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Cover Page Footnote

This project was made possible through a grant from the John C. Bock Foundation. We acknowledge the foresight of Madison Laughlin in making this project a reality. We thank two anonymous reviewers for their thoughtful feedback regarding an earlier version of this manuscript.

***Acroneuria lycorias* (Boreal Stonefly, Plecoptera: Perlidae) Emergence Behaviors Discovered in *Pinus strobus* Canopy**

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Abstract

Species of Plecoptera, or stoneflies, are known to use vertical emergence supports, and researchers believe many species of Plecoptera exploit arboreal habitats during emergence. However, the exact nature of these arboreal behaviors has largely remained a mystery. While exploring the habitat potential of *Pinus strobus* (L.) (Eastern White Pine) canopies in northern Wisconsin we observed *Acroneuria lycorias* (Newman) (Boreal Stonefly, Plecoptera: Perlidae) exuviae at heights as high as 12 m (observations at 6.6, 9, 9.5, and 12 m). Most *A. lycorias* exuviae appeared to have a strong preference for emergence sites at the underside or base of branches similar to some Odonate species. We also observed *A. lycorias*, adults climbing upwards along the main stem, post-emergence, to heights up to 22 m. To our knowledge, these heights represent the greatest heights ever documented for *A. lycorias* adults and exuviae, or any Plecopteran species. While other researchers have speculated that *A. lycorias* uses arboreal habitats during emergence, these behaviors were considered almost impossible to describe. Our observations provide us with new insights into Plecopteran emergence behaviors, especially for this species. We propose three alternative hypotheses that may explain these unique emergence behaviors.

Keywords: arboreal habitat, emergence, Plecoptera, stonefly

Species of Plecoptera, or stoneflies, are known to use vertical emergence supports, and researchers believe many species of Plecoptera exploit arboreal habitats during emergence. However, the exact nature of these arboreal behaviors has largely remained a mystery. In 2014, we began exploring the habitat potential of *Pinus strobus* (L.) (Eastern White Pine) canopies in northern Wisconsin (Laughlin et al. 2018). While climbing a large and old (>85 cm diameter at breast height, >100 years) *P. strobus* research tree on 6 June 2018, we observed *Acroneuria lycorias* (Newman) (Boreal Stonefly) exuviae and adults at various heights in the canopy (Fig. 1). We observed multiple *A. lycorias* exuviae at heights as high as 12 m (observations at 6.6, 9, 9.5, and 12 m). Most *A. lycorias* exuviae appeared to have a strong preference for emergence sites at the underside or base of branches. Laughlin et al. (2018) also observed an apparent selection for the underside or base of branches during emergence for multiple species of Odonata. We also observed multiple adult *A. lycorias* climbing upwards along the main stem and branches above the exuviae at heights up to 22 m. To our knowledge, these heights represent the greatest heights ever documented

for *A. lycorias* adults and exuviae, and any other Plecopteran species.

All exuviae and adults were observed on the southwest side of the study tree, which faced the nearby river. This tree was located approximately 10 m from the bank of the White River, a stream surrounded by tall clay banks in Ashland, Wisconsin, USA. The shore that surrounded this tree is forested with a number of old-growth and second-growth trees that have undergone minimal management under ownership of Northland College (Ashland, WI, USA) since region-wide harvests from 1890-1900. Shortly after these observations, this particular research tree was lost during a flood and no additional observations were made. Exploration of another *P. strobus* tree further from the river's edge and during the month of August yielded no additional observations.

Exuviae ($n = 5$) and adults ($n = 1$; female) were collected and identified using the keys in Hitchcock (1974), Stark and Gaufin (1976), and Stewart and Stark (2008). Specimens from the Hilsenhoff Aquatic Insect Collection at the Wisconsin Insect Research Collection (Madison, WI, USA) were also examined to verify the species. In northern



Figure 1. Adult *Acroneuria lycorias* observed climbing along main stem at 16 m. Exuviae were observed up to 12 m and adults were observed climbing along the main stem at heights up to 22 m.

Wisconsin, three *Acroneuria* species can be encountered: *A. abnormis* (Newman) (Common Stone), *A. internata* (Walker) (Lobed Stone), and *A. lycorias* (Dewalt et al. 2019). Of these, nymphs and exuviae can be readily identified using the taxonomic keys in Hitchcock (1974). Adult female *A. lycorias* can be separated from other *Acroneuria* spp. by the remnants of subanal gills, a darkened ocellar triangle, colored bands on the abdominal segments, and the shape of the subgenital plate. The shriveled anal gills can help identify adult male *A. lycorias* specimens, although examination of the paraprocts and the genitalia are required to identify some species in this group.

