, 1981; Kraemer and Swanson, 1985). Panha (1990) reported 14 species of fish that are host to glochidia. Due to inadequate knowledge of its life-cycle, the pearl mussels are being directly and indirectly obliterated e.g. by over fishing, fishing in the reproductive season and water pollution (Bauer et al., 1980; Bauer, 1988).

Thailand has great potential for producing freshwater pearl on a large scale. This is because so many of Thai species of freshwater pearl mussels are capable of making pearls

(Nakjinda et al., 1989; Panha, 1990). Apart from studies by Panha (1989 and 1990) there has not been any work done on the relationship between glochidia and host in Thailand. Glochidia are very host specific. Studying their relationship is an essential for conservation and management of both fish and pearl mussels. This will also benefit the pearl industry in the long run.

The studies on relationship of mussels and hosts in other countries can be summarized as follow :

Murphey (1942) studied the relationship of freshwater pearl mussels and trouts in U.S.A. and found that trouts are preferred as host over other fish. Tedla and Fernando (1969) studied the attachment of glochidia of *Lamsiline radiata* on *Perca flavescens* in Canada and the favourite sites were found to be around the gills. Meyers et al., (1977) investigated glochidiosis of salmon and learned that 40-50 mm long salmon are suitable host for *Margaritifera margaritifera*. Heard (1975) found that glochidia of the genus *Anodonta* would encyst on carp. Zale and Neves (1982) found 4 families of fish which can host glochidia. Kondo (1983) found that the parasitic stage of glochidia to *Anodonta woodiana* takes about 12-15 days. Morton and Dudgeon (1984) showed that glochidia of *Anodonta woodiana* would encyst around gills, abdomina and fins. Bauer and Vogel (1987) found that encystment of glochidia induced in the fish an immune system and a protective mechanism. Previously encysted fish are capable to resist new encystment. Bauer (1987b) discovered 4 species of fish which host *Margaritifera margaritifera*. They are *Salmo trutta, S. salar, Hucho hucho* and *Salvelinus fontinalis*.

The present study deals with laboratory and field investigations of glochidiosis by glochidia of the mussel *Hyriopsis (Limnoscapha) myersiana* on eleven species of fish hosts (Silver barb, *Puntius gonionotus*; Julien's carp, *Cirrhina jullieni*; Rasbora, *Rasbora sp*; Yellow mystus, *Mystus nemurus*; Iridescent mystus, *M. vittatus*; Sand Goby, *Oxyeleotris marmoratus*; Striped tiger nandid, *Pristolepis fasciatus*; Temminck's kissing gourami, *Helostoma temmincki*; Java tilapia, *Tilapia mossambica*; Common climbing perch, *Anabas testudineus*; Striped catfish, *Pangasius sutchi*).

MATERIALS AND METHODS

From September 1988 to May 1991 the females of the mussel, *Hyriopsis (Limnoscapha) myersiana* incubating with eggs or glochidia in the marsupium were continuously observed. Histological study of gonads was also done especially in female mussels. The mussels were collected from the Kwae Noi River, Karnchanaburi Province and large irrigation canals, Saraburi and Nakornsawan Province (Fig. 1).

In December 1990, 40 recently hatched specimens of each of the following species Silver barb *(Puntius gonionotus)*, Julien's carp *(Cirrhina jullieni)* ,Rasbora *(Rasbora sp.)*, Yellow mystus *(Mystus nemurus)*, Iridescent mystus *(M. vittatus)*, Striped tiger nandid *(Pristolepis fasciatus)*, Temminck's kissing gourami

(Helostoma temmincki), Java tilapia *(Tilapia mossambica)*, Common climbing perch *(Anabas testudineus)*, Striped catfish *(Pangasius sutchi)* of 12-70 mm in total length were exposed individually to 7,700, 15,400, 30,800, 61,600 glochidia for 3 hours in small aquaria containing 3,750 ml of aerated, dechlorinated tap water. The juvenile fishes had never been infected by glochidia before. Of the Sand goby *(Oxyeleotris marmoratus)* only seven juveniles were collected and exposed only to 7,700 glochidia. The fry of all species was obtained from commercial suppliers. The aeration and fish movement kept the glochidia in suspension. Ten fishes were used for each exposure level and ten for unexposed control specimens all of which were subjected to the same conditions as the test fish. After exposure, the group of infected fish and the controls were kept in separate compartments of big aquaria. The fish were fed once daily wit artificial fish pellets.

Gravid mussels from the Kwae Noi River were the major source of glochidia. During the spawning period from 21 December 1990 to 26 January 1991, these mussels were collected by local people and by dredging from the river bottom and then transported to the laboratory and held for spawning.

In the laboratory, each gravid female mussel was held in 1,000 ml of dechlorinated tap water. Glochidia were usually released within 1-2 hrs after the water warmed to 28-29°C. (4-5 hrs at room temperature, 24-25°C). The larvae were examined for viability using movement of the valves as criteria. Glochidia from different mussels were pooled and the average number per ml of suspension was determined by serial dilution so the volume of the suspension necessary for the desired exposure concentration could be calculated. Only glochidia spawned on the days of fish exposure were used.

The 48-hr mortalities were recorded and plotted against the exposure levels on semilogarithmic paper. The 48-hr LE_{50} values (the exposure levels lethal to 50 % of the fish in 48 hr) were interpolated from the graphs for each fish species.

Dead fishes were preserved in 10 % buffered formalin so that attached glochidia could be counted subsequently.

The average number of glochidia on fish from each exposure level that died between the first hour of exposure and the time of juvenile excystment, approximately 11-27 days postexposure, was determined.

The cumulative mortalities for all groups of fish were determined 30 days postexposure when parasite excystment was complete for all fish species. Dead fish were preserved for later parasite enumeration.

Results

On the basis of histological examination of 10 specimens collected and sacrificed at monthly intervals from April 1990 to March 1991, I have found a pattern of the reproductive cycle of *Hyriopsis (Limnoscapha) myersiana* living in the Kwae Noi River.

April 1990 : histological sections of both sexes show the presence of extremely reduced germinal follicles. The mother gills are empty and no glochidia are present anywhere (Fig. 2) May-June : there are no differences from April.

July : the sections in this month, present germinal follicles at an active stage of development (Fig. 3).

August : active gametogenesis occurs in specimens of both sexes.

September-October : all the individuals reached full sexual maturity and the follicles are so full of masses of gametes that they are easily detectable in histological sections with the dissecting microscope (Fig. 4).

November-December : both sexes show reduced gametogenesis. In the mother's gills, which reach a thickness of about 0.5 cm, an enormous number of glochidia are present with a few developing eggs (Fig. 5).

