

Indirect effects of introduced trout on Cascades frogs (*Rana cascadae*) via shared aquatic prey

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SUMMARY

1. The introduction of trout to montane lakes has negatively affected amphibian populations across the western United States. In northern California's Klamath–Siskiyou Mountains, introduced trout have diminished the distribution and abundance of a native ranid frog, *Rana* (= *Lithobates*) *cascadae*. This is primarily thought to be the result of predation on frog larvae. However, if trout feed on larval aquatic insects that are available to *R. cascadae* only after emergence, then resource competition may also affect this declining native amphibian.

2. Stomach contents of *R. cascadae* were compared between lakes that contained trout and those from which introduced trout were removed. Total prey mass in stomach contents relative to frog body mass was not significantly different between lakes with fish and fish-removal lakes, but in the former *R. cascadae* consumed a smaller proportion of adult aquatic insects. The stomach contents of fish included larvae of aquatic insects that are, as adults, eaten by *R. cascadae*.

3. *Rana cascadae* consumed fewer caddisflies (Trichoptera) and more grasshoppers (Orthoptera) at lakes with higher densities of fish. At lakes with greater aquatic habitat complexity, *R. cascadae* consumed more water striders (Hemiptera: Gerridae) and terrestrial spiders (Araneae).

4. We suggest that reductions in the availability of emerging aquatic insects cause *R. cascadae* to consume more terrestrial prey where trout are present. Thus, introduced trout may influence native amphibians directly through predation and, indirectly, through pre-emptive resource competition.

Keywords: aquatic macroinvertebrates, competition, introduced trout, *Rana cascadae*, restoration

Introduction

Faced with habitat loss, disease, overexploitation, climate change, pollution and the introduction of non-native species, amphibians have experienced major population declines and extinctions throughout the last

century (Stuart *et al.*, 2004; Wake & Vredenburg, 2008). In high-altitude mountain lakes, amphibians have been severely affected by the introduction of non-native trout (Salmonidae), which are stocked for recreational fisheries (Knapp & Matthews, 2000; Kats & Ferrer, 2003; Welsh, Pope & Boiano, 2006). Direct negative effects of fish introductions, such as predation on native amphibians, are commonly cited (Vredenburg, 2004; Welsh *et al.*, 2006), but indirect effects also have been demonstrated. For example, increased populations of the

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garter snake *Thamnophis atratus* (Kennicott, 1860) are associated with trout introductions in California's Klamath–Siskiyou Mountains (Pope *et al.*, 2008). *Thamnophis atratus* consumes both fingerling trout and amphibians, which may result in increased predation pressure on *R. cascadae* at lakes containing both trout and *T. atratus*.

Introduced trout may also affect amphibians indirectly through competition for prey. In the Sierra Nevada and Klamath–Siskiyou Mountains of California, non-native trout alter the abundance, biomass and emergence of aquatic insects (Knapp, Matthews & Sarnelle, 2001; Finlay & Vredenburg, 2007; Pope, Piovia-Scott & Lawler, 2009). The winged adults are consumed by terrestrial predators, including amphibians, and changes in the supply of aquatic insects have been shown to affect terrestrial consumers in other systems (Nakano & Murakami, 2001; Sabo & Power, 2002a,b; Baxter *et al.*, 2004). Comparing the diets of amphibians at lakes with and without fish could indicate the effects of fish on the consumption of aquatic prey by amphibians.

Using stable isotope analysis, Finlay & Vredenburg (2007) concluded that both mountain yellow-legged frogs (*Rana muscosa* Camp, 1917) and non-native trout rely almost entirely on benthic prey, mainly mayflies (Ephemeroptera) and caddisflies (Trichoptera). Furthermore, the stable carbon isotope signature of *R. muscosa* at lakes with fish suggested a lower contribution of benthic prey and a higher contribution of terrestrial prey. Carbon signatures are valuable, as they can show the relative contribution of various prey to tissue growth. However, stomach flushing allows for a more direct and detailed understanding of the diet and foraging behaviour. For example, stable isotope analysis does not differentiate between consumption of fewer vs. smaller benthic invertebrates, and no information is gained on the taxonomic identity of prey.

Here, we investigated the effect of introduced rainbow trout [*Oncorhynchus mykiss* (Walbaum, 1792)] and brook trout [*Salvelinus fontinalis* (Mitchill, 1814)] on the diet of *Rana cascadae* Slater, 1939 in northern California's Trinity Alps Wilderness. We compared the stomach contents of *R. cascadae* in lakes with trout and in those from which trout had been removed. We also identified the stomach contents of trout in lakes where they co-occurred with *R. cascadae*. Pope *et al.* (2009) reported that trout removal

decreased the abundance of emerging aquatic flies (Diptera), but increased the abundance of emerging mayflies, caddisflies and predatory insects (Odonata, Megaloptera and Coleoptera). Trout removal also increased the biomass of emerging insects. Based on these results, we predicted that the diet of post-metamorphic *R. cascadae* would differ between lakes with and without trout, because of a reduced availability of aquatic prey in the presence of trout.

