Introduced fish reduce the occurrence of shrews in alpine lakes

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ABSTRACT

Widespread fish introductions into originally fishless mountain lakes have had severe consequences for native biota, including aquatic macroinvertebrates, which provide important food subsidies for terrestrial and semi-aquatic insectivores like shrews (Fam. Soricidae). Since both fish and shrews rely on aquatic macroinvertebrates as food, whether in their larval or imaginal stage, we investigated if fish presence had adverse effects on shrews. Baited tubes were deployed to monitor the presence/absence of shrews by collecting their scats in lakes with and without fish in the western Italian Alps. Only two species, the Valais shrew (*Sorex antinorii*) and the Eurasian water shrew (*Neomys fodiens*), were found inhabiting the lakes' edges, where they fed on aquatic insect subsidies. The results indicate a significant pattern of exclusion between shrews and introduced fish. This negative association was especially evident in the presence of large-bodied fish (i.e., salmonids), but also of small fish (i.e., cyprinids). Consistently, compared to naturally fishless lakes, those with fish exhibit a lower availability of aquatic prey, representing a significant portion of the diet of both shrew species. Overall, our findings suggest that the impact on shrews may be mediated by a complex interplay of competition and predation between fish and shrews. Fish impacts may extend beyond the lakes to insectivorous mammals in surrounding areas. We recommend that the potential benefits to species and habitats reliant on aquatic subsidies be considered and integrated into conservation and restoration plans, and that these findings be communicated to the public to foster greater support for restoration efforts.

1. Introduction

Nutrients and organisms readily move between adjacent aquatic and terrestrial habitats and provide important ecological connections between these habitats (Nakano and Murakami, 2001; Vander Zanden and Gratton, 2011). Emergent aquatic insects represent the most important vector for lake-to-land linkages (Vander Zanden and Gratton, 2011) and are major component of both freshwater and adjacent terrestrial food-webs (Pope et al., 2009). For example, aquatic invertebrates can serve as a significant food source both for fish and other aquatic, semiaquatic and terrestrial vertebrates (e.g., newts, frogs, birds, and mammals). Semiaquatic vertebrate predators can swim or dive into the water to capture their preys, while many terrestrial predators feed on invertebrates along the shores when they emerge from the water. Evidence that terrestrial and semiaquatic vertebrates can compete with fish for aquatic invertebrates and emerging insects has been found in controlled experimental conditions (Cabrera-Guzmán et al., 2017) as well as in natural aquatic ecosystems including rivers (LeBourdais et al., 2009), wetlands (Hornung and Foote, 2006), intertidal zones (Furness et al., 1986), the open ocean (Toge et al., 2011), and lentic systems as diverse as saline mountain lakes (Hurlbert et al., 1986), ponds (Haas et al., 2007; Kloskowski et al., 2010), oligotrophic lakes (Eriksson, 1979; Epanchin et al., 2010; Hancock et al., 2023; Joseph et al., 2011; Nummi et al., 2012), and large eutrophic lakes (Winfield and Winfield, 1994).

Where fish are naturally absent, such as in high mountain lakes (Knapp et al., 2001), the only remaining vertebrates feeding on aquatic

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invertebrates should be terrestrial or semiaquatic species. These can be adapted to the harsh climatic conditions prevalent at high elevations, or they could be nomadic and migratory species that opportunistically ascend to elevated zones to exploit seasonal resources. Noteworthy among these vertebrates are amphibians, birds, and mammals (especially bats and shrews) exhibiting varying degrees of association with aquatic environments and reliance on aquatic subsidies.

However, fish have been commonly introduced into high mountain lakes, with negative direct impacts on their aquatic preys (aquatic invertebrates and amphibians; Knapp et al., 2001; Tiberti et al., 2014; Ventura et al., 2017; Osorio et al., 2022) and indirect impacts that extend throughout the entire aquatic food web (Schindler et al., 2001), ultimately influencing the adjacent terrestrial ecosystems and predators relying on aquatic prey subsidies (Matthews et al., 2002; Pope et al., 2009; Epanchin et al., 2010). For example, Matthews et al. (2002) found that alien fish lead to the disappearance of the garter snake (*Thamnopsis elegans*) from the lake shore of high mountain lakes of Sierra Nevada (CA, USA) by reducing the abundance of their shared prey (i.e., amphibians). In the same region, Finlay and Vredenburg (2007) and Joseph et al. (2011) showed that fish predation on aquatic macroinvertebrates significantly reduced prey availability for adult frogs, such as *Rana muscosa* and *Rana cascadae*. Always in the same region, a decline in abundance and fitness of nesting Grey-crowned Rosy-Finch populations (*Leucosticte tephrocotis dawsoni*; fam: Fringillidae) around mountain lakes was attributed to the same ecological mechanism, with introduced fish causing a 98 % reduction in their primary food resources, namely emerging mayflies (Epanchin et al., 2010). Nevertheless, the issue remains complex, and a comprehensive understanding is yet to be attained. Indeed, the presence of fish in mountain lakes can also increase the number of some emerging insects, particularly of tiny non-biting midges (Fam. Chironomidae; Finlay and Vredenburg, 2007; Pope et al., 2009; Tiberti et al., 2016b; Osorio et al., 2022). This phenomenon is likely associated with competitive advantages conferred upon some midge species by fish predation on their benthic invertebrate predators or competitors (Pope et al., 2009; Tiberti et al., 2016b; Osorio et al., 2022). The increase of emerging midges might alleviate competition between fish and terrestrial insectivores, potentially explaining why researchers have not been able to find negative indirect effects of alien fish on another insectivore bird (pied flycatcher, *Ficedula hypoleuca*; Milardi et al., 2019) and insectivore bats (Gruenstein et al., 2021) in boreal and mountain lakes.

Eventually, not only competition, but also direct predation could influence vertebrate species that forage on aquatic subsidies from high mountain lakes. While this relationship is well-known for amphibians, whose populations are severely affected by introduced fish predation (e. g., Miró et al., 2018), it could also be the case for other vertebrate insectivores, such as shrews. Shrews are opportunistic predators feeding on a wide variety of invertebrates (Churchfield, 1990), and, like other terrestrial insectivores, they may also be drawn to the abundance of insects emerging from aquatic habitats. Particularly, water shrews from Europe (genus *Neomys*) are closely associated with aquatic habitats, with their diet comprising at least 50 % aquatic organisms (Carter and Churchfield, 2006). Therefore, shrews and fish may compete for emerging insects and aquatic invertebrates. Yet, the small size of shrews also makes them susceptible to predation by large fish. Actually, there are several studies indicating that shrews can be part of the diet of fish; this was observed for some large, generalist fish predators, such as pikes and trout, that have the ability to ingest relatively large prey, and that can rely upon a wide range of preys, including small mammals (Teplov, 1943; Moore and Kenagy, 2004; Ohdachi and Seo, 2004; Jung et al., 2011; Lisi et al., 2014; Tiberti and Mori, 2016). Shrews may be eaten when found drowned, or directly preyed upon while walking on the shores, falling in the water, or swimming.

