REPRODUCTIVE BIOLOGY OF THREE FRESHWATER MUSSELS (BIVALVIA:UNIONIDAE) AND INCIDENCE OF PARASITIC GLOCHIDIA ON FISHES IN NAVIGATION POOL 7 OF THE UPPER MISSISSIPPI RIVER WITH OBSERVATIONS OF HOST SPECIFICITY OF LAMPSILIS VENTRICOSA (BARNES, 1823).

A Thesis

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by

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COLLEGE OF ARTS, LETTERS, AND SCIENCES

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We recommend acceptance of this thesis to the College of Arts, Letters, and Sciences in partial fulfillment of this candidate's requirements for the degree Master of Science in Biology. The candidate has completed his oral defense of the thesis.

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ABSTRACT

Three species of adult freshwater unionid mussels were examined for reproductive development on about a weekly basis from May 1982 through October 1982 and during April 1983 in Pool 7 of the Upper Mississippi River. *Lampsilis ventricosa* (Barnes 1823) was confirmed a bradytictic (long term) breeder with syngamy being exhibited during peak ambient river temperatures (24.5-26°C) in late July and early August. Developing larvae were held in marsupia until being released the following spring and early summer. *Proptera alata* (Say 1817) was also confirmed a bradytictic breeder with a similiar breeding season to *L. ventricosa*. Glochidia of *P. alata* were also contained in specialized brood chambers (marsupia). *Amblema plicata* (Say 1817) is a tachytictic or short term breeder, spawning and releasing the glochidia in the same season. Syngamy took place from late May to early July with ambient river temperatures ranging from 18 to 21°C. Glochidia of *A. plicata* were held in all four of the demibranchs and were released from early June to early August.

A sample of 1786 fish (33 species) collected in Pool 7 of the Upper Mississippi River were examined for incidence of glochidia. Of those fish examined, 74 (4.14%) had parasitic glochidia attached somewhere. *Notropis hudsonius* showed the most infection. The highest percent incidence occurred on *Stizostedion vitreum* (100%) with an average of seven glochidia per fish.

Eight species of fish were tested for their suitability as hosts of *L. ventricosa* glochidia. Species of fish infected in this study that are considered to be possible fish hosts for *L. ventricosa* glochidia by length of attachment are: *Lepomis macrochirus*, *Lepomis cyanellus*, *Micropterus dolomieui*, *Perca flavescens*, and *Stizostedion vitreum*.

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INTRODUCTION

Filter feeding unionid mussels are predominant members of the macrobenthic communities in many freshwater systems. Mussels play an important role in capturing the energy in the particulate organic matter which may otherwise be lost from streams (Wallace et al. 1977). Mussels also serve as an important food source for muskrats, racoons, birds and many game fishes. Due to their sessile-like characteristics, mussels serve as good indicators of environmental conditions (Smith et al. 1975).

Modifications of aquatic environments by human activities have significantly affected the composition and extent of the freshwater mussel fauna. Pollutants introduced by industrial, municipal and agricultural effluents have adversely affected freshwater mussel distributions and abundances (Dineen 1971, Mackie and Qadri 1973). Modification of the Mississippi River by impoundment has reduced the riverine like habitats while producing lentic environments which resulted in reduced oxygen, increased sedimentation, and also prevented the free migration of fishes (Ortmann 1909, Bates 1962, Stansbery 1971, Fuller 1974). Commercial navigation activities i.e., siltation, wingdam construction, and channel maintanance dredging operations have also contributed to mussel population decline (Fuller 1974, 1978).

The development of a commercial industry which employed products from freshwater mussels has probably been one of the most destructive factors to the mussel population of the Mississippi River Valley (Coker et al. 1921, Grier 1922, Ellis 1931, Stansbery 1971). The pearl button industry of the Upper Mississippi River began in the late 1880's near Muscatine, Iowa, and rapidly expanded along the Mississippi River (Smith 1898). The Mississippi mussel industry employed hundreds of people and in the first six months of 1898, 3641 tons of mussel shells valued at over $37,000 were purchased and used to produce 1,160,602 gross of button blanks valued at $252,570 (Smith 1898). Depletion of mussel beds coupled with the advent of the plastic button led to the decline of the mussel fishery in the late 1920's. Demand for Mississippi River mussels was revived in the 1960's when the cultured pearl industry of Japan began employing the nacre of mussel shells for cultured pearl production. A drastic increase in the harvest of freshwater mussels has occurred in the Upper Mississippi River because of depletion of southern streams from overharvest, increased demand from the cultured pearl industry, and greater demand for natural pearls in the jewelry market (UMRCC 1985).

Due to declines in the mussel fauna of various systems, studies were initiated to develop propagation methods. Leydig (1866) discovered that larvae (glochidia) of freshwater mussels are parasitic on fish. Since that time, many attempts have been made to artificially propagate mussels. Surber (1912), Lefevre and Curtis (1912), Howard (1914), Coker et al. (1921), Baker (1928) and Jones (1950) have all dealt with the subject of reproduction and propagation of freshwater mussels. Although generalizations exist regarding the propagation and reproductive biology of various mussels, specific data do not exist for many commercially and ecologically important species. Propagation and management requires more precise information concerning the actual timing of gametogenesis, fertilization, release of glochidia, and host specificity.

Freshwater mussels are classified into two very different reproductive strategies: bradytictic and tachytictic. In the Upper Mississippi River two predominant subfamilies demonstrate these strategies. The Lampsilinae are bradytictic or long term breeders. Ova are released by the female into a suprabrachial cavity in late summer or early fall and are concurrently fertilized by sperm which is emitted into the water column. After fertilization, the new embryo is transferred into the marsupial gills where they develop into the parasitic clam larvae (glochidia). The glochidia remain in the marsupia until the following summer in which they are released through the marsupial wall. The members of the sub-family Ambleminae are tachytictic or short-term breeders. Fertilization occurs in the spring and glochidia are released in the late spring or early summer.

In addition to limited information on reproductive development and timing, few data are available on the specific fish host needs by many species. Fuller (1974) has summarized most of the glochidia fish host relationships researched prior to 1972. Subsequent work to Fuller's review has been done by Kakonze (1972), Stein (1973), Wiles (1975), Weir (1977), and Zale (1980).

This study was designed (1) to examine in detail the reproductive biology of adults of three important unionid mussel species in the Upper

Mississippi River; Amblema plicata (Say, 1817), Lampsilis ventricosa (Barnes, 1823), and Proptera (Potamilus) alata (Say, 1817) on Pool 7 of the Upper Mississippi River, (2) to determine relative abundance of glochidia present on fishes collected at a Pool 7 site of the Upper Mississippi River, and (3) to determine possible fish hosts for L. ventricosa through host specificity experiments.

MATERIALS AND METHODS

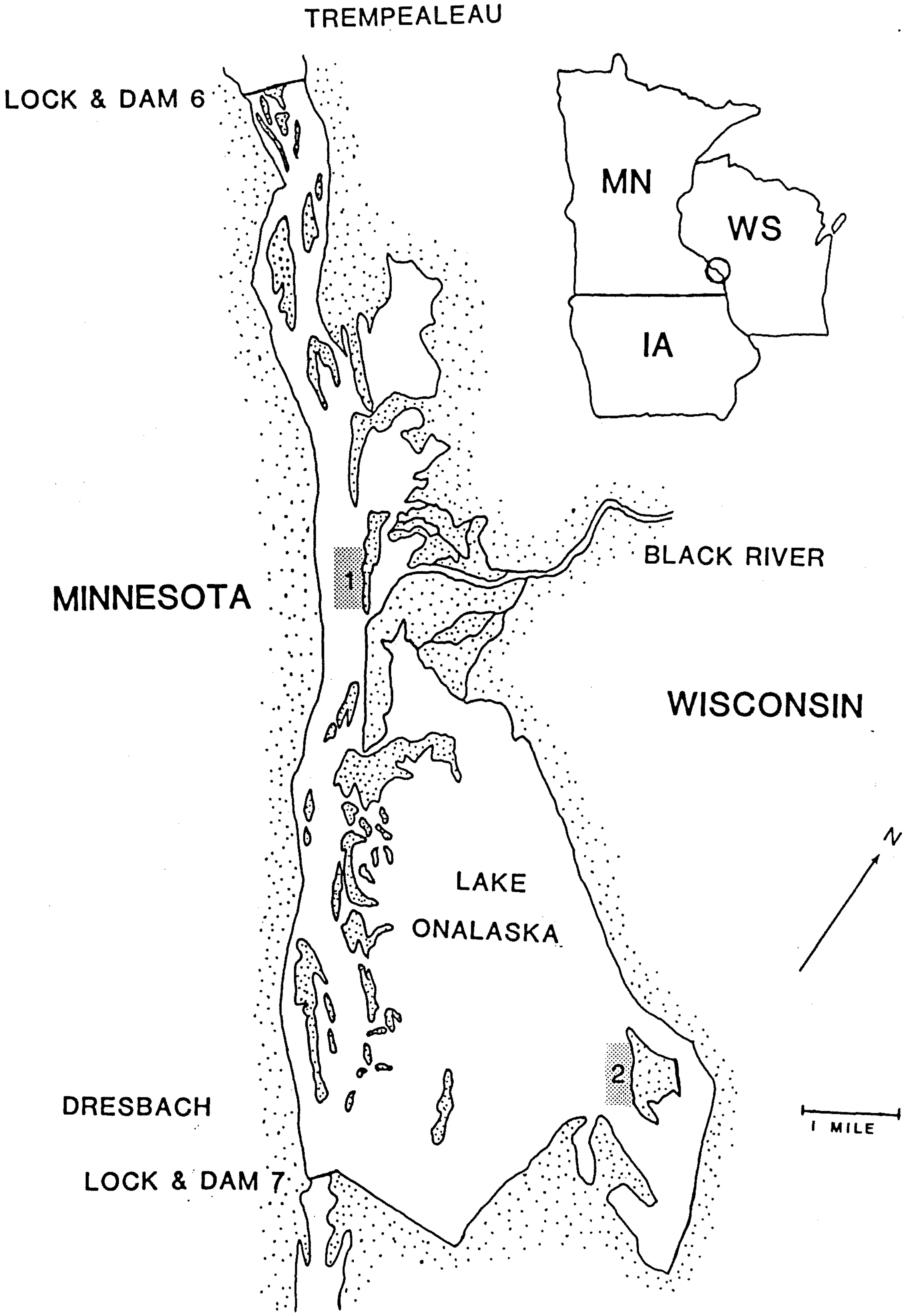
Sample Area

The Upper Mississippi River is that portion of the river which extends from Minneapolis/St. Paul, Minnesota, to Cairo, Illinois. It is made up of an impounded region consisting of a series of 27 lock and dams between Minneapolis/St. Paul, Minnesota and St. Louis, Missouri, and an open reach below that to Cairo, Illinois.

Navigation Pool 7, located in the impounded region of the Upper Mississippi River, is approximately 11.8 River Miles long stretching from Trempealeau, Wisconsin, to Dresbach, Minnesota.

Two sample areas were used for the collection of mussels for this study (Fig. 1). Station #1 was the major collection area and Station #2 was used when high water conditions made collections difficult at Station #1. Station #1 was located at River Mile 708.6 and is classified as a main channel border as defined by Rasmussen (1979). The station is characterized by a dredge spoil island with sand substrate. All mussels were collected at water depths of 0.3 to 1.5 m. The current velocity varied through the season from 3.9 cm/sec to 35.97 cm/sec. The station was a site of high recreational use such as camping and water skiing. Station #1 was chosen because of the abundance of the three targeted mussel species needed for this study. Other mussel species present during the sampling period included *Fuscionaia flava* (Rafinesque, 1820), *Quadrula pustulosa* (Lea, 1831), *Obliquaria reflexa* (Rafinesque, 1820), and *Truncilla donaciformis* (Lea, 1827). Substrate vegetation was absent in much of the study area except for the southernmost end where some *Vallisneria* was present.

Figure 1. Location of study sites, Stations 1 and 2, in Pool 7 of the Upper Mississippi River.

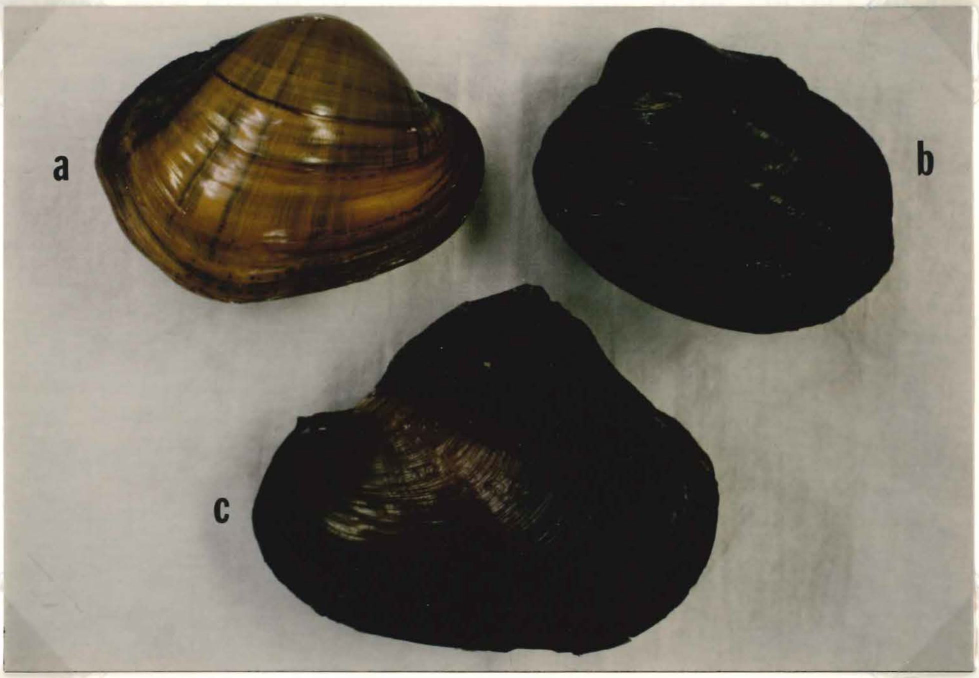

TREMPEALEAU
LOCK & DAM 6
MN
WS
IA
BLACK RIVER
1
MINNESOTA
WISCONSIN
N
LAKE
ONALASKA
2
DRESBACH
1 MILE
LOCK & DAM 7

The second sample area (Station #2) was located adjacent to Rosebud Island in Lake Onalaska (Fig. 1). The substrate was primarily coarse sand with some cobble and small rock, and a large number of dead mussel shells. Vegetation was abundant and consisted of the submergent types Ceratophyllum sp., Potamogeten crispus, and Myriophyllum sp. The current was very low (less than 3 cm/sec) during the sample period. The mussel fauna predominantly consisted of Amblema plicata (Say, 1817), Fusconaia flava (Rafinesque, 1820), Proptera alata (Say, 1817), Leptodea fragilis (Rafinesque, 1820), Lampsilis radiata luteola (Lamarck, 1819), and Lampsilis ventricosa (Barnes, 1823). A more complete list of the mussels present at this site was reported by Havlik (1983).

