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Changes in aquatic insect emergence in response to whole-lake experimental manipulations of introduced trout

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SUMMARY

1. Insects emerging from mountain lakes provide an important food source for many terrestrial predators. The amount of insect subsidy that emerges from lakes is influenced by predator composition, but predator effects could be ameliorated by greater habitat complexity. We conducted a replicated whole-lake experiment to test the effects of introduced fish predators on the abundance and composition of aquatic insects within and emerging from the littoral zone of 16 mountain lakes in the Trinity Alps Wilderness in northwestern California.
2. Study treatments matched the fisheries management options being implemented in California's wilderness areas: (i) continued stocking with non-native trout, (ii) suspension of stocking, and (iii) suspension of stocking and removal of fish. We also included four naturally fishless 'reference' lakes. We compared abundances and biomass of emerging aquatic insects before treatments were initiated and for 3 years following their establishment. Abundances of benthic insects were also compared in the third year post-treatment.
3. Trout removal rapidly increased abundances of mayflies, caddisflies, and insect predators, and overall insect biomass emerging from lakes. No significant differences were found between the suspension of stocking lakes and continued stocking lakes. Fish density was a more important predictor of aquatic insect emergence than habitat complexity.
4. Mayfly larvae responded positively to fish removal and caddisfly larvae tended to be more abundant in lakes without fish, but we did not detect effects on abundance of predatory insects. However, we found large insect predators in shallower water in lakes with fish compared to fish removal or fish-free reference lakes.
5. These results provide insights into the continuing effects of past and current fish stocking practices on the flow of insect prey from mountain lakes into the neighbouring terrestrial environment. We also show that these consequences can rapidly be reversed by removing non-native fishes.

Keywords: Klamath mountains, non-native fish, predator effects, subalpine lakes, subsidy

Introduction

Aquatic insects are major components of both freshwater communities and adjacent terrestrial habitats. Larval insects serve as prey for larger aquatic insects, amphibians and fishes, and the winged adult stages feed terrestrial predators such as birds, bats and spiders (Power & Rainey, 2000; Nakano & Murakami,

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2001; Sanzone *et al*., 2003). The amount of allochthonous insect subsidy entering a terrestrial landscape is affected by predation and environmental conditions within the aquatic habitat. We tested the effects of the widespread practice of stocking non-native fishes into mountain lakes on the amount of insect subsidy that emerges from the lakes. To make our study treatments realistic, we matched them to the fisheries management practices currently being implemented in California's mountain lakes.

The distribution of predatory fishes is an important determinant of the distribution and abundance of aquatic invertebrates (Brooks & Dodson, 1965; Wellborn, Skelly & Werner, 1996; Knapp, Matthews & Sarnelle, 2001; Baxter *et al*., 2004). Sport fish such as rainbow trout (*Oncorhynchus mykiss*, Walbaum), and brook trout (*Salvelinus fontinalis*, Mitchill) are often top predators in aquatic systems and feed heavily on larval and emerging insects (Wellborn *et al*., 1996; Carlisle & Hawkins, 1998). These fishes have been introduced to many formerly fishless lakes and streams throughout the world for recreation. Where trout are introduced, a proportion of the biomass that formerly would have emerged and supported terrestrial life is diverted to trout (Progar & Moldenke, 2002).

Within the spatially confined habitats of small lakes, these highly mobile predators can produce strong top-down effects (Vadeboncoeur *et al*., 2003; McCann, Rasmussen & Umbanhowar, 2005). Most past studies have focused on lake systems in high elevations or boreal zones (Carlisle & Hawkins, 1998; Knapp *et al*., 2001; Donald & Anderson, 2003) where predation effects are expected to be strongest. In a study in Yosemite National Park, Knapp *et al*. (2005) found widely varying effects of introduced trout on native lake biota: sites with a relatively low density of fish showed strong fish effects in alpine lakes but not at lower elevation sites. Lower elevation montane lakes often feature greater habitat complexity with differing substrates, more downed wood and aquatic macrophytes (Lacoul & Freedman, 2006) compared to alpine lakes.

Structural complexity may ameliorate predation intensity by providing refugia for insect prey (Gilinsky, 1984; Diehl, 1988, 1992; Carlisle & Hawkins, 1998; McCann *et al*., 2005). Although refuges could reduce direct predator effects, because trout are large and active predators we hypothesised that introduced trout likely still have substantial effects on the abundance and composition of aquatic insects within and emerging from the littoral zone of these lakes. Similar to other lake studies, we predicted effects would be strongest for large-bodied, mobile, and unprotected taxa (Carlisle & Hawkins, 1998; Blumenshine, Lodge & Hodgson, 2000; Nystrom *et al*., 2001; Knapp *et al*., 2005; Venturelli & Tonn, 2005).