Methods

Study system

Rana cascadae occurs from the Olympic and Cascade mountain ranges of Washington State southwards to the Cascades of northern California, and in the Klamath–Siskiyou Mountains of California (Pearl & Adams, 2005). The IUCN Red List of Threatened Species classifies *R. cascadae* as near threatened throughout its range (IUCN, 2010). Populations in the Lassen region have declined so precipitously that they are now restricted to only a handful of sites (Fellers & Drost, 1993; Fellers *et al.*, 2008), and populations in the Klamath–Siskiyou have also declined (Welsh *et al.*, 2006; Pope, 2008), though to a lesser extent. These declines have resulted in the listing of *R. cascadae* as a California Species of Special Concern (Jennings & Hayes, 1994).

This study was conducted in the Trinity Alps Wilderness, where mountain lakes were historically fishless because of physical barriers to upstream colonisation. Owing to stocking efforts, approximately 90% of mountain lakes within the wilderness area currently support introduced trout populations (Welsh *et al.*, 2006).

Sampling

We visited 12 lakes in the summer of 2007 as part of a fish-removal experiment (Pope, 2008). Three treatments had been randomly assigned to those 12 lakes: (i) fish-removal, (ii) suspension of stocking, and (iii) continued stocking. Fish had been removed in the autumn and winter of 2003 and spring of 2004 with multiple, repeated sets of gill nets following the methodology of Knapp & Matthews (1998). The remaining eight lakes continued to support trout populations. Study lakes were 3.5–11.3 m deep, 1986–

2191 m in altitude, and ranged from 0.24 to 1.44 ha in area (Table 1).

We sampled stomach contents of *R. cascadae* from all study lakes at which they were encountered, with the exception of one fish-removal lake, which had been recently sampled as part of another diet study (Monty Larson, pers. comm.). The distribution of *R. cascadae* in the Klamath–Siskiyou Mountains is strongly negatively correlated with trout presence (Welsh *et al.*, 2006). Although stomachs of non-native trout were sampled at all eight lakes with fish, we present analysis of fish stomach contents for only the four lakes at which non-native trout and *R. cascadae* co-occurred, to make any comparisons across species more relevant. At the conclusion of the study, we had obtained stomach contents of *R. cascadae* from three fish-removal lakes and four lakes with fish. We did not differentiate between lakes with fish on the basis of whether stocking had been suspended or continued, because insect emergence was not found to differ significantly between these lake types (Pope *et al.*, 2009). Samples within lakes were collected on the same day, and all 12 lakes were visited within a 12-day period (July 18–July 30) to minimise seasonal differences in prey availability and consumption.

Rana cascadae stomach contents were sampled during visual encounter surveys that were conducted at mid-day to maximise detectability (VES; Crump & Scott, 1994). Sampling starting times ranged from 11:25 to 14:05 h and continued until the entire lake perimeter had been surveyed, the time then ranging from 14:31 to 16:40 h. Stomach contents of every individual with greater than 40 mm snout-vent length were sampled at all but one lake, where densities were far higher than other lakes. At that lake, we sampled stomach contents of every other individual encountered. Stomach contents were collected via

gastric lavage using a heavy-duty syringe and 4- to 6-mm-diameter flexible catheter tubing (Solé *et al.*, 2005). Blunt forceps were used to open the mouth during insertion of the tube. If a complete bolus was not acquired after the first flush, two additional flushes were performed. Individuals were released after sampling.

At the four lakes containing both *R. cascadae* and trout, we collected stomachs from trout following a 4-h setting of a monofilament gill net, on the same day that *R. cascadae* were sampled. Of these four lakes, one supported rainbow trout, and the other three supported brook trout. Thus, we could not adequately compare diet between the two species and did not differentiate between trout species in our analysis. Five fish captured in the gill net were haphazardly selected for stomach sampling and were immediately stunned and pithed if not already dead upon net retrieval. Stomachs of these individuals were excised whole. Catch per unit effort (CPUE) was used as a relative estimate of fish density and was defined as the number of fish caught in the gill net per hour.

Stomach contents of trout and frogs were preserved in 90% ethanol and inspected under a dissecting microscope. Prey items were identified to the lowest possible taxonomic level, usually family, and body length of each item was measured. When possible, prey were categorised as terrestrial or aquatic in origin, depending on their larval habitat. Certain prey taxa, such as muscoid flies (some of which burrow in aquatic macrophytes) and parasitoid wasps (a few of which have aquatic insect hosts), have members of both terrestrial and aquatic origin. Many of these prey were not identified to species and thus were considered of uncertain origin. Prey of trout were further categorised by life stage, because larvae, pupae and adults were all well-represented in stomach contents. The majority

Table 1 Fish density and physical parameters of the seven study lakes

Lake	CPUE	Altit. (m)	Max depth (m)	Area (ha)	Aq. Veg.	Wood	Silt substratum
Adams	0	1896	4.9	0.67	0.7	0.19	0.96
Lion	8.75	2135	11.3	1.44	0.51	0.11	0.88
Little Caribou	0	2191	5.3	1.32	0.48	0.08	0.64
Luella	6.75	2117	3.8	0.94	0.31	0.03	0.44
Salmon	4	2179	4	0.66	0.51	0.09	0.84
Section Line	0	2182	4.1	0.99	0.34	0.12	0.68
Upper Stoddard	7.75	1951	3.5	0.24	0.6	0.29	0.93

Fish density [catch per unit effort (CPUE)] based on our sampling, and physical parameters from Pope *et al.* (2009). Values for aquatic vegetation, wood and silt substratum represent the proportion of littoral zone transects in which each habitat feature was encountered.