While working on the ecological consequences of fish introductions in high mountain lakes of the Western Italian Alps, we were aware of all these considerations. However, we initially considered our study area

unsuitable for examining competition and predation between shrews and introduced fish, because most study lakes approached or exceeded 2500 m, that is the upper elevational limit commonly reported for the few Soricidae species best adapted to survive at high elevations, such as *Neomys fodiens* Pennant 1771 and *Sorex antinorii* Bonaparte 1840 (Stone, 1995; Bouche and Lemmet, 2004). However, between 2014 and 2016, our perspective shifted upon observing that shrews, in particular *N. fodiens*, can inhabit altitudes well beyond 2500 m, and that introduced fish may play a role in determining their distribution in our study area: two *N. fodiens* were found in Lake Losere (2570 m) and Lake Trebecchi superiore (2723 m), two fishless lakes in the Gran Paradiso National Park (Tiberti R., personal observation); in addition, five more individuals were found entangled in fishing nets used to eradicate a brook trout population from Lake Dres (Gran Paradiso National Park), but only starting from the second year of the eradication action, when fish were already very scarce and the macroinvertebrate community recovering (Tiberti et al., 2019).

Based on these observations, we decided to investigate this issue further, examining the hypothesis that introduced fish in high mountain lakes might have a negative effect on shrew populations around the lakes. Based on our hypothesis we expected that shrews should be less frequent -or absent- in lakes with introduced fish than in naturally fishless lakes. This impact could arise through direct predation by fish on shrews or through competition for shared food resources, i.e., aquatic invertebrates and emerging insects, or from a combination of both mechanisms. To gain deeper insights into the trophic relationship between fish and shrews, we supplemented our study with data on prey availability and shrew dietary habits.

2. Material and methods

2.1. Study sites

The study was conducted in 41 lakes and ponds, 20 with and 21 without introduced fish, located within the Gran Paradiso National Park and Mont Avic Natural Park, two neighbouring protected areas in the Western Italian Alps (Fig. 1). All sites are typical mountain lakes and ponds, ranging from 0.06 to 20.34 ha in surface area, characterized by cold temperatures and ice cover duration for 4–9 months annually, located near or above the local timberline, with elevations spanning from 1802 to 2746 m. Although fish stocking is prohibited in all lakes, fish had been introduced before the enactment of these regulations. Introduced fish species included both salmonids (brown trout *Salmo trutta* L. 1758, brook trout *Salvelinus fontinalis* Mitchil 1751, arctic charr *Salvelinus alpinus* L. 1758) and small cyprinids (European minnows belonging to the *Phoxinus* sp. species complex) (Fig. 1a). Because of such collateral introductions, 6 lakes had both salmonids and cyprinids, 6 lakes cyprinids only, and 8 only salmonids (Table A.1; Fig. 1a).

2.2. Shrews sampling and analytical methods

The baited tube method (Churchfield et al., 2000) was used to assess shrew presence and frequency of occurrence. Several shrew species, including the Valais shrew (*Sorex antinorii*), pygmy shrew (*Sorex minutus*), alpine shrew (*Sorex alpinus*), Mediterranean water shrew (*Neomys milleri*), and Eurasian water shrew (*Neomys fodiens*), are reported in Gran Paradiso National Park and Mont Avic Natural Parks (Patriarca and Debernardi, 1997; Sindaco, 1999) and may be attracted to baited tubes. However, studies above 2000–2500 m are lacking, with historical records concentrated at lower elevations, leading to a limited understanding of shrew distribution in these protected areas. However, based on the known biology and distribution of these species, *Neomys fodiens* and *Sorex antinorii* were the most likely to be detected. This expectation was supported by 15 direct observations of shrews, both dead and alive: 14 *N. fodiens* and one *S. antinorii*, found around the lakes or in the fishing gear used for fish eradication (see Introduction).
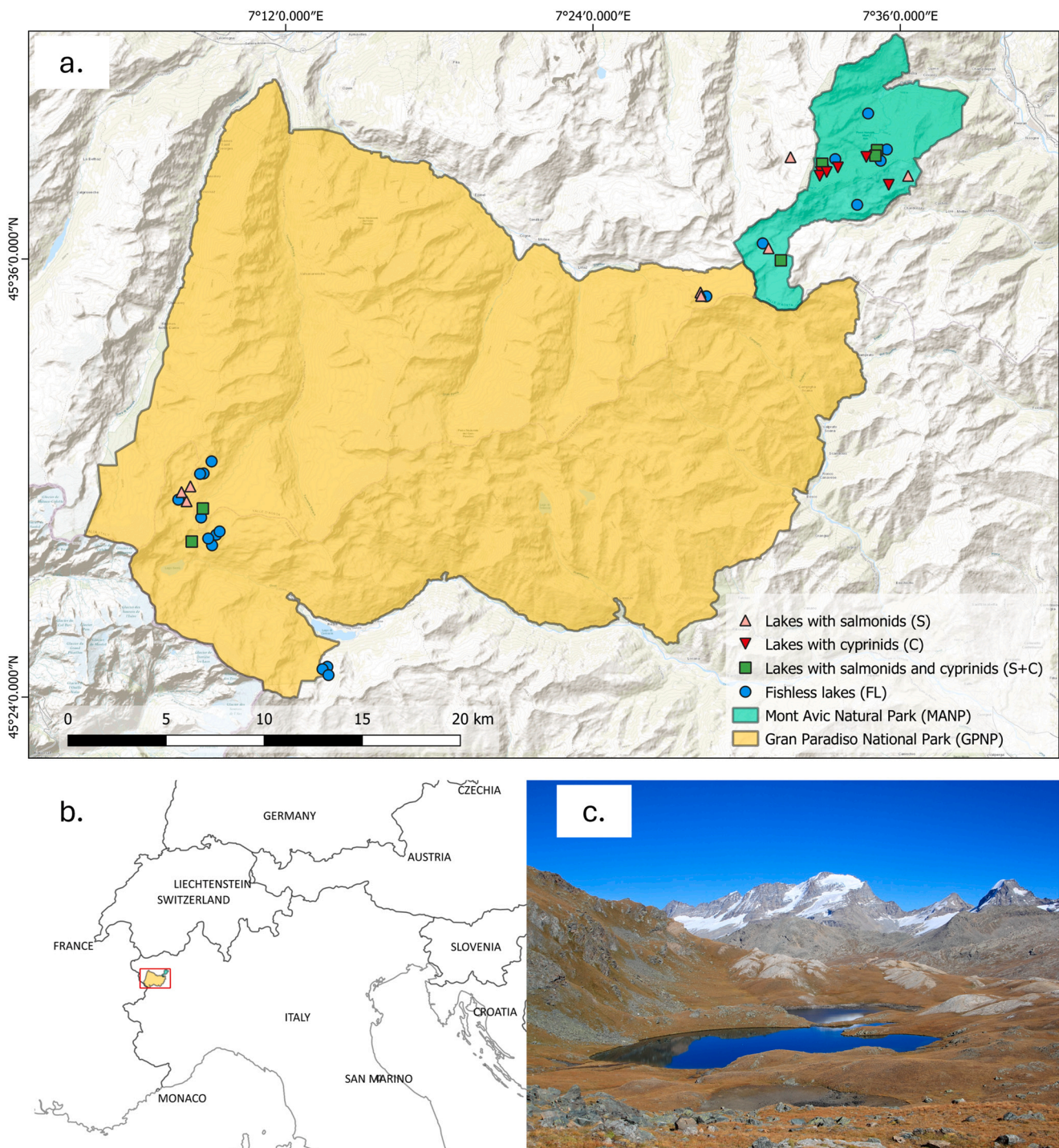
Fig. 1. Study area. Panel a: high mountain lakes sampled for the presence of shrews; Panel b: broader location of the study area (enclosed in the red frame); Panel c: a typical high mountain lake, Lake Trebecchi, Gran Paradiso National Park. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The tubes are orange plastic PVC pipes cut to lengths of 20 cm and measuring 4 cm in diameter. The tubes were baited with a small piece of sardine in oil and dry waxworm larvae and *Gammarus* sp., and covered at one end with a fine meshed net secured with a self-locking nylon cable tie. From two to six tubes were placed around the lakes, far away from paths, partially hidden among stones or in vegetation, and secured to the ground with a metal stake to protect them from possible sources of disturbance, e.g., trampling by livestock, displacement by tourists and foxes. The tubes were positioned horizontally on the ground surface, with their opening facing the water, within 30 cm from the water's edge.