Since 2000, the California Department of Fish and Game (CDFG) has reduced the number of wilderness lakes that it stocks by close to half to reduce impacts to native species, especially declining amphibians such as the mountain yellow-legged frog (*Rana muscosa*, Camp) (Knapp & Matthews, 2000) and the Cascades frog (*R. cascadae*, Slater) (Welsh, Pope & Boiano, 2006). However, the benefits of stocking cessation are unclear because fish populations may persist in a high proportion of lakes due to local recruitment (Armstrong & Knapp, 2004). Evidence from national parks in the Sierra Nevada suggests that cessation of stocking is a useful long-term management option for restoring the aquatic invertebrate fauna (Knapp *et al*., 2001, 2005), but short-term benefits of suspending stocking are unclear (Pope, 2008). In specific lake basins, State and Federal agencies have also begun to physically remove introduced fish to more actively improve conditions for sensitive amphibians (Knapp, Boiano & Vredenburg, 2007), but the recovery of the aquatic insect community after these top predators are abruptly eradicated has rarely been studied on the whole-lake scale (Finlay & Vredenburg, 2007). Additional work is also needed to demonstrate that results generalise to other geographical areas.

We conducted a replicated whole-lake experiment in a northern California wilderness area to test the effects of introduced trout at a scale relevant to ecosystem subsidies. Study treatments were matched to the fisheries management practices currently being implemented in California and include (i) continued stocking with non-native trout, (ii) suspension of stocking, and (iii) cessation of stocking and removal of fish. In addition to these three treatments, we also included fish-free lakes as 'reference' sites. We compared the biomass and abundance of emerging aquatic insects at the water surface and the abundance of larval aquatic insects in the benthos among treatment and reference lakes. Thus, we were able to assess the impacts of introduced trout on the aquatic insect community and the recovery of the insects following

implementation of treatments designed to reduce trout effects on native fauna. We also used regression analyses to assess the relative importance of fish and environmental factors on aquatic insect abundance.

Methods

Study area

The study was conducted in the Trinity Alps Wilderness in the Klamath-Siskiyou Mountains of northern California (Fig. 1). No native fishes historically occurred in the lentic habitats of the Trinity Alps Wilderness, while recent surveys indicated that introduced trout occurred in approximately 85% of the lakes greater than 2 m deep (Welsh *et al.*, 2006). For the study, we selected 16 headwater lakes distributed throughout the eastern half of the wilderness (Fig. 1), where the vast majority of lakes are found. All lakes were relatively small (<2 ha), had low recreational use, moderate elevations, and had inflows and outflows with fish barriers so treatments would not be affected by immigration of trout from outside habitats. Four of the 16 selected lakes were naturally fishless and 12 supported introduced trout. The 12 fish-containing lakes had been stocked by CDFG with brook and/or rainbow trout for over 30 years prior to the start of the study. Lakes were between 1896 and 2210 m in elevation, ranged from 0.3 to 1.98 ha, and were between 2.4 and 11.3 m deep (Table 1). Lakes occurred within mixed conifer to sub-alpine habitat zones with common trees being red and white fir, mountain hemlock, lodgepole pine and western white pine.

Study design

The 16 study basins were blocked into four groups of four lakes based on geographic location. Each group contained one historically fishless lake. The manipulative treatments – continuation of stocking, suspension of stocking, and fish removal lakes – were randomly assigned to the three fish-containing lakes in each group (Table 1). Although we tried to match the physical parameters of the fish-containing and fishless lakes, due to prior stocking of nearly all larger lakes, the fish-free sites were smaller [0.64 ha ± 0.2 (mean ± SE)] and shallower (2.67 m ± 0.15) than the fish treatment lakes (1.17 ha ± 0.15 and 5.02 m ± 0.33, respectively, Table 1). We conducted pre-treatment sampling in June–August 2003 and initiated treatments in September 2003. Crews removed trout from the four removal lakes in fall and winter of 2003 and spring of 2004 using multiple repeated gill net sets as described by Knapp & Matthews (1998). The CDFG maintained the fish treatments throughout the study

