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PRIMARY RESEARCH PAPER

Changes in the insect emergence at the water–air interface in response to fish density manipulation in high altitude lakes

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Abstract Emerging insects are important nutrient vectors for lake-to-land linkages, but they are affected by fish predation. We manipulated the fish density in four high altitude lakes in connection with a brook trout eradication project. We used a paired benthic and surface funnel trap design to test whether fish density affects abundance, size, and proportion of aquatic insects reaching the surface in the pelagic zone. The study was complemented with benthic macroinvertebrates and fish diet data. Due to sample size, we focused on midge pupae, which rely on water surface tension (water–air interface) to emerge. Conspicuous insects emerging along the shoreline (water–land interface) were rarely collected in the traps, even if they rapidly recovered through the eradication. There was a positive relationship between fish density and midge abundance, and negative relationship for size and proportion reaching the surface. The increase in the emergence of midges in the presence of fish supports several other studies, but we have provided evidence that this pattern exists in conjunction with a size reduction even when fish consume a substantial amount of midges. The inclusion of small Diptera emergence at the water–air interface is a likely keystone in understanding fish impact on insect subsidy.

Keywords Insect subsidy · *Salvelinus fontinalis* · Midge pupae · Chironomidae · Fish eradication · Gran Paradiso National Park

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Introduction

Matter and organisms readily move across the interface between aquatic and terrestrial habitats providing important ecological connections between these habitats (Nakano & Murakami, 2001; Vander Zanden & Gratton, 2011). Emergent aquatic insects represent the most potent vector for lake-to-land linkages (Vander

Zanden & Gratton, 2011) and are a major component of both freshwater and adjacent terrestrial habitats (Pope et al., 2009). For example, larvae and winged adults can serve as food for both aquatic and terrestrial predators respectively (e.g., birds, bats, amphibians, and spiders; Power & Rainey, 2000; Nakano & Murakami, 2001; Finlay & Vredenburg, 2007; Pope et al., 2009; Epanchin et al., 2010). Emergent insects are a potential biotic transport route for contaminants from lake sediments to neighboring ecosystems (Fairchild et al., 1992; Tweedy et al., 2013). However, the flux of insects entering terrestrial habitats is affected by predation on the aquatic stages (Iwakuma & Yasuno, 1983; Pope et al., 2009) and by a number of environmental factors (e.g., seasonality, weather conditions, and many lake features; Flannagan & Lawler, 1972; Iwakuma, 1992). In particular, predatory fish can strongly affect the distribution and abundance of aquatic invertebrates (Knapp et al., 2001), therefore altering the strength of trophic interactions between lakes and surrounding terrestrial habitats (Finlay & Vredenburg, 2007).

Many sport fish, feeding heavily on larval and emerging insects (Carlisle & Hawkins, 1998; Tiberti et al., 2016), have been introduced to a multitude of formerly fishless lakes, in particular high altitude lakes throughout the world, for recreational angling (Knapp et al., 2001). Here the native communities are very sensitive but highly resilient to the direct and indirect impacts of fish predation (Knapp et al., 2005). Furthermore, introduced fish are known to negatively affect the emergence of many aquatic insects (e.g., stoneflies, caddisflies, dragonflies, mayflies; Pope et al., 2009). However, fish presence can indirectly enhance the emergence rates of small Diptera, such as midges (Finlay & Vredenburg, 2007; Pope et al., 2009).

Fishless, high altitude lakes provide very good conditions for comparative and manipulative field studies concerning the impact of introduced fish. Stocked lakes can be compared with naturally fishless systems and the fish density can be more readily manipulated in relatively small lakes. Moreover, these lakes are distant from multiple local stressors occurring at lowlands and obscuring effects of the experimental treatment (Hortal et al., 2014). In the present study, the effects of density manipulation of Brook Trout (*Salvelinus fontinalis*, Mitchil 1851) an introduced fish species on the amount of insect subsidy that emerges from four high altitude lakes treated for fish eradication were investigated. The study has been carried out in the Gran Paradiso National Park (Western Italian Alps), within the framework of the fish eradication and monitoring actions provided by the LIFE + project Biodiversity Improvement of Aquatic Alpine Ecosystems (BIOAQUAE, www.bioaquae.eu). The eradication method—intensive gillnetting (Knapp & Matthews, 1998; Tiberti et al., 2014a) is non-invasive—meaning that the sampling of aquatic insects has not been disturbed, as opposed to employing chemical methods such as rotenone (Vinson et al., 2010).

The main study hypotheses are that in the presence of introduced fish, a proportion of the insects which would have formerly emerged is diverted to fish and that larger insects are selectively predated. Unlike previous studies, which have focused on more conspicuous taxa from the water–land interface (Pope et al., 2009; Epanchin et al., 2010), the present study concentrates on the emergence of small insects from the pelagic area—almost exclusively midges (Chironomidae)—and the effect of fish predation on this taxonomic group. We designed a sampling method which enabled us to calculate the proportion of insects reaching the surface. A paired benthic and surface funnel trap design was used simultaneously with the fish eradication campaign, to test if fish density affects midge abundance, their size and the proportion reaching the surface. This method enabled us to account for both within-lake and among-lake variability of emerging midge abundance and for the possible negative effects of fish density reduction on the abundance of Diptera larvae and associated emerging pupae. It was noted during observations that the amount of emergent insects vary not only within a lake (e.g., clouds of midges can be observed at thawing time) but also among apparently similar lakes, possibly depending on their trophic state (Davies, 1980). Moreover, previous results and literature data indicate that the quantity of aquatic midge larvae can be enhanced by the presence of brook trout (Carlisle & Hawkins, 1998; Finlay & Vredenburg, 2007; Pope et al., 2009; Tiberti et al., 2014b). To further examine this issue, the present study is supported by additional data on the macroinvertebrate community to examine whether fish density reduction could produce a decreasing trend on Diptera larvae and to describe the resilience of large emerging insects. We complemented the study with a description of brook trout diet to highlight the importance of midges in its feeding ecology.