Between 2021 and 2023, 243 tubes were deployed from June to September. Tubes were left in situ for 8.7 ± 4.6 days (mean ± SD). At the end of the sampling period, tubes were inspected for the presence of scats, which were sampled using sterilized tweezers and preserved in 96 % ethanol. Scat samples were examined under a binocular microscope, and assigned to Soricidae family based on the structure, size and composition of the scats following Carter and Churchfield (2006) and Churchfield and Rychlik (2006).

Because assigning species based on the appearance of scats and food remains is difficult, we analysed 13 samples, which were preserved in absolute ethanol and therefore in proper conditions to lead genetic analyses, using metabarcoding. This approach allowed us to identify which shrew species were present, characterize their diets using Molecular Operational Taxonomic Units (MOTUs), and quantify the contribution of aquatic invertebrates to their overall diet.

2.3. Shrew identification and diet using metabarcoding

The faecal DNA of the 13 samples were extracted using the QIAmp DNA Stool Mini Kit (QIAGEN), following the manufacturer's instructions and obtaining two final elution volumes of 50 μL and 100 μL. These extractions were carried out in UV-irradiated flow hood previously cleaned with 10 % bleach, sterile-water and 70 % ethanol. Extraction blanks were included so that any possible contamination could be detected at each step.

DNA extracts along with 4 extraction blanks and 2 PCR controls, were amplified using uniquely dual-tagged universal primer sets that amplify a 133 bp fragment from cytochrome *c* oxidase gene subunit 1 (Gillet et al., 2015). PCR amplifications were performed in duplicate, using a simple single-step PCR amplification protocol. Amplification reaction consisted of a 20 μL final volume containing 10 μL of AmpliTaq Gold 360 Master Mix (Applied Biosystems™), with 2 μL of each 5 μM forward and reverse 8-base tagged primers, 0.16 μL of Bovine Serum Albumin, and 2 μL of DNA extract per sample. PCR conditions consisted of 95 °C for 15 min followed by 40 cycles of 94 °C for 30 s, 45 °C for 45 s and 72 °C for 30 s, and a final extension at 72 °C for 10 min (Gillet et al., 2015). The results of these amplifications were checked on a 1.2 % agarose gel. After amplification, all labelled amplicons were pooled and purified using the MinElute PCR purification kit (QIAGEN). Their concentration was measured with the Qubit High-Sensitivity kit. Library preparation was performed using the TruSeq DNA PCR-Free kit (Illumina), and the exact library concentration was measured via qPCR using the NEBNext Library Quant kit (New England BioLabs). The libraries were sequenced as 150 Paired-end using an Illumina NOVASEC X Plus instrument by NOVAGEN Inc.

To analyse metabarcoding data, we followed the MJOLNIR pipeline (Metabarcoding Joining OBITools and Linkage Networks In R, downloaded in March 2024: https://github.com/uit-metabarcoding/MJOLNIR/tree/main) on R v4.2.0 (R Core Team, 2022). We conducted the alignment of paired-end Illumina sequences, quality filtering (retaining only sequences with an alignment quality score exceeding 40), demultiplexing, and length filtering (retaining those within the range of 123 to 143 bp) using OBITools v1.2.13 (Boyer et al., 2016). Subsequently, chimeras were removed using VSEARCH v2.23.0 (Rognes et al., 2016). We clustered the sequences into MOTUs using Swarm v3.1.4 (Mahé et al., 2014), which is based on an iterative aggregation of sequences that differ less than a given distance (d = 13). Taxonomic assignment was carried out against the BOLD database (March 2024) (Ratnasingham and Hebert, 2007) using BOLDigger v2.2.1 (Buchner and Leese, 2020). In detail, assignments to different taxonomic levels were conducted after the following similarity thresholds: 98 % species, 95 % genus, 90 % family, 85 % order, <85 % class. The taxonomic assignment of MOTUs was enhanced by comparing sequences with a reference database of invertebrate barcodes, primarily encompassing crustaceans and insects (unpublished work). Then, LULU algorithm was applied to remove erroneous MOTUs, since these are likely a result of errors during sequencing and PCR (Frøslev et al., 2017). In subsequent steps, we opted to exclude MOTUs that were not confidently assigned to Metazoa taxa. Additionally, to eliminate any remaining pseudogenes and Nuclear Mitochondrial sequences (NUMTs), we filtered out MOTUs containing stop codons and those yielding amino acid sequences of the COI-5P barcode that were deemed unlikely to originate from animal sources using coil v1.2.4 (Nugent et al., 2020). Finally, we excluded from the MOTU list all taxa smaller than the minimum prey size for Soricidae,

which is 3 mm (Churchfield, 1990). This included various planktonic species (e.g., rotifers and small crustaceans), which were probably consumed by the prey organisms of the shrews themselves.

To identify the two shrew species, we supplemented the above-mentioned protocol by constructing a phylogenetic tree. This tree included the two MOTUs from our samples and sequences from GenBank for the two relevant genera. For genus *Sorex*, we included only the closest species, while for genus *Neomys*, we incorporated the two species available with COI gene fragments (Fig. A.1). The tree was constructed using the neighbour-joining method and the Kimura-2-parameter evolutionary model in MEGA (Kumar et al., 2016).

To evaluate the trophic niche overlap between the two shrew species, we first calculated Levin's standardized index (Krebs, 1999), which ranges from 0 (indicating a specialist predator) to 1 (indicating a generalist predator). Next, we calculated Pianka's index (Pianka, 1973), which also ranges from 0 (indicating no shared resources between the two species) to 1 (indicating complete resource overlap). Both indices were estimated using the R package FSAmisc (Ogle et al., 2023).

2.4. Prey availability

Littoral macroinvertebrate data from a representative subset of lakes (29 out of 41 study sites) are provided to illustrate the variation in prey availability in lakes with or without fish or shrews. Littoral aquatic habitats were sampled for semiquantitative macroinvertebrates estimates following Knapp et al. (2001). Macroinvertebrate samples were collected focusing on three littoral habitat types categorized by clast diameter: sand shores (< 2 mm), gravel shores (2 mm ≤ clast <64 mm), and stony shores (clast ≥64 mm). Within each habitat type, ten standard sweeps (each about 1 m long) were performed with a standard d-frame net (mouth, 25 × 20 cm; mesh 0.5 mm). Benthic macroinvertebrates were separated from detritus, preserved in 70 % ethanol, and identified to the family or genus level following Campaioli et al. (1994). Sometimes sampling dates did not consistently align with the baited tube surveys, resulting in some samples being collected in preceding or subsequent periods. However, macroinvertebrates comprise many pluriannual species and are a relatively stable compartment in high-altitude lake ecosystems. Therefore, we are confident that these estimates, though asynchronous with the baited tube surveys, provide a reliable representation of prey availability. In some lakes, multiple samplings were carried out throughout the study period and macroinvertebrate estimates were therefore averaged across the various samples.

