

MALACOLOGIA, 1986, 27(1): 185-202

THE REPRODUCTIVE CYCLES AND GLOCHIDIA OF FRESH-WATER MUSSELS  
(BIVALVIA: HYRIIDAE) OF THE MACLEAY RIVER, NORTHERN NEW SOUTH  
WALES, AUSTRALIA

H. A. Jones, R. D. Simpson &amp; C. L. Humphrey

Department of Zoology, University of New England, Armidale, NSW 2351, Australia

## ABSTRACT

An investigation of the reproductive biology of five fresh-water mussel species, *Cucumerunio novaehollandiae*, *Hyridella australis*, *H. depressa*, *Hyridella* sp. and *Alathyria profuga* was undertaken in the Macleay River, northern New South Wales. Gametogenesis was studied in detail for *C. novaehollandiae* but only the cycle of larval production was described for the other species. In *C. novaehollandiae* gametogenesis occurred throughout the year. Ripe oocytes and spermatozoa were abundant in the ovaries and testes from January until August. The breeding season was highly synchronized and occurred in April, although it is possible that a second breeding season occurred during August in the upper reaches of the river. The reproductive cycle of the downstream populations lagged behind the cycle of the upstream populations of *C. novaehollandiae*. Spawning was associated with the occurrence of floods, and the resulting drop in water temperature might possibly be an important exogenous factor influencing spawning. The brooding period extended over nine weeks. Glochidial release proceeded from mid-May to the end of July in the upper reaches of the river and from June until August further downstream.

The breeding season in *Hyridella australis*, a repetitive breeder, occurred from spring to autumn but was observed only during spring and summer in *H. depressa* and *Hyridella* sp. *H. australis* produced three broods during the breeding season. The brooding period was from eight to eleven weeks depending on water temperature. Glochidia were released throughout most of the year with peak release periods in November, February and May. Females of *A. profuga* were gravid in mid-summer when most individuals were collected.

The glochidia of *Cucumerunio novaehollandiae*, *Hyridella australis* and *H. depressa* are described. Except for *H. depressa*, these are much smaller than the known glochidia of other Australian species and also differ markedly in shape.

## INTRODUCTION

The fresh-water Unionacea have highly specialized life cycles. Among the Hyriidae, the eggs are moved into specialized portions of the inner gills (marsupia) where they develop into a hooked larval stage (the glochidium). Mature glochidia are released into the water where they spend some time attached to a vertebrate host. This is generally a fish, although tadpoles (Seshaiya, 1941; Walker, 1981) and a salamander (Howard, 1951) have also been shown to be host species.

General reproductive patterns are well known for both North American unionaceans (Lefevre & Curtis, 1910, 1912; Coker *et al.*, 1921; van der Schalie, 1938; Pennak, 1953; Clarke & Berg, 1959) and European unionaceans (Bloemer, 1935, 1946; Negus, 1966; Tudorancea, 1969, 1972; Wood, 1974;

Haukioja & Hakala, 1978; Dartnall & Walkey, 1979). Detailed life histories providing information on gametogenesis, breeding seasons, periods of glochidial release, fish hosts and the duration of the parasitic period are unavailable for most species. The reproductive biology of *Margaritifera margaritifera*, however, is well known in both Europe and North America (Murphy, 1942; Roscoe & Redelings, 1964; Wood, 1974; Smith, 1976, 1979; Bauer, 1979). Trdan (1981) determined the breeding season, period of glochidial development and fish hosts for *Lampsilis radiata siliquoidea* but ignored gametogenesis. Reproductive cycles of unionaceans, including gametogenesis, have been determined in both temperate (Matteson, 1948; van der Schalie & van der Schalie, 1963; Stein, 1969; Yokley, 1972; Giusti *et al.*, 1975; Heard, 1975; Zale & Neves, 1982) and tropical (Lomte & Nagabhusanam, 1969; Ghosh & Ghose, 1972;

Nagabhushanam & Lohgaonker, 1978; Dudgeon & Morton, 1983; Humphrey, 1984) regions.

Despite the numerous morphological descriptions of glochidia in the literature (Surber, 1912; Coker *et al.*, 1921; Clarke & Berg, 1959), few of these enable identification of glochidia at the species level (Rand & Wiles, 1982). The type of glochidium (*i.e.* hooked, hookless and axehead types) is constant for the genus and in some cases the shape is also characteristic (Lefevre & Curtis, 1910). Identification of glochidia at species level, especially conspecifics, is often more difficult (Porter & Horn, 1980) but it has been achieved by using scanning electron microscopy (Rand & Wiles, 1982) and analysing glochidial morphometrics (Wiles, 1975).

Little is known of the reproductive biology of the fresh-water mussels from the Australasian region. There is, at present, only one comprehensive study of the reproductive biology of an Australian mussel and this is a tropical species (Humphrey, 1984). Fish hosts have been found for several species (Percival, 1931; Hiscock, 1951; Atkins, 1979; Walker, 1981; Humphrey, 1984). The available data indicate that the glochidia of Australian fresh-water mussels are nonspecific parasites of fish (Atkins, 1979; Walker, 1981; Humphrey, 1984). The glochidia of less than half of Australia's 17 species of fresh-water mussels have been described (McMichael & Hiscock, 1958; Atkins, 1979; Walker, 1981) although there are several unpublished records (K. F. Walker, personal communication).

The aim of the present study was to investigate reproductive strategies of warm-temperate mussels in the Macleay River, New South Wales. (Other workers are currently studying reproduction of fresh-water mussels in the Murray River.) Five and possibly six species occur in the Macleay River system although one, *Vesunio ambiguus*, is found only in the tablelands section of the Apsley River (Fig. 1). *Cucumerunio novaehollandiae* (Gray) is ubiquitous throughout the river and for this reason was chosen for a detailed investigation of its reproductive cycle, including gametogenesis, breeding season and the period of glochidial release. Upstream and downstream populations were chosen for a comparison of the reproductive cycle in different parts of the river. Less detailed study was made of the reproductive cycles of the four other hyriid species occurring in the river—

*Hyridella australis* (L.), *Hyridella depressa* (L.), *Hyridella* sp. and *Alathyria profuga* (Gould).

## MATERIALS AND METHODS

### The study area

The Macleay River is situated in northern New South Wales, with its source in the New England Tablelands. Three major tributaries, the Apsley, Chandler and Muddy Rivers drain the central catchment area via a system of deep gorges from which the river emerges near its junction with the Georges River (Fig. 1). From here, the river flows more or less directly to the sea 220 km downstream.

The Macleay is a bicarbonate river, with soft waters of low salinity and turbidity. The chemical characteristics of the river at Turner's Flat (mean values) were as follows: Calcium 9.48 mg l<sup>-1</sup>, bicarbonate 55.51 mg l<sup>-1</sup>, pH 7.7, hardness 46.0 mg l<sup>-1</sup>, chlorine 12.40 mg l<sup>-1</sup>, conductivity 143 µS cm<sup>-1</sup> and salinity 62 mg l<sup>-1</sup> T.D.S. (N.S.W. Water Conservation and Irrigation Commission; Australian Water Resources Council, 1976).

Discharge is seasonal but variable and the highest discharge rates occur during the months from January to June with a minor peak in the spring (Fig. 2). During the study period water temperatures rose to a peak of 27°C in mid-summer and began falling during March, reaching a minimum of 11°C in June (Fig. 3). This was typical of previous years. Little difference in temperature was apparent between upstream and downstream parts of the river except in May when the water temperature of the lower reaches was 2°C higher than upstream.

### Collections and species identifications

Sampling of the fresh-water mussel populations was confined to stretches of the Macleay River below Georges River since the rugged terrain and inaccessible nature of the central gorge system precluded sampling above this point. The river was regularly sampled at two stations: at Honeymoon Bend (station 1), approximately 160 km above the tidal limit and at Toorooka (station 4), 50 km above the tidal limit. Infrequent sampling was carried out at two other stations (2 and 3) along the river (Fig. 1).

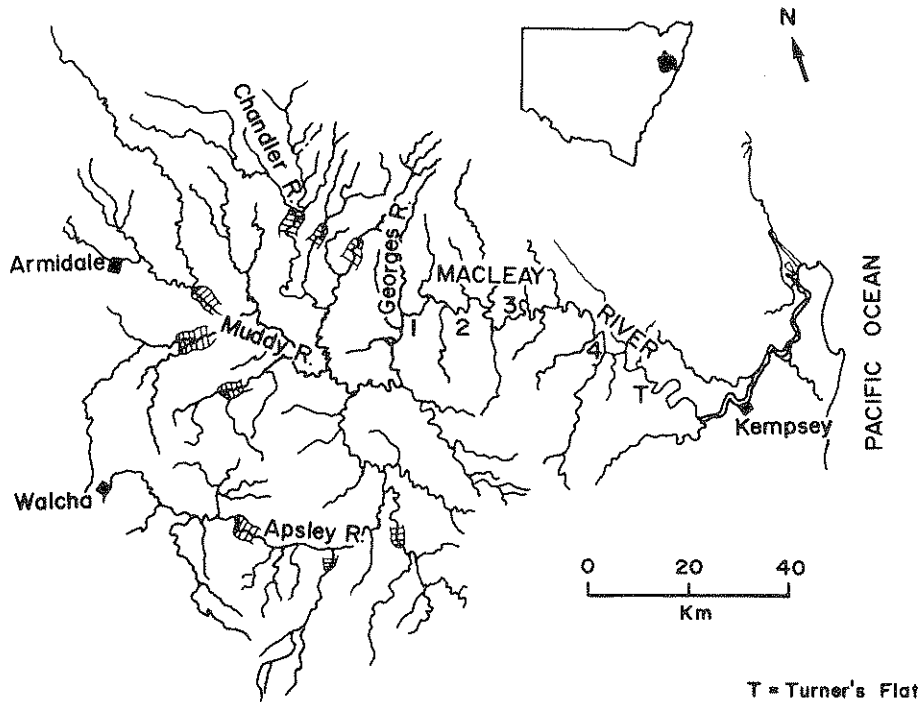


FIG.1. The Macleay River, showing the locations of the sampling stations (1, 2, 3, 4). Hatching indicates waterfalls at the top of the central gorge system.