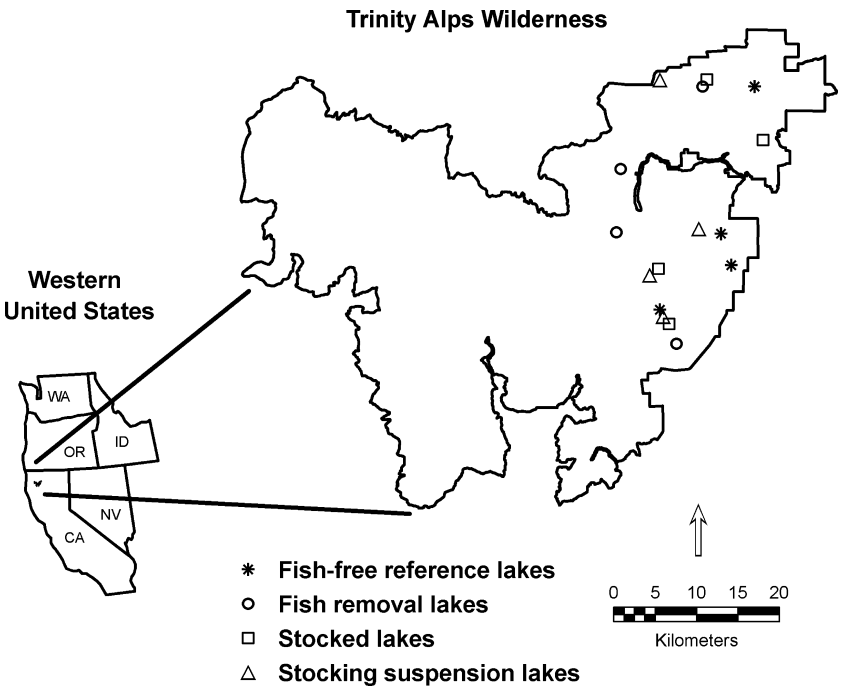

Fig. 1 Map of the Trinity Alps Wilderness study area with lakes differentiated by treatment.

Table 1 Physical parameters of the 16 study lakes

Lake	Block	Treatment	Elev. (m)	Max depth (m)	Area (ha)	pH	Lake temp.	Aq. veg.	Wood	Silt substrate
Eagle creek	1	Reference	1922	2.4	0.33	5.46	1127	0.60	0.15	0.92
Section Line	1	Removal	2182	4.1	0.99	6.38	1006	0.34	0.12	0.68
Mavis	1	Stocked	2042	4.2	1.52	6.88	1079	0.61	0.25	0.80
Hidden	1	Suspended	2050	4.6	1.51	5.99	1053	0.65	0.28	0.59
Found	2	Reference	2088	2.7	1.14	5.79	1060	0.41	0.07	0.60
Adams	2	Removal	1896	4.9	0.67	8.21	1059	0.70	0.19	0.96
Upper Stoddard	2	Stocked	1951	3.5	0.24	6.65	968	0.60	0.29	0.93
Lion	2	Suspended	2135	11.3	1.44	6.12	944	0.51	0.11	0.88
Shimmy	3	Reference	1958	3.0	0.78	6.42	1192	0.50	0.00	0.42
Little Caribou	3	Removal	2191	5.3	1.32	5.90	977	0.48	0.08	0.64
Ward	3	Stocked	2172	7.0	1.98	6.67	1013	0.75	0.15	0.68
Salmon	3	Suspended	2179	4.0	0.66	6.27	880	0.51	0.09	0.84
C26062	4	Reference	2056	2.4	0.31	7.26	764	0.43	0.01	0.83
Echo	4	Removal	2213	5.2	1.30	7.90	719	0.27	0.00	0.64
Deer	4	Stocked	2179	5.8	1.31	8.33	804	0.28	0.01	0.68
Luella	4	Suspended	2117	3.8	0.94	8.74	689	0.31	0.03	0.44

Lake temperature is the sum of the daily average water temperature for the survey period. Values for aquatic vegetation, wood, and fine substrate represent the proportion of littoral zone transects where those habitat features were encountered.

by stocking the stocked lakes yearly with rainbow trout and withholding stocking from the stocking suspension and removal lakes. Brook trout had been stocked in the lakes prior to 2002 and all fish treatments supported brook trout populations at the beginning of the project. Two of the stocked lakes (Mavis and Upper Stoddard) were accidentally left unstocked in 2004. Although these lakes still supported fish, densities were very low in 2004 and 2005 until stocking occurred in mid-summer 2005. Post-treatment sampling was conducted at the 16 study basins every 2 weeks from June to September in 2004 through 2006 (start date was dependent on spring thaw). Six survey trips were conducted during both summers of 2004 and 2005 and five were conducted in 2003 and 2006.

Fig. 2 Crew member collecting insects from an emergence trap.

Biotic variables

Emergence To quantify aquatic insect emergence from the littoral zone of each lake over the course of the summer, we set three 0.6 m diameter floating emergence traps for approximately 40 h at each lake every sampling period. The lightweight, collapsible emergence traps were composed of polyester 'No-See-um' fabric secured to a tension frame dome made from fiberglass rods and floated by an inflated bicycle tire inner tube (Fig. 2). Traps were set on the west side of each lake away from established campsites. One trap was set in silt/emergent vegetation habitat with the shallow side of the trap on the shoreline, the second trap was set in shallow (30–40 cm) water within 3 m of the shoreline over the dominant lake substrate, and the third trap was set near the second trap in about 1 m deep water; substrate was not predetermined for this location. Traps were emptied both mornings of every sampling period by inserting an aspirator into a sleeve in the trap and collecting all insects found (Fig. 2). Samples were preserved in 70% ethanol and taken back to the lab, where they were identified to family, counted and measured. For analyses of

emerging insects, we grouped the insects into four categories: trichopterans, ephemeropterans, dipterans and predators (Odonata, Megaloptera, Coleoptera). We chose these groups based on possible differences in their responses to trout. Most trichopterans have cases that help protect them from predators, ephemeropterans are relatively unprotected, and many aquatic dipterans are infaunal and thus less exposed to trout until emergence. Predatory insects differ from the other groups because they may compete with trout as well as being prey to them.