Each taxon was categorized as either non-burrowing (including epibenthic and nektonic taxa) or burrowing (living in the substrate), and as emerging (insects with terrestrial imaginal stage) or non-emerging (invertebrates spending all their life in water). Among emerging insects, we further distinguished conspicuous taxa (>10 mm) as a separate group. These categories serve to group the prey based on their accessibility and appeal to fish and shrews: non-burrower prey are more readily accessible to introduced fish, and likely also to water shrews, than those hidden within sediment (Knapp et al., 2001; Tiberti et al., 2014); emerging insects represent the only aquatic prey that are accessible for terrestrial insectivores (Milardi et al., 2019), and conspicuous emerging insects likely represent a more appealing prey for terrestrial vertebrate insectivores.

We used the non-parametric Mann-Whitney *U* test to compare the abundances of different prey groups (including burrower, non-burrower, emerging, and conspicuous emerging aquatic invertebrates) between lakes with and without fish or shrews. We used a Kruskal-Wallis test and Dunn post-hoc test for multiple comparisons to compare the abundances of the same prey groups when we considered the different fish assemblages: fishless, with salmonids, with cyprinids and with both salmonids and cyprinids.

2.5. Data analyses

To compare the distribution of shrews in lakes with or without introduced fish, we used two generalized linear mixed models (GLMM) with a Bayesian Markov Chain Monte Carlo (MCMC) approach implemented in the R package MCMCglmm (Hadfield, 2010). In Model-1, we examined the association between fish presence (regardless of fish species) and shrews, while Model-2 distinguished between lakes with salmonids, cyprinids, or both fish families. This approach allowed us to model the association between shrews (i.e., presence-absence of scats in the baited tubes) and different "fish treatments", while accounting for repeated sampling in the lakes. Lake identity (code) was fitted as random term to account for repeated measurements and avoid pseudoreplication. The presence-absence of scats of shrews was added as dependent variable and modelled with a binomial distribution (family = "Categorical").

Several independent variables were incorporated to assess the study hypothesis, while considering the temporal and spatial variability of the response variable:

- fish: a two-level factor for Model-1 indicating the presence/absence of fish, or a four-level factor for Model-2, distinguishing among fishless lakes (FL), and lakes with salmonids only (S), cyprinids only (C), or both salmonids and cyprinids (S + C);
- elevation: the elevation in m a.s.l. of each sampled lake;
- area: the surface area in ha of each sampled lake;
- days and days^2: an integer variable indicating the sampling effort per traps in terms of number of days of activity, and its quadratic effect, to assess if there is an optimal duration of the sampling using the baited tubes;
- season: an integer variable indicating the month of the summer in which the trap was recollected (from 6 to 9, i.e., June–September).

MCMC models were run for 120,000 iterations with a burn-in period of 20,000. The level of non-independence between successive samples in the chain was checked for both models with the "autocorr" function in the MCMCglmm package, while the convergence of the chains was checked by visual inspection. We considered a fixed effect to be significant if its Credibility Intervals (CI) did not include 0. We report MCMC significance *p* values (pMCMC) which correspond to twice the proportion of iterations in which the posterior distribution is positive or in which it is negative (whichever of the two is the smallest) (Baayen et al., 2008).

The model results are illustrated by plotting the predicted probability distributions of finding a shrew scat in the baited tubes. Predicted probabilities were derived from the parameters of Model-1 and Model-2, utilizing a simulated dataset comprising 1000 lakes encompassing different fish treatments (250 fishless lakes, 250 lakes with salmonids, 250 with cyprinids, and 250 with both salmonids and cyprinids), elevations (random values between 2000 and 2700 m), areas (random values between 1 and 10 ha), sampling periods (random periods from June to September, i.e., between 6 and 9), sampling efforts (random duration of the sampling from 4 to 10 days). All the statistical analyses were performed using R v4.2.0 (R Core Team, 2022).

3. Results

Out of 243 baited tubes deployed, 204 were successfully recovered, while 2 were lost and 37 were damaged or displaced by foxes, livestock, or people. Among the recovered tubes, 25 contained scats, indicating an overall sampling efficiency around 10 %. Shrews were reported from 17 lakes, including the lake situated at the highest elevation (Lake Leynir, 2746 m; Table A.1). Out of a total of 13 scats which underwent barcoding, 6 were unambiguously assigned to *N. fodiens* and the other 7 to *Sorex antinorii*.

Most scats were collected in fishless lakes and only 6 were collected

in lakes with fish (Table A.1; Fig. 2). Model-1 results indicate that there is a significant negative association between the presence of fish and that of shrews, and Model-2 specify that this negative association is more pronounced in lakes with salmonids (S) and cyprinids only (C), while no significant differences were detected between fishless lakes and those with both fish families (S + C) (Table 1; Fig. 3). Both models show a significant positive association with the sampling season, indicating that most samples were collected late in the summer, and a marginally significant association with the quadratic effect of the sampling effort, indicating that there is a likely optimal effort, i.e., number of trapping days (Table 1). Elevation is not significantly associated with the presence of shrews, suggesting that shrews are quite evenly distributed across the entire elevational range, so that the elevational gradient has little influence on the results. Predicted probabilities of detecting shrews align with the low observed efficiency of the sampling method and are consistently low across all lake categories, albeit higher in lakes without fish (see Fig. 3).

Metabarcoding analyses produced a total of 2,792,238 reads (mean ± SD: 214788 ± 399,443 per sample) which were assigned to a total of 212 prey items or MOTUs (133 for *N. fodiens* and 158 for *S. antinorii*; mean ± SD: 43 ± 11 per sample). Regarding the hosts, they were also detected in the scats, yielding a total of twice as many reads as the prey items. No significant differences were found between *N. fodiens* and *S. antinorii* in terms of the number of prey reads (U = 14, *p* = 0.37) or MOTUs (U = 19.5, *p* = 0.89) per sample. Despite the small sample size, metabarcoding analyses show that the diet of *N. fodiens* is dominated by aquatic invertebrates (81.1 % of all sequences) and that of *S. antinorii* by terrestrial invertebrates (71.0 % of all sequences), despite including 26.7 % of aquatic organisms, potentially preyed in their terrestrial imaginal stage (Table 2). A detailed description of the diet of the two species is provided in Table 2.

Levin's niche breadth index yielded values of 0.11 for *N. fodiens* and 0.02 for *S. antinorii*, indicating highly specialized diets, with *S. antinorii* showing greater specialization. Pianka's index was 0.05, confirming little resource overlap between the two species.

Macroinvertebrate communities (Table A.3 and A.4) in all lake categories, with and without fish, are dominated by comparable quantities of burrowing taxa, especially the burrowing larvae of Chironomidae (Diptera; accounting for the 66 % of all sampled organisms; Fig. 4d and e), i.e., non-significant differences of abundance (U = 91, *p* = 0.74). Non-burrowing prey, accessible for *N. fodiens*, are approximately five times more abundant in fishless lakes (mean ± SD: 258.5 ± 68.0) compared to lakes with fish (58.2 ± 25.4; U = 176, *p* < 0.001; Table A.3; Fig. 4a). This discrepancy is even more pronounced in lakes hosting either only salmonids (Z = −2.76, *p* < 0.05) or only cyprinids (Z = −2.87; p < 0.05), whereas the abundance of preys accessible for *N. fodiens* in lakes with both fish families is not significantly different from that reported in fishless lakes (Z = −2.20, *p* = 0.11; Fig. 4b). In lakes with introduced fish, several non-burrowing nektonic and epibenthic taxa are either completely absent or extremely scarce. Moreover, conspicuous non-burrowing taxa are very scarce in lakes with fish, where this category is dominated by tiny organisms such as small sized flatworms (Tricladida) and Hydracarina. The presence of shrews, instead, seems to reflect a higher abundance of non-burrowing macroinvertebrates typical of pristine fishless lakes (Fig. 4c), even if the difference between lakes with and without shrews is only marginally significant (U = 63, *p* = 0.07).

