



รายงานผลการวิจัย  
ทุนวิจัยรัชดาภิเษกสมโภช

เรื่อง

การสำรวจแหล่งและการศึกษาวงจรสี่พันธุ  
ของหอยมุกน้ำจืดในภาคกลาง

สถาบันวิทยบริการ  
จุฬาลงกรณ์มหาวิทยาลัย  
โดย

สมศักดิ์ บัญหา

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### กิตติกรรมประกาศ

ผู้วิจัยต้องขอแสดงความขอบพระคุณต่อจุฬาลงกรณ์มหาวิทยาลัย และบุคคลต่าง ๆ ที่ได้ช่วย สัตถ์สร เงินผลประโยชน์ของมหาวิทยาลัยส่วนหนึ่งจัดตั้ง เป็นเงินทุนวิจัยรัชดาภิเษกสมโภช ซึ่งทำให้ ผู้วิจัยซึ่ง เป็นผู้สอนและผู้วิจัยท่านอื่น ๆ ได้มีโอกาสได้ทำงานวิจัยให้บรรลุผลซึ่งจะทำให้เกิดประโยชน์ ต่อผู้วิจัยและส่วนรวม อันได้แก่ส่วนที่จะอ้างอิงงานวิจัยที่บางส่วนหรือนำไปประกอบการเรียนการสอน ต่อไป ผู้วิจัยก็ต้องขอขอบพระคุณเป็นอย่างสูงต่อคณะกรรมการประจำสาขาชีวภาพที่ได้พิจารณาให้ได้ รับทุนครั้งนี้ และยังได้ช่วยเหลือแนะนำในทุกเรื่องของงานวิจัย

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ท้ายสุดนี้ ขอขอบพระคุณเจ้าหน้าที่ฝ่ายวิจัยจุฬาฯ ทุกท่านที่ได้อำนวยความสะดวกในทุกเรื่อง และขอขอบพระคุณ คุณศิริลักษณ์ นาคฉาย ที่ช่วยพิมพ์รายงานวิจัย.

|                           |   |
|---------------------------|---|
| ชื่อโครงการวิจัย          | การสำรวจแหล่งและการศึกษาวงจรสืบพันธุ์ของหอยมุกน้ำจืดในภาคกลาง<br>ของประเทศไทย |
| ชื่อผู้วิจัย              | นายลุ่มศักดิ์ ปัญหา   |
| เดือนและปีที่ทำวิจัยเสร็จ | กันยายน 2532  |

### บทคัดย่อ

ได้ทำการเก็บตัวอย่างหอยมุกน้ำจืด 8 ชนิด (species) ในคลองทั่ว ๆ ไป และ  
คลองชลประทาน ที่จังหวัดนครสวรรค์ ชัยนาท ลพบุรี สระบุรี สิงห์บุรี พระนครศรีอยุธยา  
และจังหวัดปทุมธานี ในระหว่างเดือนกันยายน 2531 ถึงเดือนกันยายน 2532 พบ  
*Pilsbryochoncha exilis* เป็นปริมาณมากในแหล่งน้ำจืดและมีการกระจายอยู่ในทุกจังหวัด  
ที่ทำการสำรวจ รองลงมาได้แก่ *Ensidens ingallsianus*, *Unioandra contradens* และ  
*Scabies crispata* ส่วนชนิดที่พบอยู่แห่งเดียวคือ *Pseudodon vondembuschianus* พบ  
เฉพาะที่จังหวัดลพบุรี นอกจากนี้พบว่าชนิดที่มีขนาดใหญ่ที่สุดและอยู่ในน้ำลึกที่สุดได้แก่ *Hyriopsis*  
(*Limnoscapha*) *myersiana*

วงจรสืบพันธุ์ของหอยนั้น ได้ตรวจพบมาสู่เขี้ยว (marsupia) ของหอยตัวเมีย ระหว่าง  
เดือนกันยายน 2531 และเดือนธันวาคม 2532 พบว่ามีความแตกต่างกันเล็กน้อยในหอยแต่ละชนิด  
ได้พบปลา 14 ชนิดเป็นโฮสต์ของโกลคิเดียมของหอยมุกน้ำจืด เริ่มตั้งแต่กลางเดือนตุลาคม 2531  
จนถึงปลายเดือนมีนาคม 2532 ซึ่งสัมพันธ์กับการตั้งท้องและการปล่อยโกลคิเดียมของหอยตัวเมีย

จุฬาลงกรณ์มหาวิทยาลัย

Project Title        The Site Survey and the Study on Reproductive Cycles  
                              of Freshwater Pearl Mussels in the Central Part of  
                              Thailand

Name of the Investigator        Somsak Panha

Year                                1989

### Abstract

Eight species of freshwater pearl mussels were collected from general canals and irrigation canals in Nakornsawan Province, Chainat Province, Lopburi Province, Saraburi Province, Singburi Province, Ayutthaya Province and Pathumthani Province, from September 1988 to September 1989.

*Pilsbryochoncha exilis* was the most abundant and distributed in all provinces surveyed. *Ensidens ingallsianus*, *Unioandra contradens* and *Scabies crispata* were found in the lesser extent. *Pseudodon vondenbuschianus* was found only in Lopburi Province. The largest species which found in the deepest water was *Hyriopsis (Limnoscapha) myersiana*.

The females with marsupia were observed from September 1988 to February 1989 and the patterns were quite different from each other.

Fourteen species of fish were found as hosts of glochidium from mid-October 1988 to March 1989 which corresponded with the gestation and elimination of glochidium of female mussels.

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The Site Survey and the Study on Reproductive Cycles  
of Freshwater Pearl Mussels in the Central  
Part of Thailand

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Introduction

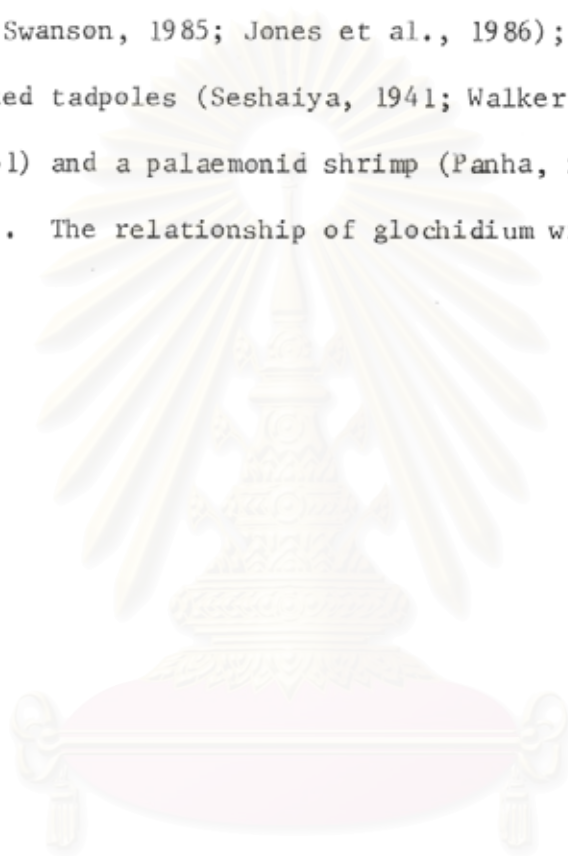
Freshwater mussels are common inhabitants of lakes, rivers, canal, stream and also swamp. There have been a number of studies on their distribution, biomass, reproductive biology particularly in Europe and U.S.A. (Bauer, 1987 b, c ; Bauer and Vogel, 1987 ; Jones et al., 1986 ; Kondo and Yamashita, 1980 ; Morton and Dudgeon, 1984 ; Tedla and Fernando, 1969 ; Zale and Neves, 1982).

However, very few works has been done on Thai freshwater mussels. Brandt (1974) reported 3 families (Margaritiferidae, Amblemidae, Unionidae) of freshwater mussels found in Thailand. Panha (1985) studied on some aspects on ecology of freshwater amblemid mussels in Thailand and showed the zonation pattern of 7 species of amblemid mussel in the canal in Ubolrachathani Province.

The amblemid freshwater mussels have highly specialized life cycles. Among the Amblemidae the eggs are moved into specialized portions of the outer gills or both inner and outer gills (marsupia)



where they develop into hooked or hookless larval stage (glochidium) (Brandt, 1974; Panha, 1989). Mature glochidia are released into the water where they spend some time attached to mostly vertebrate host, which is generally a fish (Lefevre and Curtis, 1910; Atkins, 1979; Kraemer and Swanson, 1985; Jones et al., 1986); glochidia are also found infected tadpoles (Seshaiya, 1941; Walker, 1981), a salamander (Howard, 1951) and a palaemonid shrimp (Panha, inpreparation for publication). The relationship of glochidium with fish is shown in Figure 1.



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## Materials and Methods

The irrigation canal (Fig. 2) has a length of 170 Km., and is situated (Fig. 3) from Chainat Province to Saraburi Province. It is a man-made tributary of the Chao Phraya river under the upper Chao Phraya river agricultural project.

From September 1988 to September 1989, a field study was carried out in 2 types of habitat. One was the canal, which the depth was lower than 2 metres, around the paddy field in Pathumthani Province, Ayuthya Province, Lopburi Province, Saraburi Province, Chainat Province and Nakornsawan Province. About 30 individuals of *Pilsbryochoncha exilis*, *Physunio superbus*, *Scabies crispata*, *Ensidens ingallsianus*, *Uniantra contradens*, *Pseudodon cambodjensis*, *Pseudodon vondemouschianus*, were collected by hand. The other habitat was the irrigation canal which the depth was higher than 2 metres. Nine sampling site, one in Chainat Province, 3 in Nakornsawan Province, 3 in Lopburi Province, and 2 in Saraburi Province were selected. The samples were collected by hand and bottom dredging.

Mussels collected were dissected and examined for their sexes. Shell length and incidence of females incubating eggs or glochidia in the marsupium were recorded. Some were reared for further observations and experiments in aquaria. Histological study of gonads was also done in some species.

The observation of glochidium was made both on the living materials and those preserved in 10 % formalin. Shell length and height were measured on the glochidium preserved in 10 % formalin under the microscope using an ocular micrometer.

The fish were caught monthly at all collecting sites by trawling net, floating net (Fig. 4) as well as by hands, and they were soon preserved in 10 % formalin. After which species, position, number and size of attached glochidia were examined under the binocular. Species identification of mussels was based on Brandt (1974) and fish identifications were based on Smith (1945) and the Department of Fisheries (1987).

### Results

Seasonal fluctuations in temperature, dissolved oxygen, pH, flow rate and water level were shown in Figure 5.

The distribution of Amblemid mussels over various depths of bottom of canals were presented in Figure 6 and their monthly length frequency histograms were also constructed as shown in Figure 7. These were interpreted to give an assessment of the time of larval metamorphosis and settlement as well as period of growth and reproduction.

The abundances of the six species occurring in small irrigation canals or canals near rice fields which not more than 2 meter deep with muddy bottom. The other two species, *Hyriopsis (Limnoscapha) myersiana* and *Physunio superbus* were usually found at more than 2 meter deep in the biggest irrigation canal. However *Pseudodon vondembuschianus ellipticus* was found at only Lopburi Province in a canal near rice fields and some small irrigation canals which the depth was ranged between 1.40 m to 1.95 m with muddy bottom.

The monthly mean population of 8 species was estimated and showed in Table 1. Shell length varied between 7 mm and 140 mm. The biggest species was *Hyriopsis (Limnoscapha) myersiana*, the smallest one was *Scabies crispata*. Juveniles and small individuals of mussels (< 15 mm shell length) were obtained in November, December, January and February but the frequency of occurrence was low in November and absent from March and May.

#### Sexual cycle, Female gravidity, Glochidial settlement

The ratio of males to females were as follows

*Pilsbryochoncha exilis exilis* 1.5 : 1, *Physunio superbus* 1 : 1.1, *Uniandra contradens rustica* 1 : 1.2, *Pseudodon cambodjensis* 1 : 1.6, *Ensidens ingallsianus* 1 : 1.1, *Scabies crispata* 1.2 : 1, *Hyriopsis (Limnoscapha) myersiana* 1 : 1.3, *Pseudodon vondembuschianus* 1 : 1.

The outer or inner and outer gills (demibranchs) of female were modified for the incubation of fertilized eggs (marsupium) (Fig. 8) in the suprabrachial chamber. Brooding occur in females 9-10 mm shell length in *Scabies crispata* to 140-150 mm in *Hyriopsis (Limnoscapha) myersiana* (See Table 2). On a seasonal basis, the occurrence of incubating females of 8 species was greatest in December, January and February (Fig. 9). The incidence was lowest or no female incubating marsupium in summer (March, April, May).

Glochidial settlement on fish varied from mid-October to late March (Fig. 10). The highest incidence of settlement varied with species for example *Pilsbryochoncha exilis* in February; *Scabies crispata* in January which was corresponded with the occurrence of high frequency of incubating females.