(96%) of the prey of *R. cascadae* were adult insects, so we did not group their prey by life stage.

The biomass of prey reflects its nutritional value better than the numbers consumed (MacDonald & Green, 1983), but fragmentation and partial digestion of prey precluded accurate measurements of dry mass. The biomass of adult insect prey was estimated from length–mass regressions presented in Sabo, Bastow & Power (2002), and the biomass of larval insects and non-insect prey was estimated from length–mass regressions in Benke *et al.* (1999). We used length–mass regressions for taxa that were closest to the taxonomic level to which prey had been identified. When individual prey items were so fragmented that a measure of body length was impossible, we assumed that the prey item was of average biomass for the appropriate family (when a family-based length–mass regression was used) or order (when an order-based regression was used).

Frequency of occurrence, numerical percentage and percent by estimated dry mass were calculated for each prey type at each lake (Hyslop, 1980). Frequency of occurrence (%O) was calculated as (number of stomachs containing prey type/number of stomachs containing prey) (100%). Numerical percentage (%N) was calculated as (number of individuals of prey type/total number of individuals among all prey types) 100%, and per cent by estimated dry mass (%W) as (estimated dry mass of prey type/sum of dry mass among all prey types) 100%. To reduce bias associated with using any one of these measures, we also calculated an index of relative importance (IRI), substituting mass for volume, as %O (%N + %W) (Pinkas, Oliphant & Iverson, 1971). We report IRI as a percentage to facilitate comparisons among samples and among other studies (Cortés, 1997). Finally, we calculated total relative prey mass as (sum of estimated biomass of prey in stomach/frog body mass) to estimate the total nutritional value of stomach contents for each individual. When individuals were sampled, but no stomach contents were obtained, we assumed that the stomach was empty and total relative prey mass was equal to zero.

Cumulative prey curves

Our intention was to compare frog diet between lakes with fish and those from which fish had been removed, not to describe comprehensively the diet

of *R. cascadae* and sympatric introduced trout, which would require additional sampling to account for intra- and inter-annual variation. Nevertheless, we constructed cumulative prey curves to assess whether we had completely described diet composition during our sampling period. After randomising the order of stomach contents to be analysed, the cumulative number of novel prey types was plotted against the number of stomachs examined. This process was repeated 10 times to reduce bias arising from sampling order (Ferry & Cailliet, 1996). We constructed separate curves for *R. cascadae* from fish-removal lakes and those with fish to assess completeness of diet characterisation for both lake types. To determine quantitatively whether prey curves reached an asymptote, the slope of the line generated by a linear regression on the final four curve endpoints was compared to a line of slope zero using a Student's *t*-test (Bizzarro *et al.*, 2007). The slope of the line generated from the standard error (SE) of the endpoints was similarly tested to determine whether variation stabilised, an indication of adequate precision (Bizzarro *et al.*, 2007).

Our sampling adequately characterised diet for *R. cascadae* overall and at fish-removal lakes. The cumulative prey curve for *R. cascadae* overall reached an asymptote for mean number of new prey items ($t = 3.486$, $P = 0.061$, $CV = 1.64 \pm 1.24\%$) and SE ($t = -0.3946$, $P = 0.731$). When limiting analysis to *R. cascadae* at fish-removal lakes, the prey curve also reached an asymptote numerically ($t = 4.118$, $P = 0.054$, $CV = 1.28 \pm 1.48\%$) and with regard to SE ($t = -0.306$, $P = 0.786$). For frogs at lakes with fish, the curve did not reach a numerical asymptote ($t = 9.0476$, $P = 0.012$, $CV = 2.79 \pm 2.07\%$), but did reach an asymptote with respect to SE ($t = 0.319$, $P = 0.780$). The curve for trout did not reach an asymptote ($t = 12.53$, $P = 0.005$, $CV = 1.73 \pm 1.34\%$), but variation around the mean stabilised at the endpoints ($t = 0.624$, $P = 0.596$) (Fig. 1). Thus, we concluded that we did not completely characterise the diet of trout or *R. cascadae* at lakes with fish; however, we employed a conservative, quantitative method of determining the adequacy of sample size rather than subjective and qualitative criteria more commonly used to interpret cumulative prey curves (Bizzarro *et al.*, 2007). Nevertheless, we restricted our quantitative analysis to coarse comparisons based on prey origin, and our prey-type specific analysis to