Considering emerging aquatic insects, which are available for *S. antinorii*, lakes with and without fish (U = 82, *p* = 0.46), lakes with different fish assemblages (χ^2[3] = 4.59, *p* = 0.20), and lakes with and without shrews (U = 73, *p* = 0.17) show similar abundances in total emerging insects; Fig. 5a, b and c. However, focusing the analysis to conspicuous emerging insects such as Ephemeroptera, Odonata, Plecoptera and Trichoptera (i.e., excluding small Diptera Chironomidae), they accounted for the 52 % of the sampled macroinvertebrates in fishless lakes and only the 0.2 % in lakes with fish. Their abundance was
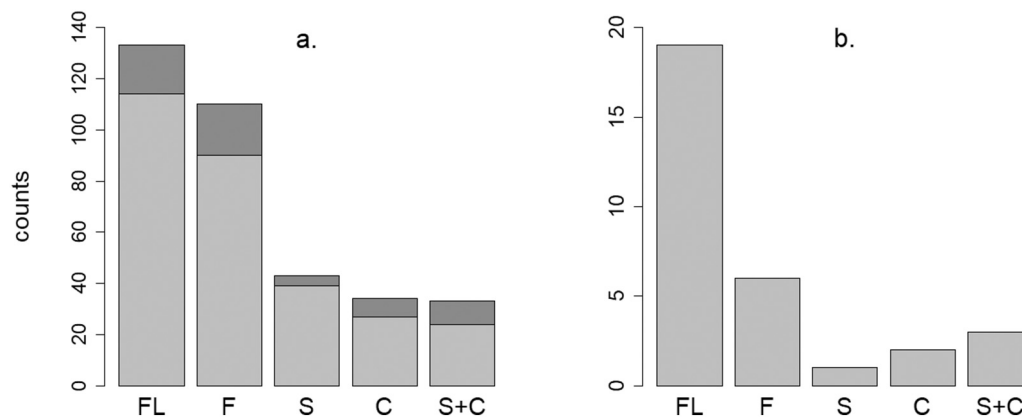
Fig. 2. Summary of baited tube monitoring for shrews in 21 naturally fishless (FL) lakes and 20 lakes with introduced fish (F), among which 8 with salmonids only (S), 6 with cyprinids only and 6 with both salmonids and cyprinids. Panel a: number of baited tubes deployed (dark grey bars) and successfully recovered (grey bars); Panel b: number of faecal samples collected in each lake category.

Table 1
Summary of the main parameters for the two generated models.

Covariates	Post mean	SD	Lower − 95 % CI	Upper - 95 % CI	pMCMC
Model-1					
(Intercept)	−31,420	15,070	−62,680	−7495	**<0.001**
Fish (No vs. Yes)	−471.0	253.0	−997.6	−30.95	**<0.05**
Elevation (m)	0.149	0.439	−0.6514	1.093	0.73
Area (ha)	−39.84	51.01	−142.5	48.61	0.41
Effort - Days	258.9	198.1	−93.10	668.6	0.15
Effort - Days^2	−15.64	10.29	−35.5	2.434	<0.10
Sampling season	3312	1679	541.3	6749	**<0.001**
Model-2					
(Intercept)	−87,680	35,420	−151,000	−26,980	**<0.001**
Fish assembly					
FL vs. S	−6892	5191	−17,700	−72.01	**<0.01**
FL vs. C	−680.0	47.0	−1531	127.5	**<0.05**
FL vs. S + C	−107.7	394.5	−853.7	678.0	0.76
Elevation (m)	0.423	0.655	−0.8066	1.610	0.44
Area (ha)	−62.31	85.57	−193.9	55.20	0.32
Effort - Days	382.5	311.4	−105.7	977.3	<0.10
Effort - Days^2	−21.94	16.24	−52.37	2.873	<0.10
Sampling season	9425	3977	2934	16,710	**<0.001**

significantly higher in lakes without fish (U = 172.5, $p < 0.001$; Fig. 5d), significantly different according to the fish assemblages ($\chi^2[3] = 12.8$, $p < 0.01$; Fig. 5e), and almost significantly higher in lakes with shrews (U = 148, $p = 0.05$; Fig. 5f).

4. Discussion

The efficiency of baited tubes was found to be low (approximately 10 %), yet consistent with findings in the literature (Churchfield et al., 2000). Our results suggest that optimizing the sampling duration could have improved the low efficiency of the baited tubes, as indicated by the marginally significant quadratic effect of day (see Table 2). However, this still resulted in a relatively low number of samples, which adds uncertainty to the interpretation of some results, despite the considerable sampling effort. We consider that this is intrinsically linked to the nature of the study, including the need for non-invasive techniques and the challenges of studying an elusive group of mammals like shrews, at the edge of their elevational distribution. However, monitoring is still ongoing, and the collection of new data and samples will hopefully address some of the remaining uncertainties.

Despite such limitation, baited tubes offer significant advantages such as low cost and the possibility to deploy multiple tubes at various sites. Altogether, these advantages enabled us to achieve a satisfactory understanding of the distribution of shrews in our study area, including several remote sites. Importantly, the absence of mortality risk was crucial for ethical and conservation considerations, especially when working with native species in protected areas. However, using signs of presence (scats), as opposed to captured animals, hindered our ability to assign the samples to species level. Consequently, we found it necessary to complement our observations with additional genetic identifications for validation and support.

According to our study hypothesis, our findings indicate that fish are negatively associated to the probability of detecting shrews. This finding aligns with a few existing studies investigating the interactions between alien fish and native insectivores in mountain lakes (Finlay and Vredenburg, 2007; Pope et al., 2009; Epanchin et al., 2010; Joseph et al., 2011). However, to the best of our knowledge this is the first study in European mountains, and the first describing a negative association between alien fish and insectivore mammals. This association was particularly pronounced in lakes exclusively inhabited by large-bodied salmonids, and, in any case, where salmonids and cyprinids do not coexist. Such negative associations may arise due to a local decline of shrews caused by the lethal effects of predation by large salmonids (as speculated by Tiberti and Mori, 2016 for water shrews), or by their avoidance of invaded sites due to the perceived risk of predation or scarcity of macroinvertebrate preys (Fig. 4). However, a clear understanding of the causal mechanisms behind this negative association remains elusive, and the relative contributions of predation and competition in driving the observed exclusion patterns are still poorly understood: both mechanisms could be involved in lakes with
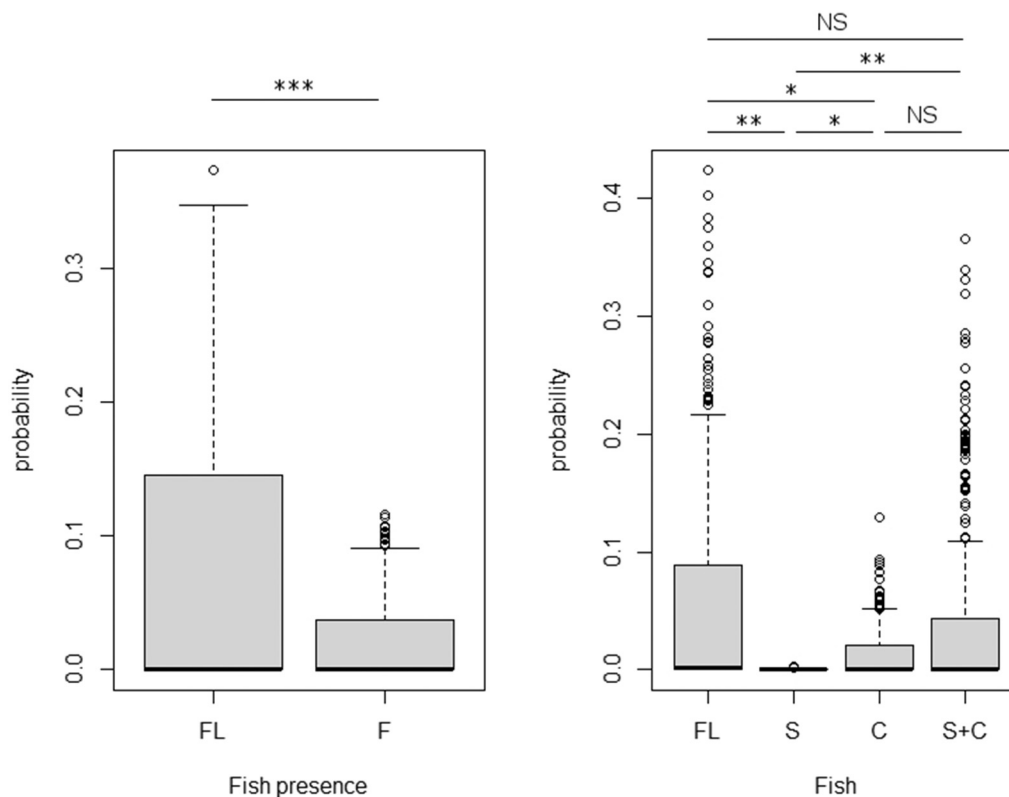
Fig. 3. Predicted probability distributions of finding a scat of shrew in the baited tubes used to monitor shrew populations in alpine mountain lakes; predicted probabilities were calculated based on Model-1 (panel on the left) and Model-2 (panel on the right) parameters (see Table 1) and based on a simulated dataset; pMCMC levels are reported. NS: pMCMC not significant; *: pMCMC<0.05; **: pMCMC<0.01; ***: pMCMC<0.001.