The glochidia, with hooks or without hook on valves settle on the fin margins or gills of fish and encyst (Fig. 11).

Twenty four collections of about one thousand five hundred fish were made from September to August, 1989 (Table 3). Of 29 fish species collected, only 14 species were infected with glochidia at various parts of the body (Fig. 12). Glochidial identification was still unknown in almost all species. Only a special observation on glochidium and host relationship of *Physunio superbis* in yellow mystus *Mystus nemurus* were made. It can be concluded that smaller fish were more heavily infected with glochidia than larger ones and the peak of infection started from November, 1988 till January, 1989 (Panha, 1989). Almost all of fish in the family Cyprinidae, Bagridae, Eleotridae found infected by glochidia. See details in Table 3.



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## DISCUSSION

The quantitative distribution of Amblemid mussels was modified in time and space by modifications of its habitat. One of the factors inducing modifications of the density and distribution of mussels in the same place was the stretch and depth of the water. In water with large stretch, mussels are more widely dispersed over the pool and as the water subsides mussels begin to withdraw as well. The zonation pattern of many species of freshwater mussels was observed (Hanex and Fernando, 1978; Panha, 1985). In fig. 6 the average density variations of each Amblemid population was investigated in the irrigation canals and canals near rice fields was recorded in relation to the depth of water from September, 1988 to September 1989. The biggest species which was also the most valuable for pearl farming, *Hyriopsis myersiana* can survive only in the large and deep irrigation canal, this species was found at the depth ranged from 2 metres to 7 metres. (Fig. 6.4).

In periods with shallow water, thus with less extensive surface, mussel density is greater and vice versa. However, the low density of *Pilsbryochoncha exilis* at Chinat Province and Pathumthani Province in April, May; June, July 1989, when the water reached its minimal depth and covered with much vegetation particularly water hyacinth was accounted by the intense degree of silting. Thus the canals became stagnant which led to an intense mortality of molluscs. It is the reason then that why samples collected in May, June, July 1989, had high percentage of empty valves (see Table 1 and Fig. 6.5).

Other factors affecting the distribution of mussels in the canals were the nature of the bottom and the presence or absence of



vegetation. In places where the bottom surface was covered by a thick layer of bare mud, the richest fauna of mussels were found. In places with silty bottom covered with vegetation (water hyacinth and others), the mussels were extremely poor. Of the Amblemidae, *Pilsbryochoncha exilis* became a dominant species while *Unicandra contradens* and *Ensidens ingallsianus* took the second place. In places with sandy, deep, well-aerated bottoms, only, *Hyriopsis myersiana* dominated.

The release of glochidia generally follows a period of incubation or gestation which the peak varies according to species. In the observation of glochidial release in *Pilsbryochoncha exilis*, *Ensidens ingallsianus*, *Unicandra contradens* and *Scabies crispata* by plankton net collecting glochidia was found the short incubation period about 1 month. The first individuals of mussels incubating marsupia was observed in September, the glochidia were found released in the water in October (Fig. 9 and Table 4), and also the host attachment beginning from October (Fig. 10). The incubation period seems rather short in all species, the further studies should be done in the near future. Most temperate zone species have long incubation periods spanning autumn and winter (Heard, 1975; Dudgeon and Morton, 1983). From the data of this study, warm and cold temperatures may speed larval development and subsequent release.

In this study, some observation results of laboratory and field were in agreement and demonstrated that some amblemid mussels exhibited a high degree of host specificity. Hosts of *Physunio superbus* were limited to two bagrid fish; yellow mystus *Mystus nemurus* and iridescent mystus *Mystus vittatus* (Panha, 1989), whereas the only host of

*Pilsbryochoncha exilis* was cyprinid fish.

Host specificity of glochidia has been observed by several authors; Lefevre and Curtis, 1910; Arey, 1921; Murphey, 1942; Tedla and Fernando, 1969; Wile 1975; Zale and Neves, 1982 and Bauer, 1987c. They showed the data of laboratory and field in details such as the susceptibility to attachment by the glochidia, host specificity, glochidial metamorphosis in hosts. Murphey (1942) observed that in nature the glochidia of *Margaritifera margaritifera* attached on the gill filament of rainbow trout and brown trout which Bauer (1987 b) presented the same results in the laboratory works.

The relationship of fish and freshwater mussel was very interesting unfortunately only 2 cases were roughly observed in this study because of time limitation of a grant period. To change the species composition and relative abundance of endemic fish fauna can result in the reproductive failure of mussel populations through loss of required fish hosts. River systems inhabited by mussels, particularly endangered species, should therefore be managed to retain their remaining endemic fish fauna, at least until appropriate fish hosts for successful reproduction are identified.

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### Conclusion

The report here is included on population study and some aspects of reproductive biology of eight species of amblymid mussels.

Of the eight species of Amblymididae present in the canals observed, the population of *Pilsbryochoncha exilis* was investigated as the species dominate in the ecosystem surveyed both in number and in distribution. *Ensidens ingallsianus*, *Unicandra contradens* and *Scabies crispata* are the second dominant. *Pseudodon vondembuschianus* was rarely found at only Lopburi Province. The biggest species which is also the most valuable for pearl farming, *Hyriopsis myersiana* can survive only the large and deep irrigation canal. It was found at the depth ranged from 2 metres to 7 metres.

Female mussels gestation was started from September 1988 to April 1989. The patterns are quite different from each other in eight species of mussels. Release of the ova into suprabranchial chamber of gills takes place from September in all species except *Pseudodon cambodjensis* and *Hyriopsis (Limnoscapha) myersiana* fertilization occurring there and developing larvae being incubated in the modified outer or both outer and inner demibranchs. From September, 1988 through March, 1989, released glochidia attach to the fins and gills of fish hosts. The peak of incidence is in December or January which depend on mussel species. The incidence of infection to fish hosts corresponds with the occurrence of high frequency of incubating females (gestation).

Fourteen species of fish were found infected by glochidia, particularly family Cyprinidae, Bagridae, Eleotridae.

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Table 2. Size range of female carrying marsupium in eight species of amblymid mussels.

| Species                                     | Size Range<br>(mm.) |
|---|---------------------|
| 1. <i>Pilsbryochoncha exilis</i>            | 63 - 136            |
| 2. <i>Physunio superbus</i>                 | 48 - 89             |
| 3. <i>Uniandra contradens</i>               | 28 - 62             |
| 4. <i>Pseudodon cambodjensis</i>            | 67 - 86             |
| 5. <i>Pseudodon vondembuschianus</i>        | 59 - 83             |
| 6. <i>Ensidens ingallsianus</i>             | 34 - 66             |
| 7. <i>Scabies crispata</i>                  | 9 - 31              |
| 8. <i>Hyriopsis (Limnoscapha) myersiana</i> | 78 - 160            |

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Table 3 Fourteen species of fish and percentage of infection by amblymid glochidia, based on collections from Canals at Saraburi Province and Ayutthaya Province, December 1988 to February 1989.

| Fish                               | No. examined | % infection |
|------------------------------------|--------------|-------------|
| Cyprinidae                         |              |             |
| A <i>Puntius schwannfeldi</i>      | 42           | 53.8        |
| B <i>Puntius gonionotus</i>        | 26           | 26.9        |
| C <i>Puntioplites proctozysron</i> | 17           | 47.0        |
| D <i>Cirrhinus jullieni</i>        | 61           | 45.9        |
| E <i>Rasbora heteromorpha</i>      | 40           | 44.4        |
| Bagridae                           |              |             |
| F <i>Mystus nemurus</i>            | 18           | 63.6        |
| G <i>Mystus vittatus</i>           | 134          | 69.4        |
| Eleotridae                         |              |             |
| H <i>Oxyeleotris marmoratus</i>    | 22           | 45.4        |
| Centropomidae                      |              |             |
| I <i>Chanda siamensis</i>          | 83           | 67.4        |
| Notopteridae                       |              |             |
| J <i>Notopterus notopterus</i>     | 40           | 60.0        |
| Nandidae                           |              |             |
| K <i>Pristolepis fasciatus</i>     | 71           | 71.6        |
| Ophicephalidae                     |              |             |
| L <i>Channa striatus</i>           | 14           | 35.7        |
| Belonidae                          |              |             |
| M <i>Xenentodon cancala</i>        | 9            | 33.3        |
| Anabantidae                        |              |             |
| N <i>Trichogaster microlepis</i>   | 67           | 43.3        |

Table 4 The numbers of glochidia collected in plankton nets from September 1988 to August 1989 at Ayutthaya Province.

| Month     | No. of glochidia collected nearest mussel bed |
|-----------|---|
| 1988      |   |
| September | -   |
| October   | 32,700  |
| November  | 69,000  |
| December  | 120,300                                       |
| 1989      |   |
| January   | 264,000                                       |
| February  | 167,000                                       |
| March     | 2,250   |
| April     | -   |
| May       | -   |
| June      | -   |
| July      | -   |
| August    | -   |

## Legend for figures

- Figure 1. General life cycle of freshwater pearl mussels.
- Figure 2. a. Irrigation canal at Takli District, Nakornsawan Province.  
b. A small irrigation canal, Inburi District, Singburi Province.
- Figure 3. The map of the irrigation canal, a man made tributary of Chaopraya river (C, Chainat Province; N, Nakornsawan Province; L, Lopburi Province; S, Saraburi Province).
- Figure 4. Trawling net (a) and floating net (b) used for fish collection.
- Figure 5. The Hydrological data of A, 193 Km. Nakornsawan Province; B, Inburi irrigation canal Singburi Province; C, Thakae, Lopburi Province; D, Ayutthaya Province; E, Rangsit, Pathumtani Province from September 1988 to September 1989 with water level, flow rate, temperature, dissolved oxygen, pH. (s1, station 1; s2 station 2; ——— surface; ----- bottom).
- Figure 6. The distribution upon different depths of bottom of eight species of Anblemid mussels from sample areas between November 1988 and January 1989. (Species 1-8 are ordered as in Table 1; a, 193 Km. canal Nakornsawan Province; b, Chainat Province; c, Inburi, Singburi Province; d, Lopburi Province; e, Banmoh, Saraburi Province; f, Ayutthaya Province; g, Pathumtani Province) (6.1, 6.2,



6.3). 6.4, The distribution of *Hyriopsis (Limnoscapha) nyersiana* in a big irrigation canal at 1, Chainat; 2, Takli; 3, Chongkae; 4, Nongmuang; 5, Banmi; 6, Thakae; 7, Lopburi; 8, Banmoh. 6.5, percentage of empty valves of *Pilsbryochoncha exilis* found at Chainat Province and Pathumtani Province (quadrat 25 m<sup>2</sup>) from September 1988 to September 1989.

Figure 7. Size frequency histograms of the population structure of eight species of Amblemid mussels from November 1988 to March 1989. (species 1-8 are ordered as Table 1). The area selected are Saraburi Province (species 1-4, 7, 8), Lopburi Province (species 5), Ayutthaya Province (species 6).

Figure 8. The marsupia of *Pilsbryochoncha exilis* (arrows) collected from Banmoh Saraburi Province, 14 November 1988.

Figure 9. The percentage frequency of females incubation larvae within the outer or both inner and outer of demibranchs from September 1988 to September 1989.

Figure 10. Incidence of infection of fourteen species of fish by glochidia of freshwater mussels from September 1988 to September 1989. Species A, B, C -----, H, N are ordered as Table 3.