January-February 1991 : the female follicles now present a few oocytes; in the lumen of most male follicles only a few ripe gamete are present; some still show large quantities of sperm. In the mother's gills, glochidia are very abundant but there is no evidence of developing oocytes.

March : the germinal follicles are empty, or almost empty. The mother s gills contain glochidia in only some areas (Fig. 6).

Panha's studies (1990) and additional researches, showed that marsupia receive secondary oocytes that will fertilized by sperms around early November. They will grow to fertilized eggs.

The embryos will go on developing into a larval stage, the glochidium, which differs totally from the adult. The glochidia have a hook on the shells, have byssus, and a group of adductor muscle. This development can be observed using a microscope. The marsupium containing unfertilized oocytes appears pale yellow while the fertilized eggs are brownish (Fig. 7). The glochidia will emerge from late November till early March. Studies from 1989-1991, showed that the peak of development of marsupia occurred between December and January. In one collection at River Kwae Noi on January 19^{th}, 1991, all the 67 female mussels, average size 12.1 ± 0.7 cm, were found to have ripe marsupia. They took about a month to release all the glochidia,they would need another one month to produce a second batch of ripe marsupia however, in the laboratory it took less than 24 hours to release all the glochidia.

Examination of the encysted fish from Natural water resources

Fishes from the same area as *Hyriopsis (Limnoscapha) myersiana* were collected by nets of different sizes and by a trawling net. They were fixed with 10 % neutral formalin before being examined for the encystment of glochidia. All the eight species of fish collected were found to have glochidia encysted at various parts. The principal sites were fins and gills (Table 1). Smaller fishes had more glochidia than the bigger ones (Fig. 8).

Host Specificity

Individual fry from 7 families and 11 species of fish were exposed to a glochidium suspension of *H. (L.) myersiana* at four concentration levels : 7,700, 15,400, 30,800 and 61,600. None of the control fish, but most of the fish exposed to large numbers of glochidia died during the first 48 hr after infection. In *Rasbora sp.* all fish were dead after exposure of every concentration of glochidia. However, no mortality at all occurred in *Anabas testudineus* (Fig. 9). Low resistance was shown by Cyprinidae e.g. *Puntius gonionotus*, *Cirrhinus jullieni*. *A. testudineus* had the highest resistance;next were *Helostoma temmincki*, *Pristolepis fasciatus* and *Mystus nemurus* respectively. In between were *Tilapia mossambica*, *Pangasius sutchi* and *Mystus vittatus* which would all die within 48 hrs at a concentration of 61,600 glochidia.

The average number of encysted glochidia in dead fish at 48 hrs post infection are shown in Table 2. The largest number of encysted glochidia were found on *Pristolepis fasciatus*, *Helostoma temmincki*, *Puntius gonionotus*, *Mystus nemurus* and *M. vittatus* in this order.

Only 7 *Oxyeleotris marmoratus* juveniles were exposed to glochidia at the lowest concentration and observed at River Kwae Noi. The same concentration was used in the natural environment with *Pristolepis fasciatus*, *Mystus nemurus* and *M. vittatus*, of which there was abundant fry available. In all 3 species the mortality rate was here lower than in the lab, especially with *Pristoleps fasciatus* where no death occurred at all with this glochidia concentration (Fig. 10).

Thirty days later at concentration 61,600 there were 100 % mortality in *Mystus vittatus*, *Pangasius sutchi* and *Tilapia mossambica* (Fig. 11). The LE_{50} of *M. vittatus* at 30 days post-infection was about 15,000. This means that at glochidial concentration of 15,000, there will be a 50 % death in *M. vittatus*, while LE_{50} of 20,000 and 50,000 were observed for *Pangasius sutchi* and *M. nemurus* respectively. Both *Pristolepis fasciatus* and *Helostoma temmincki* have a very low death rate. The most encysted areas in all species were fins and gills (Table 3) (Fig. 12).

Survived fish were kept on at a glochidium concentration of 7,700 to observe juvenile development both in actual environment and in the laboratory (Fig. 13). Mussel juveniles were found only from five species of fish : *Mystus nemurus, M. vittatus, Helostoma temmincki, Pristolepis fasciatus* and *Oxyeleotris marmoratus.* These juveniles were found with two peaks. On the 11^{th} and 12^{th} days, in *Mystus vittatus*; 13^{th} - 15^{th}, in *M. nemurus*; 18^{th} - 23^{rd} in *Helostoma temmincki* and *Pristolepis fasciatus* the latter can be found up till the 27^{th} days. In the natural environment observation on the 18^{th} - 24^{th} of *Helostoma temmincki* would have at least three to four times more juvenile mussels than those kept under laboratory conditions. In *Oxyeleotris marmoratus* the mussel juveniles were found on the 22^{th} - 26^{th} day in the same quantities as those of *Helostoma temmincki.* The juvenile mussels were continued to be cultured to observe their development onto adult mussels (Fig. 14)

Discussion

Concerning the life cycle of *Hyriopsis (Limnoscapha) myersiana*, the data collected from October 1989-April 1991 showed that the glochidia were released from November till February peaking around December and January. Similar pattern was found in two other water resources : a tributary of Chao Phraya River, Saraburi Province and River Kwae Noi, Kanchanaburi Province. So it seems that the reproductive period of thus freshwater pearl mussels would occur once in a year. During this period the water temperature was at it lowest and it was in winter time when water was clear and the current was slow or almost non-existent. In the Rainy season the water would be cloudy and have a strong current. The water temperature would be high in summer. These may be potential factors influencing the life cycle of this mussel. Cocker et al. (1921) reported that elimination of glochidia of *Anodonta* occurs in April in relation to the increasing temperature of the water; Adam (1960) reported that the glochidia of *Anodonta* developed during the winter months were eliminated in the early spring in Belgium. In Central Europe, Harms (1909); Zhadin (1952); Brodniewicz (1968) and Giusti (1975) considered the increase in water temperature as responsible for glochidium spawning of *Anodonta cygnea*.

Observation from River Kwae Noi showed that one adult female mussel may reproduce twice in one reproductive period. The release of glochidia may take as long as one month in natural surrounding while in the laboratory it only took less than

24 hours. Various possible factors come into play, in natural surroundings, these are water temperature, water current, chemical features of host, the season, salinity, and pH : in the laboratory, water temperature, pH, infection by numerous small different multi-cellular predators.

