

# What can I eat before being eaten? Notes about the diet of *Phoxinus* spp. in a high mountain lake (Monviso group, NW Italy)

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Abbà M, Fenoglio S, Bo T, 2025. What can I eat before being eaten? Notes about the diet of *Phoxinus* spp. in a high mountain lake (Monviso group, NW Italy). *Arxius de Miscel·lània Zoològica* 23, 95-102. DOI: <https://doi.org/10.32800/amz.2025.23.0095>

## Abstract

### What can I eat before being eaten? Notes about the diet of *Phoxinus* spp. in a high mountain lake (Monviso group, NW Italy)

high mountain lakes are naturally fishless and therefore particularly vulnerable to the introduction of non-native fish. In addition to salmonids, minnows *Phoxinus* spp. have recently been introduced into these environments. While the impact of salmonid introductions on biodiversity has been extensively studied, including analyses of their diet, information on the impact and diet of introduced minnows in these environments remains scarce. This study analyzes the stomach contents of minnows in a high mountain lake, demonstrating how their diet reflects the low diversity of aquatic fauna typical of these oligotrophic environments, as well as the overexploitation of natural communities resulting from the unnatural presence of fish. This information can be useful for better understanding the complex ecological dynamics that arise when multiple predators are introduced into sensitive and unique environments such as high mountain lakes.

**Key words:** High mountain lakes, Fish introductions, Fish diet, Minnow, Predation

## Resumen

### ¿Qué puedo comer antes de ser comido? Notas sobre la dieta de *Phoxinus* spp. en un lago de alta montaña (grupo Monviso, noroeste de Italia)

Los lagos de alta montaña carecen de ictiofauna por naturaleza y, por lo tanto, son particularmente vulnerables a la introducción de peces alóctonos. Además de los salmónidos, recientemente se han introducido piscardos *Phoxinus* spp. en estos entornos. Si bien el impacto de la introducción de salmónidos en la biodiversidad se ha estudiado ampliamente, incluyendo análisis de su dieta, la información sobre el impacto y la dieta de los piscardos introducidos en estos entornos sigue siendo escasa. Este estudio analiza el contenido estomacal de piscardos en un lago de alta montaña, demostrando cómo su dieta refleja la baja diversidad de fauna acuática típica de estos entornos oligotróficos, así como la sobreexplotación de las comunidades naturales resultante de la presencia no natural de peces. Esta información puede ser útil para comprender mejor la compleja dinámica ecológica que surge cuando se introducen múltiples depredadores en entornos sensibles y únicos como los lagos de alta montaña.

**Palabras clave:** Lagos de alta montaña, Introducción de peces, Dieta de los peces, Piscardo, Depredación

## Resumen

### Què puc menjar abans que em mengin? Notes sobre la dieta de *Phoxinus* spp. en un llac d'alta muntanya (grup Monviso, nord-oest d'Itàlia)

Els llacs d'alta muntanya no tenen peixos de manera natural i, per tant, són particularment vulnerables a la introducció de peixos no autòctons. A més dels salmònids, recentment s'han introduït piscards *Phoxinus* spp. en aquests ambients. Tot i que l'impacte de les introduccions de salmònids en la biodiversitat s'ha estudiat àmpliament, incloent-hi anàlisis de la seva dieta, la informació sobre l'impacte i la dieta dels peixos petits introduïts en aquests ambients continua sent escassa. Aquest estudi analitza el contingut estomacal dels piscards en un llac d'alta muntanya, demostrant com la seva dieta reflecteix la baixa diversitat de fauna aquàtica típica d'aquests ambients oligotròfics, així com la sobreexplotació de les comunitats naturals resultant de la presència no natural de peixos. Aquesta informació pot ser útil per comprendre millor les dinàmiques ecològiques complexes que sorgeixen quan s'introdueixen múltiples depredadors en ambients sensibles i únics com els llacs d'alta muntanya.