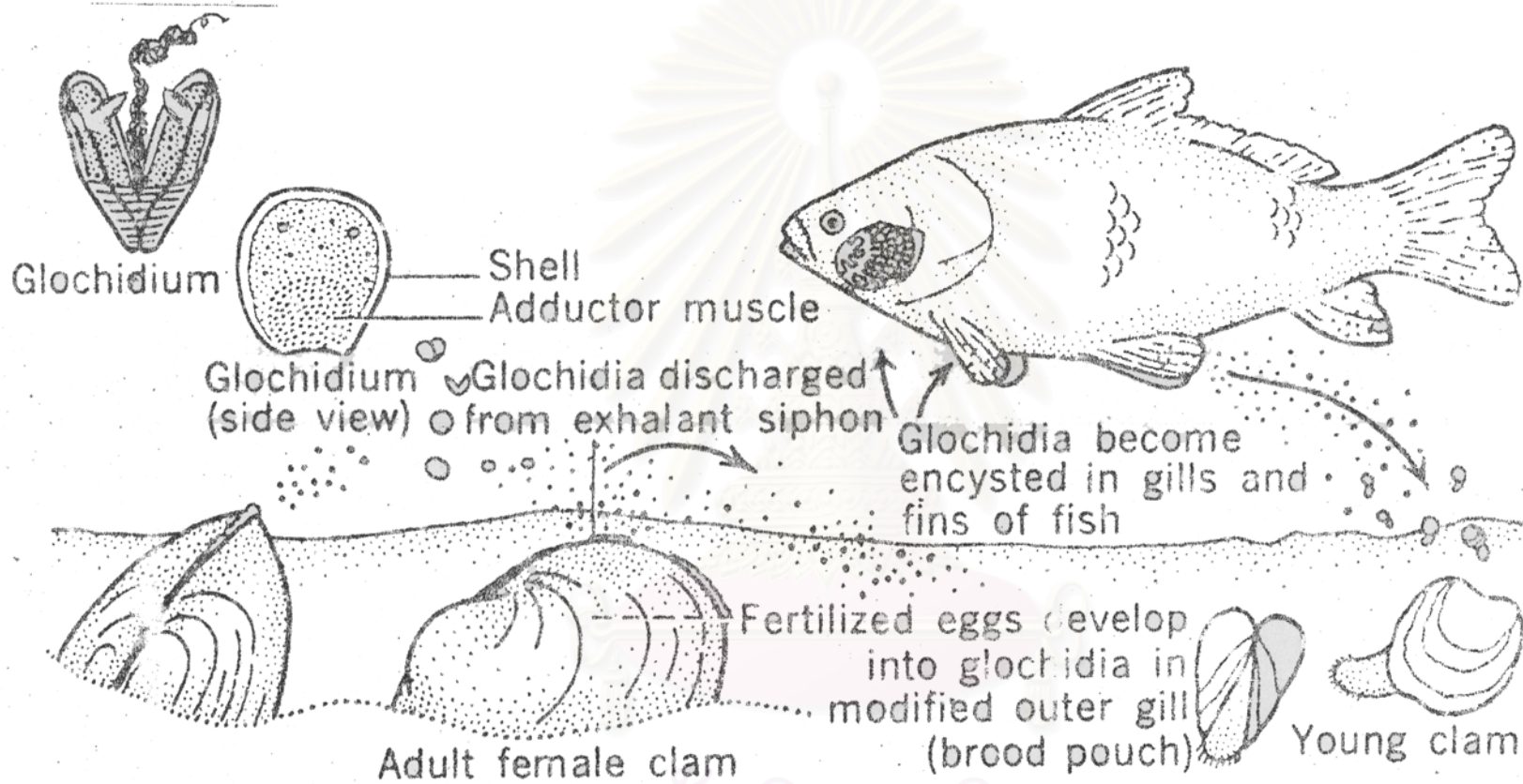
Figure 11. Glochidial characteristic of *Unioendra contradens*.



Figure 12. The glochidia of *Imindra contradens* settled on tail of small cyprinid fish *Cirrhinus jullieni* (A), and on pectoral fin, abdomen (arrow) position (B).



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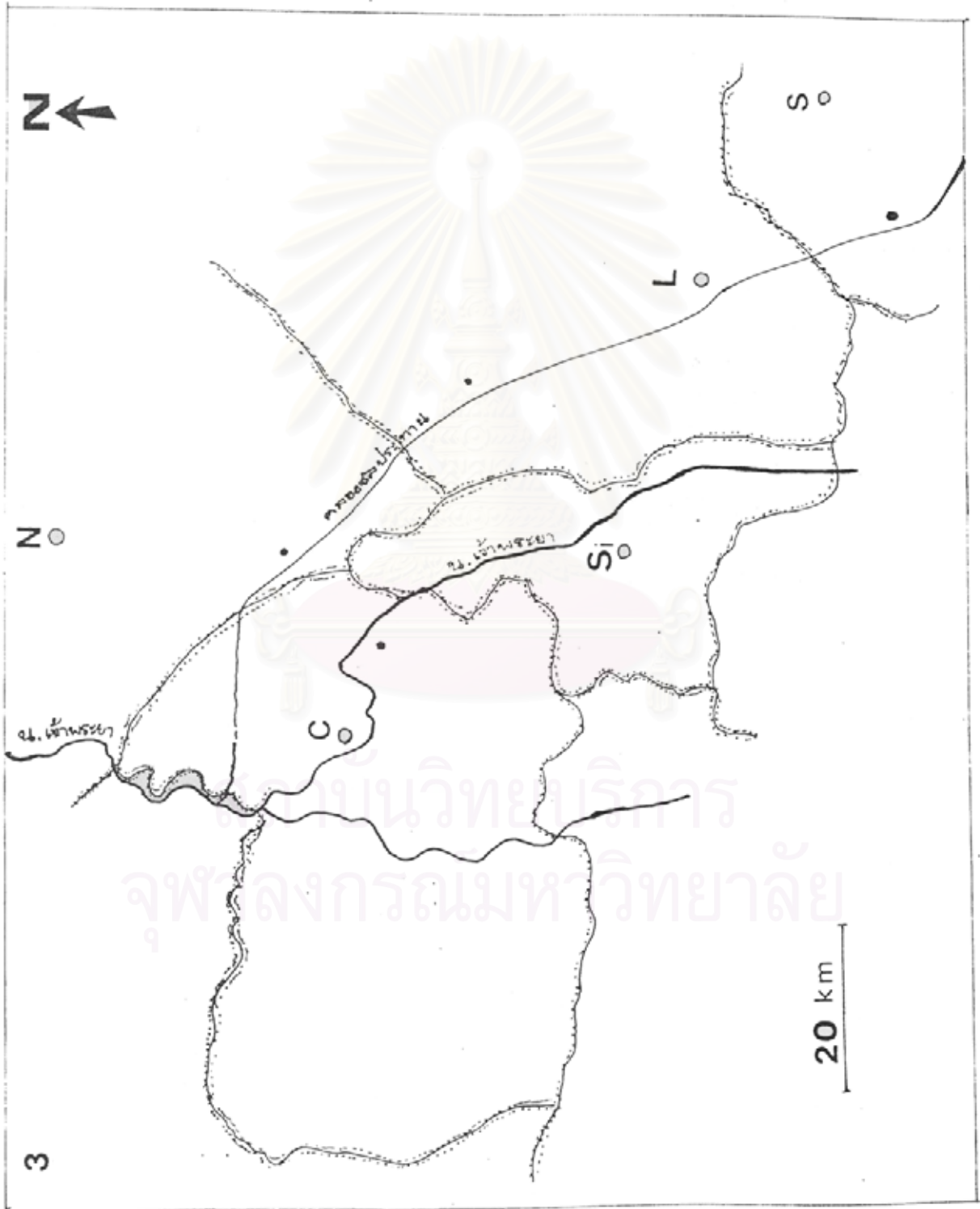
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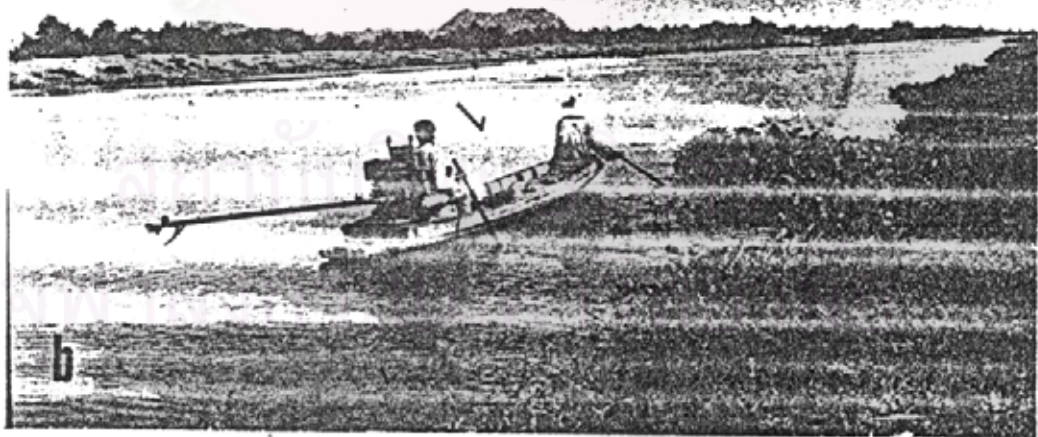


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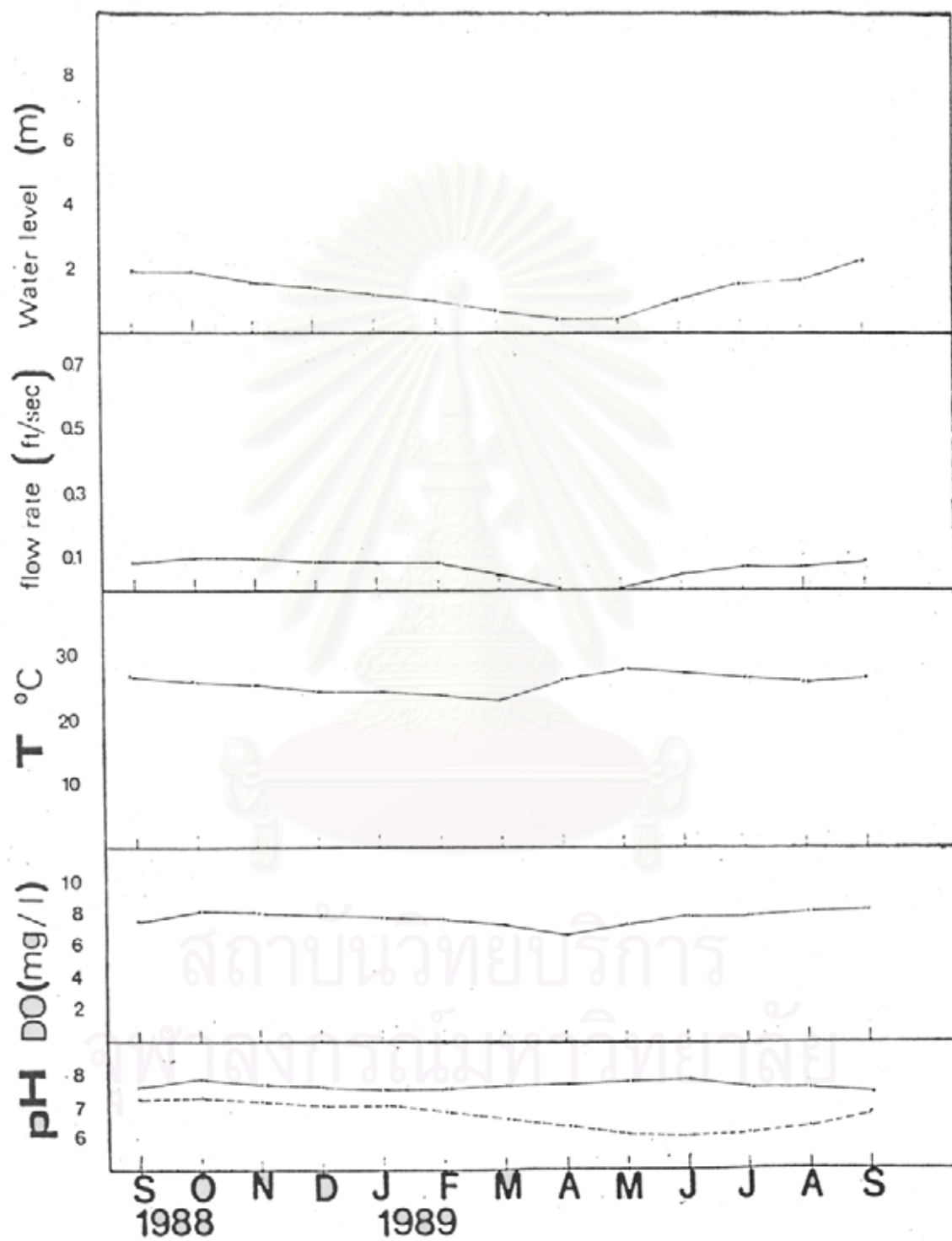