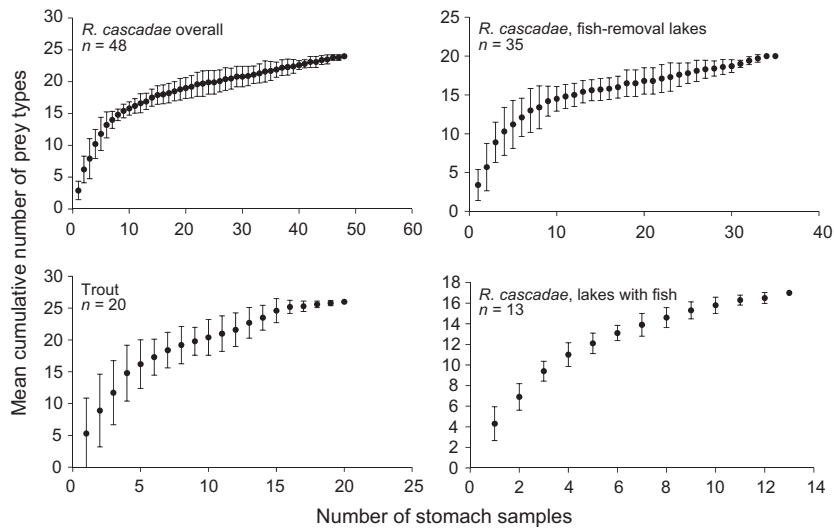


Fig. 1 Mean \pm standard deviation for cumulative number of prey types per stomach sample for *Rana cascadae* overall, separated between fish-removal lakes and those with fish, and for trout. Asymptotes were reached for the curves of *R. cascadae* overall and *R. cascadae* at fish-removal lakes, but not for trout or *R. cascadae* at lakes with fish.

common prey – those occurring in stomach contents at over half of all lakes – that are more likely to be detected given limited sampling (Ferry & Cailliet, 1996). Prey of uncertain origin were excluded to conserve statistical power and ensure interpretability of results.

Statistical analyses

We compared total relative prey mass and the numerical proportion of aquatic prey among fish-removal lakes and those with fish using a linear mixed-effects model. Fish presence or absence was a fixed factor, and lake was a random factor. Because proportions can only vary between 0 and 1, we used an angular transformation on the proportion of aquatic prey response variable (Steel, Torrie & Dickey, 1997). Mixed-effects model analyses were conducted using the nlme package (Pinheiro *et al.*, 2009) in R v2.7 (R Development Core Team, Vienna, Austria).

We used canonical correspondence analysis (CCA) to test whether the number of common prey items consumed per frog was related to fish density (CPUE) and aquatic habitat complexity. Aquatic habitat complexity was chosen based on the finding of Pope *et al.* (2009) that it was an important predictor of the abundance of large insect predators and was constructed in the same manner described therein. Specifically, we used principal components analysis to combine three highly correlated features of the littoral zone (proportion of aquatic vegetation, woody

debris and silt substratum) for each lake (Pope *et al.*, 2009) and used the canonical scores from the first axis, which accounted for 85% of the explained variance, as values for the aquatic habitat complexity variable. CCA was conducted with 1000 Monte Carlo simulations and a significance level of $\alpha = 0.05$. Following significant CCA results, we conducted univariate regressions with fish density and/or aquatic habitat complexity as predictor variables for each prey type to assess the relative importance of each environmental variable. We ranked these models with Akaike's Information Criterion corrected for small sample sizes (AIC_c; Burnham & Anderson, 2002). CCA was conducted in XL STAT version 2010.3.02 (Addinsoft, New York, NY, U.S.A.).

Results

Frog stomach contents

The proportion of aquatic prey in frog stomachs was five times higher in lakes from which fish had been removed than lakes containing fish ($F_{1,5} = 9.4$, $P = 0.03$, Fig. 2). However, total relative prey mass did not differ between lake types ($F_{1,6} = 0.20$, $P = 0.67$). The proportion of individuals with empty stomachs did not differ among fish-removal lakes and those with fish ($t = 0.354$, $P = 0.74$). Fifty-eight individuals were sampled among the seven study sites, and stomach contents were retrieved from the majority (83%, $n = 48$). All aquatic insects found in stomach contents of *R. cascadae* were adults.

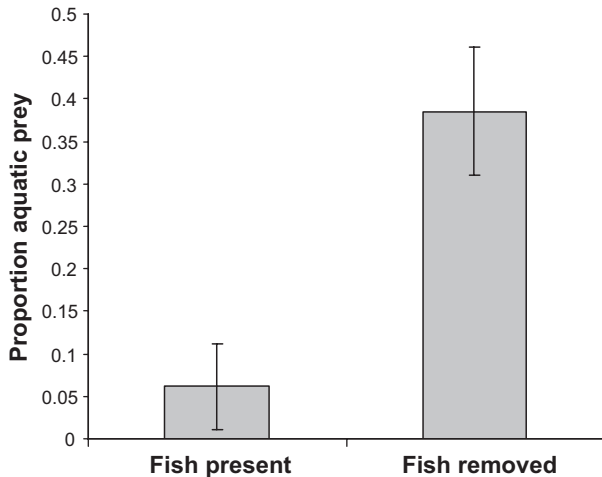


Fig. 2 The proportion of aquatic prey in *Rana cascadae* stomachs at lakes with fish ($n = 4$) and those from which fish were removed ($n = 3$; mean \pm standard error). Individual stomach samples were averaged for each lake.