Species identifications were based on McMichael & Hiscock (1958) and shell collections held by the Australian Museum (Sydney). We are confident about the identifications made of four species that occur in the main stem Macleay River. These species are *Cucumerunio novaehollandiae*, *Hyridella australis*, *H. depressa* and *Alathyria profuga*. (The occurrence of *A. profuga* in the Macleay River drainage is a new distributional record.) There is a likelihood, moreover, that a fifth species occurs although uncommonly; some shells collected during the present study match closely, published descriptions (McMichael & Hiscock, 1958) and museum identifications of *H. drapeta*. However, the glochidia (found in only one of the specimens collected from the Macleay River) do not match published descriptions of the glochidia of this species (Atkins, 1979). In morphology they are similar to the glochidia of *H. de-*

*pressa*, although from the scant data available they are smaller (see Table 1). More collections will need to be made to determine whether these shells are merely ecophenotypic variants of *H. depressa* or whether in fact they represent individuals of an undescribed species of *Hyridella*. For now, individuals of this type are referred to as *Hyridella* sp.

The abundances of the five species occurring in the main stem Macleay River were, in decreasing order, *Cucumerunio novaehollandiae*, *Hyridella australis*, *H. depressa*, *A. profuga* and *Hyridella* sp. Specimens were collected monthly from July 1982 to July 1983 by snorkelling or by hand. Supplementary collections were made in January 1985. Care was taken to process mussels quickly after collection since gravid females are known to abort the larvae from the marsupia when under stress (Lefevre & Curtis, 1910, 1912; Hiscock, 1951). Animals were either packed

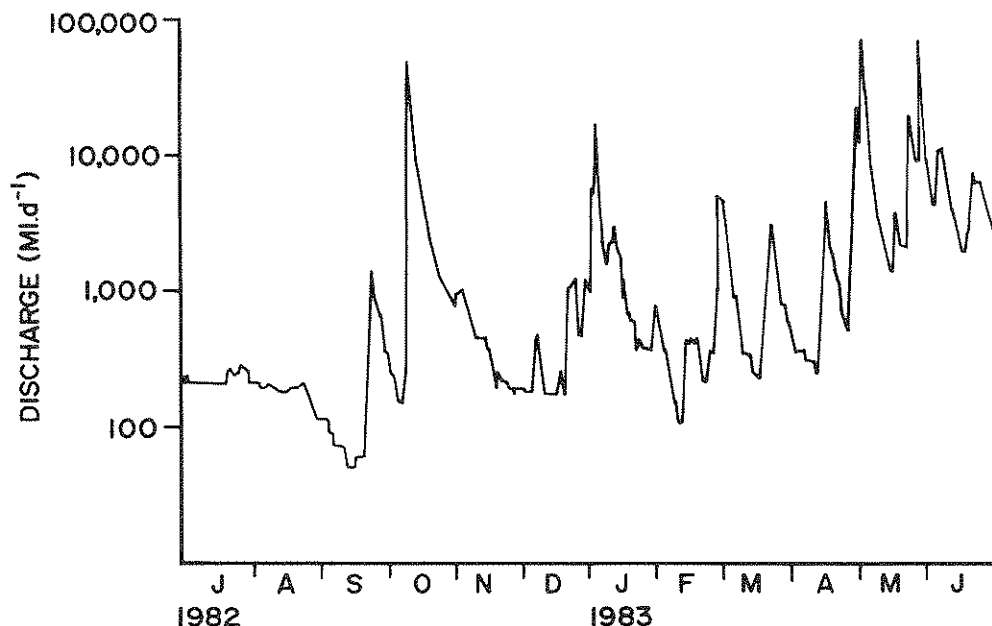


FIG. 2. Daily discharge of the Macleay River during the study period. (Based on data supplied by the New South Wales Water Conservation and Irrigation Commission).

in ice or fixed in 10% formalin made up from river water. Approximately 50 *C. novaehollandiae* were collected monthly from each of these stations, but the other species were collected in similar numbers from station 1 only. Only *C. novaehollandiae* was abundant at station 4.

#### Examination of gonads

Adult *C. novaehollandiae* ranging from 100–160 mm in length were sectioned at 6  $\mu\text{m}$  through the central region of the visceral mass and stained with Masson's trichrome or Mayer's haematoxylin and counterstained with eosin. Quantitative data of the stages of spermatogenesis were obtained from microscopical examination of stained sections through the central region of the visceral mass. In each of five individuals from each month, ten acini were selected at random and the proportion of each cell type, along a line through each acinus, was calculated using an ocular micrometer.

Oocyte sizes were measured with an ocular micrometer from visceral smears of 10 individuals per month and seasonal changes in

the mean oocyte size determined. 4500 oocytes were measured. The number of oocytes (sample size) required to give a representative mean oocyte size in each individual was determined by plotting the means against sample size (Fig. 4) until the mean value ceased to fluctuate (Elliott, 1977).

The inner demibranchs of the gills of females from all four species were examined to determine gravidity. Small portions from gravid gills were removed and examined under the microscope so that the stage of development of the larvae could be determined. Four stages were recognized:

Stage I. Marsupium empty and undeveloped.

Stage II. Eggs or embryos present in the marsupia. Embryos included all stages of development from zygotes to individuals in which the larval shell had not formed.

Stage III. Glochidia present in the marsupia. Glochidia were characterized by the development of the adductor muscle and the larval shell. This included individuals in which hooks were unformed or rudimentary to fully developed larvae, free of their vitelline membrane.

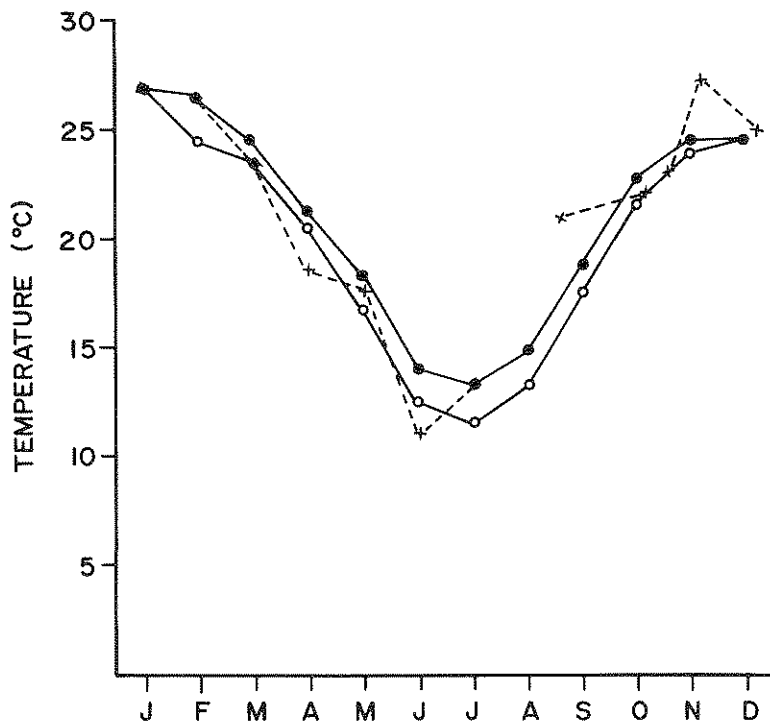


FIG. 3. Monthly water temperatures for the Macleay River, showing readings taken during the study period (broken line) and the mean monthly water temperatures for Georges River junction (closed circles) and Turner's Flat (open circles) since 1976. (Data supplied by the New South Wales Water Conservation Irrigation Commission)

Stage IV. Post-glochidial release phase. Some glochidia may be retained in the marsupium, which often has a bubbly appearance immediately after glochidial release. The water tubes are clearly visible in the inner demibranchs of *C. novaehollandiae* that have recently released glochidia. The dorsal two-thirds of the marsupia in *H. australis* retains a mass of tissue after the glochidia have been released, which imparts a false impression of gravidity. Aborted glochidia could be distinguished from those normally released at stage IV only if they were not mature glochidia.

The stages of glochidial development were further subdivided into early glochidia, intermediate glochidia and mature glochidia after Heard (1975). Dimensions of length (maximum valve diameter in the hinge plane), hinge length and height (depth from hinge to hook) of mature glochidia were measured. Refer-

ences made to glochidial dimensions are in the order of height  $\times$  length.