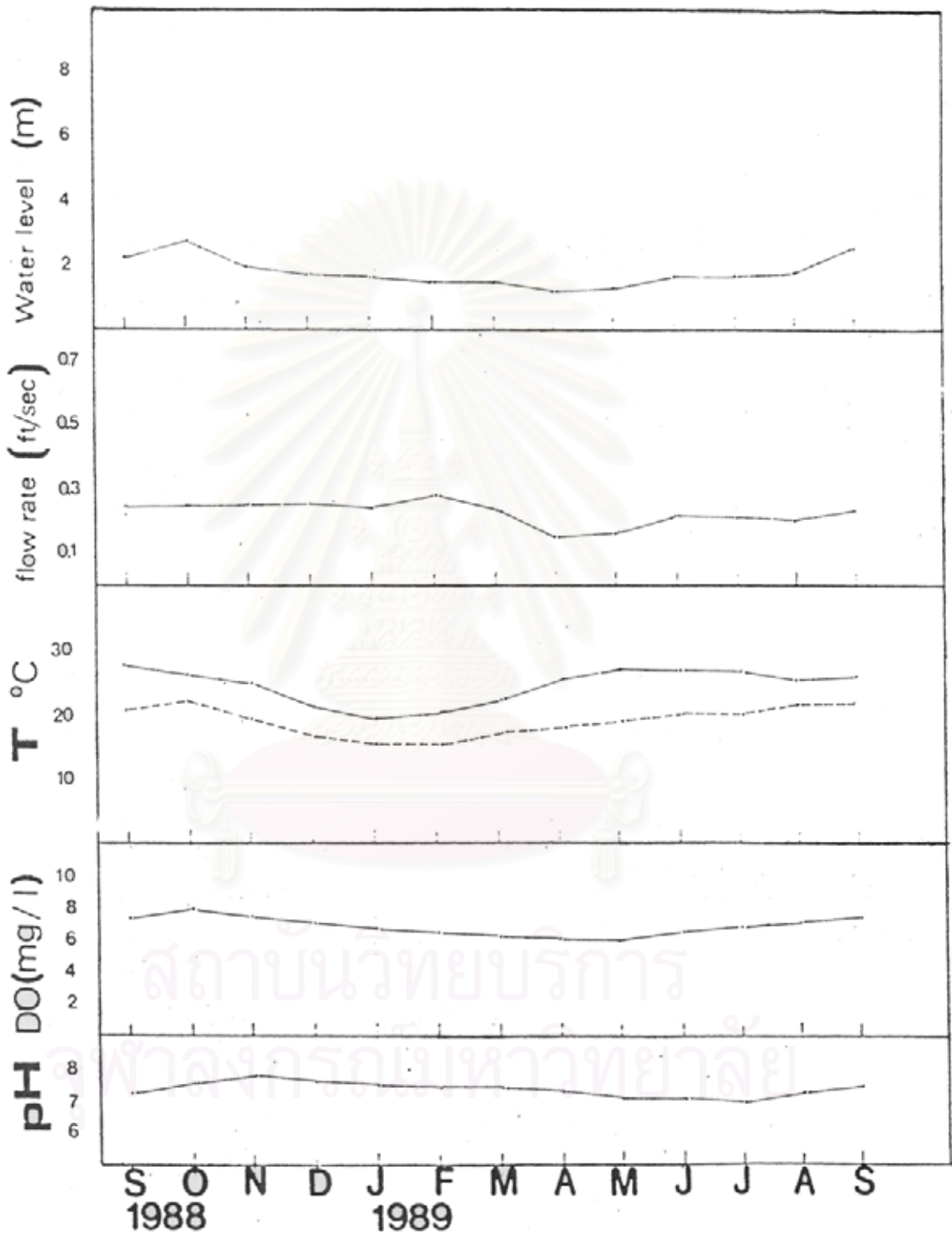


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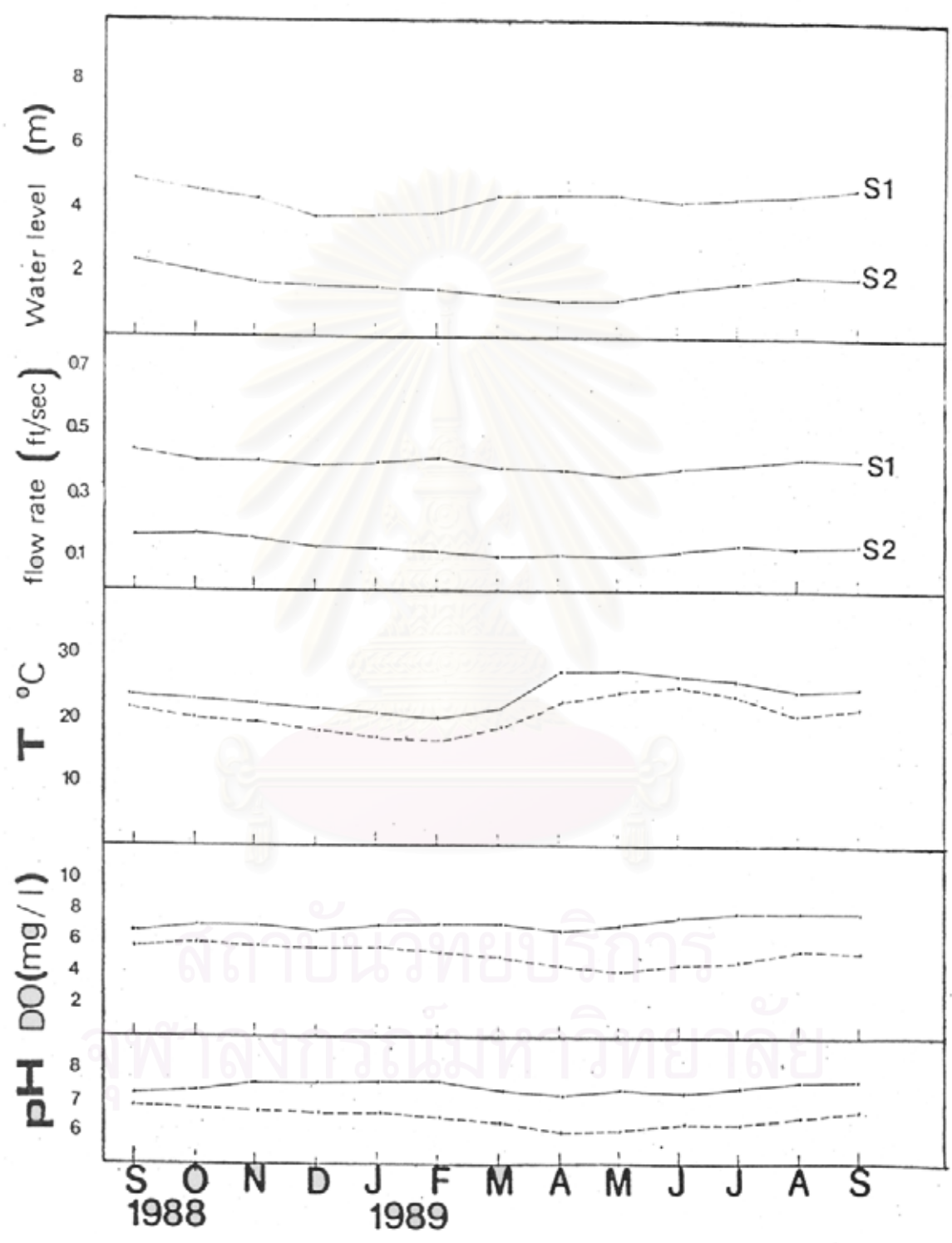




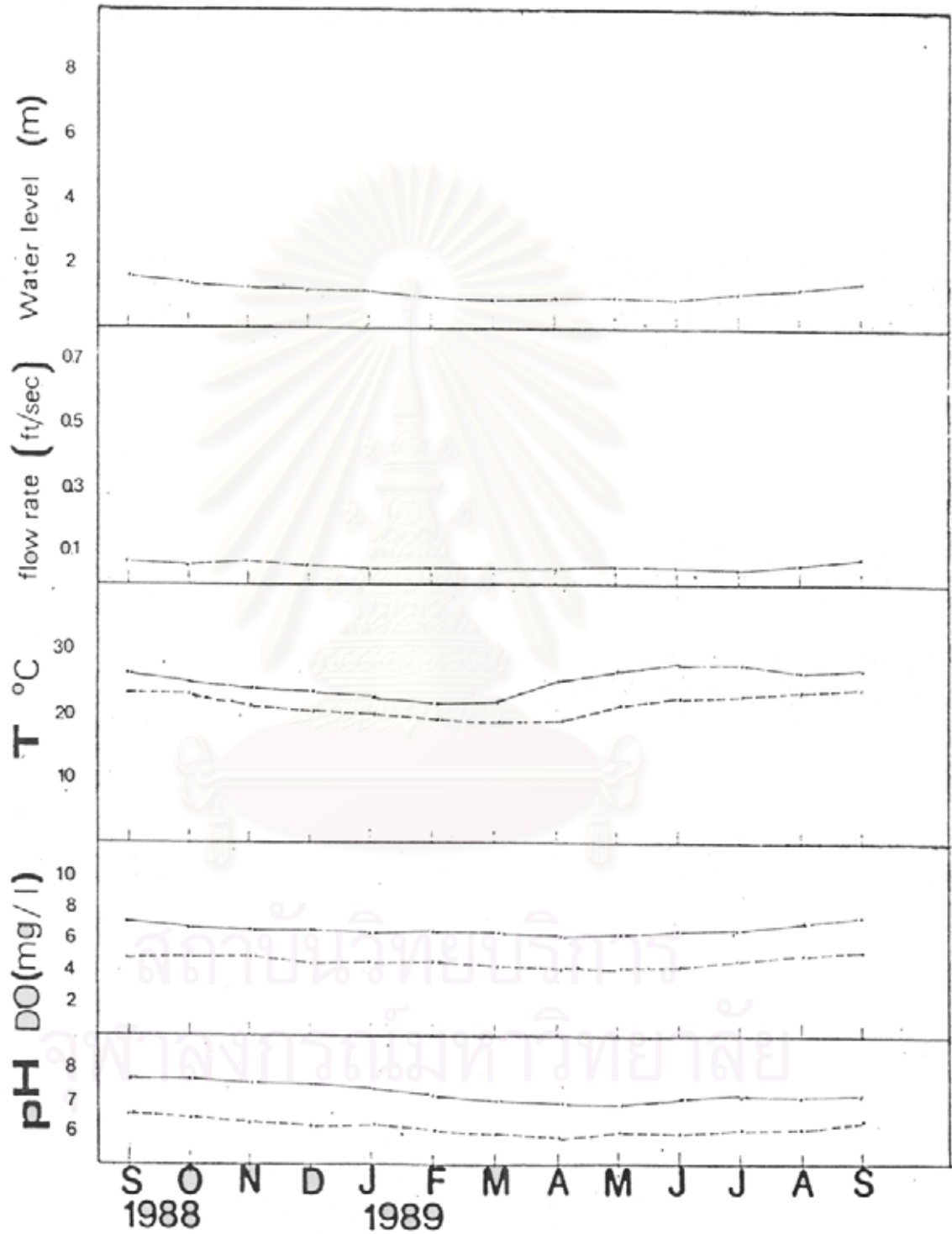
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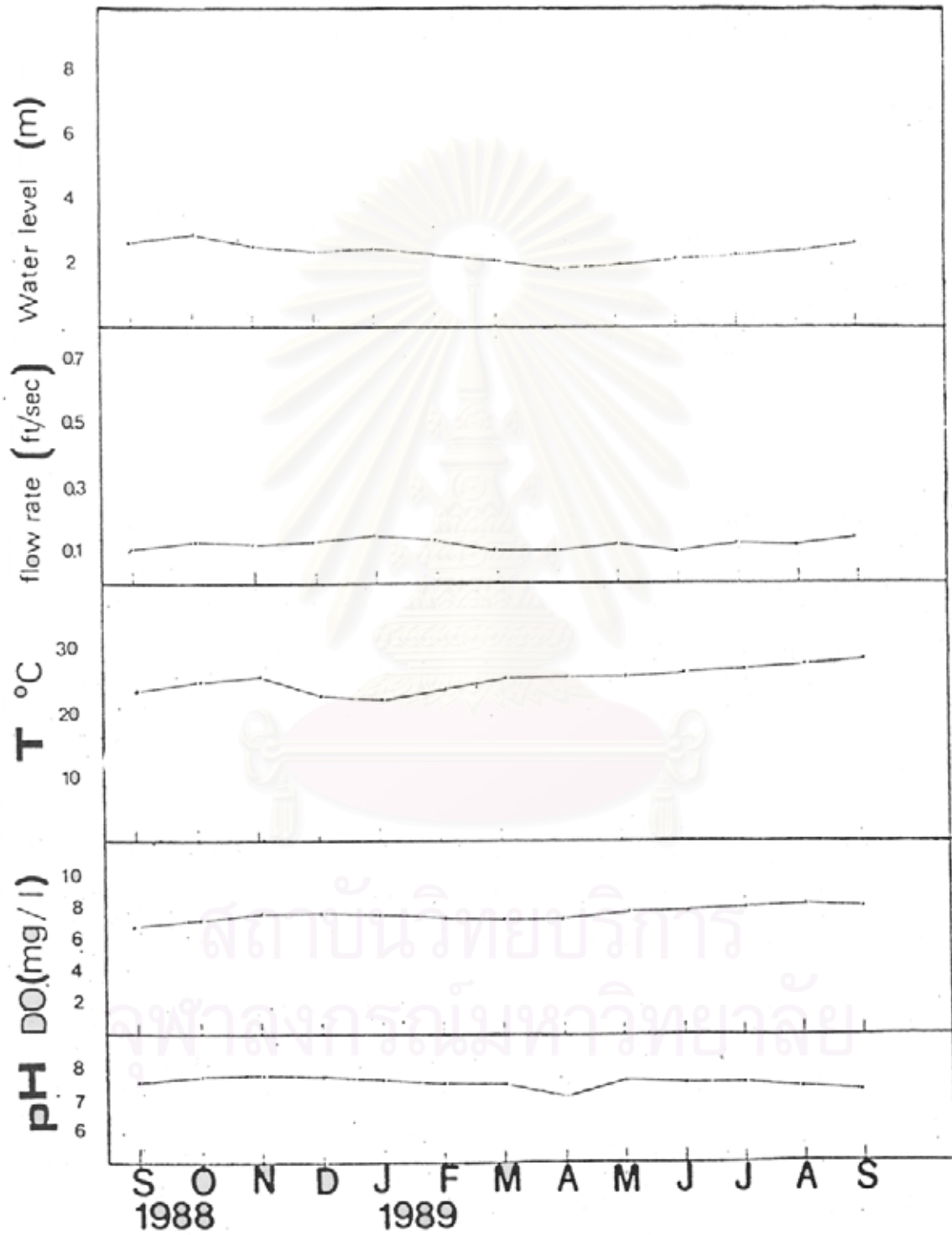