Trichoptera were the most important prey for *R. cascadae* at fish-removal lakes (39% IRI), while aquatic (including pleustonic) Hemiptera (all of which were water striders, hereafter Gerridae) and terrestrial Lepidoptera were the most important prey at lakes with fish, each accounting for approximately 15% of the total IRI. At fish-removal lakes, Trichoptera, Odonata and Araneae accounted for the highest numerical percentage of prey; terrestrial Hymenoptera (mostly Formicidae), Orthoptera and Araneae accounted for the highest numerical percentage at lakes with fish (Table 2).

In the CCA relating fish density and aquatic habitat complexity to the consumption of common prey, the eigenvalues for the first two axes accounted for 68% and 32% of the explained variance ($P = 0.001$). The first axis was most highly correlated with CPUE (Fig. 3), which best predicted and correlated positively with consumption of terrestrial Hymenoptera (adj. $R^2 = 0.36$) and Orthoptera (adj. $R^2 = 0.72$), and correlated negatively with consumption of Trichoptera (adj. $R^2 = 0.59$). Aquatic habitat complexity best predicted and correlated positively with consumption of Araneae (adj. $R^2 = 0.58$), Gerridae (adj. $R^2 = 0.64$) and Odonata (adj. $R^2 = 0.25$), and correlated negatively to consumption of terrestrial Lepidoptera (adj. $R^2 = 0.35$). Consumption of terrestrial Diptera and Coleoptera was not highly correlated with either variable (adj. $R^2 \leq 0$ and 0.004, respectively) (Table 3).

Trout stomach contents

Trout stomach contents (we did not distinguish between rainbow and brook trout) included immature and adult aquatic insects that were found only as adults in *R. cascadae* stomachs, terrestrial insects that were also consumed by *R. cascadae*, and cladoceran zooplankton. Cladocera, aquatic pupae of Diptera and terrestrial Hymenoptera, which were mostly winged ants (Formicidae), were the most numerous prey in trout stomach contents (Table 4). Terrestrial Hymenoptera and Hymenoptera of uncertain origin, but mostly parasitoid wasps, accounted for the majority (53% combined) of estimated prey mass. Although Cladocera were the most abundant prey numerically, they accounted for only 0.34% of estimated prey mass. Pupae of aquatic Diptera were the most common prey, occurring in 15 of 20 stomachs analysed. Cladocera and aquatic dipteran pupae accounted for the greatest percentage IRI (25% and 22%, respectively).

Discussion

Trout removal increased the consumption of aquatic prey by *R. cascadae*. Non-native trout fed on both immature and adult aquatic insects, but *R. cascadae* consumed only the adults. Thus, at lakes with both trout and frogs, trout are able to consume pre-emptively the potential prey for *R. cascadae* before it has emerged, putting *R. cascadae* (and possibly other terrestrial predators) at a competitive disadvantage for aquatic insect prey. Pre-emptive competition is not the only possible mechanism by which trout could reduce the availability of aquatic insects to *R. cascadae*. For example, adult insects sometimes avoid oviposition in or near waterbodies with fish (Binckley & Resetarits, 2008; Resetarits & Binckley, 2009). In addition, post-metamorphic frogs may reduce the risk of predation or harassment by trout by hunting further away from the shore at lakes with fish.

The lack of a difference in total relative prey mass could be because of a compensatory increase in the consumption of terrestrial prey, which accounted for a considerable portion of fish and frog stomach contents by all measures at both lake types. Given our limited sampling (one visit per lake, one sampling per individual), we cannot conclude whether there is a diet-mediated reduction in fitness for *R. cascadae* in the presence of trout in general. If a difference in total