Reproductive Biology

Three species of freshwater unionid mussels were examined for reproductive development during the 1982 and 1983 season. The three-ridge, Amblema plicata (Say, 1819) (Fig. 2b), was selected because it was reported as the most abundant mussel in the Upper Mississippi River (Havlik 1983, Fuller 1978, Theil 1981), and it is a commercially important species for the Japanese pearl culture industry (Utterback 1915). The pocketbook mussel, Lampsilis ventricosa (Barnes, 1823) (Fig. 2a), was chosen because it is a congener of the endangered Lampsilis higginsi (Lea, 1857). Information gathered on this closely related mussel could possibly provide a model for studies related to recovery of the endangered Higgins' Eye mussel. The pink heel splitter, Proptera alata (Say, 1817) (Fig. 2c), another Lampsilinae and bradytictic breeder, is a relatively abundant mussel species in Pool 7 of the Upper Mississippi River (Havlik 1983).

Figure 2. Representative adult specimens of *Lampsilis ventricosa* (a), *Amblema plicata* (b), and *Proptera alata* (c).


a
b
c

The three species of freshwater mussels were collected by hand in water ranging 0.3 to 1.5 m in depth. Mussels were collected from Station #2 from 8 April to 29 April 1983 because of high water at Station #1. Mussels were collected from Station #1 on about a weekly basis from 27 May to 1 October 1982. Attempts were made to collect at least six individuals of each species on each of the 18 sampling dates. Specimens were placed in 70% ethyl alcohol after collecting and were transported to the laboratory for further observation and histological examination. A total of 124 L. ventricosa, 229 A. plicata and 82 P. alata were collected and examined microscopically to identify sex, gonadal condition, and the presence or absence of glochidia in the demibranchs in the female specimens. A section of the gonadal-visceral mass was dissected out and preserved in 70% ethyl alcohol in separate vials. Each individual mussel was aged (by counting growth annuli on valves) and marked with a corresponding identification number. If glochidia were present in the demibranchs, one of the demibranchs was preserved in a separate vial and measurements were taken of the glochidia.

The dissected sections of the gonadal-visceral mass were run through a standard dehydration series (Humason 1967) and put into parafin for histological sectioning. Serial sections (0.8μm) were made with a microtome and placed on precleaned microscope slides which were coated with albumin. Sections were fixed to the slides by wetting with 5% formalin. Slides were then run through a standard Eosin Hematoxylin staining series. Specimens were observed microscopically for the size and amount of sex material present and the stage of gamatogenesis was recorded. Stages of gametogenesis were divided into four catagories based on data from Yokely (1972) for both males and females as follows:

Males

Stage 1 - Acini widely spaced, lumina with some spermatogonia and nutritive material present (space present)

Stage 2 - Space between acini less, more spermatogonia present and some spermatids present, lumina becoming filled

Stage 3 - Acini closely packed, lumina filled with tightly spaced spermatogonia, spermatids, and spermatozoa.

Stage 4 - Acini widely spaced with no gametic materials present.

Females

Stage 1 -Acini widely spaced with lumina relatively empty, some small ova present surrounded by nutritive material.

Stage 2 -Acini spaced more closely with more and larger ova present, some in lumina.

Stage 3 -Acini closely situated with lumina filled with many large mature ova. Acinal walls appear much thinner.

Stage 4 - Spent individual, no ova present, only some nutritive material.

If demibranchs contained glochidia, developmental stage was observed and recorded. The condition of the marsupia was divided into four stages as described below:

1. No glochidia present
2. Few embryos and glochidia present
3. Full of embryos or glochidia
4. Evidence of the release of glochidia

Parasitic Period

Fishes were collected at Station #1 at two-week intervals during the 1982 sampling season to determine the incidence of natural infection by the parasitic glochidia. Fishes were collected with a 9-m bag seine (64-mm mesh lead and 32-mm mesh bag). Collections were taken at each of the nine biweekly sampling periods at 0600, 1200, 1800, and 2400 hours. All samples were fixed and preserved in 10% buffered formalin. Samples were inspected in the lab with the aid of a binocular field scope. Identification, size, place of glochidial attachment, and the number of glochidia were recorded. A total of 1,786 fish were examined.

One species of mussel was chosen for preliminary studies of host specificity. *Lampsilis ventricosa* was chosen because it is the congener of the endangered *L. higginsi*. The hosts for *L. ventricosa* could be tested with the glochidia of *L. higginsi* and could in turn be used for the recovery and mitigation of this species.

The laboratory component of this investigation involved the exposure of eight species of fish to *L. ventricosa* glochidia. The fish species selected represented the families Cyprinidae, Ictaluridae, Centrarchidae, and Percidae. Gravid female *L. ventricosa* were procured by hand and were transported immediately to the laboratory in one gallon plastic jugs containing ambient temperature river water. The females were maintained in aerated 40 liter aquaria at near ambient river temperatures and were fed zooplankton daily (mainly cladocerans and rotifers).

Infective glochidia were obtained from the female marsupia by using a hypodermic syringe puncturing the outer edge of the marsupia and flushing glochidia out with water into a petri dish. The glochidia were allowed to sit in a petri dish of water for approximately 15 minutes before being checked for viability with the use of a weak (1%) saline solution. Viable glochidia snapped shut when introduced to the saline solution. Approximately 10 fish of each of the eight different species were exposed to viable glochidia. All fish were hatchery/laboratory raised fish thus eliminating the possibility of previous infection or possible immunization to the glochidia (Arey 1923, Rueling 1919). The eight species chosen for this investigation were Cyprinus carpio, Pimephales promelas, Ictalurus melas, Lepomis macrochirus, Lepomis cyanellus, Micropterus dolomeiui, Perca flavescens, and Stizostedion vitreum. Each fish was exposed to approximately 50 glochidia. Glochidia were injected into the right opercular cavity using a hypodermic syringe. Fish were then placed in 40 liter aquaria at 14-15°C, the ambient temperature of the laboratory water supply. Commercial dry food pellets were fed to the fish on a daily basis throughout the extent of the investigation. Fish were anethesized in Tricaine methanesulfanate to reduce possible harm and inspected daily for attachment and development of glochidia. Presence or absence and approximate number of glochidia were recorded daily. The bottoms of the aquaria were siphoned and material inspected daily for sloughed off glochidia or transformed juveniles.

RESULTS

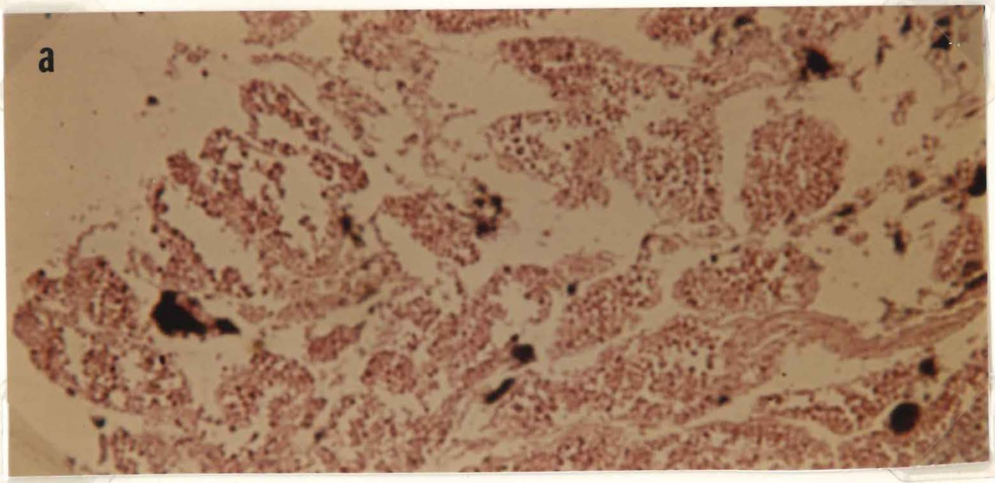
Gametogenesis

Lampsilis ventricosa. Gonadal activity was evident in both sexes of *L. ventricosa* throughout the year. Only mature adult mussels 3-15 years of age (average age, 7.5 years) were inspected. Acini (alveoli) of males from March through early June were widely spaced and contained of some spermatogonia and nutritive material (Stage 1; Fig. 3a,4a). Acini were fuller and contained more spermatogonia and some spermatids from early June 1982 to 10 July 1982. Not only were the lumina becoming more filled, but the space between acini was being reduced (Stage 2; Fig. 3b). Gonadal visceral mass was also more swollen in specimens collected at this stage of development. Late July samples were quite swollen and acini were full and distended with the lumina filled with many tightly packed spermatids and mature sperm (Fig. 3c). Male specimens collected from 21 July to 26 August 1982 were dominated by the prescence of sperm (Fig. 4a). Samples collected in early September no longer had a swollen gonadal visceral mass and acini were again widely spaced and contained nutritive granules with some spermatogonia. Peak sperm production seemed to be correlated with peak ambient river temperatures (Fig. 4a,4d).

Nearly all of the ovarian tissue examined during spring months (8 April through 23 June 1982) was classified in Stage #1 (Fig. 4b,5a) with small eggs surrounded by nutritive material. July specimens contained

Figure 3. Histological section of Lampsilis ventricosa testis in (a) Stage 1: Acini widely spaced with some spermatogonia and nutritive material (specimen collected 8 April 1983), (b) Stage 2: acini more closely spaced and lumina becoming filled with spermatids (specimen collected 23 June 1982), and (c) Stage 3: acini crowded and lumina filled with many spermatids and sperm (specimen collected 21 July 1982).