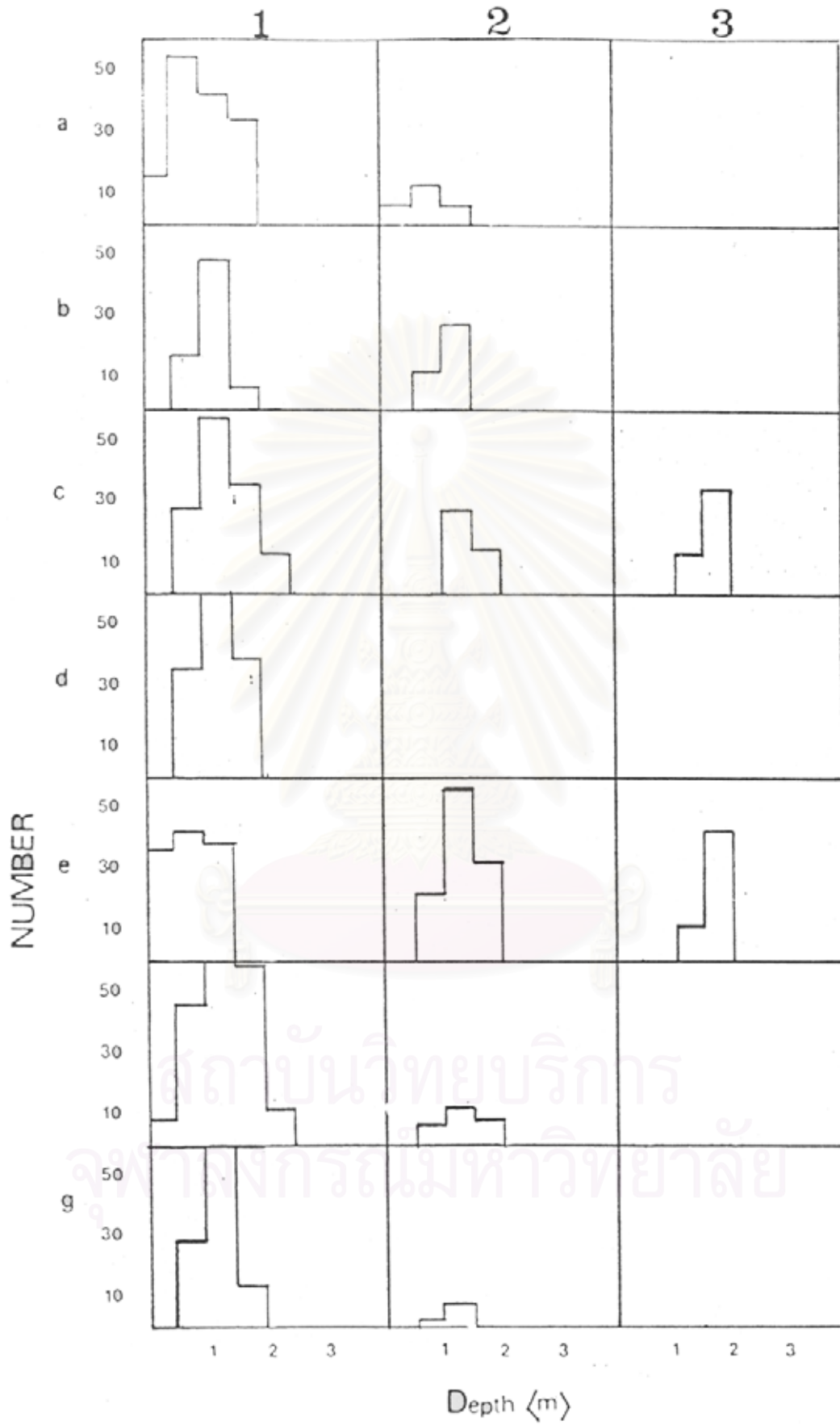
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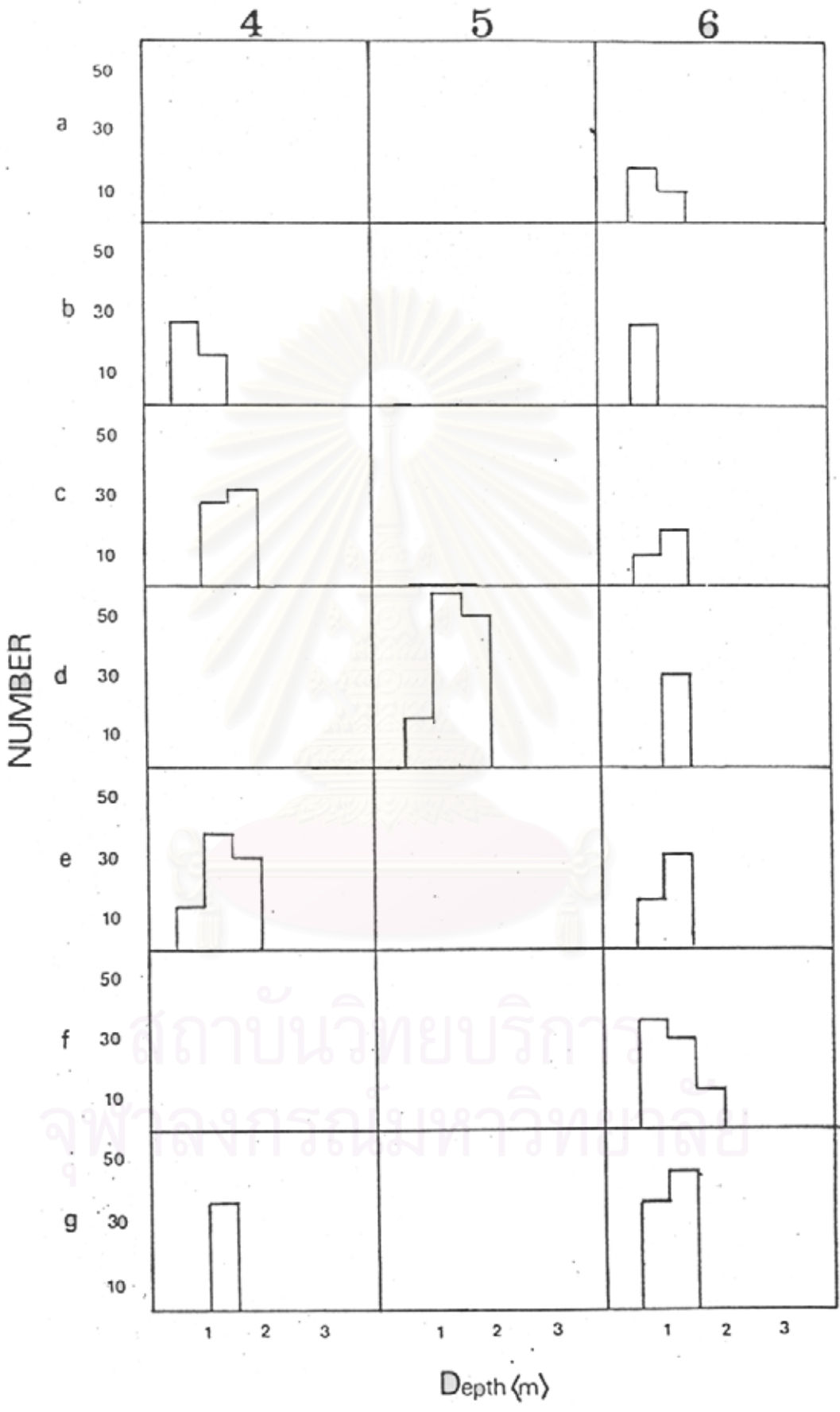
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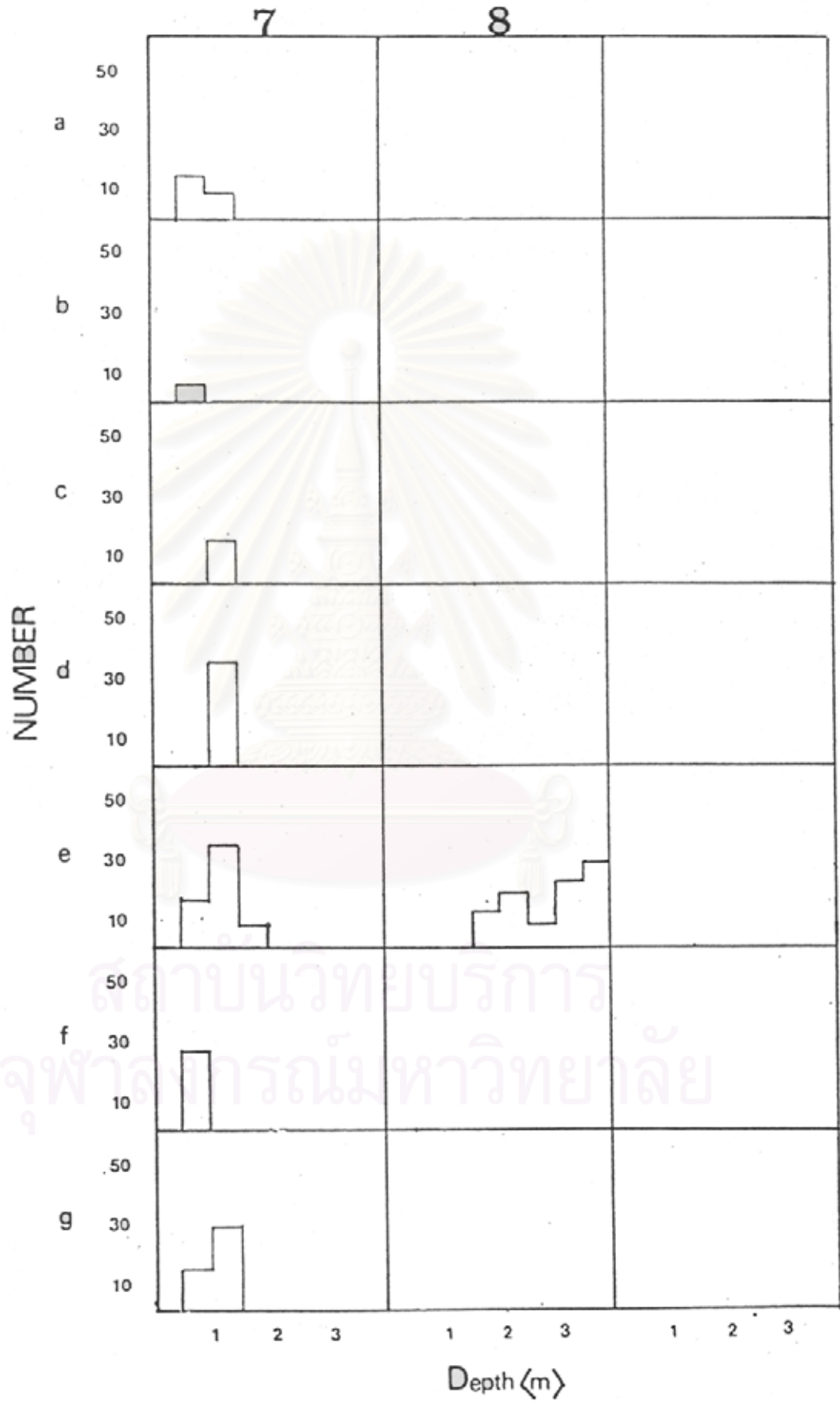
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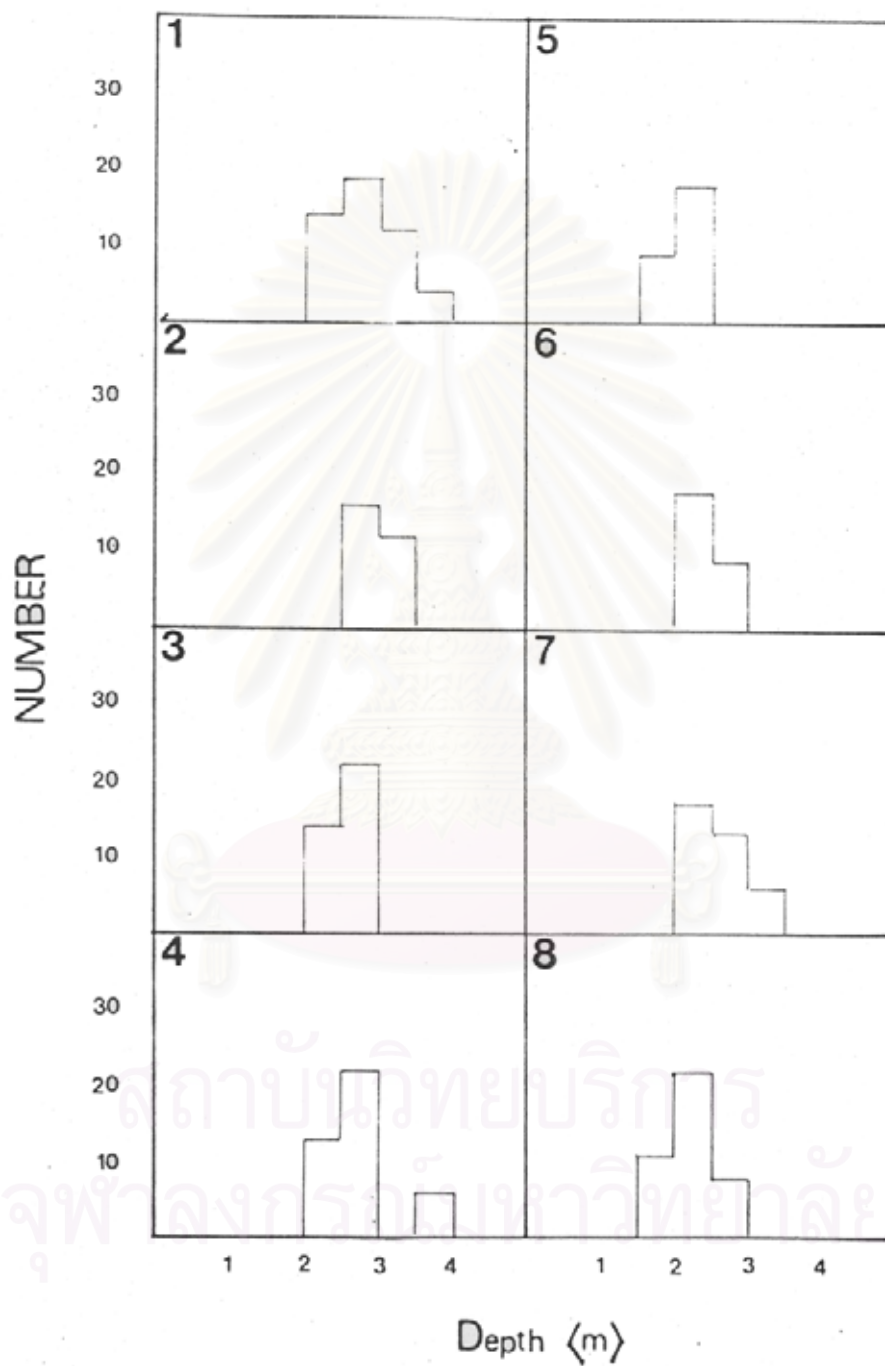