**Paraules clau:** Llacs d'alta muntanya, Introducció de peixos, Dieta de peixos, Piscards, Depredació

Received: 12/11/2024; Conditional acceptance: 27/05/2025; Final acceptance: 19/06/2025; Published: 03/07/2025

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## Introduction

Alpine, and in general, high mountain lakes are remote ecosystems considered extreme for life due to their harsh environmental characteristics (Catalan et al 2006). These include low temperatures and highly diluted, oligotrophic waters, as well as a marked seasonal alternation between the extended winter period, characterized by snow and ice cover and limited light conditions, and the short summer period, typified by extremely high solar radiation (Sommaruga 2001, Catalan et al 2006, Füreder et al 2006). These conditions result in high mountain lakes hosting a few dominant, well-adapted organisms that support relatively simple communities, making these environments unique and distinctive (Sommaruga 2001, Füreder et al 2006). Despite their remoteness from more populated areas, these ecosystems are so sensitive and vulnerable to external and anthropogenic pressures that they are considered sentinels of environmental change (Pastorino and Prearo 2020, Pastorino et al 2024).

The isolation of high mountain lakes has resulted in a natural absence of fish, and one of the main threats to these environments is the introduction of non-native species for angling, mainly salmonids (Pastorino and Prearo 2020, Pastorino et al 2024). The impacts of introduced salmonids on faunal assemblages in high mountain lakes are well documented and relate to predatory pressure and consequent ecological effects on zooplankton, macroinvertebrates, and amphibians (Knapp et al 2001, Parker et al 2001, Perilli et al 2020, Tiberti et al 2014, Tiberti and von Hardenberg 2012, Miró and Ventura 2020). More recently, minnows (for example, *Phoxinus* spp.) have also been introduced into high mountain lakes. They have been introduced accidentally as surviving live bait for salmonids, as a result of contamination of released salmonid stocks, or intentionally to provide forage fish for trout or char (Museth et al 2007). Minnows have an omnivorous and unspecialized diet, feeding mainly on aquatic insect larvae and benthic crustaceans, and occasionally on the larvae and eggs of other fish. In lacustrine environments, they also feed on zooplankton (Zerunian 2004). The effects of minnows on biodiversity in high mountain lakes have been less studied than those of salmonids, but some authors have pointed out that they also prey on zooplankton, amphibians, and benthic macroinvertebrates (Miró et al 2018, Miró and Ventura 2020, Næstad and Brittain 2010), with negative effects on the latter even greater than those of trout (Osorio et al 2022). Moreover, despite minnows being part of trout diets (Museth et al 2003), they can cause the decline (Museth et al 2007, Tiberti et al 2022) or disappearance (Miró and Ventura 2015, 2020) of previously introduced salmonids, mainly through competition for common trophic resources (Museth et al 2007) and predation on salmonid eggs (Miró and Ventura 2020). Some authors have investigated the diet of salmonids introduced to high mountain lakes in northern and southern Europe (Cavalli et al 1998, Hesthagen et al 1992, Museth et al 2010, Tiberti et al 2016). To our knowledge, information on the diet of minnows in these environments is only available for northern Europe, particularly Norway (Hesthagen et al 1992, Museth et al 2010). The aim of this study was to analyze the diet of introduced minnows in sympatry with introduced salmonids in an Italian high mountain lake, to increase our understanding of their feeding habits in these peculiar environments.

## Materials and methods

### Study area

Lake Fiorenza (fig. 1) is a small high mountain (2,113 m a.s.l.) oligotrophic lake of glacial origin located in the Po Valley (NW Italy), within the Monviso Natural Park (44.6971°N, 7.0927°E; WGS84). It has a surface area of 2.3 ha and a maximum depth of 15 m. During summer, the average surface temperature is 12.5°C. From a depth of 6 m downward, the temperature gradually drops to 6°C. This temperature decrease is also accompanied by a slight decline in pH and oxygen levels from the surface to the bottom, along with a slight increase in conductivity (Gentili et al 2002, Conseil Supérieur de la Pêche 2006).