a



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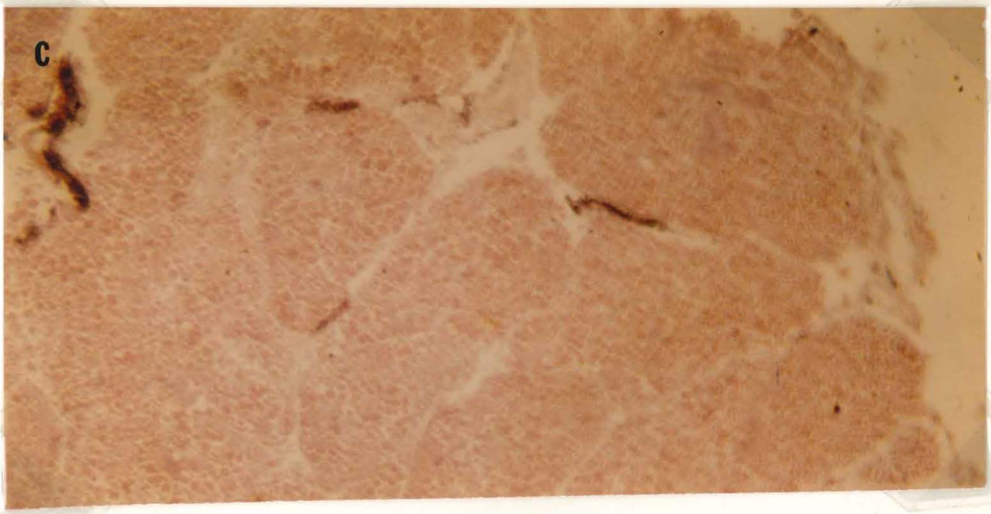
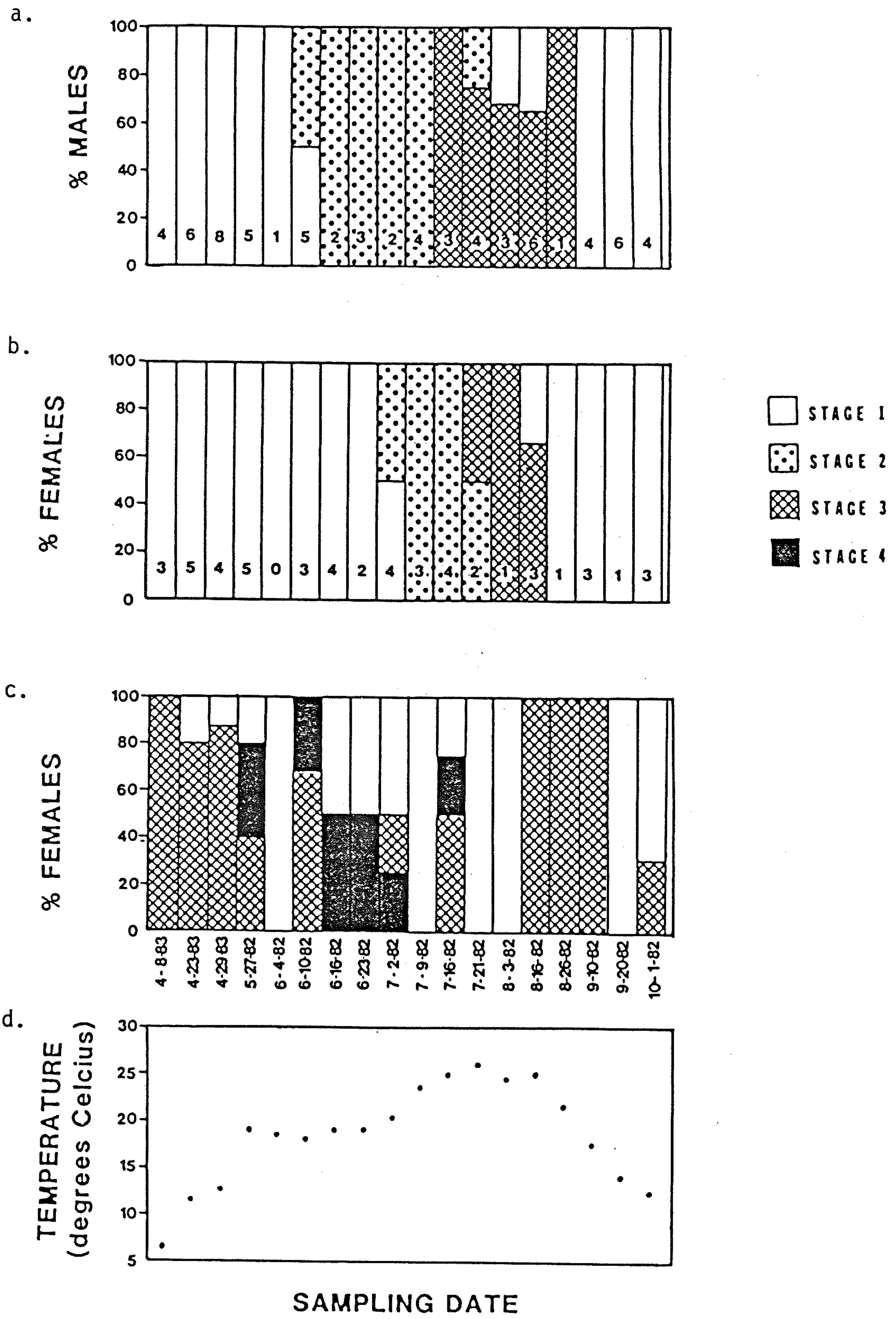
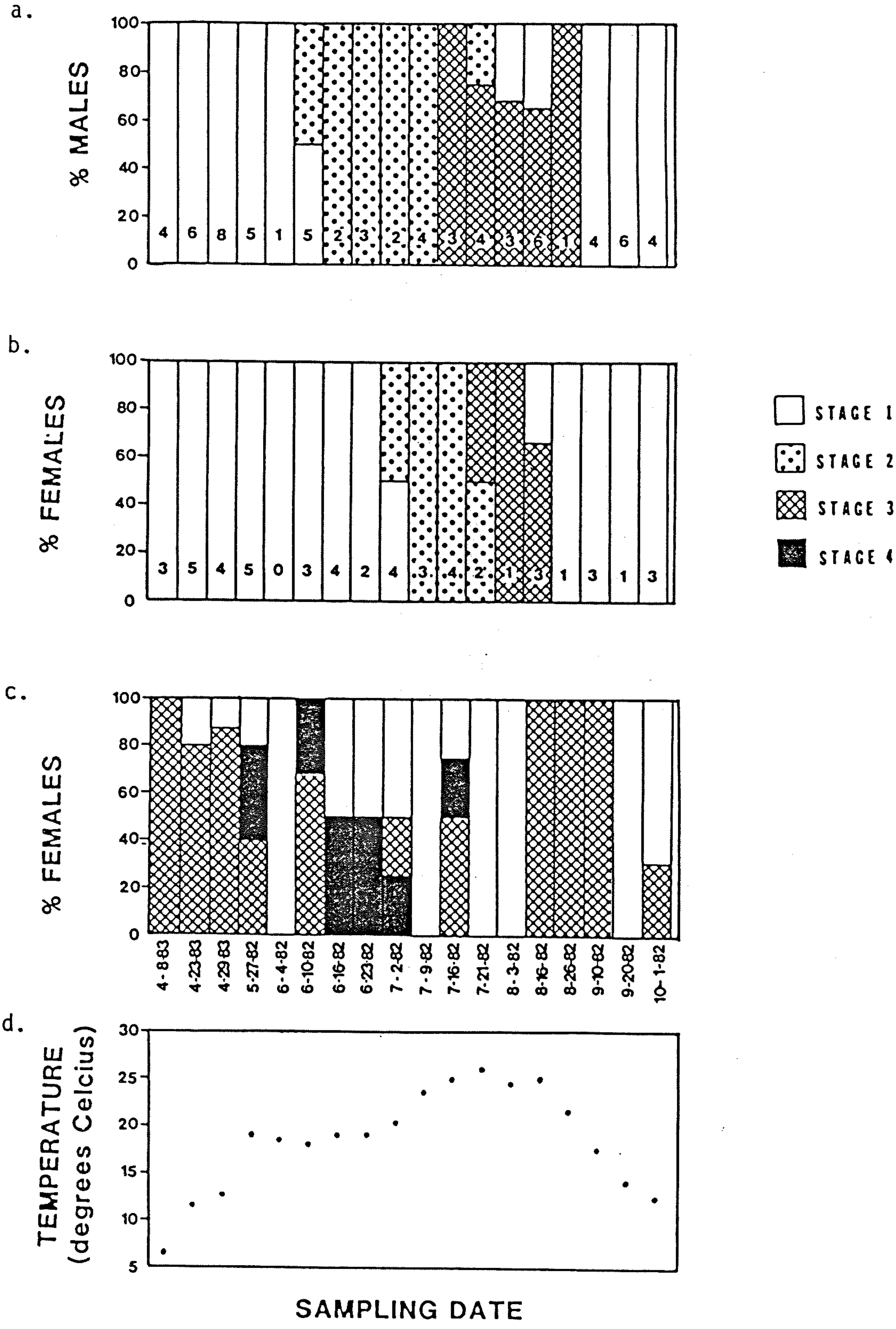


Figure 4. The condition of *Lampsilis ventricosa* specimens collected during the 18 sampling dates. Male gonadal tissue (a), female gonadal tissue (b), and marsupia (c) are grouped by percent on the relative developmental stages present. The numbers in each column represent the total number of individuals examined. Ambient river temperature is also shown (d).



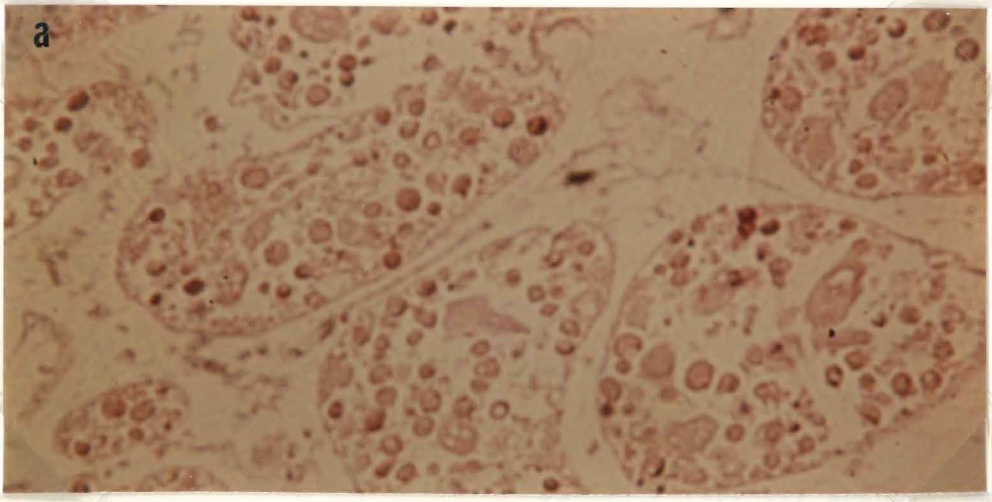
a.
% MALES
100
80
60
40
20
0
4 6 8 5 1 5 2 3 2 4 3 4 3 6 1 4 6 4
b.
% FEMALES
100
80
60
40
20
0
3 5 4 5 0 3 4 2 4 3 4 2 1 3 1 3 1 3
STAGE 1
STAGE 2
STAGE 3
STAGE 4
c.
% FEMALES
100
80
60
40
20
0
4-8-83
4-23-83
4-29-83
5-27-82
6-4-82
6-10-82
6-16-82
6-23-82
7-2-82
7-9-82
7-16-82
7-21-82
8-3-82
8-16-82
8-26-82
9-10-82
9-20-82
10-1-82
d.
TEMPERATURE (degrees Celcius)
30
25
20
15
10
5
SAMPLING DATE

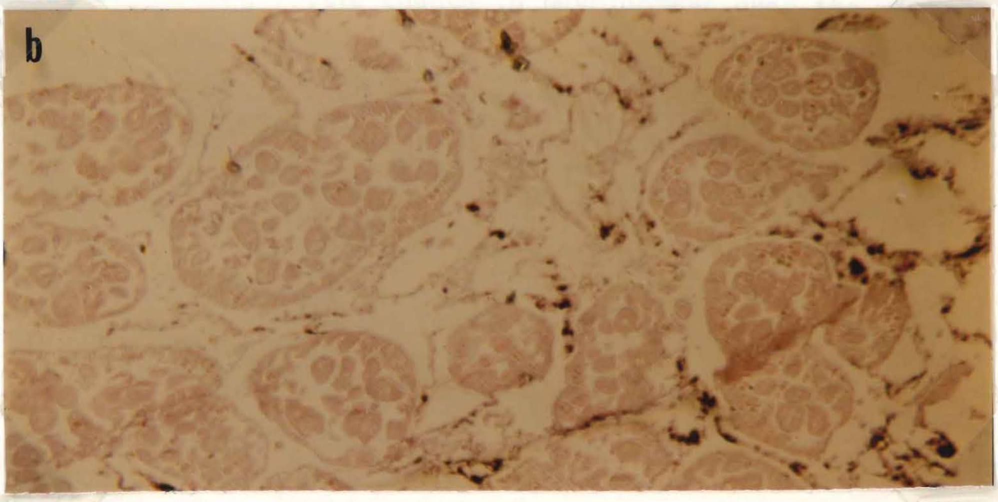
Figure 4. The condition of _Lampsilis ventricosa_ specimens collected during the 18 sampling dates. Male gonadal tissue (a), female gonadal tissue (b), and marsupia (c) are grouped by percent on the relative developmental stages present. The numbers in each column represent the total number of individuals examined. Ambient river temperature is also shown (d).

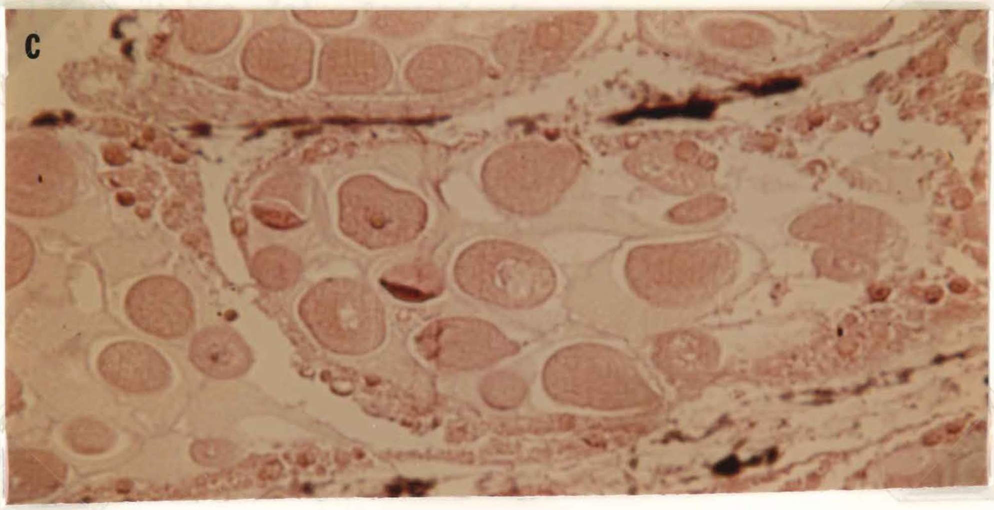


a.
% MALES
b.
% FEMALES
c.
% FEMALES
d.
TEMPERATURE (degrees Celcius)
STAGE 1
STAGE 2
STAGE 3
STAGE 4
4-8-83
4-23-83
4-29-83
5-27-82
6-4-82
6-10-82
6-16-82
6-23-82
7-2-82
7-9-82
7-16-82
7-21-82
8-3-82
8-16-82
8-26-82
9-10-82
9-20-82
10-1-82
SAMPLING DATE

Figure 5. Histological section of Lampsilis ventricosa ovary in (a) Stage 1: acini are widely spaced and acinal walls thick containing small eggs surrounded by nutritive material (specimen collected 10 September 1982), (b) Stage 2: developing eggs moving into acinal lumina (specimen collected 2 July 1982), and (c) Stage 3: acini closely packed and containing fully developed eggs enclosed by thin acinal walls (specimen collected 16 August 1982).


a


b


c

larger ova with some ova present in the lumina (Stage 2; Fig. 5b). The first date that Stage #3 occurred was also the point of the highest ambient river temperature (Fig. 4b,4d). Female gonadal visceral mass was quite swollen and the lumina of the acini contained many large mature eggs. Acinal walls appeared much thinner than Stage 1 and 2 specimens. Only one female was collected and inspected on 26 August 1982 and that specimen looked as if eggs were recently spent. September and October samples were similar in developmental stage to spring samples, suggesting little change in development over the winter months.

