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Fishing for hosts: Larval spurting by the endangered thick-shelled river mussel, *Unio crassus*

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Understanding the life-history characteristics of endangered species is crucial to their conservation, management, and predicting their responses to environmental change (Stark et al., 2004). Host specificity is central to the evolutionary diversification and conservation of the Unionida (Barnhart et al., 2008; Modesto et al., 2018). In the North American mussels of the Ambleminae subfamily, specificity toward a restricted host fish range that shares the same microhabitat as the mussel has resulted in remarkable morphologies and behaviors that dramatically increase the likelihood of attachment and successful transmission of larvae (glochidia). This includes modification of mantle flaps into lures and packaging of glochidia into conglutinates that resemble insect larvae upon which the host fishes preferentially feed (Barnhart et al., 2008). Conversely, more generalist mussel species, that use a wide range of fish hosts, instead typically release glochidia freely into the water, or upon mucus threads in which passing fish become entangled (Aldridge & McIvor, 2003). The likelihood of successful transmission of glochidia onto the host may be further enhanced through female mussels releasing more glochidia into the water in the presence of fish (Jokela & Palokangas, 1993).

Little attention has been paid to glochidial release in European mussels that have a limited range of host fishes, such as the endangered *Unio crassus* (Lopes-Lima et al., 2014). In laboratory exposures, glochidia of *U. crassus* metamorphosed successfully on minnows (*Phoxinus phoxinus*) and chub (*Squalius cephalus*), but >90% failed to metamorphose on six other native and non-native fish species (Taeubert et al., 2012). Douda et al. (2012) found that, under experimental conditions, at least some *U. crassus* glochidia developed on 14 of 27 potential hosts tested, but that only three species (rudd, *Scardinius*

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erythropthalmus; *P. phoxinus*; and bullhead, *Cottus gobio*) enabled the majority of the attached glochidia to metamorphose successfully. Similar host specificity was reported in natural populations, with *P. phoxinus* and *C. gobio* carrying large numbers of glochidia in French rivers, but other species carrying few or none (Lamand et al., 2016).

During the springtime, some populations of *U. crassus* display a remarkable behavior, where mussels move to the river bank before regularly ejecting long jets of water back into the river. This spurting behavior in *U. crassus* was first remarked upon in 1913 and was interpreted as the result of feeding behavior, with the water jet used to expel feces (Israel, 1913). In 1926, it was suggested the animals were simply stranded during falling water levels (Mentzen, 1926). More recently Vicentini (2005) noted that the behavior happens only in the spring and summer when mussels are gravid, and confirmed the observations reported by Gelei (1932) that the spurted water contained glochidia. This reaffirmed Gelei's (1932) proposal that spurting served as a way of directing glochidia toward host fishes. To date, reports on the spurting behavior of *U. crassus* remain anecdotal and qualitative. The objective of this study was to quantitatively examine the spurting behavior of *U. crassus*, microscopically observe the spurted material, assess the behavioral response of fishes in the river to the spurted material, and to make inferences on the adaptive significance of this behavior.

Studies were conducted during May 2018 in the Biała Tarnowska River, Poland. Fifteen mussels were monitored throughout an entire spurting cycle (3–6 h in length). During monitoring, the time interval between spurts and spurt distance was recorded, and a subsample of six spurts was collected from each mussel by holding a tube over the exhalant aperture. The volume of spurted water per spurt was recorded, and the number and viability of glochidia in the spurt was measured. We also tested whether potential host fish were preferentially attracted to mussel spurts, by comparing mussel spurts with paired control spurts delivered via a syringe. For more detailed methods, see Appendix S1: Section S1.

Brooding females migrated to the water's edge where they anchored into the riverbed. With their posterior margin raised above the waterline, water jets were spurted from the exhalant siphon (Figure 1A, Video S1) to a distance up to 100 cm (Figure 1B) and carried up to 1127 glochidia in up to 3.1 mL water (Figure 2A). All observed spurting mussels positioned themselves approximately perpendicular to the river margin, with spurts always landing in the water. Spurts were released every 91 s ±3 (SE), with spurt frequency and distance being more variable at the start and end of the cycle (example of a typical spurting sequence shown in Figure 1C). Non-destructive inspection of marsupia showed that spurting ceased when all glochidia had been released. Greatest spurting activity occurred from mid-morning to mid-afternoon. A significantly greater proportion of benthic and pelagic fish (*Barbatula barbatula*, *Gobio gobio*, 0+ Cyprinidae) inspected the spurted water compared with paired controls (Figure 1D). Salt exposure tests showed the glochidia to be viable (93% ± 2 [SE] after 5–12 h, Figure 2B).