Table 2 Stomach contents of *Rana cascadae*

Prey type	<i>R. cascadae</i> at fish-removal lakes				<i>R. cascadae</i> at lakes with fish			
	%N	%W	%O	%IRI	%N	%W	%O	%IRI
Araneae ^T	10.11	3.73	34.66	7.30	14.70	7.71	39.17	13.03
Chilopoda ^T	0.69	0.54	4.76	0.24	0.00	0.00	0.00	0.00
Coleoptera ^A	0.00	0.00	0.00	0.00	0.76	0.04	5.00	0.17
Coleoptera ^T	6.76	5.11	29.87	9.46	3.18	0.89	16.25	1.67
Coleoptera ^U	5.16	3.41	12.78	1.61	10.35	17.00	57.92	13.83
Diptera ^A	2.41	1.17	9.09	1.05	0.00	0.00	0.00	0.00
Diptera ^T	3.98	1.19	13.85	1.18	3.42	0.64	19.58	1.25
Diptera ^U	8.31	0.77	33.36	4.48	3.33	0.28	12.50	2.71
Ephemeroptera ^A	0.67	0.06	1.96	0.06	0.00	0.00	0.00	0.00
Gastropoda ^U	0.00	0.00	0.00	0.00	0.76	1.91	5.00	0.58
Hemiptera ^A	6.68	17.43	29.06	14.31	7.88	10.46	32.50	15.09
Hemiptera ^T	1.87	0.22	11.05	0.47	0.00	0.00	0.00	0.00
Homoptera ^T	0.40	0.01	3.03	0.04	0.00	0.00	0.00	0.00
Hymenoptera ^T	8.95	3.81	33.59	5.38	15.86	6.57	62.92	12.08
Hymenoptera ^U	2.16	3.30	12.78	0.95	9.33	5.51	33.33	6.98
Lepidoptera ^T	0.00	0.00	0.00	0.00	6.83	27.48	39.58	14.96
Megaloptera ^A	1.33	0.18	3.92	0.27	0.00	0.00	0.00	0.00
Neuroptera ^T	0.40	0.10	3.03	0.05	0.76	0.11	5.00	0.19
Neuroptera ^U	0.67	0.09	1.96	0.07	0.00	0.00	0.00	0.00
Odonata ^A	10.99	9.53	41.15	12.45	3.03	1.61	5.00	1.02
Orthoptera ^T	1.39	2.62	9.52	1.57	12.71	13.85	51.67	13.21
Phalangida ^T	0.00	0.00	0.00	0.00	2.00	0.93	8.33	0.81
Trichoptera ^A	21.16	46.73	37.33	39.07	2.42	5.00	11.25	2.41
Unknown ^U	5.90	–	13.67	–	2.67	–	14.58	–

Stomach contents of *R. cascadae* averaged among fish-removal lakes ($n = 3$ lakes, 35 frogs) and those with fish ($n = 4$ lakes, 13 frogs) expressed as per cent by number (%N), per cent by estimated weight (%W), frequency of occurrence (%O), and per cent of index of relative importance (%IRI). Superscripts indicate terrestrial, aquatic or uncertain origin.

relative prey mass had been detected, additional questions of seasonality, costs of foraging and the nutritional quality of prey items, biomass aside, would remain.

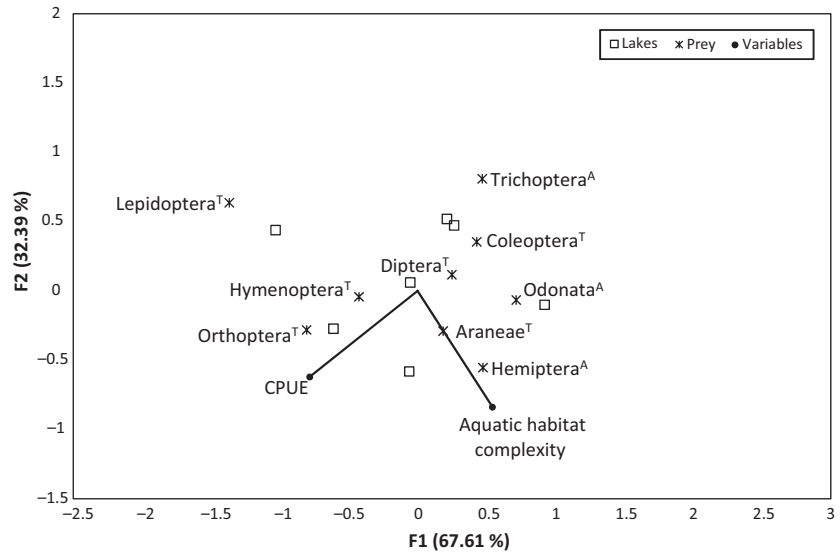
Trout reduce the emergence and availability of adult Trichoptera (Pope *et al.*, 2009), which we found to be one of the most important prey items for *R. cascadae* in fish-removal lakes. Trichoptera did not account for a large part of trout diet overall at the time of our sampling (Table 4); however, two fish stomachs contained only Trichoptera. Such specialisation is consistent with other observations indicating that individuals may preferentially take certain prey items, in some instances for long periods of time (Bryan & Larkin, 1972).

In addition to reducing the abundance of Trichoptera, non-native trout also reduce Ephemeroptera abundance at these sites (Pope *et al.*, 2009). Although poorly represented in *R. cascadae* stomach contents, Ephemeroptera may be important during pulsed

mass-emergence events occurring prior to our sampling.