Definition of some terms is needed here. The breeding season, for animals that fertilize externally, spans the coincident periods of spawning of eggs and spermatozoa (Giese, 1959; Simpson, 1977). For those animals that do not fertilize externally, spawning and fertilization can be two separate events. The breeding season of fresh-water mussels is when the spermatozoa are spawned by the males to fertilize ova being moved into the gill chambers. The gestation period then follows and refers to the time elapsing between the movement of the oocytes into the marsupia until the development of mature glochidia. The gestation period is part of the total brooding period, which spans the time of placement of oocytes in the marsupia to the release of glochidia.

TABLE 1. Morphometric data for glochidia of the five species of fresh-water mussels found in the Macleay River.

Species	n*	Mean length ± SD (µm)	Mean height ± SD (µm)	Hinge length ± SD (µm)	Ht/Lth ratio (%)	Hinge/Lth ratio (%)
<i>C. novaehollandiae</i>	50	52.2 ± 0.6	64.1 ± 0.2	35	116	64
<i>H. australis</i>	50	73.9 ± 0.5	94.7 ± 0.3	40	128	68
<i>H. depressa</i> <sup>1</sup>	50	253 ± 5	244 ± 5	152 ± 6	97	60
<i>Hyridella</i> sp. <sup>1</sup>	20	239 ± 4	233 ± 4	136 ± 2	97	57
<i>A. profuga</i> <sup>1</sup>	20	239 ± 5	204 ± 2	165 ± 4	85	69
<i>A. profuga</i> <sup>2</sup>	—	245	200	—	82	—
<i>H. drapeta</i> <sup>3</sup>	—	330 ± 10	230 ± 10	248	71	75

\*From five individuals (*C. novaehollandiae*, *H. australis* and *H. depressa*) and one individual each (*Hyridella* sp. and *A. profuga*).

<sup>1</sup>Measurements made from preserved material.

<sup>2</sup>From McMichael & Hiscock (1958).

<sup>3</sup>From Atkins (1979). Hinge length is estimated from an illustration.

## RESULTS

### Spermatogenic cycle

The pattern of spermatogenesis in male *Cucumerunio novaehollandiae* from the upstream station between July 1982 and July 1983 was determined (Fig. 5). Spermatogenesis occurred throughout the year but at reduced tempo during the colder months of June and July as indicated by the reduced numbers of cells in the earlier spermatogenic stages (Table 2). The period between August and November was a recovery period in which

unspawned gametes from the previous season were either resorbed or released and during which a build-up of spermatogonia and sperm-morulae occurred (Fig. 6). Typical spermatogenesis was almost completely absent during this time, most of the activity being directed towards the production of sperm-morulae. An increase in the tempo of spermatogenesis occurred from late November until late April during which intensive production of spermatozoa and enlarging of the acini occurred (Figs. 7–8). This phase was also characterized by large clusters of spermatids that were absent in the months prior to November.

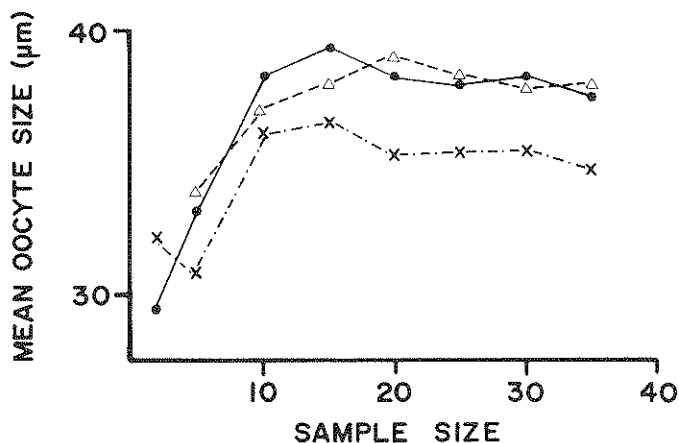


FIG. 4. Relationship between number of oocytes measured (sample size) and mean oocyte size in *Cucumerunio novaehollandiae* from the Macleay River: 26 January 1983, Honeymoon Bend (triangles); 26 March 1983, Toorooka (crosses); 15 July 1983, Toorooka (closed circles).

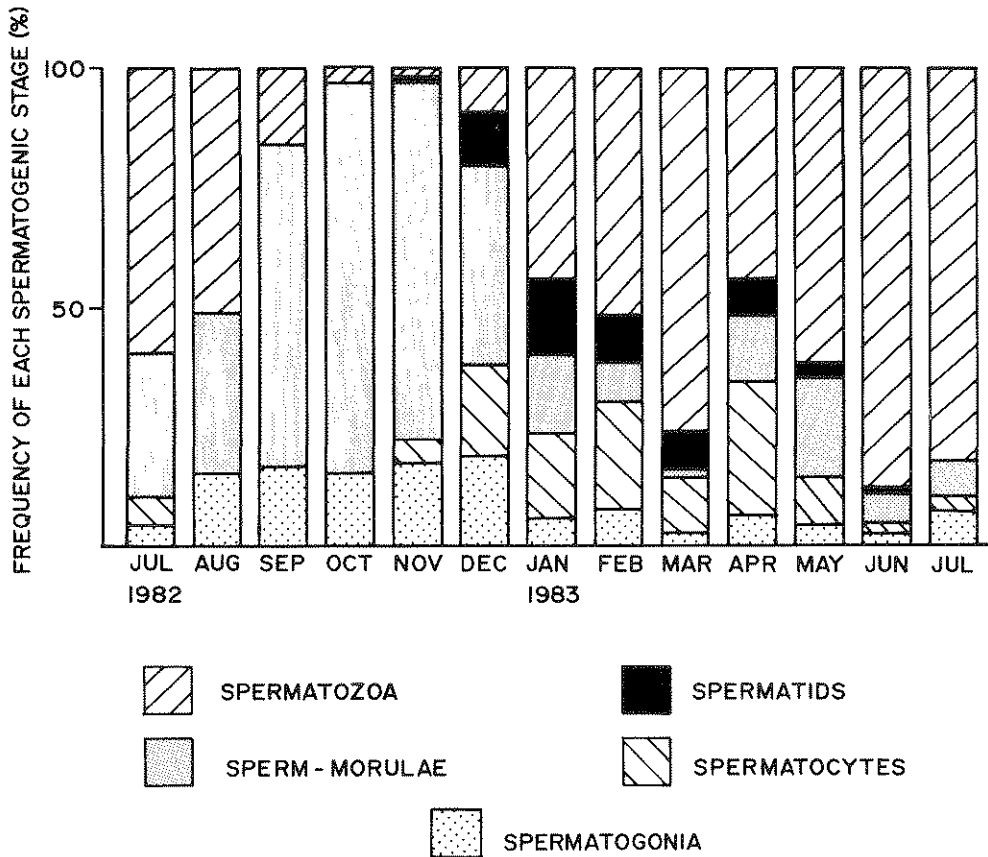


FIG. 5. Monthly changes in the proportion of the spermatogenic stages represented in the testes of *Cucumerunio novaehollandiae* from the upper reaches of the Macleay River.

Spawning occurred in the period of late March and April but was incomplete and many spermatozoa remained in the acini. After spawning, spermatogenesis continued from the remaining spermatogenic cells and the acini were again packed with spermatozoa in June and July (Fig. 9). Hence, three phases of spermatogenesis were recognized:

1) a recovery phase characterized by nests of spermatogonia and a high proportion of sperm-morulae;

2) an active phase characterized by nests of primary spermatocytes, secondary spermatocytes and spermatids. Spermatozoa were present in the lumen of the acini, and

3) a maturation phase in which sperma-

tozoa were abundant but spermatogenic activity was reduced.

#### Female cycle

The following description of seasonal oocyte production is for females from the upper reaches of the river. Both developing oocytes, connected to the follicle walls by a stalk, and mature oocytes were absent from the ovary between August and November. Mature oocytes remaining from the previous reproductive period were resorbed early during this period (Fig. 10). Nutritive granules were prolific along the follicle walls which, by late October, were thickened with oogonia (Fig. 11).

TABLE 2. Monthly descriptions of spermatogenesis in *Cucumerunio novaehollandiae*.

---

JULY: Both spent and mature individuals were present. The acini of mature individuals were large and closely spaced. The acini were filled with mature spermatozoa and very few of the earlier spermatogenic stages were present. The acini of spent individuals were small and widely spaced. Few spermatozoa were present in the lumen of the acini and spermatogonia and sperm-morulae were abundant.

AUGUST: Acini were slightly reduced in size when compared with individuals from July and fewer spermatozoa were present. Acini walls were thicker and there were numerous clusters of spermatogonia. Sperm-morulae were common.

SEPTEMBER: Acini were small, widely spaced and completely filled with sperm-morulae. Nests of spermatogonia were common and very few spermatozoa were present.

OCTOBER: Very little change from the previous month.

NOVEMBER: Clusters of primary spermatocytes appeared in some individuals and many of the sperm-morulae appeared to be metamorphosing into clumps of spermatozoa.

DECEMBER: Spermatozoa began to build up in the acini lumina and clusters of spermatocytes and spermatids were abundant. Sperm-morulae still dominated the acini.

JANUARY: In all individuals, spermatozoa were abundant and large clusters of spermatids in various stages of metamorphosis into spermatozoa were present. Spermatocytes were very common but there were fewer sperm-morulae.

