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A little story about river pollution, predation, and leg regeneration in Serratella ignita (Ephemeroptera: Ephemerellidae)

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Abstract:

We found significantly different percentages of malformed nymphs in two contiguous populations of *Serratella ignita* along a NW Italy stream. We suggest that this may be due to a marked difference in the environmental conditions and especially to the effect of pollution on the functional structure of the insect community.

Keywords: environmental pollution; autotomy; feeding functional groups; trophic interactions

Introduction

Among Insects, the regeneration of a lost body part is an interesting phenomenon that, contrary to what happens in some other invertebrate groups, typically concerns limbs and not parts of the trunk, tissues, or other organs (Maruzzo and Bortolin 2013). Even if most of the studies focused on terrestrial insects, the capacity to regenerate a limb lost during the immature stage has also been documented in a few aquatic insects, including Odonata and Ephemeroptera (Saxton et al. 2020). In some mayflies, limbs seem to show a "breakage point" (Almudi et al. 2019), which allows the easy detachment of the leg when subject to pressure, a self-defense mechanism called autotomy that facilitates escape when attacked. The capacity to break off legs and then regenerate them likely allows these insects to survive predators better (Bely and Nyberg 2010). In this study, we report and discuss the different occurrences of *Serratella ignita* (Poda, 1761) (Ephemeroptera: Ephemerellidae) nymphs with malformed/regenerated legs in two reaches of the same stream characterized by contrasting environmental conditions.

Materials and Methods

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In the frame of an extensive study about the combined impact of climate change and wastewater treatment discharge (WWT) on the environmental quality of lotic systems, we monthly collected quantitative aquatic insect samples from the Malone stream (NW Italy). We sampled two stations, the first located 100 m upstream of a WWT (45° 16′ 54.0′′ N, 7° 40′ 20.0′′ E) and the second located 150 m downstream. Up- and downstream sections had similar streambed and channel characteristics. Using a Surber net (22 x 23 cm; 500 micron mesh), we collected five samples/month in each station to assess the taxa presence and abundance of the natural population of benthic insects. In this study, we focused on the samples collected in April and March 2022 (N = 20 surber), as in this period, the nymphs of *S. ignita* reach their largest size before emerging. To evaluate environmental quality we employed the widely used BMWP and ASPTe assessment methods (Buss et al. 2015; Monge-Salazar 2021).

Results

Analyzing the samples, we found several *S. ignita* specimens with not regularly developed, i.e. malformed legs (**Fig. 1**). Interestingly, specimens with regenerating legs were almost exclusively found in the upstream section. Here, on a total number of 494 specimens, 99 reported malformed legs. Among them, 7 nymphs showed a malformation in the first right leg, 14 in the first left leg, 22 in the second left leg, 28 in the second right leg, 14 in the third left leg, and 14 in the third right leg. In the downstream section, we found 314 *S. ignita* nymphs, among which only one showed a malformed leg (the mesothoracic right one). In general, no missing or torn structures are noted; this suggests that the limb has been completely detached, and the one found is in its regrowth phase. No organism belonging to another species reported similar malformations.

We detected a marked difference in the environmental quality between the two stream reaches. In the upstream section, BMWP values reached 185, corresponding to a first ecological class (excellent quality, with an ASPT value equal to 5.1). In contrast, in the downstream section, the BMWP reached a 69 score, corresponding to an impacted environment (ASPT = 1.76). We also detected a marked difference in the functional structure of the two benthic communities. While the upstream community was characterized by a more balanced presence of Functional Feeding Groups (Collectors Cg = 56.1%, Filterers F =

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40.1 %, Scrapers Sc = 1.15 %, Predators P = 1.92 %, Shredders Sh = 0.71), downstream the community was dominated by taxa feeding on fine particulate organic matter (Cg = 78.7%, F =19.8 %, Sc = 0.40 %, P = 0.40 %, Sh = 0.50). In particular, for predators (represented in this stream mainly by *Rhyacophyla* sp., *Chloroperla* sp., and *Onychogomphus forcipatus* immature stages), we highlighted a significant reduction in the polluted reach (t-test among quantitative Up and Downstream samples = - 2.31, $p < 0.05$).

Discussion

This study suggests that the presence of predators could influence the frequency of prey with limb regenerating, highlighting still little-known effects of pollution on the features of aquatic insect populations. According to Nilson (1986), we hypothesized that the highest presence of nymphs with regenerating legs might be linked to higher predation pressure in the upstream station; in the downstream station, the deteriorated environmental quality led to a reduction of predator presence and then limited this phenomenon. The study of trophic interactions between species is a fundamental component of aquatic entomology. Predators are found in nearly all major aquatic insect groups (Fenoglio et al. 2009; Fukuoka 2023). They are known to play an important role in freshwater communities, for example conditioning prey abundance and their distribution within microhabitats (Bo et al. 2010), influencing community composition and trophic cascades (Start and Gilbert 2017) and, as in this case, determining the occurrence of malformations in preys. It is well known that pollution can alter the functional structure of stream insect communities (Merritt et al. 2017), also influencing prey availability and predator diet (Bo et al. 2020) but our findings suggest that pollution can indirectly also affect the frequency of malformations in a prey population. Finally, this study highlights how the ability to regenerate legs among mayflies can be more widespread than what has been known so far.

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Caption to figure

Figure 1. Three *Serratella ignita* nymphs from Malone stream (NW Italy) showing regenerating legs.

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