

GILL EVACUATION AND RELEASE OF GLOCHIDIA BY *UNIO PICTORUM* AND *UNIO TUMIDUS* (BIVALVIA: UNIONIDAE) UNDER THERMAL AND HYPOXIC STRESS

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ABSTRACT

This study aimed to elucidate the mechanism of glochidial release in *Unio pictorum* (L., 1758) and *U. tumidus* Philipsson, 1788, and thus interpret the confused reports of unionine reproduction in the literature. Both species release mature, hooked glochidia on mucous threads, resembling a pearl necklace. Individual threads can reach 15 cm in length and carry *c.* 500 glochidia. Under hypoxic conditions both species released conglutinate-like structures comprising *c.* 500 eggs and immature, unhooked glochidia. The conglutinate-like structures released by *U. pictorum* and *U. tumidus* are coloured yellow and white, respectively. Increased temperature did not induce the release of conglutinates. The conglutinate-like structures are unlikely to be involved in increasing reproductive success by attracting host fishes. Rather, the premature evacuation of the gill contents may relieve the respiratory burden on adult mussels under hypoxic stress. Ambient hypoxia during the reproductive season may help explain the irregular recruitment success of relatively long-lived mussels, such as *U. pictorum* and *U. tumidus*.

INTRODUCTION

The glochidia of almost all freshwater unionoid mussels are obligate ectoparasites on fish hosts (Kat, 1984; Hoggarth, 1999). Following attachment, the glochidia become encysted by host tissue and then undergo metamorphosis, becoming juvenile mussels and generally excysting some three to four weeks later. The primary selective advantage of such a strategy is that the glochidia are dispersed through the system, which may be particularly advantageous for upstream dispersal and in lentic systems where passive dispersal is poor because of limited water currents. In some cases, the mussels form an association with a very narrow range of host fishes that share the same habitat requirements, resulting in a greater chance of the juvenile mussels excysting in a suitable habitat. The protective cyst may also enable dispersal across saline waters. For some species, such as those belonging to the Margaritiferidae, the glochidia derive nutrients from the host and thus grow considerably while encysted (Ziuganov, Zotin, Nezhlin & Tretiakov, 1994).

Young & Williams (1984) estimated that the probability of a glochidium of *Margaritifera margaritifera* (L., 1758) surviving to metamorphose is just 0.000001%. Consequently, adaptations can be seen in many species that enhance the chances of the glochidia attaching to an appropriate host. At the simplest level, glochidia may be armed with hooks and spines, which enable them not only to attach to the gills of the host, as seen in the unhooked Margaritiferidae (Ziuganov *et al.*, 1994; Araujo, Bragado & Ramos, 2001), but also to the fins, scales and eyes, as seen in the Anodontinae (Aldridge, 1997). Other species have evolved mechanisms that increase the chances of the glochidia encountering a host fish. For example, the extended mantle flaps of some species in the subfamily Lampsilinae mimic a fish that forms a central part of the diets of the host fishes (Kraemer, 1970). The mussel thereby induces an attack from the host fish and concurrently releases its glochidia (Haag & Warren, 1999).

Many North American species release the entire glochidial contents of each gill water tube packaged in a mucous matrix, termed a conglutinate, that mimics fish food items such as

leeches, worms, insect larvae, or small fishes (Kat, 1984; Watters, 1999). When a potential host consumes a conglutinate, the conglutinate breaks apart, enabling the glochidia to attach to the gill filaments. A small number of *Lampsilis* species release the entire glochidial load from a gill demibranch as a single superconglutinate, which resembles a fish in shape and coloration. The superconglutinate remains attached to the mussel with a mucous thread that can reach up to 4 m long and darts in the water column, further enhancing the fish mimicry (Haag, Butler & Hartfield, 1995).

The structure of the glochidium and the mechanism of glochidial release in the subfamily Unioninae is poorly understood. For example, the glochidia of the British *Unio* species were considered to be hookless by Wood (1974), while numerous studies have described the presence of hooks (e.g. Pekarinen & Englund, 1995).