Proptera alata. Gonadal activity was present in both sexes of *P. alata* throughout the year. Mature specimens between 5 and 15 years of age (average age, 9.3 years) were inspected. Acini of males were widely spaced and spermatogonia and nutritive material were loosely packed within the lumina until June (Stage 1; Fig. 6a). At this time acini became fuller and contained many spermatogonia and some spermatids (Stage 2; Fig. 6b). Gonadal visceral mass at Stage 2 development appeared swollen. Peak sperm production took place during the month of July (Stage 3; Fig. 7a). Gonadal visceral mass was very swollen, little space was evident between acini, the lumina were very full, and many spermatids and sperm were present (Fig. 6c). August samples showed acini of males appearing to be in Stage 1 with spaces between acini and in lumina. Males remained in this particular stage throughout the remainder of the sampling period and appeared to be in a similar condition in the spring.

Figure 6. Histological section of *Proptera alata* testis in (a) Stage 1: acini widely spaced, some spermatogonia and nutritive material present (specimen collected 29 April 1983), (b) Stage 2: acini more closely spaced, lumina becoming filled with developing spermatids (specimen collected 27 May 1982), and (c) Stage 3: acini crowded and lumina filled with many spermatid and sperm (specimen collected 16 July 1982).

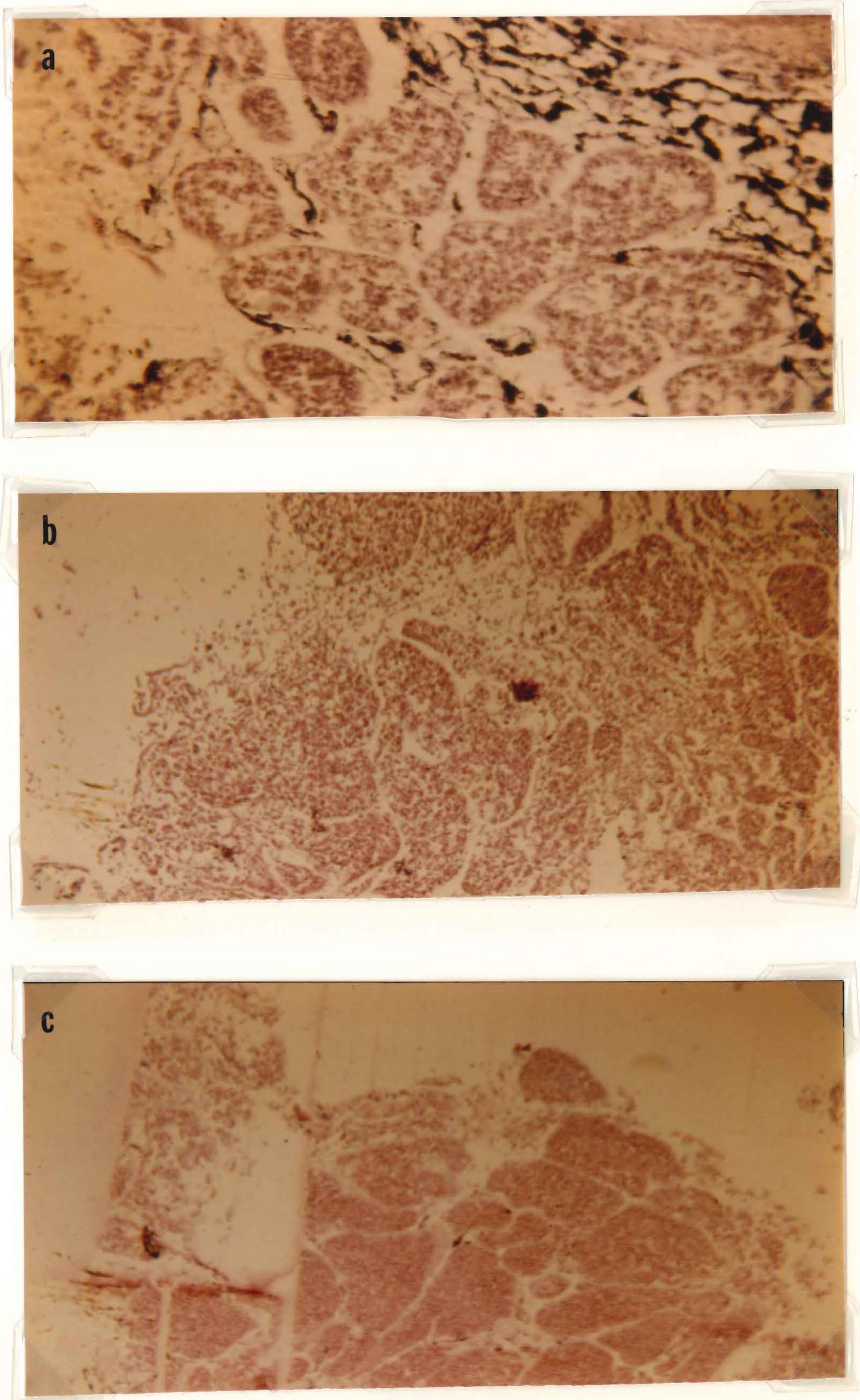
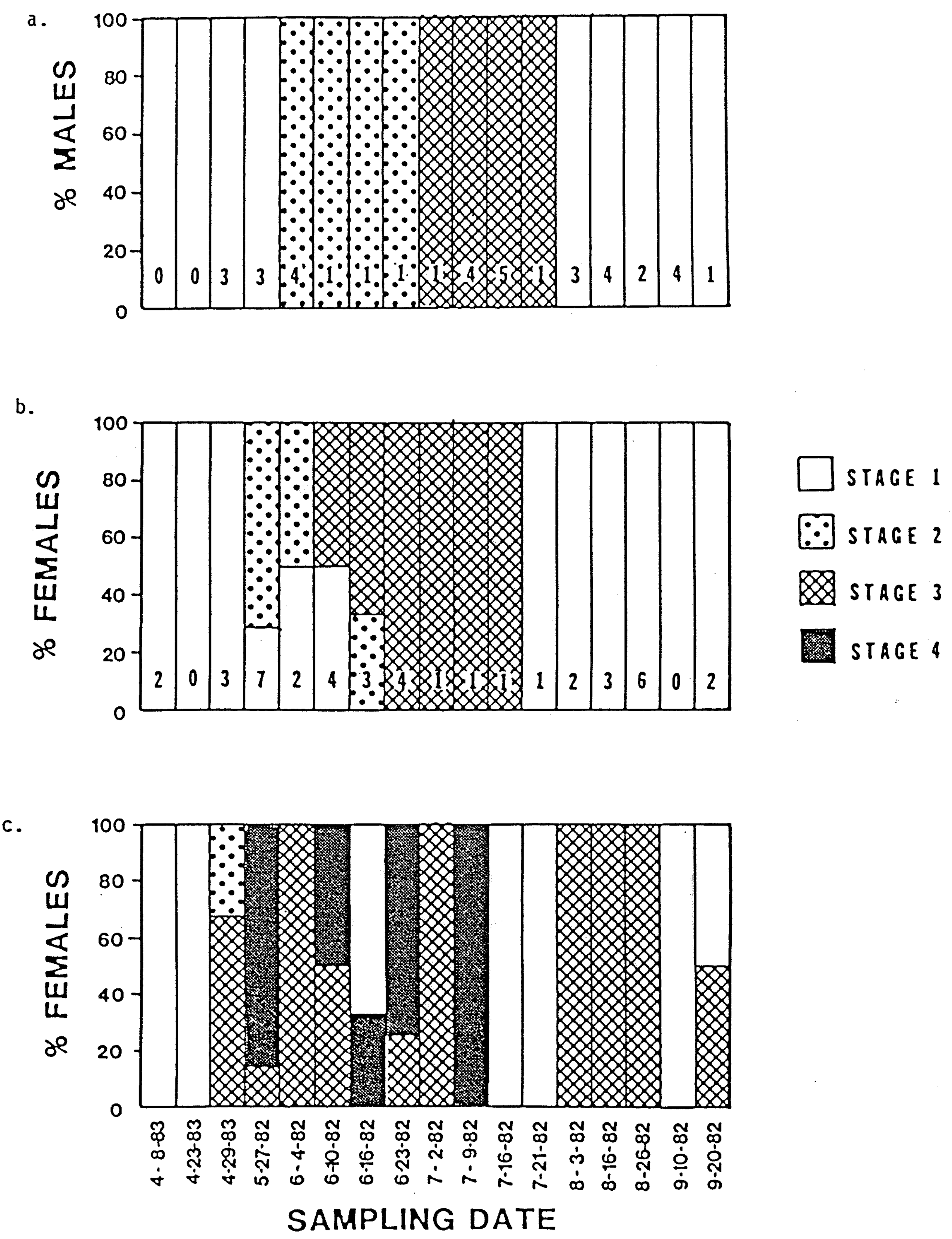

a
b
c

Figure 7. Condition of *Proptera alata* specimens collected during the 18 sampling dates. Female gonadal tissue (a), male gonadal tissue (b), and marsupia (c) are grouped by percent on the relative developmental stages present.

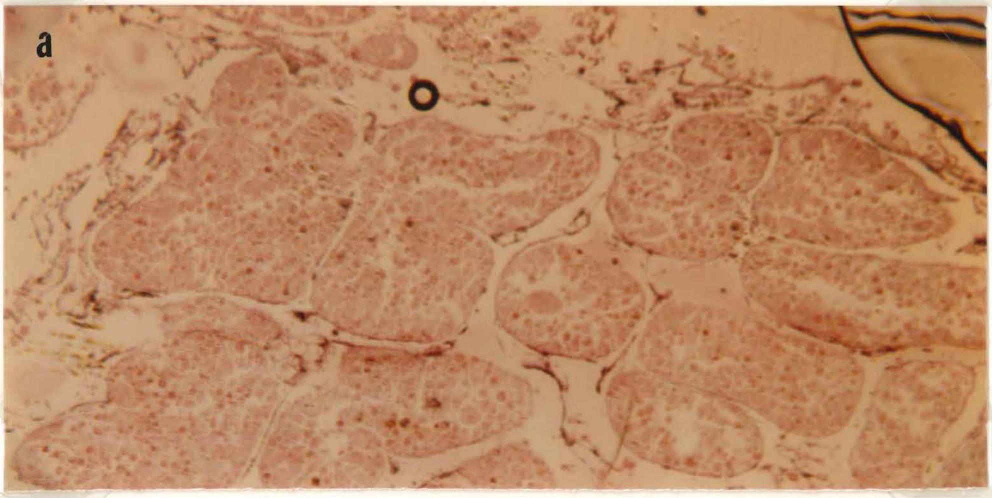


a.
% MALES
0
20
40
60
80
100
0 0 3 3 4 1 1 1 1 4 5 1 3 4 2 4 1
b.
% FEMALES
0
20
40
60
80
100
2 0 3 7 2 4 3 4 1 1 1 1 2 3 6 0 2
STAGE 1
STAGE 2
STAGE 3
STAGE 4
c.
% FEMALES
0
20
40
60
80
100
4-8-83
4-23-83
4-29-83
5-27-82
6-4-82
6-10-82
6-16-82
6-23-82
7-2-82
7-9-82
7-16-82
7-21-82
8-3-82
8-16-82
8-26-82
9-10-82
9-20-82
SAMPLING DATE

Ovarian tissue of P. alata collected in April indicated that females were in Stage 1 condition with some small oocytes present and surrounded by nutritive material. The lumina were somewhat empty and the spaces between the acini and the acinal walls were relatively thick (Fig. 8a). Some May samples showed development of gonadal tissue to Stage 2, with ova present in the lumina (Fig. 8b). Specimens in this condition had expanded or swollen gonadal visceral masses. June samples varied in development from Stage 1 to Stage 3. Specimens in Stage 3 development had very swollen gonadal visceral masses and large mature ova present in the lumina of acini (Fig. 8c). July and August specimens were mainly in the Stage 3 phase with mature ova predominating, although August specimens seemed to have fewer eggs present. Late August samples appeared to have only a few eggs present and resembled Stage 1 specimens (Fig. 7b). This condition was predominant throughout the rest of the year. These specimens resembled spring samples, suggesting little change over the colder winter months.