The experiments on host specificity of glochidia of *Hyriopsis (Limnoscapha) myersiana* in 11 species of 7 families of fishes showed that *Pristolepis fasciatus* and *Oxyeleotris marmoratus* (Nandidae and Eleotridae respectively), gave the highest number of juvenile mussels and glochidial encystment. Experiments on *O. marmoratus* were limited to 7 juvenile fishes and done in the River Kwae Noi only. All data of *P. fasciatus*, were from the laboratory. This species may prove to be a suitable host of *H. (L.) myersiana*, thus could be of great potential in producing small mussels in large quantity under laboratory condition.

The mortality of the small fishes at 48 hours post-infection may be due to physiological stress from encysted glochidia. Mortality after 30 days may result from chronic physiological stress, due to enlarging glochidia as well as infection by fungi and bacteria once weakened. Glochidial infection may be the cause of fish epidemics, Black (1981) found that glochidia were among the parasites of the anadromous Brook Charr (*Salvelinus fontinalis*) and may hinder fish movements. Some charr undergoing smoltification do not go to sea whilst others do. There are many studies on the quantity, and specific identity of parasitic glochidia found on many species of economic fish as well as their pathology. (for example, Molnar et al., 1974; Hare and Frantsi, 1974; Hare and Burt, 1976; Cone and Anderson, 1977; Hanex and Fernando, 1978; Watson and Dick, 1980).

Host specificity proves very important in these experiments. Bagrid fish have low to moderate resistance to glochidia. The production of juveniles in the mussels parasitic on Bagridae is low to moderate. Still the peak was high in *Mystus nemurus* but juvenile mortality was also rather high. In the parasites of Bagridae, the transition from glochidia to juveniles is not very successful. This may be due to host resistance. Glochidia may be expelled from the host during encystment resulting in poorly developed or even diseased. Futish and Millemann (1978) showed that the Coho Salmon *(Oncorhynchus kisutch)* has a better immunity to glochidia of *Margaritifera margaritifera* than the Chinook Salmon *(Oncorhyncus tshawytscha)*. The glochidia would be ejected from the gills by 4.5 days post-infection. Bauer (1987a) concluded that the pattern of glochidial mortality suggests that less susceptible hosts respond to infection with a rapid tissue response whereas the susceptible host shows a delayed response presumably due to a humoral factor.

In our experiments we found that the numbers of encysted glochidia depend on the size of the host and the positions of the parasite on the host body. On fish which are the specific host to the glochidia will mostly develop to juveniles. Figure 7 shows that *Pristolepis fasciatus* gives the highest juvenile yield of the species studied in the laboratory. While in the natural environment, *Helostoma temmincki* and *Oxyeleotris mormoratus* are capable of producing juveniles. Furthermore the appearance of these juveniles occurs in two intervals : The Bagridae : *Mystus vittatus* and *M. nemurus* appear on the 11^{th} - 19^{th} days post-infection, peaking on the 12^{th} and 15^{th} day respectively. Unfortunately, these juveniles according to our observations have a high mortality after further cultivation. The Anabantidae : *Helostoma temmincki*; and the Nandidae : *Pristolepis fasciatus*, these juveniles occur on the 18^{th} - 27^{th} days. Both in the field and in the laboratory experiments yield the same result for the *Helostoma temmincki* even though in the field the juvenile yield is many times more than in the laboratory.

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Table.1. Position and quantities of encysted glochidia on 8 species of fish collected from natural resources 1. *Puntius gonionotus* 2. *Cirrhinus julleni* 3. *Rasbora* sp. 4. *Mystus nemurus* 5. *M. vittatus* 6. *Oxyeleotris marmoratus* 7. *Pristolepis fasciatus* 8. *Puntioplites proctozysron*

Position / fish species	Fin				Mouth	Gill	Abdomen	n
	Tail	Dorsal	Pectoral	Anal				
1.	2.01±5.9	0.42±0.6	0.7±1.06	-	-	2.3±0.85	1.6±2.1	14
2.	0.57±0.85	0.55±0.78	0.78±0.63	-	-	-	-	26
3.	0.75±0.5	0.61±0.96	-	-	0.63±0.76	-	-	31
4.	1.9±1.4	0.87±0.8	2.7±3.8	1.9±3.6	0.84±1.2	4.5±4.3	2.09±1.3	12
5.	4.3±3.5	2.9±1.5	2.1±1.3	2.1±1.1	0.4±0.7	4.8±5.1	2.0±1.9	41
6.	5.2±6.4	-	2.1±3.8	-	2.7±2.3	8.3±2.1	2.5±2.0	8
7.	4.4±6.4	3.9±3.1	2.3±0.9	3.6±1.3	3.0±1.9	8.0±3.5	1.6±0.5	27
8.	0.8±0.83	-	1.5±1.4	1.8±2.4	-	1.3±2.3	-	11

*n = number of fries examined

Table 2. Average encysted glochidia in dead fries 48 hours after experiment (15,400 concentration in *Puntius gonionotus* and *Cirrhinus jullieni*, 61,600 in the other 6 species). Glochidia were counted after fixed in 10 % neutral formalin for 1-2 months.
(n = number of fires examined).

Fries Species	X ± SD glochidia found	n
1. *Puntius gonionotus*	60.3 ± 17.9	10
2. *Cirrhinus jullieni*	25.4 ± 14.0	10
3. *Mystus vittatus*	53.2 ± 28.8	10
4. *Mystus nemurus*	58.6 ± 8.7	5
5. *Pangasius sutchi*	34.5 ± 16.2	6
6. *Tilapis mossambica*	37.8 ± 13.6	6
7. *Pristolepis fasciatus*	76.5 ± 10.6	2
8. *Helostoma temmincki*	63.5 6.4	2

Table 3. Mean ± SD of glochidia of *Hyriopsis (Limnoscapha) myersiana* in 6 fries species.

Position / Fish numbers and species	Fin				Mouth	Gill	Head	Abdomen
	Tail	Dorsal	Pectoral	Anal				
(10) *Puntius gonionotus*	10.7±3.7	5.3±3.5	9.2±4.1	1.3±1.2	0.5±1.3	15±5.3	1.2±1.4	1.0±1.5
(10) *Cirrhinus jullieni*	7.3±5.7	2.6±3.4	2.3±2.6	0.3±0.7	0.5±0.5	11.1±3.7	2.3±2.0	1.4±1.5
(5) *Mystus vittatus*	15.6±4.7	8.8±4.3	6.4±7.4	1.0±1.2	1.4±1.1	22.4±7.5	1.6±1.5	3.2±1.9
(5) *Mystus nemurus*	9.4±3.4	5.0±2.7	4.6±2.9	5.6±2.5	3.2±1.9	11.6±7.8	2.8±1.6	2.8±1.3
(7) *Pristolepis fasciatus*	23.6±3.9	6.0±4.4	7.7±4.4	20.4±6.0	7.3±3.6	17.1±6.2	1.6±1.5	0.9±0.9
(5) *Helostoma temmicki*	8.6±3.5	2.4±1.1	1.8±0.8	1.2±2.3	1.0±1.4	7.8±4.2	3.4±2.9	2.0±1.0

Table 4. A verified diagram of resistance of 11 species of fries versus production of juveniles mussel, *Hyriopsis (Limnoscapha) myersiana* is shown below. (At, *Anabas testudineus*; C, *Cirrhina jullieni*; Ht, *Helostoma temmincki*; Mn, *Mystus nemurus*; Mv, *M. vittatus*; Om, *Oxyeleotris marmoratus*; P, *Puntius gonionotus*; Pf, *Pristolepis fasciatus*; Ps, *Pangasius sutchi*; R, *Rasbora sp.*; Tm, *Tilapia mossambica*).