Latter (1891) suggested that '*Unio*' releases its glochidia in 'long slimy masses' that can extend 'a distance of 2 or 3 inches' and that these masses can be withdrawn back into the shell of the mussel. However, when *Unio pictorum* (L., 1758) and *U. tumidus* Philipsson, 1788, are held in the laboratory during the reproductive season, they are often seen to release glochidial masses that resemble closely the conglutinates of *Actinonaias ligamentina* (Lamarck, 1819) as figured in Coker, Shira, Clark & Howard (1921, as *A. carinata*). Furthermore, these conglutinate-like structures can be coloured, and could be interpreted as resembling a leech or flatworm.

The aim of this study was to elucidate the mechanisms of glochidial release in *U. pictorum* and *U. tumidus*, and thus interpret the confused reports of unionine reproduction.

MATERIAL AND METHODS

Twenty-one gravid *U. pictorum* and five gravid *U. tumidus* were collected from the Old West River, Cambridgeshire (National Grid Reference TL438722) during May 2001. In this river system *Unio* species carry mature glochidia during May and June (Aldridge, 1999). The possession of maturing eggs and glochidia can be observed in the field by gently prising the valves apart by approximately 5 mm, when the mussel is removed from

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Figure 1. Mucous strands carrying mature glochidia of *Unio pictorum*. Scale bar = 1 cm.

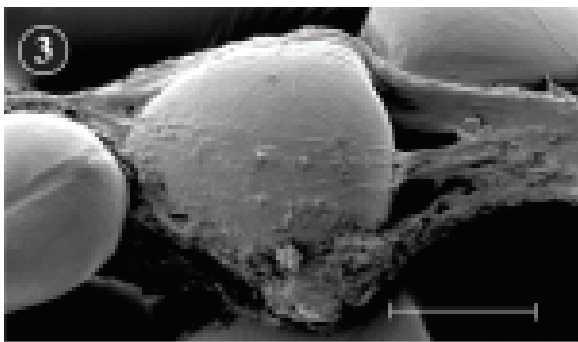


Figure 3. SEM detail of mucous thread surrounding mature glochidia of *U. pictorum*. Scale bar = 100 μ m.



Figure 5. Yellow conglutinate-like structures released by stressed *U. pictorum*. Scale bar = 1 mm.

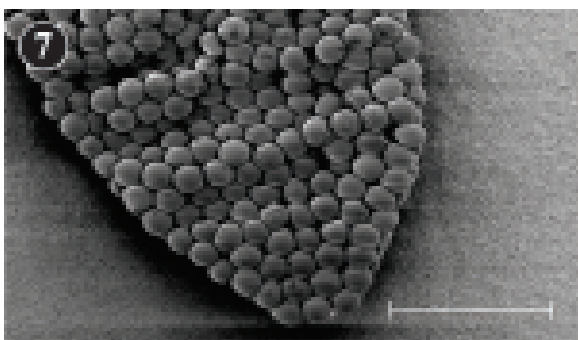


Figure 7. SEM of conglutinate-like structure of *U. tumidus*, composed of tightly-packed developing eggs. Scale bar = 1 mm.

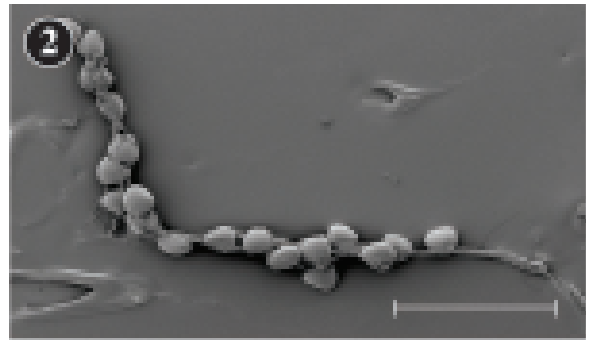


Figure 2. SEM of mature glochidia on a mucous thread released by *U. pictorum*. Scale bar = 1 mm.

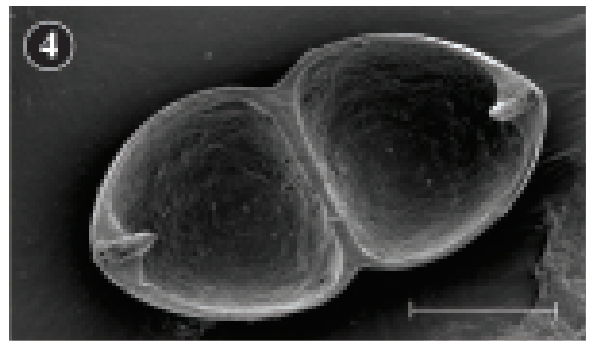


Figure 4. Mature, hooked glochidium of *U. pictorum*. Scale bar = 100 μ m.