In accordance with Gelei (1932) and Vicentini (2005), we interpret spurting in *U. crassus* as a glochidial dispersal strategy that increases the likelihood of attachment to host fishes and avoidance of non-hosts. We significantly extend these previous observations by quantifying key characteristics of the mussel spurts, confirm that glochidia in the spurts are viable and demonstrate that potential host fish are attracted to these spurts, thus providing for the first time a convincing interpretation of this remarkable larval transmission behavior. By spurting into the air and not water, glochidia can travel greater distances from the mussel. Surface water disturbance attracted surface-foraging hosts, such as 0+ chub (Vicentini, 2005), in both spurts and control squirts, but fish showed greater interest in the spurted material that delivered glochidia into the water column. It is likely that disturbance of the surface water is especially detected by and attractive to fish in water with little or no movement, as was the case in our study site (see Video S1). By being mistaken for food items, glochidia are subsequently inhaled and attach to the gills (Modesto et al., 2018) where they encyst and metamorphose. 0+ fishes, including the cyprinid *S. cephalus* that is known to serve as a host to *U. crassus* (Taeubert et al., 2012) and which is known to be present in our study site (T. Zając, unpublished data), can serve as especially suitable hosts as they have not acquired induced immunity to glochidial parasitism (Barnhart et al., 2008). The glochidia in our study remained viable for 5–12 h which means that further transmission to hosts may occur through downstream drifting and also through settlement on the river bed, which could bring the glochidia into contact with benthic foraging fish. In our study, glochidia were no longer viable after 50 h, although laboratory studies by Benedict and Geist (2021) found that *U. crassus* glochidia remained viable for up to 144 h at cooler temperatures (15°C), which may further enhance the likelihood of successful encystment of glochidia that do not immediately attach to fish attracted to the spurted material.

Glochidial spurting has not been documented in any other unionid and has rarely been reported in *U. crassus* (but note Gelei, 1932; Vicentini, 2005). The short duration time of spurting could result in the behavior being overlooked in some populations, although given the high research intensity on *U. crassus* in many localities,