Methods

Study lakes

All the study lakes are included in the GPNP, a large protected area located between 45°25′ and 45°45′N and between 7° and 7°30′W in the Western Italian Alps. The GPNP shows a large altitudinal extension (between 800 and 4,061 m) and a typical alpine climate. In this paper, toponyms of the lakes will be substituted by abbreviations: Leynir-LEY; Nero-NER; Djouan-DJO; Dres-DRE. The main geographical, morphological, and chemical features of the study lakes are reported in Table 1. The lakes are natural (non-dammed) and are all located above or at the local tree line with watersheds belonging to the Alpine and nival belts. Thermal stratification occurs only in the late summer in the deepest lake LEY with the ice-covered season lasting for 7–9 months per year (Tiberti et al., 2010). Based on nutrient content, the studied lakes are oligotrophic or ultraoligotrophic. The presence of brook trout dates back to the 1960s. Currently, a strict fishing ban prohibits angling and fish stocking; the BIOAQUAE project provided the eradication effort of brook trout from the study lakes.

Table 1 Main geographic, morphometric, and chemical data of the studied lakes

	DJO	DRE	NER	LEY
Latitude N	45°33′27″	45°24′45″	45°33′06″	45°30′28″
Longitude E	07°10′43″	07°13′25″	07°10′07″	07°09′08″
Altitude (m)	2515	2087	2671	2747
D_{max} (m)	3.0	7.4	6.0	22.1
A (ha)	1.33	2.6	1.7	4.5
Geology	CS	AG	CS	CS
pH	8.7 ± 0.4	7.0 ± 0.5	8.0 ± 0.3	8.0 ± 0.3
TP (μg l^{-1})	3.5 ± 1.3	4.3 ± 2.5	2.3 ± 1.6	3.0 ± 0.9

Chemical variables are expressed as mean ± SD from 22-28 repeated sampling (between 2008 and 2015)

D_{max} maximum depth, *A* area, *Geology—AG* catchment entirely composed by Acidic Gneiss, *Geology—CS* catchment dominated by thick covering of Calcareous Schists, *TP* total phosphorus

Sampling and analytical methods

Emergent insects

To sample the emergent insects, we used submerged funnel-traps made of transparent PVC (Fig. 1A) similar to the model designed by Hamilton (1965) and described by Davies (1984). From 2013 to 2015, we performed 8 repeated sampling sessions per lake (June 25th–September 7th) during the ice-free season. During each sampling session, four plots of traps were placed along the two meter isobaths and left in the lakes for 4–15 days. Each plot consists of one trap pair. One trap was placed just below the water surface and the other suspended just above the lake bottom, there was approximately 1.5 m between the mouth of the two traps (Fig. 1B). Each trap pair was suspended from a floating rope anchored to the lake shore and intersecting the two-meter isobaths at the sampling point. The water level of lakes DRE, LEY, and DJO was constant, while lake NER can undergo a water level reduction (approximately 1 m) during the summer. However, the water level fluctuation was not detectable over a daily or weekly period and did not affect the vertical displacement of the traps. The position of each trap pair along the 2-m isobaths was randomly generated in the R environment (R 3.1.1) using the function "readshapeSpatial" (package "maptools") to import the shape file of the 2-m isobaths and the function "runifpontOnLines" (package "spatstat") to generate four random points (R Development Core Team, 2013). The point generation was repeated eight times in each lake to generate a sufficient number of random points to change the position of the trap pairs at each sampling session.

The sampled insects were removed from the traps and fixed in 70% ethanol directly in the field. The samples were sorted under a stereomicroscope at a magnification of 10×. Emergent insects were separated from other aquatic invertebrates (e.g., aquatic Coleoptera and Heteroptera), classified at the family level using the identification key proposed by Campaioli et al. (1994) and enumerated. The maximum length of all the non-fragmented emergent insects was measured.

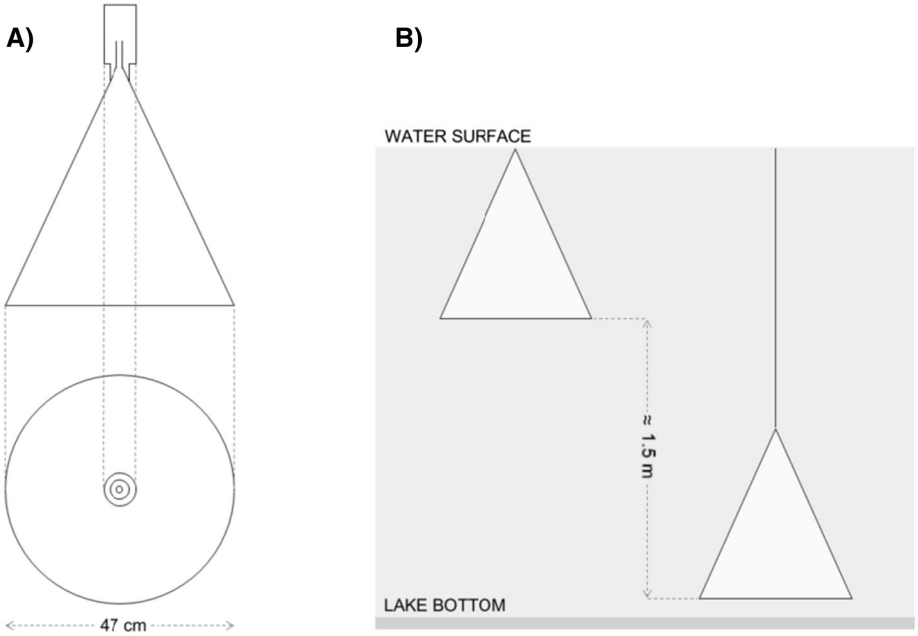

Fig. 1 Projection of the insect emergence trap (**A**) and positioning scheme of a plot/trap pair along the two meters isobaths, the traps are suspended to a floating rope fixed between the lakes' shore (**B**)