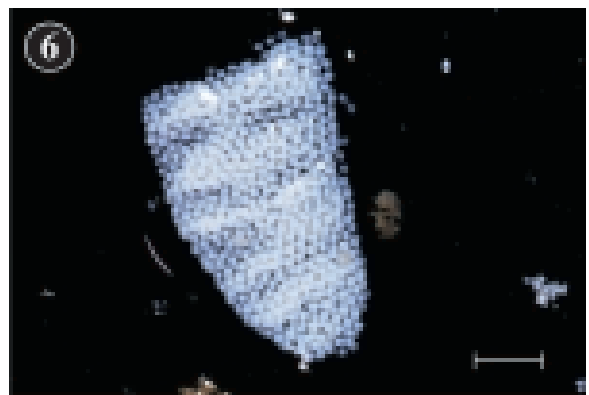


Figure 6. White conglutinate-like structure released by stressed *U. tumidus*. Scale bar = 1 mm.



Figure 8. SEM of hookless, immature glochidium of *U. pictorum*. Scale bar = 100 μ m.

the water, and observing whether the outer gill demibranchs are swollen (McIvor, 1999). All mussels were returned to the lab and held in aerated (87% DO₂), dechlorinated tap water at a constant 17°C, matching the ambient river conditions.

Release of 'conglutinates' and glochidia under low stress

Five *U. pictorum* and five *U. tumidus* were held individually in aerated, 17°C aquaria containing 500 ml dechlorinated tap water, and monitored regularly for the release of 'conglutinates' and glochidia.

Release of 'conglutinates' and glochidia under stressed conditions

Sixteen *U. pictorum* were placed individually into aquaria containing 500 ml dechlorinated tap water. Batches of four mussels were assigned to four treatments: 17°C with aeration; 17°C without aeration; 25°C with aeration; and 25°C without aeration.

Each beaker was monitored on a regular basis and the dissolved oxygen concentration measured. Any 'conglutinates' in the bottom of the beaker were counted and removed. Monitoring was terminated when no mussel had released 'conglutinates' or glochidia for at least four days. All mussels were once again assessed for the presence of remaining eggs/glochidia, before being returned to the field.

RESULTS

Release of 'conglutinates' and glochidia under low stress

Within three days, both species of mussel had released long mucus threads lined with glochidia, resembling pearl necklaces. The threads reached up to 15 cm in length and often became entwined with one another (Fig. 1). A single thread from *U. pictorum* carried approximately 50–60 glochidia in each 1-cm length of mucus (Figs 2 and 3). The presence of spined hooks (Fig. 4) and the active snapping action of the glochidia confirmed that the glochidia were mature.

A number of the mussels also released large numbers of yellow (*U. pictorum*) or white (*U. tumidus*), conglutinate-like structures that fell to the bottom of the aquaria (Figs 5 and 6). Light microscopy and scanning electron micrographs (Fig. 7) revealed that each of these structures was composed of approximately 500 developing eggs and immature glochidia. The immature glochidia did not have hooks (Fig. 8) and did not show the characteristic snapping activity of mature glochidia.

Release of 'conglutinates' and glochidia under stressed conditions

DO₂ levels in the aerated aquaria remained at ~55% throughout the experimentation, while DO₂ in unaerated aquaria fell to 0% within 80 h. The gill demibranchs of all mussels were found to be empty at the termination of the experiment. Two mussels did not release 'conglutinates' or glochidia, and were subsequently found to have been non-gravid.

Over the course of the experiment, individual mussels released an average of 30.0 ± 3.6 (SE) conglutinate-like structures (excluding those mussels which did not release 'conglutinates'; Fig. 9). Mature glochidia were also released by half of the mussels that received aeration.

The time taken for each mussel to release half its 'conglutinates' (T_{50}) was determined (Fig. 10). Two-way ANOVA showed that 'conglutinates' were released significantly more quickly in the absence of aeration ($F = 11.29$, $df = 1,10$, $P = 0.007$), while temperature, and the interaction between aeration and tempera-

ture had no significant effect ($F = 0.34$, $df = 1,10$, $P = 0.574$; $F = 0.34$, $df = 1,10$, $P = 0.572$, respectively).