Benthos Littoral benthic macroinvertebrates were sampled intensively during the 2006 field season. We collected 12, 1 m benthic sweep samples at each lake during all five sampling trips (one fish removal lake was not sampled during the first trip due to a nearby forest fire), using a 30 cm wide D-frame dip net. At four equally spaced transects around the lake shore, three sweeps were taken at approximately 0.1, 0.5 and 1.0 m deep. In the field, insects ≥4 mm were sorted and identified to order except Odonata which was identified to suborder. Within each taxon, individuals were grouped by size classes, counted and measured. For benthic analyses we used the same categories described for the emerging insects but separated the predator group into small predators (<12 mm) and large predators (>12 mm), because trout are visual predators that preferentially prey on large-bodied, active invertebrates (Diehl, 1992; Carlisle & Hawkins, 1998). These size categories were not used for emerging predators because they were all large at emergence. Because benthic insects could be reliably identified in the field, we were able to use non-destructive sampling and returned the insects to the lake following processing.

Trout In mid-summer of all four study years, we sampled trout presence and density at each fish removal, continue stocking, and suspend stocking lake. A single 36 m long, variable mesh, monofilament gill-net was set perpendicular to the shoreline for approximately 4 h during the daytime in each lake. Captured trout were identified to species and counted. Trout densities were estimated as catch per unit effort (CPUE: number of fish captured per hour of net set). A linear regression comparing summer 2003 CPUE values to actual density of fish removed from the four fish removal lakes in the fall of 2003 showed that CPUE and density are highly correlated ($r = 0.97$, $P < 0.01$). No fish other than trout were present in any of the study lakes.

Environmental variables

We recorded water temperature at each study lake every 2 h during the survey season at 0.5 and 1.25 m depth with Onset© (Pocasset, MA, U.S.A.) water temperature loggers. Lake temperatures were quantified by calculating average daily temperatures during the 57 sampling days when temperatures were collected at all lakes and then summing these daily averages for each lake. We also recorded pH with handheld pH meters. Littoral zone habitat characteristics were measured by sampling from approximately 25 evenly spaced transects around the perimeter of each study lake. At each transect, depth, substrate, and presence of aquatic vegetation and large woody debris were recorded at three distances from shore (0.1, 0.5 and 1.0 m). Littoral zone substrate categories were defined by dominant particle size (e.g. fines included silt and sand particles and were <2 mm in diameter, gravels ranged from 2 to 32 mm in diameter; and boulders were >256 mm in diameter). Estimated littoral zone slope was calculated by obtaining the slope of a least-squares line through the three depths at each specific distance from shore. The slopes for each transect were averaged to obtain a mean littoral zone slope for each lake.

Data analyses

We compared abundances of fish, emerging and benthic insects and the biomass of emerging insects among treatments with analysis of variance. We first compared trout densities in the 12 treatment lakes in 2003 to ensure that pre-treatment densities were not significantly different among treatments. We used repeated measures ANOVA to compare catch per net-hour in stocked lakes versus stocking suspension lakes from 2004 to 2006 to see if differences occurred post-treatment. Removal lakes were not included in this analysis because after 2003 we did not catch any trout in the removal lakes during the 4-h gill-net samples. We also compared mean length of trout in stocked lakes versus stocking suspension lakes from 2004 to 2006.

To analyse the effect of the fish treatments on the biomass of emerging aquatic insects, we first converted insect length data to biomass following order-specific length-weight regressions given in Sabo, Bastow & Power (2002). A preliminary model included 2003 biomass (prior to implementation of treatments) and lake area as covariates and average emergent biomass from 2004, 2005, and 2006 as the response variables. The covariates were not significant and were removed from the model for the final analysis. Biomasses were log-transformed to meet ANOVA assumptions.

Separate MANOVAS were run on the emergence and benthic data sets with abundances from each insect group as the response variables to test for differences in relation to the four treatments. When the MANOVAS produced significant results or strong trends ($P < 0.1$), we followed with a series of 'protected' ANOVAS on each response variable to evaluate which variables contributed significantly to the multivariate responses (Scheiner, 2001). We analysed the abundances of emerging insects during the 3 years of the experiment using repeated measures ANOVAS. The 2003 abundance covariate was significant for Ephemeroptera ($P = 0.005$) and the lake area covariate was significant for Trichoptera ($P = 0.02$). These covariates were not significant for other groups and were removed from their models. Benthic data was only collected in 2006. We used repeated measures ANOVAS to compare treatment effects over the course of the sampling season, with survey period as the repeated factor and depth as an additional within-lake predictor. All abundances were log-transformed to meet normality assumptions.