Resistance	Juvenile Production		
	Hight	Low	NO
High	Om, Pf	Ht, Mn	At
Low		Mv	Ps, C, P, Tm
None			R

Legend for Figures

Figure

1 Map showing main study area, Kwae Noi River, Karnchanaburi Province (arrows).

2 The gills of *Hyriopsis (Limnoscapha) myersiana* are empty, no glochidia are present.

3 The section of testis (a) showing the spermatocytes, and ovary (b) showing the oocytes.

4 The section of testis, showing spermatozoa.

5 The gills of *H. (Limnoscapha) myersiana*, showing developing eggs (arrow).

6 The gills contain glochidia in only some area (arrow).

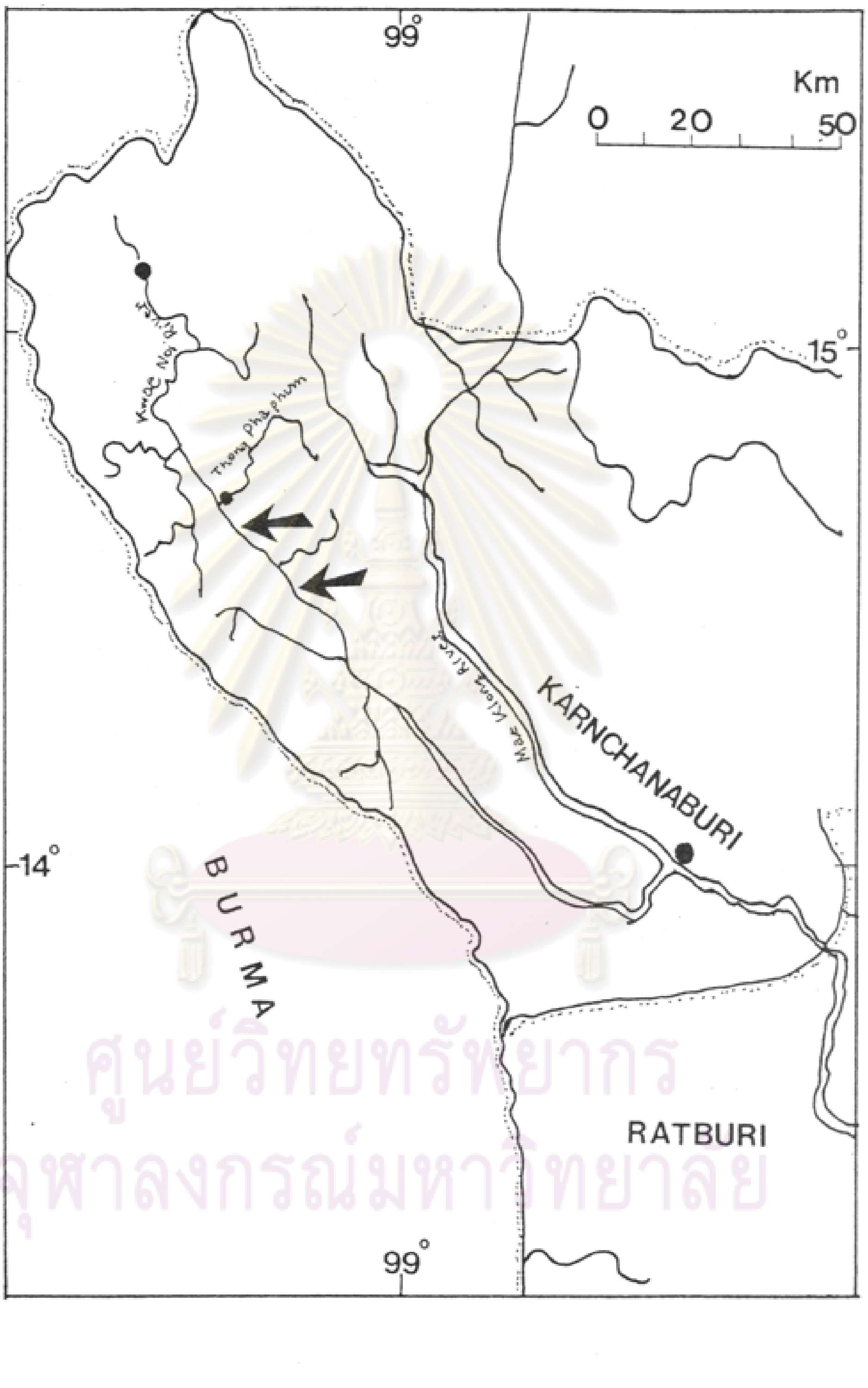
7 The marsupium of *H. (Limnoscapha) myersiana* containing pale yellow unfertilized oocytes (U) and brownish fertilized oocytes (B).

8 Numbers of encysted glochidia of *Hyriopsis (Limnoscapha) myersiana* on 5 size categories of 8 species of fish collected from irrigation canal, Chainat Province. (the species of fish are numbered as Table 1).

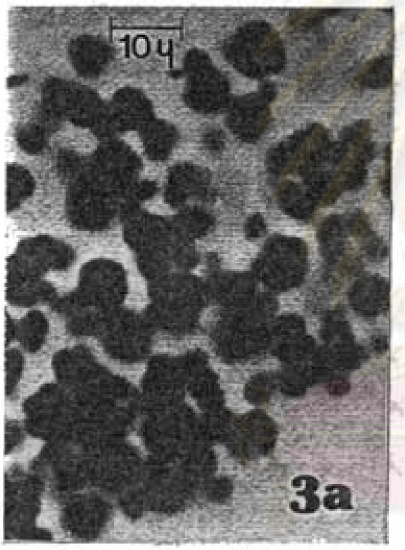
9 The percentage of mortality of fry exposed during 48 hours to a known numbers of *Hyriopsis (Limnoscapha) myersiana* glochidia (Ht, *Helostoma temmincki*, Pf, *Pristolepis fasciatus*; Tm, *Tilapia mosswambica*; Mn, *Mystus nemurus*; Mv, *M. vittatus*; Ps, *Pangasius sutchi*; C, *Cirrhinus jullieni*; P, *Puntius gonionotus*).