DISCUSSION

The fact that the conglutinate-like structures consisted of immature, unviable glochidia suggests that the structures are not adapted to increasing infestation rates on fish; even if a fish host were to eat such structures the glochidia would be incapable of attaching. Rather, the reproductive strategy of *Unio* species appears to resemble that of the anodontines (Aldridge, 1997) and rely on host fishes becoming passively entangled in their mucus threads.

It appears that *Unio* species have a tendency for releasing conglutinate-like structures when held in the laboratory, whereas the anodontines *Anodonta cygnea* (L., 1758), *A. anatina* (L., 1758) and *Pseudanodonta complanata* (Rossmässler, 1835) never release conglutinate-like structures (D. Aldridge, personal observation). The propensity of unionines to release immature glochidia may explain the conflicting reports in the literature; the hookless glochidia described by Wood (1974) were presumably immature glochidia that had been released by stressed mussels. Similar confused descriptions have been given for the glochidia of *Velesunio ambiguus* (Philippi, 1847), which have been described as being both hookless (Hiscock, 1951) and hooked (McMichael & Hiscock, 1958), presumably reflecting immature and mature glochidia, respectively.

The unhooked larvae released by *Unio* species in this study were particularly notable because they were not constrained within the vitelline membrane (Fig. 8). This contrasts with the descriptions of glochidial development by Wood (1974) who found that the glochidia of *A. cygnea* matured with fully formed hooks while still inside the vitelline membrane. Presumably, the order of developmental processes, such as hook formation and loss of the vitelline membrane, is different between the unionines and anodontines.

The adaptive significance of the release of eggs and immature glochidia in *Unio* species may be explained by the physiological costs associated with brooding. Tankersley & Dimock (1993) demonstrated that the respiration rate in *Pyganodon catarracta* (Say, 1817) was notably lower in brooding females than in non-brooding females and males. They suggested that this was in part a consequence of reduced functional gill surface area. By evacuating the gill contents, the mussels may benefit from increased gill surface area and thus increased rates of oxygen uptake. Such a strategy may be particularly important when ambient dissolved oxygen levels fall or when temperatures rise, resulting in reduced solubility of oxygen.

Premature release of larvae may improve the lifetime reproductive success of species, such as *U. pictorum* and *U. tumidus*, which can live for up to 22 years (Aldridge, 1999). It may well pay to forego reproduction in a single year if by doing so the mussel improves its chances of surviving through to the next reproductive season. Additionally, it may pay *Unio* spp. to evacuate their gills if ambient conditions fall to levels below which maturing glochidia would die; even if only a small number of mature glochidia are present in the demibranchs, the mussel would be maximizing its reproductive success.

The finding that all mussels evacuated their gills prematurely when under stress may help to explain the highly variable annual recruitment success years in unionid mussels (Aldridge, 1999). For example, if riverine oxygen levels fall below a threshold during May or June, an entire population may prematurely evacuate their gills resulting in no recruitment during that year. Environmental pollutants may induce similar gill evacuation. For example, Reynolds & Guillaume (1998) showed that individuals of *A. anatina* ejects embryos of bitterling fish from their