To test for the specific effect of fish removal and stocking suspension on aquatic insect biomass, we included *a priori* contrasts between these treatments and stocked lakes. ANOVAS were consistent with assumptions of homoscedasticity, normality of residuals (with the exception of emerging Ephemeroptera), and additivity between block and treatment effects. Two-tailed *P*-values were used in all instances. Analyses of treatment effects were conducted with PROC GLM and PROC MIXED in SAS 8.0 (SAS Institute, Carey, NC, U.S.A.).

We used multiple regression and ordination to determine the relative contributions of fish density and environmental variables to the abundance of benthic and emerging aquatic insects. We used abundances of the insect groups from 2006 at each lake as dependent variables and density of trout (catch per net hour), pH, lake temperature, and habitat complexity as continuous predictor variables. Predictor variables were selected *a priori* based on existing literature or field observations that suggested their importance in affecting aquatic insect abundances. We created the habitat complexity variable by combining the variables for aquatic vegetation, large woody debris, and substrate in a principal components analysis and using the canonical scores from the first axis, which accounted for 72% of the explained variance. The eigenvectors for the three variables were positively correlated along the first axis so that lakes with a high proportion of aquatic vegetation also tended to have a high proportion of wood and silt substrate. We removed littoral zone slope as a predictor variable due to its high collinearity with lake water temperature ($r = 0.82$).

To assess how the aquatic insect community related to the environmental variables (including fish density), we first ran a multivariate multiple regression on both the emergence and benthic data using canonical correspondence analysis (CCA). Results of the CCAs were tested for relationships between environmental and insect community matrices with 1000 Monte Carlo simulations. If significant ($P \leq 0.05$), we followed with univariate multiple regressions to calculate the relative importance of predictor variables influencing the abundances of insects in the four insect groups. For each group, we ran all-possible-subsets regressions and ranked the 15 models with AIC_c (Burnham & Anderson, 2002). To provide a metric for the importance of each variable in the context of the set of variables considered, we computed Akaike weights for all models within four AIC_c units of the top model and then summed the weights of models containing the particular variable (Burnham & Anderson, 2002). The CCA analyses were performed with PCord 4.0 (MjM Software Design, Gleneden Beach, OR, U.S.A.) and univariate regressions were performed with SAS.

Results

Trout

In 2003, pre-treatment densities of trout were similar in the three fish treatment categories ($F_2 = 0.25$,

$P = 0.784$). We removed 672 trout (94% *S. fontinalis* and 6% *O. mykiss*) from the four fish removal lakes in the fall and winter of 2003 and did not catch trout again in the 2004–06 4-h gill net sets. Across all post-treatment years, stocking suspension lakes did not differ from stocked lakes in terms of trout densities ($F_{1,9} = 1.31$, $P = 0.32$) or mean sizes ($F_{1,9} = 1.28$, $P = 0.3$), although fish densities tended to be greater and sizes tended to be smaller in the stocked lakes than stocking suspension lakes (Pope, 2008).

Emergence

In 2006, 3 years after fish removal, fish removal lakes had substantially greater emerging biomass than stocked lakes, which had the lowest of all treatments (Tukey's HSD: $P = 0.04$). Stocking suspension lakes did not differ from stocked lakes (Tukey's HSD: $P = 0.66$). Emerging insect biomass tended to be greater at removal lakes compared to stocked lakes over all post-treatment years (Fig. 3, $F_{1,9} = 4.07$, $P = 0.07$), even though two stocked lakes showed significant biomass increases in 2005 coincident with a failure to stock those lakes in 2004 and a subsequent reduction in fish densities. Biomass was more variable in reference and stocking suspended lakes throughout the experiment, and including these lakes in the analysis weakened the overall difference between treatments ($F_{3,9} = 2.67$, $P = 0.11$). Predators represented the majority of emerging aquatic insect biomass caught in traps (89%) and over 95% of the emerging predators were odonates, primarily damselflies of the family Coenagrionidae. Aeshnidae, Libellulidae, Corduliidae and Lestidae were also represented as were Megaloptera, family Sialidae. Emerging ephemeropterans were of the family Baetidae and trichopterans were primarily from the families Leptoceridae, Limnephilidae and Sericostomatidae. Midges from the families Chironomidae and Ceratopogonidae made up most of the dipteran group.

Abundance patterns were similar to biomass for emerging insects, with an increasing number of Trichoptera, Ephemeroptera, and predators emerging from the removal lakes compared to the other treatment lakes (Fig. 4). By 2006, more insect predators and Trichoptera emerged from removal lakes than stocked lakes (removal-stocked contrast, $F_{1,9} = 5.97$, $P = 0.04$; and $F_{1,8} = 13.40$, $P = 0.006$, respectively). In contrast, there was a tendency for fish removal lakes to have fewer Diptera emerging than stocked lakes ($F_{1,9} = 3.79$, $P = 0.08$). By 2006, removal lakes did not significantly differ from the reference lakes for any of the individual insect groups (Tukey's HSD: $P > 0.22$ in all cases), although combined abundances of Trichoptera, Ephemeroptera, and predators at the removal lakes surpassed the reference lakes (Fig. 4). There were no differences between stocking suspension lakes and stocked lakes for any of the insect groups ($P > 0.18$ in all cases).