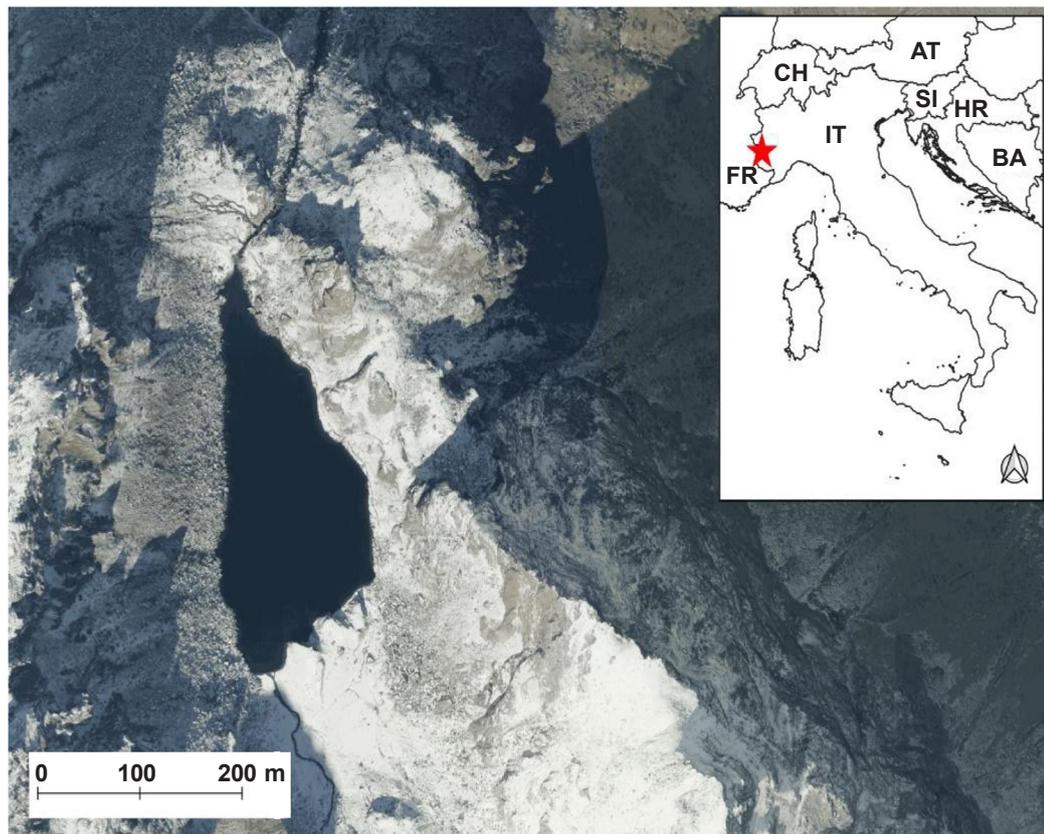
Originally, this lake was fishless and has undergone a series of fish introductions, dating back to the 1960s. Most of these introductions were carried out by individual fishermen or local fishing societies, resulting in a lack of a clear historical record.

Today, the fish community in the lake includes brown trout *Salmo trutta* Linnaeus, 1758, minnow *Phoxinus* spp., and occasional rainbow trout *Oncorhynchus mykiss* Walbaum, 1792 (Gentili et al 2002, Fenoglio 2010). Recent genetic analyses indicate that *Phoxinus lumaireul* (Schinz, 1840), native to the Central Balkans, co-occurs in Lake Fiorenza with *Phoxinus csikii* (Hankó, 1992) (De Santis et al 2021). Since minnow species show minimal morphological differences and phenotypic plasticity (Rampler et al 2016), and no genetic analysis was conducted in this study, we refer to all minnows as *Phoxinus* spp.

### Fish sampling and analysis of stomach contents

On August 19, 2019, 46 minnows were collected via electrofishing using a Scubla IG200/2 device. The fish were euthanized with an overdose of clove oil and preserved in 80% ethanol.

In the laboratory, each fish was measured to an accuracy of 0.1 mm (total length). The digestive tracts were then extracted, and their contents analyzed using a Nikon SMZ 1500 light microscope (magnification 60-100x) coupled with



**Fig. 1.** Study area and location of Lake Fiorenza (NW Italy, 44.6971°N, 7.0927°E; WGS84).

**Fig. 1.** Área de estudio y ubicación del Lago Fiorenza (noroeste de Italia, 44.6971°N, 7.0927°E; WGS84).

a Sony HD video camera and a Samsung LCD monitor. Prey identification and counting were based on sclerotized body parts, such as head capsules, mouth parts, wings, and leg fragments. According to Stewart and Stark (2002), counting sclerotized fragments (e.g., head capsules or legs) can provide a reasonably accurate estimate of prey consumed. This method has been employed in various scientific studies on the diets of both vertebrates (e.g., Bo et al 2012) and invertebrates (e.g., Fenoglio et al 2007, 2008).