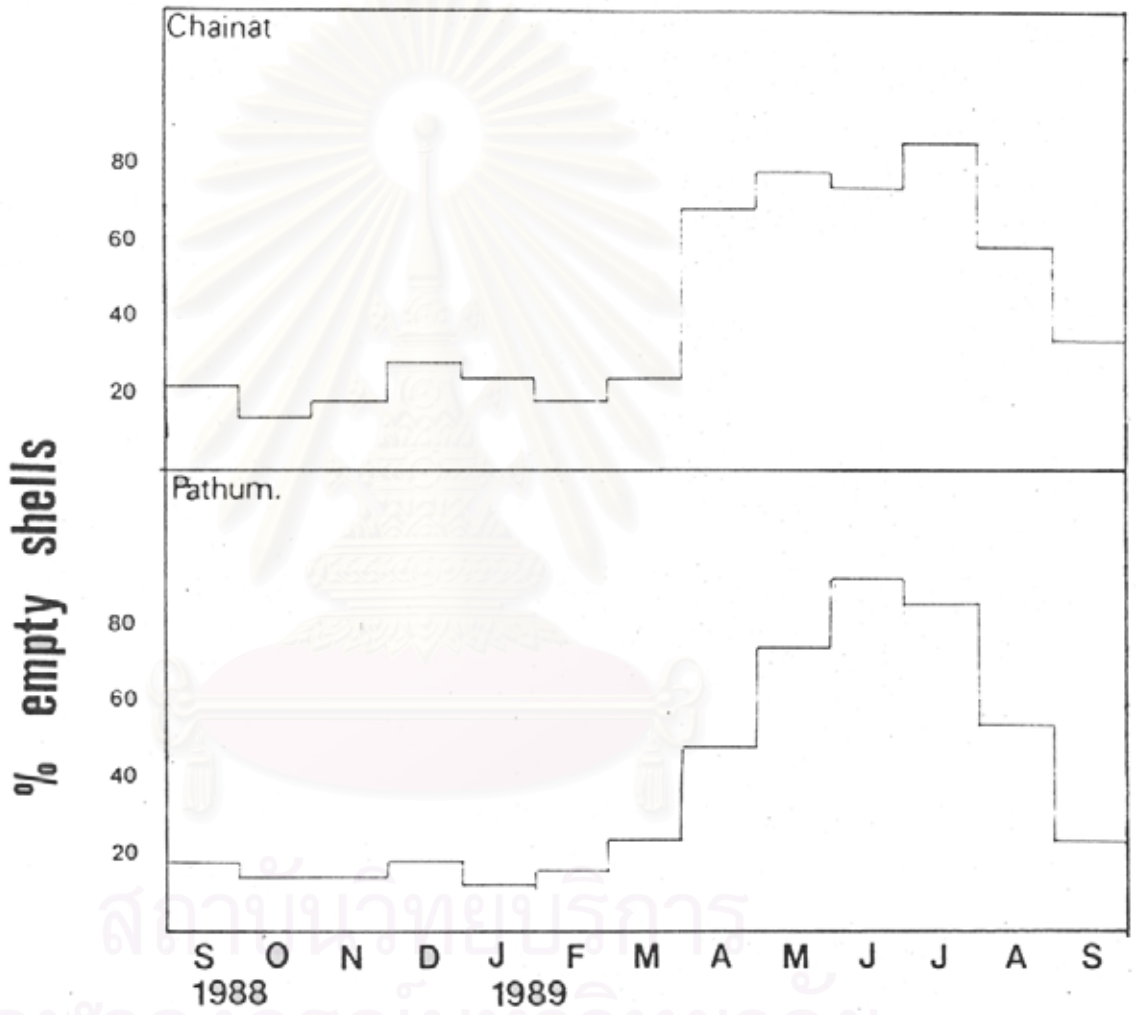
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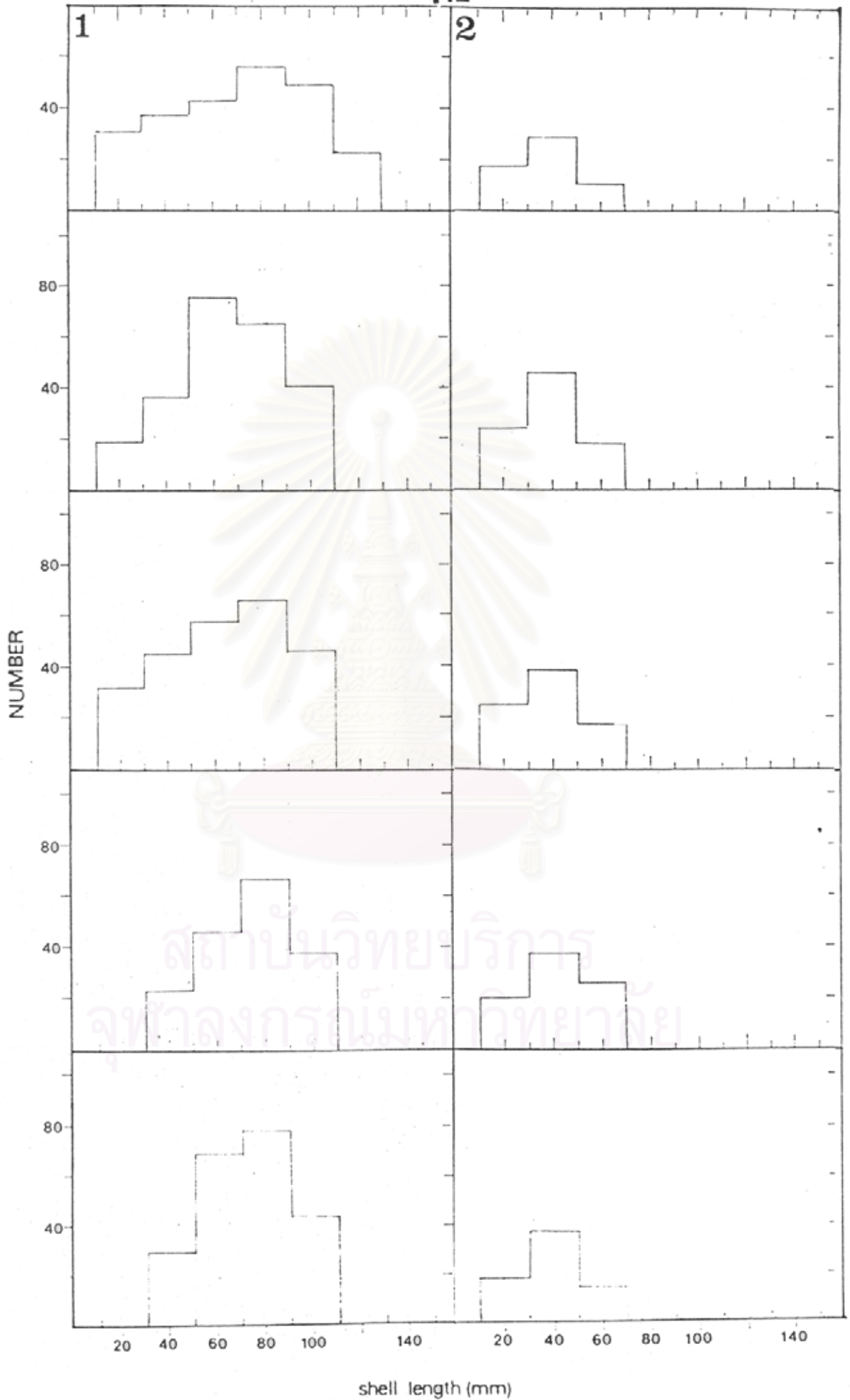
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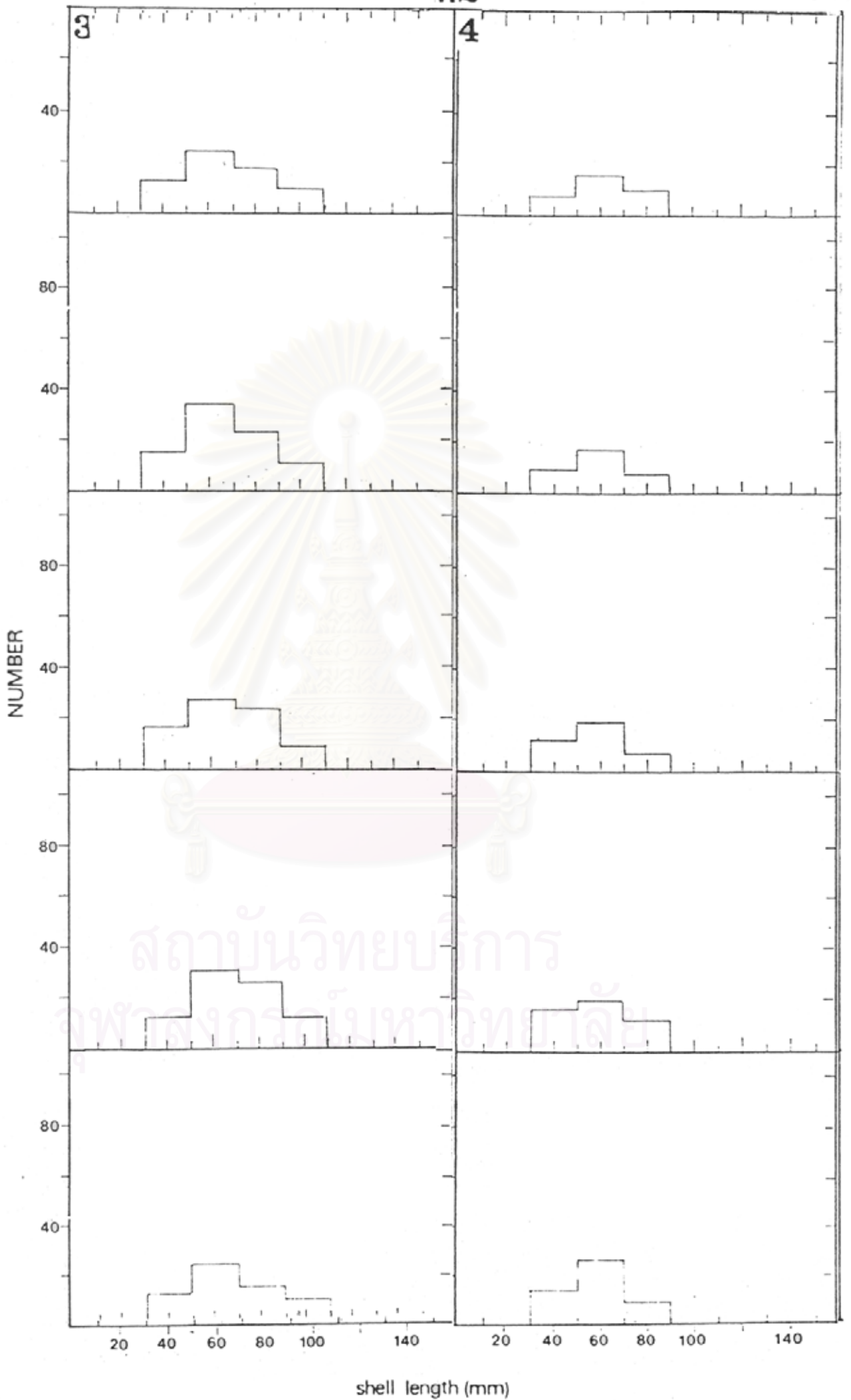
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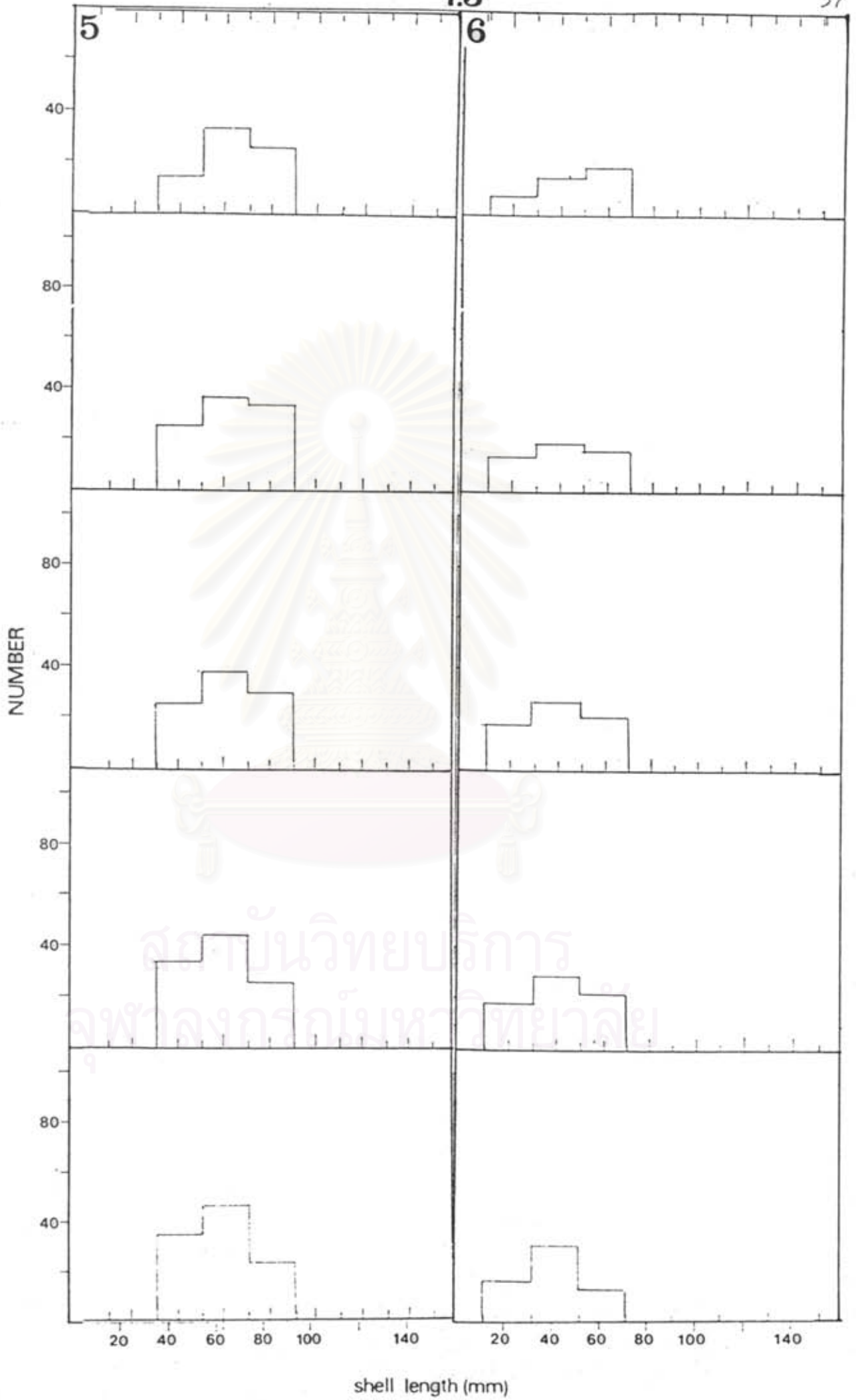
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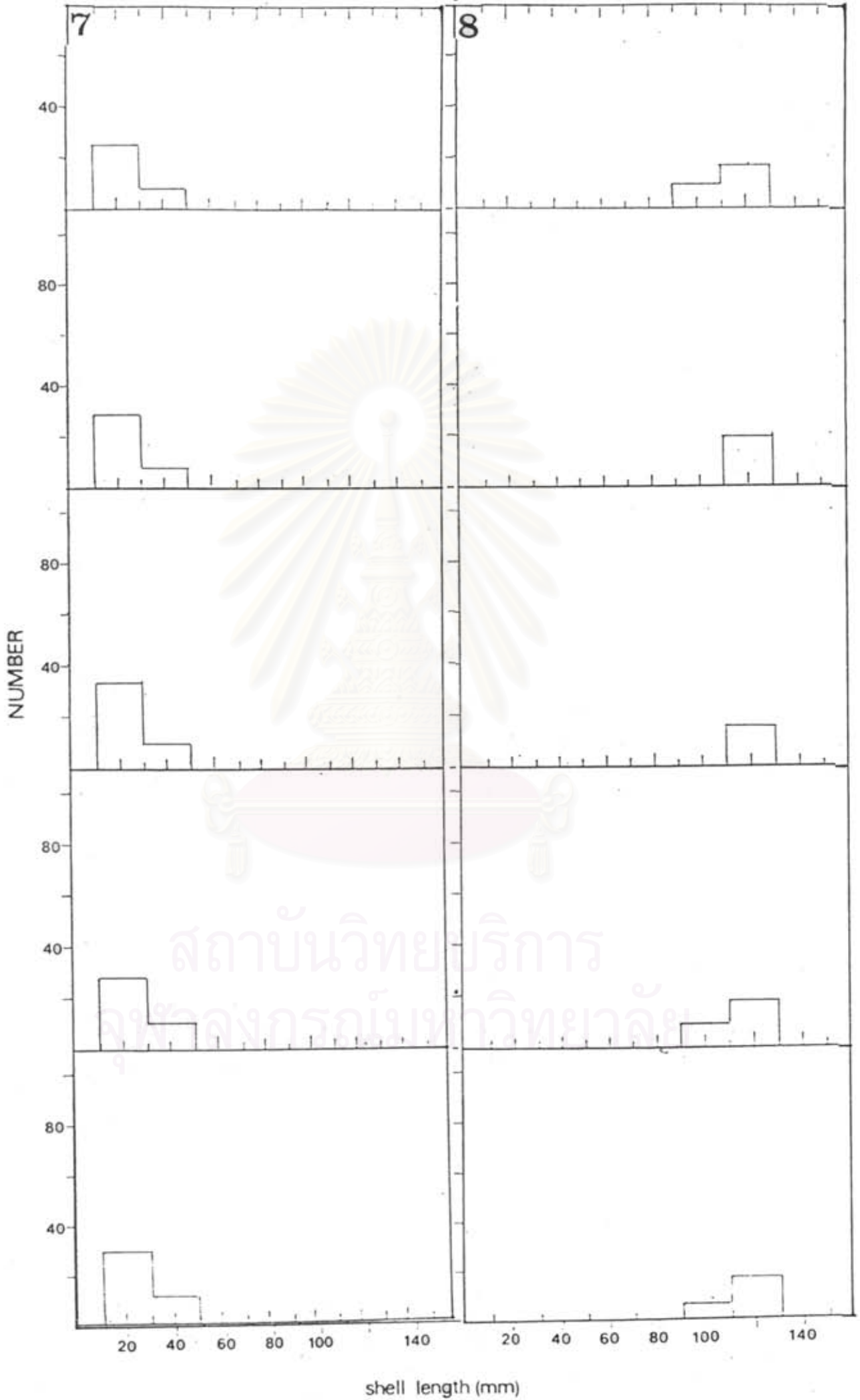