salmonids, while competition for food resources is the likely leading factor in lakes with cyprinids only.

Although shrews were never found in the diet of the fish from the study sites, their absence could be attributed to their scarcity, which may, in turn, be a consequence of the presence of fish. This is indeed a common finding for many other prey groups, such as amphibians and conspicuous aquatic invertebrates, when they experience significant declines or local extirpation because of fish predation (Tiberti et al., 2016a). Predation may contribute significantly to the exclusion of *N. fodiens* owing to its aquatic habits, which subject it to a higher risk of predation compared to terrestrial shrews. On the other hand, *S. antinorii* might also be preyed by introduced fish. Actually -and quite surprisingly- evidence of fish predation on shrews primarily involves small terrestrial Soricidae rather than semiaquatic water shrews of the genus *Neomys* (Teplov, 1943; Moore and Kenagy, 2004; Ohdachi and Seo, 2004; Jung et al., 2011; Lisi et al., 2014). While the latter might be too large to be consumed or capable of escaping from fish, terrestrial shrews found along the shores or accidentally falling into the water could be more susceptible to predation due to their smaller size and limited swimming abilities.

Considering competition, it likely plays a role in shaping the negative association between fish and shrews. This becomes evident when considering that comparable exclusion patterns are observed in lakes hosting large predatory salmonids or small cyprinids, which are incapable of preying on shrews due to their diminutive size. A case of competitive exclusion was observed by Browett et al. (2023) in their study focused on the dietary overlap between an introduced (greater white toothed shrew *Crocidura russula*) and a native shrew (pygmy shrew *Sorex minutus*). In our case of study, competition is likely associated to the dietary composition of the investigated shrew species and the observed prey depletion, which is likely associated to introduced fish, i. e., reduction of accessible non-burrowing taxa in the aquatic habitat and

of conspicuous emerging insects in the terrestrial habitat (Figs. 4 and 5). Indeed, aquatic invertebrates dominate the diet of *N. fodiens* and emerging insects are an important component of the diet of *S. antinorii*. Despite the small sample size and high individual variation in shrew diets (Browett et al., 2023), our dietary data, though incomplete, still offers a useful distinction between the two shrew species and provides valuable insights for interpreting our results. The strong dependence of *N. fodiens* on non-burrowing aquatic invertebrates such as Coleoptera, Ephemeroptera, Plecoptera, and Trichoptera (Table 2) supports the hypothesis of competitive exclusion by introduced fish. This may occur because fish often eradicate these prey from the environment, reducing their availability for native predators like shrews, as well as their contribution to the fish's own diet, where they are indeed almost absent (Tiberti et al., 2016a). At the same time, the depletion of conspicuous emerging insects, may also impact *S. antinorii*, but probably to a lesser degree, because *S. antinorii* can rely on many other terrestrial preys (Table 2). However, it is likely that the influence of fish extends beyond shrews and aquatic macroinvertebrates to encompass and impoverish the entire insectivore community living along the shoreline (Baxter et al., 2004; Knight et al., 2005; Epanchin et al., 2010). For example, the alterations of insect subsidies induced by fish (e.g., reduced emergence of conspicuous insects) can lead to a reduction of terrestrial invertebrate predators such as spiders (Baxter et al., 2004), which constitute a significant portion of the diet of *S. antinorii* (Table 2). In this regard, the emergence of conspicuous insects transfers a substantial quantity of energy from aquatic to terrestrial environments due to their size and consequent higher energy yield per prey (Bartels et al., 2012; Schindler and Smits, 2017).

Although counterintuitive, the same negative association between fish and shrews was not as evident as in lakes where both salmonids and cyprinids coexist, i.e., where the effects of predation and competition should sum up. This could be explained by the antagonistic interaction

Table 2

Diet composition retrieved from metabarcoding analyses for the two shrew species, *Neomys fodiens* and *Sorex antinorii*. Prey are clustered by class/order and divided into aquatic (including all aquatic taxa and emerging insects with aquatic larvae) or terrestrial; prey with unresolved taxonomy are grouped apart. A %: percent abundance of the prey taxa averaged over six samples from *N. fodiens* or 7 samples of *S. antinorii*; F %: percent frequency of occurrence of each prey group. Extended results with the maximum achieved taxonomic resolution is provided in Table A.2.