In contrast to the patterns seen for aquatic insects overall and for Trichoptera, habitat complexity rather than fish density best predicted *R. cascadae* consumption of Gerridae and Araneae. Gerridae accounted for 60% of the aquatic Hemiptera found in trout stomach contents (the other aquatic Hemiptera belonged to the families Corixidae, Notonectidae and Macroveliidae), but trout apparently do not reduce the availability of gerrids to *R. cascadae*. Comparing the per cent IRI values across species at lakes with fish, Gerridae were relatively more important for *R. cascadae* than for trout. Frogs are perhaps better able to capture gerrids, which tend to occur in shallow water near the shore with emergent macrophytes and woody debris that may be more difficult for trout to access. Shrub cover along the lake shore is positively correlated with aquatic habitat complexity at these sites (Pope, unpubl. data) and may provide habitat for terrestrial

Fig. 3 Canonical correspondence analysis of the relationship between commonly occurring prey of known origin found in *Rana cascadae* stomach contents and two environmental variables, fish density (catch per unit effort) and aquatic habitat complexity. The direction of each environmental variable vector indicates the relation to F1 and F2, the first and second canonical axes, which accounted for 67.61% and 32.39% of the variance explained. The length of each vector represents the strength of the correlation between that environmental variable and *R. cascadae* prey consumption. The locations of prey symbols relative to the vectors indicate the strength and direction of the relationship with each vector.



insects that are eaten by Araneae, perhaps increasing spider density and availability to *R. cascadae*.

The positive relationship between fish density and frog consumption of Orthoptera (adj. $R^2 = 0.72$), and to a lesser extent terrestrial Hymenoptera (adj. $R^2 = 0.36$), demonstrates that certain terrestrial prey are consumed in greater numbers at lakes with reduced availability of aquatic insects. Given that these groups are not more abundant along the shore at lakes with fish (Pope, unpubl. data), this finding suggests that frogs may forage further from lake

margins in the presence of fish. If so, then there may be a fitness cost as frogs move away from the lake margin and from an aquatic refuge from terrestrial predators.

Trout may reduce the transfer of energy and nutrients from lakes to terrestrial food webs by reducing aquatic prey availability to *R. cascadae*, a species that is likely to facilitate such transfers because it feeds at the aquatic–terrestrial interface (Ballinger & Lake, 2006). Although the emergence of aquatic Diptera is greater in lakes with fish than fish-removal lakes (Pope *et al.*, 2009), aquatic Diptera were absent from stomach contents of *R. cascadae* at lakes with fish (Table 2). Considering that terrestrial insects, particularly Hymenoptera, were well represented in trout stomach contents, energetic transfers from terrestrial food webs to lakes are also likely to be altered by trout.

Differential digestion and fragmentation affected our ability to identify prey, leading to uncertainty about the origin of some. For example, identification of Coleoptera was often based on the presence of elytra in stomach contents. Based on the fact that *R. cascadae* is not known to forage while completely submerged in water and the abundance of aquatic Coleoptera is negatively related to fish density (Pope *et al.*, 2009), Coleoptera of uncertain origin, which tended to be more abundant in stomach contents at lakes with fish (Table 2), were probably terrestrial. While digestive rates are likely to vary among prey types, affecting the amount of time that prey are identifiable after their consumption, we

Table 3 Results of regressions predicting consumption of common prey by *Rana cascadae*

Prey type	Akaike weights			Adj. R^2	P-value
	CPUE	Aquatic HC	CPUE and Aquatic HC		
Araneae ^T	0.03+	0.94+	0.03	0.58	0.029
Coleoptera ^T	0.63-	0.35+	0.02	0.004	0.358
Diptera ^T	0.48-	0.50+	0.02	≤0	0.514
Hemiptera ^A	0.01+	0.96+	0.03	0.64	0.018
Hymenoptera ^T	0.88+	0.09-	0.03	0.36	0.089
Lepidoptera ^T	0.18+	0.63-	0.19	0.35	0.095
Odonata ^A	0.28-	0.62+	0.1	0.25	0.143
Orthoptera ^T	0.96+	0.01+	0.03	0.72	0.010
Trichoptera ^A	0.94-	0.02-	0.04	0.59	0.027

Akaike weights and directionality of the relationship for each model that was assessed as a predictor for the consumption of each common prey type of known origin for *R. cascadae*.

P-values and adjusted R^2 are provided for the best models, shown in bold, based on AIC_c. Superscripts indicate terrestrial, aquatic origin.

CPUE, catch per unit effort; HC, habitat complexity.