Amblema plicata. Gonadal activity was evident in both sexes of A. plicata throughout the sampling season (Fig. 9). The average age of individuals was 11.4 years with a range of 5 to 20 years. Peak gamete production occurred from 27 May through 9 July 1982. The ambient river temperatures ranged from 18-23.5°C during this period. Acini of males were varied in development in the April and May specimens, with Stage 2 being predominant (Fig. 10b). There appeared to be a shift of from Stage 1 in early April to Stage 3 in early June (Fig. 9a). All specimens collected from 10 June to 2 July 1982 were in the

Figure 8. Histological section of *Proptera alata* ovary in (a) Stage 1: acini widely spaced with space present in lumina and acinal walls thick containing rudimentary eggs and nutritive material (specimen collected 3 August 1982), (b) Stage 2: developing eggs moving into acinal lumina (specimen collected 27 May 1982), and (c) Stage 3: acini closely packed containing fully developed eggs (specimen collected 23 June 1982).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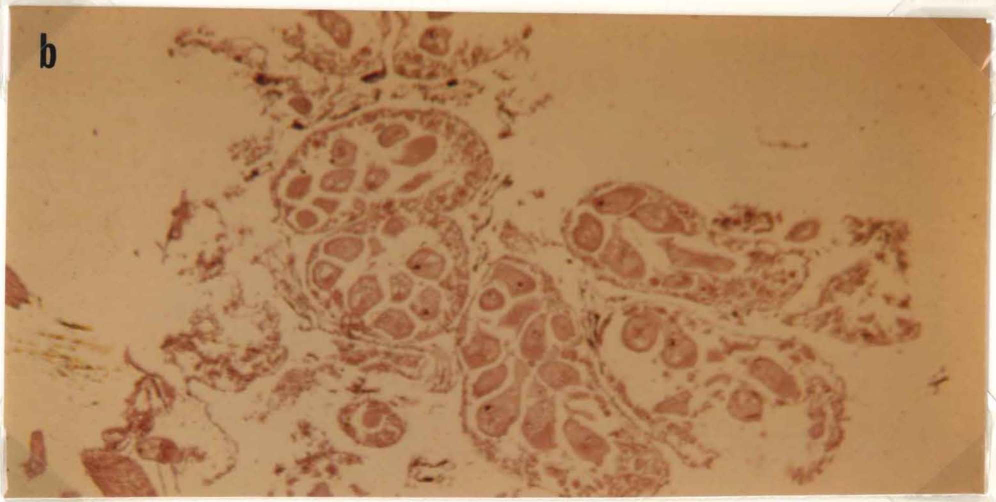
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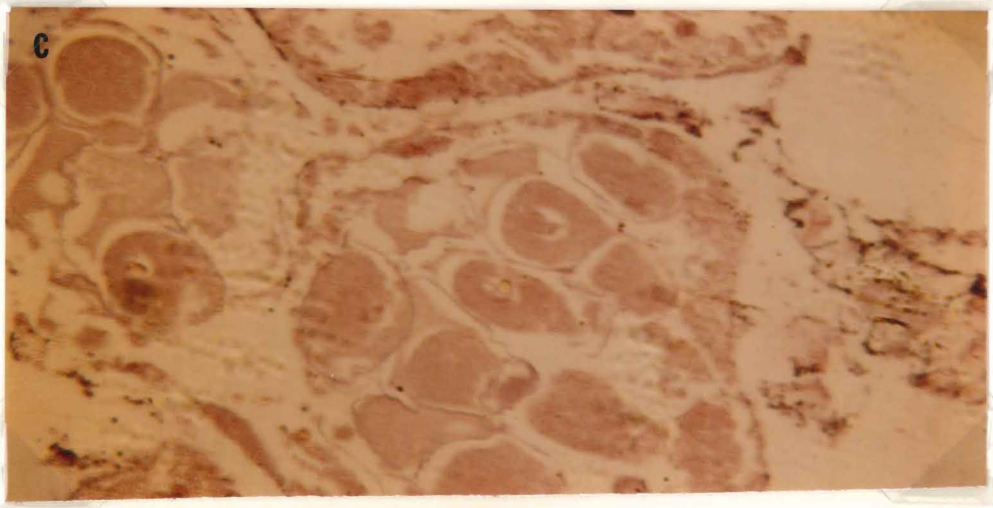
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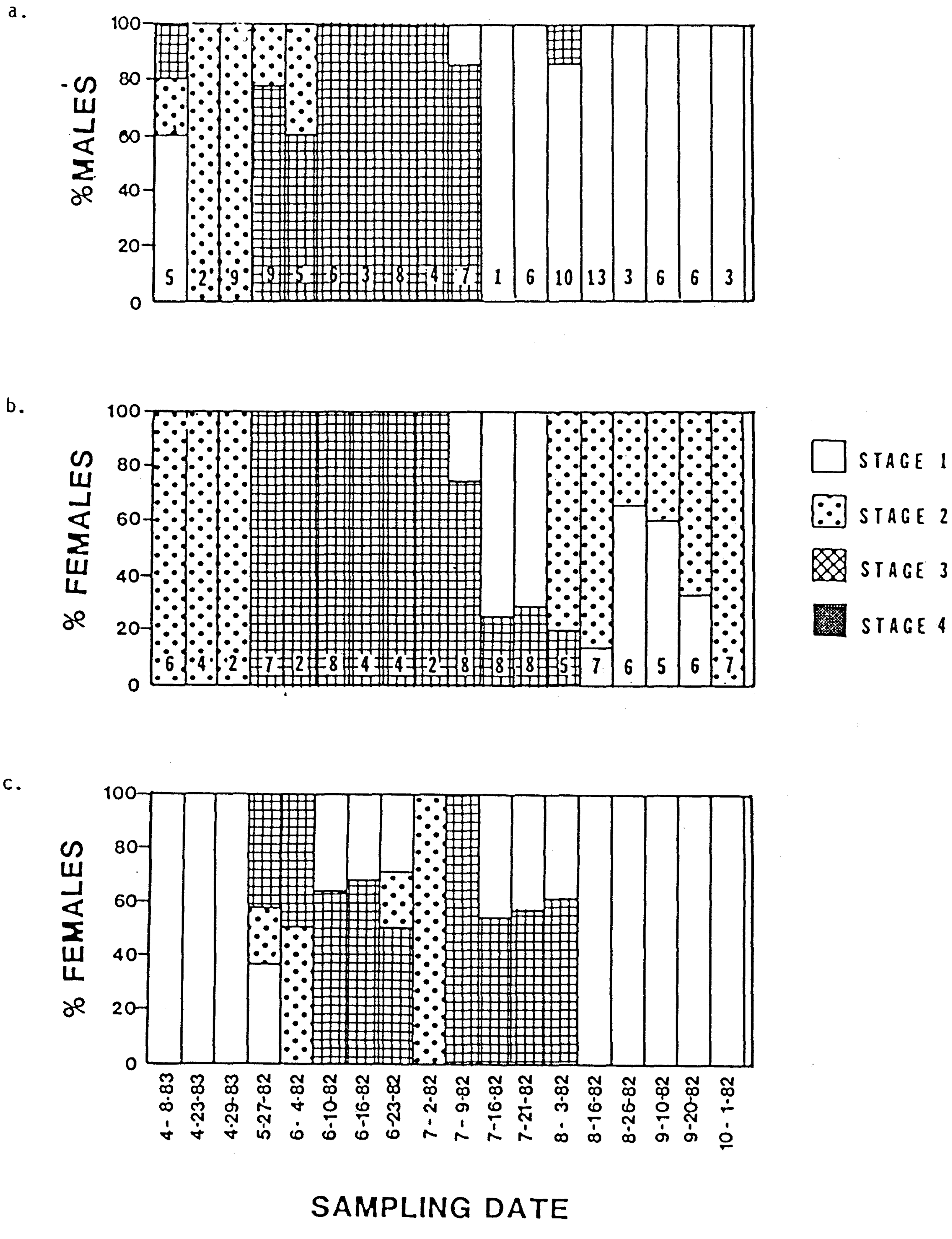
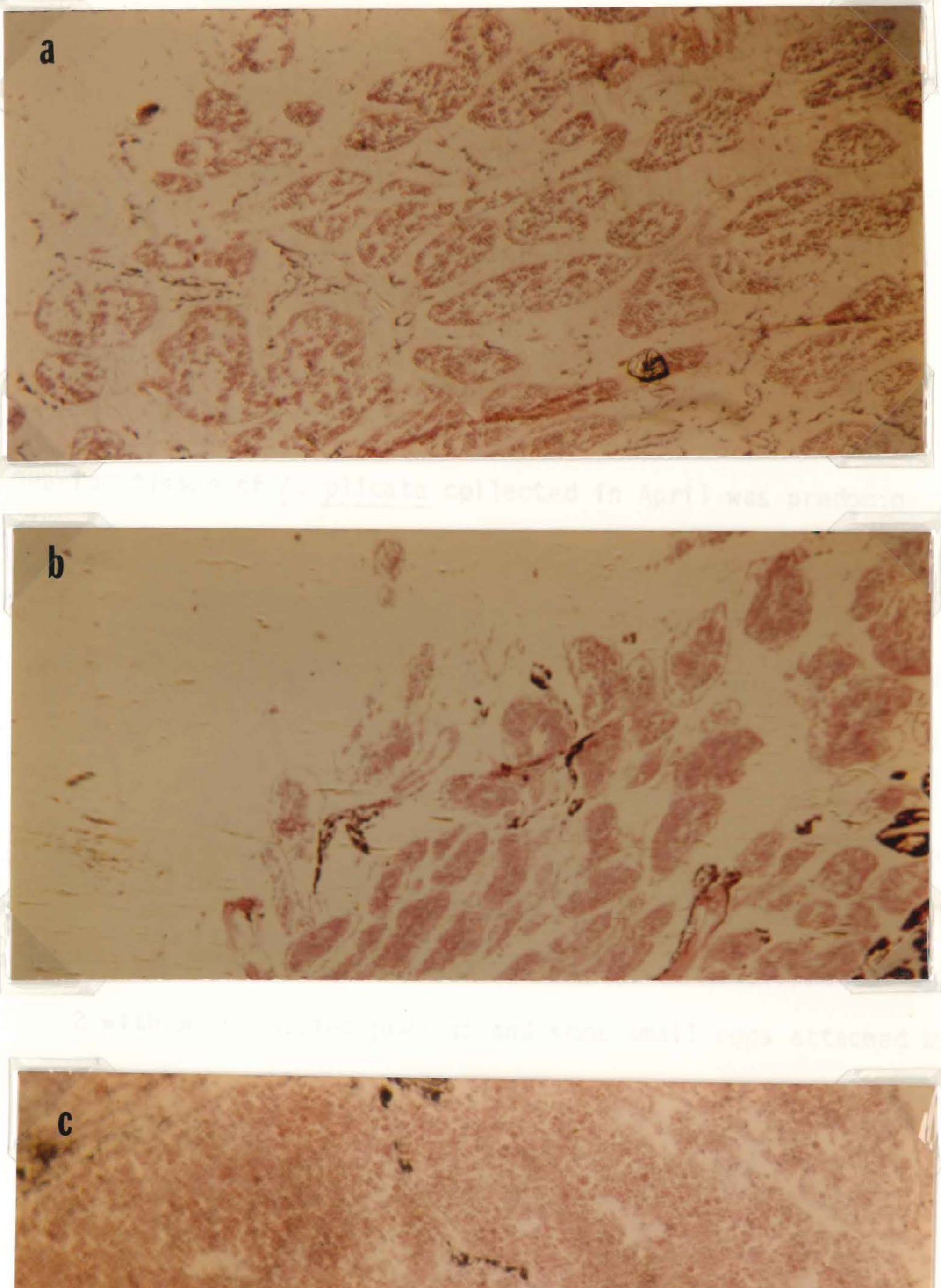
Figure 9. The condition of *Amblema plicata* specimens collected during the 18 sampling dates. Male gonadal tissue (a), female gonadal tissue (b), and marsupia (c) are grouped by percent on the relative developmental stages present. The numbers in each column represent the total number of individuals examined.



a.
100
80
60
40
20
0
% MALES
5
2
9
9
5
6
3
8
4
7
1
6
10
13
3
6
6
3
b.
% FEMALES
6
4
2
7
2
8
4
4
2
8
8
8
5
7
6
5
6
7
STAGE 1
STAGE 2
STAGE 3
STAGE 4
c.
% FEMALES
4-8-83
4-23-83
4-29-83
5-27-82
6-4-82
6-10-82
6-16-82
6-23-82
7-2-82
7-9-82
7-16-82
7-21-82
8-3-82
8-16-82
8-26-82
9-10-82
9-20-82
10-1-82
SAMPLING DATE

Figure 10. Histological section of *Amblema plicata* testis in (a) Stage 1: acini widely spaced with some spermatogonia and nutritive material present (specimen collected 3 August 1982), (b) Stage 2: acini more closely arranged and lumina becoming filled with spermatids (specimen collected 29 April 1983), and (c) Stage 3: acini crowded with lumina filled with many spermatids and sperm (specimen collected 23 June 1982).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Stage 3 condition with mature sperm and many spermatid present (Fig. 10c). This condition was somewhat detectable from the outward appearance of the swollen gonadal visceral mass. The acini were spaced well apart with space present in the lumina in male specimens collected from 10 July to 1 October 1982. Spermatogonia were the prominent gametic materials present (Fig. 10a). Little change in development was evident between fall and spring samples.

Ovarian tissue of A. plicata collected in April was predominantly in Stage 2 development (Fig. 9b). These specimens had many small ova present with some large ova in the lumina of the acini (Fig. 11b). All specimens inspected from 27 May through 2 July 1982 contained mature ova in the lumina of the acini and were in developmental Stage 3 (Fig. 11c). Individuals in this stage were considered to be sexually mature and ready for syngamy. Specimens collected from 9 July to 3 August 1982 varied in developmental stage from 1 to 3 (Fig. 11a). Specimens collected from 3 August to 1 October 1982 varied in developmental stage from 1 to 2 with many oocytes present and some small eggs attached by stalks to the acinal wall. Specimens collected in October resembled those specimens collected in April.

Syngamy and Embryogeny

Lampsilis ventricosa. It was reaffirmed in this study that L. ventricosa is a long term or bradytictic breeder. Fertilization of the gametes took place between late July and mid August during peak river temperatures (25.5-26.6°C). Embryos were first observed in the marsupia (posterior enlargement of the outer demibranch, Fig. 12a) in mid

Figure 11. Histological section of _Amblema plicata_ ovary in (a) Stage 1: acini widely spaced with small eggs surrounded by nutritive material (specimen collected 10 September 1982), (b) Stage 2: developing eggs moving into acinal lumina (specimen collected 10 September 1982), and (c) Stage 3: acini closely arranged containing fully developed eggs enclosed by thin acinal walls (specimen collected 4 June 1982).