FEBRUARY: Acini were large and closely spaced. Spermatogenesis was slightly more advanced than the previous month.

MARCH: Acini were densely packed with spermatozoa with peripheral bands of spermatocytes and spermatids.

APRIL: Acini were reduced in size and contained fewer spermatozoa than in March. The incidence of sperm-morulae increased.

MAY: Little change from April.

JUNE: Acini were again filled with spermatozoa but very few earlier spermatogenic stages were present.

JULY: Little change from the previous month except for the appearance of bands of spermatogonia and sperm-morulae around the periphery of acini.

---

Rapid growth of primary oocytes owing to vitellogenesis occurred from November (Fig. 12) and continued until the end of March when the acini were packed with mature oocytes. A sharp decrease in mean oocyte diameter occurred between March and April, coinciding with the movement of eggs into the marsupia. Following spawning, there was a second rapid build-up in the numbers of mature oocytes and acini were again packed with oocytes by mid-July (Fig. 13). Oogenesis in the downstream population lagged behind the upstream population and spawning did not occur until late-April or early-May (Fig. 14). Hence, three phases could be recognized in the oogenic cycle:

1) a recovery period from the end of the previous breeding season until late October (upstream) or December (downstream). Ripe oocytes remaining from the previous reproductive period were resorbed or passed out of the ovaries and the follicle walls thickened owing to a build-up of oogonia and nutrient reserves;

2) a growth phase from late October until about the end of March (upstream) and from December until about May (downstream).

During this phase vitellogenesis occurred and the acini became swollen as they filled with primary oocytes, and

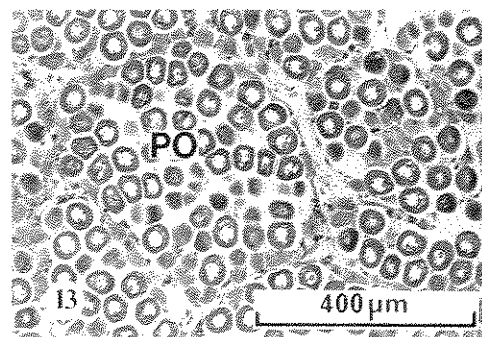
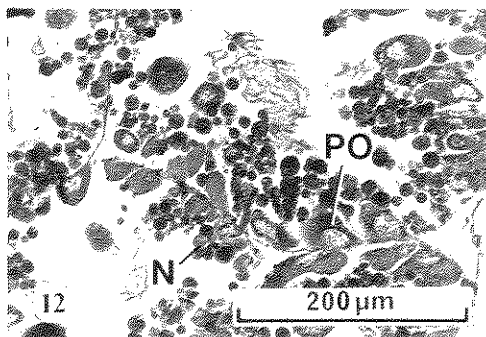
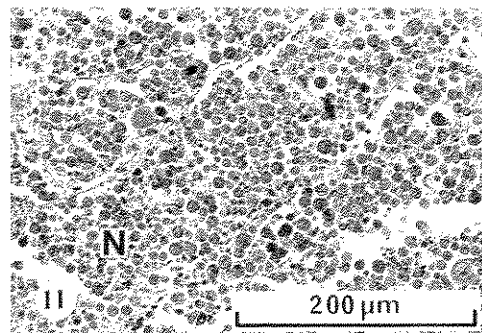
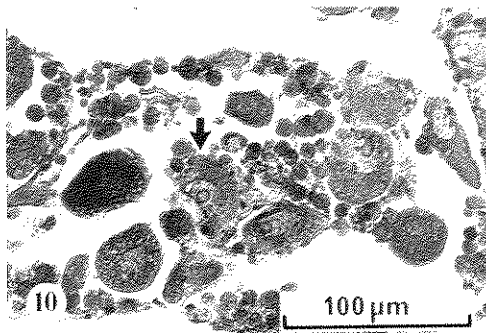
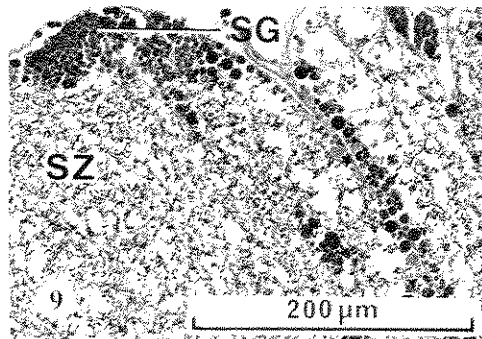
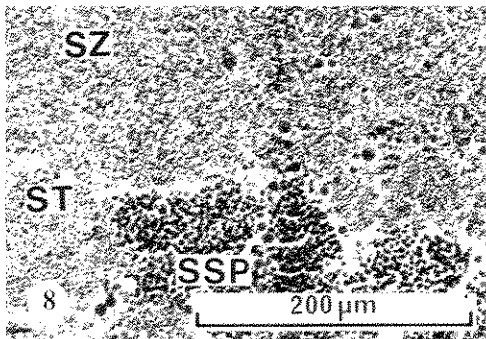
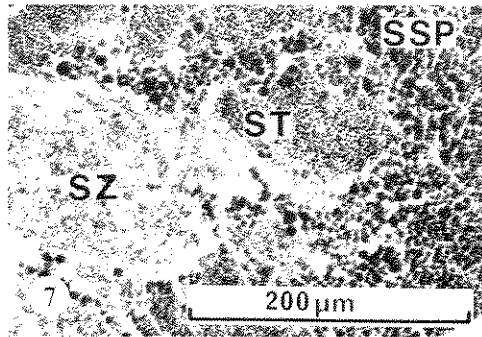
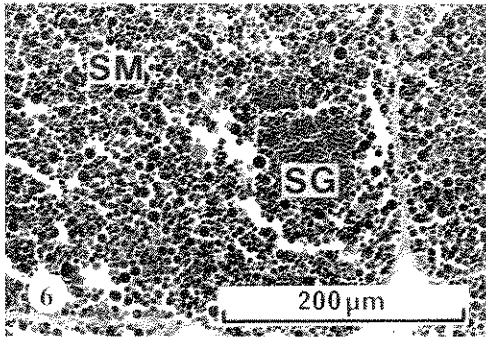
3) a spawning phase from late March (upstream) until early May (downstream).

#### Description of the glochidia

Mature glochidia of all species were collected, although these were found in the marsupia of only one female each of *A. profuga* and *Hyridella* sp. The glochidial morphometrics of these species are summarized in Table 1.

The glochidia of *C. novaehollandiae* (Figs. 15-17) are extremely small ( $64 \times 55 \mu\text{m}$ ), globose and of suboval outline. Two short hooks are present on the inside of each shell valve. Scanning electron microscopy has revealed a convoluted surface structure of the shell valves (K. F. Walker, personal communication). Glochidia of *H. australis* (Figs. 18-20) are small in comparison with other hyriids, measuring  $95 \times 74 \mu\text{m}$ . The glochidia are slightly elongate, subtriangular and the shell valves are perforated by small pores. The larvae are double-hooked and the teeth are recurved and set on a common base. The





glochidia of *H. depressa* (Figs. 21–25) are large ( $244 \times 253 \mu\text{m}$ ), subtriangular and possess a single, bifurcated hook set slightly off-centre on the ventral surface of each valve. The glochidia of *Hyridella* sp. also possess a bifurcated hook; they are very similar in morphology to *H. depressa* but are slightly smaller ( $233 \times 239 \mu\text{m}$ ). The glochidia of *A. profuga* compare well with published descriptions for this species (McMichael & Hiscock, 1958) (see Table 1).

#### Brooding period

##### *Cucumerunio novaehollandiae*

*C. novaehollandiae* is a winter breeder with a highly seasonal cycle (Figs. 26–27). The brooding period extends from April to August. The reproductive cycle of downstream populations, however, lags slightly behind that of the populations upstream (Table 3).

When collected on 9 May 1983, immediately after a major flood (Fig. 2), most of the females from the downstream population had just moved eggs into their marsupia. Five weeks later these were at an intermediate glochidial stage and mature glochidia were being released in mid-July, 9 weeks after spawning. Mussels from the upstream station were releasing glochidia during mid-May and had finished by mid-July. On the basis of a similar progression in development, females in the upstream population probably spawned in late March or early April.

##### *Hyridella australis*

The reproductive activity in this species extended throughout most of the year except for the coldest month, July (Fig. 28). Most of the November collection from the upstream population aborted their larvae. Consequently, it was not possible to accurately separate females with mature glochidia in their gills (stage III) from those which had released their larvae (stage IV).

There were at least two, probably three, brooding periods during 1982/1983, indicated by the high proportion of females carrying glochidia at different times of the year. The four week intervals between sampling precluded accurate assessment of the time elapsing between fertilization and glochidial release. However, the gestation period appears to have been about eight weeks during the summer months when the water temperature was in the vicinity of  $27^\circ\text{C}$ , and about 11 weeks during the autumn when the water temperature was lower ( $11^\circ\text{C}$ ). Breeding periods must have occurred between 26 November and 22 December 1982, and again between 28 February and 26 March in 1983, that is, approximately 13 to 14 weeks apart.