Figure

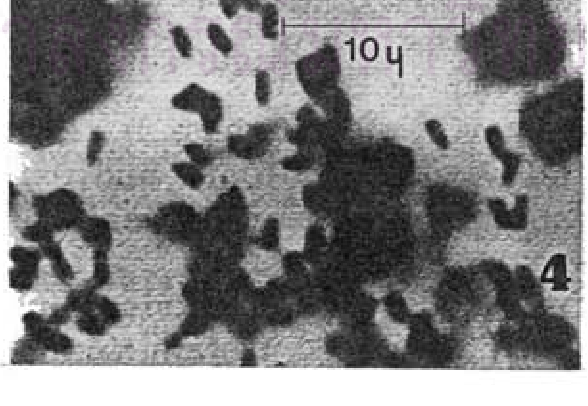
1

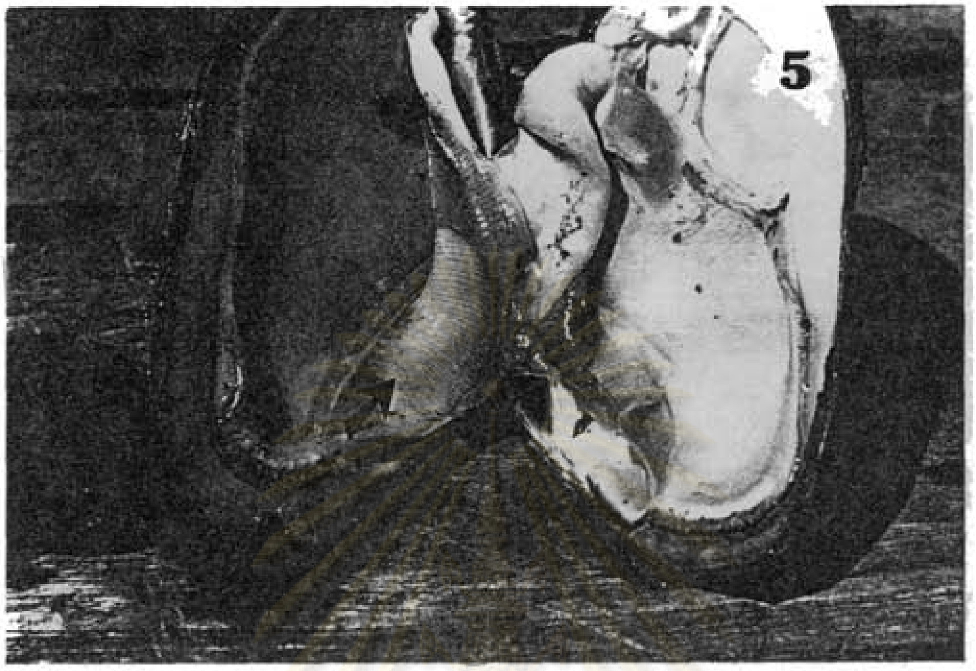

99°
Km
0 20 50
15°
Kwae Noi River
Thong Pha Phum
Mae Klong River
KARNCHANABURI
-14°
BURMA
RATBURI
99°