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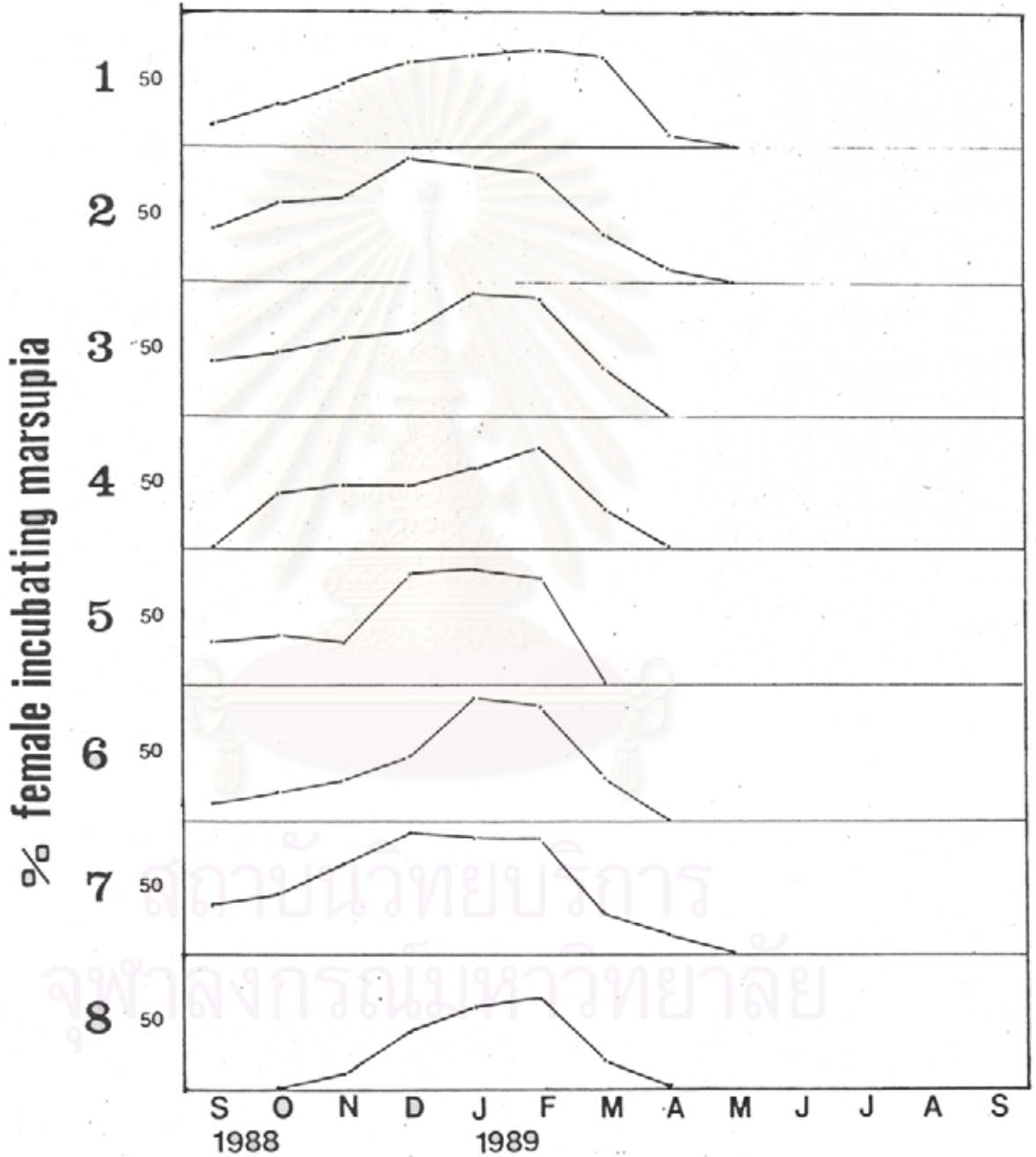