One of us (C.L.H.) has observed the release of glochidia of *H. australis* while diving in clean river conditions in January 1985. Glochidia are extruded from the exhalent siphon of mature females in a wormlike conglutinate. The conglutinate, approximately 4 cm in length, is tan coloured and bears white, transversely striated bands along one side. Laboratory examination revealed that the tanned material is composed entirely of mature glochidia bound together in a mucous matrix with the white striations comprising a loosely binding tissue. Distinct, rhythmical pumping actions of the exhalent siphon were noted that caused the wormlike mass to wave and fall about the siphon where it was posteriorly inserted or attached. Discharged conglutinates were also found lying free and intact on the sediments adjacent to adult females.

##### *Hyridella depressa*, *Hyridella* sp. and *Alathyria profuga*

Scant data were obtained for the brooding periods of these species (Table 4). However, *H. depressa* and *Hyridella* sp. were gravid during the spring and summer. It is possible that both species breed more than once per year, as in *H. australis*. Females of *A. profuga* bearing glochidia were present only in mid-

FIGS. 6–13. Stages of gonadal activity in *Cucumerunio novaehollandiae* collected from the upper reaches of the Macleay River. FIG. 6. Sperm-morulae in an inactive testis, 26 October 1982. FIG. 7. Maturing testis, 26 February 1982. FIG. 8. Mature testis immediately prior to spawning, 26 March 1983. FIG. 9. Mature spermatozoa in a testis, 15 July 1983. FIG. 10. Deteriorating oocytes (arrow) from an ovary, 27 July 1982. FIG. 11. Ovary in the resting phase and filled with nutrient matter, 26 October 1982. FIG. 12. Early oogenesis, 26 November 1982. FIG. 13. Mature ovary, 15 July 1983, N, nutrient granules; PO, primary oocytes; SG, spermatogonia; SM, sperm-morulae; SSP, secondary spermatocytes; ST, spermatids; SZ, spermatozoa.

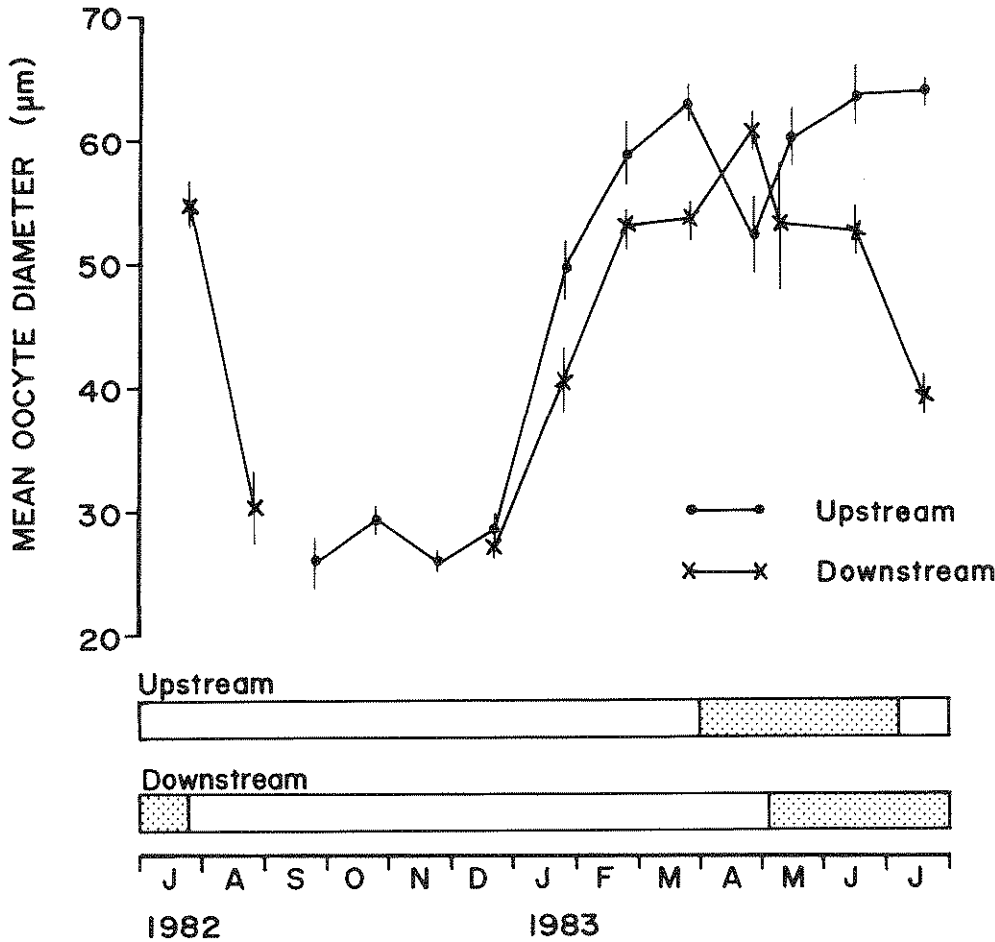


FIG. 14. Seasonal variation in primary oocyte size of *Cucumerunio novaehollandiae*. The bars are equal to 1 standard error of the mean. Brooding periods (stippled areas) are shown for upstream and downstream populations.

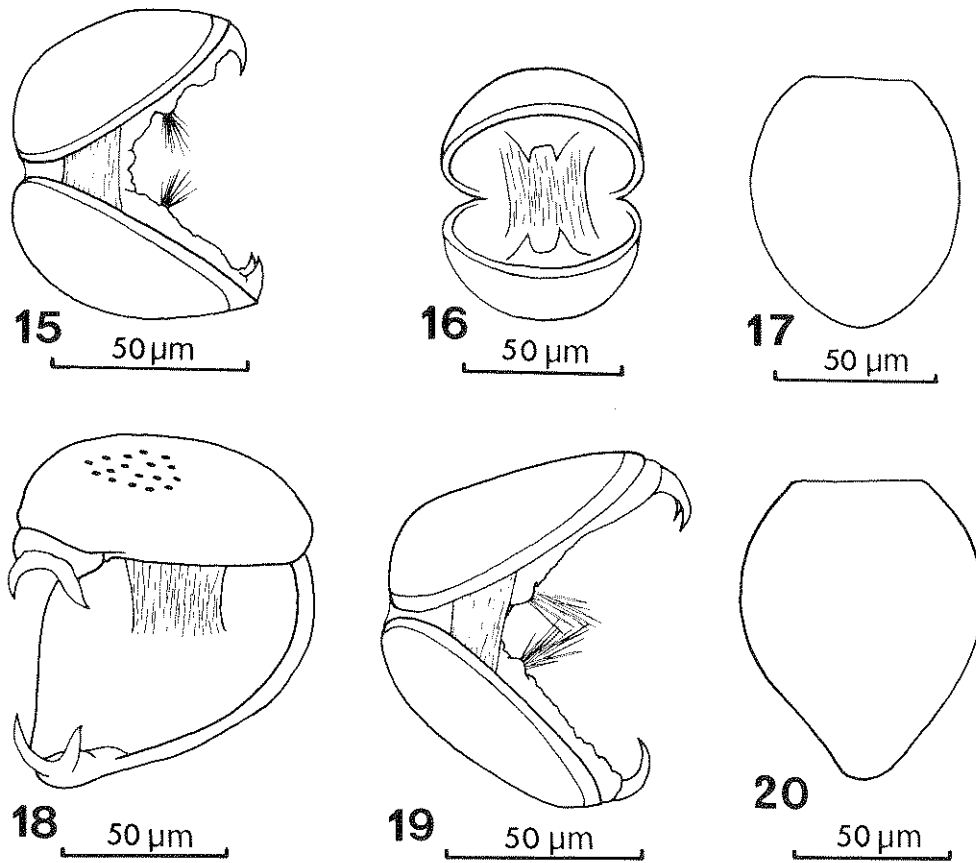
-summer, although very few specimens were collected at other times of the year.

DISCUSSION

Gametogenesis occurred throughout most of the year in *Cucumerunio novaehollandiae*, with peak activity during the hottest months of the year. This is typical of unionaceans from temperate climates (van der Schalie & van der Schalie, 1963; Yokley, 1972; Giusti *et al.*, 1975; Heard, 1975; Smith, 1979; Zale &

Neves, 1982). Mature ova were produced during the summer and autumn, coinciding with the peak in spermiogenesis.