2


10 μ
3a


100 μ
3b


10 μ
4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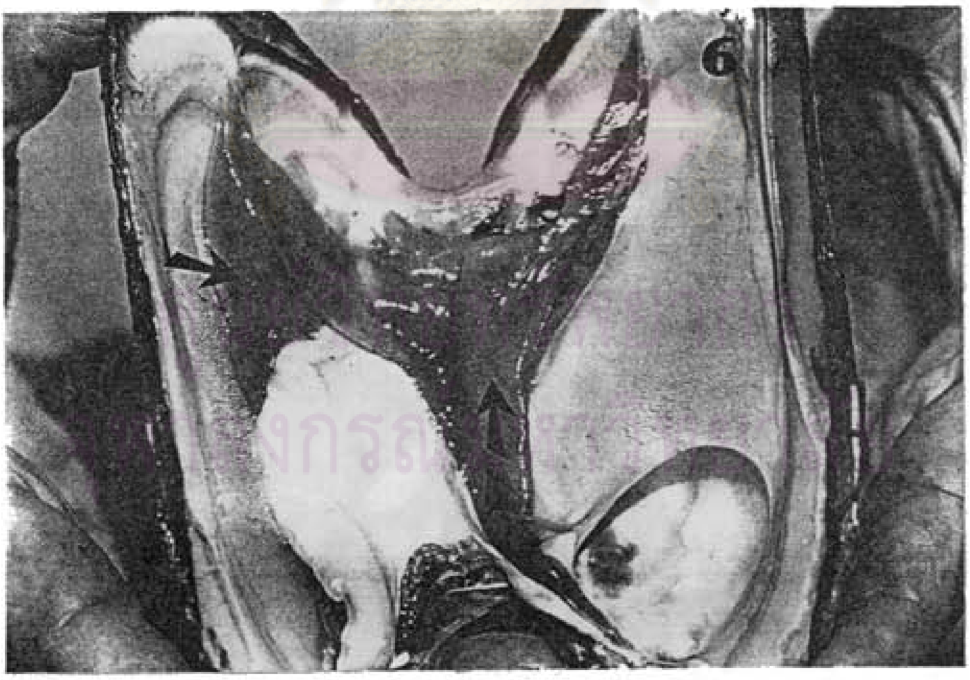
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