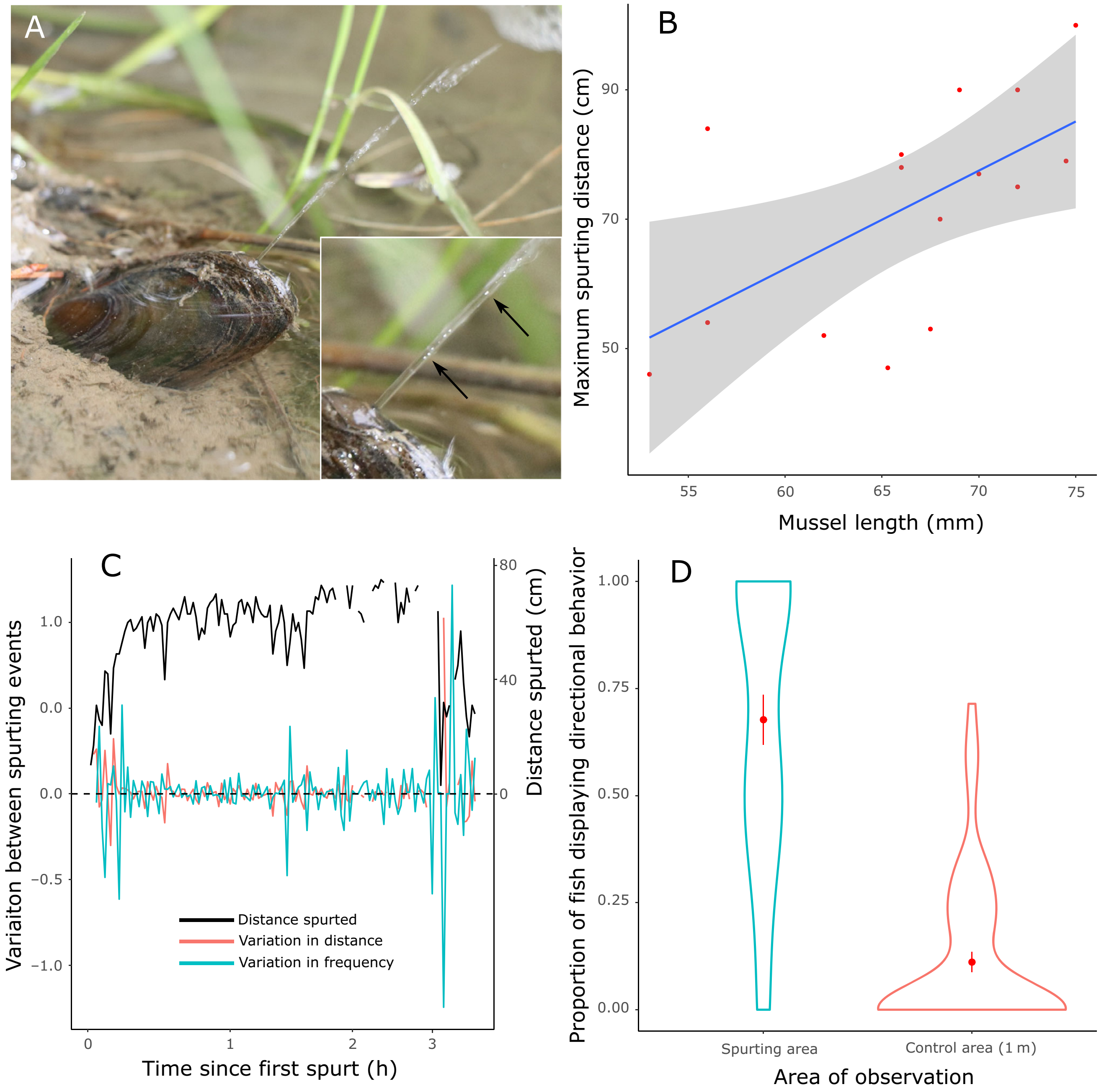

FIGURE 1 Characteristics of *Unio crassus* spurting events. (A) The mussels release intermittent jets of water from the exhalant siphon, containing glochidia (arrows, inset). Photo credit: David C. Aldridge. (B) Larger mussels have a greater maximum spurting length (regression, $F_{1,13} = 7.231$, $p = 0.019$, $R^2 = 0.36$; shading shows 95% confidence interval [CI]). (C) Time-series of the entire spurting sequence for one mussel (length 72 mm), lasting 3 h 27 min and containing 137 spurts in total. Variation in distance or frequency between consecutive spurting events is expressed as the log ratio of the spurt at time t to the spurt at time $t-1$ (e.g., a value of 0 indicates the distance of the spurt was equivalent to the distance of the spurt before that). The spurting sequence begins and ends erratically, but features consistently-distanced spurts at regular intervals in the middle of the sequence. To better highlight variation, each spurting event is plotted an equal distance apart, but the time between spurts gradually becomes longer toward the end of the sequence, leading to an uneven distribution of spurts per hour on the x-axis. (D) Possible host fish for glochidia were attracted to mussel spurts, but less so to control jets of glochidia-free water (2 mL) delivered by syringe, with a higher proportion of fish swimming or redirecting toward the landing area of the spurt (Wilcoxon signed-ranked, $W = 10$, $p < 0.001$). Red dots and error bars indicate mean ± 1 SE.

we suggest the behavior is not found across all populations. There is emerging molecular evidence that *U. crassus* populations across Europe in fact comprise numerous species (Lopes-Lima, unpublished data), and it is possible that not all these species spurt. Moreover, spurting may play an important isolating role that could facilitate