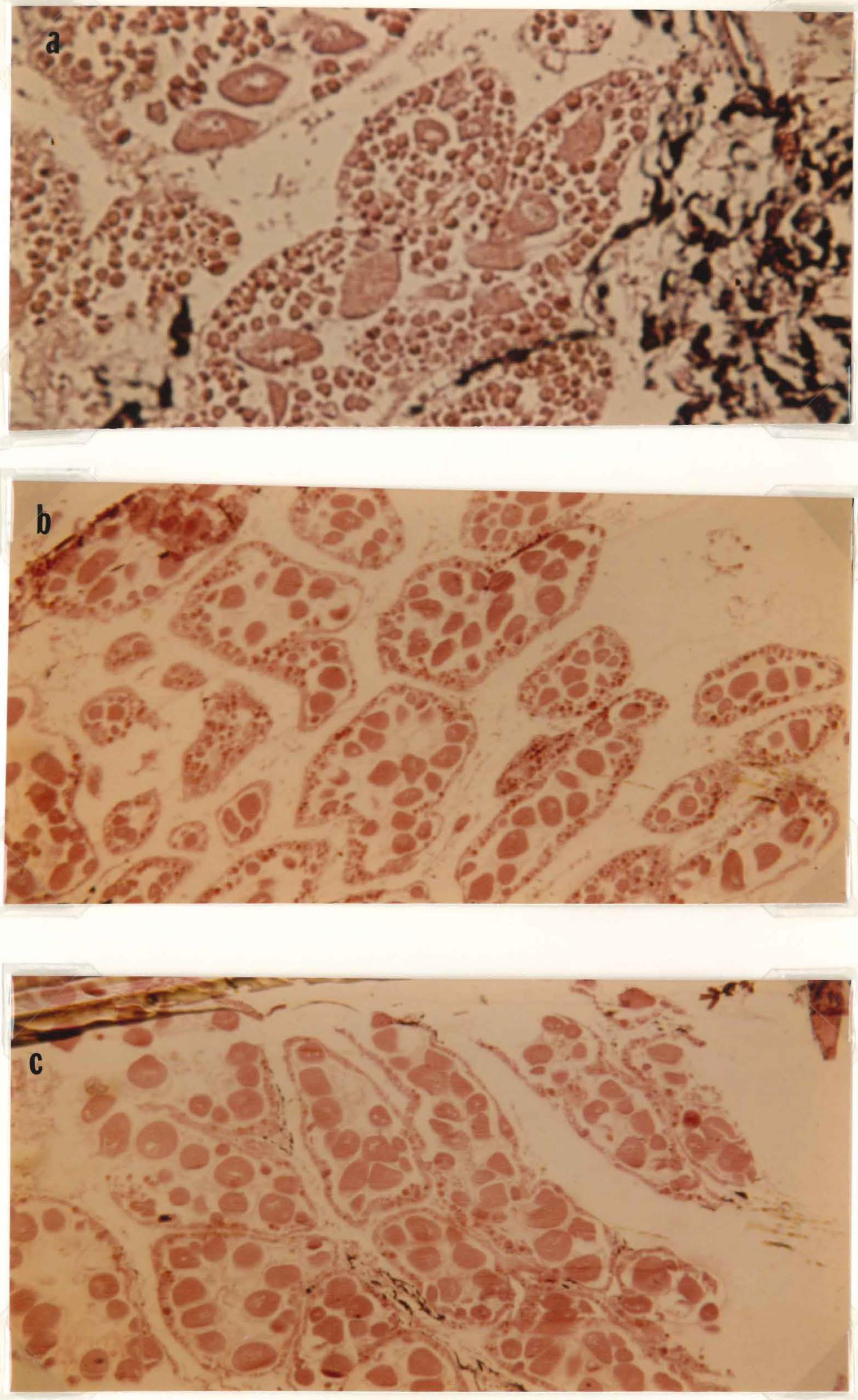
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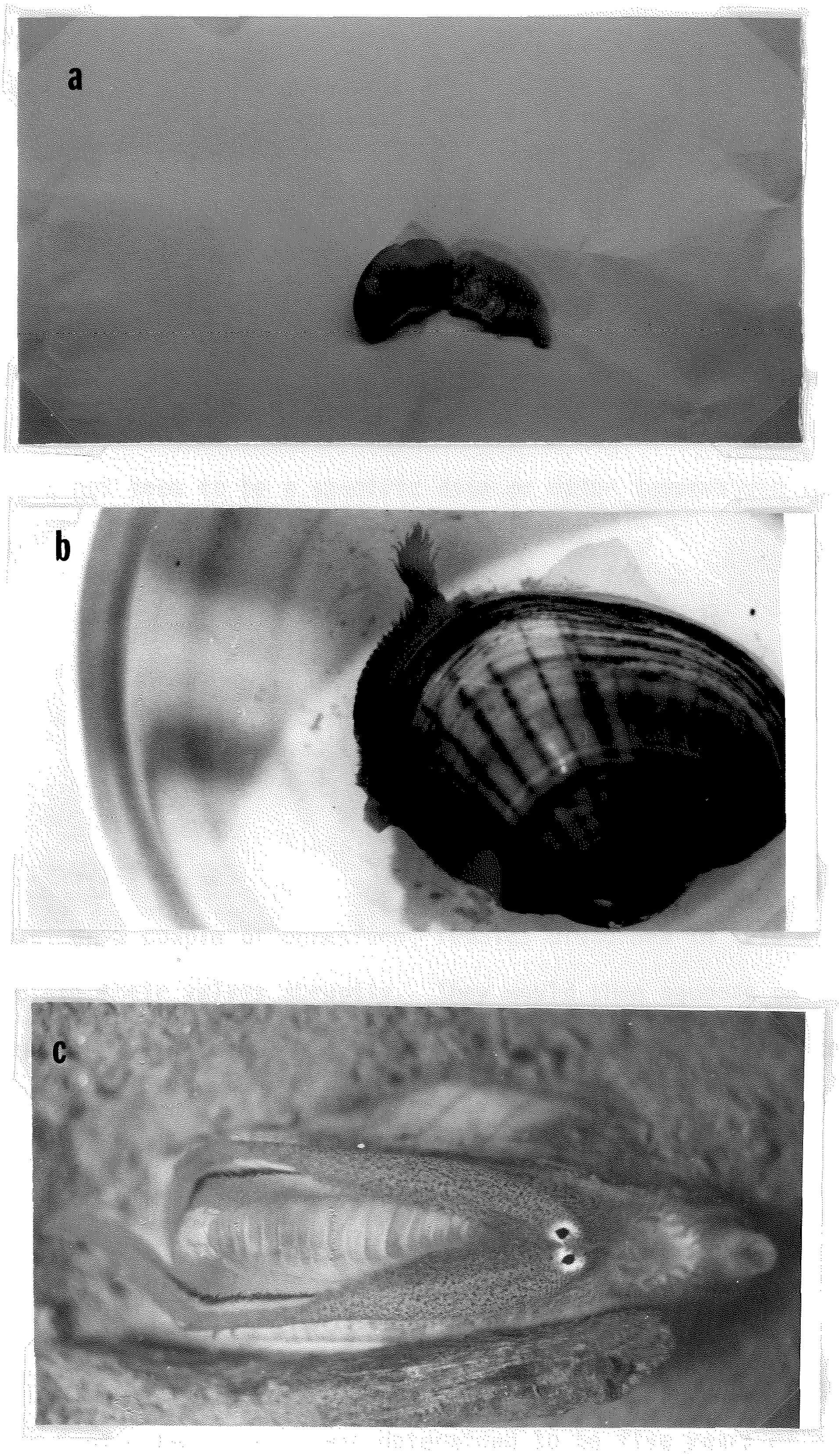
Figure 12. Morphological characteristics of adult female *Lampsilis ventricosa*. A gravid outer demibranch showing enlarged marsupia (a), side view of extended mantle flap (b), and top view of gravid female with extended mantle flap and extruded marsupia (c).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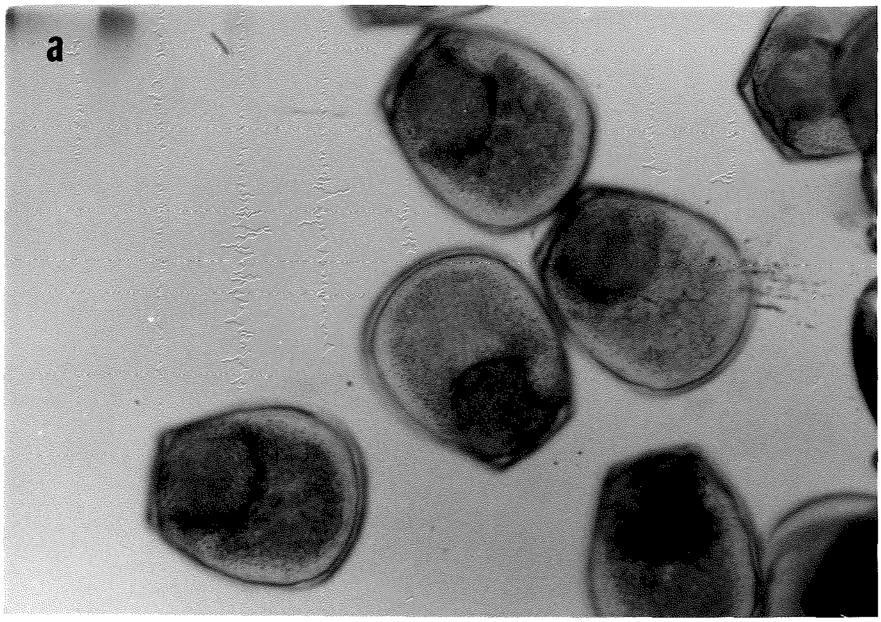
August. Embryos developed quickly into mature glochidia (Fig. 13). Data suggest that this development took place in approximately two weeks at these water temperatures. Females that successfully completed the fertilization process stored the developed glochidia in the marsupia over winter. No apparent changes in the glochidia were noticed between fall collections and spring collections. The glochidia averaged 24.4μm in length, 20.7μm in width, and mean hinge length was 10μm. The glochidia were released at different times by different individuals. There did not seem to be a specific date or water temperature that promoted glochidial release. Evidence of released glochidia (ruptured marsupia, reduced glochidial numbers) was recorded from as early as 27 May 1982 to as late as 16 July 1982 (Fig. 4c). Glochidia were released through the outer margins of the marsupial wall. It was observed that females with mature glochidia (recognizable by their swollen marsupia) had their mantle flap extended and producing undulating motions in the water while exposing the outer margins of the swollen marsupia (Fig. 12b, c). On a couple of occasions, females disturbed in this condition would close their valves abruptly. They would then squeeze out the swollen marsupia between the mantle flaps and squeeze tighter, rupturing the outer margin of the marsupia releasing glochidia in almost an explosive manner. The released glochidia were free to attach themselves to a host fish where metamorphosis could begin. After successful metamorphosis on the host fish, the glochidia release themselves and begin life as a juvenile. The actual time sexual maturity is reached was not determined from this study, although mature gonadal material was observed from a female that was determined to be five years of age.

Figure 13. Mature glochidia of *Lampsilis ventricosa* snapped shut (a) and gapping (b).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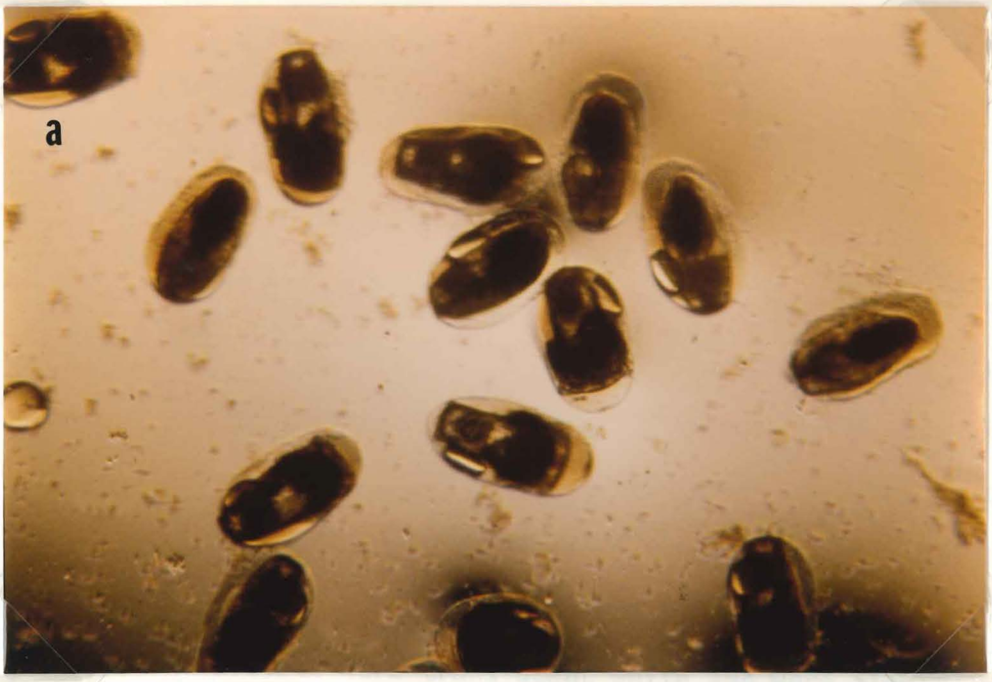
b

<u>Proptera alata</u>. <u>P</u>. <u>alata</u> was confirmed to have a bradytictic reproductive cycle. Fertilization of gametes occurred during peak river temperatures of 25.5 to 26°C (21 July to 3 August 1982) (Fig. 4d, 7). Ova were fertilized somewhere between oviducts and the demibranchs in the female. Zygotes were transferred to the posterior end of the outer demibranch. This area, known as the marsupial cavity, becomes somewhat enlarged and swollen as it fills with developing embryos. The embryos develop into mature glochidia in approximately three weeks. Females inspected on 26 August 1982 had glochidia in very advanced stages of development. Glochidia were stored in marsupia over the winter months and released through the outer margins of the marsupia. No noticeable differences were evident between fall and spring glochidia. On 27 May 1982, 82% of the females inspected showed signs of recent release of glochidia (Fig. 7c). Mature glochidia were found in specimens as late as 9 July 1982. Glochidia averaged 36.2µm in length and 13.7µm in width. No female specimens were found to contain glochidia from mid July to late July. Glochidia of this species remained in a vitelline membrane until released, at which time the action of the young parasite ruptures the membrane (Fig. 14a). The glochidia of <u>P</u>. <u>alata</u> have four hooks (two in each valve) to aid in the attachment to a particular fish host (Fig. 14b).