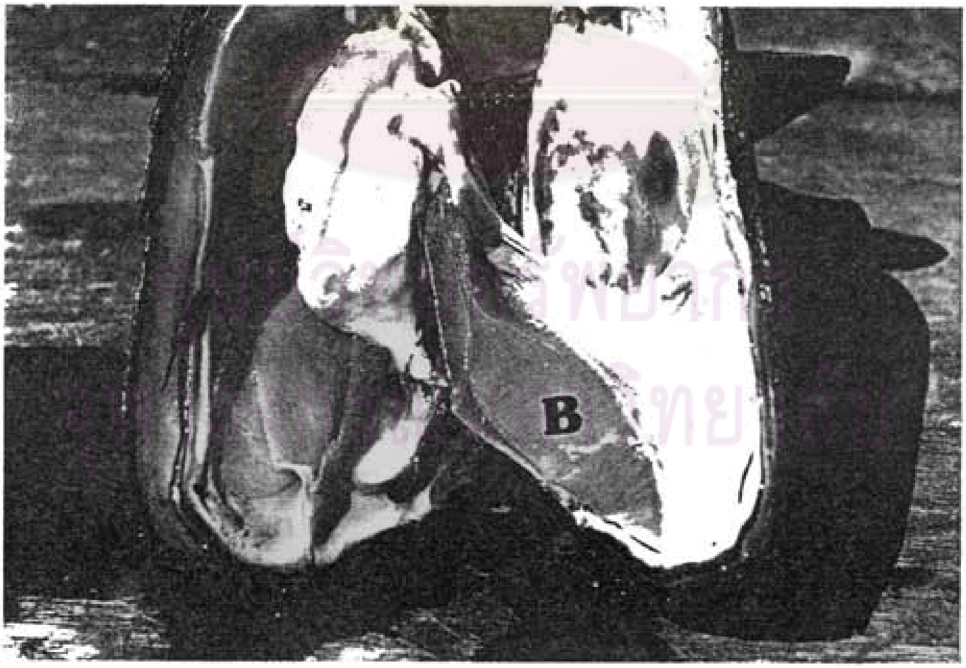
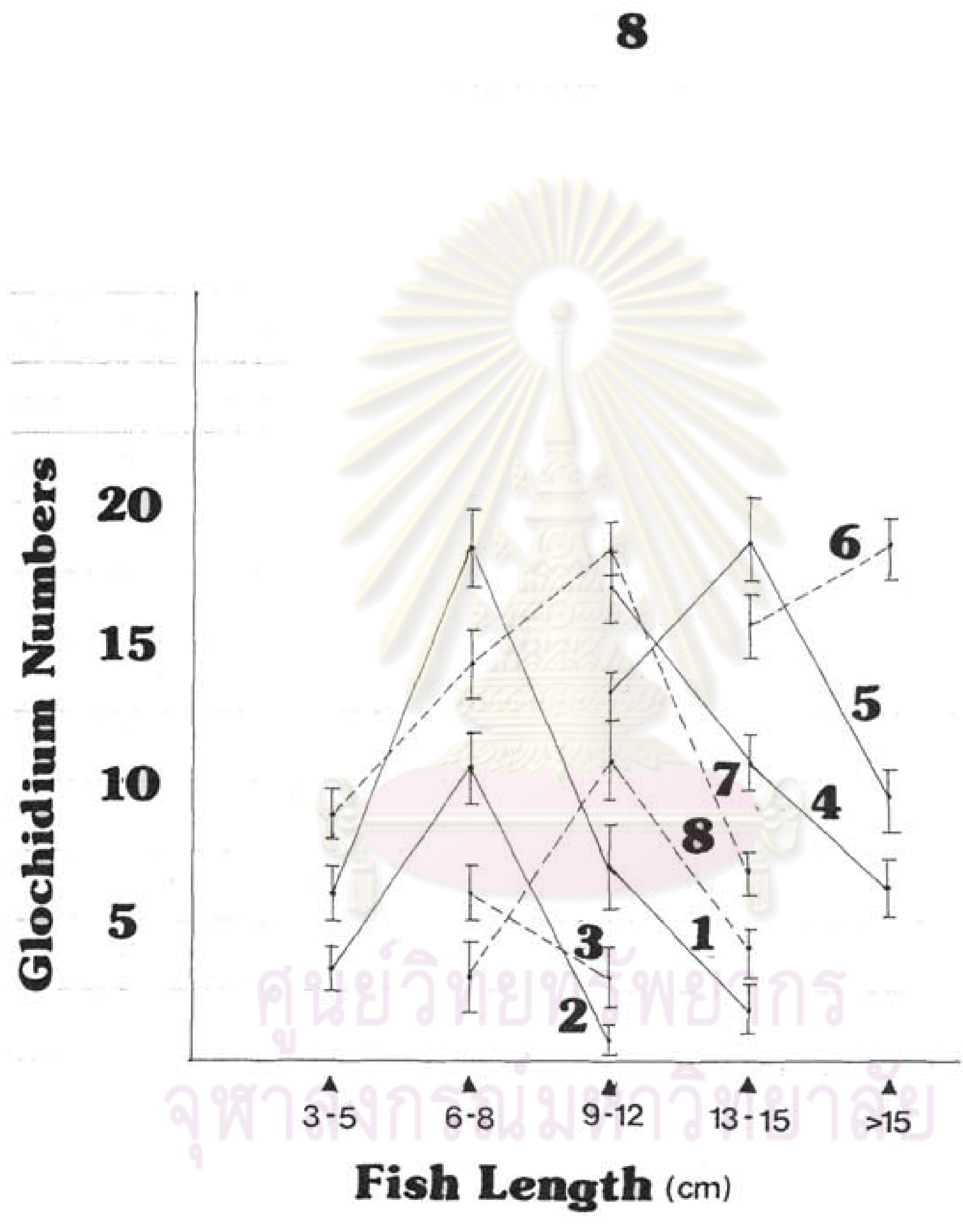
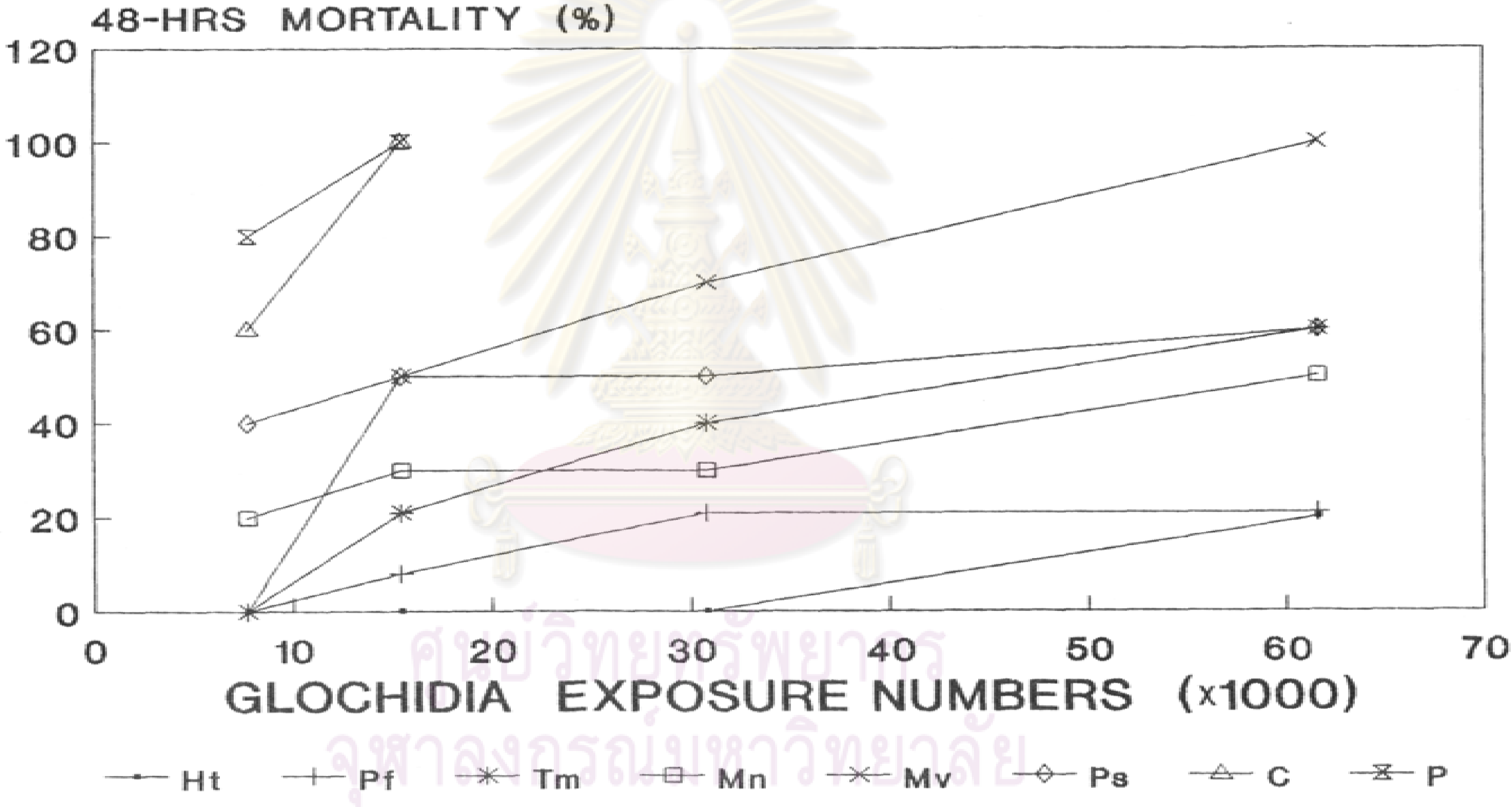
7