Benthic macroinvertebrates

Macroinvertebrate samples were collected twice each year (2013–2015) during the ice-free period. The first sample was collected at the beginning of summer (June or July) and the second later in the season (August or September), to take into account the seasonal variations within the macroinvertebrate community. All shore-accessible habitats were sampled for semi-quantitative macroinvertebrate estimates. Three littoral habitats were chosen according to clast diameter: sand shores (clast diameter < 2 mm), gravel shores ($2 \text{ mm} < \text{clast} < 64$ mm), and stony shores (clast > 64 mm). In each of these habitats, ten standard sweeps, each approximately 1 m long, were conducted with a standard d-frame net (mouth, 25×20 cm; mesh 0.5 mm). Benthic macroinvertebrates were separated from detritus and sediments in the field and preserved in 70% ethanol. Macroinvertebrates were identified to a family or genus level using the identification key proposed by Campaioli et al. (1994), and counted.

Fish diet

Between 2013 and 2015, we collected stomach contents from 186 fish (150 in 2013, 32 in 2014 and 4 in 2015; 13 from lake DJO, 83 from lake DRE, 74 from lake LEY, 16 from lake NER). Stomachs were checked for prey presence/absence analysis with a subsample of 125 stomachs used for counting prey items and a subsample of 91 stomachs (from fish larger than 15 cm) for the analysis of prey biovolumes. Most of the data were included in Tiberti et al. (2016) with the specific aim to describe the factors influencing the diet of brook trout. Fish maximum length was converted into six size classes encompassing values from 0 to $\geq$25 cm at five-centimeter intervals. Analyzed stomachs represented all of the present size classes. Stomachs were preserved in 70% ethanol. Larger prey items were identified and enumerated under low magnification (under a stereomicroscope), while we used a closed counting chamber and a binocular dissecting microscope at ×40 (Olympus CH-BI45-3) for microscopic preys such as zooplankton. Biovolumes were measured by gently pressing the prey items belonging to each prey group in a graduated cylinder. Fragmented or partially digested items were recognized using body parts resistant to digestion (e.g., cephalic capsule) or recorded as non-identified prey. For the description of the diet, data are offered on frequency of occurrence of the prey items ($F_i = N_i/N \times 100$, where N_i is the number of fish with prey i in their stomach and N is the total number of analyzed stomachs), relative abundance ($A_i = S_i/S_t \times 100$, where S_i is the sum of the counts of prey i in all the stomachs and S_t is the total sum of all the prey items counted), and relative biovolume ($B_i = V_i/V_t \times 100$, where V_i is the sum of the biovolumes of prey i in all the stomachs and V_t is the total sum of all the measured biovolumes).

Fish density manipulation and estimate

Intensive gill-netting and electrofishing were used to reduce the fish density. Two types of nets were used: multi-mesh gillnets (36 × 1.8 m; divided into 6 panels with increasing mesh sizes from 10 to 38 mm), and pelagic gillnets (from 36 × 1.8 m to 50 × 10 m depending on the lakes depth; mesh-size = 25 mm). All the nets were held vertically and fixed to the shore with ropes along several transects, bearing 1–6 nets. Since their initial positioning, the nets were left in the lakes for the duration of the project, including the ice-cover season (October–May). During the 2013–2015 ice-free seasons, the fish were regularly collected from the nets by rowing a dinghy along the nets and pulling up sections to remove the trapped fish. At the same time, electrofishing (with a ELT62 II 160 GI backpack equipment) and additional movable multi-mesh gillnets were used to a varying degree, dependent on the lakes features in regard to the littoral area (e.g., littoral vegetation) or along the tributaries to support the eradication efforts. A complete presentation of the outputs of the eradication actions is beyond the aims of the present study. However, eradication will be considered after 1 year without fish captures, in lakes DRE and LEY this time has not yet expired.

To test the effect of fish presence and removal on the proportion of emergent insects reaching the surface, we assessed the relative fish density. The relative fish density (D) along the eradication campaign gradually decreased from 1 to 0 and was calculated on a monthly base as

$$D_i^n = \frac{\mathrm{CPUE_i}}{\mathrm{CPUE_1}} \qquad (1)$$

$$\mathrm{CPUE}_i^n = \frac{N_i}{S_i \times t_i}, \qquad (2)$$

where the capture per unit effort (CPUE) calculated during the first month of eradication ($\mathrm{CPUE_1}$) was used as the reference value to calculate relative fish density values in the following n months [1], while its relative density value was set to 1. The *CPUE* is the ratio between N (number of fish captured each month) and the capture effort [$S \times t$ = total surface of the nets (m^2) × fishing time (hours)] [2]. Relative fish densities have been used as a covariate in the following statistical models.

Statistical analyses

Since the sampled fraction of the emergent insects community was composed almost exclusively of midge pupae (Chironomidae, Diptera; see "Results" section), we limited our analyses to this dominant family. All statistical analyses were implemented under the "lme4" (Pinheiro & Bates, 2006) and "nlme" (Bates, 2013) packages of the statistical environment R v.3.1.1 (R Development Core Team, 2013). We ran three mixed models to test if fish density affects the daily abundance of emergent midge pupae (Table 2, Model 1), their size (Table 2, Model 2), and the proportion of midge pupae reaching the surface (Table 2, Model 3). An exhaustive summary of how the models were fitted is provided in Table 2. Model adequacy was verified by examination of residuals and Pearson residuals as described in Zuur et al. (2009). Because of overdispersion, a traditional approach of modeling count data with a Poisson error distribution was not appropriate for Model 1, therefore we used a negative binomial error distribution (Zuur et al., 2009; Table 2). For Model 3, we followed the indications of Crawley (2012) for Generalized Linear Mixed Effects Models with proportion data. Besides the relative fish density (D), which was added as an explanatory variable in all the models, in Models 1 and 2 we added the number of days elapsing from thaw (to take into account the possible seasonal variations of the insect taxonomic composition and emergence phenology)