Prey were categorized into groups and subgroups at different taxonomic levels. Insects were divided into orders ('groups') and, where possible, into suborders or families ('subgroups'). Other prey items were identified at the most reliable taxonomic level available. For each prey, the life stage (larva, nymph, pupa, or adult) and environment (terrestrial or aquatic) were recorded. For each group, subgroup, and variable (life stage and environment), a percentage frequency was calculated as the ratio between the number of fish containing that prey and the total number of fish examined. Since the life stage of insects is closely related to their environment, and the results for these two variables were redundant, we focused on and presented the results for the environment variable (terrestrial/aquatic).

## Results

In total, 46 minnows were analyzed. Their mean length was  $6.15 \pm 0.41$  cm (range: 5.3-7.0 cm). All stomachs contained prey. Mites (subclass Acarina), bivalves (class Bivalvia) of the genus *Pisidium*, and several orders of insects (Coleoptera, Diptera, Heteroptera, Homoptera, Hymenoptera, Plecoptera, Thysanoptera, Trichoptera) were identified. On average, there were  $34.3 \pm 22.2$  (range: 9-112) prey items per minnow (fig. 2). Additionally,  $0.85 \pm 1.09$  (range: 0-5) prey items per minnow could not be identified. No plant material or zooplankton specimens were found or identified. The table showing the stomach contents of each minnow can be found in the supplementary material (table 1s).

Diptera were the most frequent prey, present in 100% of the fish. They were followed by Coleoptera, Homoptera, Hymenoptera, and Thysanoptera. Diptera were also the most abundant prey, with a total of 1,204 individuals counted, followed by Homoptera with 107 individuals. Among Diptera, Brachycera were the most frequent prey (95.7%), followed by Chironomidae (80.4%). However, Chironomidae were the most abundant subgroup, with 843 individuals counted.

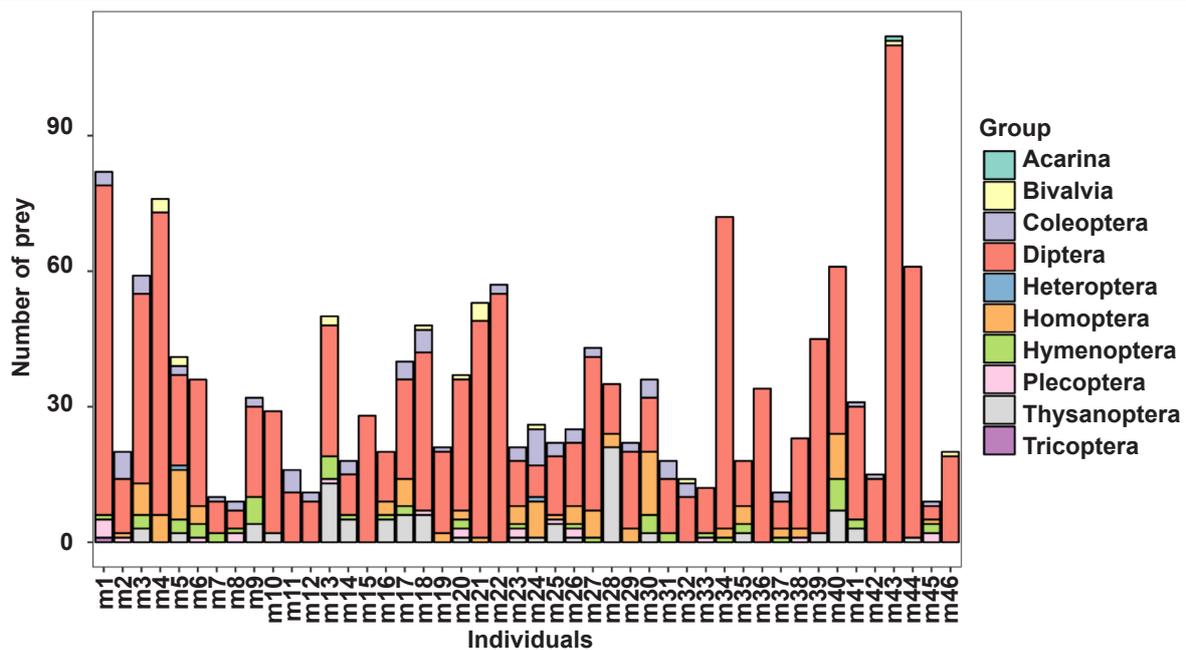


Fig. 2. Number of prey items divided by group identified in each minnow.

Fig. 2. Número de presas clasificados por grupo, identificados en cada piscardo.

