Breeding cycle of the freshwater mussel *Anodonta grandis* Say

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Received April 18, 1985

LEWIS, J. B. 1985. Breeding cycle of the freshwater mussel *Anodonta grandis* Say. Can. J. Zool. **63**: 2477–2478.

The gonads and marsupia of a population of *Anodonta grandis* Say in a small, eutrophic lake in the Laurentians were examined for the presence of eggs and glochidia over a period of three summers. The species was found to be a tachytictic (short-term) breeder, contrary to the general opinion that the Anodontinae are bradytictic (long-term) breeders.

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L'examen des gonades et des marsupiums prélevés au cours de trois étés chez une population d'*Anodonta grandis* Say d'un petit lac eutrophe des Laurentides a permis de déterminer le nombre d'oeufs et de glochidies présents. L'espèce s'est révélée un reproducteur tachytictique (à court terme), contrairement à l'opinion générale qui veut que les Anodontinae soient des reproducteurs bradytictiques (à long terme).

[Traduit par le journal]

There are two types of breeding cycles in the Unionidae, tachytictic or short term and bradytictic or long term (Heard and Guckert 1970; Kat 1984). The Anodontinae, which include *Anodonta grandis* Say, are regarded as long-term breeders and retain their glochidia in the marsupium throughout winter until spring (Clarke 1973). In the following account the breeding cycle of a population of *A. grandis* is described and shown to be tachytictic.

Twenty-five to 40 specimens (5–10 cm in length) of *Anodonta grandis* were collected at approximately 2-week intervals from depths of 0.5 to 2 m in Lac Yvan, a small eutrophic lake in the Laurentians about 50 km north of Montreal. Animals were preserved in 70% alcohol without fixing and stored for later examination. Gonads were examined by cutting open the visceral mass and carefully noting the presence or absence of eggs under a dissecting microscope. Glochidia were observed in the same manner by slitting open the marsupial demibranchs. The results of gonad and marsupia analysis between June and September over three seasons, 1981–1983, are shown in Fig. 1.

Anodonta grandis females were gravid between the end of June and the end of August of each year. The peak of egg production occurred about mid-July and the highest percentages of animals carrying glochidia occurred 3 to 5 weeks later. By the middle of September no traces of eggs or glochidia were found in the population.

These results are contrary to the general pattern of breeding cycles in the Anodontinae (Mackie 1984) and to the observations of Clarke (1973) who found that in *Anodonta grandis*, the breeding season in Pennsylvania lasted from early August to the following April or May. In North Dakota and Vermilion Bay, Ontario, gravid specimens with eggs were collected on August 3 and 22, respectively (Clarke 1973).

Reproductive strategies in freshwater bivalves has been reviewed by Mackie (1984) who confirmed that both short- and long-term development occur in the Unionidae and noted that a mixing of reproductive strategies appears to be common in pisidiid clams. Heard (1975) reported both bradyticity and tachyticity in several species of *Anodonta* with one species (*A. peggyae*), showing either bradyticity or undergoing two breeding cycles in a year. He concluded that the Anodontinae exhibit greater plasticity in life histories than do other unionid subfamilies.

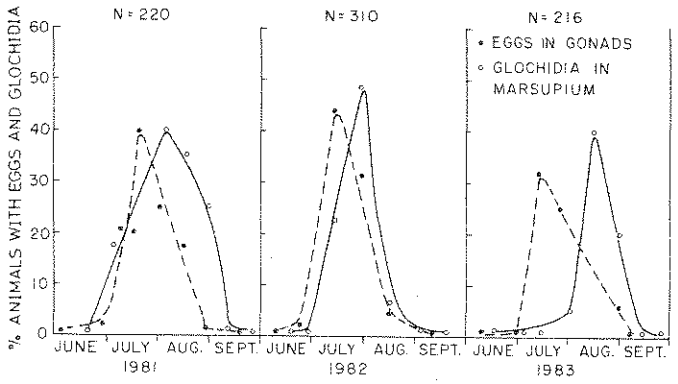

FIG. 1. Breeding cycle in *Anodonta grandis* Say, 1981–1983. Curves were fitted by eye; *N* is the total number of animals examined each summer.

The advantage of bradyticity as a life history strategy has been discussed by Kat (1984) and was considered in part to be a strategy for ensuring settlement and growth of juveniles during a short growing season. Bradyticity would allow a longer growing season before the onset of the first winter (Negus 1966). On this basis one might expect animals at the northern end of a species range to have prolonged breeding cycles and southern populations to be tachytictic. A second possible cause of the modification of the breeding term may be that glochidia release is synchronized to correspond to predictable periods of host availability (Kat 1984).