A highly synchronized breeding season occurred during the autumn. However, males only partially spawned. This could reduce the risk of mistiming the release of gametes by the sexes. In other temperate fresh-water mussels both males and females spawn at the same time (Zale & Neves, 1982). Giusti *et al.* (1975), however, noted that not all male *Anodonta cygnea* spawned at the same time and *Elliptio complanatus* males release spermatozoa over a time span of about one



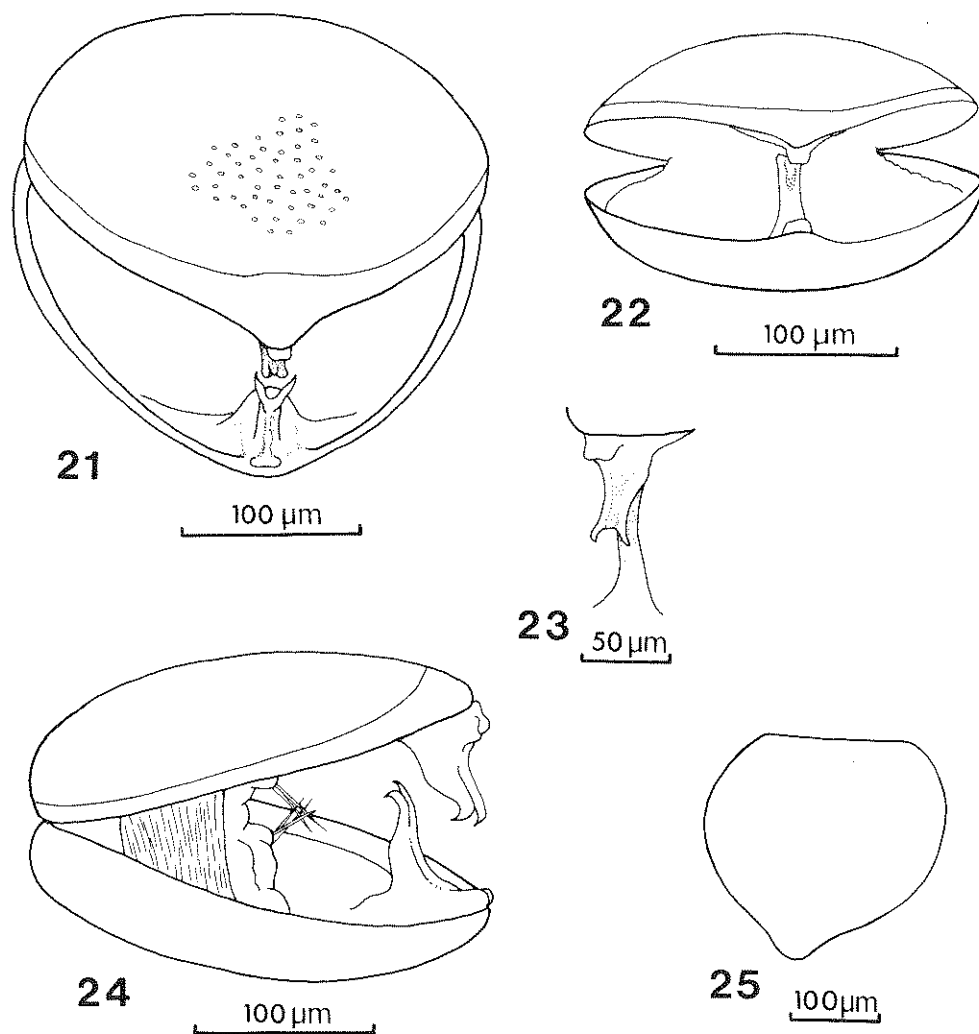
FIGS. 15-17. Glochidium of *Cucumerunio novaehollandiae*. FIG. 15. Lateral view. FIG. 16. Ventral view. FIG. 17. Outline of the shell valves. FIGS. 18-20. Glochidium of *Hyridella australis*. FIG. 18. Oblique ventral view. FIG. 19. Lateral view. FIG. 20. Outline of the shell valves.

month, which overlaps the period when females are likely to be receptive to fertilization (Matteson, 1948).

In contrast to *C. novaehollandiae*, other members of the southeastern Australian fresh-water mussel fauna may have much broader breeding seasons. Atkins (1979), in a study of a coastal Victorian stream, found *Hyridella drapeta* glochidia on fish throughout the year, with peak infections during the spring. Similarly, Hiscock (1951) found *Velesunio ambiguus* glochidia on fish throughout the year except between May and September, when no fish were examined. In agree-

ment with Hiscock's data, Walker (1981) found that glochidia may be present in the marsupia of *Velesunio ambiguus* throughout the year, although two peaks in gravidity were recognized; one in spring and the other during late summer/early autumn. The results of the present study indicate that *H. australis*, *H. depressa* and *Hyridella* sp. breed throughout much of the warmer part of the year. This also appears to be the case for the New Zealand species, *H. menziesi* (Percival, 1931) and prolonged breeding seasons may well prove to be characteristic of the genus *Hyridella*.

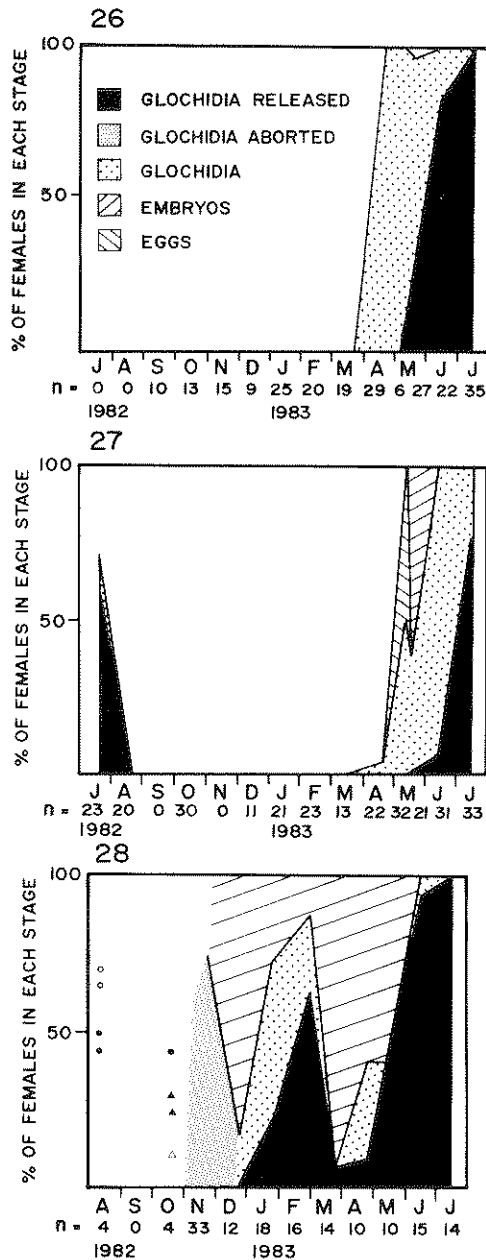
A series of broadly synchronized reproduc-



FIGS. 21–25. Glochidium of *Hyridella depressa*. FIG. 21. Oblique view. FIG. 22. Ventral view. FIG. 23. Lateral view of the forked teeth and the protuberances at their bases. FIG. 24. Structure of the teeth. FIG. 25. Outline of the shell valves.

tive cycles, such as occurs in *H. australis*, have been infrequently reported for freshwater mussels from temperate regions (Allen, 1924, cited by Heard, 1975) but asynchronous, repetitive breeding cycles occur throughout the year in some tropical unionaceans (Kenmuir, 1981b; Humphrey, 1984). More detailed investigations of the reproductive biology of temperate zone unionaceans may find repetitive breeding to be more wide-

spread than presently indicated; especially since some tachytictic species such as *Unio* spp. have the potential to produce many broods per year (Dudgeon & Morton, 1983). There are, however, several cases of freshwater mussels breeding twice per year (Lefevre & Curtis, 1910, 1912; Ortmann, 1912; Wood, 1974; Heard, 1975). Successive build-ups of large numbers of mature gametes indicated that *C. novaehollandiae* may breed



FIGS. 26–28. Seasonal distribution of the reproductive stages in the gills of freshwater mussels from the Macleay River. FIG. 26. *Cucumerunio novaehollandiae*, upstream population. FIG. 27. *C. novaehollandiae*, downstream population. FIG. 28. *Hyridella australis*, upstream population. For individual animals, open circles = non-gravid females; closed circles = embryos in marsupia; closed triangles = glochidia in marsupia; open triangles = non-gravid but glochidia recently released.

TABLE 3. Proportion of *Cucumerunio novaehollandiae* with different stages of developing larvae in gills throughout the Macleay River, 9 May 1983.

Stage of development	Station			
	1	2	3	4
I	0.0	5.3	5.0	0.0
II	0.0	0.0	0.0	50.0
III Early glochidia	0.0	94.7	0.0	0.0
Intermediate glochidia	100.0	0.0	95.0	50.0
Mature glochidia	0.0	0.0	0.0	0.0

twice per year: once in autumn and again in late winter/early spring. This pattern only occurred in the upper reaches of the river. Similarly Porter & Horn (1980) detected area variation in the reproductive cycle of a North American mussel in Lake Waccamaw. Also, variation in reproductive patterns within the one species of fresh-water mussel has been previously shown between rivers (Bauer, 1979) and at different latitudes (Matteson, 1948; Smith, 1976; Kenmuir, 1981a).

Sperm-morulae are of widespread occurrence in the Bivalvia (Bloomer, 1935, 1936, 1939; Coe & Turner, 1938; Ropes & Stickney, 1965; van der Schlie & Locke, 1941), including the Hyriidae (Heard, 1975; Peredo & Parada, 1984; Humphrey, 1984). Sperm-morulae occurred seasonally in *C. novaehollandiae* and were absent during the period of spermiogenesis. Seasonality of occurrence of sperm-morulae has also been described for other bivalves (Heard, 1975; Smith, 1979). Bloomer (1946) inferred that sperm-morulae metamorphosed into spermatozoa in *Anodonta cygnea* from the observation that sperm-morulae disappeared prior to the appearance of spermatozoa. This was also the case for *C. novaehollandiae* in which the process of atypical spermatogenesis was observed to the stage where the sperm-morulae consisted of elongate spermatids immediately prior to active spermatogenesis. Coe & Turner (1938) thought that sperm-morulae in *Mya arenaria* underwent cytolysis but there was no evidence of this in *C. novaehollandiae*.