All minnows had fed on prey of terrestrial origin (remains of which were present in 100 % of the stomachs examined), while 82 % of the fish had consumed aquatic prey. Of the subgroups identified, 12 are terrestrial and six are aquatic. The most frequent terrestrial prey were winged forms of Brachycera (Diptera), Nematocera (Diptera), Coleoptera, Homoptera, Hymenoptera, and Thysanoptera (fig. 3). Among aquatic prey, Chironomidae larval stages were the most common, followed by *Pisidium* bivalves (fig. 3). In terms of abundance, a total of 727 terrestrial prey and 852 aquatic prey items were counted. Aquatic Chironomidae dominated with 829 individuals, while the most abundant terrestrial prey were adults of Brachycera and Nematocera (fig. 4).

## Discussion

The introduction of non-native species always causes changes and disruptions to local biological communities, and this is particularly true for freshwater fish, which are probably the most displaced and introduced group of animals by humans (Britton 2023). Examples of introductions between catchment areas (e.g., Welsh catfish in Southern Europe, Mancini et al 2022) or even between continents (e.g., brown trout in New Zealand, Jones and Closs 2017) are well documented. However, within-catchment introductions –such as those in high mountain lakes– are perhaps less studied. In these naturally fishless environments, there is a long history of salmonid introductions for angling purposes (Miró and Ventura 2020, Tiberti et al 2014). The subsequent introduction of small cyprinids (mainly *Phoxinus* spp.) is linked, on one hand, to live bait fishing and, on the other, to the need to provide food for salmonids (Museth et al 2007). While studies on the diet of trout in high mountain lakes are available (e.g., Cavalli et al 1998, Tiberti et al 2016), research on the diet of minnows in these environments remains scarce.

One of the main findings of our work is that the analyzed minnows exploit a broader range of non-aquatic than aquatic trophic resources, particularly winged insects that fall onto the lake's surface. In fact, the diversity of terrestrial prey is twice that of aquatic prey. Moreover, terrestrial prey are consumed more frequently than aquatic prey, to the extent that some specimens had only terrestrial prey in their stomachs. Another significant finding is the low diversity of aquatic prey, with chironomids dominating the minnows' diet in terms of both frequency and abundance among the aquatic component. Several factors may explain these results. First, high mountain lakes are oligotrophic environments where the scarcity of nutrients such as phosphates and nitrates limits phytoplankton presence, thereby preventing the development of rich and abundant aquatic communities (Füreder et al 2006). This results in chironomids, which are well adapted to challenging environments, becoming the most abundant and available component of the macrobenthos in these lakes (Čiamporová-Zaťovičová et al 2010, Fjellheim et al 2009, Füreder et al 2006, Gabetti et al 2024). Consequently, minnows in Lake Fiorenza likely feed mainly on *Chironomidae* larvae because they are the most abundant and accessible part of

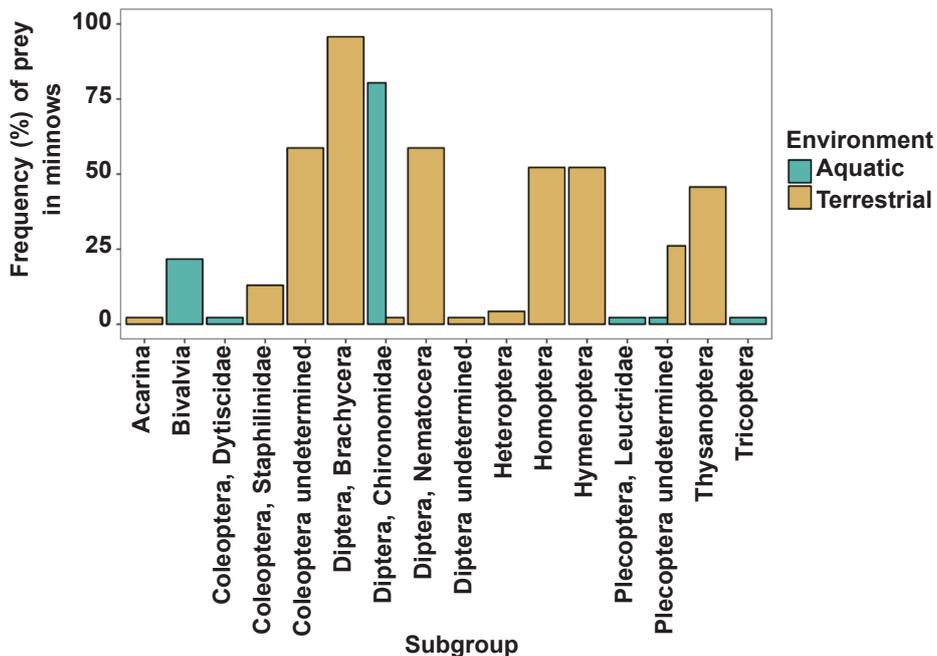


Fig. 3. Frequency (%) of prey in minnows by type of environment (aquatic/terrestrial).