Multiple regression analyses indicated that fish density (CPUE) was the most important predictor of abundances of emerging Ephemeroptera, Trichoptera

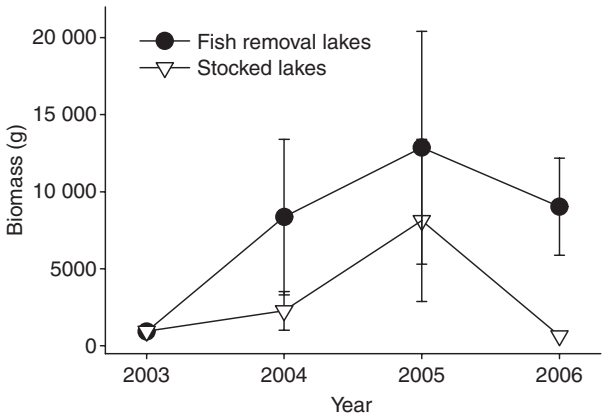

Fig. 3 Mean annual biomass (±SE) of emerging insects that were caught in emergence traps each year for the fish removal lakes and stocked lakes. Two stocked lakes were accidentally not stocked in 2004 with a subsequent decrease in fish density and increase in emerging insect biomass in the 2005 sampling year.

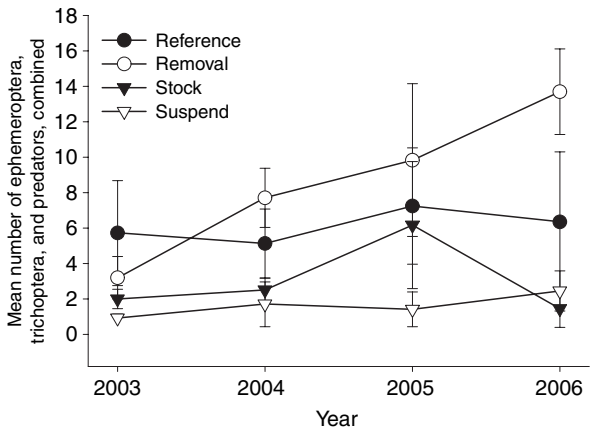

Fig. 4 The combined mean number (±SE) of Ephemeroptera, Trichoptera, and insect predators that were caught in emergence traps each year by treatment category. 2003 was prior to implementation of treatments.

Table 2 Results of all-possible-subsets regressions predicting the abundance of emerging and benthic insect groups

Insect group	Survey	Variable weights				Adj. R^2	No. of models
		Habitat	CPUE	Lake temp.	pH		
Ephemeroptera	Emergence	0.15+	*1.0−	0.19−	0.52+	0.50	6
	Benthic	0.05+	*0.92−	0.10−	0.38+	0.57	5
Trichoptera	Emergence	0.14−	*1.0−	0.11−	0.12+	0.43	4
	Benthic	0.06−	*0.83−	0.45−	*0.50+	0.33	7
Diptera	Emergence	0.11+	*1.0+	0.11−	0.11−	0.46	4
	Benthic	0.18+	*0.49+	0.30−	0.19−	0.13	8
Predators	Emergence	0.36+	0.11−	*0.90+	0.09+	0.45	5
Lg. predators	Benthic	*0.61+	0.16−	0.37+	*0.88−	0.51	7
Sm. predators	Benthic	0.17+	*1.0−	0.15+	0.53+	0.23	6

For each variable, we report the sum of AIC_c weights for all models within 4 AIC_c units of the top model in which the variable occurred. Signs indicate the relationship of the environmental variable with the dependent variable. The adjusted R^2 value is for the top model based on AIC_c. The number of additional models within 4 AIC_c units of the top model is also reported.
CPUE, catch per unit effort.
*Variable was in top model based on AIC_c.

and Diptera, while lake temperature was most important for predicting abundance of predators (Table 2). Fish had a negative effect on the abundance of Ephemeroptera, Trichoptera and predators and a positive effect on the abundance of Diptera. The first two axes interpreted by CCA were significant (axis 1: $P = 0.009$, axis 2: $P = 0.006$) and explained 35% and 18% of the explained variance (70% of the total variance), respectively. The axes were most strongly correlated with fish density and lake temperature (Fig. 5a).

Benthos

The abundance of benthic insects was similarly affected by fish treatment (MANOVA: $F_{15,14} = 2.41$, $P = 0.05$), with significant treatment effects on Ephemeroptera ($F_{3,9} = 3.98$, $P = 0.05$) and Trichoptera ($F_{3,9} = 3.83$, $P = 0.05$). Trichoptera were more abundant in reference lakes than lakes in which stocking was suspended (Tukey's HSD, $P = 0.05$), and the tendency was similar for Ephemeroptera (Tukey's HSD, $P = 0.09$). Neither Diptera nor large and small predators showed differences in abundance among treatments ($P > 0.18$ in all cases). Because we only counted insects ≥4 mm, this analysis did not include most dipterans, especially the small midges that were more abundant emerging from lakes with fish. When we focused on the comparison of fish removal lakes and lakes that continued to be stocked with fish, we found that fish removal tended to increase the abundance of Ephemeroptera ($F_{1,9} = 4.36$, $P = 0.07$), but effects were non-significant for other groups ($P > 0.23$ in all cases).