<u>Amblema plicata</u>. <u>A</u>. <u>plicata</u> is a tachytictic breeder, spawning and releasing the glochidia in the same season. Syngamy appeared to start in Pool 7 sometime during late May with spawning evident from 27 May to 9 July 1982 (Fig. 9). Ova were present in the suprabrachial cavity

Figure 14. Glochidia of *Proptera alata*. Glochidia still in vitelline membrane (a) and glochidia free of membrane (b).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and/or zygotes were present in the demibranchs. This was a longer spawning season than observed for the bradytictic breeders studied. Fertilization took place in the suprabrachial chambers and possibly in the demibranchs. Sperm assembled in small volvox like colonies were observed in the suprabrachial cavity in a few female specimens. Zygotes were stored in all four of the demibranchs. Presence of the zygotes was barely noticeable, with only a relatively small amount of swelling taking place. The demibranchs did appear a little different in color because of their contents. The evidence of mature glochidia in the demibranchs was noticed on 10 June 1982 and as late as 3 August 1982. No glochidia were found in demibranchs after 3 August 1982. The glochidia of A. plicata were translucent white and were almost semicircular in shape (Fig. 15). The awaiting parasite lies with valves open at 180° to each other. The abissal thread was not evident in preserved specimens. Glochidia averaged 21.6µm in length, 20.9µm in width and the mean hinge length was 13.1µm.

Incidence of Glochidia

A sample of 1786 fish (33 species) were examined for incidence of glochidia. Of those examined, 74 (4.14%) had parasitic glochidia attached somewhere on the body (Table 1). Fish were found to possess glochidia from 10 June through 19 August 1982. No infections were observed on fishes collected on 27 May, 2 September, and 16 September 1982. The highest incidence of infection occurred on 21 July and 3 August 1982. Notropis hudsonius showed the most infection. The highest percent incidence occurred on Stizostedion vitreum (100%) with an average of seven glochidia per fish.

Figure 15. Mature glochidia of *Amblema plicata*. Shut glochidia showing semi-circular shape (a) and gapping glochidia with valves open at $180^{0}C$ (b).

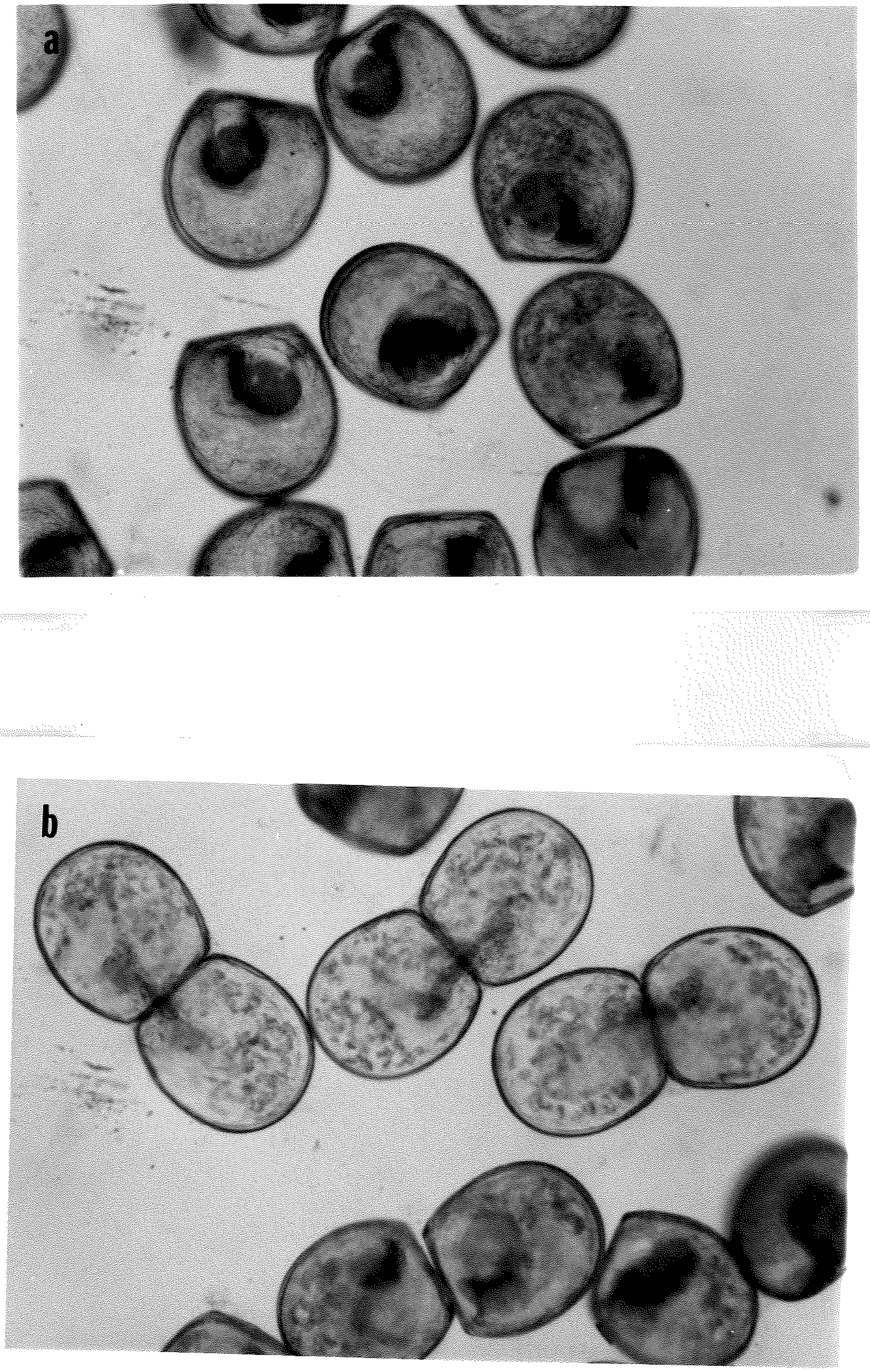
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Table 1. Incidence of glochidial infections on fishes collected from Navigation Pool 7 of the Upper Mississippi River.

Date and species	Number of fish checked	% incidence	Average number of glochidia/ fish
06-10-82			
Esox lucius	5	40	1.0
Hybognathus nuchalis	0	-	-
Notropis atherinoides	152	0	-
N. blennius	16	0	-
N. hudsonius	2	0	-
N. spilopterus	3	0	-
N. texanus	0	-	-
Pimephales vigilax	3	0	-
Carpiodes sp.	0	-	-
Morone chrysops	14	0	-
Micropterus salmoides	0	-	-
Stizostedion vitreum	0	-	-
06-23-82			
Esox lucius	8	0	-
Hybognathus nuchalis	0	-	-
Notropis atherinoides	35	0	-
N. blennius	6	0	-
N. hudsonius	20	65	1.5
N. spilopterus	46	0	-
N. texanus	0	-	-
Pimephales vigilax	27	0	-
Carpiodes sp.	4	0	-
Morone chrysops	0	-	-
Micropterus salmoides	0	-	-
Stizostedion vitreum	1	100	10.0
07-07-82			
Esox lucius	0	-	-
Hybognathus nuchalis	0	-	-
Notropis atherinoides	120	0	-
N. blennius	7	0	-
N. hudsonius	17	53	4.0
N. spilopterus	88	0	-
N. texanus	0	-	-
Pimephales vigilax	54	0	-
Carpiodes sp.	1	100	2.0
Morone chrysops	0	-	-
Micropterus salmoides	7	0	-
Stizostedion vitreum	0	-	-

Table 1. continued

Date and species	Number of fish checked	% incidence	Average number of glochidia/ fish
07-21-82			
Esox lucius	0	-	-
Hybognathus nuchalis	2	50	1.0
Notropis atherinoides	5	0	-
N. blennius	18	0	-
N. hudsonius	39	35.8	1.6
N. spilopterus	13	23	1.7
N. texanus	0	-	-
Pimephales vigilax	50	0	-
Carpiodes sp.	1	0	-
Morone chrysops	13	46	4.2
Micropterus salmoides	2	50	1.0
Stizostedion vitreum	1	100	4.0
08-03-82			
Esox lucius	1	0	-
Hybognathus nuchalis	0	-	-
Notropis atherinoides	13	38.5	1.2
N. blennius	25	16	1.0
N. hudsonius	20	35	4.4
N. spilopterus	7	0	-
N. texanus	71	5.6	1.3
Pimephales vigilax	7	14	1.0
Carpiodes sp.	0	-	-
Morone chrysops	12	0	-
Micropterus salmoides	4	0	-
Stizostedion vitreum	0	-	-
08-19-82			
Esox lucius	0	-	-
Hybognathus nuchalis	3	0	-
Notropis atherinoides	28	0	-
N. blennius	22	0	-
N. hudsonius	8	12.5	1.0
N. spilopterus	4	0	-
N. texanus	42	0	-
Pimephales vigilax	17	0	-
Carpiodes sp.	8	0	-
Morone chrysops	0	-	-

Table 1. continued

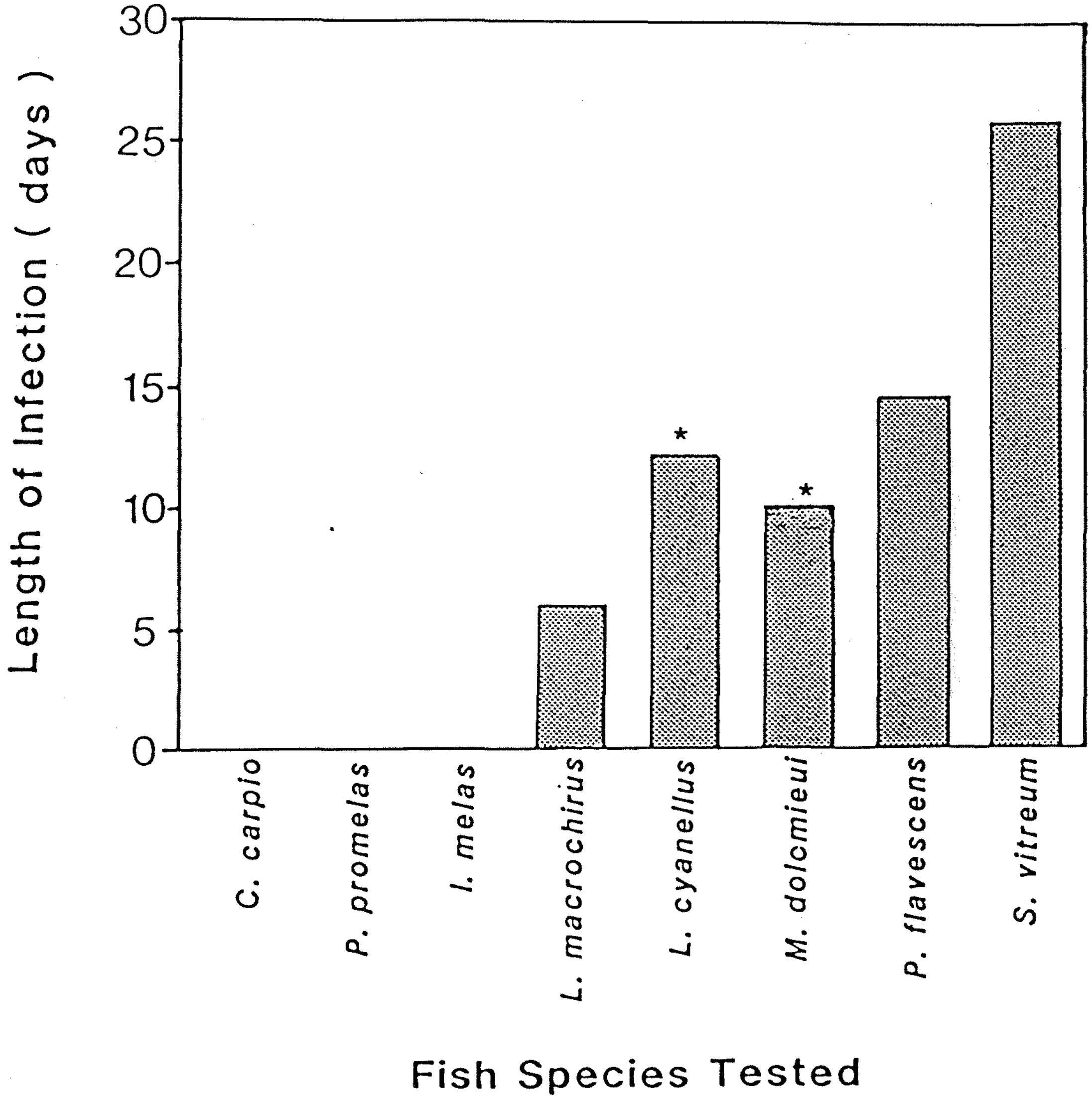
Date and species	Number of fish checked	% incidence	Average number of glochidia/ fish
Micropterus salmoides	1	0	-
Stizostedion vitreum	0	-	-

**The following is a list of species that were checked but no glochidia were present any time during the sampling season. The species name is followed by the number of fish that were examined. Lepisosteus osseus (4), Dorosoma cepedianum (9), Hiodon tergisus (1), Notemigonus crysoleucus (2), Notropis emiliae (1), Catastamid sp. (6), Moxostoma sp. (3), Noturus gyrinus (1), Aphredoderus sayanus (13), Fundulus notti (1), Labidesthes sicculus (13), Culaea inconstans (1), Ambloplites rupestris (12), Lepomis macrochirus (38), Pomoxis annularis (1), Pomoxis nigromaculatus (81), Ammocrypta clara (110), Etheostoma nigrum (4), Perca flavescens (3), Percina caprodes (8), Aplodinotus grunniens (3).