| Prey group	Phylum	Class	Order	*N. fodiens* (N = 6)		*S. antinorii* (N = 7)	
				A %	F %	A %	F %
				81.1	**100.0**	**26.7**	**100.0**
	Annelida	Clitellata	Crassiclitellata	<0.1	33.3	<0.1	57.1
	Annelida	Clitellata	Enchytraeida	–	–	1.1	14.3
	Annelida	Clitellata	Lumbriculida	13.5	33.3	1.2	14.3
	Arthropoda	Arachnida	Trombidiformes	–	–	<0.1	14.3
	Arthropoda	Collembola	Entomobryomorpha	–	–	<0.1	14.3
	Arthropoda	Insecta	Coleoptera	3.9	100.0	1.3	85.7
Aquatic	Arthropoda	Insecta	Diptera	18.7	100.0	4.8	100.0
	Arthropoda	Insecta	Ephemeroptera	11.8	100.0	2.1	100.0
	Arthropoda	Insecta	Hemiptera	<0.1	33.3	6.1	71.4
	Arthropoda	Insecta	Neuroptera	<0.1	16.7	–	–
	Arthropoda	Insecta	Odonata	<0.1	16.7	0.6	14.3
	Arthropoda	Insecta	Plecoptera	13.3	100.0	5.4	100.0
	Arthropoda	Insecta	Trichoptera	19.8	100.0	4.2	100.0
	Chordata	Amphibia	Anura	<0.1	16.7	<0.1	14.3
	Mollusca	Gastropoda		<0.1	16.7	–	–
				18.8	**100.0**	**71.0**	**100.0**
	Annelida	Clitellata	Crassiclitellata	<0.1	66.7	14.4	42.9
	Arthropoda	Arachnida	Araneae	7.2	50.0	8.7	71.4
	Arthropoda	Arachnida	Opiliones	0.3	50.0	<0.1	42.9
	Arthropoda	Arachnida	Sarcoptiformes	<0.1	16.7	<0.1	57.1
	Arthropoda	Chilopoda	Geophilomorpha	<0.1	16.7	<0.1	14.3
	Arthropoda	Chilopoda	Lithobiomorpha	<0.1	16.7	<0.1	14.3
	Arthropoda	Collembola	Entomobryomorpha	<0.1	33.3	<0.1	14.3
	Arthropoda	Collembola	Poduromorpha	–	–	<0.1	14.3
	Arthropoda	Diplopoda	Chordeumatida	0.7	33.3	12.3	42.9
	Arthropoda	Diplopoda	Glomerida	<0.1	16.7	13.0	14.3
Terrestrial	Arthropoda	Diplopoda	Julida	0.1	50.0	10.7	85.7
	Arthropoda	Diplopoda	Polydesmida	0.1	33.3	–	–
	Arthropoda	Insecta	Coleoptera	<0.1	50.0	2.0	57.1
	Arthropoda	Insecta	Diptera	10.1	100.0	1.7	100.0
	Arthropoda	Insecta	Hemiptera	<0.1	33.3	0.7	28.6
	Arthropoda	Insecta	Hymenoptera	<0.1	50.0	1.6	100.0
	Arthropoda	Insecta	Lepidoptera	<0.1	66.7	0.4	42.9
	Arthropoda	Insecta	Orthoptera	–	–	<0.1	14.3
	Arthropoda	Insecta	Psocodea	–	–	<0.1	14.3
	Arthropoda	Malacostraca	Isopoda	0.1	16.7	<0.1	14.3
	Mollusca	Gastropoda	Stylommatophora	<0.1	50.0	5.4	42.9
				0.16	**50.0**	**2.25**	**85.7**
	Annelida	Clitellata	Enchytraeida	<0.1	16.7	–	–
	Annelida	Clitellata		–	–	<0.1	14.3
	Arthropoda	Arachnida	Sarcoptiformes	0.1	16.7	2.1	14.3
Unresolved	Arthropoda	Arachnida	Trombidiformes	<0.1	16.7	–	–
	Arthropoda	Arachnida		<0.1	16.7	–	–
	Arthropoda	Insecta	Diptera	–	–	<0.1	28.6
	Arthropoda	Insecta		<0.1	33.3	–	–
	Arthropoda	Malacostraca		–	–	<0.1	57.1

between salmonids and cyprinids, which typically results in an overall decrease in the density of both fish families (Tiberti et al., 2022), but we cannot rule out the possibility that this is due to the small sample size when considering the different fish assemblages separately. Similar ecological patterns were however observed for other taxa such as zooplankton, where the presence of both salmonids and cyprinids resulted in less severe ecological effects (Schabetsberger et al., 2006).

The results also indicate that the probability of finding shrews increased as summer progresses. The seasonal trends in shrew detection probability could be attributed to the strong seasonal fluctuations of the resident populations, with maximum population size/density in late summer (Churchfield, 1990). Alternatively, in a somewhat speculative perspective, the same seasonal pattern could be due to the seasonal variations in habitat suitability and use, associated to dispersal or nomadic movements (Stone, 1995). The scarcity of food during winter and the limited access to aquatic prey due to the thick ice covering lakes and watercourses -despite shrews being potentially able to forage in the subnivean space (Churchfield, 1990)- could restrict the use of higher

elevations to summer months. This would align shrews with the rest of the insectivores inhabiting high-altitude lakes, which are present (or active, in the case of hibernating amphibians) only in summer.

In conclusion, the present study demonstrates that fish introductions in mountain lakes are negatively associated with the presence of native shrews. Direct predation and competition for aquatic preys and emerging insects are the factors likely contributing to this exclusion pattern. Though understanding the precise underling ecological mechanisms remain complex, we depict all potential trophic connections among shrews, introduced fish, and their prey in a conceptual model, which could serve as a basis to plan further development of the present study (Fig. 6). Clearer insights could come from a manipulative approach, involving the removal of fish, the recovery of invertebrate prey, followed by a putative recovery native insectivore, such as the shrews. Currently, a study involving approximately twenty lakes treated for fish eradication is underway as part of the Life RESQUE ALPYR project (www.liferesquealpyr.eu), and we are confident that this project will help to shed light on these patterns in the coming years.
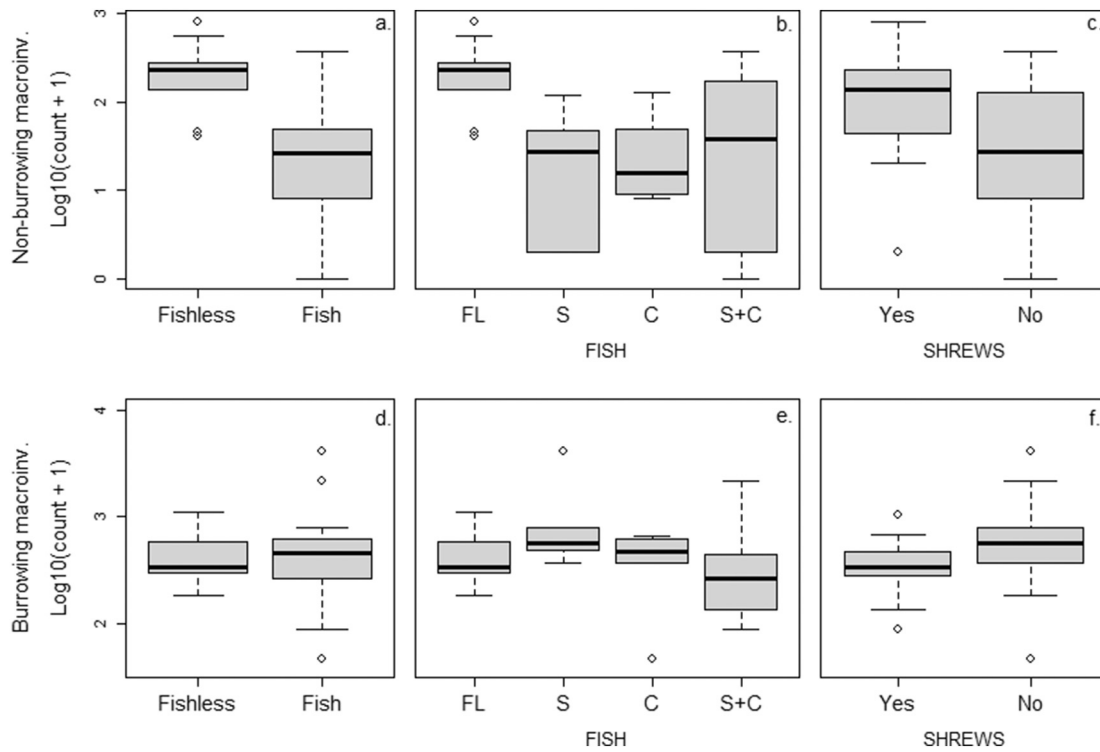
Fig. 4. Boxplots showing the distribution of log10 + 1 counts of non-burrowing (Panels a-c) and burrowing (Panels d-f) littoral macroinvertebrate in 29 high mountain lakes with and without introduced fish or shrews. FL: Fishless (*N* = 11); S: lake with salmonids only (*N* = 6); C: lakes with cyprinids only (N = 6); S + C: lakes with both salmonids and cyprinids (*N* = 6).
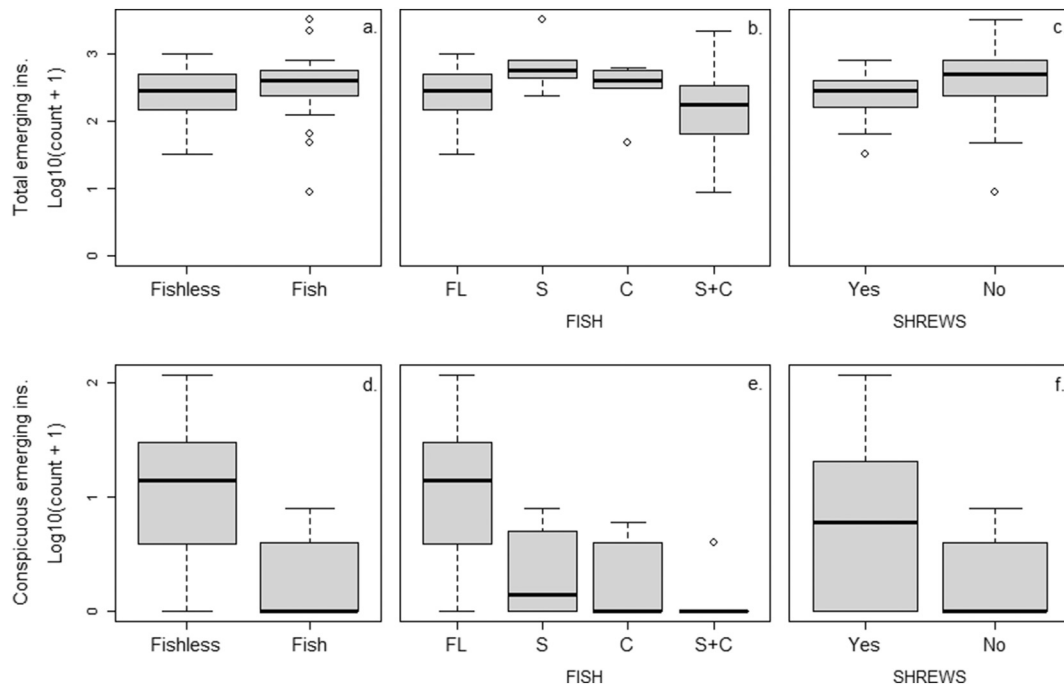
Fig. 5. Boxplots showing the distribution of log10 + 1 counts of total emerging (Panels a-c) and conspicuous emerging (Panels d-f) insects in 29 high mountain lakes with and without introduced fish or shrews. FL: Fishless (N = 11); S: lake with salmonids only (N = 6); C: lakes with cyprinids only (N = 6); S + C: lakes with both salmonids and cyprinids (N = 6).