Although there was no indication that fish treatment affected the overall abundance of larval insect predators, treatments did affect their distribution within lakes. Predators ≥12 mm were more common in deeper water in fish removal lakes compared to stocked lakes (depth by treatment interaction: $F_{3,6} = 3.43$, $P = 0.03$). This group was dominated by Odonata (81%), equally represented by damselflies and dragonflies, with Megaloptera and Coleoptera constituting the remainder.

The first axis of the CCA relating insect abundances to fish and environmental variables was the only significant axis ($P = 0.03$) and explained 49% of the variance. The axis was most highly correlated with fish density ($r = 0.70$, Fig. 5b). Fish density was an important negative predictor of abundances of Ephemeroptera, Trichoptera, and small predators based on the univariate multiple regression models (Table 2). Large predators were most strongly influenced by habitat complexity and pH. They were more abundant in the benthos of complex littoral zones and in lakes with moderate to low pHs.

Discussion

This whole-lake, replicated experiment showed that the presence of introduced trout was the most important factor affecting the emergence of insects from mid-elevation lakes in northern California. The abundance of ephemeropterans, trichopterans and

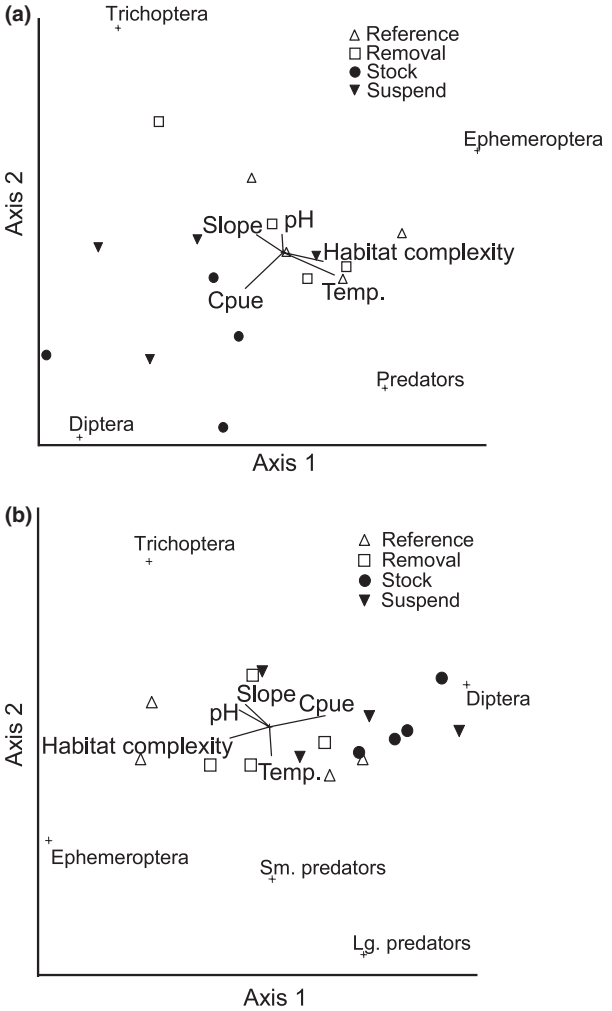

Fig. 5 Ordination of sites along the first two axes of the Canonical Correspondence Analysis regressing (a) emerging and (b) benthic aquatic insect abundances on environmental data, using LC scores. The bi-plot overlay shows vectors related to the environmental variables. The longer the vector, the stronger the relationship with the insect community matrix. The position of the insect groups (+) relative to the environmental vectors can be used to interpret the relationship between insect groups and environmental variables. Axes are scaled by standard deviates.

insect predators emerging from lakes was inversely related to densities of trout. This is important because changes in insect emergence can have cascading consequences for terrestrial communities. For example, Knight *et al.* (2005) found that fish reduced dragonfly emergence with subsequent consequences for terrestrial plants. The overall reduction of insect biomass due to fish is also important for terrestrial predators (McCarty, 1997; Nakano & Murakami, 2001; Power *et al.*, 2004; Fukui *et al.*, 2006; Finlay & Vredenburg, 2007; Marczak & Richardson, 2007). Nakano & Murakami (2001) showed that emerging insects constituted on average 25% of the annual energy budget for riparian birds and up to 90% for some species seasonally, while McCarty (1997) documented strong increases in swallow foraging rates over experimental ponds where insect emergence had increased in response to fish removal. Changes in aquatic insect emergence can have similar effects on bats (Fukui *et al.*, 2006).