Table 2 Summary of the general and linear mixed effects models features

Models	Models features	
Model 1	Dependent variable	Daily abundance of emerging midges per trap (214 observations)
Midge abundance	Fixed effects	Fish relative density (D) + Days from thaw + Trap position (Surface vs. Bottom)
	Random effects	Lake (4 groups)
	Error distribution	Negative binomial
Model 2	Dependent variables	Midges maximum length (4407 observations)
Midge size	Fixed effects	Fish relative density (D) + Days from thaw + Trap position (Surface vs. Bottom)
	Random effects	Lake (4 groups)
	Error distribution	Gaussian
Model 3	Dependent variables	Proportion of midge pupae reaching the surface (107 observations)
Proportions	Fixed effects	Fish relative density (D)
	Random effects	Lake (4 groups)
	Error distribution	Binomial

and the position of the funnel traps (surface vs. bottom). The exact thaw date was determined by direct observation (in 2013) or using data collected from underwater temperature loggers (2014 and 2015).

Results

Fish density manipulation

In Fig. 2 (1st row panels), we show the brook trout CPUE trends between the Jun-01, 2013 and the Sep-30, 2015. By the September 30th, 2015 we had removed 1,403 fish from lake DJO (0.11 fish $\times$ m^{-2}), 15,138 from lake DRE (0.58 fish $\times$ m^{-2}), 3,438 from lake LEY (0.08 fish $\times$ m^{-2}), and 201 from lake NER (0.01 fish $\times$ m^{-2}). During the study period, the fish CPUE rapidly decreased in all the lakes treated for fish eradication. In lakes DJO and NER reached zero by the second year of the eradication campaign; in lakes DRE and LEY, by the third year. In lake DRE, the CPUE was also relatively high during the second field season due to a recruitment spike resulting from the density reduction of adult and potentially cannibalistic, brook trout.

Insect emergence

We collected 214 samples (107 trap pairs) for emergent insects, 42 samples (21 pairs) were discarded because at least one of the two traps had problems (one of the samples was totally or partially lost during the collection, or the oxygen, due to lakes primary production, filled and floated the traps). The non-discarded traps contained a total of 5,060 midge pupae (mean ± SD per trap: 23.6 ± 52.5) representing close to the totality of the emergent insects (except two caddisflies and three dragonflies). We also captured many other invertebrates (bycatch), in particular: $\approx$1000 midge larvae, $\approx$330 corixidae (aquatic Heteroptera), 36 acarina (Arachnidae), 15 dytiscidae (aquatic Coleoptera, both larvae and adults), and 11 Hirudinea. The mean ± SD number of emergent midges from the studied lakes was 2.9 ± 7.0 midges $\times$ trap^{-1} $\times$ day^{-1} (17.0 = midges $\times$ m^{-2} $\times$ day^{-1}) in the traps at the lakes surface and 4.8 ± 11.7 midges $\times$ trap^{-1} $\times$ day^{-1} (27.5 = midges $\times$ m^{-2} $\times$ day^{-1}) in the traps at the lake bottom (Fig. 2; 2nd row panels).

The GLMM results show a positive relationship between the number of midge pupae captured per day in the traps and the fish density (Table 3). This general trend is significant in spite of the fact that lake NER shows the opposite trend (Fig. 2). The mean size of the midge pupae is negatively influenced by the relative fish density and is larger at the beginning of the ice-free season (Table 3). There is also a negative relationship between the proportion of midge pupae reaching the surface and the fish density (Table 3). Validation of the model through analysis of the