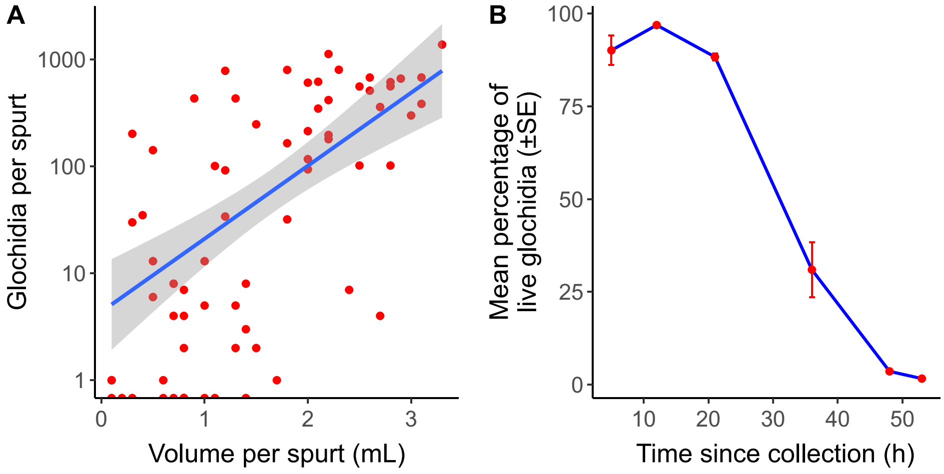

FIGURE 2 Characteristics of spurted glochidia. (A) Relationship between volume of water in a spurt and the number of glochidia in the spurted water. More glochidia were carried in larger spurt volumes (with number of glochidia logged, $F_{1,67} = 57.67$, $p < 0.001$, $R^2 = 0.46$; shading represents 95% CI). (B) Percentage of live glochidia at different times after collection, as determined by valve closure on exposure to salt. Glochidia were held in river water at ambient river water temperature. Material from 10 spurts per mussel was combined, with replicates representing three individual mussels. Closure responses were determined from three replicates of approximately 100 glochidia at each time point.

speciation; spurting may affect the taxa and life stage of fish that glochidia encounter and this may in turn affect the microhabitats into which metamorphosed juveniles enter after excystment from the host. Further isolation may arise through site-specific co-evolution between the mussels and hosts; Douda et al. (2014) demonstrated that there are often differences in the suite of fish hosts used by *U. crassus*, even across genetically and geographically close populations.

U. crassus populations have declined globally by 90% since the 1970s, and this has been attributed largely to habitat degradation, pollution, invasive species, disease, and loss of host fishes (Lopes-Lima et al., 2014). Host specificity can increase vulnerability of mussels as survival is intrinsically linked to that of their hosts. Spurting may provide an additional explanation for declines; even if water quality is high, increasing embankment of rivers precludes spurting through loss of shallow, muddy margins. Furthermore, increasing deforestation of catchments has resulted in more frequent springtime flooding (Šilhán, 2015), making females at river margins vulnerable to displacement. Daylight spurting increases the visual detection of glochidia by potential hosts but may also elevate exposure to increasing marginal foragers, including invasive muskrat (*Ondatra zibethicus*) and more recently mink (*Neovison vison*) (Brzeziński et al., 2010). Our study highlights how species-specific behaviors can expose some organisms to unexpected and disproportionately large effects from anthropogenically-induced environmental stressors, such as increased floods, destruction of marginal habitats, and introduction of non-native predators; the interplay between animal behavior and global change deserves closer attention not only for freshwater mussels but also for wider conservation initiatives.

Closer inspection of glochidia release mechanisms of apparently non-spurting *U. crassus* populations, coupled with population genetic studies, may reveal undescribed behaviors and discrete phylogenetic units. Spurting populations may deserve particular conservation attention. Studying glochidia release in other Unionida may provide important insights into host specificity and help identify drivers of local and global declines.

AUTHOR CONTRIBUTIONS

David C. Aldridge, Adam Ćmiel, Anna Lipińska, Manuel Lopes-Lima, Ronaldo Sousa, Amilcar Teixeira, Katarzyna Zając, Tadeusz Zając designed and conducted fieldwork and sample analyses. David C. Aldridge and Joshua I. Brian analyzed the data and produced the figures/video. David C. Aldridge drafted the manuscript and all authors contributed to revisions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data and code (Brian, 2023) are available in Zenodo at https://doi.org/10.5281/zenodo.7654858.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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