There may be a particular advantage to *Anodonta grandis* in adopting a short-term strategy in Lac Yvan. Because the lake is long and narrow in shape (1–200 m × 1000 m) there is a strong directional flow towards the outlet during the spring runoff. Thus, glochidia released in the spring by long-term breeders would likely be carried out of the lake whereas glochidia released in the late summer by short-term breeders would be more likely to be retained in Lac Yvan. It would be of interest to follow the breeding cycles in other northern lakes to determine whether or not the Lac Yvan population is an unusual example of tachyticity.

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Isolation and purification of *Eimeria bovis* (Apicomplexa: Eimeriidae) first-generation merozoites[1]

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Received January 7, 1985

REDUKER, D. W., and C. A. SPEER. 1985. Isolation and purification of *Eimeria bovis* (Apicomplexa: Eimeriidae) first-generation merozoites. Can. J. Zool. **63**: 2478–2480.

By using a simple procedure involving meront disruption, centrifugation, and a nylon wood column, large numbers of viable *Eimeria bovis* first-generation merozoites were obtained from experimentally infected calves. More than 10^9 highly purified merozoites were obtained from a single calf inoculated 15.5 days earlier with 1.5×10^6 sporulated oocysts of *E. bovis*.

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Une technique simple (rupture du méronte, centrifugation, utilisation d'une colonne de nylon et laine) a permis d'obtenir un grand nombre de mérozoïtes bien vivants de première génération d'*Eimeria bovis* chez des veaux infectés expérimentalement. Un prélèvement très pur contenant plus de 10^9 mérozoïtes a été ainsi obtenu chez un seul veau injecté, 15,5 jours plus tôt, de $1{,}5 \times 10^6$ oocystes en sporulation d'*E. bovis*.

[Traduit par le journal]

Introduction

Difficulties in obtaining large numbers of purified and viable eimerian merozoites have hampered *in vitro* cultivation and biochemical and immunological investigations of these economically important organisms. Of the few procedures devised for this purpose, most outline techniques for purification of sporozoites (Wagenbach 1969; Stotish *et al.* 1977; Schmatz *et al.* 1984) and merozoites (Witlock and Danforth 1982; Fernando *et al.* 1984) of *Eimeria* spp. infecting chickens. We have found that a method described by Hammond *et al.* (1965) for isolating and purifying *Eimeria bovis* merozoites from cattle is tedious, time consuming, and results in a final preparation that contains relatively large numbers of host intestinal cells. Herein, we report a relatively rapid and simple procedure for recovering large numbers of highly purified and viable first-generation merozoites of *E. bovis*.

Materials and methods

One Holstein bull calf (1.0–1.5 months old), free of intestinal coccidia, was given 1.5×10^6 sporulated *E. bovis* oocysts orally. Exactly 15.5 days after inoculation, the calf was killed by electrocution and the small intestine was removed and cut longitudinally. Intestinal contents were washed from the intestinal surface with cold tap water. Meront-containing portions of the ileum and jejunum were cut into 20- to 25-cm strips, washed in three 1-L changes of Hank's balanced salt solution (calcium and magnesium free; HBSS) (pH 7.4), and vigorously stirred in 1.5 L of 1.0 m*M* dithiothreitol (Sigma, St Louis, MO) in HBSS. The mucosal surface of each strip was removed by scraping with a glass microscope slide, rinsed with HBSS into a 500-mL beaker, suspended in HBSS, and passed through two layers of coarse-mesh cheesecloth. The parasite suspension was then divided equally among six to eight 100-mL glass pharmaceutical graduates (tapered bases), resuspended in HBSS, allowed to sediment for 15 min (Hammond *et al.* 1965), and the supernatant was removed by aspiration. Sedimentation and aspiration were repeated 3–5 times, after which numerous meronts were clearly visible at the bottom of the pharmaceutical graduates. During the sedimentation–aspiration process, samples were combined and then divided equally into four phar-

TABLE 1. Mean number of *Eimeria bovis* merozoites, host cells, and percent merozoites obtained from four groups after each step in the purification procedure ($\bar{x} \pm$ SD; $n = 4$)

Cell category	Step 1	Step 2	Step 3
Merozoites ($\times 10^6$)	1107.0±139.6	547.0±40.9	302.0±27.1
Erythrocytes ($\times 10^5$)	495.0±110.0	50.0±11.6	12.8±14.7
Nonerythrocytic host cells ($\times 10^5$)	1575.0±431.2	340.0±65.3	0.0±0.0
Total host cells ($\times 10^5$)	2070.0±381.7	400.0±74.8	12.8±14.7
% merozoites in sample	84.5±1.3	93.3±0.9	99.5±0.6

NOTE: Step 1, after meront disruption; step 2, after centrifugation for 1 min at 200 × *g*; step 3, after passage through the nylon wool column. Mean values within each category are all significantly different at $p \leq 0.05$.

[1]Journal Series No. J-1591, Montana Agricultural Experiment Station, Bozeman, MT.