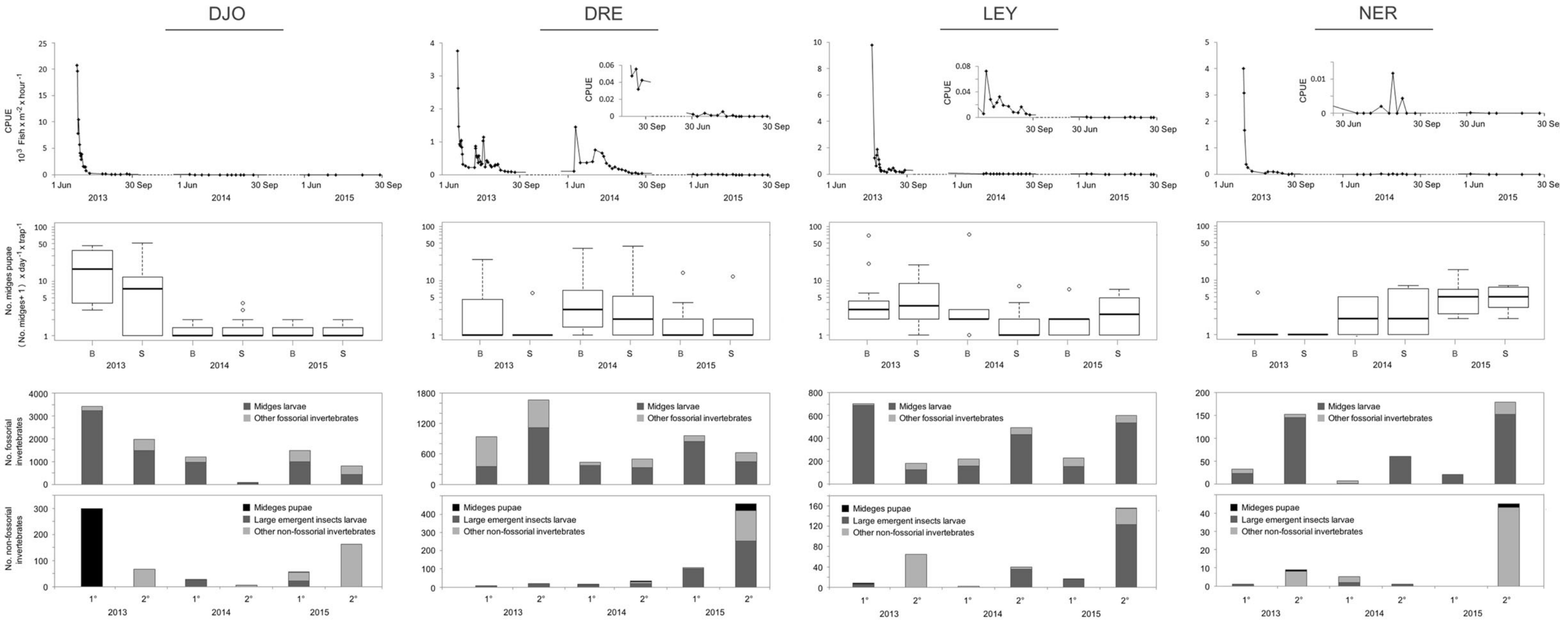

Fig. 2 *First row panels* fish capture per unit effort (CPUE 10^3 Fish $\times$ m^{-2} $\times$ h^{-1}) from the June 1st, 2013 to the September 30th, 2015; the *y* axis have been rescaled in the small additional graphs to highlight the trends approaching zero in lakes DRE, LEY, and NER. *Second row panels* distribution of the midge pupae abundance in the surface (S) and bottom (B) traps in the 2013–2015 period. *Third row panels* number of midges larvae and other fossorial invertebrates in the macroinvertebrate community samples collected during the 1° (June/July) and 2° (August/September) sampling period. *Fourth row panels* number of midge pupae, large emergent insects (stoneflies, mayflies, caddisflies, and dragonflies), and other benthic or nektonic invertebrates in the macroinvertebrate community samples collected during the 1° and 2° sampling period

Table 3 General and Linear Mixed Effects Models results summary: estimates β, standard errors SE (β), associated Wald z score [=β/SE(β)] and significance level P for all fixed terms in the analysis

Model	Fixed terms	Coef. β	SE (β)	z	P
Model 1					
Midge abundance	Intercept	1.64	0.40	4.16	<0.001
	Fish relative density (D)	0.91	0.36	2.54	<0.05
	Trap position (bottom *vs.* surface)	−0.31	0.27	−1.17	0.24
	Days from thaw	−0.01	0.01	−1.94	0.05
Model 2					
Midge size	Intercept	4.83	0.15	32.99	<0.001
	Fish relative density (D)	−0.85	−0.04	−20.91	<0.001
	Trap position (bottom *vs.* surface)	−0.27	0.03	−10.66	<0.001
	Days from thaw	−0.01	0.00	−11.12	<0.001
Model 3					
Proportions	Intercept	−0.27	0.09	−2.91	<0.01
	Fish relative density (*D*)	−0.36	0.09	−3.99	<0.001

Table 4 Diet of *Salvelinus fontinalis* from lakes DJO, DRE, LEY, and NER (Gran Paradiso National Park) expressed as frequency of occurrence (*F* %), relative abundance (*A* %), and relative biovolume (*B* %). Values in bold provide total percentages for prey groups 1–7

Prey groups	*F* % *N* = 186	*A* % *N* = 125	*B* % *N* = 91
1. Zooplankton[a]	**19.9**	**22.6**	**0.3**
2. Nectonic invertebrates	**45.2**	**47.8**	**13.5**
Nectonic Coleoptera, i	0.3	0.1	0.3
Nectonic Diptera, p[b]	41.4	47.5	11.3
Aquatic Heteroptera	7.5	0.1	2.0
Isopoda	0.1	0.1	0.0
3. Benthic invertebrates	**38.2**	**1.1**	**9.4**
Plecoptera, l	10.2	0.1	0.2
Tricoptera, l	30.1	1.0	6.0
Odonata, l	2.2	0.0	3.2
Hydracarina	0.1	0.0	0.0
4. Aquatic fossorial invertebrates	**42.5**	**19.2**	**14.9**
Mollusca—*Pisidium* sp.	7.5	0.1	0.2
Fossorial Diptera, l[c]	38.7	19.0	14.8
Oligochaeta	0.5	0.0	0.0
5. Terrestrial invertebrates[d]	**68.8**	**9.0**	**31.2**
6. Vertebrates[e]	**3.8**	**0.0**	**28.1**
7. Undetermined rests	**9.7**	**0.3**	**2.6**

i imaginal stage; *l* larvae, *p* pupae

[a] Copepoda, Ostracoda and Cladocera less than 3 mm long

[b] Chironomidae pupae

[c] Almost exclusively Chironomidae larvae

[d] Including terrestrial Coleoptera, Diptera, Imenoptera, Orthoptera, Lepidoptera, Plecoptera, Tricoptera, Hemiptera, Aranea, Miriapoda, Acarina

[e] Brook trout (*Salvelinus fontinalis*) and common frog (*Rana temporaria*)

residuals did not show any systematic trend in the goodness of fit.

Benthic macroinvertebrates

We report the complete list of macroinvertebrate taxa and the number of sampled individuals in Table S1 (online supplementary materials). The frequency of occurrence and the abundances of midge pupae in the macroinvertebrate samples were low (Fig. 2, 3rd row panels). Midge larvae were always represented in the samples but they did not clearly reflect the positive relationship observed between midge pupae and fish density, with the exception of lake DJO where a decreasing trend was observable. Through the eradication process, the macroinvertebrate communities included an increasing number of nektonic and benthic taxa including several emergent insects, in particular stoneflies and caddisflies, but also dragonflies and mayflies (Fig. 2, 4th row panels).

Fish diet

Brook trout feed on a large variety of prey including aquatic and terrestrial invertebrates and vertebrates. Midge pupae were found in 41.4% of the analyzed stomachs (Table 4).