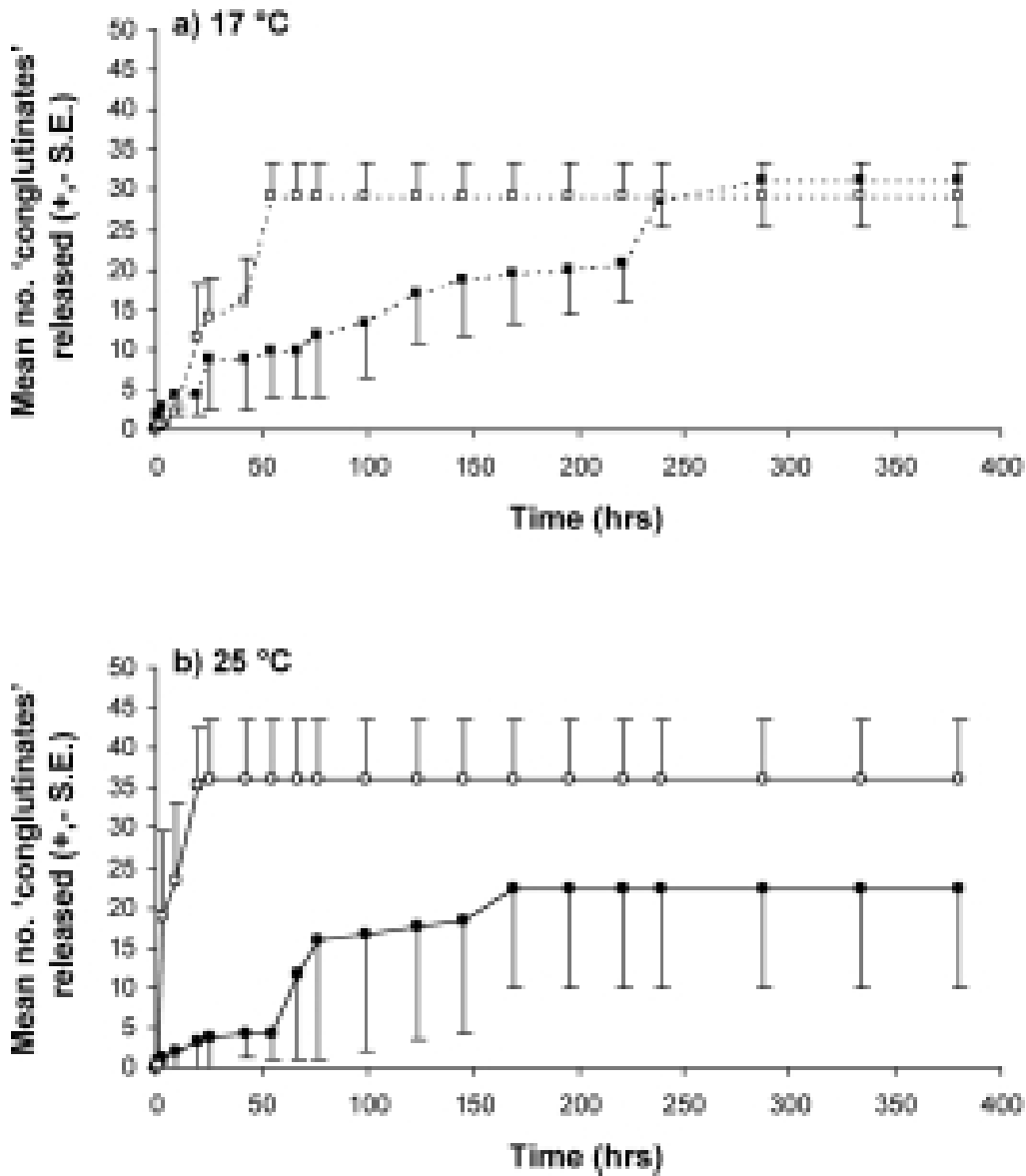


Figure 9. Cumulative mean number of conglomerate-like structures released by *U. pictorum* over a period of 15 days at 17°C (a) and 25°C (b). Solid symbols = with aeration; open symbols = without aeration.

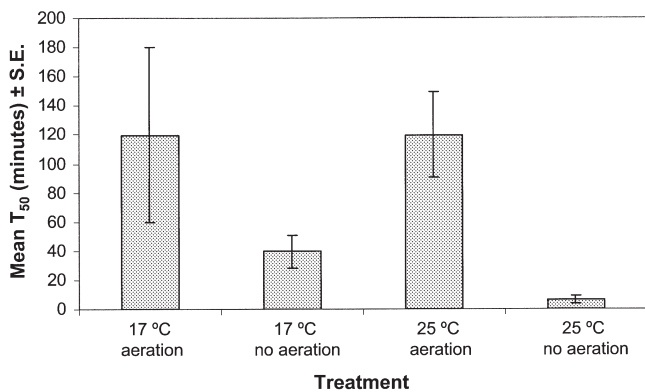


Figure 10. Mean time (T_{50} , min) for *U. pictorum* to release half of their conglomerate-like structures under different experimental treatments.

gill demibranchs when exposed to high concentrations of phosphate ($\geq 500 \mu\text{g l}^{-1}$).

These results suggest that gill evacuation by *Unio* species under stressed conditions can be viewed as an adaptive strategy to reduce hypoxic stress on the adult, rather than to increase the chance of glochidia attaching to a suitable host. It is possible that other species of unionid show a similar response, in which case the interpretation of the selective pressures underlying conglomerate release may need to be re-evaluated.

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