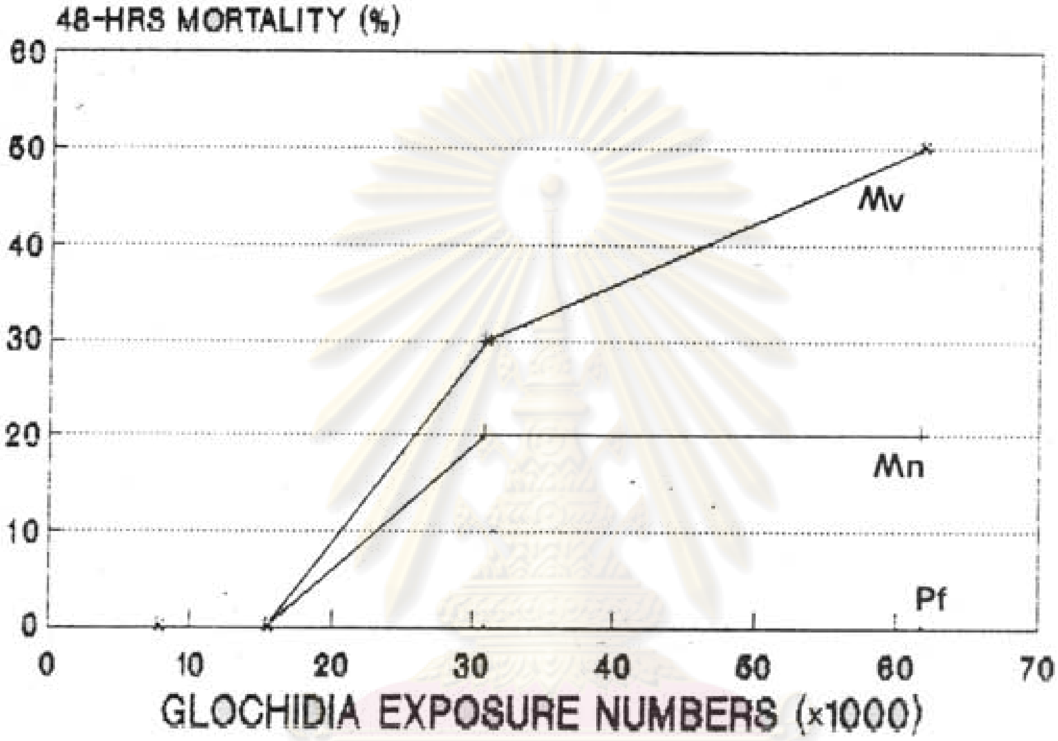



8
Glochidium Numbers
20
15
10
5
6
5
7
4
8
3
1
2
3-5
6-8
9-12
13-15
>15
Fish Length (cm)

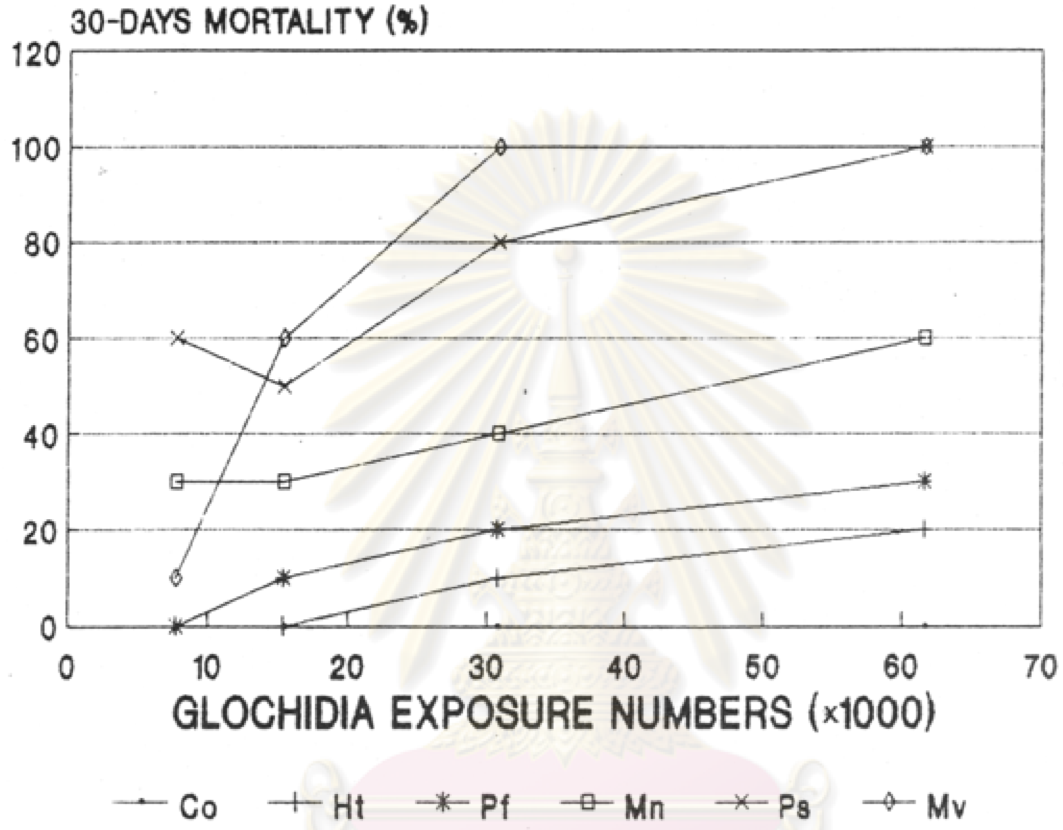
9


48-HRS MORTALITY (%)
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70
GLOCHIDIA EXPOSURE NUMBERS (x1000)
Ht
Pf
Tm
Mn
Mv
Ps
C
P

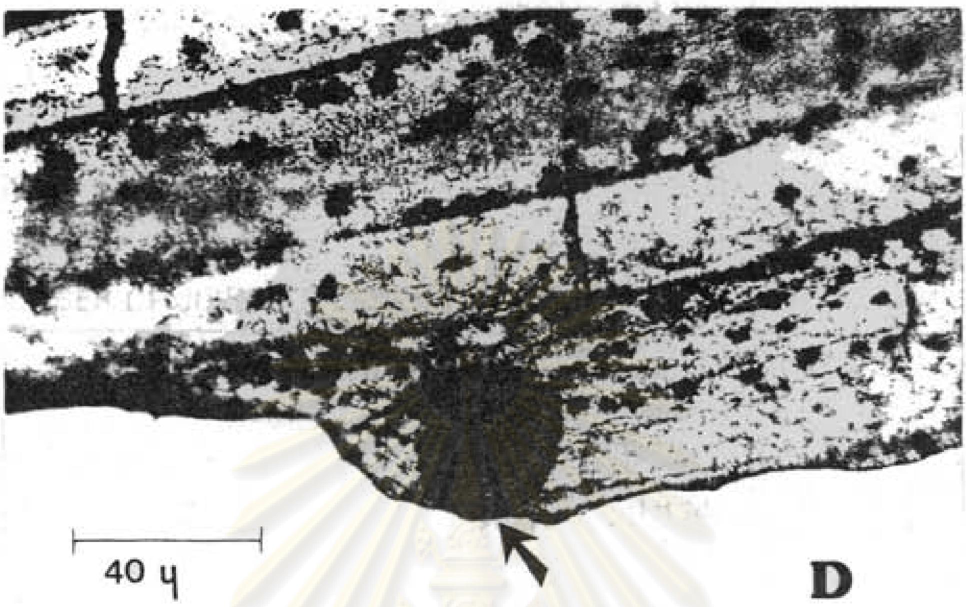
10


48-HRS MORTALITY (%)
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
GLOCHIDIA EXPOSURE NUMBERS (x1000)
Mv
Mn
Pf

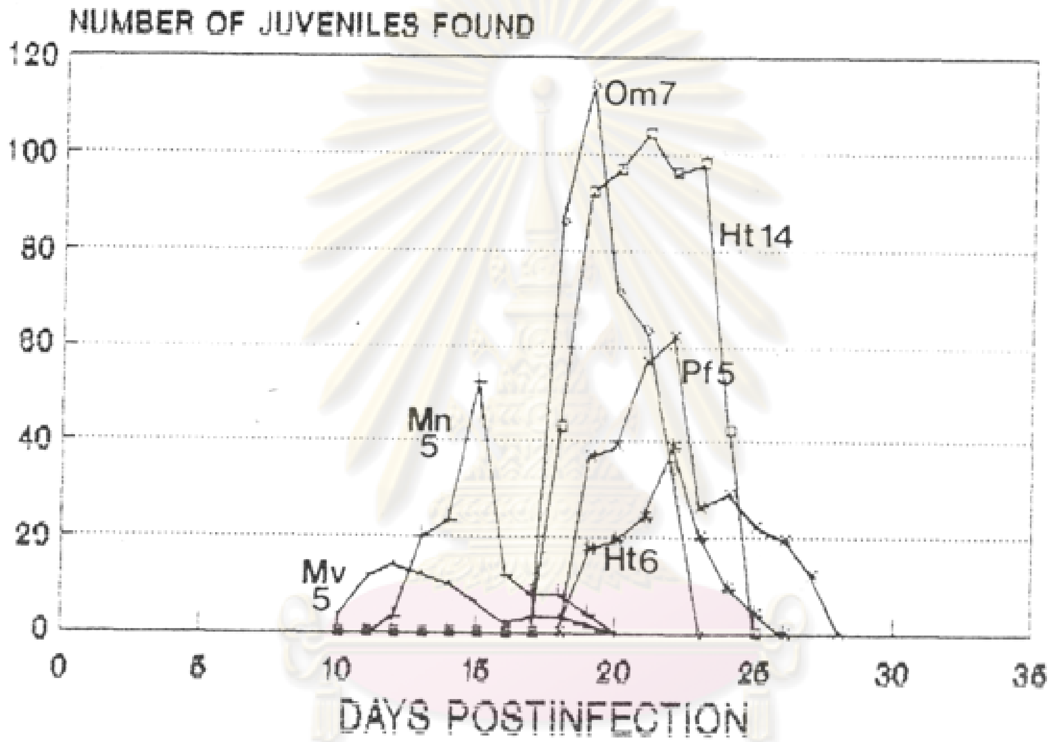
11


30-DAYS MORTALITY (%)
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
GLOCHIDIA EXPOSURE NUMBERS (×1000)
Co
Ht
Pf
Mn
Ps
Mv

12



13


NUMBER OF JUVENILES FOUND
120
100
80
60
40
20
0
Om7
Ht 14
Pf 5
Mn 5
Ht6
Mv 5
0
5
10
15
20
25
30
35
DAYS POSTINFECTION

14