Table 4 Stomach contents of introduced trout

Prey type	Introduced trout			
	%N	%W	%O	%IRI
Araneae ^T	0.21	0.04	25.00	0.09
Cladocera ^A	47.29	0.34	35.00	25.44
Coleoptera adults ^A	0.03	0.11	5.00	0.01
Coleoptera adults ^T	1.29	3.10	35.00	2.34
Coleoptera larvae ^A	0.90	2.82	15.00	0.85
Coleoptera larvae ^U	0.03	0.11	5.00	0.01
Diptera adults ^A	0.35	0.20	20.00	0.17
Diptera adults ^T	0.03	0.06	5.00	0.01
Diptera adults ^U	0.35	0.08	30.00	0.20
Diptera larvae ^A	8.35	1.21	35.00	5.11
Diptera pupae ^A	17.47	1.62	75.00	21.85
Gastropoda ^A	0.03	3.03	5.00	0.23
Hemiptera ^A	0.49	6.74	20.00	2.21
Hemiptera ^T	0.31	0.28	15.00	0.14
Hemiptera ^U	0.03	0.02	5.00	0.00
Homoptera ^T	2.51	0.20	30.00	1.24
Hymenoptera ^T	13.01	34.96	25.00	18.31
Hymenoptera ^U	5.11	18.34	45.00	16.11
Neuroptera ^T	0.14	4.40	10.00	0.69
Odonata adults ^A	0.10	1.98	10.00	0.32
Odonata larvae ^A	1.04	9.34	10.00	1.58
Orthoptera ^T	0.07	2.61	10.00	0.41
Plecoptera ^A	0.03	0.17	5.00	0.02
Trichoptera adults ^A	0.17	7.01	20.00	2.19
Trichoptera larvae ^A	0.28	1.25	20.00	0.47
Unknown ^U	0.35	–	25.00	–

Stomach contents of 20 trout sampled from four lakes with fish where *Rana cascadae* stomach samples were also acquired, expressed as per cent by number (%N), per cent by estimated weight (%W), frequency of occurrence (%O) and per cent of index of relative importance (%IRI). Superscripts indicate prey of terrestrial, aquatic or uncertain origin. Lifestage is specified for taxa that were taken at multiple lifestages; if not specified, insect prey were consumed as adults.

assumed that differences in these rates would be consistent between fish-removal lakes and those with fish and thus would not confound comparisons between lake types.

Aquatic insect assemblages appear to be similarly affected by introduced trout in the Klamath–Siskiyou and Sierra Nevada Mountains: conspicuous aquatic macroinvertebrates are generally less abundant in lakes with fish (Knapp *et al.*, 2001; Finlay & Vredenburg, 2007; Pope *et al.*, 2009). However, terrestrial insects accounted for a large portion of stomach contents for *R. cascadae* at all lakes in this study, whereas Finlay & Vredenburg (2007) found that carbon isotope signatures indicated almost complete reliance on aquatic prey for *R. muscosa* at fishless lakes, and an

increased reliance on terrestrial prey in the presence of trout. Furthermore, terrestrial insects accounted for a large portion of trout stomach contents at our study lakes, but not in the Sierra Nevada, where there is little riparian canopy cover (Finlay & Vredenburg, 2007). The terrestrial zone surrounding lakes in the subalpine environment of the Klamath–Siskiyou Mountains may be more productive than the resource-poor alpine environment surrounding high-altitude lakes in the Sierra Nevada, leading to increased availability of terrestrial insect prey.

Other amphibians that forage in or near lakes in the Klamath–Siskiyou Mountains also experience a reduction in the availability of aquatic insect prey in the presence of non-native trout (Pope *et al.*, 2009). This may not result in a pronounced diet shift for species that spend a considerable time away from lakes, such as the pacific chorus frog [*Pseudacris regilla* (Baird & Girard, 1852)] and western toad [*Anaxyrus* (= *Bufo*) *boreas boreas* (Baird & Girard, 1852)]. Long-toed salamanders (*Ambystoma macrodactylum* Baird, 1849) may be more affected by a loss of aquatic prey because they breed in permanent waterbodies, and their larvae feed on aquatic insects and zooplankton (Anderson, 1968), but *A. macrodactylum* is highly palatable to trout and rarely co-occurs with them (Welsh *et al.*, 2006). For the rough-skinned newt [*Taricha granulosa* (Skilton, 1849)], which is unpalatable (Welsh *et al.*, 2006), reduced availability of aquatic prey raises the possibility of a negative indirect effect of trout because both the larval (Chandler, 1918) and adult (Taylor, 1984) newts feed on aquatic insects. However, the distribution of *T. granulosa* is not related to trout presence (Welsh *et al.*, 2006), suggesting that if such an effect is present, it is not so strong that it drives a distributional response.

Our results also indicate the potential for introduced trout to reduce aquatic subsidies to other (non-amphibian) terrestrial predators, such as birds and bats that forage at or near lake margins. For example, grey-crowned rosy finches (*Leucosticte tephrocotis dawsoni* Grinnell, 1913) in the Sierra Nevada are less abundant at lakes with fish than lakes without fish during the period of mayfly emergence, which coincides with the provisioning of young nestlings and fledglings (Epanchin, Knapp & Lawler, 2009). If terrestrial insects are more abundant and available in the Klamath–Siskiyou, trout-mediated loss of aquatic insect prey may be less likely to drive distributional patterns of terrestrial predators.

A change in the diet of frogs resulting from competition with non-native trout is only one of many effects of trout introductions to previously fishless lakes. Our results highlight the fact that indirect effects of trout introductions in mountain lakes have not been characterised fully, particularly with respect to the impacts of trout on terrestrial consumers of aquatic prey. This lack of understanding could lead to an underestimate or inaccurate assessment of the impacts of non-native trout on amphibians and other terrestrial predators.

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