Host Specificity

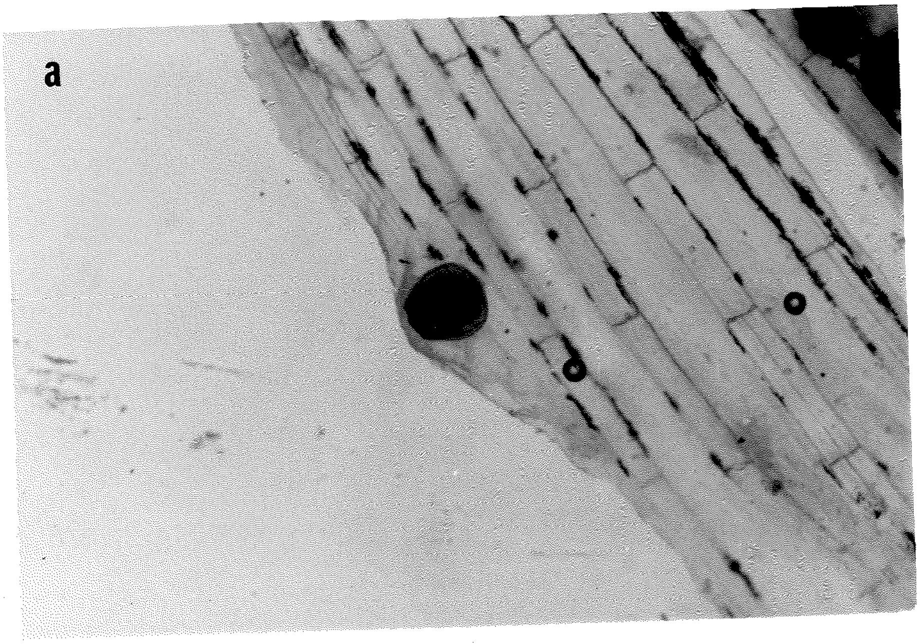
Lampsilis ventricosa glochidia attached themselves readily to the gill lamellae of all fish species infected. The glochidia dropped off individuals of the Cyprindae and Ictaluridae families within a couple of hours and remained attached to members of the Centrarchidae family for more than three days and as long as 12 days. Members of the Percidae family were infected for the longest period of time (14-21 days) (Fig. 16). Any fish species that remained infected for more than three days was considered to be a possible fish host. Species of fish infected in this study that were considered to be possible fish host for *L. ventricosa* by length of infection are: *Lepomis macrochirus*, *Lepomis cyanellus*, *Micropterus dolomieui*, *Perca flavescens*, and *Stizostedion vitreum*. However, no juveniles were recovered by the termination of the study on day 26. Attached parasitic glochidia did not undergo any noticeable changes in size, but did become more opaque as the mantle and the foot developed. Encystment of glochidia was complete after approximately 4-5 days (Fig. 17).

Figure 16. Period of attachment of *Lampsilis ventricosa* glochidia to eight species of fish at 14-15 ^{0}C. Asterisk (*) indicates fish died while glochidia were still attached. Study terminated on day 26.



Length of Infection (days)
30
25
20
15
10
5
0
*
*
C. carpio
P. promelas
I. melas
L. macrochirus
L. cyanellus
M. dolcmieui
P. flavescens
S. vitreum
Fish Species Tested

Figure 17. Encysted *Lampsilis ventricosa* glochidia on fin of *Stizostedion vitreum* on 10th day of exposure (a) and on gill lamellae of *Stizostedion vitreum* (b).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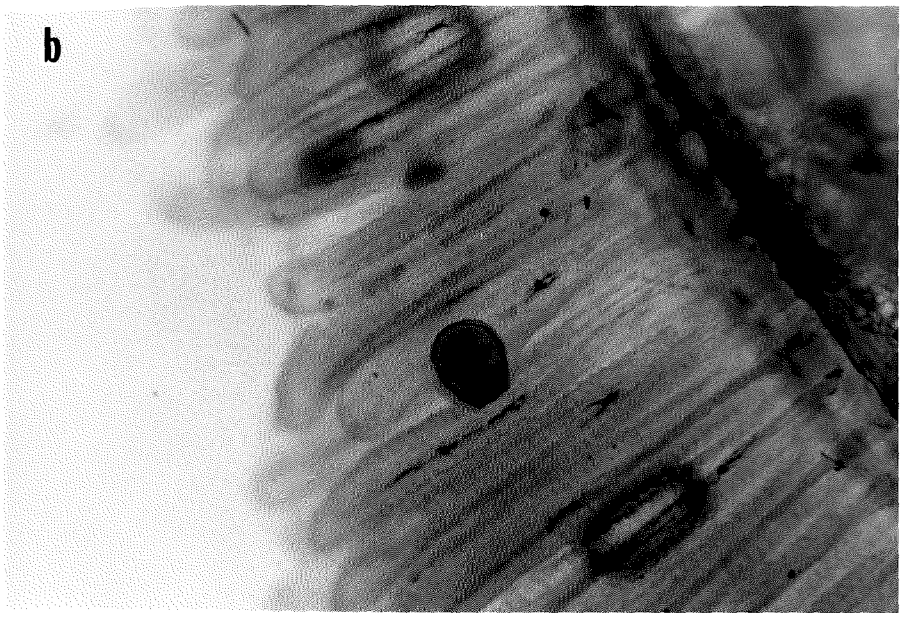
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DISCUSSION

Most research has identified mussel species as either long term (bradytictic) or short term breeders (tachytictic). The findings of this investigation confirm that *L. ventricosa* and *P. alata* are both bradytictic while *A. plicata* is tachytictic. Although these general breeding seasons have been reported for many mussels (Sterki 1895, Surber 1912, Lefevre and Curtis 1912, Howard 1914, 1922, Coker et al. 1921), the actual timing of gametic release in relation to ambient water temperature has been reported rarely. Significant stages in the reproductive cycles of freshwater mussels are directly dependent on the temperature of the surrounding waters (Matteson 1948). Retarded temperatures in the spring correlate to both retarded development and release of spermatozoa and the rate of oogenesis. Although reference to the specific dates of breeding seasons of *A. plicata* have been made by many researchers (Sterki 1895, Ortmann 1909, Surber 1912, Utterback 1916, Baker 1928, van der Schalie 1936, Clarke and Berg 1959, Stein 1973), the relationships of temperatures to the various reproductive events has not been reported. The breeding season of *A. plicata* has been observed from May to early August with most researchers reporting gravid females during the month of July. Stein (1973) reported gravid females from 11 June to 1 August. No gravid individuals were found below 20°C. She suggested that it appeared that 20°C was critical to the initiation of breeding in *A. plicata*. However, my results differ slightly from Stein in that gravid females were found at water temperatures between 18 and 20°C. No gravid individuals were observed in late samples, although water temperatures were still above 20°C. It seems probable that a particular water temperature could trigger the onset of breeding in A. plicata, but different factors determine the cessation. It has been reported by some researchers that tachytictic breeders can produce more than one brood per season (Coker et al. 1921). Further investigations with A. plicata from the Upper Mississippi River will be needed before this can be proven. Maturation of glochidia appeared to take as little as two weeks. Therefore, it is possible for successive broods to be produced.

The breeding season of Lampsilis sp. is spread out over several months (Surber 1912, Lefevre and Curtis 1912, van der Schalie 1938). Gravid female L. ventricosa were collected during every month of the present study (April through October). Although release of the glochidia varied between individuals, syngamy seemed to take place during a relatively short period and at near peak ambient river temperatures (24.5-26.0°C). Similar results were found for P. alata. Development of the glochidia seemed to be rapid and release of the glochidia seemed to begin for P. alata and L. ventricosa at 19°C. The present study did not investigate between river temperatures of 12.8 to 19°C, therefore the critical temperature could possibly be somewhat less than 19°C.

Females of L. ventricosa and P. alata both possess specialized structures for the containment of the developing embryos and glochidia whereas A. plicata uses a more primitive structure. The glochidia of

P. alata and L. ventricosa are stored in the posterior part of their outer branchiae which are transformed into branchial uteri (Sterki 1895). The mussels of these two species show sexual dimorphism not only in valve shape but in the form of the marsupial pouch. The individuals of A. plicata show no sexual dimorphism in either valve shape or in the demibranchs. Glochidia in this species are stored in all four of the female's demibranchs. Evidence of embryos and/or glochidia is easily observed by the inspection of marsupia in L. ventricosa and P. alata but it is very difficult to see any difference between a gravid or a non-gravid A. plicata. Review of the literature suggests that there may be physiological advantages to this specialized structure found in bradytictic individuals such as better water circulation to increase the supply of oxygen for developing embryos (Ortmann 1910, Coker et al. 1921, and Baker 1928).

Many observations of the mantle flap of Lampsilis sp. have been reported (Kirtland 1851, Ortmann 1910, Wilson and Clark 1912, Utterback 1915, Coker et al. 1921, Kraemer 1970, and Fuller 1971). It has been suggested that the chances of attachment of glochidia to fish might be increased by the flapping motion of the posterior mantle flap. When larvae are mature and ready to be released, predatory fish are attracted and receive a discharge of glochidia. L. ventricosa possess such a mantle flap and females demonstrating this flapping or undulating motion were always gravid with the extended gravid marsupia often protruding from the exterior margin of the mussel cavity (Fig. 12). I have on many occasions observed this flapping behavior and agree that it resembles the appearance of a small fish. Individuals disturbed when in this state would often extrude the swollen marsupia and clamp their valves tightly, causing a violent expulsion of the glochidia through the exterior margin of the marsupia. The glochidia released were not in the conglutinated form, but instead were masses of many separate individuals. Ortmann (1910) observed this same behavior in Lampsilis luteola (Lamarck), L. ventricosa and L. multiradiata (Lea). Ortmann went on to state that he observed evidence of this discharge through observations in shape of the marsupiua of preserved P. alata (as well as some other species not here mentioned). I, too, have observed this disturbed and destroyed marsupial edge in samples of alcohol preserved female P. alata, but have not observed the actual discharge of glochidia. This was reported in my results for L. ventricosa and P. alata by the marsupial Stage 4 (Fig. 4 and 7). Evidence of release in A. plicata was not noticeable because their mode of release was not evidenced by any change in the demibranchs. Ortmann (1910) describes the release as the "natural way" and involves the following pathway: the glochidia go from the water-tubes (ovisacs) into the suprabranchial canal, from this into the cloacal chamber, and out by way of the anal opening. No release of glochidia in this species was observed in this investigation and a marsupia Stage 4 was not reported respectively (Fig. 17).

Five species of Perciform fishes were found to be potential hosts of L. ventricosa through artificial laboratory infections. "Contact with any part of the fish affords the stimulus which causes the adductor muscle of the glochidia to contract and close the valves" (Lefevre and Curtis 1912). The hookless glochidia of *L. ventricosa* were attached to the gill lamellae, but some became attached to opercular and fin membranes. Cyprinid and ictalurid species became only initially infected but glochidia dropped or sloughed off within a few hours as also seen by Lefevre and Curtis (1912) for carp. Glochidia are quite unselective in their initial attachment (Lefevre and Curtis 1912, Arey 1921 and 1924, Davenport and Warmuth 1965), but persistent attachment is dependent on substances present in fish mucous (Wood 1974). The fishes used in many experiments have shown differences in their ability to retain glochidia (Lefevre and Curtis 1912, Howard 1914, 1922). Persistence of glochidia in this study ($\geq$ 5 days) was used to confirm potential fish hosts. Fuller (1978) reported *L. macrochirus* (Coker et al. 1921), *M. dolomieui* (Coker et al. 1921), *M. salmoides* (Coker et al. 1921, Lefevre and Curtis 1912, Reuling 1919), *P. annularis* (Coker et al. 1921, Wilson 1916), *P. flavescens* (Coker et al. 1921), and *S. canadense* (Coker et al. 1921, Wilson 1916) as suspected hosts for *L. ventricosa.* In the current investigation, persistent attachment occurred on many of these species and also *S. vitreum* and *L. cyanellus.* In a subsequent study by Waller et al. (1984), a similar host specificity experiment was performed except with an "elevated temperature" (21-22°C as compared to 14-15°C). Similar results were obtained with juvenile mussels being retrieved after day 13 of infection from *M. salmoides* and *S. vitreum.* Length of infection has been shown in many studies to be affected by ambient water temperature (Lefevre and Curtis 1912). Tedla and Fernando (1969) infected Lampsilis radiata siliquoidea on yellow perch (P. flavescens) and had a duration of infection of 40-50 days at 15°C. Walleye in my study were still infected heavily (100%) at day 26 at which time the study was terminated.

Artificial infection not only can be used to determine a probable fish host, but it can also be used as a method of propagation of mussels. A test of feasibility of such an operation was performed in La Crosse, Wisconsin, on the west channel of the Mississippi River in November 1907. In this experiment seven species of fish and glochidia from four Lampsilis sp. were used. C. carpio and the bullheads were unsuccessfully infected. Successfully infected individuals were retained in tanks and later released. This experiment was one of many that investigated the possibilities of making a large scale mussel propagation operation. Previous studies such as those by Coker et al. (1921) and Howard (1914) investigated the methods of propagation for several mussel species. Methods of artificial propagation were updated by Jones (1950). Currently, work is being done with the artificial propagation of glochidia in medium that involves the elimination of the intermediate fish hosts (Isom and Hudson 1982, Ellis and Ellis 1926). Isom and Hudson (1982) reported transformation of glochidia in "in vitro" culture that may prove to be beneficial in reestablishing populations of threatened or endangered mussels.

The incidence of natural infections of mussel glochidia on fishes is generally quite low (Lefevre and Curtis 1912, Surber 1912, Everman and Clark 1918, Coker et al. 1921, Wiles 1975, Trdan 1981, Zale and Neves 1982). Surber (1912) examined 2,815 fish of 38 different species and found only 41 were infected from June to November (fish were presumably collected in the Fairport, Iowa area). This is a 1.5% rate of infection. In the present study, 1,786 fish (33 different species) were inspected and an incidence of 4.1% was determined. This low incidence of natural infection suggests even more strongly that major gains in mussel populations could be obtained by the successful practices of artificial propagation. Information such as that presented in this study could be useful for the successful mitigation of these species and possibly their congeners.

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