Loosanoff & Davis (1952) and Sastry (1963) have demonstrated from their experiments with marine bivalves the importance of temperature as an activator of spawning. Environmental cues responsible for initiating

TABLE 4. Miscellaneous brooding records for individuals of the minor species found in the Macleay River.

Date	State of marsupia								
	Embryos			Glochidia			Empty		
	Hd*	Hs*	Ap*	Hd	Hs	Ap	Hd	Hs	Ap
3 Aug. 1982	1	—	—	—	—	—	—	—	—
26 Oct. 1982	6	2	—	3	—	—	5	—	—
7 Nov. 1982	—	—	—	—	—	1	—	—	—
26 Nov. 1982	2	—	—	—	—	—	—	—	—
22 Dec. 1982	4	—	—	—	—	—	—	—	1
22 Jan. 1983	—	—	—	—	—	—	—	—	2
26 Feb. 1983	2	—	—	—	—	—	—	—	—
26 Mar. 1983	—	—	—	—	—	—	2	—	—
26 Apr. 1983	—	—	—	—	—	—	1	—	1
16 May 1983	—	—	—	—	—	—	1	—	—
1/2 Jan. 1985	11	—	12	6	—	3	—	1	9
16 Jan. 1985	4	—	6	5	—	2	6	3	6

\*Hd = *Hyridella depressa*; Hs = *Hyridella* sp.; Ap = *Alathyria profuga*

spawning in fresh-water mussels have not been experimentally identified but numerous authors have brought attention to the correlation between breeding season and water temperature (Harms, 1909; Tudorancea, 1969; Yokley, 1972; Kenmuir, 1981b; Zale & Neves, 1982). Reproductive activity in *H. australis* may well be limited by low temperatures since gravid females were absent during the coldest months of the year. Similarly, low water temperatures were found to reduce breeding activity in *Velesunio angasi*, a tropical northern Australian species (Humphrey, 1984). Conversely, spawning in *C. novaehollandiae* appears to be related to falling water temperatures. Spawning in this species took place immediately after a flood which resulted in a sudden drop in water temperature. Sudden temperature changes brought about by the Danube high floods were thought to initiate breeding in *Unio tumidus* (Tudorancea, 1969, 1972) and a similar mechanism may be responsible for the highly synchronous breeding season in *C. novaehollandiae*.

The duration of the brooding periods in *C. novaehollandiae* and *H. australis* are comparable with many temperate unionaceans. Organogenesis is completed in two weeks and glochidia are mature in one month in *Elliptio complanatus* (Matteson, 1948). Development took two months in *Anodonta cygnea* (Wood, 1974; Giusti *et al.*, 1975), one month for *Unio tumidus* (Tudorancea, 1969) and *Margaritifera margaritifera* (Smith, 1976) and seven to eight weeks for four species of fresh-

water mussels from the Upper Tennessee River drainage, U.S.A. (Zale & Neves, 1982). Yokley (1972) found that the brooding period in *Pleurobema cordatum* took four to six weeks depending on water temperature. Water temperature also affects the gestation period in *M. margaritifera* (Smith, 1976). The results of the present study suggest that the gestation periods of *H. australis* and *C. novaehollandiae* are similarly influenced by water temperature.

Glochidial release by way of worm-like conglutinates such as occurs in *H. australis* has not previously been reported in hyriid unionaceans, although this mode of release is common to members of several genera of North American unionids (Kat, 1984). The larval conglutinates reported in unionids resemble various vermiform food items of the fish host and thereby enhance the likelihood of host contact (Chamberlain, 1934; Kat, 1984). At this early stage of investigation the appearance and rhythmical waving action of the conglutinates of *H. australis* suggest a similar mimicry of host food items as is displayed by the North American species.

Of the species examined in the present study, only the glochidia of *H. australis* and *A. profuga* have been previously described (McMichael & Hiscock, 1958). However, the descriptions by McMichael & Hiscock for the glochidia of *H. australis* match in size and general outline those for the glochidia of *H. depressa*, described here for the first time. In the present study, identifications of adults

were carefully checked and therefore it appears that McMichael & Hiscock incorrectly assigned a description of *H. depressa* glochidia to *H. australis*.

The glochidia of the Australian hyriids can no longer be viewed as a group which vary only slightly in size and shape (Atkins, 1979; Walker, 1981). *C. novaehollandiae* and *H. australis* produce much smaller glochidia than any other Australian fresh-water mussel. Indeed, in relation to other unionaceans the glochidia of *C. novaehollandiae* are amongst the smallest known, exceeded only by *M. margaritifera* (47  $\mu\text{m}$  diameter) (Roscoe & Redelings, 1964) and *Margaritana* (= *Margaritifera*) *monodonta* (50  $\times$  52  $\mu\text{m}$ ) (Lefevre & Curtis, 1912). Moreover, the morphology of the hooks of *H. australis* and *C. novaehollandiae* differ markedly from previous descriptions of glochidia of Australian fresh-water mussels. Typically, the Hyriidae possess a single, curved hook on each valve which may, or may not, have a forked point (Parodiz & Bonetto, 1963). These hooks, however, have been greatly modified in *H. australis* and *C. novaehollandiae*. The glochidia of *H. australis* bear a protruding double-hook on each valve. *H. glenelgis* also produces double-hooked glochidia (K. F. Walker, personal communication). In *C. novaehollandiae* the glochidia possess a pair of short, recurved hooks on each valve. Further, finer details of the hook morphology of *H. depressa* glochidia show clear differences between this species and its congeners.

*H. australis*, *H. depressa* and *H. drapeta* have almost identical geographical ranges and possess only slight conchological and anatomical differences (McMichael & Hiscock, 1958). The distinctive characteristics of the glochidia of each (namely, size and shape of the shell and structure of the hooks) are strong evidence that these mussels are separate species and not ecophenotypic variants of the one species as hinted at by Walker (1981). On the contrary, the limited evidence presented in this study suggests that the *Hyridella* complex may need to be subdivided even further. The hooks of *H. depressa* and *H. australis* appear to be modifications of a single hook in which a forked point has evolved. In *H. australis* this is more advanced. Even in *C. novaehollandiae* it appears as though the two hooks have evolved from a single hook through to the stage where they are now almost separate.

#### ACKNOWLEDGEMENTS

Thanks are due Dr. K. F. Walker who provided information on fresh-water mussels of southeastern Australia. We are grateful also for the services of Ms. L. Bridges (illustrations), Mr. R. Hobbs and Ms. L. Keogh (photography).

#### REFERENCES CITED

- ATKINS, L. G., 1979, Observations of the glochidial stage of the fresh-water mussel *Hyridella* (*Hyridella*) *drapeta* (Iredale) (Mollusca: Pelecypoda). *Australian Journal of Marine and Freshwater Research*, 30: 411-416.
- AUSTRALIAN WATER RESOURCES COUNCIL, 1976 ["1975"], *Review of Australia's water resources*. Department of Natural Resources, Australian Water Resources Council, Canberra: Australian Government Publishing Service, 170 p.
- BAUER, G., 1979, Untersuchungen zur Fortpflanzungsbiologie der Flussperlmuschel (*Margaritana margaritifera*) im Fichtelgebirge. *Archiv für Hydrobiologie*, 85: 152-165.
- BLOOMER, H. H., 1935, A further note on the sex of *Anodonta cygnea* L. *Proceedings of the Malacological Society of London*, 21: 304-321.
- BLOOMER, H. H., 1936, A note on the sex of *Anodonta anatina*. *Proceedings of the Malacological Society of London*, 22: 129-134.
- BLOOMER, H. H., 1939, A note on the sex of *Pseudanodonta Bourguignat* and *Anodonta Lamarck*. *Proceedings of the Malacological Society of London*, 23: 285-297.
- BLOOMER, H. H., 1946, The seasonal production of spermatozoa and other notes on the biology of *Anodonta cygnea* (L.). *Proceedings of the Malacological Society of London*, 27: 62-68.
- CHAMBERLAIN, T. K., 1934, The glochidial conglomerates of the Arkansas Fanshell, *Cyprogenia aberti* (Conrad). *Biological Bulletin*, 66: 55-61.
- CLARKE, A. H. & BERG, C. O., 1959, The fresh-water mussels of central New York. *Memoirs of the Cornell University Experimental Station*, 367: 1-79.
- COE, W. R. & TURNER, H. J., 1938, Development of the gonads and gametes in the soft-shell clam (*Mya arenaria*). *Journal of Morphology*, 62: 91-111.
- COKER, R. E., SHIRA, A. F., CLARK, H. W. & HOWARD, A. D., 1921, Natural history and propagation of freshwater mussels. *Bulletin of the United States Bureau of Fisheries*, 37: 77-181.
- DARTNALL, H. J. G. & WALKEY, M., 1979, The distribution of glochidia of the swan mussel, *Anodonta cygnea* (Mollusca) on the three-spined