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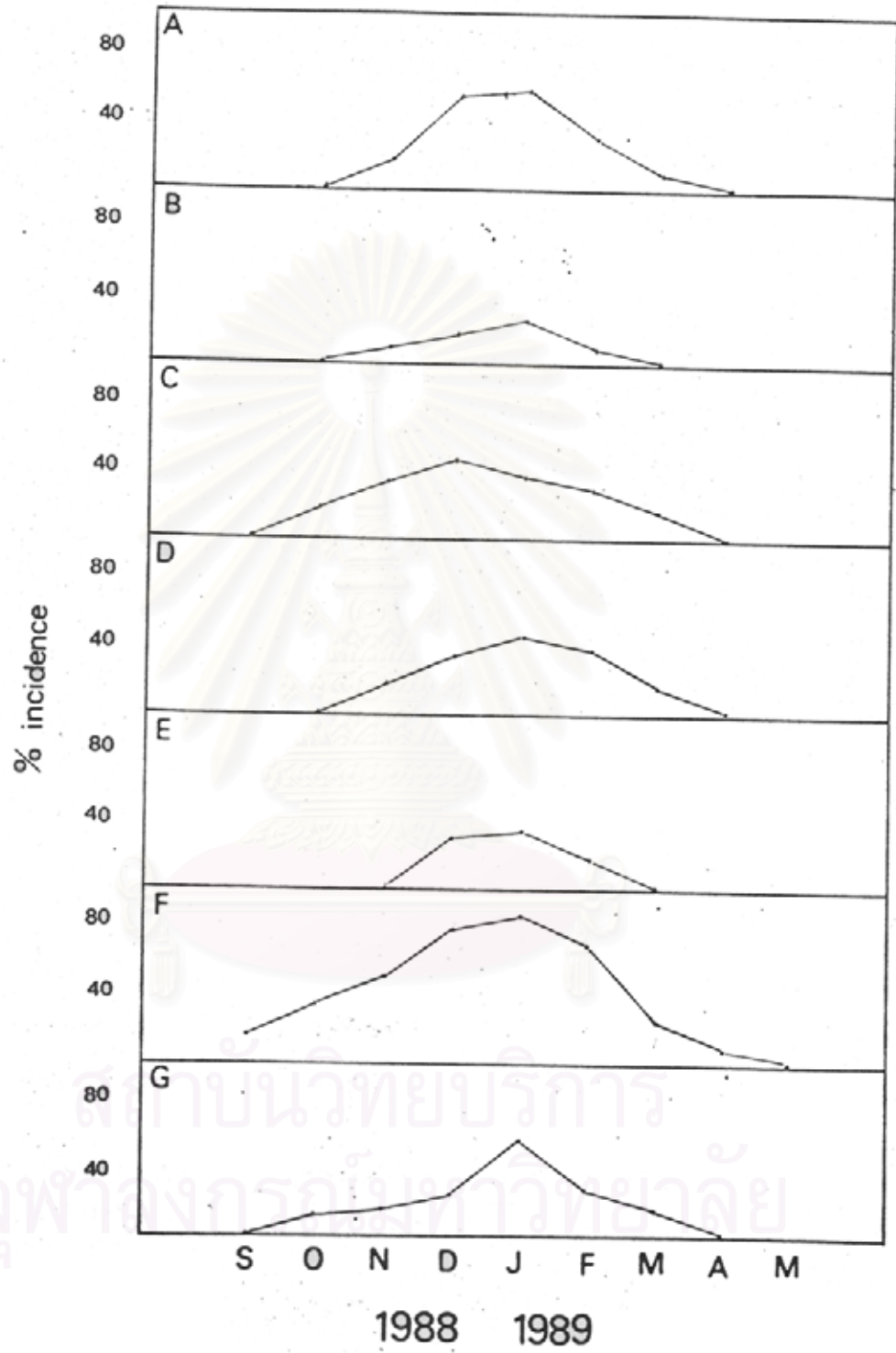
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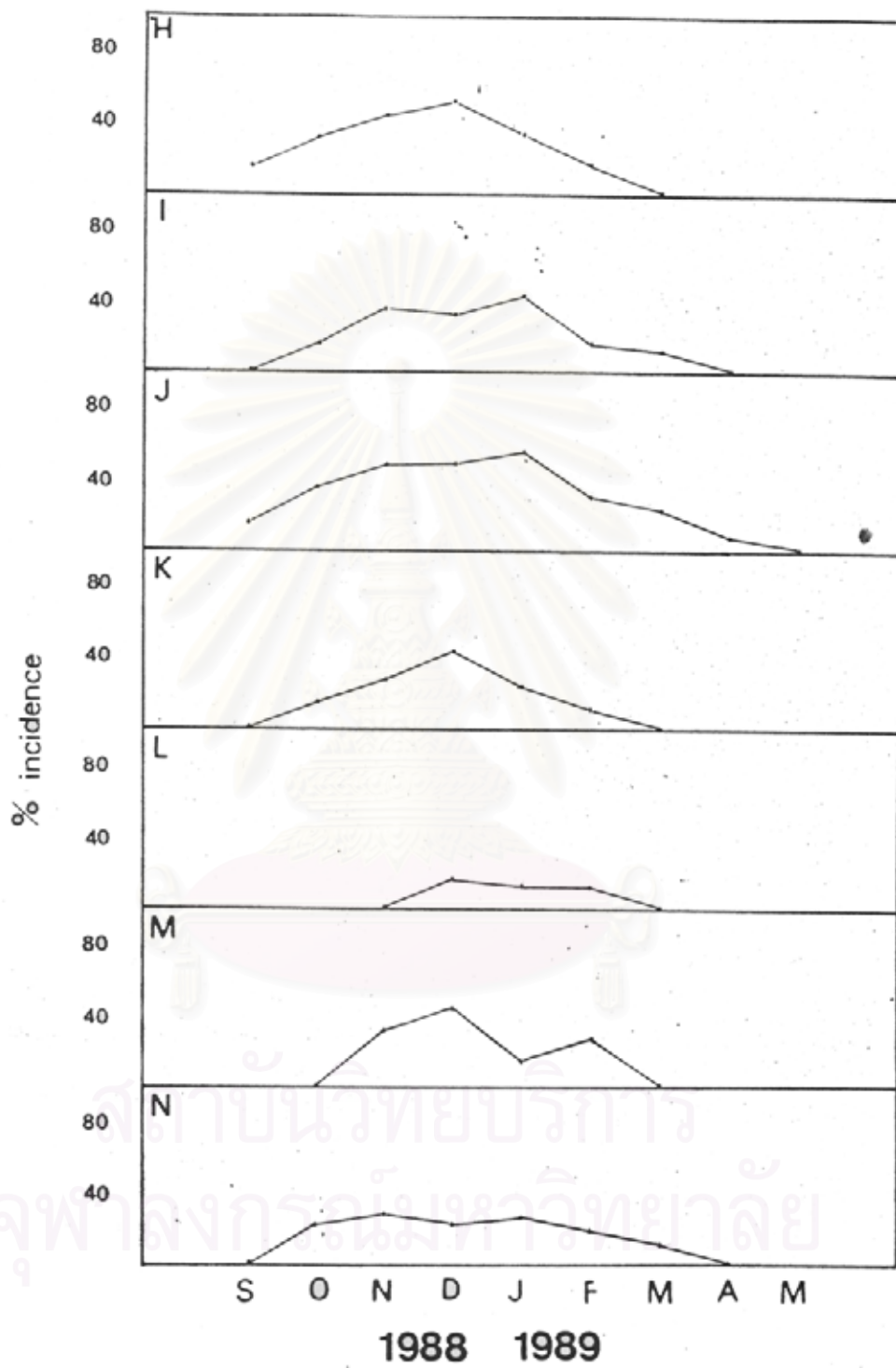




# 10.1



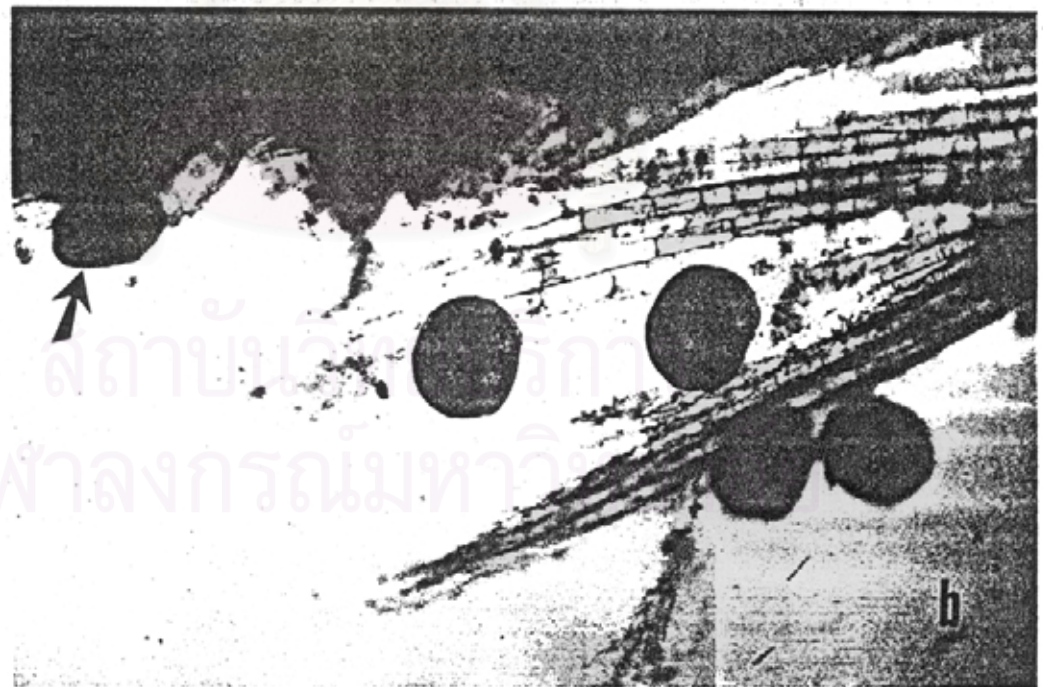
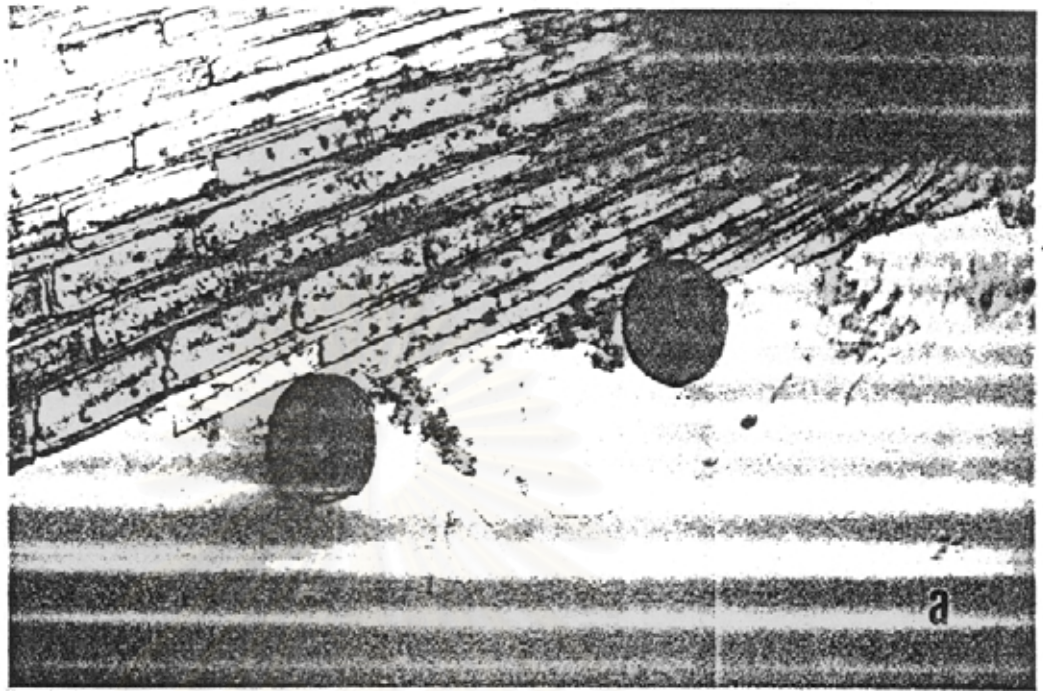
## 10.2





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