Fig. 3. Frecuencia (%) de presas en piscardos según el tipo de entorno (acuático/terrestre).

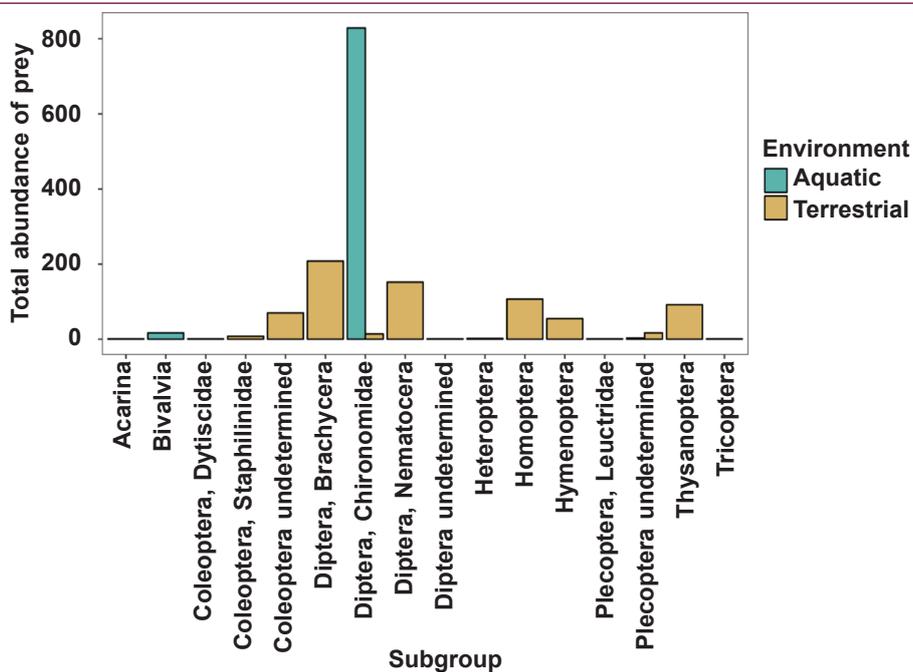


Fig. 4. Total abundance of prey by type of environment (aquatic/terrestrial).

Fig. 4. Abundancia total de presas según el tipo de entorno (acuático/terrestre).

the macrobenthic community, rather than due to a specific preference. This aligns with findings by Museth et al (2010), who observed in a Norwegian lake where *Phoxinus* spp. and *Salmo trutta* coexisted that both species showed a marked preference for feeding on chironomid larvae.

Another important factor to consider is that the long-term presence of non-native fish has led to a decline in the lake's natural biodiversity (Osorio et al 2022, Schabetsberger and Jersabek 1995), which is likely reflected in the minnows' diet. This could explain the low diversity of aquatic prey, which is directly influenced by predation pressure from aquatic predators. In contrast, terrestrial prey reach the lake surface incidentally from the surrounding environment, and their overall diversity is not directly affected by minnow predation.

Finally, it should be noted that the sample analyzed in this study was collected during a single sampling event. Therefore, while the results provide valuable insights into the diet of introduced minnows in high mountain lakes, some aspects of their trophic habits may have gone undetected. For example, the absence of zooplankton and plant material in the sample may reflect this limitation; it is possible that the minnows were collected at a time when these components were not part of their diet.

In conclusion, although the data are limited to a single high mountain lake, they contribute to the growing body of knowledge in trophic ecology, which has become a central element in freshwater sciences. There is a crucial need to expand our understanding of the foraging behavior of aquatic organisms, as this is essential for describing ecological interactions, analyzing trophic webs, investigating the impacts of invasive species, and better understanding the functional dynamics of inland water ecosystems.

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**Acknowledgements**

We would thank M. Cammarata and A. Candiotta for help during sampling activities.

**Author's contributions**

All authors contributed to the observations and study conception.

**Conflicts of interest**

No conflicts declared.

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