The present study does not clarify whether the observed issue is a widespread conservation problem for the shrews, partially because shrews, including *S. antinorii* and *N. fodiens,* are broadly distributed at lower elevations (Churchfield, 1990) and high mountains likely represent marginal habitats for them. Nevertheless, the effect of alien fish on small mammals such as Soricidae could have passed unnoticed at lower elevations, and our finding could be part of a more general conservation problem. We therefore suggest that the effects of alien fish
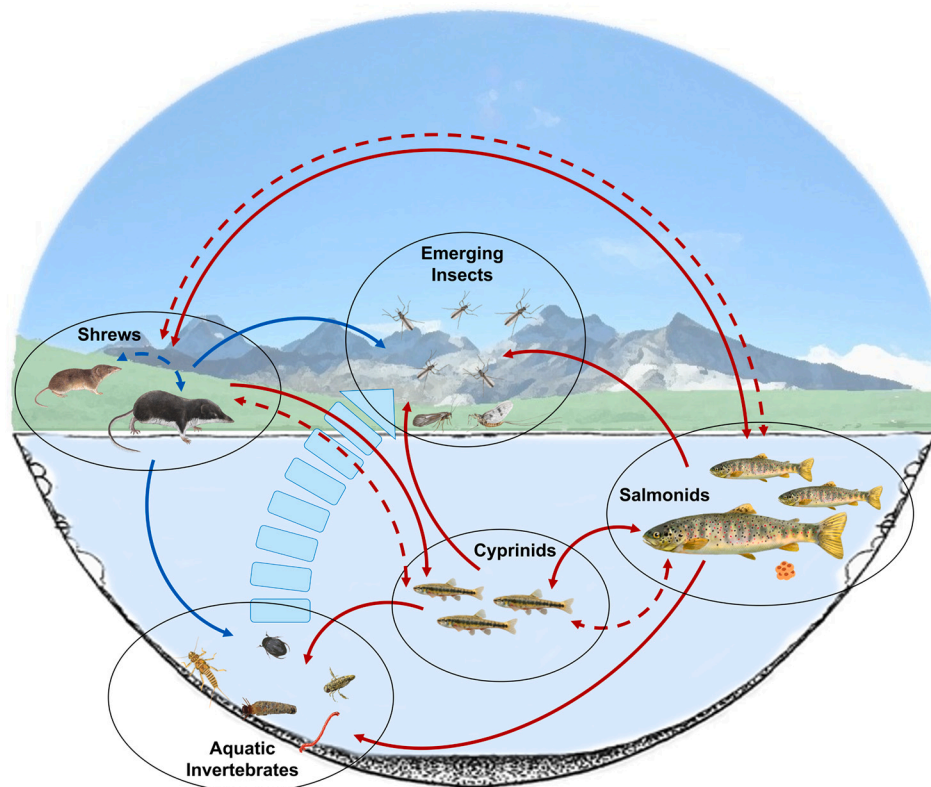
Fig. 6. Conceptual scheme representing the trophic interactions among shrews, introduced fish, and their shared preys (aquatic macroinvertebrates and emerging insects) in high mountain lakes. Blue arrows illustrate natural interactions among native taxa, while red ones represent interactions involving alien fish. Solid arrows indicate predation and can be uni- or bi-directional, while dashed arrows represent competition and are always bidirectional. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

introductions on shrews should be further investigated, also in low elevation areas, and especially for semiaquatic shrews, which could be particularly prone to the impact of fish.

Finally, our findings should be considered within the broader context where fish presence significantly disrupts high-altitude aquatic ecosystems, alters aquatic subsidies, and extends impacts beyond the lake itself, serving as a vivid example of the pervasive effects introduced fish have on these fragile environments. Hopefully, this example may also contribute to fulfil a communication gap that likely weaken high mountain lake conservation strategies, that is the lack of charismatic species garnering public sympathy. Compared to other native species (e. g., tiny invertebrates and some amphibians evoking either fascination or indifference and even aversion), shrews exhibit the typical characteristics of a "cute" species, one of the traits that identify charismatic species (Albert et al., 2018). Therefore, even if they are relatively unknown to the public and often mistaken for mice, shrews could work as a flagship species against fish introductions in high mountain lakes. In this context, we believe that shrews possess enough charisma for attracting public attention, advocating improved regulation of high-altitude fisheries management, and undertaking the necessary measures and actions to stop and reverse the fish invasion in mountain lakes.

CRediT authorship contribution statement

Declaration of competing interest

The authors declare no competing interests.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2024.110830.

Data availability

Data will be made available on request.

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