Discussion

Water–air and water–land interfaces and the composition of the emergent insects samples

Due to our sampling method, midge pupae effectually represent the totality of the sampled emergent insects in GPNP lakes. Compared to the studies conducted in similar habitats (Pope et al., 2009; Epanchin et al., 2010; Gruenstein, 2014), larger insects such as caddisflies, stoneflies, and mayflies were not sampled, regardless of rapid recovery during the eradication (Fig. 2). Sampling at a depth of two meters, rather than closer to the shoreline, can produce important differences in the composition of the emergent insect samples. Indeed, many insect nymphs (e.g., caddisflies, stoneflies, dragonflies) need to climb onto banks or aquatic vegetation to emerge and usually the shoreline (water–land interface) is the recipient of a large number of emergent insects from the whole lake. On the contrary small dipterans such as midges can emerge relying solely on the surface tension of water (water–air interface; Nayar & Ali, 2003; Armitage et al., 2012) and can be found in large numbers emerging from the whole lake surface. Also, many mayfly and some caddisfly species can emerge at the water surface but mayflies were very rare or absent in the study lakes (Tiberti et al., 2014b; Table S1 supplementary online materials) and significant cases of caddisfly emergence from the pelagic area of the lakes were not observed. The flux of biomass between aquatic and terrestrial environments can be strongly affected by the emergence of larger, but less numerous taxa crossing the water–land interface (Pope et al., 2009; Finlay & Vredenburg, 2007). However, ecosystem geometry can greatly influence the biomass exchanges. When the pelagic area is larger or proportionally larger (e.g., in those lakes with a low shoreline development, such as sub-circular lakes) the widespread—and occasionally massive—insect emergence at the water–air interface can become important. This could be critical not only in terms of abundance but also in terms of biomass (Vander Zanden & Gratton, 2011; Gratton et al., 2008) and could compete in magnitude with the insect emergence at the water–land interface. Since emerging insects can enter the terrestrial environment through two alternative routes, (1) the water–air interface (which extends all over the lakes surface and which is crossed by the insects able to emerge relying on the surface tension of water) and (2) the water–land interface (which approximately match the perimeter of the lakes and which is usually crossed by larger insects), to achieve a complete picture of the insect subsidy to the terrestrial environments, it would be necessary to combine the data from both the interfaces. Generally, the studies concerning insect emergence are based on samples from the littoral area, which do not account for the insects that emerge in the pelagic zone (Vander Zanden & Gratton, 2011). The present study should be considered representative of the mechanisms driving the impact of introduced fish on the insect emergence in the pelagic area. Irrespective of the fact that these findings do not take into account the measurement of insect emergence at the water–land interface, the macroinvertebrate samples clearly show that introduced fish depress the abundance of their larval stages and, therefore, their emergence rates (in accordance with the existing literature: Finlay & Vredenburg, 2007; Pope et al., 2009; Epanchin et al., 2010). Moreover, having focused on the water–air interface and on midge emergence, the present study has the merit to cope with an overlooked aspect of the impact of introduced fish on the lake-to-land relationship.

Abundances, size, and proportions of the emergent midge pupae

Our results show a positive relationship between fish density and midge abundance. Fish stocking in once fishless lakes usually depresses the emergence rates of large insects (Pope et al., 2009) but can indirectly enhance the emergence of small bodied Diptera and in general, their abundance (Carlisle & Hawkins, 1998; Knapp et al., 2001; Wissinger et al., 2006; Finlay & Vredenburg, 2007; Pope et al., 2009; Tiberti et al., 2014b). Midge pupae represent a very important food resource for brook trout (present study; Tiberti et al., 2016). The indirect mechanism enabling the midges to withstand strong predatory impact—and therefore providing a stable food resource for brook trout—is probably of pivotal importance for the survival of the introduced fish populations. In particular this mechanism should be strong enough to overcome the strong predation on the midge pupae, which negatively affects the proportion of emergent pupae reaching the lakes surface. The mechanisms driving the impact of fish predation on small emergent Diptera are rather unclear; usually the authors describe an increase of emergent Diptera followed by focus on the impact on the most conspicuous taxa (Finlay & Vredenburg, 2007; Pope et al., 2009). On the contrary, the present study shows that midges can have higher emergence rates, even under a strong predatory pressure; as also shown by the brook trout diet. This indirect effect of fish stocking is believed to be determined, both by a competitive and predatory release within the invertebrate community by the selective-predation on large-bodied invertebrate grazers and predators (Gilinsky, 1984; Blumenshine et al., 2000; Tolonen et al., 2003), and, or by an increase of the flow of nutrients to the lake bottom mediated by introduced fish (Leavitt et al., 1994; Knapp et al., 2001). We also observed a negative impact of fish density on the size of emergent midges and therefore added another hypothesis: that increased midge emergence could be caused by a competitive release within the midge larvae community. Under the size selective predation exerted by fish, the larval community could be dominated by smaller (with lower metabolic demand) and more abundant taxa, while in naturally fishless lakes, fewer but larger taxa (with higher metabolic demand) could better compete for resources.

The negative impact of fish density on the size of midge pupae is consistent with the literature findings concerning visual predators and size selective predation (e.g., Blumenshine et al., 2000; Knapp et al., 2001; Eby et al., 2006). The observed effect of fish density manipulation on the mean size of midge pupae is small [the model estimate of the difference between the midges size at maximum and minimum relative fish density (1 and 0) is equal to 0.36 ± 0.06 mm (mean ± SE)], but statistically significant. This could indicate an improved survival of larger individuals or a taxonomic shift towards larger species. In the absence of a detailed taxonomic analysis, with such a small statistical effect size, it is challenging to distinguish between the two causes. The result underlies important consequences for the nutrient subsidies to the terrestrial environment. A shift towards the emergence of larger insects implies a general increase of the single biomass units entering the terrestrial environment, which could offset (to a variable extent) the effect of reduced abundance emergent midges.