These observations provide us with new insights into Plecopteran species emergence behavior, which is poorly understood, especially for this species (Narf and Hilsenhoff 1974, Poulton and Stewart 1988, Sheldon 1999). Mature Plecopteran nymphs are known to crawl out of streams and cold lakes where eggs are laid, and climb vertical surfaces such as logs or the base of trees as emergence supports (Hynes 1976). There, they transform to the adult stage, leaving exuviae at the emergence site. In Wisconsin, adults are known to emerge during a two-week period in early spring while water temperatures remain below 10 °C (Krzysztof

and Szczytko 1984) and mate while resting on a horizontal substrate (Peckarsky 1979). Most species appear to select for emergence sites near the ground (Hynes 1976). Thus, our observations of *A. lycorias* exuviae at heights up to 12 m substantially expands the known range of heights for Plecopteran emergence. Laughlin et al. (2018) also documented that certain species of odonates use emergence sites at great heights in *P. strobus* canopies. Additionally, our observations of post-emergence behavior by adult *A. lycorias* (i.e., climbing up the stem following emergence to heights exceeding 22 m) is indicative of a life cycle-related behavior that has been previously undocumented. Narf and Hilsenhoff (1974) speculated that *A. lycorias* adults inhabit tree canopies in Wisconsin, but they dismissed collection of specimens in the canopy as “almost impossible” (p.124). Szczytko and Kondratieff (2015) also acknowledged the difficulty of collecting adult Plecopterans. Our observations indicate that *A. lycorias* may have a two-step process of canopy utilization where nymphs climb to heights well above the forest floor in preparation for emergence. Post-emergence, *A. lycorias* adults then climb up the stem of the structure to the upper canopy.

Why *A. lycorias* uses the canopy for these life-cycle related behaviors, remains

an open question. Adult *Acroneuria* have a short lifespan and are not known to feed (Peckarsky 1979), so utilization of the canopy for the consumption of organic material is unlikely. This life history trait (i.e., upward movement of adults following emergence) may be beneficial for maintaining genetic diversity by facilitating long-distance dispersal, for reducing competition between species through spatially-constrained niche partitioning, or for hastening growth and life stage development via exploitation of warmer and drier microclimates associated with the canopy.

As Plecoptera are considered weak-flying or non-flying insects (Marden and Kramer 1994), climbing to great heights may facilitate dispersal. Plecopteran dispersal is difficult to study directly and “dispersal events that are biologically important may remain undetected” (Winterbourn et al. 2007, p. 1). Adult Plecoptera numbers tend to decline with increasing distance from stream, with 90% of adults caught within 11 m of a stream channel, suggesting that only a limited number of individuals are likely to disperse between streams (Briers et al. 2002). In few cases, adults have been observed > 40 m away from their natal streams (Briers et al. 2004); these few long-distance dispersers may play a significant role in maintaining genetic diversity (Winterbourn 2005). Limited by poor flight, upward movement of adult Plecoptera to the upper canopy may allow greater dispersal distances.

Alternatively, species of Plecoptera exhibit temporal displacement of life cycles to reduce interbreeding and competition (Peckarsky 1979, Dewalt and Stewart 1995). *Acroneuria lycorias* may co-occur with species such as *A. abnormis*, and in such cases, *A. lycorias* nymphs have been observed emerging slightly earlier than *A. abnormis* individuals (Harper and Pilon 1970). This temporal segregation of emergence may prevent interbreeding and reduce competition for food resources and space for drumming and courtship behavior (Peckarsky 1979). The climbing behavior we observed may provide a secondary mechanism of reproductive isolation and reduce competition for drumming sites, allowing multiple species to coexist in close proximity to their natal stream through separation along a vertical gradient.

Finally, the use of canopies may also be beneficial for hastening growth and life stage development which can be linked to drier and warmer conditions (Ernst and Stewart 1985, Poulton and Stewart 1988). Sites in the canopy may be warmer and drier due to increased solar exposure and greater air flow relative to sites near the ground.

Plecoptera are intolerant of environmental stressors and have been speculated to be the insect order most threatened by human encroachment (Hynes 1993). Thus, it is important to understand the distribution and diversity of Plecoptera for the maintenance and restoration of aquatic biodiversity. Our research draws additional ecological connections between riparian forest canopies and aquatic life that can have important conservation implications; best management practices for timber harvest often include protection of riparian corridors. Our understanding of the role riparian forest canopies may have in the maintenance of aquatic systems is still expanding, and our work further supports efforts to conserve riparian corridors. With ongoing research and monitoring, we can better understand the ecological importance of forest canopies and forested riparian corridors to Plecoptera and other aquatic species.

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