- stickleback *Gasterosteus aculeatus* (Pisces). *Journal of Zoology (London)*, 189: 31–37.
- DUDGEON, D. & MORTON, B., 1983, The population dynamics and sexual strategy of *Anodonta woodiana* (Bivalvia: Unionacea) in Plover Cove Reservoir, Hong Kong. *Journal of Zoology (London)*, 201: 161–183.
- ELLIOTT, J. M., 1977, Some methods for the statistical analysis of samples of benthic invertebrates. *Freshwater Biological Association, Scientific Publications*, No. 25: 1–144.
- GHOSH, C. & GHOSE, K. C., 1972, Reproductive system and gonadal activities in *Lamellidens marginalis* (Simpson, 1900). *Veliger*, 14: 283–288.
- GIESE, A. C., 1959, Comparative physiology: annual reproductive cycles of marine invertebrates. *Annual Review of Physiology*, 21: 547–576.
- GIUSTI, F. L., CASTAGNOLO, L., FARINA, M. & RENZONI, A., 1975, The reproductive cycle and glochidium of *Anodonta cygnea* L. from Lago Trasimeno (central Italy). *Monitore Zoologico Italiano*, 9: 99–118.
- HARMS, W., 1909, Postembryonale Entwicklungsgeschichte der Unioniden. *Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere*, 28: 325–386.
- HAUKIOJA, E. & HAKALA, T., 1978, Life history evolution in *Anodonta piscinalis* (Mollusca, Pelecypoda). *Oecologia*, 35: 253–266.
- HEARD, W. H., 1975, Sexuality and other aspects of reproduction in *Anodonta* (Pelecypoda: Unionidae). *Malacologia*, 15: 81–103.
- HISCOCK, I. D., 1951, A note on the life history of the Australian freshwater mussel, *Hyridella australis* Lam. *Transactions of the Royal Society of South Australia*, 74: 146–148.
- HOWARD, A. D., 1951, A river mussel parasitic on a salamander. *Natural History Miscellanea, Chicago*, No. 77: 1–6.
- HUMPHREY, C. L., 1984, Biology and ecology of the freshwater mussel *Velesunio angasi* (Bivalvia: Hyriidae) in the Magela Creek, Alligator Rivers Region, Northern Territory. Ph.D. Thesis, University of New England, Armidale, 479 p.
- KAT, P. W., 1984, Parasitism and the Unionacea (Bivalvia). *Biological Reviews*, 59: 189–207.
- KENMUIR, D. H. S., 1981a, Seasonal breeding activity in freshwater mussels (Lamellibranchiata: Unionacea) in Lake Kariba and Lake Mcllwaine. *Transactions of the Zimbabwe Science Association*, 60(4): 18–23.
- KENMUIR, D. H. S., 1981b, Repetitive spawning behaviour in two species of freshwater mussels (Lamellibranchiata: Unionacea) in Lake Kariba. *Transactions of the Zimbabwe Science Association*, 60(4): 49–56.
- LEFEVRE, G. & CURTIS, W. C., 1910, Reproduction and parasitism in the Unionidae. *Journal of Experimental Zoology*, 9: 79–115.
- LEFEVRE, G. & CURTIS, W. C., 1912, Studies on the reproduction and artificial propagation of freshwater mussels. *Bulletin of the United States Bureau of Fisheries*, 30: 105–201.
- LOMTE, V. S. & NAGABHUSHANAM, R., 1969, Reproductive cycle in the freshwater mussel *Parreysia corrugata*. *Marathwada University Journal of Science*, 8: 113–118.
- LOOSANOFF, V. L. & DAVIS, H. C., 1952, Temperature requirements for maturation of gonads of northern oysters. *Biological Bulletin (Woods Hole, Massachusetts)*, 103: 80–96.
- MATTESON, M. R., 1948, Life history of *Elliptio complanatus* (Dillwyn, 1817). *American Midland Naturalist*, 40: 690–723.
- McMICHAEL, D. F. & HISCOCK, I. D., 1958, A monograph of the freshwater mussels (Mollusca: Pelecypoda) of the Australian region. *Australian Journal of Marine and Freshwater Research*, 9: 372–508.
- MURPHY, G., 1942, Relationship of the freshwater mussel to trout in the Truckee River. *California Fish and Game*, 28: 89–102.
- NAGABHUSHANAM, R. & LOHGAONKER, A. L., 1978, Seasonal reproductive cycle in the mussel, *Lamellidens corrianus*. *Hydrobiologia*, 61: 9–14.
- NEGUS, C. L., 1966, A quantitative study of growth and production of unionid mussels in the River Thames at Reading. *Journal of Animal Ecology*, 35: 513–532.
- ORTMANN, A. E., 1912, Notes upon the families and genera of the naiades. *Annals of the Carnegie Museum*, 8: 222–365.
- PARODIZ, J. J. & BONETTO, A. A., 1963, Taxonomy and zoogeographic relationships of the South American naiades (Pelecypoda: Unionacea and Mutelacea). *Malacologia*, 1: 179–213.
- PENNAK, R. W., 1953, *Freshwater invertebrates of the United States*. Ronald Press, New York, p. 694–707.
- PERCIVAL, E., 1931, A note on the life history of *Diplodon lutulentus* Gould. *Transactions and Proceedings of the New Zealand Institute*, 62: 86–91.
- PEREDO, S. & PARADA, E., 1984, Gonadal organization and gametogenesis in the freshwater mussel *Diplodon chilensis chilensis* (Mollusca: Bivalvia). *Veliger*, 27: 126–133.
- PORTER, H. J. & HORN, K. J., 1980, Freshwater mussel glochidia from Lake Waccamaw, Columbus County, North Carolina. *American Malacological Union Bulletin*, 1980: 13–17.
- RAND, T. G. & WILES, M., 1982, Species differentiation of the glochidia of *Anodonta cataracta* Say, 1817 and *Anodonta implicata* Say, 1829 (Mollusca: Unionidae) by scanning electron microscopy. *Canadian Journal of Zoology*, 60: 1722–1727.
- ROPEL, J. W. & STICKNEY, A. P., 1965, Reproductive cycle of *Mya arenaria* in New England. *Biological Bulletin (Woods Hole, Massachusetts)*, 128: 315–327.
- ROSCOE, E. J. & REDELINGS, S., 1964, The ecology of the freshwater pearl mussel,

- Margaritifera margaritifera* (L.). *Sterkiana*, 16: 19–32.
- SASTRY, A. N., 1963, Reproduction of the bay scallop. *Aequipecten irradians* Lamarck. Influence of temperature on maturation and spawning. *Biological Bulletin (Woods Hole, Massachusetts)*, 125: 146–153.
- SCHALIE, H. van der, 1938, The naiad fauna of the Huron River, in south-eastern Michigan. *University of Michigan Museum of Zoology Miscellaneous Publications*, 40: 1–83, 12 pl., map.
- SCHALIE, H. van der & LOCKE, F., 1941, Hermaphroditism in *Anodonta grandis*, a freshwater mussel. *Occasional Papers of the Museum of Zoology, University of Michigan*, 432: 1–7.
- SCHALIE, H. van der & SCHALIE, van der, A., 1963, The distribution, ecology and life history of the mussel, *Actinonaias ellipsiformis* (Conrad), in Michigan. *Occasional Papers of the Museum of Zoology, University of Michigan*, 663:1–17.
- SESHAIYA, R. V., 1941, Tadpoles as hosts for the glochidia of the freshwater mussel. *Current Science*, 10: 535–536.
- SIMPSON, R. D., 1977, The reproduction of some littoral molluscs from Macquarie Island (sub-Antarctic). *Marine Biology*, 44: 125–142.
- SMITH, D. G., 1976, Notes on the biology of *Margaritifera margaritifera* (Lin.) in central Massachusetts. *American Midland Naturalist* 96: 252–256.
- SMITH, D. G., 1979, Sexual characteristics of *Margaritifera margaritifera* (Linnaeus) populations in central New England. *Veliger*, 21: 381–383.
- STEIN, C. B., 1969, Gonad development in the three-ridge naiad *Amblema plicata* (Say, 1817). *Annual Report of the American Malacological Union*, 1969: 30.
- SURBER, T., 1912, Identification of the glochidia of freshwater mussels. *United States Bureau of Fisheries Document*, No. 771: 1–10, 3 pl.
- TRDAN, R. J., 1981, Reproductive biology of *Lampisilis radiata siliquoidea* (Pelecypoda: Unionidae). *American Midland Naturalist*, 106: 243–248.
- TUDORANCEA, C., 1969, Comparison of the populations of *Unio tumidus* Philipsson from the complex of Crapina-Jijila marshes. *Ekologia Polska*, A, 17: 185–204.
- TUDORANCEA, C., 1972, Studies on Unionidae populations from the Crapina-Jijila complex of pools (Danube zone) liable to inundation. *Hydrobiologia*, 39: 527–561.
- WALKER, K. F., 1981, The ecology of freshwater mussels in the River Murray. *Australian Water Resources Council Technical Paper*, No. 63.
- WILES, M., 1975, The glochidia of certain Unionidae (Mollusca) in Nova Scotia and their fish hosts. *Canadian Journal of Zoology*, 53: 33–41.
- WOOD, E. M., 1974, Development and morphology of the glochidium larva of *Anodonta cygnea* (Mollusca: Bivalvia). *Journal of Zoology (London)*, 173: 1–13.
- YOKLEY, P., 1972, Life history of *Pleurobema cordatum* (Rafinesque, 1820) (Bivalvia: Unionacea). *Malacologia*, 11: 351–364.
- ZALE, A. V. & NEVES, R. J., 1982, Reproductive biology of four freshwater mussel species (Mollusca: Unionidae) in Virginia. *Freshwater Invertebrate Biology*, 1: 17–28.