The seasonal variable (days elapsed from thaw) negatively affects both the midge abundance and size, confirming the general finding that the strong seasonal patterns dominating alpine habitats are a major ecological force also for high altitude lakes.

The trend of midge abundance discussed so far is related to the GLMM results, but this trend can vary among lakes, as well as macroinvertebrate resilience (Fig. 2) and the number of captured fish. In particular, lake NER shows an increasing trend of midge abundance during the eradication process which is unlikely to be attributed to chance; at the same time, macroinvertebrate resilience was less pronounced in lake NER than in other lakes (Fig. 2) and the number of fish inhabiting lake NER was much smaller than in other lakes (only 200 fish captured, compared to the thousands of fish captured in the other lakes). These differences may have a relation with the opposite trend in the midge abundance observed in lake NER. For example, due to the weak resilience of the macroinvertebrates in lake NER, the midges may just show the direct positive effects of a reduced fish predation pressure and not the indirect negative effects of an enhanced competition/predation pressure within the benthic community. Alternatively, due to a small initial fish density, the indirect ecological mechanisms enhancing midge abundance could be weaker; for example, if the midge abundance is enhanced by an increased flow of nutrients (in the form of fish feces) to the lake bottom (as suggested by Knapp et al., 2001), a low fish density can affect the strength of this mechanism.

Conservation implications

The fact that the impact of introduced fish can overcome the lake's perimeter can have important conservation implications in the framework of invasion and restoration ecology (Eby et al., 2006). A number of terrestrial predators which rely on prey from aquatic ecosystems (spiders, amphibians, snakes, birds, and perhaps bats; Matthews & Knapp, 2002; Fukui et al., 2006; Lawler & Pope, 2006; Finlay & Vredenburg 2007; Epanchin et al., 2010; Benjamin et al., 2011; Joseph et al., 2011; Gruenstein, 2014) can be affected by the presence of introduced fish, with detectable, indirect cascading effects in terrestrial habitats (Knight et al., 2005; Gratton et al., 2008). Moreover, the areas surrounding the lakes can include protected habitats. For example, alpine prairies and mountain peat bogs are included in the Annex I of the "Habitat Directive," Council Directive 92/43/EEC and host endangered species, which could be negatively impacted by the direct and indirect effects of fish predation on the emergent insect community. Therefore, a better understanding of lake-to-land linkages can also direct the conservation strategies of habitats and species, providing a scientific background for the implementation of conservation actions such as the eradication of introduced fish.

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References

Armitage, P. D., L. C. Pinder & P. Cranston, 2012. The Chironomidae: Biology and Ecology of Non-biting Midges. Springer, Berlin.

Bates, D. M., 2013. Linear Mixed Model Implementation in lme4. University of Wisconsin, Madison.

Benjamin, J. R., K. D. Fausch & C. V. Baxter, 2011. Species replacement by a nonnative salmonid alters ecosystem function by reducing prey subsidies that support riparian spiders. Oecologia 167: 503–512.

Blumenshine, S. C., D. M. Lodge & J. R. Hodgson, 2000. Gradient of fish predation alters body size distributions of lake benthos. Ecology 81: 374–386.

Campaioli, S., P. F. Ghetti & A. Minelli, 1994. Manuale per il riconoscimento dei macroinvertebrati delle acque dolci italiane. Provincia autonoma di Trento, Trento.

Carlisle, D. M. & C. P. Hawkins, 1998. Relationships between invertebrate assemblage structure, 2 trout species, and habitat structure in Utah mountain lakes. Journal of the North American Benthological Society 17: 286–300.

Crawley, M. J., 2012. Proportion data. In Crawley, M. J. (ed.), The R Book. Wiley, Hoboken: 569–591.

Davies, I. J., 1980. Relationships between dipteran emergence and phytoplankton production in the Experimental Lakes Area, northwestern Ontario. Canadian Journal of Fisheries and Aquatic Sciences 37: 523–533.

Davies, I. J., 1984. Sampling aquatic insect emergence. In Downing, J. A. & F. H. Rigler (eds), A Manual on Methods for the Assessment of Secondary Productivity in Fresh Water. Blackwell Scientific Publications, Oxford: 161–227.

Eby, L. A., W. J. Roach, L. B. Crowder & J. A. Stanford, 2006. Effects of stocking-up freshwater food webs. Trends in Ecology and Evolution 21: 576–584.

Epanchin, P. N., R. A. Knapp & S. P. Lawler, 2010. Nonnative trout impact an alpine-nesting bird by altering aquatic-insect subsidies. Ecology 91: 2406–2415.

Fairchild, W. L., D. C. Muir, R. S. Currie & A. L. Yarechewski, 1992. Emerging insects as a biotic pathway for movement of 2, 3, 7, 8-tetrachlorodibenzofuran from lake sediments. Environmental Toxicology and Chemistry 11: 867–872.

Finlay, J. C. & V. T. Vredenburg, 2007. Introduced trout sever trophic connections in watersheds: consequences for a declining amphibian. Ecology 88: 2187–2198.

Flannagan, J. F. & G. H. Lawler, 1972. Emergence of caddisflies (Trichoptera) and mayflies (Ephemeroptera) from Heming lake, Manitoba. The Canadian Entomologist 104: 173–183.

Fukui, D. I., M. Murakami, S. Nakano & T. Aoi, 2006. Effect of emergent aquatic insects on bat foraging in a riparian forest. Journal of Animal Ecology 75: 1252–1258.

Gilinsky, E., 1984. The role of fish predation and spatial heterogeneity in determining benthic community structure. Ecology 65: 455–468.

Gratton, C., J. Donaldson & M. J. Vander Zanden, 2008. Ecosystem linkages between lakes and the surrounding terrestrial landscape in northeast Iceland. Ecosystems 11: 764–774.

Gruenstein, E., 2014. The Response of Bats to Introduced Trout in Naturally Fishless Lakes of the Sierra Nevada, California. Master Thesis, Department of Biological Sciences, San José State University, San José, CA.