When trout were removed from four lakes, overall insect biomass quickly increased compared to lakes still containing trout, and after 3 years the removal lakes surpassed the fish-free reference lakes in abundances of large-bodied insects and biomass of emerging insects. Removal lakes may have surpassed reference lakes because reference lakes were smaller and less complex than the treatment lakes; perhaps such habitats cannot support the same amount of insects as the larger lakes. Alternatively, a lack of alternative vertebrate predators following trout removal may have allowed aquatic insects to reach higher densities in the short-term. For example, fish are negatively correlated with some predatory salamanders in this area (e.g. Welsh *et al.*, 2006). Additionally, the fish removal lakes may have had a buildup of nutrients (Schindler, Knapp & Leavitt, 2001) that fueled better insect breeding and survival in the recovery period.

Even though most lakes had complex littoral zones that might have ameliorated predator effects, our results are consistent with the results of several studies conducted in less complex alpine or high latitude habitats (Carlisle & Hawkins, 1998; Knapp *et al.*, 2001; Nystrom *et al.*, 2001; Venturelli & Tonn, 2005). In general, large-bodied insects were less abundant in lakes with trout, while dipterans, which were predominantly small and occurred in the substrate, tended to be more abundant. The increase in dipterans may be due to decreased competition from larger grazing or detritivorous insects or to predator release if fish preferentially preyed on large-bodied invertebrate predators (Gilinsky, 1984; Blumenshine *et al.*, 2000; Tolonen *et al.*, 2003). Habitat complexity was associated with greater abundance of large predatory insects. However other groups did not show this pattern, leaving trout as the most important driver of insect abundance. These results are important in showing that results of earlier studies

generalise to lower elevations and a different geographical area.

The single year of benthic data showed similar, but not identical patterns of fish effects on large-bodied taxa. Ephemeroptera larvae responded positively to fish removal and Trichoptera larvae tended to be more abundant in lakes without fish, but we did not detect treatment effects on insect predators or dipterans. We did, however, find indications that the presence of trout changed the distribution of large predaceous insects in the benthos. More large predators occurred in shallow water in stocked lakes compared to fishless lakes. This difference in spatial distribution could result from habitat-specific predation rates, behavioral changes to avoid fish, or both (Crumrine, 2006; Wohlfahrt *et al.*, 2006). We did not find similar effects on small predators. This is consistent with previous studies showing that the behavioral avoidance response of older instars is greater than that of young instars (Crumrine, 2006). The lack of significant differences across treatments with regard to abundance of large predators seemed to be influenced by habitat complexity, pH and water temperature. Our multiple regression analyses showed that predator abundances were more influenced by these factors than by trout. We believe that we inadvertently sampled across the altitudinal limits of Odonates because they were far less abundant at higher altitude lakes than at lower altitude lakes. Thus, environmental factors superseded fish treatment effects for the large predator group. Given our low replication and high variability across lakes, we likely lacked power to elucidate the more subtle effects of fish on large insect predators in the benthos of complex littoral zones.

Overall, effects on benthos tended to be slightly weaker compared to emergence, which could be due to the more limited time over which samples were collected or because benthic samples collected early in the season may have contained larvae that would have been eliminated by predation later in the season. Many taxa would be especially vulnerable to fish predators as they leave structural refuges (i.e. caddisfly cases, detritus) or simply move within the aquatic habitat to accomplish emergence. Alternatively, fish effects may have been equally strong in the benthos, but noise in the data due to sampling issues may have contributed to the reduced effects. Sweep sampling is difficult in habitats with woody debris or dense aquatic vegetation.

Recovery of some insect taxa apparently took as little as 1 year in fish removal lakes, and, in general, insect communities in fish removal lakes did not differ from fishless reference lakes at the end of the 3-year recovery period. However, suspension of stocking was not effective in restoring insect abundance or biomass for most of the lakes in our study. The lack of effect was not surprising because stocking suspension did not significantly affect trout density during the experiment. By 2006, however, one of the stocking suspension lakes appeared to have gone fishless. Four times as many large-bodied insects emerged from that lake in 2006 compared to 2003, when fish density was highest. The results of our regression analyses suggest that insect abundances respond to fish density and not simply presence/absence. Additional monitoring will be necessary to reveal if and when fish densities decrease enough in the stocking suspension lakes to see significant changes in aquatic insect populations.

In conclusion, we were able to assess the short-term ramifications of changes in fisheries management practices on aquatic insect subsidies. We showed rapid recovery of large-bodied insect taxa in mid-elevation lakes that were restored to a fishless condition, whereas recovery was slower or absent in lakes where stocking was suspended. The vast majority of mountain lakes in the western United States have been stocked with trout for several decades. Impacts of this large-scale fish stocking effort on aquatic-to-terrestrial ecosystem subsidy have just begun to be assessed (Finlay & Vredenburg, 2007), and insights from in-depth studies following lake restoration to a fishless condition will help scientists and wildlife managers understand the impacts of past actions and the consequences of future management changes.

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