Hamilton, A. L., 1965. An Analysis of a Freshwater Benthic Community with Special Reference to the Chironomidae. PhD dissertation, University of British Columbia, Vancouver, British Columbia.

Hortal, J., J. C. Nabout, J. Calatayud, F. M. Carneiro, A. Padial, A. Santos, T. Siqueira, F. Bokma, L. M. Bini & M. Ventura, 2014. Perspectives on the use of lakes and ponds as model systems for macroecological research. Journal of Limnology 73: 46–60.

Iwakuma, T., 1992. Emergence of Chironomidae from the shallow eutrophic Lake Kasumigaura, Japan. Hydrobiologia 245: 27–40.

Iwakuma, T. & M. Yasuno, 1983. Fate of the univoltine chironomid, *Tokunagayusurika akamusi* (Diptera: Chironomidae), at emergence in Lake Kasumigaura, Japan. Archiv für Hydrobiologie 99: 37–59.

Joseph, M. B., J. Piovia-Scott, S. P. Lawler & K. L. Pope, 2011. Indirect effects of introduced trout on Cascades frogs (*Rana cascadae*) via shared aquatic prey. Freshwater Biology 56: 828–838.

Knapp, R. A. & K. R. Matthews, 1998. Eradication of nonnative fish by gill netting from a small mountain lake in California. Restoration Ecology 6: 207–213.

Knapp, R. A., K. R. Matthews & O. Sarnelle, 2001. Resistance and resilience of alpine lake fauna to fish introductions. Ecological Monograph 71: 401–421.

Knapp, R. A., C. P. Hawkins, J. Ladau & J. G. McClory, 2005. Fauna of Yosemite National Park lakes has low resistance but high resilience to fish introductions. Ecological Application 15: 835–847.

Knight, T. M., M. W. McCoy, J. M. Chase, K. A. McCoy & R. D. Holt, 2005. Trophic cascades across ecosystems. Nature 437: 880–883.

Lawler, S. P. & K. L. Pope, 2006. Non-native fish in mountain lakes: effects on a declining amphibian and ecosystem subsidy. University of California Water Resources Center, Davis.

Leavitt, P. R., D. E. Schindler, A. J. Paul, A. K. Hardie & D. W. Schindler, 1994. Fossil pigment records of phytoplankton in trout-stocked alpine lakes. Canadian Journal of Fisheries and Aquatic Sciences 51: 2411–2423.

Matthews, K. R. & R. A. Knapp, 2002. Garter snake distributions in high-elevation aquatic ecosystems: is there a link with declining amphibian populations and nonnative trout introductions? Journal of Herpetology 36: 16–22.

Nayar, J. K. & A. Ali, 2003. A review of monomolecular surface films as larvicides and pupicides of mosquitoes. Journal of Vector Ecology 28: 190–199.

Nakano, S. & M. Murakami, 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proceeding of the National Academy of Sciences 98: 166–170.

Pinheiro, J. & D. Bates, 2006. Mixed-effects models in S and S-PLUS. Springer Science & Business Media, Berlin.

Pope, K. L., J. Piovia-Scott & S. P. Lawler, 2009. Changes in aquatic insect emergence in response to whole-lake experimental manipulations of introduced trout. Freshwater Biology 54: 982–993.

Power, M. E. & W. E. Rainey, 2000. Food webs and resource sheds: towards spatially delimiting trophic interactions. In Hutchings, M. J., M. J. John & A. J. A. Stewart (eds), The Ecological Consequences of Environ mental Heterogeneity. Blackwell Science, Malden: 291–314.

R Development Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org/. Accessed 1 September 2015.

Tiberti, R., E. Acerbi & R. Iacobuzio, 2014a. Preliminary studies on fish capture techniques in Gran Paradiso alpine lakes: towards an eradication plan. Journal of Mountain Ecology 9: 61–74.

Tiberti, R., A. von Hardenberg & G. Bogliani, 2014b. Ecological impact of introduced fish in high altitude lakes: a case of study from the European Alps. Hydrobiologia 724: 1–19.

Tiberti, R., S. Brighenti, C. Canedoli, R. Iacobuzio & M. Rolla, 2016. Factors influencing the diet of introduced brook trout (*Salvelinus fontinalis*; Mitchill, 1814) in an Alpine area. Journal of Limnology, in press.

Tiberti, R., G. A. Tartari & A. Marchetto, 2010. Geomorphology and hydrochemistry of 12 Alpine lakes in the Gran Paradiso National Park, Italy. Journal of Limnology 69: 242–256.

Tolonen, K. T., H. Hamalainen, I. J. Holopainen, K. Mikkonen & J. Karjalainen, 2003. Body size and substrate association of littoral insects in relation to vegetation structure. Hydrobiologia 499: 179–190.

Tweedy, B. N., R. W. Drenner, M. M. Chumchal & J. H. Kennedy, 2013. Effects of fish on emergent insect-mediated flux of methyl mercury across a gradient of contamination. Environmental Science and Technology 47: 1614–1619.

Vander Zanden, M. J. & C. Gratton, 2011. Blowin'in the wind: reciprocal airborne carbon fluxes between lakes and land. Canadian Journal of Fisheries and Aquatic Sciences 68: 170–182.

Vinson, M. R., E. C. Dinger & D. K. Vinson, 2010. Piscicides and invertebrates: after 70 years, does anyone really know? Fisheries 35: 61–71.

Wissinger, S. A., A. R. Mcintosh & H. S. Greig, 2006. Impacts of introduced brown and rainbow trout on benthic invertebrate communities in shallow New Zealand lakes. Freshwater Biology 51: 2009–2028.

Zuur, A. F., E. N. leno, N. J. Walker, A. A. Saveliev & G. M. Smith, 2009. Mixed effects models and extensions in ecology with R. Springer Science & Business Media, New York.